EFFECTS OF AN ACUTE BOUT OF MODERATE INTENSITY EXERCISE ON POSTPRANDIAL LIPEMIA AND AIRWAY INFLAMMATION

by

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B.S., Arizona State University, 2012

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

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KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

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Abstract

Obesity and asthma often coexist in the same people. Both are characterized by the presence of low-grade systemic inflammation. A high-fat diet may contribute to concurrent development of both conditions by promoting a pro-inflammatory postprandial environment leading to a transient accumulation of blood lipids (postprandial lipemia; PPL) and acute airway inflammation. Previous results from our lab have shown an ~20% increase in airway inflammation two hours after consuming a high-fat meal (HFM) that was significantly associated with increased plasma triglycerides. While acute exercise has been shown to attenuate PPL, it is unknown whether these protective effects will translate to reduced airway inflammation after a high-fat meal. PURPOSE: To determine the effects of an acute bout of exercise on airway inflammation after a HFM. We tested the hypothesis that an acute bout of exercise 12 hours before a high-fat meal would protect against subsequent airway inflammation in healthy men and would be related to the decreased PPL and systemic inflammatory markers. METHODS: In a randomized cross-over study, 12 healthy college-aged men consumed a HFM (1g fat/1kg body weight) 12 hours following exercise (EX; 60 min at 60% VO2max) or without exercise (CON). Exhaled nitric oxide (eNO; measure of airway inflammation), blood lipid profiles (venous sample; total cholesterol, HDL, LDL, triglycerides, glucose), inflammatory markers (hsCRP, TNF-α, IL-6) and pulmonary function tests (PFT) (forced expiratory volume in 1-s,forced vital capacity, forced expiratory flow at 25-75% of vital capacity) were measured pre-HFM, two hours, and four hours post-HFM. RESULTS: Baseline eNO was not different ($p>0.05$) between trials. eNO increased ($p<0.05$) post HFM at two hours in the both CON and EX conditions. eNO between trials was not different ($p>0.05$). Triglycerides were significantly increased two and four

hours post HFM but were not different ($p>0.05$) between conditions. There was no relationship ($p>0.05$) between eNO and triglycerides or systemic inflammatory markers for any time point in either condition. Pulmonary function did not differ ($p>0.05$) between any condition.

CONCLUSION: These results demonstrate that an acute bout of moderate intensity exercise 12 hours before a HFM does not attenuate postprandial airway inflammation or lipemia in healthy college-aged men.

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Acknowledgements

First and foremost I would like to thank my fellow graduate students in Kinesiology for their support and comradery. You all have made my time during this endeavor enjoyable and unforgettable. I am extremely blessed to have made so many life-long friendships here. Thank you to my advisor, Dr. Craig A. Harms for your patience and support throughout my project. You have truly helped me to apply my knowledge and to think critically about any problem. Thank you to my committee members Drs. Tom Barstow and Sara Rosenkranz for your advice and effort they have put into this project. Next I would like to thank Dr. Mark Haub for the use of his lab space and for his time spent training me to administer intravenous catheters. I would also like to thank Dr. Brian Lindshield for the use of his lab equipment and space. Lastly, thank you to Kristin Noriega for teaching me how to process ELISA kits. It has been a wonderful privilege to work with such an amazing group of scientists.

Chapter 1 - Introduction

The prevalences of asthma and obesity have risen concurrently over the past few decades. Additionally, asthma and obesity are commonly coexisting in the same people. However, the pathophysiological mechanisms involved in the concurrent development of asthma and obesity are not fully understood. One theory is that both conditions are marked by underlying chronic inflammatory states. Both obesity and asthma are associated with chronically increased levels of low-grade systemic inflammation, specifically increased inflammatory cytokines TNF-α, IL-6, and CRP (26, 83, 88). A high fat diet is known to contribute to obesity and moreover may increase levels of circulating inflammatory cytokines. Traditionally, airway inflammation, a characteristic symptom of asthma, has been thought to be primarily influenced by environmental factors such as allergens and pollutants (26). Recent research however has reported a transient increase (~20%) in airway inflammation two hours following the consumption of a single high fat meal in non-asthmatic subjects (78). This increase in airway inflammation was associated with an increase in plasma triglycerides (78). Repeated exposure to an airway inflammatory stimulus (i.e. a HFM) therefore, may contribute to pathogenesis of asthma.

Insight into potential mechanisms for the increase in airway inflammation following a HFM can be gained from previous research showing that, after three weeks of dietary fish oil supplementation, the postprandial increase in inflammation was abolished (2). This decreased inflammatory response after fish oil supplementation is thought to be due to changes in arachadonic acid metabolism and alterations in the composition of inflammatory cell membranes (12). In addition to decreasing the postprandial airway inflammation via decreased sensitivity to a stimulus, potentially an increase in triglycerides, it may be possible to attenuate airway

inflammation by decreasing the triglyceride response to a high fat meal. It is well established that a single bout of exercise can attenuate postprandial lipemia (34, 41). This attenuation is generally thought to be an acute response as opposed to a training adaptation and is dependent on both the timing of the exercise bout and the energy expended during the bout. Exercise performed ~12-16 hours before consumption of a high fat meal has been shown to be most effective in reducing postprandial lipemia (103). This timing corresponds with peak lipoprotein lipase activity stimulated by the exercise bout. Additionally, an acute bout of exercise stimulates a unique anti-inflammatory response. Exercise stimulates an anti-inflammatory cascade of cytokines beginning with an increase in IL-6 from the contracting muscle that, unlike systemic inflammatory response to sepsis or injury, is free of an initial pro-inflammatory phase, typically marked by an increase in TNF-α (73). With both its lipid lowering and anti-inflammatory effects, exercise performed before a high fat meal may provide a protective effect in the airways as well. However, this hypothesis has not been tested. Therefore, the primary purpose of this study was to investigate the effect of a single continuous bout of aerobic exercise on airway inflammation after a high fat meal.

Chapter 2 - Literature Review

Asthma and obesity are commonly coexisting conditions (8). The pathophysiological mechanisms involved in the concurrent development of asthma and obesity are not fully understood. One hypothesis is that both conditions involve underlying inflammatory and immune maladaptations that may alter the processing of lipids in the postprandial state. The following review of literature outlines the pathophysiological characteristics common to both asthma and obesity and their relationships with lipid metabolism in the postprandial period. Additionally, the mediation of these processes via diet and exercise interventions will be discussed.

Asthma

Asthma is a chronic inflammatory disease of the airways marked by airway hyperresponsiveness, airway obstruction, and airway inflammation (26). About 8% of American adults suffer from asthma, reflecting a 15% increase in prevalence over the last decade (16). Asthma is associated with an increased risk for mortality and a decreased quality of life. Additionally, asthma is a costly disease. Over $56 billion each year is spent on healthcare costs of asthma in the United States including nearly two million emergency department visits and half a million hospitalizations in 2009 (16). People suffering from asthma may experience clinical symptoms such as shortness of breath, recurring wheezing, and sudden-onset bronchoconstriction as well as sudden exacerbation of any of these symptoms (26). Asthma is commonly diagnosed by presence of symptoms such as episodic airflow obstruction or hyperresponsiveness. Additionally, spirometry may be used to assess the reversibility of the obstruction using an inhaled bronchodilator. Other diagnostic tools include subjecting the patient

to a bronchoconstrictor challenge, usually using methacholine and measurements of exhaled nitric oxide (eNO) which are elevated in asthmatics (26).

The asthmatic airway has immunological, structural, and inflammatory properties that differ from those in the healthy airway. In the asthmatic airway, the airway wall is infiltrated with inflammatory cells, most commonly T cells and eosinophils, along with activated mast cells and macrophages. Typically, more severe forms of asthma also show increased neutrophil cells in the airway. In addition to the increased presence of immunoregulatory cells, asthmatics experience structural airway remodeling including increases in size and number of mucous-secreting globular cells and variable increases in wall thickness (19). Additionally, smooth muscle hyperplasia may occur in the airways increasing the smooth muscle area by up to three times (6). Inflammation and remodeling of the airways contribute to bronchial hyperresponsiveness, an exaggeration of the bronchoconstrictor response to pathogens or other stimuli contacting the airway epithelium (26). Together, airway inflammation, remodeling, hyperresponsiveness, and possible airway edema contribute to overall airway narrowing (6, 19).

Adults with asthma may experience accelerated loss of lung function compared to non-asthmatic adults. In an eighteen year longitudinal study, rate of decline of forced expiratory volume in one second (FEV_1) was compared between non-asthmatics and asthmatics. Overall, both male and female asthmatics demonstrated a steeper decline in FEV_1 compared to their healthy counterparts (70).

