The Impact of Lifestyle, Age, and Sex on Systemic and Airway Inflammation and Oxidative Stress

by

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B.S., University of Mary Washington, 2009
M.S., Florida Atlantic University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Abstract

The overall aim of this dissertation was to determine the impact of lifestyle (i.e. habitual and acute physical activity and diet), age, and sex on systemic and airway inflammation and oxidative stress. In study 1 (Chapter 2) we examined the impact of habitual physical activity level on the post-prandial airway inflammatory response following an acute bout of moderate intensity exercise. Results indicated that the mean exhaled nitric oxide (eNO; marker of airway inflammation) response increased for all groups at two hours post high-fat meal (HFM) (~6%) and returned to baseline by four hours post-HFM. However, there was a varying eNO response from baseline to four hours in the group that exercised in the post-prandial period compared to the group that remained sedentary. These findings suggest airway inflammation occurs after a HFM when exercise is performed in the post-prandial period, regardless of habitual physical activity level. In study 2 (Chapter 3) we investigated the post-prandial oxidative stress response to meals of varying calories and fat. Specifically, we assessed the post-prandial airway and systemic 8-isoprostane (a marker of oxidative stress) responses to meals with moderate-fat (8.5 kcal/kg of bodyweight) and high-fat content (17 kcal/kg of bodyweight) from baseline to six hours post-meal in a randomized crossover design. This study revealed that systemic 8-isoprostane increased from baseline to six hours post-meal (38.3%), but there was no difference between the moderate-fat meal (MFM) and HFM conditions. There were no changes in airway 8-isoprostane from baseline to six hours post-MFM or HFM, or between the MFM and HFM conditions. Lastly, in study 3 (Chapter 4), we were interested in examining 8-isoprostane responses in older adults, since 8-isoprostane has been reported to increase with age. Previous research also suggests that older women (OW) and older men (OM) have differences with regard to prevalence and severity of late-onset asthma. In this study, we sought to determine whether

the airway 8-isoprostane response to a strenuous bout of exercise was different in OW compared to OM. A secondary aim was to determine whether post-exercise 8-isoprostane generation was correlated with decrements in lung function. Our results showed that the generation of 8-isoprostane from pre- to post-exercise increased ~74±77% in OW and decreased ~12±50% in OM. The decrease in 8-isoprostane generation was not correlated with improvements in lung function from pre- to post-exercise. These findings collectively contribute to the literature by enhancing our understanding of the impact of lifestyle factors, age and sex on modifying and potentially mitigating the risk of developing chronic diseases.

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Approved by:

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Acknowledgements

The first person I have to thank immensely is my advisor, Dr. Craig Harms. When I came to Kansas State University originally as an instructor with little research experience, you took me on as your PhD student when I wanted to return full-time to graduate school. Thank you for taking a chance on me, letting me figure things out for myself along the way, and for being the best role model in both the personal and professional aspects of life. Next, I need to give my most heartfelt thank you to Dr. Sara Rosenkranz. Your passion for science and mentoring students has rubbed off on me and enabled me to succeed. Thank you for your daily enthusiasm, thorough edits, collaboration, mentorship, and friendship. Next, thank you to my dissertation committee, Drs. Rozell, Barstow, Harms, Rosenkranz, and Fong, for their feedback and help through this process. Also, many people opened the doors to their offices and/or laboratories to help in many ways, thanks to Drs. Barstow, Chapes and Levitt. A special thanks to an amazing group of faculty and graduate students in the Departments of Kinesiology and Food, Nutrition, Dietetics and Health. I have had so much fun living my academic life with you! Sam Emerson, thanks for spending the past years discussing research ideas and finding things to laugh about daily. Also, a special group of people who made this process easier through friendship and collaboration, thank you to Brooke Cull, Colby Teeman and Josh Smith.

Allison Kurti, thank you for your selfless guidance. You have always had the most wonderful gift for writing, and because you are the older sister, your experiences in Academia have led the way. You have been the strongest female role model, and you have always given advice and a listening ear to make sure I had the best chances of succeeding. Next, thank you to my amazing fiancé, Matt Sidener, who is as proud of me as I am of myself. Without your daily support, kind words, and heartfelt hugs, the process would have been a lot less fun and a lot more stressful. You have been my greatest blessing.

Additionally, I lost several people close to me along this journey. My grandma Betty was so proud of my sister and me, and saved every paper we published. Whenever I am nervous or anxiety-stricken, her memory and spirit bring me comfort and ease. We miss her fiercely. Finally, the two people that supported me with all the love in this world, but just as importantly, taught me from a young age that when you start something, you finish it; thank you to my parents Dr. Dave and Wendy Kurti. Completing my PhD and being internally motivated to have a productive experience took discipline, which is a vital quality to success that I learned from

you both. Above all else, thank you for having faith in me in everything I have chosen to pursue. Sometimes, when I lost faith in myself, I chose to remember how much you two believe in me.

Dedication

I dedicate these words to my older sister, Allison Kurti. At the time you read this, it will be too late to get out your red pen. Still, your words, written and spoken, have lifted me up, made me laugh, motivated and inspired me.

I also dedicate these words to every teacher, mentor, professor, friend, and family member that have reminded me to always follow my heart. That advice has never let me down.

Chapter 1 - Introduction

Overview:

Many factors may influence the risk of developing respiratory disease, including environmental triggers and genetic predisposition. Additionally, lifestyle factors such as high-fat diets and physical inactivity increase the risk for developing respiratory diseases, including asthma (1, 2). The rates of asthma have increased to over 10% in Westernized countries over the past few decades (3), and may be due, in part, to the typical diet that is calorically dense and nutrient poor (4). Another contributing factor, beyond poor dietary choices, is the alarming statistic that only 5% of adults meet the American College of Sports Medicine Physical Activity (PA) guidelines of accumulating greater than or equal to 150 minutes per week of moderate-to-vigorous PA or 60 minutes of vigorous PA per week (5). In addition to low PA levels and poor nutrition, aging is a risk factor for the development of respiratory diseases. Specifically, the development of late-onset asthma has been reported to be more common and severe in post-menopausal women compared to age-matched men (6). The experiments included in this document focus on elucidating the impact of various combinations of lifestyle factors (including acute and chronic exercise and meals of varying caloric and fat content), as well as age and sex on airway and systemic inflammation and oxidative stress. Inflammation and oxidative stress have been shown to elicit structural changes in the airways (7), which may lead to the development of asthma and other respiratory complications.

High-fat Meals and the Airways

Our laboratory has shown that a single high-fat meal (HFM) increases airway inflammation (8), and this has been confirmed in both asthmatics (9) and non-asthmatics (10). Findings from previous research indicate that pulmonary function does not decline post-HFM in

healthy subjects, however previous researchers have reported that repeated HFMs might increase the likelihood of developing asthma (11). In fact, in the United States and other Westernized countries, the prevalence of asthma cases have been increasing, which may be partly due to the calorically dense and nutrient poor content of the food consumed (3). The poor nutritional content of the Westernized diet may also be a factor in the increasing rates of asthma and obesity in the same individual (12). Due to the alarming rise in asthma prevalence, researchers have utilized a variety of different methods to attempt to attenuate the post-prandial increases in airway inflammation and oxidative stress, potentially mitigating the risk for asthma development. Specifically, researchers have utilized dietary supplementation (i.e. fish oils) to modify the post-prandial airway inflammatory response (13), as well as other therapeutic strategies (14), to reduce systemic and airway inflammation. Since exercise is a natural anti-oxidant and anti-inflammatory, our laboratory was interested in determining whether either an acute bout of exercise or habitual PA level influenced the post-prandial inflammatory response to a high-fat meal.

Exercise: A Natural Anti-oxidant and Anti-inflammatory

Chronic exercise training increases antioxidant compounds and enzymes that may combat reactive oxygen species (ROS) production, resulting in less oxidative stress and reduced oxidative damage (15). In addition to the higher antioxidant status in active individuals, Merrill et al. (1989) reported that exercise-trained individuals display an attenuated lipemic response post-HFM compared to untrained individuals (16). Furthermore, even an acute bout of exercise from 16 hours pre- to 1.5 hours post-HFM may also result in an attenuated post-prandial lipemia (PPL) (17). However, the impact of acute exercise on post-prandial airway inflammation in chronically active versus insufficiently active individuals has not been investigated. Considering

habitual physical activity is a natural anti-oxidant and anti-inflammatory, and an acute bout of exercise may reduce PPL, we sought to ascertain the impact of a single bout of moderate intensity exercise on the post-prandial lipemic and airway inflammatory response to a HFM in participants who were either physically active or insufficiently active. This project is included as the first project of my dissertation (Chapter 2).

Assessment of Airway inflammation and Oxidative Stress

Our laboratory has previously assessed post-prandial changes in exhaled nitric oxide, which is a validated measure to non-invasively assess airway inflammation. In Chapter 2 of this dissertation, we have added to the existing literature by reporting which inflammatory processes were involved in the post-prandial increase in airway inflammation by quantifying neutrophils and eosinophils via sputum cell differentials. There has been considerable debate about the possible post-prandial mechanisms inducing the increase in exhaled nitric oxide. There is currently a controversy in the literature regarding which mechanisms are activated to elicit an increase in eNO. Specifically, possible mechanisms leading to the increase in eNO may be either toll-like receptor 4 (TLR4) dependent or TLR4-independent (18, 19). Previous research has shown that lipolysaccharide (LPS) may increase TLR messenger RNA expression and lead to an increase in sputum neutrophils (9). Yet there are existing data that suggest that dietary fats may lead to an upregulation of I-kappa B kinase beta and nuclear factor kappa B (NFkB) due to the post-prandial increase in reactive oxygen species (ROS) (20). The TLR4-independent pathways converge on the inducible nitric oxide synthase (iNOS) pathway, which may increase eNO post-HFM (21). Therefore, it is possible that both LPS and ROS will contribute to post-prandial airway inflammation. While airway inflammation transiently increases and then returns back to

baseline by 4 hours post-prandially, no existing studies have investigated the airway and systemic oxidative stress response to a high-fat meal.

The gold-standard for assessing oxidative stress in the lungs and airways is 8-isoprostane, which is associated with the development and progression of asthma (22). In addition, the increase in 8-isoprostane in the systemic circulation may impair flow-mediated dilation (23). Also, repeated high-fat meals are associated with increased risk of developing cardiovascular disease (24). However, in the assessment of PPL, many researchers have used meals that are much larger than the typical individual consumes, with acute high-fat meal challenges that may contain greater than 1000 kcals. There are no existing studies, to our knowledge, which assess the post-prandial airway and systemic 8-isoprostane responses to meals of varying caloric and fat content. Therefore, with the second study of this dissertation, we were interested in determining the airway and systemic 8-isoprostane responses to a typical HFM and a more "true-to-life" moderate fat meal (MFM. In addition, we sought to determine whether PPL was associated with airway and systemic 8-isoprostane in the HFM and MFM condition (Chapter 3).

Aging and Cardiopulmonary Disease Risk

In the two prior studies included in this dissertation, we used a HFM challenge as a stimulus to transiently increase inflammation and/or oxidative stress. However, an acute bout of strenuous exercise is also a physiological stressor that transiently increases oxidative stress generation (24). Consequently, older adults may experience cardiopulmonary complications after activities that require strenuous exertion (25). These activities may include lifting a heavy suitcase, mowing the lawn, shoveling snow from the driveway, or other common actions performed in daily life. Throughout the aging process, older adults are at an increased risk for developing pathologies, including chronic obstructive pulmonary disease and late-onset asthma

(6). Risk for developing these diseases differs by sex (26), with asthma prevalence and severity more commonly reported in post-menopausal women compared to age-matched men. While many mechanisms may be responsible for increasing the risk of respiratory disease development in post-menopausal women, we were interested in investigating sex differences in airway 8-isoprostane generation after a strenuous bout of exercise in older adults (Chapter 4). In addition, we were interested in determining whether the changes in generation of 8-isoprostane from pre- to post-exercise were associated with changes in lung function.

The studies included in this dissertation were designed to contribute to the existing literature by elucidating the impact of lifestyle factors (i.e. habitual and acute physical activity and diet), age and sex on airway and systemic inflammation and oxidative stress. Ultimately, these experiments were designed to enhance understanding of the role of lifestyle factors, age, and sex on disease development. In addition, we sought to add to the existing literature by contributing methods to potentially mitigate the risk of developing various cardiorespiratory respiratory diseases. Each chapter in this dissertation represents a separate study (Abstract, Introduction, Methods, Results, Discussion, Conclusion and References).

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Chapter 2 - Does chronic physical activity level modify the airway inflammatory response to an acute bout of exercise in the post-prandial period?

Now in publication as Kurti SP, Rosenkranz SK, Chapes SK, Teeman CS, Cull BJ, Emerson SR, Levitt MH, Smith JR, Harms CA. Does chronic physical activity level modify the airway inflammatory response to an acute bout of exercise in the post-prandial period? *Applied Physiology, Nutrition and Metabolism* 2017, 42(2): 173-180, 10.1139/apnm-2016-0335

Abstract

Recent studies have confirmed that a single high-fat meal (HFM) leads to increased airway inflammation. However, exercise is a natural anti-inflammatory and may modify post-prandial airway inflammation. The post-prandial airway inflammatory response is likely to be modified by chronic physical activity (PA) level. **PURPOSE**: To investigate whether chronic PA modifies the airway inflammatory response to an acute bout of exercise in the post-prandial period in both insufficiently active and active subjects. **METHODS**: Thirty-nine non-asthmatic subjects (twenty active (ACT), 13M/7F) who exceeded PA guidelines ($\geq$150 min moderate-vigorous PA/week) and nineteen insufficiently active ((IN), 6M/13F) underwent an incremental treadmill test to exhaustion to determine VO_2peak. Subjects were then randomized to a condition (COND), either remaining sedentary (CON) or exercising (EX) post-HFM. Exercise was performed at the heart rate corresponding to 60% VO_2peak on a treadmill one-hour post-HFM (63% fat, 10kcal/kg bw). Blood lipids and exhaled nitric oxide (eNO: marker of airway inflammation) were measured at baseline, 2 h and 4 h post-HFM. Sputum differential cell counts were performed at baseline and 4 h post-HFM. **RESULTS** The mean eNO response for all groups increased at 2 h post-HFM (~6%) and returned to baseline by 4 h (p=0.03). There was a time*COND interaction (p=0.04), where EX had a greater eNO response at 4 hours compared to CON. Sputum neutrophils increased at 4 hours post-HFM (p<0.05). **CONCLUSION**: These findings suggest that airway inflammation occurs after a HFM when exercise is performed in the post-prandial period, regardless of habitual activity level.

Introduction

High-fat diets may lead to respiratory complications (Butler et al. 2006), as well as an increase in risk of developing asthma (GINA Executive and Science Committees, 2007). Even after a single high-fat meal (HFM), airway inflammation (measured via exhaled nitric oxide (eNO) (Rosenkranz et al. 2010; Johnson et al. 2015)) and sputum neutrophils increase in non-asthmatic individuals (Kurti et al. 2015). Researchers have also reported an increase in airway inflammation in asthmatic individuals (Wood et al. 2011**)**. The increase in airway inflammation that occurs post-HFM may be due to mechanisms that are both toll-like receptor-4 (TLR4) independent and TLR4-dependent (Zhang et al. 2005; Teng et al. 2014). Previous research has shown that lipopolyssacharide (LPS) may increase TLR messenger RNA (mRNA) expression and lead to the increase in sputum neutrophils (Wood et al. 2011). However, there is also evidence that suggests that dietary fats may increase reactive oxygen species (ROS), leading to the upregulation of I-kappaB kinase beta and nuclear factor kappa-B (NFkB) (Kim and Sears 2010). These pathways converge on the inducible nitric oxide synthase (iNOS) pathway and may increase eNO (Gaston et al. 1994) post-HFM. The increase in eNO may also be reflective of eosinophilic airway inflammation, however changes in eNO and the percentage increase in eosinophils may not be strongly associated with each other in non-asthmatics (Berlyne et al. 2000). Therefore both LPS, as well as ROS may contribute to post-prandial airway inflammation.

Exercise is both a natural antioxidant and an anti-inflammatory agent, and an acute bout of physical activity may be a potential method for attenuating the post-prandial airway inflammatory response to a HFM. However, post-prandial HFM modifications in airway inflammation will likely be dependent on energy expenditure during and in response to the bout

of physical activity, as well as the participant's chronic physical activity level. A single bout of exercise has been shown to reduce post-prandial lipemia (PPL) (Gill and Hardman 2003; Freese et al. 2014). However, results have been conflicting, likely because energy expenditure may need to be high in order to elicit reductions in lipemia. In a review written by Katsanos and Moffatt (2004), the authors estimated that between 600 and 700 kcals may need to be expended to produce an attenuated lipemic response. However, physically inactive individuals may achieve a reduction in lipemic response to a HFM with a smaller energy expenditure compared to physically active individuals (Katsanos and Moffatt 2004; Brandauer et al. 2013). In fact, an energy expenditure as minimal as 300 to 500 kilocalories may elicit a reduction in lipemia in inactive participants when performed without caloric replacement following the exercise bout (Murphy et al. 2000).

While an increase in lipemia has been reported to increase oxidative stress systemically (Tushuizen et al. 2006), physically trained individuals have increased antioxidant defenses to combat oxidative stress (Gomez-Cabrera et al. 2008). We recently reported that active, non-asthmatic individuals experience airway inflammation post-HFM, and an acute bout of exercise does not modify this response (Kurti et al. 2015; Johnson et al. 2015). However given that ROS can increase after a single HFM (Mohanty et al. 2002; Tushuizen et al. 2006) and may result in airway inflammation, individuals who are insufficiently active, with reduced antioxidant defenses, may have a greater magnitude of the cellular oxidative stress and inflammatory response to a HFM as compared to physically active individuals.

Therefore the current study sought to determine whether chronic PA modified the airway inflammatory response to an acute bout of exercise in the post-prandial period. We hypothesized that the reduction in airway inflammation after post-prandial exercise would be more apparent in

insufficiently active participants (<150 minutes of moderate to vigorous PA per week)) compared to active (≥150 minutes of moderate to vigorous PA) participants.

Methods

Subjects

Forty participants between the ages of 18 and 45 were recruited for the study. Participants were separated by chronic physical activity level by guidelines in the Physical activity guidelines advisory committee report (2008). If participants were meeting or exceeding PA guidelines (acquiring greater than or equal to 150 minutes of moderate to vigorous PA/week), they were placed in the active group (ACT). If they were not, they were placed into the insufficiently active (IN) group. The amount of PA per week was determined by International Physical Activity Questionnaire (IPAQ) screening tool. Physically active participants (13M/7F: age: 24.3±5.5 years) and insufficiently active participants (6M/13F: age: 25.9±5.4 years) completed the study. There was one dropout due to dislike for the HFM and inability to complete the protocol in the time allotted for meal consumption. Prior to the study, all subjects completed a medical history questionnaire to ensure they did not have any cardiovascular disease risk factors, were non-smokers and non-asthmatics. Subjects were excluded if they were taking any medications for heart respiratory, or metabolic diseases. Before the protocol was performed, subjects were briefed regarding the purpose of the study and written and verbal consent were obtained. The study was approved by the Institutional Review Board at Kansas State University and conformed to the ethical principles set forth in the *Declaration of Helsinki.*

Experimental Design

A pretest-posttest parallel groups design was utilized, and all participants visited the laboratory two times with one week in between sessions. The full experimental protocol has been previously published and is outlined here briefly (Kurti et al. 2015; Teeman et al. 2016).

Session 1: Initial Measurements

During the initial visit, subjects completed the required paperwork including a medical history questionnaire and the international physical activity questionnaire (IPAQ). This questionnaire has been validated to assess PA by requiring subjects to recall their PA behavior for the previous seven days (Dinger et al. 2006). The PA is categorized into sedentary time, walking, moderate or vigorous activity each day and is representative of a typical week of PA. Subjects then underwent initial measurements, including height, weight, dual X-ray absorptiometry (DEXA) scan and pulmonary function testing. Subjects then performed an incremental exercise test to exhaustion in order to determine peak aerobic capacity (VO_2peak) as well as to identify the duration of the acute exercise bout if randomized into the exercise (EX) group. Following the exercise test, insufficiently active and physically active subjects were randomized into either the post-meal exercise condition (IN EX or ACT EX, respectively) or the no exercise condition, in which subjects remained sedentary in the post-meal period (IN CON or ACT CON).

Experimental Measurements at session 1: Initial assessment

Anthropometrics

Anthropometrics were assessed according to standard guidelines. Height was measured to the nearest 0.1 cm with a portable stadiometer (Invictus Plastics, Leicaster, England) and weight was measured to the nearest 0.1 kg with a digital scale (Pelstar LLC, Alsip, IL, USA). A DEXA

scan was then performed to assess body composition, including body fat percentage and lean mass (GE Lunar Prodigy, Madison, WI, USA).

Pulmonary function testing

Standardized pulmonary function tests, according to American Thoracic Society criteria, were performed to ensure that subjects were non-asthmatic and had normal pulmonary health (MIR Winspiro Pro, Waukesha, WI, USA). Subjects completed the standardized pulmonary function tests evaluated by the maximum flow volume loop (MFVL) (Miller et al. 2005). The MFVL assesses peak expiratory flow (PEF), forced vital capacity (FVC), forced expiratory volume in 1-s (FEV_1), and forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$). The MFVL was performed until three attempts were within 10% of one another and the highest values derived from the MFVL were used for analyses. An acceptable MFVL was achieved if the subject refrained from coughing, refrained from hesitation, no extra breath was taken, did not inhale too early, there was no obstruction or leak in the mouthpiece, or the expiratory volume (FEV_1) was <5% of the FVC or within 0.150 L, whichever was greater. Percent of predicted values were calculated according to reference values (Knudson et al. 1983).

Incremental test to exhaustion (peak aerobic oxygen consumption: VO_2peak)

An incremental test to exhaustion was performed on a treadmill (Precor 932i) using a standardized protocol for establishing peak oxygen consumption (VO_2peak) previously utilized in our laboratory (Kurti et al. 2015; Teeman et al. 2016). During the test, heart rate (HR) was monitored using a Polar WearLink Coded chest strap HR monitor, and rating of perceived exertion (RPE) was reported using a modified Borg scale (0-10) to assess effort at the end of each stage. Throughout the entire test, metabolic and ventilatory data were recorded by breath-by-breath analysis (Parvomedics TrueOne 2400 Metabolic Cart, Sandy, Utah). At the end of each

stage and completion of the protocol, HR and VO_2 data were recorded. Thirty seconds into each stage, RPE was recorded. The VO_2peak test started at a 2% incline at the subjects perceived fastest 5k pace per mile. Every 2 minutes the speed increased by 0.5 miles per hour (mph), and increased 1% incline and 0.5 mph every two minutes following the third stage. The test was terminated when the subject could not maintain the speed and chose to stop the test. All subjects reported an RPE indicating they perceived their work to be very hard (greater than 7 on the 1-10 Borg scale), and achieved a HR at termination of the test that was within 10 beats per minute of their age-predicted maximum HR.

Experimental measurements performed at session 2: High-Fat meal testing session

High-fat meal

Thirty-nine of the forty subjects initially recruited were able to finish the HFM testing session, with one dropout who did not finish the entire HFM protocol. The HFM consisted of a Jimmy Dean's Meat Lover's breakfast bowl (10kcal/kg/body weight: 63% fat, 15% carbohydrate). The nutritional make-up of the meal was 460 calories per bowl, 33 grams of fat, 13 grams of saturated fat, 265 milligrams of cholesterol, 17 grams of total carbohydrate and 24 grams of protein. Subjects had 20 minutes to consume the HFM, and time began from the first bite of the meal. Time for the session began at the last bite of the HFM. Energy consumed was determined according to subject body weight, and ranged from 450 to 1113 kcals. Calories from fat ranged from 280 to 700 fat kcals.

Exercise bout

One hour following the last bite of the HFM, the subjects randomized to the EX group completed a bout of treadmill exercise (a brisk walk on a moderate incline) at HR equal to 60% VO_2peak to expend half of the calories they consumed from the HFM. Exercise time ranged

from 25 to 70 minutes in participants randomized to the EX condition. The subject who exercised for more than one hour interrupted their exercise bout to perform the 2-hour blood draw, and then completed their last 10 minutes of exercise.

Exhaled nitric oxide

The measurement of exhaled nitric oxide via chemiluminescence is a validated method for assessment of airway inflammation (Borland et al. 1993) (Sievers Nitric Oxide Analyzer 280, Sievers Instruments Inc, Boulder, CO, USA). Measurements were performed before the HFM, and at two hours and four hours post-meal. The test was performed according to American Thoracic Society guidelines (Kharitonov et al. 1997). Subjects were instructed to sit up straight with their feet flat on the floor without wearing a nose-clip. Subjects first performed a maximal inhalation, and then performed a steady exhalation that lasted approximately 6 seconds at a constant flow rate while data were recorded in real time. Testing was performed three times, with measurements within 5% of one another, and the average value was used for analysis. Exhaled nitric oxide data were positively skewed and required a square root transformation of the raw data prior to analysis.

Sputum induction, processing and analyses

Twenty-one of the thirty-nine subjects (53.8%) performed sputum induction to confirm which inflammatory process was involved in the post-prandial airway inflammatory response. Previous research has reported that an allergen-induced T-helper 2 (T_H2) immune activation increases the influx of eosinophils into the airway, whereas a neutrophilic pattern is caused by an innate immune dysfunction (Gleich 2000; Simpson et al. 2006; Simpson et al. 2007). Therefore both eosinophils and neutrophils were measured in the subset of subjects that underwent sputum induction to confirm which inflammatory processes were involved. Hypertonic saline (5%) was

administered using an ultrasonic nebulizer (Omron Healthcare, Lake Forest, IL, USA) with an aerodynamic diameter of 5µm and a 0.7 mL/min output. The same methods for sputum induction have been previous published in work from our laboratory (Kurti et al. 2015). The test was completed when a selective plug could be obtained, or was terminated if the subject was not able to expectorate within 30 minutes of breathing in the nebulized saline (Pin et al. 1992**)**. Processing of sputum samples was completed within two hours of induction, and processing methods for these samples from our work have also been previously published (Kurti et al. 2015). Briefly, treatment of samples was performed with 0.1% dithiothreitol (DTT) in diluted water (4 times by weight) as well as phosphate-buffered saline (PBS). The typan blue exclusion method was performed to assess cell viability and counts were done on the hemacytometer. The remainder of the filtrate was resuspended, and then three cytospins (Shandon CytoSpin 2) were prepared with 100-150 µL sample at 500 rpm for 15 minutes. A modified Wright's stain (Harleco Hemacolor, Gibbstown, NJ) was used to stain slides, and all were then mounted with Permount (Fisher Scientific, Fairlawn, NJ). Salivary contamination was evaluated using methods published by Pizzichini et al. (1996). A second investigator blinded to condition performed sputum cell differentials, and differentials were performed as according to previously validated methods (Telenga et al. 2012; Kurti et al. 2015).

Blood sampling

An intravenous catheter was inserted into the antecubital vein and blood samples were drawn during fasting before the HFM, and at 2 hours and 4 hours post-meal. Blood samples were assessed for triglycerides. Triglycerides were significantly different at baseline between ACT and IN, and were not normally distributed. Triglycerides at baseline were added as a covariate and were log transformed prior to analysis. Methods for the assessment of triglycerides have

been previously published (Kurti et al. 2015; Teeman et al. 2016). The current study was part of a larger study, and the purpose of the present study was to determine the airway inflammatory response post-HFM, therefore all blood lipid (other than triglycerides) and glucose values for these subjects can be found in Teeman et al. (2016) and are not reported here.

Statistical Analyses

Data were analyzed using SPSS Statistical Software v.23 (IBM, Armonk, NY). Descriptive data are expressed as mean±SD. All data were checked for normality using the Shapiro-Wilk test and to verify that parametric assumptions were met. Data were log10 transformed or transformed by computing the square root of the absolute values when parametric assumptions were not met. Three-way analyses of variance ((within-subject factor= time: baseline, 2 hours, 4 hours) and (between-subjects factors =activity level (AL): active (ACT) or inactive (IN); condition (COND): no exercise (CON) or exercise (EX)) were performed. Interaction effects are included for AL*time, COND*time, and AL*COND*time for blood analytes and exhaled nitric oxide. Airway inflammation via sputum cell counts was assessed with a one-way analysis of covariance, using baseline neutrophils and eosinophils as the covariate, to identify which inflammatory processes were involved. To assess correlations between sputum cell counts and eNO, Pearson Product moment correlation coefficients were used. For all analyses, significance was set at $p<0.05$.

Results

Subject characteristics

Baseline subject characteristics are displayed in Table 2.1. There was significantly higher baseline blood pressure ($p=0.04$), heart rate ($p<0.01$), waist circumference ($p=0.03$), body fat percentage ($p<0.01$) and absolute ($p<0.01$) and relative VO_2peak ($p<0.01$) in IN compared to

ACT. The only significant difference in resting pulmonary function was a significantly lower FEV_1 in IN participants compared to ACT participants. However FEV_1 in both groups was still above 100% of age and height-predicted values. Also, FVC was smaller in IN compared to ACT. However IN participants were also significantly shorter (p=0.03), therefore differences in FEV_1 were likely due to the fact that they had smaller lungs. All subjects had never been diagnosed with asthma, which they indicated on the medical history questionnaire and was confirmed via pulmonary function testing in which all subjects exhibited an FEV_1/FVC of >70%.

Triglycerides

Data for triglycerides are displayed in Figure 2.2. Triglycerides significantly increased over time for ACT and IN subjects at 2 hour (61±48%) and were further increased at 4 hours post-HFM by (27±32%; p<0.001). The overall increase from baseline to four hours in ACT and IN was 100±76%. There were no significant differences based on AL (p=0.97) or COND (p=0.82). There were no significant interactions for time*AL (p=0.296), time*COND (p=0.74), or time*AL*COND (p=0.62).

Energy Balance

Energy balance information is displayed in Table 2.2. There were no significant differences between ACT CON and IN CON based on energy consumed (p=0.70), energy balance at four hours (p=0.89), or caloric balance between food and exercise (p=0.19). However, for ACT EX as compared to IN EX, there was a significant difference in the minutes of exercise required to expend half of the calories of the HFM, where the IN EX walked longer than the ACT EX (d=1.61, p<0.01). In the IN EX compared with IN CON, the IN EX had significantly lower caloric balance after the HFM compared with IN CON (d=-1.40, p=0.01), however the amount of food consumed was not significantly different (p=0.98). In ACT EX compared with

ACT CON, the exercising group had a greater energy deficit at 4 hours (d=-1.77, p<0.01) due to greater energy expenditure. The amount of food consumed was not significantly different between groups (p =0.68).

Airway inflammation- eNO

Exhaled nitric oxide was our primary dependent variable; however, to determine which inflammatory processes were involved, sputum induction was performed in a subset of subjects. Not all subjects completed eNO testing due to technical difficulties with the flow sensor towards the end of the study, but the following completed eNO in each group: ACT CON, n=9; IN CON, n=9, ACT EX, n=7; IN EX, n=9. At baseline, eNO was not significantly different based on AL (p=0.77) or COND (p=0.77). However, eNO was significant as a quadratic function over time for all subjects (p=0.03), increasing at 2 hours and returning to baseline at 4 hours post meal. There was also a significant time*COND interaction (p=0.04), where the eNO response in EX compared to CON was different over the 4 hours, increasing in EX and remaining elevated, while it increased and returned to baseline in CON. There were no significant time*AL (p=0.37) or time*AL*COND (p=0.58) interactions. The mean eNO response in each cohort post-HFM is displayed in Figure 2.3.

Confirmatory tests-sputum induction

To assess the neutrophil response post-HFM, analyses were performed using the number of neutrophils at baseline as a covariate because the percentage change in neutrophils was impacted by the percentage present at baseline. The percentage of neutrophils increased significantly in the post-prandial period in a subset of 21 subjects who underwent sputum induction. The mean increase in neutrophils was 10.9±4.5% (95% CI: 1.5-20.4%). The study was not powered to detect differences in neutrophils and eosinophils for each group, given the small

sample available (IN CON, n=6; ACT CON, n=5; IN EX, n=4; ACT EX, n=6). When performing a post-hoc sample size calculation, we found a moderate effect for the increase in neutrophils by COND alone (d=0.44), and 8 subjects in each group would be needed to show a significant increase in neutrophils in the walking condition compared to the no walking condition. By AL alone, there was a small to moderate effect (d=0.36) and 12 subjects would be needed in each group to show an increase in neutrophils in ACT compared to IN subjects.

The ACT CON and ACT EX data, however, have been previously published as the main outcome in a preliminary study from our laboratory (Kurti et al. 2015). Eosinophils were also assessed post-HFM to determine which inflammatory processes were involved. Eosinophils did not significantly increase from baseline (0.5±0.5%) to four hours (0.5±0.6%) post-HFM (p=0.89). Percent change from baseline to 4 hours post-HFM was not significant (0.0±0.9%; 95% CI: -0.4-0.4%).

Associations between airway inflammation and eNO

To assess associations between markers of airway inflammation, bivariate correlations were performed. Eighteen of twenty-one subjects that performed sputum induction also had performed eNO at all time points. The percentage change in neutrophils from baseline was not associated with the percentage change in eNO from baseline to 2 hours (r=0.36, p=0.16) or baseline to four hours (r=0.26, p=0.31). The percentage change in eosinophils was also not associated with the percentage change in eNO from baseline to 2 hours (r=-0.03, p=0.92), or baseline to 4 hours (r=-0.12, p=0.65). The percentage change in neutrophils was not associated with the change in eosinophils from baseline to 4 hours (r=0.09, p=0.70).

Discussion

Main findings

The major finding in this study was that physically active and insufficiently active participants in the walking condition had a larger eNO response compared with the no walking condition. This finding was in contrast to our hypothesis that IN subjects would have a greater attenuation in post-prandial eNO compared to CON. There may be many factors contributing to the lipemic and airway inflammatory response post-exercise and post-meal, many of which may interact with one another, therefore these factors will be the focus of our discussion.

Airway inflammation post-HFM

In many of our previous studies, as well as the current study, eNO significantly increased as a main effect of time after a high-fat meal (Rosenkranz et al. 2010; Ade et al. 2014; Johnson et al. 2015). We have previously shown that eNO increases post-prandially from baseline to 2 hours with a subsequent return to baseline by 4 hours post-HFM (Kurti el al. 2015, Johnson et al. 2015). Wood et al. (2011) showed that at 4-hours post-HFM in non-asthmatic subjects, there was not an increase in eNO, but there was a 15.3±6.2% increase in neutrophils. Wood and colleagues did not measure eNO at 2 hours, and therefore at 4 hours post-meal eNO may have started to return to baseline values. The difference in time course for changes in eNO and neutrophils may be why there are not significant associations between markers of airway inflammation in Wood and colleagues work as well as the present study. Additionally, the change in eNO was not associated with changes in eosinophils. The airway inflammatory response in neutrophils, eosinophils, and eNO indicated independence of the responses of these markers of airway inflammation. Therefore, it is possible that the increase in eNO is not only due to inflammatory processes, but may suggest that oxidative processes are involved, where eNO is increased

because there is a reduced clearance in NO systemically. The competition of antioxidant enzymes following a HFM may inhibit the reduction of NO production, leading to an increase in exhaled NO due to the attenuation or absence of an increase in iNOS activity (Bonini et al. 2014).

We are confident that oxidative stress and inflammatory processes were involved in the eNO response following a HFM. Possible mechanisms contributing to the eNO response may be either TLR4 dependent or independent. Wood and colleagues showed that 4 hours post-HFM, expression of TLR4 mRNA significantly increases via a NFkB-driven cascade (Wood et al. 2011). However the increase in exhaled NO may also occur due to the direct upregulation of iNOS in the airways (Gaston et al. 1994). For example, in rats injected with lipopolyssaccharide (LPS), iNOS expression has been shown to increase (Sugita et al. 2002). Dietary fats also increase reactive oxygen species (ROS), which may increase neutrophils in the airway. Dietary fats increase NFkB and I-kappaB kinase beta (Ricciardolo et al. 2004), upregulating iNOS and contributing to the increase in exhaled NO (Gaston et al. 1994).

Impact of physical activity level of post-prandial airway inflammation

Basal eNO in asthmatics can be lowered by physical activity interventions (Mendes et al. 2011). Additionally, a recent study by Scott et al. (2015) indicated that even an acute bout of physical activity lowers eNO in insufficiently active asthmatics. However in the current study, we did not find differences based on activity level (active or insufficiently active), but did find differences based on whether subjects exercised or remained sedentary (EX or CON) in the post-prandial period. Given that all of our subjects were non-asthmatic, the post-prandial eNO response following a HFM was unexpected.