Asthma and Obesity

Asthma prevalence is markedly different between obese and non-obese individuals. According to 2005-2006 NHANES data, the prevalence of asthma in the non-obese population of

the United States is 6.1% and the prevalence of asthma in the obese population is nearly double at 11.9% although more recent data suggests that this figure may be closer to 15% (56, 61). Obesity is defined as excess adiposity and is often assessed using body mass index (BMI) with a cut point of greater than or equal to 30 kg/m^2 in adults (15). According to the Center for Disease Control (CDC), in 2012, 35.7% of Americans were obese (15). As the prevalence of obesity has risen, the prevalence of asthma has risen concurrently. In addition to the higher prevalence of asthma in the obese population, obese asthmatics also experience more severe symptoms (80). Obesity is associated with less manageable asthma symptoms requiring a greater dependency on pharmaceutical interventions when compared to non-obese asthmatics (80). In fact, 75% of emergency room visits due to asthma are accounted for by obese patients (92).

While asthma and obesity appear to be epidemiologically and clinically related, the physiological link between the two conditions is unclear. There are two common hypotheses regarding the link between asthma and obesity. The first is that excess adiposity places a greater mechanical burden on the chest wall and airways. Two main alterations to lung function in obese subjects are a decreased tidal volume and an increased functional residual capacity (49). The decreased tidal volume is compensated for by an increased breathing frequency. This decreased tidal volume allows for less airway stretch, an important bronchodilating mechanism, and thus, predisposes obese individuals to increased airway responsiveness (83, 87). Additionally, the decreased functional residual capacity may unload airway smooth muscle and allow for excessive shortening when the smooth muscle is activated (33, 83). Lastly, the excess weight imposed on the chest wall and abdomen increase the work of breathing. Due to this increased work of breathing, obese subjects may experience more discomfort and/or difficulty breathing

during exercise. This discomfort may be comprehended as asthma symptoms by a clinician or patient (88).

The second hypothesis for the underlying link between asthma and obesity involves inflammation and immune modification. Low-grade systemic inflammation is characteristic of both asthma and obesity (26, 83, 88). C-reactive protein (CRP), an indicator of systemic inflammation, is elevated in both obesity and asthma (30, 32, 90). Additionally, obese individuals may have increased plasma levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (88). Specifically, TNF-α secretion from adipose tissue is likely increased in obese individuals (48, 52). A pro-inflammatory environment may be important in the pathogenesis of asthma (26). It has been shown that asthmatic individuals also have increased levels of IL-6 and TNF-α (47). Roles of these cytokines in both systemic and airway inflammation are discussed below.

Acute and Low-Grade Systemic Inflammation

When the body sustains an injury or infection, a short-term state of inflammation occurs locally and is accompanied by a systemic response. This systemic response is called an acute-phase inflammatory response. This response consists of a cascade of inflammatory signaling molecules known as cytokines. In humans, the typical inflammatory response begins with a rise in pro-inflammatory cytokines, TNF-α then IL-1β. Next, plasma IL-6 is increases potentiating a series of anti-inflammatory cytokines (IL-10, IL-1 receptor antagonist, soluble TNF receptors) (73). In a study by Steensberg et al. (2003), this anti-inflammatory cascade was reproduced by injecting a bolus of recombinant IL-6 into healthy human subjects (85). The overall inflammatory response is reflected by an increase in the hepatocyte-derived acute phase protein, CRP which is delayed about 16-24 hours from the initial stimulus.

When the body can no longer regulate this inflammatory balance, chronic low-grade inflammation develops. Chronic low-grade inflammation is generally evidenced by 2-3 times greater systemic TNF-α, IL-1, IL-6, IL-1ra, sTNF-R, or CRP concentrations than normal resting values and often accompanies aging and many chronic diseases such as atherosclerosis, heart disease, type 2 diabetes, and obesity (73). In asthmatic subjects, systemic inflammation measured by high sensitivity CRP (hs-CRP) has been shown to be associated with airway inflammation as assessed by sputum eosinophil cell count (90).

Low-grade inflammation, Obesity, and Asthma

Adipose tissue is a metabolically active tissue. In fact, recent evidence suggests that adipose tissue can be a potent endocrine organ (20, 62). For example, TNF-α is synthesized and secreted by adipose tissue (20). Increased systemic levels of TNF-α may be important to the development and perpetuation of asthma. In a study by Chen et al. (2003), murine tracheal rings were exposed to either a solution of TNF-α or diluent alone. Rings exposed to TNF-α *in vitro* had greater contractility in response to both carbochol- and KCl-induced contraction as well as attenuated relaxation ability when exposed to isoproterenol (17). Similar increases in contractile strength occurred when TNF-α treated bronchial segments were exposed to acetylcholine *in vitro* (86). *In vivo*, this airway responsiveness is also present; however, effects seem to be less immediate (91). Furthermore, TNF-α may induce airway inflammation via induction of adhesion molecules and recruitment of inflammatory cells in the bronchial epithelium (91).Together, these studies suggest that TNF-α may pay a role in potentiating airway hyperresponsiveness and airway inflammation associated with asthma.

Airway Inflammation

Chronically elevated levels of airway inflammation are a key characteristic of asthma. Airway inflammation that is not associated with disease can occur acutely in all people in response to a variety of stimuli and are not always associated with hyperresponsiveness (26); however, when multiple cells and mediators interact repeatedly, airway inflammation may eventually result in pathological features of asthma (26). The airway epithelium functions as a regulator of airway smooth muscle and attracts and regulates inflammatory cells in response to injury. The epithelium itself contains epithelial immune cells such as mast cells, macrophages, and lymphocytes. Damage to this epithelium significantly contributes to inflammation (54). Airway inflammation can be induced by a number of factors including exposure to allergens, pollutants, and infection (26). When a pathogen comes in contact with the airway epithelium, a local inflammatory response ensues. Epithelium cells release immune moderators such as IL-1 which in turn activate mast cell and T- and B- cells in the submucosal layer. Together, these cells release inflammatory cytokines causing tissue inflammation and activating inducible nitric oxide synthase (iNOS). iNOS converts L-arginine into nitric oxide (NO), a bronchodilator. Upregulation of iNOS can be induced by a number of pro-inflammatory cytokines including TNF-α, IL-1β, and oxidants and results in an increased concentration of nitric oxide that is released into the airways and subsequently exhaled (54). eNO can be used as a non-invasive, quantitative index of airway inflammation as the two as closely associated (37, 43). eNO is often used to diagnose chronic airway inflammatory diseases such as asthma and is specifically used as a marker of eosinophilic airway inflammation (25). Measurement and evaluation of eNO has been standardized for clinical use by the American Thoracic Society (ATS) guidelines. An eNO value of >50ppb that is sustained over time is indicative of the presence of sustained eosinophilic inflammation (25). In addition to allergens, infection, and pollutants, modifiable lifestyle factors

such as adiposity and diet can also influence airway inflammation. In non-asthmatic subjects, BMI is associated with airway inflammation as measured by eNO (23, 51).

Inflammation and High-Fat Meals

Consumption of a high fat meal (HFM) results in acute airway inflammation. A study by Rosenkranz et al. (2010) showed that there was a 20% increase in eNO two hours after consumption of a single high-fat meal in healthy subjects (78)(2).While the mechanisms linking HFM consumption are not known, the resulting airway inflammation is thought to be produced via a similar pathway to airway inflammation induced by an allergen or infection. One mechanism proposed by Rosenkranz et al. (2010) is that saturated fats in the HFM suppress the anti-inflammatory properties of HDL cholesterol allowing an increase in eNO. In addition to an acute increase in airway inflammation, a high-fat, high-carbohydrate meal has been shown to prolong the generation of reactive oxygen species (ROS) and increase activity of nuclear factor–KappaB (NF-κB) suggesting an increased systemic inflammatory response (68). Furthermore, after a six hour fat challenge blood leukocyte counts were significantly increased from 2-6 hours post meal in healthy participants. Neutrophil and monocyte activation were increased after consumption of the meal (97). There is some evidence that IL-6 is also increased after consumption of a high fat meal (9, 66, 102). TNF-α has been shown to increase around 1-4 hours after the consumption of a high-fat meal (27, 66). However, this is not a consistent finding (9, 69). The postprandial increase in eNO found by Rosenkranz et al. was significantly correlated with the rise in blood triglycerides (78). It is possible that this relationship could be mediated by changes in systemic pro-inflammatory cytokine concentrations. IL-6 and TNF-α have each been shown to increase lipolysis from adipose tissue resulting in increased plasma lipids (28, 48). Furthermore, the increase in airway inflammation after a high fat meal has been shown to be

modifiable (2). A recent study from our lab has demonstrated that three weeks of fish oil supplementation attenuated the postprandial increase in eNO (2). This attenuation is likely due to alterations in arachadonic acid (AA) metabolism that promote an anti-inflammatory cellular environment (2). Increased proportion of omega-3 derived fatty acids to omega-6 derived AA in cell membranes decreases the production of AA derived inflammatory cytokines and a decrease in inflammatory response of the immune cells (12). While this mechanism may decrease the sensitivity of the immune response to a stimulus, potentially an increased plasma triglycerides from a high fat meal, it is unknown whether postprandial airway inflammation can be attenuated by interventions intended to decrease the postprandial lipemic response.