Researchers have reported that physically active and trained subjects have greater antioxidant defenses with which to combat ROS (Gomez-Cabrera et al. 2008). Reactive oxygen species increase post-HFM (Mohanty et al. 2002), therefore we hypothesized that active individuals with a greater antioxidant status would have an attenuated airway inflammatory response, due to the ability to clear ROS produced and lead to less activation of iNOS. Stewart and colleagues showed that active individuals have an attenuated inflammatory response when injected with LPS systemically, and this is likely due to lower activation of TLR4 (Stewart et al. 2005). The active individuals in our study did not display an attenuation of the eNO response post-HFM, and therefore it is possible that there was not a reduction in ROS due to chronic PA level. It is also possible the insufficiently active participants had lower oxidative stress because they were young; since it has been shown that younger individuals have lower oxidative stress compared to older individuals (Cadenas and Davies 2000). Therefore measuring the airway inflammatory response after performing post-prandial exercise may be an important topic to investigate in an aging population.

While chronic effects of training did not reduce post-prandial airway inflammation in the present study, there were acute effects on airway inflammation and cellular stress after the bout of exercise. The participants were walking to expend half the calories of the HFM, and it is possible that sheer rate via increases in ventilation could have increased activation of NO isoforms (Sheel and McKenzie 1999). However, eNO typically decreases immediately after exercise when ventilation is higher (Mehta et al. 1997). It is possible the IN EX subjects had a more reduced eNO response than the ACT EX subjects because they had higher ventilation during the exercise bout at 60% VO_2peak, and exercised longer, which they were not accustomed to. The mechanisms contributing to airway inflammatory and oxidative processes during and

after an acute bout of exercise should be explored further, and specifically researchers should determine the origin of eNO at different intensities to better understand the post-prandial and post-exercise responses.

Exercise: acute or chronic, and post-prandial lipemia

Chronic physical activity has been shown to result in lower triglycerides after a high-fat meal (Merrill et al. 1989) compared to low physical activity levels, and this has been confirmed in many other studies (Hardman and Aldred 1995; Katsanos and Moffatt 2004). However the impact of an acute bout of physical activity on PPL is somewhat unclear. We recruited two cohorts of subjects (IN and ACT) because we hypothesized that the reduction in PPL is likely to be dependent on chronic activity level. The fact that there was not a reduction in PPL in either group, further contributes to a large body of conflicting literature. Several systematic reviews suggest that higher energy expenditures are required to see reductions in PPL (Katsanos and Moffatt 2004), and more than what individuals may realistically perform following a high-fat meal. The bout of physical activity utilized in our study represented a more true-to-life exercise bout (a brisk walk), that an individual may perform one hour after consuming a HFM. In light of recent studies, we believed the bout would lead to a reduction in PPL, particularly in the insufficiently active group. The average energy expenditure in the EX group was approximately 370 kcal. This level of energy expenditure has recently been shown to reduce PPL in sedentary overweight men (Chu et al. 2016). In fact, the researchers report that exercise duration of only 20 minutes at 60% power elicited a 20% reduction in PPL. Interestingly, subjects in the study performed by Chu and colleagues had a higher VO_2max (40.4 ±8.6 mL/kg/min) and lower body fat percentage (19.0 ±5.3%) compared to participants in the present study. Gabriel and colleagues (2012) found a trend for reductions in PPL after an exercise energy expenditure of

103.2±5.1 kcals during a high-intensity interval trial, and subjects were recreationally active but not endurance trained. Conversely, active participants may need as much as 600 to 700 kilocalories of energy expenditure to see a reduction in PPL. We previously reported that this group of active subjects did not have a reduction in PPL following acute exercise (Kurti et al. 2015), and it is possible that the insufficiently active subjects in the current study engaged in more PA as compared to previous studies where a relatively low exercise energy expenditure elicited a reduced lipemic response. Most of our subjects were living on a college campus, and therefore even the short amount of time that they walk to and from class may be sufficient to have beneficial training adaptations. Subjects in our study reported getting only 29±41 minutes of moderate to vigorous activity over 7 days, which is lower than the ACSM guidelines. Nonetheless, insufficiently active subjects reported walking for 220±280 minutes per week, which may be enough to show beneficial training adaptations with regard to post-prandial responses to a HFM.

Limitations

There are several factors that may have influenced our results. Given that active individuals have an increased antioxidant capacity compared to insufficiently active individuals, antioxidant status should be considered. If the antioxidant status of subjects was not different at baseline and eNO may be reflective of oxidative and inflammatory processes, total antioxidant status and/or capacity may account for the eNO responses seen in the current study. We have previously shown that increasing fruit and vegetable consumption (Chenoweth et al. 2015) or vitamin supplementation (Kurti et al. 2015), which likely improves total antioxidant capacity, improves post-exercise lung-function. Therefore measurement of antioxidant capacity in our subjects would allow for further exploration of potential mechanisms from our current results.

Additionally, it would have been ideal to perform sputum analysis in all of the subjects enrolled to determine differences by activity level and condition. Considering our study was intended to determine whether chronic PA level modified the post-prandial eNO response, detecting differences in sputum cell differentials by AL and COND was not a primary aim. Still, additional information on inflammatory cell presence by chronic PA level and condition would be ideal and will be included in future studies. It would have also been useful to assess airway hyper-responsiveness after sputum induction, however this was not possible in our protocol. We have previously reported that 25% hypertonic saline elicited airway hyper-responsiveness in healthy non-asthmatic subjects (Smith et al. 2015), while a lower hypertonic saline concentration (5%) has not been shown to be effective in eliciting airway hyper-responsiveness in this population. Lastly, the triglyceride levels of our subjects were low, and even the inactive individuals did not have a large magnitude of increase in the post-prandial period. Therefore, the low levels of triglycerides in our subject pool may have prevented a further attenuation of lipemia, particularly considering the fact that triglycerides didn't increase above 150 mg/dL post-prandially.

Future research

Future research should elucidate the mechanisms contributing to the post-prandial increase in eNO. Given that several inflammatory and oxidative stress pathways may contribute to the effects of both a HFM and a bout of exercise on the airways in the post-prandial period, investigating longer or more intense bouts of exercise, and utilizing different timing around the meal is important in future research. Also, the modification of the post-prandial airway inflammatory response should be investigated in populations such as obese individuals or asthmatics. Researchers should focus on determining what happens to the airway inflammatory response post-HFM in these individuals when a reduction in PPL is evident.

Conclusions

Results from the current study indicate that an acute bout of moderate intensity exercise following a HFM does not affect PPL in either insufficiently active or active non-asthmatics, but does impact the airway inflammatory response. In individuals who are chronically physically active, eNO increases when exercise is performed post-prandially compared to those not engaging in physical activity in the post-prandial period. Additionally, results from this study suggest that mechanisms contributing to post-prandial airway inflammation are independent, with no associations between the eNO, neutrophilic and eosinophilic responses following a HFM.

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Table 2.1 Subject Characteristics

	ACTIVE (13M/7F)			INACTIVE (6M/13F)			sex difference (IN)	sex difference (ACT)
	Value		SD	Value		SD	(*p*-value)	(*p*-value)
Age (years)	24.3	±	5.5	25.9	±	5.4	0.07	0.66
Height (cm)	174.3	±	9.5	167.7	±	10.4*	<0.01^	<0.01^
Weight (kg)	76.1	±	14.2	74.1	±	19.3	0.21	<0.01^
Body mass index (BMI) (kg/m^2)	24.9	±	2.8	26.3	±	6.7	0.75	0.27
Body Fat (%)	20.5	±	9.8	34.1	±	11.5*	0.02^	<0.01^
Waist circumference (cm)	85.7	±	5.4	92.9	±	13.0*	0.91	0.53
Systolic (mmHg)	119.3	±	12.9	110.8	±	11.1*	0.05^	0.08
Diastolic (mmHg)	67.3	±	9.4	71.5	±	8.7*	0.56	0.52
VO_{2peak} (L/min)	4.2	±	1.0	2.9	±	1.0*	<0.01^	<0.01^
VO_{2peak} (ml/kg/min)	54.5	±	7.7	38.9	±	9.5*	<0.01^	<0.01^
Resting Heart Rate (bpm)	64	±	10	75	±	9.0*	0.76	0.34

Baseline Pulmonary Function Tests

	ACTIVE (13M/7F)			INACTIVE (6M/13F)			sex difference (IN)	sex difference (ACT)
	Value		SD	Value		SD	(*p*-value)	(*p*-value)
PEF (L/s)	9.0	±	2.7	7.7	±	1.9	<0.01^	<0.01^
FVC (L)	5.5	±	1.7	4.5	±	2.0	0.01^	<0.01^
FEV_1 (L)	4.7	±	1.4	3.7	±	1.2*	0.03^	<0.01^
FEV_1/FVC (%)	86.0	±	5.9	86.3	±	12.5	0.08	0.53
$FEF_{25-75\%}$ (L/s)	5	±	1.4	4.3	±	1.4	0.24	<0.01^

Values are expressed as mean ± SD. *Significance difference between active (ACT) and inactive (IN) participants $p<0.05$

^shows sex difference between males and females in ACT and IN groups

bpm, beats per minute; PEF, peak expiratory flow; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-second

$FEF_{25-75\%}$, forced expiratory flow rates between 25-75% of FVC

Table 2.2 Energy Balance

	ACT CON			ACT EX			IN CON			IN EX		
Energy Consumed	774.4	±	164.6	747.5	±	123.7	741.8	±	199.3	739.3	±	199.3
Energy expended on treadmill	N/A	±	N/A	373.7	±	61.9^	N/A	±	N/A	369.7	±	99.6^
Caloric Balance (Food + EX)	874.4	±	395.7	373.7	±	61.9^	666.3	±	282.6	369.7	±	99.6^
Energy Balance at 4 hr	458.7	±	87.6	103.6	±	43.5^	466.3	±	139.1	146.2	±	72.93^
Exercise time (minutes)	N/A	±	N/A	32.1	±	6.2*^	N/A	±	N/A	47.1	±	11.6^

All data are represented as kilocalories (kcals) unless otherwise noted.

*p<0.05. Indicates significant difference between ACT CON and ACT EX, IN CON and IN EX

^Denotes significance between ACT EX and IN EX or ACT CON and IN CON

Figure 2.1 Schematic of the study

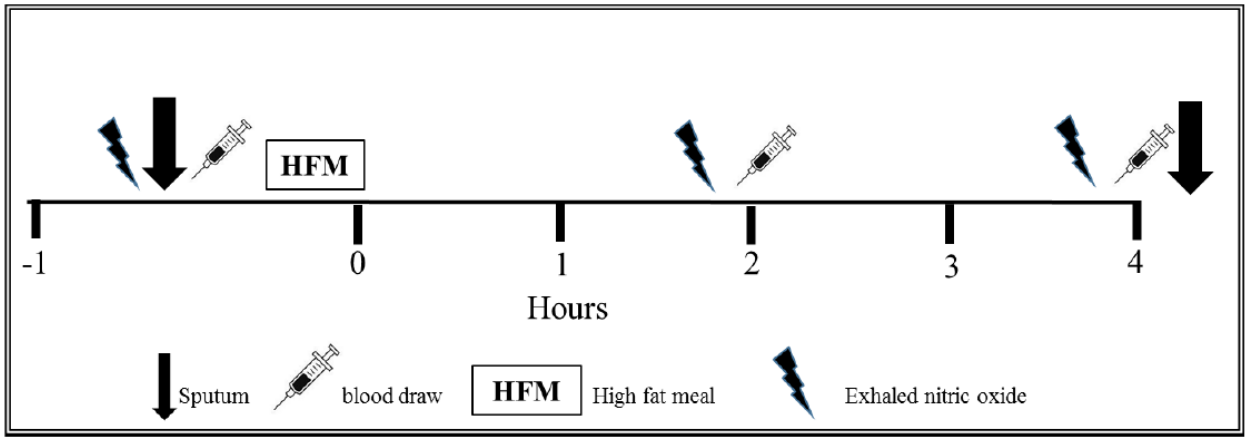
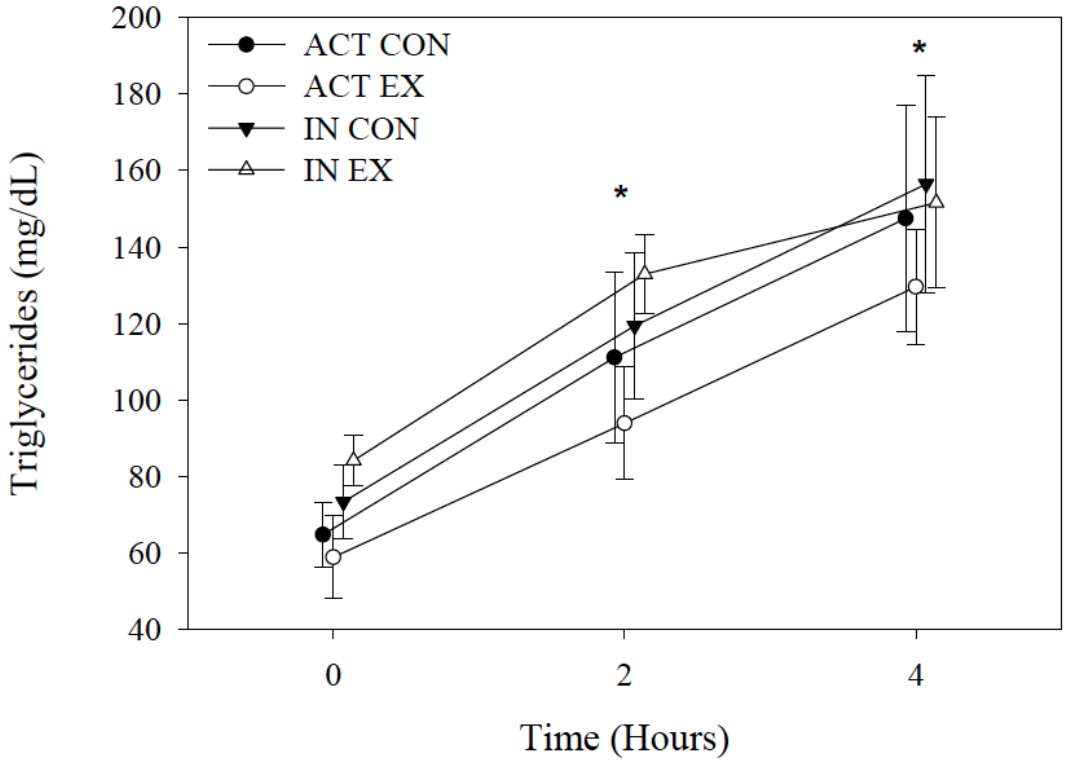
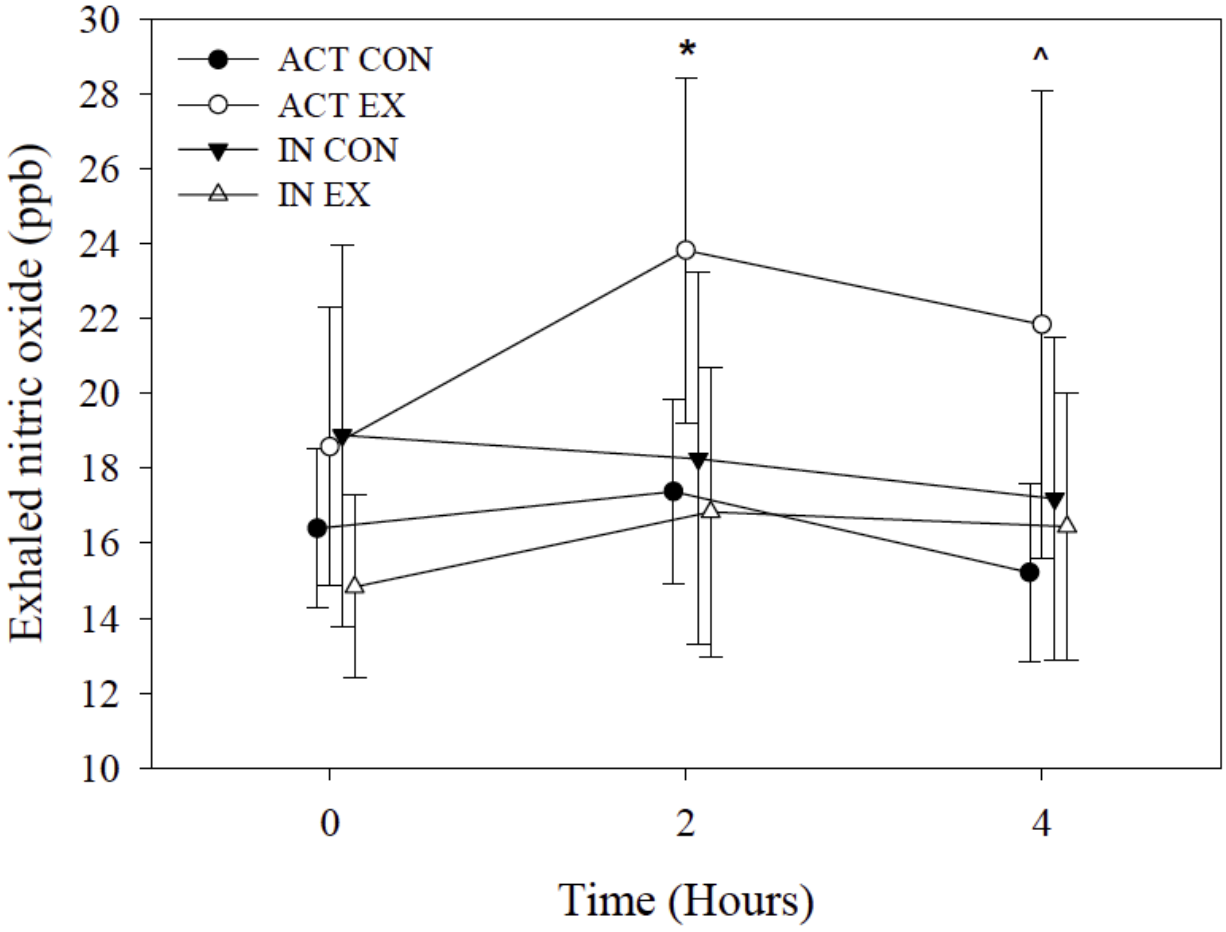


Figure 2.2 Triglyceride response post-HFM



Triglyceride response post-HFM in ACT CON (●), ACT EX (○), IN CON (▼), IN EX (Δ). Data are shown as absolute means±SD. Triglycerides increased significantly from baseline to 2 hours and 4 hours (*), with no differences by activity level (AL) or condition (CON). Data points are offset to more clearly display the data. Significance was set at $p<0.05$.