Lipid Metabolism

In addition to inflammatory changes, consumption of a high-fat meal leads to changes in blood lipids and glucose in the postprandial period including transient hyperlipidemia. This rise in blood lipids typically peaks at four hours post-meal and returns to normal values by eight hours. During this period, known as postprandial lipemia (PPL), lipid appearance from the small intestine competes with lipoprotein remnants secreted from the liver and lipolysis from adipose tissue to be cleared from the blood via LPL at the muscle and adipose tissue. The degree of PPL is affected by such factors as meal composition, energy balance, physical activity, and disease status (24, 41).

Effect of Excess Lipids on the Epithelium

Studies in the vascular endothelium have shown detrimental effects on vascular smooth muscle function after a high-fat meal (67, 98, 100). Nitric oxide (NO) bioavailability is a key player in the vasodilatory ability of the smooth muscle. Reduction of NO bioavailability via oxidative stress from excess lipids seems to play an important role in the attenuation of vascular

control (100). As excess free fatty acids are oxidized by the mitochondria, superoxide (O_2^-) molecules accumulate. These free radicals are scavenged by NO; however, this process also results in the production of peroxynitrite ($ONOO^-$), another strong oxidant (4, 100). In addition to reduction of NO via direct scavenging, production of NO via NOS is also diminished. Oxidation and uncoupling of necessary cofactors results in a decrease of NO production and instead, additional O_2^- is produced (100). Increased oxidative stress has been proposed to produce an inflammatory response (67). Overall, NO bioavailability in the vascular endothelium seems to be diminished as a result of a high fat meal. In contrast, NO seems to be increased in the airway epithelium ((2). The differing responses to a high fat meal in airway and vascular endothelium can most likely be attributed to the differing NOS isoforms present. While vascular endothelium is predominantly controlled by endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) seems to be the key enzyme responsible for increased NO production in the airways. Even so, antioxidant supplementation has been shown to have a protective effect on both vascular function and airway inflammation after a high-fat meal (2)). Improved endogenous antioxidant status via dietary interventions may reduce pro-inflammatory cytokine production in the airways and vasculature (13, 53, 104). Exercise interventions have also been shown to stimulate similar enhancement of endogenous antioxidants and have similar anti-inflammatory effects (99, 100).

Anti-Inflammatory Effects of Exercise

An acute bout of exercise stimulates a unique anti-inflammatory response. Unlike the systemic inflammatory response to sepsis or tissue injury, exercise does not cause an initial elevation of TNF-α (73, 89). Instead, the contracting muscle functions as an endocrine organ, directly producing and secreting inflammatory cytokines, called myokines, into the blood. The

most notable cytokine increase in response to exercise is IL-6. Blood concentrations of IL-6 have been shown to increase by 20- to 100-fold during and immediately following exercise (31, 73). The extent of the increase in IL-6 concentration is dependent upon the intensity, duration, and muscle mass involved in a given exercise bout (60, 71). In contrast to the typical inflammatory response, this increase in IL-6 in absence of an initial increase in TNF-α appears to have an overall anti-inflammatory effect. In an *in vitro* study of human mononuclear cells, IL-6 suppressed the expression of TNF-α (81). A true anti-inflammatory effect of exercise has been demonstrated *in vivo* in healthy human subjects who received a low dose of intravenous endotoxin. Subjects who did not exercise prior to injection of the endotoxin experienced an approximately 250% increase in TNF-α. Subjects who cycled for a total of three hours and were injected after 2.5 hours of cycling did not see this increase in TNF-α. Additionally, direct injection of IL-6 without exercise produced results similar to the exercising group (84). This finding suggests that IL-6 plays an important mediating role in the anti-inflammatory role of exercise. Additionally, anti-inflammatory cytokines IL-10, IL-1ra, and sTNF-R subsequently increase following the increase in IL-6 released by the contracting muscle. Stimulated production of the sTNF-R in absence of a rise in TNF-α further supports a systemic anti-inflammatory effect (71, 73, 85).

Exercise and Lipid Metabolism

In addition to direct anti-inflammatory effects of myokines released from contracting muscle, it is well established that a single bout of exercise can attenuate postprandial lipemia (10, 35, 41, 42, 44, 46, 74, 75, 103). This attenuation has been shown to be dependent on energy expended during a bout of exercise, not on exercise intensity per se (29, 95, 96). Timing of the exercise bout also affects the degree of attenuation of PPL. Optimum attenuation of PPL appears

to be delayed by 12-16 hours after exercise (29, 41, 103). Additionally, energy replacement after exercise diminishes the protective effect on PPL (10, 35, 45). A single 60-90 minute bout of moderate-intensity exercise (~60% VO_2max) has been shown to decrease PPL in both trained and untrained subjects (42, 46). Although many studies have shown that trained individuals exhibit lower PPL, when asked to refrain from exercise for greater that 60 hours, PPL was not different from sedentary individuals. Additionally, some differences in attenuation of PPL may be explained by increased energy expended during exercise due to a higher workload for a required intensity (41). It is likely that acute exercise reduces PPL by increasing LPL activity at the skeletal muscle and VLDL secretion by the liver. Studies have shown that LPL activity is higher in active subjects compared to untrained subjects, enhancing lipid clearance (50); however, no benefit is seen with respect to postprandial lipemia when the last exercise bout of the trained subject is more than 48 hours prior to the meal (46). Additionally, LPL activity and protein mass peak at greater than eight hours post-exercise, coinciding with the delayed maximum beneficial effects on PPL (82). Along with an increase in LPL activity, very low density lipoprotein (VLDL) secretion from the liver may be reduced by an acute exercise bout (41). In humans, reductions in postprandial plasma concentration of VLDL-TG has been shown to be reduced following exercise (39, 59) and in mice, reduced postprandial secretion of VLDL from the liver has been observed after exercise (36, 65). Resistance training and high-intensity interval training have also been shown to be effective for reducing PPL; however, energy expenditure does not seem to predict the attenuation as well as with aerobic exercise (5, 22, 93). Sex differences may exist in the postprandial response after exercise as well. Exercise seems to be more efficacious in lowering PPL for women than for men (34). This may be attributed to lower baseline concentrations of VLDLs in women than in men and a slower rate of chylomicron

clearance in men (55, 57). However, when differences in baseline TG are controlled for, there does not seem to be a sex difference with regard to PPL (34).

Protective Effect of Exercise on Endothelial Function

An acute bout of aerobic exercise has been shown to be effective in protecting vascular endothelial function following a high-fat meal (67). Along with the postprandial reduction in blood lipids, three additional mechanistic hypotheses have been made by Padilla et al. (2006) regarding this protective effect. First, shear stress from exercise may be sufficient to increase the release of NO and improve vasodilation. Secondly, exercise may diminish oxidative stress and its resulting inflammation from excess lipids associated with a high-fat meal. Although exercise itself can induce oxidative stress, this oxidative stress has been shown to increase production of endogenous antioxidants (63). This increased synthesis of antioxidants following exercise may protect against free radicals and subsequent inflammation associated with a high-fat meal. Thirdly, previously discussed direct anti-inflammatory effects of the contracting muscle may protect the vascular endothelium (67). It is unknown whether these protective effects, specifically antioxidant and anti-inflammatory effects of exercise translate from the vascular endothelium to the airway epithelium.

Effect of Exercise Timing on Intervention Efficacy

Efficacy of acute exercise interventions appears to be moderated by the timing of the exercise bout for both protection of endothelial function and attenuation of postprandial lipemia. With regard to endothelial function, exercise 16-18 hours prior to an HFM resulted in 15% higher microvasculature function compared to no exercise (40). In the conduit arteries, FMD was increased above baseline when an acute exercise bout was performed two hours post-HFM (67). Vascular dysfunction after a HFM is positively correlated to the degree of PPL. Presumably,

decreasing this PPL would enhance endothelial function (100). In a study by Zhang et al. (1998), moderate aerobic exercise 12 hours before consumption of an HFM was more effective in reducing PPL than exercise one hour before or one hour after the HFM. Exercise performed one hour before the HFM also decreased PPL compared to no exercise. Exercise one hour after consuming an HFM was not effective in reducing PPL (103). In a recent quantitative review, the average effect size of exercise from 8-24 hours before an HFM on PPL was -0.66 compared to -0.44 for exercise performed less than eight hours before an HFM indicating that exercise from 8-24 hours before a HFM may be more efficacious in lowering PPL compared to exercise preformed less than 8 hours preprandially (34).

Conclusion

In summary, acute inflammation associated with a high-fat meal may provide a link between the associated pathogeneses of obesity and asthma. Specifically, a high-fat meal has been shown to increase systemic and airway inflammation. Evidence suggests that exercise has anti-inflammatory effects that may protect against systemic inflammation following a high-fat meal. While it is well established that exercise protects vascular endothelial function postprandially, it is unknown whether these protective effects are extrapolated to the airway epithelium. The purpose of this study was to investigate the effect of a single prior continuous bout of aerobic exercise on airway inflammation after a high fat meal. Additionally, we were interested in identifying whether any protective effect of exercise on airway inflammation was associated with a reduction in PPL or systemic inflammatory markers. We hypothesized that an acute bout of aerobic exercise 12 hours prior to a high-fat meal would protect against subsequent airway inflammation in healthy men. Furthermore, we expected the decrease in airway

inflammation to be associated with a decrease in PPL and plasma concentrations of pro-inflammatory cytokines.