Figure 2.3 The mean eNO response post-HFM



The mean eNO response in ACT CON (●), ACT EX (○), IN CON (▼), IN EX (Δ). There was a significant increase as a main effect of time (*), where eNO increased from baseline to 2 hours and then started to return to baseline by 4 hours post-HFM. There was a higher eNO response over time by CON (^). Data points are offset to more clearly display the data. Significance was set at $p<0.05$.

Chapter 3 - Post-prandial systemic 8-isoprostane increases after consumption of moderate- and high-fat meals in insufficiently active males

Now in publication as Kurti SP, Emerson SR, Rosenkranz SK, Teeman CS, Emerson EM, Cull BJ, Smith JR, Harms CA. Post-prandial systemic 8-isoprostane increases after consumption of moderate and high-fat meals in sufficiently active males. *Nutrition Research* 2017, 39: 61- 68. DOI: http://dx.doi.org/10.1016/j.nutres.2017.02.003

Abstract

A single high-fat meal (HFM) leads to an increase in triglycerides and oxidative stress. Oxidative stress can be assessed via 8-isoprostane generation, which is associated with the development of asthma and cardiovascular disease. No previous research has investigated whether airway and systemic 8-isoprostane increases post-prandially in non-asthmatics according to the caloric and fat content of a meal. Our purpose was to assess airway and systemic 8-isoprostane following a HFM and a true-to-life moderate-fat meal (MFM). We hypothesized that airway and systemic 8-isoprostane would increase after a HFM and MFM, with the greatest increase in the HFM condition. Eight non-asthmatic men (25.8±6.9 years) completed the HFM and MFM trials in a randomized crossover design. Following a 10-hour fast, participants consumed either a HFM (17 kcal/kg body mass, 60% fat, 23% CHO) or a MFM (8.5 kcal/kg body mass, 30% fat, 52% CHO). Exhaled breath condensate to assess airway 8-isoprostane was collected at baseline, 3 and 6 hours post-meal. Venous blood samples were collected at baseline and hourly until 6 hours post-meal to assess triglycerides, and every 3 hours for systemic 8-isoprostane. Airway 8-isoprostane responses were not significant as a main effect of time (p=0.072), between conditions (p=0.365), or between time and condition (p=0.319) post-meal. Systemic 8-isoprostane increased over time (p<0.001), but not between conditions (p=0.124) or between time and condition (p=0.649) post-meal. Triglycerides incremental area under the curve increased over time in the HFM compared to the MFM condition (p=0.013). Following a HFM and MFM, 8-isoprostane increases systemically, however airway 8-isoprostane does not change.

Introduction

High-fat diets have shown to be associated with many negative clinical outcomes, including cardiovascular [1] and respiratory diseases [2]. Mechanisms that may contribute to the development of these diseases include the post-prandial inflammatory and oxidative stress responses resulting from the consumption of high-fat diets. High-fat diets may result in oxidative stress by promoting lipid peroxidation, which can be described as the process in which excess oxidants (i.e. free radicals) attack the composition of lipids [3]. Specifically, dietary fats may impact a specific and reliable marker of oxidative stress, 8-isoprostane, by modifying the lipid composition of tissues and increasing the enzymatic oxidation of arachidonic acid [4]. The oxidation of arachidonic acid may result in the increase in 8-isoprostane through the prostaglandin pathway [4]. It is well established that dietary fats also impact triglycerides, which increase after a single high-fat meal (HFM) [5]. Furthermore, the post-prandial lipemic response to a HFM is exaggerated in individuals who are physically inactive compared to physically active individuals [6].

In addition to HFM induced increases in post-prandial lipemia (PPL), our laboratory has reported changes in the airway, with an increase in exhaled nitric oxide (eNO: a validated marker of airway inflammation) [7-9] and sputum neutrophils following consumption of a HFM [10, 11]. While the post-prandial airway inflammatory response is important to consider, airway oxidative stress may also lead to airway remodeling and increased asthma risk [12]. Therefore post-prandial airway oxidative stress may be important to assess, however airway oxidative stress has scarcely been investigated after the consumption of a HFM. The gold standard for assessment of airway oxidative stress is 8-isoprostane, which is associated with the development and progression of asthma [13].

Systemically, post-prandial oxidative stress develops from post-prandial lipemia and is associated with a higher risk for diabetes, obesity and atherosclerosis [14]. Since 8-isoprostane may be produced by oxidation of unsaturated fatty acids [15], which are incorporated into low-density lipoproteins and then oxidized in the vascular endothelium, post-prandial systemic 8-isoprostane may be an important factor modulating the risk for developing cardiovascular disease (CVD). The elevation in 8-isoprostane has been reported to be associated with CVD development [16], impair flow-mediated dilation (FMD) [17] and contribute to impaired vascular function [18]. Additionally, assessment of PPL, inflammation and oxidative stress following the consumption of a HFM in the existing literature, often included HFMs containing more than 15 kcal/kg of body weight, commonly exceeding 1000 kcal with greater than 80 grams of saturated fat. While these meals may elicit PPL and oxidative stress [19], they may not represent oxidative stress responses following a more true-to-life MFM. Based on this, we hypothesized that airway and systemic 8-isoprostane would increase post-MFM and HFM, with the greatest magnitude of increase in the post-HFM condition. Therefore, our primary objectives were to assess the post-prandial airway and systemic 8-isoprostane responses to a HFM and a more true-to-life MFM in insufficiently active male participants.

Methods

Study population

Eight college-aged males who did not engage regularly in any planned physical activity per week, and did not meet physical activity guidelines (moderate-to-vigorous physical activity (MVPA) less than 150 minutes per week [20]) participated. Insufficient physical activity was confirmed via the International Physical Activity Questionnaire (IPAQ) [21]. Participants were non-asthmatics, and had never been diagnosed with any pulmonary or metabolic diseases, which

was confirmed via the medical history questionnaire. All participants completed both the HFM and MFM trials in a randomized crossover design, however one participant required early removal of the catheter during the post-HFM assessment period. Investigators were able to assess blood lipids and glucose via finger sticks, however 8-isoprostane was not measured systemically in one subject during the HFM condition. Prior to the protocol, all participants signed the informed consent document. The study was approved by the Institutional Review Board at Kansas State University and conformed to the principles set forth in the *Declaration of Helsinki*.

Experimental design

Participants visited the laboratory on three occasions, with at least one week between visits. The initial visit consisted of completion of the medical history questionnaire, the IPAQ, and a briefing about the study protocol. Participants then rested for 15 minutes and blood pressure was measured in triplicate using an automated blood pressure cuff (Omron Healthcare, Lake Forest, IL). Then height was measured to the nearest 0.1 cm with a portable stadiometer (Invictus Plastics, Leicaster, England), weight was assessed to the nearest 0.1 kg with a digital scale (Pelsar LLC, Alsip, IL, USA), and waist circumference was determined using a Gulick spring loaded measuring tape (Accufitness, Greenwood Village, CO, USA). Following these measurements, a dual-x-ray absorptiometry (DEXA) scan was performed to measure body composition (GE Lunar Prodigy, Madison, WI, USA). For the meal testing sessions, participants visited the laboratory following at least an overnight fast of 10-hours, with no exercise or alcohol for 24 hours prior to the meal testing session. When participants arrived at the laboratory, they were randomly assigned into the HFM or MFM condition. The other condition was completed at the subsequent visit, and all meal sessions were at least one week apart, but not longer than four weeks apart. Prior to the meal, resting blood pressure was assessed. After this, exhaled breath

condensate (EBC) was assessed, followed by insertion of the indwelling catheter and a fasting blood draw. Participants then had 20 minutes to consume the meal, according to previously published protocols from our laboratory [7-11]. After the final bite of the meal, time started for the session. Blood draws were performed every hour post-meal for 6 hours to determine triglyceride response, however systemic 8-isoprostane was only assessed at the 3 and 6-hour time points post-meal. Participants completed the EBC tests at baseline, 3 and 6 hours post-meal for analysis of airway 8-isoprostane. Participants were required to remain sitting through the entire post-prandial period, and were only allowed to stand to use the bathroom, which is located less than 20 feet from where the participants were required to sit. The experimental protocol is displayed in Figure 3.1.

Meal conditions

In the MFM condition, participants consumed sausage, egg and cheese Lean Pockets (8.5 kcal/kg bodyweight, 30% fat, 52% CHO) with 11 g of protein, 60 mg cholesterol, and 370 mg of sodium per serving. The range of calories participants consumed in the MFM condition was from 475-993 kcals. The HFM consisted of a sausage, egg and cheese breakfast bowl (17 kcal/kg body weight, 60% fat and 23% CHO) with 17 grams of protein, 255 mg cholesterol, and 730 mg sodium. This test meal was similar to meals previously used in our laboratory [10, 11, 22]. The range of calories participants consumed in the HFM condition was from 950-1986 kcals.

Airway 8-isoprostane

Participants performed exhaled breath condensate measurements before, 3 hours and 6 hours post-HFM and MFM. Commercially available RTubes were used for EBC measurements (Respiratory Research, Inc., Austin, TX, USA). Participants performed tidal breathing for 10 minutes while seated, with feet flat on the floor and wearing a nose-clip. After 10 minutes of data

collection, ~1.5 mL of condensate was collected and immediately frozen at -60°C in the RTubes and later analyzed in triplicate for exhaled 8-isoprostane with a commercially available enzyme linked immunosorbent assay kit (Cayman Chemical, Ann Arbor, MI, kit#516351). The samples only went under one freeze-thaw cycle. Prior to the assay, EBC samples were concentrated five-fold by performing C-18 solid-phase extraction (SPE) purification. To perform the purification, EBC samples were loaded onto C-18 SPE cartridges that had been previously conditioned with 5 mL of MeOH followed by one mL of water. The 8-isoprostane was eluted from each column using the EIA Buffer. A standard curve was then established by serial dilution of the 8-isoprostane standard. Intra-assay coefficients of variation for airway 8-isoprostane was 7.5%.

Blood lipids and plasma 8-isoprostane

An intravenous catheter was inserted into an antecubital vein, and blood samples were drawn at baseline, and hourly for 6 hours post-HFM to assess plasma triglycerides. Blood draws were completed by first removing saline with a 3 mL syringe, flushing out the sample line. The whole blood sample was then taken through a 5 mL syringe (BD, Franklin Lakes, NJ, USA). Afterwards, the entire blood sample was transferred into vacutainer tubes (6 mL K2 EDTA BD Vacutainer, Franklin Lakes, NJ, USA). Alere Cholestech capillary tubes were used to draw blood from the vacutainers for lipid analysis (Cholestech LDX Analyzer, Alere San Diego Inc., San Diego, CA), while the remaining whole blood sample was centrifuged for 15 minutes using a CxR Centrifuge (LW Scientific, Lawrenceville, GA, USA). The plasma was stored in cryovials (Fisher, Hanover Park, IL, USA), stored at -60°C, and later analyzed in triplicate for systemic 8-isoprostane using a commercially available assay kit (Cayman Chemical, Ann Arbor, Michigan, USA, kit #516351). Prior to the assay, each plasma sample was extracted using a C-18 solid-phase extraction SPE purification. To perform the SPE, 200 µL of each sample was loaded onto

the C-18 SPE cartridges that had already been purified by 5 mL of MeOH. The samples were dried under liquid nitrogen, and then resuspended to the original sample volume using EIA Buffer. The samples were diluted so that the absorbances would fall within the linear range of the standard curve. Intra-assay coefficients of variation for systemic 8-isoprostane was 8.3%.

Statistical Analyses

An *a priori* sample size calculation was performed with post-prandial triglycerides as the dependent variable. In order to determine statistical significance with 80% power at an α-level of 0.05 for post-prandial triglycerides, six participants were needed. For systemic 8-isoprostane, the power analysis concluded the same number of participants were needed. However, eight participants completed the testing sessions to increase power to detect differences in other outcome measures. Data were analyzed using SPSS Statistical Software v.23 (IBM, Armonk, NY, USA) and GraphPad Prism v6 for TG AUC calculations. Data are expressed as means ± SD. Prior to analysis, data were checked for normality and to verify that parametric assumptions were met. A 3 (time: baseline, 3 hours, 6 hours) x 2 (condition: HFM or MFM) repeated measures analysis of variance (ANOVA) was used to assess post-prandial changes in airway and systemic 8-isoprostane. Triglyceride area under the curve (AUC), incremental AUC (iAUC), peak triglycerides and triglyceride time to peak were analyzed with a one-way ANOVA with condition (MFM or HFM) as the between subjects factor. Correlations were assessed between triglycerides, airway and systemic oxidative stress with the Pearson Product moment correlation coefficient. For all analyses, significance was set to $p<0.05$. Bonferroni adjustments were made to reflect multiple comparisons.

Results

Participant characteristics

Characteristics for participants are displayed in Table 3.1. All eight participants who enrolled in the study completed the initial assessment and both testing sessions. Participants were not hypertensive and reported normal cardiopulmonary and metabolic health on their medical history questionnaires.

Airway 8-isoprostane

Group level airway 8-isoprostane results can be seen in Figure 3.2. Airway 8-isoprostane did not change over time (p=0.072), by condition (p=0.365), or as an interaction between time and condition (p=0.319). In the HFM session, baseline airway 8-isoprostane (1.794±0.387 pg/mL) remained relatively similar until 3 hours post-HFM (Δ-0.044; 3 hr=1.750±0.365 pg/mL, p=0.999) and then decreased to 1.383±0.258 pg/mL at 6 hours post-HFM, (Δ-0.367; p=0.232). In the MFM condition, baseline airway 8-isoprostane (2.029±0.625 pg/mL) decreased at 3 hours post-HFM (Δ-0.440; 3 hr= 1.589±0.399 pg/mL, p=0.080), and then stayed relatively similar from 3 to 6 hours post-HFM (Δ+0.088; 6 hr= 1.676±0.429 pg/mL, p=0.937).

Systemic 8-isoprostane

Mean plasma 8-isoprostane results can be seen in Figure 3.3. Plasma 8-isoprostane significantly increased as a main effect of time (p<0.001), however did not differ between conditions (p=0.124), or by time and condition (p=0.649). In the HFM condition, baseline plasma 8-isoprostane (33.253±8.95 pg/mL) stayed similar from baseline to 3 hours post-HFM (32.310±5.283 pg/mL, p=0.999), and then increased from 3 to 6 hours post-HFM (41.153±8.918 pg/mL, p=0.015). In the MFM condition, baseline plasma 8-isoprostane (27.705±4.708 pg/mL) remained similar from baseline to 3 hours post-HFM (26.223±4.324 pg/mL, p=0.752) and then increased from 3 to 6 hours post-HFM (38.143±8.201 pg/ml, p=0.014).

Blood lipids

Fasting triglycerides were 113.0±61.3 mg/dL (range: 45.0-271.0 mg/dL). There was a significant increase in post-prandial triglycerides in both the MFM and HFM conditions (Table 3.2). In a one-way ANOVA with condition as the between subjects factor, total triglyceride AUC (F=3.641, p=0.077) and peak triglycerides (F=3.076, p=0.101) were not different between trials**.** The incremental AUC was larger in the HFM compared to the MFM condition (465.97±213.18 mg/dL x 6 hours; F=3.377, p=0.013). Also, the time to peak was longer in the HFM compared to the MFM (1.38±0.57 hours; F=5.762, p=0.031). In a two-way ANOVA with change in triglycerides over time as the within-subjects factors and condition as the between subjects factor, there was a significant main effect of time (p<0.001) and a time x condition interaction (p=0.018), however no main effect for condition alone (p=0.098).

Associations between triglycerides, systemic and airway 8-isoprostane

When analyzing the entire HFM and MFM conditions together, there were no significant associations between airway 8-isoprostane, systemic 8-isoprostane and triglycerides at any time point (p-values>0.05). When conducting exploratory analysis by condition, total triglycerides AUC, iAUC for triglycerides, peak triglycerides, and time to triglycerides peak, were not significantly associated with the change in plasma 8-isoprostane at any time-point in the MFM condition (p-values>0.05). However, in the HFM conditions, from baseline to 6 hours post-meal, systemic 8-isoprostane was associated with total triglyceride AUC (r=0.874, p=0.010), as well as iAUC for triglycerides (r=0.812, p=0.026), peak triglycerides (r=0.896, p=0.006) and time to triglycerides peak (r=0.804, p=0.029). Baseline triglycerides were also associated with changes in plasma 8-isoprostane from baseline to 6 hours post-HFM (r=0.837, p=0.019). There were no associations between changes in triglycerides and systemic 8-isoprostane in the MFM condition.

In both the MFM and HFM conditions, body fat percentage was associated with the change in plasma oxidative stress (r=0.511, p=0.051).

Discussion

Our main findings were that (1) airway 8-isoprostane did not change from baseline to 3 or 6 hours post-MFM or HFM in insufficiently active, non-asthmatic men, and (2) systemic 8-isoprostane increased post-prandially in both the HFM and MFM conditions. Accordingly, we rejected our hypothesis that airway 8-isoprostane would increase after the consumption of a MFM and a HFM. However, the data were consistent with our second hypothesis that systemic 8-isoprostane would increase post-HFM, but with a similar increase for the MFM and HFM conditions. In our recent work, we showed that increased inflammation occurs two hours post-HFM when measured via exhaled nitric oxide [7-11], but starts to return to baseline at four hours post-HFM. We have also recently showed that sputum neutrophils increase at four hours post-HFM in non-asthmatics [10, 11], and this has also been previously shown in asthmatics [23]. While we did not find any changes in oxidative stress at 3 hours post-HFM in the present study, it is possible we missed the peak 8-isoprostane response and airway 8-isoprostane was already returning to baseline values. It is also possible the oxidative stress response at baseline was elevated due to the fact that subjects were fasted when they arrived at the laboratory [24], however this is speculative since the only supporting data that are available, to our knowledge, come from experiments completed in animal models. Since the current study was the first to assess post-prandial airway 8-isoprostane, it will provide a possible time course for researchers to consider in the future.

Additionally, several researchers have reported that vitamin supplementation attenuates post-exercise bronchoconstriction [25, 26], likely because of increased antioxidant defenses in

the airways. Also, younger individuals have greater antioxidant defenses [27], which have the ability to neutralize and clear free radicals. Therefore it is possible that non-asthmatic younger individuals are able to clear reactive oxygen species (ROS) generated post-HFM and handle the oxidative load of a meal challenge in the airways, in contrast to the systemic 8-isoprostane response. However, this preliminary finding of airway oxidative stress responses needs to be investigated in more detail. High-fat diets may contribute to the development of asthma [2], and oxidative stress is involved in airway remodeling and inflammation [28], which may lead to the development of asthma and respiratory disease. Therefore the post-prandial airway oxidative stress response should be examined using a different time course (possibly every hour post-HFM), with multiple high-fat meals in succession to determine whether there might be an additive effect. Additional investigations are needed for individuals who have a greater chance of developing asthma, such as older and obese individuals.

A surprising and important finding in this study was that systemic oxidative stress increased to a similar extent in the MFM and HFM trials. This could provide important information for insufficiently active populations who are at increased risk for developing CVD into adulthood, considering that systemic oxidative stress contributes to endothelial dysfunction [16, 18, 29]. Hall and colleagues have reported that post-prandial lipid peroxidation increases at three hours and six hours post-HFM [30], however this was when EPA was added to the test meal and it appears that changes in post-prandial systemic 8-isoprostane response do not consistently increase [31-33]. Therefore, the post-prandial oxidative stress response may vary based on the type of fat consumed. To put the present study in context with the existing literature, the percentage increase in systemic 8-isoprostane was ~40% in the MFM condition alone. Hall et al. reported a post-prandial increase of 48% in systemic 8-isoprostane and a

reduction in nitric oxide [30]. Reduction in nitric oxide bioavailability could impair arterial function. Berry and colleagues reported that when post-prandial 8-isoprostane increased ~10 ng/L, which was similar to that reported in the present study of ~9 ng/L, FMD decreased by 3% [17]. Therefore it is important to examine whether even a true-to-life MFM typical of what individuals consume daily could elicit decreases in FMD and nitric oxide bioavailability when completely sedentary in the post-meal period. Such findings would suggest compromised arterial function following typical meal consumption patterns, in healthy college-aged participants who are inactive.

Neri and colleagues also reported circulating markers of oxidative stress increased after a high-fat meal in healthy controls [19]. However, researchers used a much higher fat composition with 80 grams of saturated fat and over 1100 kcal in the meal. The range of calories in our HFM was 950.3-1985.6 kcal with a mean of 1342.8±353.3 kcal and in our MFM was 475.2-992.8 kcals with a mean of 671.4±176.6 kcals. Therefore, our MFM represents a more true-to-life meal with a caloric content that may elicit a lipemic response [10, 11, 22], but is lower in total fat content than meals used in many studies intending to represent a typical Western meal. On another note, Neri et al. reported that the increase in oxidative stress was completely ameliorated when antioxidant supplementation was added [19]. The participants in our study were insufficiently active, which may indicate they had lower total antioxidant capacity (TAC). Higher antioxidant status protects against the oxidative stress that may elicit vascular damage [34], therefore younger, but insufficiently active participants may be more likely to see the increase in oxidative stress systemically post-HFM and post-MFM, as long as there is increased PPL. An interesting finding in the present study was the participants with the greatest body fat percentage had the greatest systemic 8-isoprostane response to the meals. Considering

cardiovascular disease and endothelial dysfunction may be the result of chronic elevation in triglycerides and oxidative stress [35] and obese individuals are at higher risk for developing CVD [36], post-prandial studies of varying fat and calories should be included in the nutrition literature in more "at-risk" and clinical populations.

As an exploratory analysis, we wanted to determine whether there were correlations between systemic 8-isoprostane and triglycerides. The generation of systemic 8-isoprostane was associated with the triglyceride response after the HFM, but not after the MFM. Considering that the triglyceride time to peak response was longer in the HFM period compared to the MFM period, but peak triglycerides were not significantly different, the mechanisms contributing to the post-prandial 8-isoprostane may also be different with regard to timing. In the MFM condition, the lipemic response was not associated with the increase in airway and systemic 8-isoprostane, however the change in systemic 8-isoprostane from baseline to 6 hours was associated with PPL in the HFM condition. These associations between systemic 8-isoprostane and PPL in the HFM condition alone was a surprising finding, and the differences in post-prandial oxidative stress by meal type may be due to different contributing mechanisms. The mechanisms contributing to post-prandial dysmetabolism are associated with cardiovascular disease risk [37], and may be mediated by oxidative stress [38]. Peak triglycerides commonly occur 3-4 hours post-HFM [39], which is why we chose to measure oxidative stress at 3 hours post-HFM. We also wanted to determine oxidative stress at 6 hours, because triglycerides may continue to rise or peak at this time point [40] or return to baseline. Post-prandial generation of oxidative stress occurs due to the increase in triglycerides and carbohydrates after a meal [41]. Considering that the time to peak for triglycerides was longer in the HFM condition compared to the MFM conditions, this

may explain why triglycerides were associated with plasma 8-isoprostane in HFM condition only.

There are several limitations in the present study that may have influenced our results. It is likely that the post-prandial airway and systemic 8-isoprostane responses are dependent on the total antioxidant status (TAS) of the participants. Therefore, it would be ideal to include TAS when reporting changes in 8-isoprostane in future studies. Additionally, since airway and systemic 8-isoprostane have not been reported in the same participant pool after consumption of a HFM and MFM, it would be ideal to have measurements taken every hour after the meal. This would give future researchers more information for the time course of changes in markers of lipid peroxidation post-prandially. Also, it would have been beneficial to perform pulmonary function testing in the post-prandial period to (1) compare possible changes in pulmonary function between meal types and (2) confirm our previous findings that pulmonary function does not change after the consumption of a HFM [7]. Finally, no specific measures of lipid peroxidation were recorded (i.e. TBARS, HNE, MDA, NT), and should be examined with regard to post-prandial changes in oxidative stress in future work. Also, we need to consider that the current results may not be generalizable to other populations including: older adults, active individuals, and those with pulmonary or metabolic diseases.

Meals of varying caloric and fat content do not seem to impact the airway 8-isoprostane response in young, non-asthmatic males at three and six hours post-meal. However, a HFM meal with 17 kcal/kg of body weight and a MFM of 8.5 kcal/kg of bodyweight both led to increased 8-isoprostane systemically. There were no differences in airway 8-isoprostane over time or between meals in the MFM and HFM condition. This may be due to antioxidant capacity in the

airways of the subjects in our study to protect against lipid peroxidation, and therefore total antioxidant status may be important to report along with 8-isoprostanes in future studies. Considering that even a single true-to-life MFM elicited increases in systemic 8-isoprostane, the impact of repeated MFM's and HFM's on 8-isoprostane could be of importance for clinical assessment of risk for developing CVD. However, whether the increase in 8-isoprostane translates to decreases in arterial function in insufficiently active males has yet to be determined.

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Table 3.1 Subject Characteristics

	Means		SD
Age (years)	25.8	±	6.9
Height (cm)	173.8	±	6.3
Weight (kg)	79.0	±	20.1
Waist circumference (cm)	37.9	±	5.8
Body fat (%)	21.0	±	12.7
Systolic BP (mmHg)	118.9	±	6.7
Diastolic BP (mmHg)	68.5	±	8.1

Participant characteristics of insufficiently active males (n=8) who completed both the HFM and MFM conditions
centimeters, cm; blood pressure, BP; millimeters of mercury, mmHG

Table 3.2 Post-prandial blood lipid values in MFM and HFM conditions

	MFM (n=8)		HFM (n=8)	
	Means ± SD		Means ± SD	P[a]
Total TRG AUC (mg/dL x 6 hrs)	819.1 ± 491.5		1409.3 ± 815.0	0.106
iAUC TRG (mg/dL x 6 hrs)	213.5 ± 201.7		679.5 ± 414.9*	0.013
Peak TRG (mg/dL)	164.1 ± 102.3		300.6 ± 174.6	0.082
Time to peak (hrs)	2.8 ± 1.2		4.1 ± 1.1*	0.031

[a]P values indicate comparison between the MFM and HFM trials

Triglycerides, *TRG;* area under the curve, *AUC;* incremental area under the curve, *iAUC*

Figure 3.1 Schematic of the study

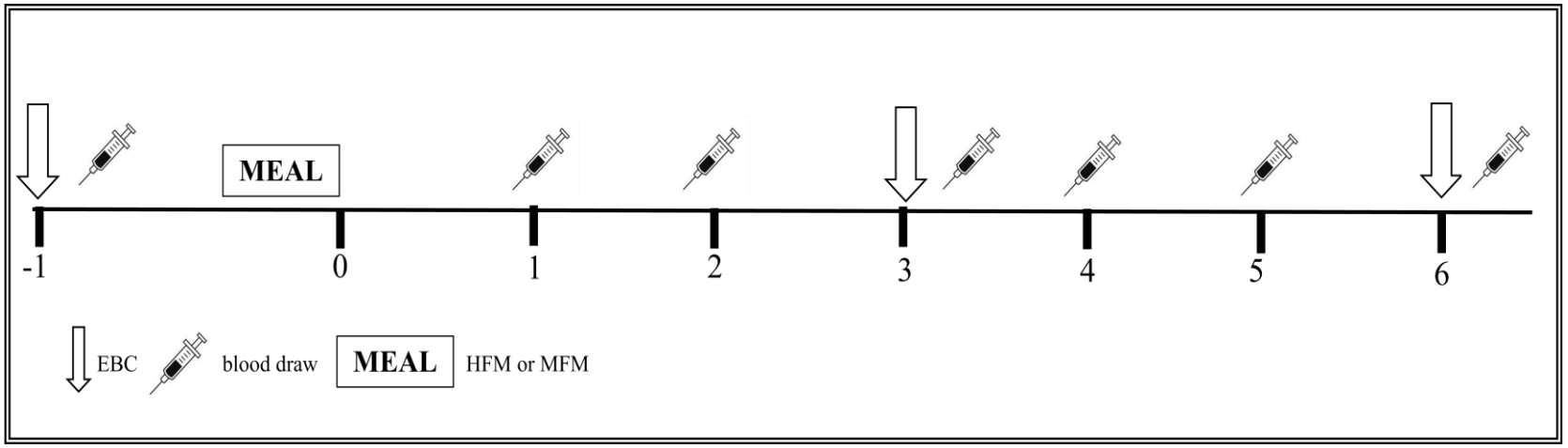
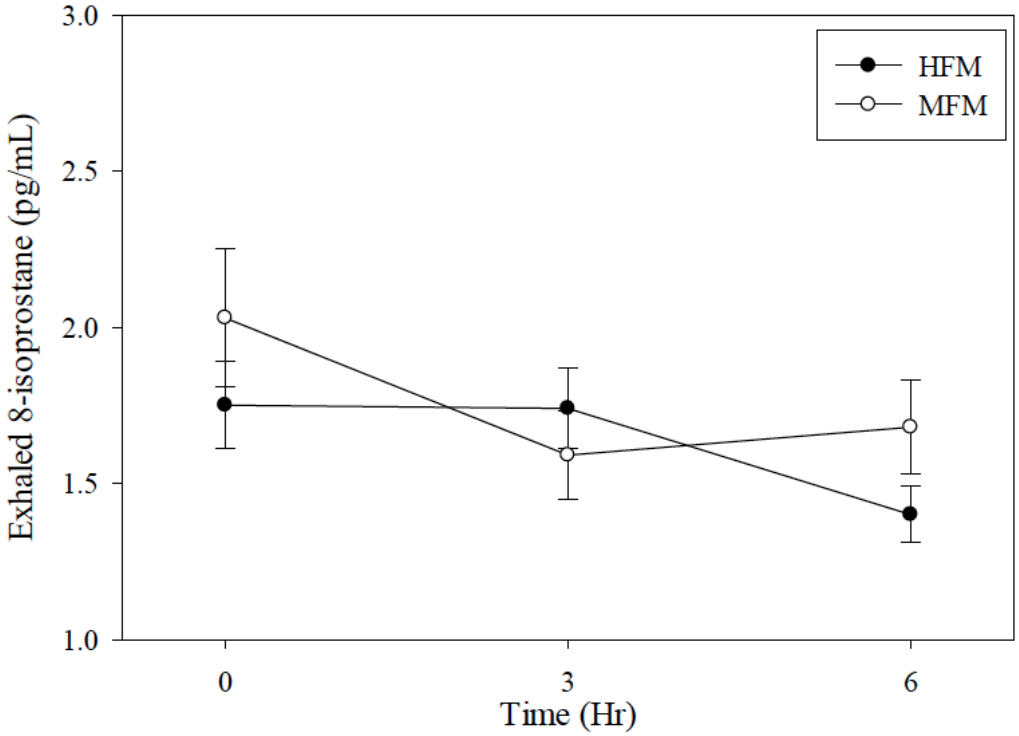
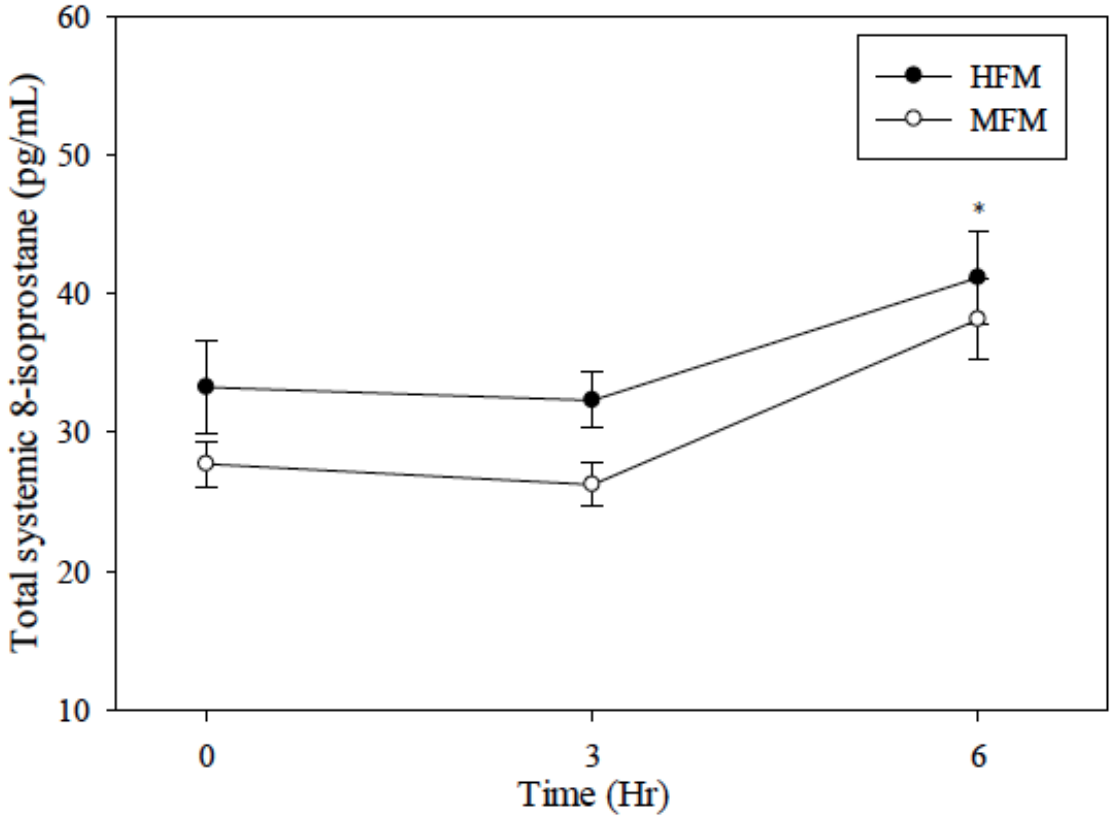


Figure 3.2 Mean airway 8-isoprostane responses in HFM and HFM



Mean post-prandial airway 8-isoprostane (n=8) responses with standard error bars in the HFM (•) and MFM (o) conditions are shown. There were no significant changes in airway 8-isoprostane over time in either condition and no significant difference between conditions.

Figure 3.3 Mean systemic 8-isoprostane responses in HFM and MFM



Mean post-prandial systemic 8-isoprostane (n=8) with standard error bars in the HFM (•) and MFM (o) conditions are shown. There was a significant increase over time in the HFM and MFM conditions (*), with no significant difference between conditions. Significance was set at $p<0.05$.

Chapter 4 - Older women exhibit greater airway 8-isoprostane responses to strenuous exercise compared to older men

In preparation as Kurti SP, Emerson SR, Smith JR, Rosenkranz SK, Alexander SA, Lovoy GM, Harms CA. Older women exhibit greater airway 8-isoprostane responses to strenuous exercise compared to older men.

Abstract:

INTRODUCTION: Development of respiratory diseases is associated with elevated 8-isoprostane in the airways. However, sex differences exist in development of these diseases. Using an exhaustive exercise bout as a physiological stressor may elucidate whether there is a sex difference in post-exercise airway 8-isoprostane in older adults. **PURPOSE**: To determine whether sex differences exist in airway 8-isoprostane responses to exhaustive exercise and establish whether changes in airway 8-isoprostane generation are correlated with lung function from pre- to post-exercise. **METHODS:** Subjects aged 65±4 years (12 OW/12 OM) came to the laboratory for one testing session. Baseline measurements included exhaled breath condensate (EBC) for assessment of airway 8-isoprostane and standard pulmonary function testing (PFTs) to assess forced expiratory volume in 1-second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$). Subjects then performed a VO_{2peak} test on a cycle ergometer. Immediately post-exercise, PFTs and EBC were performed. **RESULTS:** The generation of airway 8-isoprostane from pre- to post-exercise was different between OW and OM (p=0.003), increasing ~74±77% in OW and decreasing ~12±50% in OM. In OW, FEV_1 increased post-exercise (p=0.02), but was not associated with 8-isoprostane. In OM, FEV_1, FEV_1/FVC and $FEF_{25-75\%}$ increased post-exercise (p<0.05), however the decreased 8-isoprostane was not associated with improvements in FEV_1/FVC and $FEF_{25-75\%}$ (p>0.05). **CONCLUSIONS:** The OW exhibited greater airway 8-isoprostane responses to exhaustive exercise compared to OM, suggesting that sex differences in oxidative stress may play a role in the airway remodeling that is associated with disease development.

Keywords: Respiratory airway; Exercise science; Airway oxidative stress; Aging; Sex-based difference

Introduction

The prevalence of late-onset asthma diagnoses in individuals over 60 years of age is increasing, with a greater percentage of women diagnosed between 65 and 75 years of age compared to age-matched males (10). Oxidative stress and inflammation increase with age (6), which may contribute to airway remodeling and changes in airway structure (18). Recent investigations have also confirmed that lipid peroxidation, which is damaging to the airways, increases with age (5). Specifically, one particular marker of lipid peroxidation, 8-isoprostane, is elevated in asthmatics (23) as well as individuals with chronic obstructive pulmonary disease (COPD) (22). The elevation in airway 8-isoprostane is positively associated with decrements in lung function (32). Additionally, the generation of 8-isoprostane in the airways is associated with airway hyper-responsiveness, which has been reported in asthmatics (2) and more recently in non-asthmatic subjects (24). There is some dispute regarding the contribution of oxidative stress to the aging process (31), yet several studies have confirmed that aging and respiratory diseases are associated with increased 8-isoprostane (3, 29, 37).