Chapter 3 - Methods

Subjects

Twelve college-aged men volunteered to participate. Subjects were recreationally active but none were competitively training. All subjects were non-smokers and had apparently healthy pulmonary, metabolic, and cardiovascular function as determined by medical questionnaire. All subjects had normal pulmonary function and normal fasting blood lipid and glucose levels as determined by standard pulmonary function tests and intravenous blood sampling. Subjects were not using any medications or dietary supplements including antioxidant and fish oil supplementation throughout the course of the study. Each participant was informed of any risks and signed informed consent forms. All procedures were approved by the Institutional Review Board at Kansas State University, Manhattan, KS.

Experimental Design

Subjects visited the laboratory on three separate occasions. On the first visit, height, weight, and body composition via dual-energy x-ray absorbtiometry (DXA) were measured. Subjects then performed the VO_2max test on an electronically braked cycle ergometer to determine the appropriate intensity for sub-maximal exercise in the experimental condition. On two subsequent visits, subjects performed one of two trials separated by at least seven days in a randomized cross-over study design: control trial (high-fat meal only; CON) and an exercise trial 12 h before the high fat meal (EX). In the control condition, subjects reported to the lab after a 12 h fast. Subjects abstained from exercise, caffeine and alcohol consumption for 24 h before the trial. In addition, subjects were asked to record their diet for the 24 h period before the trial. They repeated this diet in the 24 h prior to baseline measurements for the experimental trial. Upon

reporting to the lab, a battery of baseline pulmonary tests and blood analyses were performed. These are described in detail below. After completion of baseline testing, subjects consumed the high-fat meal (HFM). Measures identical to those at baseline were repeated at 2- and 4-h postprandially.

For the exercise trial, subjects reported to the laboratory on two separate occasions, 12 h apart. Subjects refrained from caffeine and alcohol consumption and exercise with the exception of the prescribed bout for 24 h prior to baseline. On the first visit, 12 h before baseline measurements, subjects performed one hour of cycling at 60% of VO_2max. Metabolic and ventilatory data were collected breath-by-breath continuously throughout the bout (Sensormedics 229 Metabolic Cart, Sensormedics Corp., YorbaLinda, CA). Heart rate was recorded every three minutes using a chest strap heart rate monitor (Polar T31-Uncoded, Polar). Subjects fasted for 12 hours immediately after exercise. An HFM identical to the control trial was consumed 12 hours after the start of the exercise bout. Baseline pulmonary measurements and blood analyses were performed. All measures were repeated at 2- and 4-h postprandially.

Maximal Aerobic Capacity

An incremental exercise test was performed on an electronically braked cycle ergometer (Sensormedics 800, Sensormedics Corp., YorbaLinda, CA). Metabolic and ventilatory data were collected and analyzed on a breath-by-breath basis (Sensormedics 229 Metabolic Cart, Sensormedics Corp., YorbaLinda, CA). Heart rate was monitored continuously using a chest strap heart rate monitor (Polar T31-Uncoded, Polar). Arterial oxygen saturation (SpO_2) was estimated via a pulse oximeter (Datex-Ohmeda 3900P, Madison, WI). A modified Borg's rating of perceived exertion (RPE) scale, values of 1-10, were recorded at each exercise stage. After three minutes of rest and baseline measurements, subjects maintained a pedaling frequency of

60-80 rpm beginning at a work rate of 50W and increasing 25W every minute until exhaustion. Subjects were verbally encouraged to achieve the highest work rate possible. The highest O_2 consumption averaged over 15 seconds was considered the VO_2max. After resting for 15 min, VO_2max was verified (76). by setting the work rate at 105% of the work rate corresponding to VO_2max. Subjects pedaled at 60-80 rpm until exhaustion. If subjects failed to pedal for at least two minutes, or did not achieve a VO_2 within the range of 10 mL/1W, validation criteria were not met and a repeated full test was scheduled for a later date. Gas exchange threshold was estimated by three blinded researchers using the V-slope method (7) to determine if subjects exercised above or below their threshold and whether this influenced their results.

High Fat Meal

The high-fat meal (HFM) consisted of ice cream (Edy's Grand Vanilla) and whipping cream (Reddi Wip original). Serving size was be determined by body weight (1 g fat/ 1 kg body weight). The amount of ice cream was calculated as body weight in kg x 4.0625 = g of ice cream. Whipping cream was measured as body weight in kg x 1.5 = ml of whipping cream. The HFM was consumed within 20 minutes. Nutritional makeup of the HFM was 4.5 g saturated fat, 30 mg cholesterol, and 16 g carbohydrate per serving.

Experimental Measures

Pulmonary Function Measures

Pulmonary tests carried out at baseline and 2- and 4-h postprandially in all conditions consisted of standard pulmonary function tests (PFTs; forced vital capacity, forced expiratory flow in 1-s, forced expiratory flow at 25-75% of vital capacity) (SensorMedics 229 Metabolic Cart, Sensor MedicsCorp., Yorba Linda, CA), exhaled nitric oxide tests (eNO) as a marker of airway inflammation via chemiluminescence (Sievers Nitric Oxide Analyzer 280, Sievers Instruments

Inc, Boulder, CO, USA), and impulse oscillometry (IOS, Jaeger, Germany) as a measure of airway resistance. All PFTs were performed in triplicate with maximal values used in analysis according to ATS guidelines (72). eNO tests were performed in triplicate with average values used in analysis.

Blood Sampling and Biochemical Assays

Hs-CRP, TNF-α, IL-6, blood lipid profiles, and blood glucose were determined at baseline and 2- and 4-h after the consumption of the high-fat meal in all conditions via serial blood draws using an intravenous catheter. Insertion site was cleaned with alcohol and dried. The catheter was inserted into the median cubital or cephalic vein using a 21-gauge needle. The catheter was connected to a 3-way stopcock and an IV administration set. A 1L saline bag containing 0.9% NaCl was allowed to flow into the vein at a drip rate of one per second. A waste sample of 3mL was extracted in order to clear any saline from the sampling line. At each time point, 5mL blood samples were collected in a disposable syringe. 40μL samples were collected from the syringe in sterile lithium heparin coated capillary tubes within 10 seconds. The remainder of the sample was transferred to a 6 mL K2EDTA vacutainer and centrifuged to separate plasma from red blood cells. Plasma was removed and frozen at -60°C.

Total cholesterol, HDL, LDL, triglycerides, and blood glucose were analyzed using a Cholestech LDX analyzer (Alere San Diego Inc., San Diego, CA). The 40 μL whole blood sample was applied to a lipid profile plus glucose cassette (Alere Cholestech LDX Lipid Profile·Glu cassette, Alere San Diego, Inc., San Diego, CA). The cassette was placed in the analyzer where plasma was separated from the blood cells. LDL was precipitated out of the sample using dextran sulfate and magnesium acetate precipitating reagent. Total cholesterol and HDL were measured using three enzymatic reactions catalyzed by cholesterol esterase and

cholesterol oxidase. Triglycerides were measured using a series of enzymatic reactions via lipase, glycerol kinase, and glycerol phosphate oxidase. Lastly, glucose was measured using an enzymatic reaction catalyzed by glucose oxidase. Following each enzymatic process, a color reaction using horseradish peroxidase allowed a proportional amount of dye to be formed to the desired substance. Color was measured by reflectance photometry. A magnetic strip on each cassette allowed the analyzer to calibrate and convert the reflectance reading (%R) to the total cholesterol, LDL, HDL, triglyceride, and glucose concentrations (mg/dL).

Hs-CRP, IL-6, and TNF-alpha were measured by serial blood samples via intravenous catheter. ELISA kits were used to analyze the samples. For the hs-CRP assay, 5µL of plasma was diluted 1:100 with provided sample diluent. 10µL of provided standards and diluted samples were dispensed into appropriate wells coated with monoclonal antibody (MAb) to CRP. Anti-CRP-HRP conjugate was added to all wells and incubated for 60 minutes at room temperature on an orbital shaker. CRP in the sample bound to anti-MAb on the well and the anti-CRP second antibody then bound to CRP. Liquid was then removed from all wells and unbound protein and HRP conjugate were washed out with prepared wash buffer. Next, 100µL of TMB substrate was added to each well and incubated for 15 minutes in the dark at room temperature allowing the intensity of color to be proportional to the concentration of CRP. Lastly, 50µL of provided stop solution was added to each well and a standard curve related color intensity to CRP concentration. Absorbance was read using a microplate reader (Synergy HT Multi-Mode Microplate Reader, Biotek Instruments Inc., Winooski, VT) at 450nm. Similar technique was used to determine the concentration of plasma IL-6. 100µL of sample and standard were added to the appropriate wells which were coated in rat monoclonal antibody specific for IL-6. IL-6 was bound by the antibody during a 60-minute incubation period at room temperature on an orbital

shaker. Next, the liquid in the wells was rinsed and 100µL of a second, non-overlapping biotin-conjugated rat monoclonal antibody specific for human IL-6 was used to detect the bound IL-6 during a second 60-minute incubation period on an orbital shaker. Wells were then rinsed with wash buffer to remove any residual protein and 100µL of HRP-conjugated streptavidin solution was added to the plate and incubated for 30 minutes at room temperature on an orbital shaker. Wells were rinsed again with wash buffer and 100µL of TMB substrate solution was added to each well and incubated in the dark at room temperature for 15 minutes. 100µL of HRP stop solution was added to each well. The absorbance was determined using a microplate reader at 405nm. Prior to assaying for TNF-α, reconstituted non-specific mouse serum was added to plasma samples and standard in a 1:20 ratio in order to compensate for the effects of human anti-mouse IgG. 100µL of the sample and standard mixtures were added to the appropriate wells. Each well was coated with monoclonal antibody specific for TNF-α. 100µL of human TNF-α AChE-Fab' conjugate was also added to each well with the exception of the blank wells. The plate was incubated overnight at 4°C. Wells were emptied and rinsed with wash buffer before adding 200µL of Ellman's Reagent and incubating in the dark. Absorbance was measured after 15, 30, 60, 120, 240, and 260 minutes of incubation at 405nm. For each assay, standards were plated in duplicate and samples were plated singly.

Statistical Analysis

SigmaStat 10 Statistical Software (Systat Software, Chicago, IL) was used for data analysis. Data is expressed as mean ± SD. Two-way repeated measures ANOVA with time (baseline, 2-, and 4- hour time points) and condition (CON, EX) as independent factors was used. Tukey post-hoc tests were carried out for significant effects. Magnitudes of responses were

quantified as peak and area under the curve (AUC) responses. Relationships were determined via linear regression. Significance was set at $p < 0.05$ for all analyses.

Chapter 4 - Results

Subjects

Subject characteristics are shown in Table 1. All subjects were non-obese (BMI<30). The average VO_2max was 46.9 ± 7.9 ml/kg/min. During the exercise bout, three subjects exercised below their ventilatory threshold (VT) and nine exercised above VT but this did not significantly affect results.

Table 1: Subject Characteristics

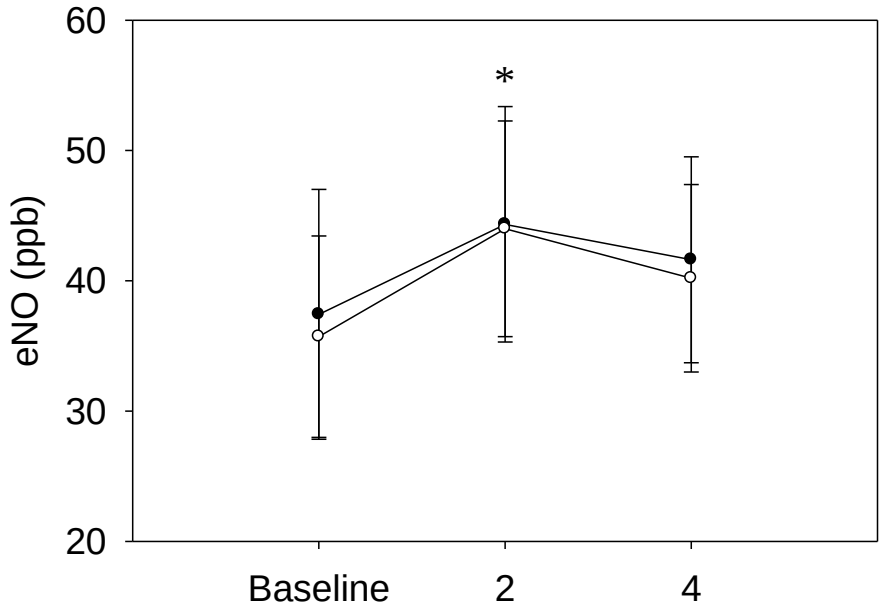
Table 1: Subject Characteristics		
	Mean ± SD	Range
Age (years)	23.0 ± 3.2	18.4 - 29.2
Weight (kg)	78.5 ± 11.7	68 – 108
Height (cm)	178.9 ± 5.5	170 – 191
BMI	24.5 ± 2.7	21.4 - 29.7
Body Fat %	14.3 ± 4.5	8.2 - 22.8
VO_2max (ml/kg/min)	46.9 ± 7.9	30.2 - 59.3
VT (%VO_2max)	55.3 ± 8.2	43.1-71.6

Exhaled Nitric Oxide

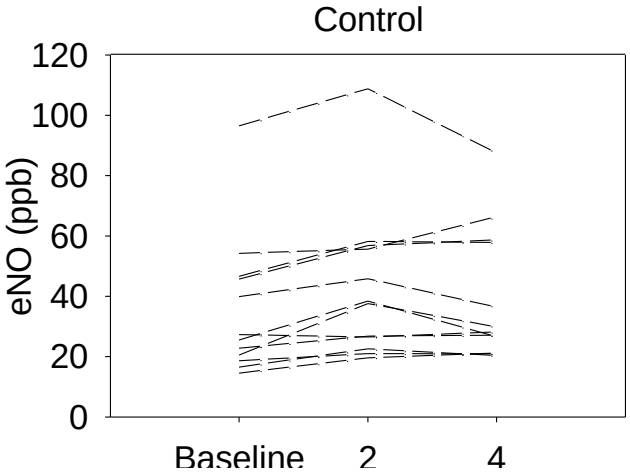
Mean and individual eNO responses are shown in Figure 1. Baseline eNO was not different between CON and EX conditions ($p>0.05$). ENO increased ~18% two hours after consuming the HFM in both conditions ($p= 0.003$). In each condition, 11 of the 12 subjects increased eNO 2 hours postprandially. Four hours postprandially, eNO was not different ($p>0.05$) from baseline or two hours in either condition. There was no difference ($p>0.05$) in eNO between conditions.

Figure 1: Exhaled Nitric Oxide Response to HFM

A



B



C

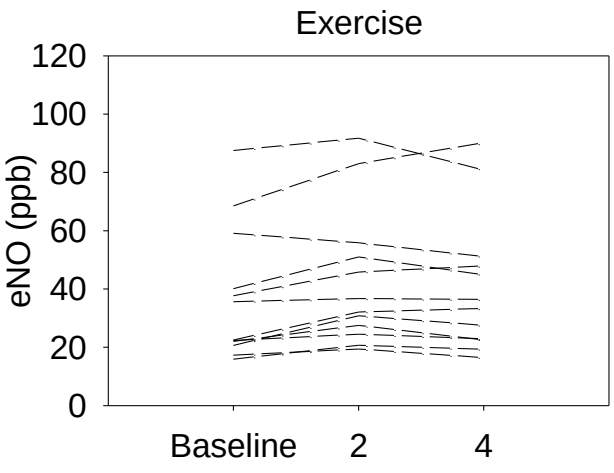


Fig. 1: Group mean (±SE) for CON (filled circles) and EX (open circles) for eNO after HFM (A) and individual eNO responses for CON (B) and EX (C) conditions. An increase in eNO from baseline was seen at 2 h in both conditions.

* Significantly different from baseline; $p<0.05$

Blood Lipids

Mean blood lipid values for each condition and time point are shown in Table 2. EX blood lipid profiles were not significantly different than CON. TG response is shown in Figure 2A. Individual responses are shown in Figures 2B and 2C. Average fasting TG were 52 and 70 mg/dl in the CON and EX conditions respectively and were not different between trials ($p>0.05$). In both conditions, TG were significantly elevated at 2- and 4 h postprandially. TG AUC did not differ between conditions (CON=126.4 ± 70.8, EX=77.5 ± 56.4; $p>0.05$). Additionally, LDL was decreased at two hours compared to baseline in both conditions ($p=0.12$). CON and EX values did not differ from each other ($p>0.05$). Blood glucose was significantly lower than baseline at two hours in both conditions ($p=0.005$). There were no changes in TC or HDL across time for either condition ($p>0.05$). There were no significant relationships between changes in blood lipids or glucose and eNO ($p>0.05$).