The literature has shown that older adults have a varying healthspan (amount of years they are healthy in their lifespan), and several studies have reported sex differences in oxidative stress in older adults (38), which are associated with virtually all pathologies explored. The prevalence of respiratory diseases differs between older men and post-menopausal women, and previous investigations have shown that post-menopausal women are at higher risk for negative respiratory health outcomes as compared to older men. Also, older men and post-menopausal women have varying prevalence in the development of respiratory diseases (36), and previous investigations have shown that post-menopausal women are at higher risk for negative respiratory system health outcomes compared to older men (7). Older adults (60 and over) are

likely to have complications as a result of physical exertion, which causes a transient increase in reactive oxygen species (ROS) generation (26, 30). Therefore, is important to determine whether older men and women respond differently to various physiological stressors that elicit oxidative stress. A single bout of maximal exercise is a good model to investigate differences in 8-isoprostane generation. Also, the airway 8-isoprostane responses to an exhaustive exercise bout could provide more mechanistic insight into sex differences in the development of chronic diseases by using a specific biomarker that is associated with disease development.

There are no existing studies, to our knowledge, that have investigated whether sex differences exist in the airway 8-isoprostane response to exhaustive exercise in older adults. In addition, no previous research has elucidated whether changes in airway oxidative stress are associated with changes in lung function from pre- to post-exercise in older women (OW) and older men (OM). The primary purpose of the present investigation was to determine whether OW and OM exhibit differences in airway 8-isoprostane generation following a bout of exhaustive exercise. Further, we sought to determine whether changes in airway 8-isoprostane generation were correlated with changes in lung function in older adults. We hypothesized that OW would have elevated airway 8-isoprostane responses post-exercise compared to OM. We also hypothesized that in both OW and OM, an increased generation of airway 8-isoprostane would be negatively associated with lung function changes from pre to post-exercise.

Methods

Subject characteristics

Twenty-four individuals over the age of 60 and of varying physical activity levels completed the study (12 OW, 12 OM). Subjects visited the laboratory after 24 hours of no exercise and a 2-hour fast. They were also asked to refrain from antioxidants or dietary supplementation for at least 3

days prior to their testing session. When subjects first came to the laboratory, they were briefed on the study protocol, signed an informed consent document, and then completed the medical history questionnaire. The medical history questionnaire was used as a health-screening tool and also to ensure that all OW participating were post-menopausal. To ensure subjects were ready to engage in vigorous physical activity, they were required to complete the physical activity readiness-questionnaire (PAR-Q) (1). Subjects were not hypertensive (Systolic BP<142: Diastolic BP< 92) per the PAR-Q guidelines. If a subject had greater than two risk factors for cardiovascular disease, but did not have any signs and/or symptoms of disease, they were still able to participate under the recommendation of their primary care physician. The study protocol was approved by the Institutional Review Board at Kansas State University and conformed to the principles set forth in the *Declaration of Helsinki*.

Experimental Design

After completion of the required questionnaires and paperwork, subjects reclined in the supine position for 15 minutes and blood pressure was measured a total of three times with five minutes in between each measurement (28). Subjects then had height recorded on a portable stadiometer to the nearest 0.1 cm and weight recorded to the nearest 0.1 kg by an investigator using a standard physician's scale. After the initial measurements, subjects underwent exhaled breath condensate (EBC) testing, followed by baseline pulmonary function testing (PFTs). After PFTs, subjects performed the incremental exercise to exhaustion, followed by PFTs within two minutes after completion/termination of the exercise test. Subjects then underwent EBC, followed by the last round of PFTS at 20 minutes post-exercise. Figure 4.1 represents the study methods. The experimental measures are elaborated in the section below.

Experimental Measurements

Pulmonary function testing

Pulmonary function was assessed using standard PFTs and according to the American Thoracic Society (ATS)/European Respiratory Society guidelines (21). The maximum flow volume loop was used to assess pulmonary function, measuring forced vital capacity (FVC), forced expiratory volume in 1-second (FEV_1), forced expiratory flow between 25% and 75% of FVC ($FEF_{25-75\%}$), and peak expiratory flow (PEF) (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). Subjects performed PFTs until three measurements were within 10% of one another, and averaged for analysis. This procedure is more stringent than ATS guidelines, and has been used many times previously in our laboratory (18, 19, 35). Percent of predicted lung function was calculated using reference values from Knudson et al. (1983) (16). Lung function measurements were assessed at baseline, immediately post-exercise, and approximately 20 minutes post-exercise following the EBC testing.

Incremental exercise test to exhaustion

Subjects performed an incremental cycle ergometer **(**SensorMedics 800**)** test to exhaustion to determine peak oxygen consumption (VO_{2peak}) according to similar methods published in our laboratory (9). During the entire test, subjects were required to maintain a cadence of 60-80 revolutions per minute (rpm). The incremental test to exhaustion began with three minutes of resting data collection where metabolic and ventilatory responses were recorded. Afterwards, subjects began the test with a 3-minute unloaded warm-up where subjects cycled at 60-80 rpm. After the warm-up, the incremental test began at a load of 25 watts and increased by 25 watts per minute until either the subjects reached volitional fatigue, or investigators terminated the test due to subject failure to maintain a cadence of at least 50 rpm for at least 5 revolutions. Heart rate

was recorded the final 10 seconds of every minute and breath-by-breath data for ventilation, carbon dioxide production (CO_2) and oxygen (O_2) consumption were recorded through the entire test. The criteria that were used to determine whether subjects reached VO_2peak were a respiratory exchange ratio (RER) of greater than 1.15 and an achieved heart rate max (HR_{max}) within 10 beats per minute of an age-predicted HR_{max}.

Assessment of Airway 8-isoprostane

Subjects performed tidal breathing without a noseclip for 10 minutes into an RTube (Respiratory Research, Austin, TX) while seated in a chair, and with their feet flat on the floor for 10 minutes. This protocol collects ~1.0-1.5 mL of exhaled breath condensate sample. The samples were then plunged from the RTubes, aliquoted into microcentrifuge tubes for freezing, and immediately frozen in a -60 degree Celsius freezer. Samples were analyzed within six months of data collection. Samples from EBC were concentrated by C-18 solid phase extraction prior to analysis and analyzed using a commercially available ELISA kit. Samples were analyzed in triplicate with an intra-assay coefficient of variation of 5.2%.

Statistical Analyses

Data were analyzed using SPSS Statistical Software v.24 (IBM, Armonk, NY, USA). The data are expressed as mean±SD. Data were checked for normality prior to the analysis. Two-way analysis of variance (ANOVA) was used where the within-subjects factor was time (pre- and post-exercise) for airway 8-isoprostane generation and time (pre-, post-, and 20-minutes post-exercise) for lung function. The between-subjects factor was sex (male or female). A one-way repeated measures ANOVA was subsequently used to determine time point differences in lung function within each sex. Correlations were assessed between changes in airway 8-isoprostane and lung function with the Pearson Product-moment correlation coefficient. Bonferroni

adjustments were made to reflect multiple comparisons. For all analyses, significance was set to $p<0.05$.

Results

Subject characteristics

Baseline subject characteristics and exercise responses are displayed in Table 4.1. OW and OM were not different in age ($p=0.23$). The OW had greater body fat percentage, and were shorter than OM ($ps<0.01$). Data from the incremental exercise test to exhaustion are also displayed in Table 4.1. The OW had lower absolute VO_{2peak}, relative VO_{2peak}, and peak power at VO_{2peak} compared to OM ($ps<0.01$). Women had a higher RER compared to OM ($p=0.03$), and a lower peak ventilation (V_E) ($p<0.01$). The maximum heart rate achieved at VO_{2peak} was not different between OW and OM ($p=0.96$).

Generation of Airway 8-isoprostane

There was no difference in airway 8-isoprostane pre-exercise between OW (11.3±7.9 pg/mL) and OM (11.3±3.8 pg/mL) ($p=0.99$). Figure 4.2 displays the mean airway 8-isoprostane responses in OW and OM. In OW and OM combined, there was no significant main effect of time ($p=0.18$), however there was a significant interaction between time and sex ($p=0.003$), where OW and OM differed in the airway 8-isoprostane responses to the bout of exhaustive exercise. Figure 4.3A displays the individual responses of the OW while Figure 4.3B displays the responses of the OM. As can be seen by the individual responses, 9/12 OW elicited an increase in 8-isoprostane while the other three OW remained relatively unchanged, or showed a small decrease in the generation of airway 8-isoprotane. In contrast, 9/12 men showed decreased generation of 8-isoprostane in response to the strenuous bout of exercise, while only 3/12 had increased 8-isoprostane. When analyzing OW alone, 8-isoprostane generation significantly

increased by ~74% (p<0.01), while OM alone decreased by ~12%, yet the decrease in OM was not statistically significant (p=0.19). There was one large increase in 8-isoprostane generation in OM that was an outlier when we performed the outlier labeling technique in SPSS, and without the subject included in the analysis, the 8-isoprostane generation in OM decreased significantly from pre- to post-exercise (p=0.03).

Lung function

Lung function data are displayed in Table 4.2. When analyzing sex differences in percent of predicted lung function, there were no significant differences between males and females for FVC, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$ or PEF (p>0.05), however there were significant increases in percent of predicted lung function from pre- to 20-minutes post-exercise as a main effect of time in FEV_1 (p=0.02), FEV_1/FVC (p<0.01) and $FEF_{25-75\%}$ (p<0.01). Also, changes in lung function between OW and OM were different across time (time* sex interaction) from pre-to 20-minutes post-exercise in FEV_1/FVC (p=0.04) and $FEF_{25-75\%}$ (p=0.03). When further examining pair-wise comparisons within each sex, there was a significant increase in FEV_1 from baseline to post-exercise (p=0.02) that returned to baseline by 20 minutes post-exercise (p>0.90) in OW. There were no significant changes in FVC, FEV_1/FVC, $FEF_{25-75\%}$, or PEF from baseline, post- or 20-minutes post-exercise (all p-values >0.10) in OW. In OM, there was no change in FVC from baseline to immediately post-exercise (p>0.90) or baseline to 20 minutes post-exercise (p=0.91). However, there was a significant increase in FEV_1 post-exercise (p=0.03) that returned back to baseline 20 minutes post (p=0.25). There was also an increase in FEV_1/FVC post-exercise (p=0.01) and 20 minutes post (p<0.01). $FEF_{25-75\%}$ increased post-exercise (p=0.03) and remained higher at 20 minutes after the exercise bout (p<0.01) compared to baseline. There were no changes observed in PEF immediately or 20-minutes post-exercise in OM or OW (p>0.90).

Associations between variables

There were no correlations between airway 8-isoprostane generation from pre- to post-exercise with changes in lung function in OW and OM combined. In OW and OM, there were no associations between absolute or percentage changes in 8-isoprostane and lung function (p>0.05).

Discussion

Major Findings

The primary purposes of this study were to determine whether OW and OM varied in airway 8-isoprostane responses after exhaustive exercise, and to elucidate whether changes in airway 8-isoprostane were correlated with changes lung function. The results from the current study supported our hypothesis that airway 8-isoprostane generation was greater post-exercise in OW compared to OM. Our second hypothesis was not supported, as changes in 8-isoprostane were not correlated with changes in lung function in OW or OM.

Airway 8-isoprostane generation

In the present study, airway 8-isoprostane increased following exhaustive exercise in the OW and not in the OM, which is a novel finding. The generation of 8-isoprostane, the most abundant of the F2-isoprostanes, in the airways is derivative of arachidonic acid metabolism (35), resulting primarily from free radical-induced peroxidation. 8-isoprostane is a validated marker of lipid peroxidation and oxidative stress (25). While our hypothesis was that older post-menopausal women would have a higher airway oxidative stress response compared to age-matched men, our next step was further elucidating the mechanisms in aging that contribute to the elevated response in the OW. Post-menopausal women have age-related immune changes, which may be due to the loss of estrogen, that make them especially susceptible to oxidative

stress generation (8). In fact, Kos-Kudla et al. (2000) showed that serum estrogen was lower in postmenopausal asthmatics compared to non-asthmatic post-menopausal women. When post-menopausal asthmatics were given hormone replacement therapy (HRT), asthma symptoms improved (16). This is in agreement with Carlson et al. (2001) who reported that post-menopausal women using HRT exhibited a higher FEV_1 compared to post-menopausal women not using HRT (4). In addition, Huh (1994) and colleagues reported that males have higher lipid peroxidation compared with females, however the response was attributed to higher estradiol concentration in females (11). Therefore the loss of estrogen through menopause coupled with age-related changes in immune cells may provide a mechanistic explanation for the present findings, although more research needs to be conducted to test these hypotheses. Future research should examine the influence of sex hormones on airway oxidative stress.

Changes in airway 8-isoprostane and lung function

Previous research indicates that 8-isoprostane in EBC has negative impacts on the airways, and that in asthmatics 8-isoprostane is associated with the clinical severity of asthma (5). More recent literature has reported that 8-isoprostane generation after a strenuous bout of exercise was significantly associated with the degree of hyper-responsiveness in non-asthmatics (7). Considering that research has shown that elevated 8-isoprostane is detrimental to the respiratory system, and lipid peroxidation increases with both age and acute exercise, there is a need to elucidate the 8-isoprostane responses in the airways after exhaustive exercise. Bronchodilation occurs after deep inspirations (i.e. post-exercise) in healthy, non-asthmatics (12), yet this bronchodilatory effect of deep inspirations (such as during exercise) diminishes with age (33). In the current study, post-exercise bronchodilation (increase in FEV_1) was more pronounced, and lasted until 20 minutes post-exercise in OM, while in OW it returned back to

baseline values by 20 minutes post-exercise. While 8-isoprostane generation was not associated with lung function in either group, OW did not bronchodilate as much from pre- to post-exercise, and it is possible oxidative stress could be moderating changes in airway function. Women exhibited an increase in FEV_1, but it did not last as long nor was it associated with 8-isoprostane. These sex differences in lung function following exercise in older adults should be explored further to determine whether the onset of changes in airway structure could be a contributing factor to our results.

Limitations

While we have presented the most relevant data, there are many studies referenced previously that show conflicting results with regard to changes in 8-isoprostane generation. Many issues may contribute to the conflicting findings in the current literature, and we were not able to account for several of those variables. Antioxidant status may impact the generation of 8-isoprostanes, however the results are inconsistent and some studies show no changes after antioxidant supplementation, while others show an attenuation of 8-isoprostane based on antioxidant status (26). We had our subjects refrain from supplementation or vitamins for 3 days, however, we were not able to measure total antioxidant status in our subjects. Finally, previous studies have suggested that habitual physical activity level (24) as well as body fat percentage (15) may impact 8-isoprostane production. While we were not able to match OM and OW for body fat percentage and fitness level, neither factor was correlated with changes in airway 8-isoprostane from pre- to post-exercise. Further, to verify there was not an interaction between body fat percentage or peak oxygen consumption and changes in airway 8-isoprostane, we included both as covariates in an ANCOVA (results not presented). Neither body fat percentage (p=0.91) nor absolute VO_2peak (p=0.54) impacted 8-isoprostane generation in this model.

Therefore, we are confident that the changes in airway 8-isoprostane from pre- to post-exercise were primarily due to sex difference, and were not impacted by body fat percentage or peak oxygen consumption.

Conclusions

In summary, this study suggests that sex differences exist in airway 8-isoprostane responses following an exhaustive bout of exercise in older adults. In our study, OW had a greater airway 8-isoprostane response following exhaustive exercise compared to age-matched OM. Post-exercise bronchodilation was smaller in OW compared to OM. Generation of airway 8-isoprostane was not correlated with improvements in lung function in OW or OM. Given these novel findings regarding oxidative stress responses in the airways of older adults, further research should explore the mechanisms behind these sex differences as well as the possibility of modification of these responses through lifestyle intervention.

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Table 4.1 Subject characteristics for older women (OW) and older men (OM)

	OW (n=12)			OM (n=12)		
	Value		SD	Value		SD
Age (years)	64.1	±	4.1	66.2	±	4.1
Height (cm)	160.1	±	5.6*	174.6	±	7.2
Weight (kg)	76.7	±	20.7	85.6	±	16.4
Body mass index (BMI) (kg/m^2)	30.2	±	9.2	27.6	±	4.3
Body Fat (%)	41.5	±	11.5*	25.8	±	9.9
Systolic (mmHg)	128.1	±	11.3	132.9	±	9.0
Diastolic (mmHg)	80.1	±	4.4	81.6	±	4.8
Peak Exercise Data						
VO_{2peak} (L/min)	1.5	±	0.3*	2.5	±	0.6
Relative VO_{2peak} (mL/kg/min)	20.6	±	6.7*	29.7	±	9.4
RER	1.4	±	0.2*	1.2	±	0.1
Ventilation (L/min)	67.3	±	11.2*	102.8	±	33.8
Power (watts)	129.2	±	23.4*	216.7	±	63.4
HRmax (bpm)	147.4	±	8.8	146.9	±	30.3

mmHg, millimeters of mercury; VO_{2peak}, peak oxygen consumption

RER, respiratory exchange ratio; HRmax, maximum heart rate

*Significantly different compared to OM ($p<0.05$)

Table 4.2 Pulmonary Function Data before exercise, immediately post-exercise, and 20 minutes post-exercise

	OW						OM					
	Pre-Exercise		Post-Exercise		20 minutes post		Pre-Exercise		Post-Exercise		20 minutes post	
	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD
n=	12		12		12		12		12		12	
PEF (L/s)	5.60 ±	0.67	5.63 ±	0.78	5.75 ±	0.63	8.95 ±	1.71	8.80 ±	1.72	9.05 ±	1.76
FVC (L)	2.87 ±	0.31	2.93 ±	0.29	2.86 ±	0.32	4.51 ±	0.96	4.54 ±	0.94	4.46 ±	0.92
FEV_1 (L)	2.15 ±	0.22	2.21 ±	0.20*	2.21 ±	0.36	3.25 ±	0.72	3.39 ±	0.73*	3.32 ±	0.69*^
FEV_1/FVC (%)	75.08 ±	4.76	75.67 ±	4.72	75.61 ±	4.54	72.22 ±	5.56	74.68 ±	5.78*	74.75 ±	5.05*^
$FEF_{25-75\%}$ (L/s)	1.78 ±	0.54	1.87 ±	0.55	1.81 ±	0.50	2.34 ±	0.89	2.72 ±	1.06*	2.68 ±	0.86*^

PEF, peak expiratory flow; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1-second; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of FVC

*Significantly different change from baseline within-group ($p<0.05$)

^Significantly difference between OW and OM over time ($p<0.05$)

Values are expressed as mean ± SD.