Table 2: Blood Lipids and Inflammatory Markers

Table 2: Blood Lipids and Inflammatory Markers						
	Baseline		**2 h**		**4 h**	
	CON	**EX**	**CON**	**EX**	**CON**	**EX**
TG (mg/dl)	52 ± 9	70 ± 20	97 ± 38*	100 ± 29*	89 ± 23*	87 ± 16*
TC (mg/dl)	143 ± 24	145 ± 20	150 ± 25	143 ± 19	146 ± 25	147 ± 22
HDL (mg/dl)	44 ± 17	45 ± 11	46 ± 15	44 ± 14	47 ± 13	45 ± 13
LDL (mg/dl)	88 ± 14	88 ± 18	84 ± 15*	79 ± 18*	83 ± 19	84 ± 19
Glucose (mg/dl)	81 ± 7	78 ± 7	70 ± 17*	70 ± 11*	76 ± 7	78 ± 5
IL-6 (pg/ml)	29.8 ± 38.0	34.3 ± 39.5	30.8 ± 35.5	33.0 ± 38.6	33.4 ± 38.7	30.6 ± 35.2
TNF-α (pg/ml)	6.48 ± 2.13	6.66 ± 2.62	6.94 ± 2.08	6.72 ± 2.78	7.75 ± 2.91	7.44 ± 2.27
hsCRP (mg/L)	0.25 ± 0.32	0.60 ± 0.96	0.26 ± 0.43	0.70 ± 0.83	0.27 ± 0.45	0.74 ± 0.92

Table 2: Values are presented as mean ± SD, TG: triglycerides; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; IL-6: interleukin 6; TNF-α: tumor necrosis factor-alpha; hsCRP: high sensitivity C-reactive protein
* Significantly different from baseline; $p<0.05$

Figure 2: Plasma Triglyceride Response to HFM

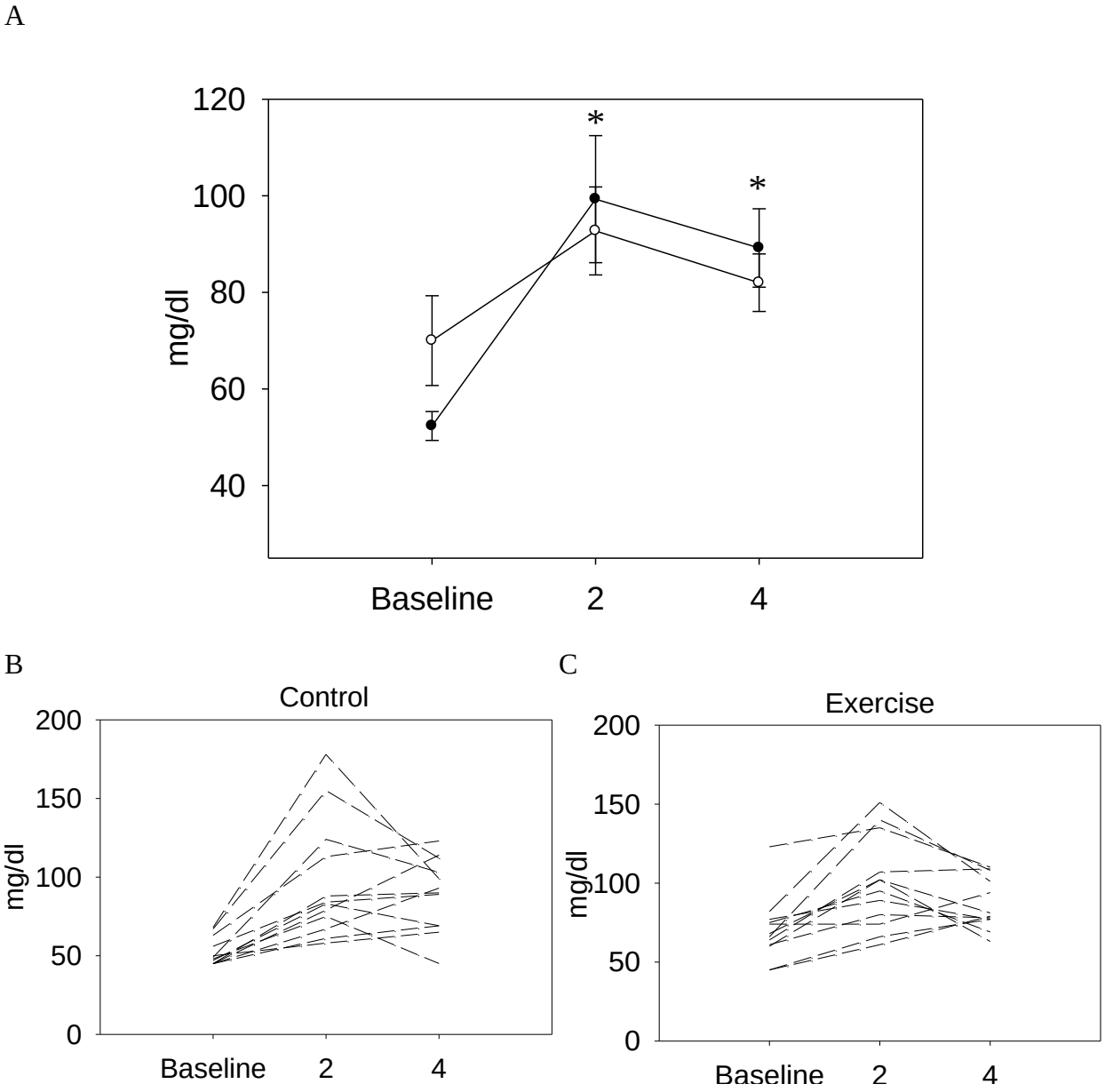


Fig. 2: Group mean (±SE) for CON (filled circles) and EX (open circles) for TG after HFM (A) and individual TG responses for CON (B) and EX (C) conditions. An increase in TG from baseline was seen at 2- and 4 h in both conditions.

* Significantly different from baseline; $p<0.05$

Inflammatory Markers

Mean values for hsCRP, TNF-α, and IL-6 are shown in Table 2. Mean baseline hsCRP was not different between conditions (CON=0.25 pg/ml, EX=0.60 pg/ml; $p>0.05$). All subjects were within the healthy range (<3.0 pg/ml) for the CON condition. Only one subject fell outside of this range in the EX condition (hsCRP=3.37pg/ml). There were no changes in hsCRP between time points or conditions (see Figure 3A).

Mean baseline concentration of TNF-α in the CON and EX conditions were 6.48 and 6.66 pg/ml respectively and were not different from each other ($p>0.05$). Mean IL-6 concentrations at baseline were 30.5 pg/ml for CON and 34.3pg/ml for EX. Baseline values were not significantly different. There was no change in concentration of IL-6 or TNF-α over time or condition (see Figures 3B and 3C; $p>0.05$). There were no significant relationships between inflammatory markers and eNO or TG.

Figure 3: Inflammatory Cytokines

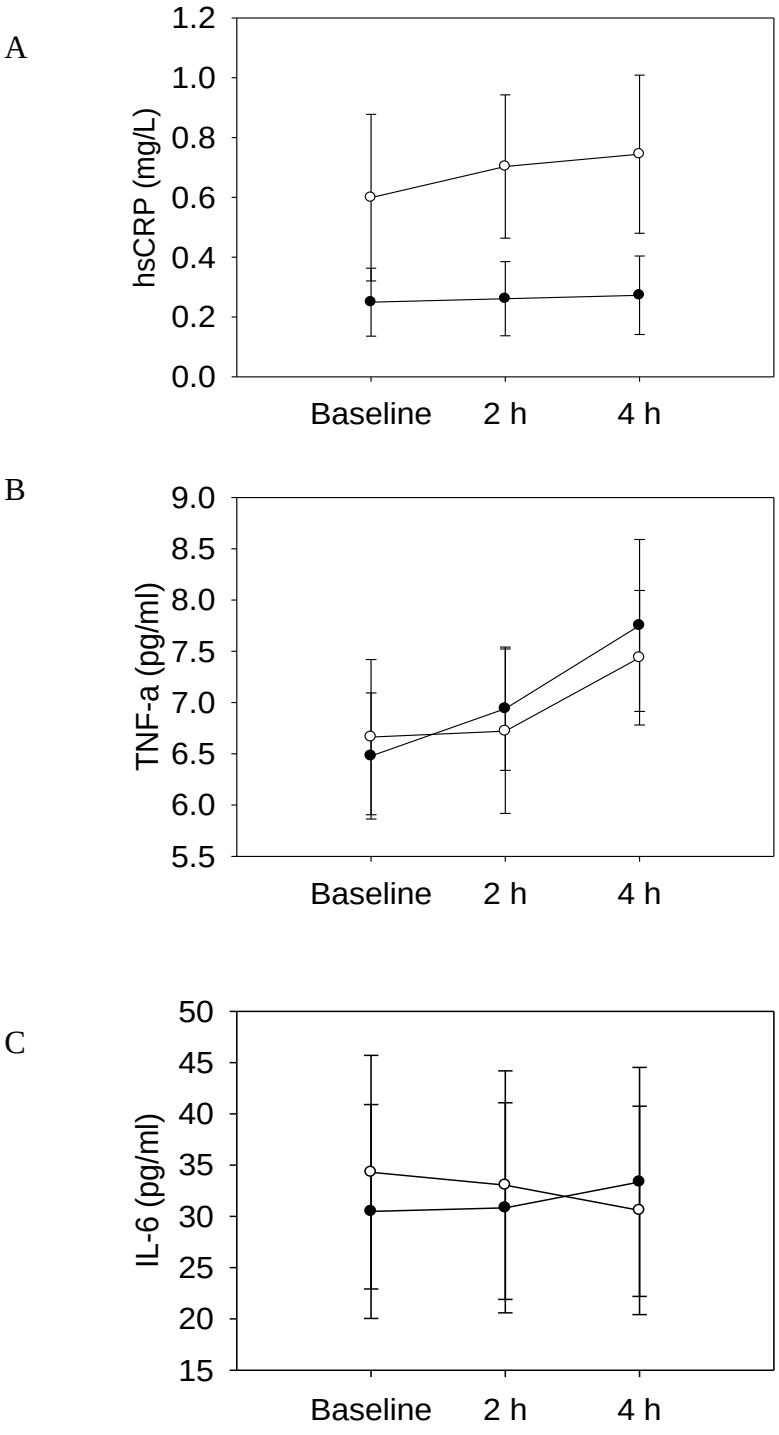


Fig. 3: Group mean (±SE) for CON (filled circles) and EX (open circles) for hsCRP (A), TNF-α (B), and IL-6 (C) after HFM. No significant differences within or between conditions were found.

Pulmonary Function

Mean data for pulmonary function is shown in Table 3. In the EX condition, FEV_1/FVC was significantly higher than CON at 4 h only (CON=80.7% EX=82.7%; p=.001). Additionally, within the EX condition, FEV_1/FVC was higher at 4 h compared to 2 h (2h=81.0% 4h=82.7%; p=.025); however, there were no differences in FEV_1 or FVC individually within or between conditions (p>0.05). FEF_{25-75} for EX was significantly higher than CON at 4 h only (CON=4.54L EX=4.83L; p=.005). There were no changes $R_{central}$ or R_{periph} between condition or time points (p>0.05). There were no significant relationships between pulmonary function and eNO or TG.

Table 3: Pulmonary Function Tests

Table 3: Pulmonary Function Tests						
	CON			**EX**		
	Baseline	**2 h**	**4 h**	**Baseline**	**2 h**	**4 h**
FVC (L)	5.89 ± 0.82	5.97 ± 0.96	5.84 ± 0.87	6.00 ± 0.88	5.87 ± 0.80	5.86 ± 0.86
FEV_1 (L)	4.71 ± 0.62	4.74 ± 0.60	4.64 ± 0.57	4.78 ± 0.60	4.68 ± 0.57	4.77 ± 0.59
FEV_1/FVC (%)	81.3 ± 8.5	81.1 ± 7.4	80.7 ± 7.7	81.3 ± 8.7	81.0 ± 7.7 **	82.7 ± 8.7*
FEF_{25-75} (L/s)	4.54 ± 1.09	4.55 ± 0.80	4.43 ± 0.84	4.58 ± 0.97	4.57 ± 0.96	4.78 ± 1.03
$R_{central}$ (KPa/(L/s))	0.15 ± 0.06	0.15 ± 0.07	0.14 ± 0.07	0.15 ± 0.07	0.12 ± 0.05	0.13 ± 0.05
R_{periph} (KPa/(L/s))	0.19 ± 0.08	0.17 ± 0.09	0.16 ± 0.08	0.18 ± 0.10	0.17 ± 0.09	0.17 ± 0.10
eNO (ppb)	35.7 ± 23.3	43.1 ± 25.1ǂ	40.1 ± 22.0	37.4 ± 23.0	43.3 ± 23.6ǂ	41.1 ± 23.7

Table 3: Values are presented as mean ± SD. FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; FEF_{25-75}: forced expiratory flow during 25-75% of vital capacity; $R_{central}$: central resistance of the airways; R_{periph}: peripheral resistance of the airways; eNO: exhaled nitric oxide.
* Significantly different from CON; p<0.05
**Significantly different from 4 h; p<0.05
ǂ Significantly different from baseline; p< 0.05

Chapter 5 - Discussion

The purpose of this study was to investigate the effects of a single continuous bout of aerobic exercise on airway inflammation which occurs following a high a fat meal. We also were interested in identifying whether any protective effect of exercise on airway inflammation was associated with a reduction in postprandial lipemia (PPL) or systemic inflammatory markers. Our data suggests that there was no protective effect of an acute bout of moderate exercise twelve hours prior to consumption of a high fat meal on subsequent airway inflammation in active, healthy men. Additionally, the increase in airway inflammation after a high fat meal was not significantly associated with the degree of PPL or inflammatory markers in the blood. Therefore, an acute bout of exercise may not be an effective means of protecting against postprandial airway inflammation in non-asthmatic, active subjects.

Airway Inflammation

Previous research has shown that a HFM leads to a significant increase in airway inflammation (exhaled nitric oxide; eNO) two hours postprandially (2, 78). Additionally, airway inflammation has been shown to return to baseline values four hours postprandially in healthy subjects after consumption of similar meal composition (102). Our data is in agreement with these findings. The percent increase in eNO relative to baseline values in the current study (~18%) was also in agreement with previous studies. Of note, although still within the normal range, baseline eNO values of our subjects (~36 ± 23.3 ppb) were considerably higher compared to previously reported eNO values for healthy, non-asthmatic, normal weight men (~17-20 ppb) (2, 78, 102). Given that our subjects were free of asthmatic symptoms, we are not sure of the reason for these increased baseline eNO values. Possible explanations for the elevated eNO include exposure to allergens or acute infections (25, 26). Nevertheless, despite the elevated

baseline, a HFM appears to provide a sufficient stimulus to increase airway inflammation during the postprandial period.

Negative effects of a HFM on vascular endothelial function have been shown to be modifiable. For example, moderate exercise two hours following a HFM reverses the vascular endothelial dysfunction see with the HFM alone (67, 100). Airway inflammation and thus, eNO also reflects dysfunction in the airway epithelium. The airway epithelium functions as a regulator of airway smooth muscle and inflammatory cells in response to injury similar to the vascular endothelium. Damage to this epithelium significantly contributes to inflammation (54). Our data suggests that moderate exercise 12 hours before a HFM does not have beneficial effects on inflammatory responses in the airway epithelium. In contrast to these previous findings, an acute bout of moderate intensity exercise on the day before a HFM was ineffective in attenuating inflammation in the bronchial epithelium as measured by eNO. Two major differences between the vascular and bronchial endothelium could contribute to this discrepancy. First, bronchial epithelium is physically separated from TG-rich blood whereas the vascular endothelium comes into direct contact with the blood. Second, isoforms of NOS differ between production of NO in the vasculature and production of NO in the airway. While vascular NO is predominantly formed by eNOS, eNO is predominantly formed via iNOS. The lack of a protective effect of acute exercise on eNO could be attributed to the differences in regulation of the two isoforms. Specifically, eNOS activity is dependent upon intracellular calcium concentrations and the iNOS isoform is independent of intracellular calcium and instead its activity is induced by inflammatory cytokines, bacterial products, or infection (4). Additionally, as discussed below, our exercise intervention did not decrease postprandial lipemia. Without a change in the presumed stimulus for airway inflammation, we would not expect to see a change in the airway

inflammatory response. However, our data does not suggest a causal relationship between triglycerides and airway inflammation (discussed below).

Postprandial Lipemia

In previous research, a single bout of moderate exercise performed 12 hours before a high fat meal was effective in attenuating postprandial lipemia (103). Interestingly, the acute exercise pre-HFM in our study did not show similar results. One possible explanation for this discrepancy may be due to the physical activity habits of our subjects. Although we did not directly assess physical activity, all of our subjects reported planned exercise bouts on at least three days per week. Previous studies have compared the effects an acute bout of exercise on PPL between exercisers and non-exercisers where exercisers were only asked to refrain from vigorous exercise for 0-36 hours prior to a HFM. These studies reported that exercisers demonstrated low levels of postprandial lipemia compared to inactive subjects (64, 79, 101, 105). However, other studies comparing trained and untrained individuals when exercise was restricted for a longer period prior to a HFM (>60 hours) showed no differences in PPL between trained and untrained subjects (46, 94). Due to these findings, it has been recommended to study active subjects at least 60 hours after their last bout of exercise, corresponding with recent evidence that LPL activity may remain elevated for 48-60 hours after an exercise bout (41). While we encouraged our subjects to refrain from exercise for >24 hours before consumption of a HFM, we cannot exclude the effects of recent exercise that may have occurred. Consequently, it is difficult to separate the effects of chronic exercise from those of an acute bout. In this case, it is possible that the last bout of exercise prior to the 24 hour restriction may have affected the control condition; however, it has also been shown that caloric replacement after a bout of exercise diminishes the protective effect of that bout on subsequent meals (10, 35). This suggests that energy balance

may be more important that the gross energy expended in an exercise bout (34). It is unlikely that subjects exercised and remained fasted from their last previous bout prior to the experimental trials until consuming the test HFM (>24 h). Therefore, we would expect minimal influence from a subject's last previous bout on PPL in our control condition. Furthermore, suppression of postprandial VLDL secretion from the liver has been demonstrated after two hours of 60% VO_2max exercise (59) but not after one hour of 60% VO_2max exercise (58). Although we did not directly measure VLDL, exercise duration may not have been sufficient to reduce plasma triglycerides via this mechanism.