Figure 4.1 Schematic of the study design

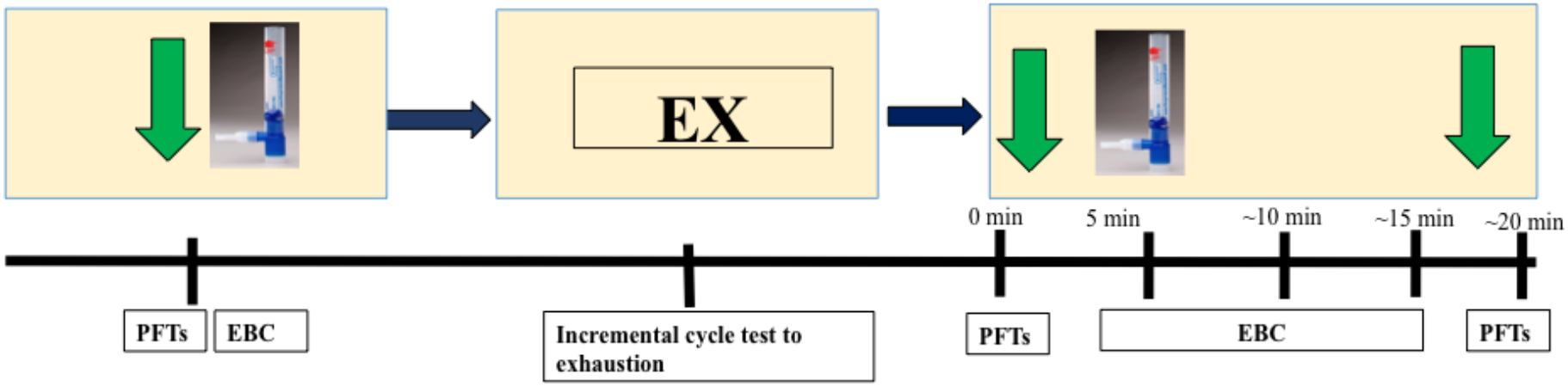
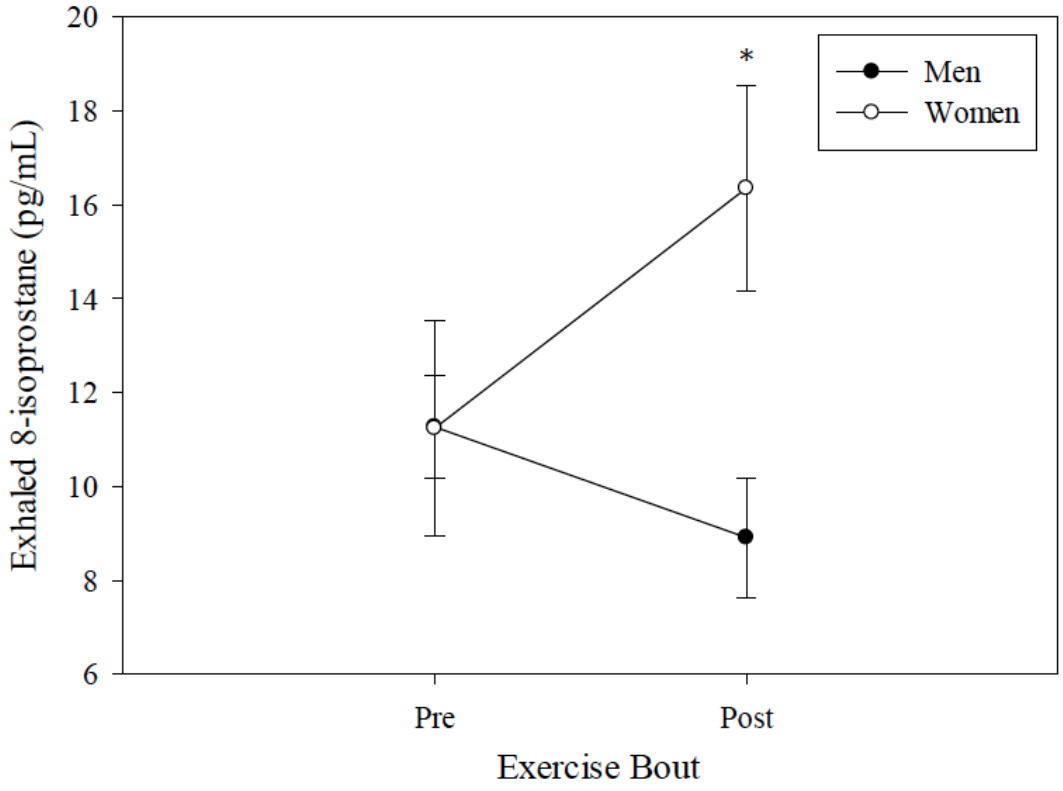


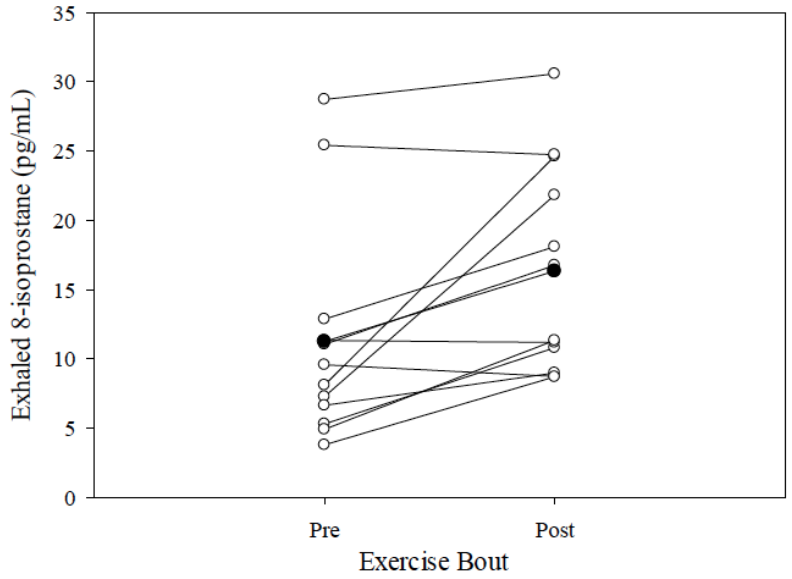
Figure 4.2 Mean airway 8-isoprostane generation in OM and OW



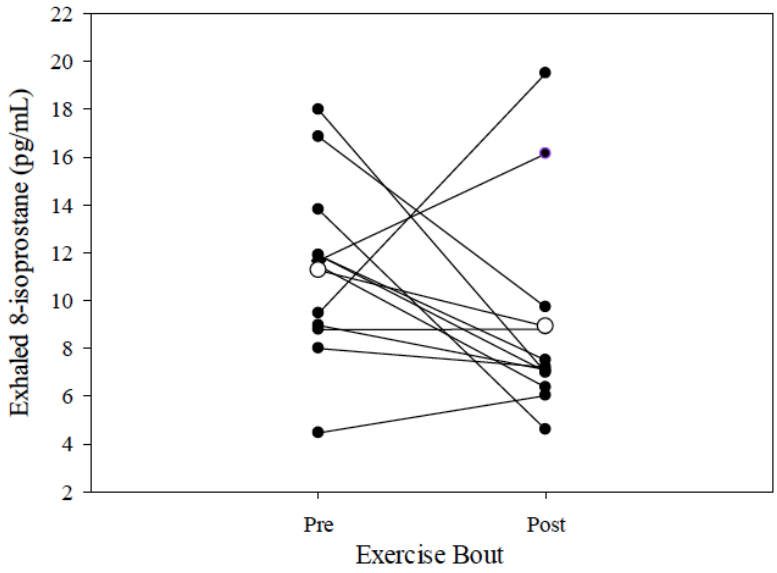
Data are displayed as mean with standard error bars. The mean airway 8-isopostane generation in OM (•) and OW (o) is shown. Airway 8-isoprostane significantly increased in older women (*p<0.05) but did not significantly change in OM.

Figure 4.3 Individual airway 8-isoprostane responses in OW (A) and OM (B)

A. OW



B. OM



Individual airway 8-isoprostane responses are shown in OW and OM. The mean data point for OW is displayed with a filled in black circle and with an open circle for the OM. As can be seen by the individual responses, 9/12 women exhibited an increase in the generation of exhaled 8-isoprostane from pre- to post-exercise, while 9/12 men exhibited a decrease in 8-isoprostane generation from pre- to post-exercise.

Chapter 5 - Conclusions

When considering the implications of this dissertation, each study adds important information to the existing literature. The overall hypothesis of this work was that exercise, both an acute bout as well as chronic physical activity level, and high-fat meals, would alter physiological biomarkers that are associated with asthma development and progression. The repeated increase in inflammation and oxidative stress after repeated high-fat meals may potentially increase the likelihood of disease development and progression. In the first study of this dissertation, we reported that a single-high fat meal (HFM) increases airway inflammation in non-asthmatics, measured non-invasively via exhaled nitric oxide, and more invasively via an increase in airway neutrophils. However, chronic physical activity level did not modify the airway inflammatory response to the HFM, while exhaled nitric oxide increased to a greater extent post-HFM in the acute exercise condition compared to remaining sedentary in the post-prandial period (Chapter 2). While these were surprising findings, the results made us question whether we could better assess changes in biomarkers of oxidative stress that are associated with structural changes in the airways. We thought it would be worthwhile to investigate the oxidative stress responses to meals of varying caloric and fat content.

This led to our next study, where we sought to determine the airway and systemic 8-isoprostane responses to moderate and high-fat meals of different caloric and fat content (8.5 kcal/kgbw and 17 kcal/kgbw). Interestingly, insufficiently active adults exhibited greater systemic 8-isoprostane responses after both moderate-fat and high-fat meals, without a difference in airway 8-isoprostane generation. In addition, the increase in post-prandial systemic 8-isoprostane was similar to the reported post-prandial increases in other research studies when a

decrease in flow-mediated dilation was present (Chapter 3). These findings suggest that even after a single true-to-life meal, there could be impaired arterial function in insufficiently active males. While we were interested in following up on these post-prandial results, the oxidative stress response is also associated with development and progression of late-onset respiratory diseases. Specifically, airway 8-isoprostane has been reported to increase with age. Researchers have also reported a sex difference in the prevalence of respiratory disease and severity in older adults, therefore the oxidative stress (8-isoprostane) response to a physiological stressor was the focus of our final investigation. The results from this study showed that older women had a greater airway 8-isoprostane response to a strenuous bout of exercise than age-matched men. Interestingly, men exhibited decreased airway 8-isoprostane from pre- to post-exercise. The generation of 8-isoprostane was not associated with changes in lung function in OW or OM (Chapter 4).

In conclusion, this series of studies furthers the information currently available in the post-prandial lipemic and respiratory literature by elucidating the impact of lifestyle factors, sex, and age on physiological biomarkers associated with the development of respiratory diseases. It appears the assessment of post-prandial systemic 8-isoprostane responses in insufficiently active males and post-exercise airway 8-isoprostane responses in older women may provide important clinical information that might be useful in determining the potential risk for development of respiratory disease and other chronic illness.

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EDUCATION

Graduate:	2013-2017	Ph.D. Kinesiology Kansas State University Mentor: Dr. Craig A. Harms *Dissertation title: The impact of lifestyle, age, and sex on systemic and airway inflammation and oxidative stress*
	2009-2011	M.S. Exercise Science and Health Promotion (*Specialization: Exercise Physiology*) Florida Atlantic University
Undergraduate:	2005-2009	B.S. Biology University of Mary Washington

POSITIONS

2013-present	Graduate Teaching Assistant- *Department of Kinesiology, Kansas State University*
2012-2013	Faculty Instructor- *Department of Kinesiology, Kansas State University*
2011-2012	Advanced Placement Chemistry Teacher- *Oasis High School, Cape Coral, FL*
2009-2011	Graduate Teaching Assistant, *Department of Exercise Science and Health Promotion, Florida Atlantic University*

PROFESSIONAL MEMBERSHIPS

2015-present	American Society for Nutrition
2010-present	American College of Sports Medicine
2014-present	American Physiological Society
2010-2012	International Society of Sports Nutrition
2011-2012	Co-Founder, Slow Foods FAU Nutrition Club

GRANT SUPPORT

2016-present	University Distinguished Professors Graduate Student Research Grant Awarded: $5,000 Title: "*The impact of lifestyle and age on systemic and airway inflammation and oxidative stress"*

	Central States American College of Sports Medicine Doctoral Student Research Award Awarded: $1,000 Title: *"Does chronic physical activity level impact baseline and postprandial oxidative stress in asthmatics?"*
	Dr. Rick Scheidt Graduate Student Award for Excellence in Research in Aging Awarded: $1,700
2015-2016	Small Grant program for KSU Graduate Students in Arts, Humanities and Social Sciences Awarded: $1,000 Title: *"Does physical activity status modify airway oxidative stress associated with aging?"*
	College of Human Ecology Doctoral Dissertation Award Awarded: $1,000 Title: *"The impact of lifestyle on systemic and airway oxidative stress and inflammation"*
2014-2015	Undergraduate Research Excellence Grant for funding for Kourtney Foley Awarded: $1,000 *"Title: Does the consumption of fish oil attenuate painful symptoms associated with Raynaud's phenomenon?"*

PUBLICATIONS

Peer-Reviewed Publications:

1. Emerson SR, **Kurti SP**, Teeman CS, Emerson EM, Cull BJ, Haub MD and Rosenkranz SK. Realistic Test-Meal Protocols Lead to Blunted Postprandial Lipemia but Similar Inflammatory Responses Compared to a Standard High-fat Meal. (2017). *Current Developments in Nutrition (in press).*

2. Emerson, S.R., **Kurti, S.P.,** Harms, C.A., Melgarejo, T., Logan, C., Rosenkranz, S.K. (2017). Magnitude and Timing of the Postprandial Inflammatory Response to a High-fat Meal in Healthy Adults: A Systematic Review. *Advances in Nutrition;* 3:213-225. DOI: 10.3945/an.116.014431.

3. **Kurti, S.P**., Emerson, S.R, Rosenkranz, S.K., Teeman, C.S, Emerson, E.E, Cull, B.J, Smith, J.R, Harms, C.A. (2017). Post-prandial responses of systemic and airway 8-isoprosane to meals of varying caloric and fat content in non-asthmatic, insufficiently active men. *Nutrition Research.* 39;61-68.

4. **Kurti, S.P,** Rosenkranz, S.K, Chapes, S.K, Levitt, M.L, Cull, B.J, Teeman, C.S, Emerson, S.R, Smith, J.R., Harms, C.A. (2017). Does Chronic Physical Activity Level Modify the Airway Inflammatory Response to an Acute Bout of Exercise in the Post-Prandial Period? *Journal of Applied Physiology, Nutrition and Metabolism.* 42(2): 173-180.

5. Smith, J.R, Broxterman, R.M, Hammer, S.M, Alexander, A.M, Didier, K.D., **Kurti, S.P**, Barstow, T.J, Harms, C. (2017). Cardiovascular Consequences of the Inspiratory Muscle Metaboreflex: Effects of Age and Sex. *American Journal of Physiology- Heart and Circulatory Physiology.*

6. Teeman, C.S., **Kurti, S.P.**, Cull, B.J., Emerson, S.R., Haub, M.D., Rosenkranz, S.K. (2016). Postprandial Lipemic and Inflammatory Responses to High-Fat Meals: A Review of the Roles of Acute and Chronic Exercise. *Nutrition and Metabolism*. 13:80. DOI: 10.1186/s12986-016-0142-6.

7. Smith, J.R., **Kurti, S. P.**, Meskimen, K., Harms, C.A. (2016). Expiratory flow limitation and operating lung volumes during exercise in older and younger adults. *Respiratory Physiology and Neurobiology.*

8. **Kurti, S.P**., Smith, J.R., Murphy, J.D., Ferguson, C.S., Brown, K.R., Harms, C.A. (2016). Improved Lung Function following Dietary Antioxidant Supplementation in Exercise-Induced Asthmatics. *Respiratory Physiology and Neurobiology*. 220:95-101.

9. Emerson, S.R., Haub, M.D., Teeman, C.S., **Kurti, S.P.,** Rosenkranz, S.K. (2016) Summation of blood glucose and TAG to characterise the 'Metabolic Load Index'. *British Journal of Nutrition.*

10. **Kurti, S.P,** Kurti, A.N., Rosenkranz S.K., Emerson S.R., Smith, J.R., Harms C.A. (2016). Indoor Air Pollution Exposure and Influence of Lifestyle on Respiratory Health and Lung Function in Belizean Adults and Children. *International Journal of Environmental Research and Public Health.* 13(7): 643.

11. Berrones, A., **Kurti, S.**, Kilsdonk, K., Cortez, D., Melo, F, Whitehurst, M. (2016). Barefoot Running Reduces the Submaximal Oxygen Cost in Female Distance Runners. *Journal of Strength and Conditioning Research*. 30(8), 2348-53.

12. Teeman, C, **Kurti, S.P**, Cull, B.J., Emerson, S.R., Haub, M.D., Rosenkranz, S.K. (2016). Does moderate intensity exercise in the postprandial period attenuate the inflammatory response to a high-fat meal? *Nutrition Journal. 15:24.* doi: 10.1186/s12937-016-01340-4.

13. Emerson, S.R, **Kurti, S.P**, Haub, M.D, Snyder, B.S, Rosenkranz S.K. (2016). Effects of thirty and sixty minutes of moderate-intensity aerobic exercise on postprandial lipemia and inflammation in overweight men. *Journal of the International Society of Sports Nutrition.* 13:26. doi: 10.1186/s12970-016-0137-8.

14. McEntire, S.J., Smith, J.R., Ferguson, C.S., Brown, K.R., **Kurti, S.P**., Harms, C.A. (2016). The Effect of Exercise Training with Inspiratory Muscle Training on Inspiratory Muscle Fatigue and Time-Trial Performance. *Respiratory Physiology and Neurobiology.* 230:54-9.

15. Smith, J.R., Broxterman, R.M., Barstow, T.J., Ade, C.J., Evans, K.K., **Kurti, S.P.** (2016). Hammer, S, Harms, C.A. The Effect of *N*-acetylcysteine on Blood Flow and Muscle Oxygenation Characteristics during Handgrip Exercise. *Physiological Reports.*

16. Smith, J.R, Broxterman, R.M, Hammer, S.M, Alexander, A.M, Didier, K.D., **Kurti, S.P,** Barstow, T.J, Harms, C. A. (2016). Sex Difference in the Cardiovascular Consequences of the Inspiratory Muscle Metaboreflex. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology.* 311(3): R574-81. doi: 10.1152/ajpregu.00187.2016.

17. **Kurti, S.P,** Rosenkranz, S.K., Levitt, M., Cull, B.J., Teeman, C.S., Emerson, S.R., Harms, C.A (2015). Does Moderate Intensity Exercise Attenuate the Post-prandial Lipemic and Airway Inflammatory Response to a High-fat Meal? *BioMed Research International: Special Issue on Modulation of Lung Inflammation.* Article ID: 647952. doi: 10.1155/2015/647952. Epub Apr 27.

18. **Kurti, S.P**., Smith, J.R., Emerson, S.R., Castinado, M., and Harms, C.A. (2015). Absence of Respiratory Muscle Fatigue in High-Intensity Continuous or Interval Cycling Exercise. *Journal of Strength and Conditioning Research*. 29(11):3171-6.

19. Johnson, A.M., **Kurti., S.P**., Smith, J.R., Rosenkranz, SK., Harms, C.A. (2015). Effects of an Acute Bout of Moderate Intensity Exercise on Airway Inflammation and Postprandial Lipemia. *Journal of Applied Physiology, Nutrition and Metabolism.* 41(3):284-91.

20. Smith, J.R., **Kurti, S.P.,** Johnson, A.J., Kolmer, S,. Harms, C.A. (2015). Impact of varying physical activity levels on airway sensitivity and bronchodilation in healthy humans. *Journal of Applied Physiology, Nutrition, and Metabolism.* 40(12):1287-93.
21. Smith, J.R., Emerson, S., **Kurti, S.P**, Kirti, G., Harms, C.A. (2015). Lung volume and expiratory flow rates from pre- to post-puberty. *European Journal of Applied Physiology.* 115(8):1645-52.

22. Skutnik, B., Smith, J.R., Johnson, A.J., **Kurti, S.P.,** Harms, C.A. (2015). The effect of low volume interval training on resting blood pressure in pre-hypertensive subjects. *Physician and Sports Medicine.* 44(2):177-83. doi: 10.1080/00913847.2016.1159501.

23. Emerson, S.R., Rosenkranz, S.K., Rosenkranz, R.R., **Kurti, S.P**., Harms, C.A. (2015). "Is there a link between sugar-sweetened beverage consumption and post-exercise airway narrowing across puberty?" *Public Health Nutrition.* 19(13):2435-40. doi: 10.1017/S1368980015003109.

24. Emerson, S.R**.**, **Kurti, S.**, Rosenkranz, S.K., Smith, J.R., and Harms, C.A (2014). Decreased prevalence of exercise expiratory flow limitation from pre- to post-puberty. *Journal of Medicine and Science in Sports and Exercise.* Jul;47(7):1503-11.

Published Book Chapter:

1. Harms, C.A., Smith J.R., **Kurti S.P**. Sex Differences in Normal Pulmonary Structure and Function. In: Hemnes A editor. Gender, Sex Hormones and Respiratory Disease – A Comprehensive Guide. 2015.

Manuscripts Under Revision/Review:

1. **Kurti, S.P**, Smith, J.R., Rosenkranz, S., Jurrens, K., Laughlin, A., Harms, C.A. Deep Inspirations Attenuate Postprandial Airway Inflammation in Non-Asthmatic Adults: A Randomized Crossover Study**.** *Submitted to Respiratory Physiology and Neurobiology* (Under Review)

2. Smith, J. R, Didier, K.D., Hammer, S.M., Alexander, A.M., **Kurti, S.P.,** Barstow, T.J., Harms, C.A. Effect of Cyclooxygenase Inhibition on the Inspiratory Muscle Metaboreflex-Induced Cardiovascular Consequences. *Journal of Applied Physiology*. (Under Revision)

3. Smith, J.R, Broxterman, R.M, Hammer, S.M, Alexander, A.M, Didier, K.D., **Kurti, S.P,** Barstow, T.J, Harms, C. The Effect of Aging on the Inspiratory Muscle Metaboreflex. *Submitted to the Journal of Physiology.*

4. Emerson, S.R., **Kurti, S.P.,** Emerson, E.M., Cull, B.J., Casey, K., Fees, A., Haub, M.D., Rosenkranz, S.K. Postprandial metabolic responses vary in age and level of physical activity". Submitted to the *Journal of Nutrition, Health and Aging*. (Under Revision).

Manuscripts in preparation:

1. **Kurti, S.P.,** Allen, J., Abello, J., Wood, J, Rosenkranz, S.A., Harms, C.A. The impact of chronic physical activity level on the diversity of the lung microbiome. To be submitted to the *Journal of Applied Physiology*.

2. **Kurti, S.P.,** Emerson, S., Smith, J, Rosenkranz, S.A., Alexander, S., Lovoy, G., Harms, C.A. Sex differences exist in the airway 8-isoprostane response to strenuous exercise. To be *Submitted to Respiratory Medicine.*

3. **Kurti, S.P.,** Simpson, R., Lovoy, E., Agha, N., Baker, F., Harms, C.A. Body fat percentage, but not fitness level or infection history, is associated with post-exercise oxidative stress in younger adults. To be submitted to *Brain, Behavior and Immunity.*

4. **Kurti, S.P**., Simpson, R.J, LaVoy, E., Emerson, S.R, Rosenkranz, S.K., Agha, N. Baker, F. Harms, C.A. Systemic 8-isoprostane responses are dependent on cytomegalovirus serostatus in older adults. To be submitted to *Brain, Behavior and Immunity*.

5. **Kurti, S.P.**, Emerson, S.R., Rosenkranz, S.K., Smith, J.R., Harms, C.A. Habitual Physical Activity Level Modifies Lung Function, But Not 8-isoprostane Generation, in Older Adults. To be submitted to *Respiratory Physiology and Neurobiology.*

POSTER PRESENTATIONS

1. Smith, J.R., Alexander, A.M., Hammer, S.M., Didier, K.D., **Kurti, S.P**., Broxterman, R.M., Barstow, T.J., Harms, C.A. Effect of Aging on Sex Differences in the Inspiratory Muscle Metaboreflex. Accepted at the American College of Sports Medicine Annual Meeting (Denver, CO, 2017)

2. Emerson, S.R., **Kurti, S.P.,** Emerson, E.M., Cull, B.J., Casey, K., Fees, A., Haub, M.D., Rosenkranz, S.K. Postprandial Triglyceride Responses in Younger versus Older Active Adults. Accepted at the American College of Sports Medicine Annual Meeting (Denver, CO, 2017)

3. **Kurti, S.P.**, Emerson, S.R., Rosenkranz, S.K., Smith, J.R., Harms, C.A. Habitual Physical Activity Level Modifies Lung Function, But Not 8-isoprostane Generation, in Older Adults. Accepted at Experimental Biology (Chicago, IL, 2017)

4. Smith, J. R, Didier, K.D., Hammer, S.M., Alexander, A.M., **Kurti, S.P.,** Barstow, T.J., Harms, C.A. Contribution of Prostaglandins to the Inspiratory Muscle Metaboreflex-induced Cardiovascular Consequences. Accepted at Experimental Biology (Chicago, IL, 2017)

5. **Kurti, S.P.,** Emerson, S., Smith, J, Rosenkranz, S.A., Alexander, S., Lovoy, G., Harms, C.A. Older women exhibit higher airway, but not systemic, 8-isoprostane responses to exhaustive exercise compared to older men. Accepted at the American College of Sports Medicine Annual Meeting (Denver, CO, 2017)

6. Emerson, S.R., **Kurti, S.P.,** Harms, C.A., Melgarejo, T., Logan, C., Rosenkranz, S.K. Magnitude and Timing of the Postprandial Inflammatory Response to a High-fat Meal in Healthy Adults: A Systematic Review. Submitted to American Society of Nutrition/Experimental Biology (Chicago, IL, 2017)

7. **Kurti, S.P**., Emerson, S.R, Rosenkranz, S.K., Teeman, C.S, Emerson, E.E, Cull, B.J, Smith, J.R, Harms, C.A. Post-prandial exhaled 8-isoprostane responses to meals of varying caloric and fat content in non-asthmatic, insufficiently active men. Experimental Biology (San Diego, CA, April, 2016)

8. **Kurti, S.P.,** Smith, J.R., Rosenkranz, S.K., Jurrens, K., Laughlin, A., Harms, C.A. Deep Inspirations Attenuate Postprandial Airway Inflammation in Non-Asthmatic Adults: A Randomized Crossover Study**.** American College of Sports Medicine Annual Meeting (Boston, MA, May, 2016)

9. Emerson, S.R., **Kurti, S.P.,** Teeman, C.S., Emerson, E.M., Cull, B.J., Haub, M.D., Rosenkranz, S.K. Size and Timing Matter: Differential Triglyceride Responses to Three Meal Conditions. Experimental Biology (San Diego, CA, April, 2016)

10. Foley, K.R., **Kurti, S.P.,** Mailey, E.L. Do dietary fish oil consumption and physical activity participation impact the duration, frequency and perceived severity of attacks in patients with Raynauds phenomenon? Experimental Biology (San Diego, CA, April, 2016)

11. Smith, J.R, Broxterman, R.M., Hammer, S.M., Alexander, A.M., Didier, K.D., Barstow, T.J., **Kurti, S.P.,** Harms, C.A. Sex Differences in the Inspiratory Muscle Metaboreflex. American College of Sports Medicine Annual Meeting (Boston, MA, May 2016)

12. Smith, J.R, Broxterman, R.M., Hammer, S.M., Alexander, A.M., Didier, K.D., Barstow, T.J., **Kurti, S.P.,** Harms, C.A. Effect of Aging on the Inspiratory Muscle Metaboreflex. Experimental Biology (San Diego, CA, April 2016)

13. Teeman, C.S., Cull, B.J., **Kurti, S.P.**, Emerson, S.R., Haub, M.D., Rosenkranz, S.K. Does VO_{2peak} moderate the association between dietary fat intake and post-prandial fat oxidation? Central States American College of Sports Medicine regional conference (University of Kansas, October) and Annual American College of Sports Medicine national meeting (San Diego, CA., May, 2015) and KSU Research Forum (Manhattan, KS, March, 2015)

14. **Kurti, S.P**, Rosenkranz, S.K, Chapes, S.K, Cull, B.J, Teeman, C.S, Emerson, S.R. Harms, C.A. Does Moderate Intensity Exercise Attenuate The Post-prandial Lipemic And Airway Inflammatory Response To A High-fat Meal? Integrative Physiology of Exercise Conference, Eden Roc, Miami (September 2014)

15. Emerson, S.R., Rosenkranz, S.K., Rosenkranz, R.R., **Kurti, S.P**., Harms, C.A. "Is there a link between sugar-sweetened beverage consumption and post-exercise airway narrowing across puberty?" Annual American College of Sports Medicine national meetings (San Diego, CA, May 2015)
16. Emerson, S.R, Haub, M.D, Snyder, B.S, **Kurti, S.P,** Rosenkranz S.K. 60 minutes of moderate-intensity walking improves fasting insulin sensitivity in overweight non-diabetic men. *Accepted* at the Integrative Physiology of Exercise Conference, Eden Roc, Miami (September 2014) and Central States American College of Sports Medicine regional Conference (University of Kansas, October)

17. Smith, J.R, **Kurti, S.P,** Johnson, A.M, Kolmer, S.A, Harms, C.A. Impact of Physical Activity on Airway Responsiveness and Bronchodilation in Healthy Subjects. Integrative Physiology of Exercise Conference, Eden Roc, Miami (September 2014). Presented as an oral presentation at the American College of Sports Medicine National Conference (San Diego, CA, May 2015)

18. **Kurti, S.P**, Emerson, S.R., Smith, J.R., Castinado, M., and Harms, C.A. The effect of a high-intensity interval training session on respiratory muscle fatigue. Central States American College of Sports Medicine and the American College of Sports Medicine National Conference (October 2013; May 2014)
19. Emerson, S.R**.**, **Kurti, S.**, Rosenkranz, S.K., Swain, K., and Harms, C.A (2013). Changes in cardiopulmonary function from pre to post adolescence. American College of Sports Medicine National Conference (May 2014)

20. **Kurti, S.P.** (2010). Does an acute bout of Tai Chi exercise improve balance in young and old individuals? The College of Education Outstanding Graduate Achievement Council Symposium, Boca Raton, FL (November 2010)

21. **Kurti, S.P**. (2009). Maintaining maximal grip strength over consecutive trials in female elite tennis players and female non-athletes. Selected by student achievement council to present in the Annual Graduate Student Research symposium

ORAL PRESENTATIONS

1. **Kurti, S.P,** Rosenkranz, S.K, Chapes, S.K, Levitt, M.L, Cull, B.J, Teeman, C.S, Emerson, S.R, Klaassen, T, Harms, C.A. The Effect of Physical Activity on Post-Prandial Triglycerides and

Airway Inflammation Following a High-Fat Meal. Slide presentation *presented at* the annual American College of Sports Medicine national conference (May 26-May 30)

2. **Kurti, S.P**, Emerson, S.R., Smith, J.R., Castinado, M., and Harms, C.A. The effect of a high-intensity interval training session on respiratory muscle fatigue. Thematic poster *presented at* the annual American College of Sports Medicine national conference (May 27-June 1)

3. Johnson, A.M., **Kurti., S.P**., Smith, J.R., Rosenkranz, SK., Harms, C.A. Effects of an Acute Bout of Moderate Intensity Exercise on Airway Inflammation and Postprandial Lipemia. Thematic poster *presented at* the annual American College of Sports Medicine national conference (May 27-June 1)

4. **Kurti, S.P,** Kurti, A.N., Rosenkranz S.K., Emerson S.R., Harms C.A. The Effect of Indoor Air Pollutants on Lung Health and Reported Respiratory Symptoms among Rural Belizean Adults. Talk *presented at* The Global Health and Innovative Conference, Yale University, (April 12-13)

5. Kurti, A.N., **Kurti, S.P,** Rosenkranz S.K., Emerson S.R., Harms C.A. Environmental Contributors to Decreased Lung Function in Belizean Children. Talk *presented at* The Global Health, Yale University, (April 12-13)

6. Emerson, S.R**.**, **Kurti, S.**, Rosenkranz, S.K., Swain, K., and Harms, C.A (2013). Changes in cardiopulmonary function from pre to post adolescence. *Presented at* the Central States American College of Sports Medicine Regional conference, Warrensburg, MO (October 2013)

7. Herold, A., **Kurti, S.**, Rew S., Pereira, F. The Importance of Proper Posture. *Presented at* the American College of Sports Medicine Annual Meeting to the Council of Healthy Aging, Baltimore, MD (June 2010)

TEACHING EXPERIENCE

2015-present	KIN335	Exercise Physiology lecture, Kansas State University
2013-present	KIN336	Exercise Physiology laboratory, Kansas State University
2013-present	KIN360	Anatomy and Physiology laboratory, Kansas State University
2012-2013	KIN520	Undergraduate and Graduate Practicum in Kinesiology
2012-2013	KIN792	Internships in Kinesiology
2012-2013	KIN360	Anatomy and Physiology lecture/laboratory, Kansas State University
2009-2011	ESHP220	Health and Fitness for Life, Florida Atlantic University
2010-2011	ESHP100	Introduction to Nutrition, Florida Atlantic University
2011	ESHP300	Weight Management, Florida Atlantic University
2009-2011	ESHP340	Undergraduate Practicum, Florida Atlantic University

Scores for overall effectiveness as an instructor (out of 5.0): average score: Anatomy and Physiology (4.8), Exercise Physiology (4.6)

HONORS AND AWARDS

2017 Presidential Award for Excellence in Undergraduate Teaching ($5,000)

2016	K-State Alumni Association Graduate student award for Excellence in Research and Academics
	1st place College of Human Ecology graduate research and creative inquiry forum
	Department of Kinesiology Distinguished Doctoral student of the year
	College of Human Ecology Outstanding Graduate Student of the Year
	Graduate Student Council Award for Excellence in Teaching (Finalist)
	Presidential Award for Excellence in Undergraduate Teaching (Finalist)
2015	Graduate Student Council Award for Excellence in Teaching (Finalist)
	Presidential Award for Excellence in Undergraduate Teaching (Finalist)
2013-present	Full Graduate Teaching Assistantship, Kansas State University
2011	Summa Cum Laude, Florida Atlantic University
2009-2011	Full Graduate Assistantship, Florida Atlantic University
2009	Cum Laude Society, University of Mary Washington
2009	*Who's Who* award for outstanding attitude, leadership and service, University of Mary Washington
2005-2009	Dean's List/Athletic Honor Roll, University of Mary Washington

MENTORSHIP AND TRAINING

2015-2017	Samantha Alexander, Rebekah Shirley, Garrett Lovoy, Ian Bower, Luke Wheeler *Completing Independent Projects*
2014-2016	Kourtney Foley- Completed independent project, "*Title: Does the consumption of fish oil attenuate painful symptoms associated with Raynaud's phenomenon*?"
2014-2015	Tory Klaasen, assisted in counting of sputum cell differentials
	Currently at: Doctoral program in Physical Therapy at Wichita State
2013-2014	Matthew Castinado- Developing Scholars Program, Completed independent project "*Title: The effect of high-intensity exercise on respiratory muscle fatigue"*
2013-2015	Eric Vargas- assisted in data collection
	Kayla Jurrens- assisted in data collection and manuscript preparation
	Currently at: Doctoral program in Physical Therapy at Regis University
	Anna Laughlin- assisted in data collection and manuscript preparation
	Currently at: Masters program in Occupational Therapy at St. Marys

PEER-REVIEWER

European Journal of Applied Physiology
Journal of Science and Medicine in Sport
Journal of Applied Physiology, Nutrition and Metabolism
Journal of Applied Physiology

PROFESSIONAL SERVICE

2016-present	College of Human Ecology graduate student ambassador
2016	Invited Speaker- College of Human Ecology Kappa Omicron Nu Society
2016	Undergraduate Office of Research and Creative Inquiry forum judge
2015-2016	Department of Kinesiology Anatomy and Physiology instructor search committee graduate student representative

2014	Department of Kinesiology Faculty search committee graduate student representative
2014	Graduate student representative for the Human Ecology Faculty Council

ADDITIONAL SKILLS

Laboratory Skills	Use of metabolic cart to perform VO_2 maximum tests and graded exercise tests
	Body composition measurements
	Proficient in administering pulmonary function tests
	Phlebotomy (venous blood draw and intravenous catheter)
	Sputum induction and processing (create cytospins and count inflammatory cells)
	Perform ELISA analysis
	Cell cultures, DNA extraction, basic flow cytometry