It has been previously demonstrated that the change in airway inflammation after a HFM was correlated with plasma triglycerides (2, 78). Specifically, Rosenkranz et al. (2010) and Ade et al. (2013) showed that eNO and TG from baseline to two hours post-HFM were associated suggesting a dose-response relationship between triglycerides and airway inflammation. However, we did not find this relationship at two or four hours postprandially or in regard to the total exposure to TG and eNO over the four hour period, suggesting that the two variables are not directly related as a large increase in triglycerides was not necessarily accompanied by a large increase in airway inflammation. The absence of a relationship between postprandial lipemia and airway inflammation in our study may be explained by several factors. First, the overall TG response of our subjects was markedly lower than the TG responses reported in previous studies. While Rosenkranz et al. (2010) and Ade et al. (2013) report mean fasting TG from 75-96 mg/dl and two hour TG increasing up to 126-220 mg/dl, our subjects, consuming an identical HFM began with lower fasting TG (52 mg/dl) and only increased to 97 mg/dl in the CON condition. The peak TG response of subjects in the current study was nearly the same as fasting TG reported by Rosenkranz et al (2010). Again, this may be attributable to the likely higher physical

activity level of our subjects. Subjects in previous studies from our lab were generally inactive. Fasting TG and PPL were similar to previously reported values for trained subjects in both the CON and EX conditions (18). It is possible that our subjects experienced a "floor" effect for PPL. According to the American Heart Association, 12 h fasting TG of <100mg/dl is considered "Optimal" and <150mg/dl is still considered "Normal." Even after consumption of a HFM, mean TG peak in both the CON and EX conditions never exceeded 100 mg/dl, an "Optimal" fasting TG value. Additionally, only two individuals exceeded 150 mg/dl during the postprandial period. Because TG remained at homogenously low concentrations within our population, the ability to detect a correlation with eNO may have been diminished. However, this is an unlikely explanation because our subjects exhibited similar relative increases in eNO in response to a smaller change in TG compared to previously reported data. In fact, in absolute terms, our subjects had a greater increase in eNO than the increase for the sedentary subjects in previous studies (2, 78).

A second explanation for the variable inflammatory responses to the triglyceride load involves the dietary status of the subjects. Although we did not measure the chronic dietary habits or endogenous antioxidant status of the individuals, variation in eNO response is likely at least in part attributable to diet (2). Increased consumption of omega-3 fatty acids has been shown to completely mitigate the postprandial airway inflammation induced by a HFM (2). By alterations in arachadonic acid content of inflammatory cell membranes, production of several pro-inflammatory cytokines is inhibited. Thus, the degree of incorporation of omega-3 fatty acids into the inflammatory cell membranes could regulate the sensitivity per se of the airway inflammatory cells to the lipid increase. Variation in this sensitivity could explain the dissociation between TG concentration and eNO. Given the significant effect of dietary omega-3

consumption on airway inflammatory response to a HFM shown by Ade et al (2014), this hypothesis is likely to explain at least part of the variability in airway inflammatory response to the HFM.

Another plausible explanation for the disconnect between TG and eNO in our study is that there is a physical separation of the pulmonary epithelium and blood triglycerides. Whereas pathogen recognition receptors on macrophages in contact with the blood recognize dietary lipoproteins as pathogen-associated molecular patterns and induce an inflammatory cytokine cascade (including TNF-α and IL-6), immunoregulatory cells in the airways are physically separated from TG-rich blood (11). The disconnection between the plasma TG concentrations and the inflammatory response within the pulmonary system is likely linked by one or more mediating processes. For instance, a HFM has been shown to increase endotoxin (lipopolysaccharide; LPS), a molecule capable of inducing acute lung injury (38). The increases in LPS may activate inflammatory cascades in the airway epithelium independently via innate immune mechanisms.

Inflammatory Cytokines

Although several studies have assessed the effects of a HFM on inflammatory cytokines, there is a lack of agreement on the changes in TNF-α and IL-6. While some studies show an increase in TNF-α after a HFM (66), others show a decrease (9, 69, 102). Our data suggests that there is no change in TNF-α four hours after a HFM. Additionally, our finding that IL-6 was unchanged four hours after a HFM is in agreement with some (9), but not all previous findings (66, 69, 102). Although there was no evidence of an effect of a HFM on IL-6 in our study, other findings suggest that IL-6 is not increased until eight hours postprandially (9, 69). Because we only measured IL-6 up to four hours postprandially, it is possible that there was a delayed

increase in IL-6 that was not accounted for in our study. Hs-CRP is a delayed response to whole body changes in inflammatory state. Typically, alterations in hs-CRP are detected 16-24 hours after a stimulus (14, 73). Notably, due to the delayed response of hs-CRP, total systemic inflammatory response to the HFM is not detectable at our sampling time points.

Why was there a lack of effect of exercise on baseline or postprandial cytokine concentrations? Due to the timing of blood sampling, only hs-CRP would reflect inflammatory changes due to the exercise bout. No significant change in hs-CRP between CON and EX indicates that there was no overall change in systemic inflammatory status in response to our exercise bout. In our study, the 4 h postprandial time point was ~16 hours post-exercise. This sampling time is at the minimum time any changes would be observable; however, it is unlikely that hs-CRP may have been elevated if sampled at a later time. Although increases in CRP have been shown 16 hours after marathon running (14), there is no evidence for similar increases after shorter (~1 hour) exercise bouts (21, 77).

Furthermore, the increase in airway inflammation with a lack of change in systemic inflammation in our study suggests that inflammation in the pulmonary system can occur independently of systemic inflammation. While TNF-α, and IL-6 are important mediators of systemic inflammation, other cytokines such as IL-4, IL-5, or granulocyte-macrophage colony-stimulating factor (GM-CSF) may be more sensitive indicators of inflammation within the pulmonary system (26). When an antigen is present in the airway, IL-4, IL-5, and GM-CSF are released first from mast cells and Th2 cells. IL-4 and IL-5 stimulate differentiation and activation of eosinophils in the airway epithelium. GM-CSF prolongs the activation of eosinophils. Additionally GM-CSF can be released by the activated eosinophils, further potentiating the inflammatory response (11). While TNF-α and IL-6 can indicate the overall systemic

inflammatory environment, they are not direct mediators of the airway inflammatory response. However, IL-4, IL-5, and GM-CSF are not typically measured cytokines with regard to systemic inflammation. Our intention was to compare airway inflammation (eNO) with the systemic inflammatory environment, commonly assessed by TNF-α and IL-6.

Pulmonary Function

How is pulmonary function affected by an increase in airway inflammation? An increase in airway inflammation would be expected to be accompanied by a detriment in pulmonary function; however, a lack of change in pulmonary function tests despite an increase in airway inflammation in our study is consistent with previous reports in non-asthmatics (2, 78, 102). Although FEV_1/FVC at four hours during the exercise condition was statistically significantly higher than the two hour time point and the four hour time point during the CON session, the difference was <2% improvement and still within normal ranges. Additionally, all FEV_1/FVC values were considered normal according to American Thoracic Society guidelines (72). Slight increases in FEV_1/FVC spirometry measurements are often caused by submaximal inspiratory or expiratory efforts by subjects and are representative of airway restriction in less than half of all cases (72). Lack of change in FEV_1 or FVC individually and no changes in airway resistance further imply that airway function was unaffected by the intervention and that the small statistically significant difference in FEV_1/FVC did not represent a physiologically significant improvement in pulmonary function.

Implications

This study further confirms that the airways are sensitive to dietary intake; specifically, consumption of a HFM. Although pulmonary function was unaffected in our subjects by acute high-fat intake, repeated exposure to inflammatory stimuli has been implicated in the

pathogenesis of asthma (26). Overall, evidence from the current and previous studies from our lab (2, 71) suggests that dietary status and interventions targeting anti-inflammatory status or omega-3 content of may be more efficacious in reducing airway inflammation than exercise interventions following a HFM. As discussed previously, our data provides evidence that the sensitivity of the airways to an increase in plasma triglycerides may be more important than the magnitude of the triglyceride increase in an active population. The effectiveness of fish oil supplementation in preventing postprandial airway inflammation (2) also supports this idea. Increased proportion of omega-3 fatty acids to the omega-6 fatty acid, arachadonic acid (AA), leads to replacement of AA in cell membranes by omega-3 fatty acids. AA is a precursor to many inflammatory cytokines, prostaglandins, and leukotrienes. Specific to airway inflammation, production of AA-derived mediators prostaglandins-D_2 (PGD_2) and –E_2 (PGE_2) , as well as leukotrienes C,D, and E, is inhibited (12). PGE_2 is important in differentiation of T helper cells to the Th2 phenotype which is disproportionally expressed in asthma (12, 26). Overall, inhibition of the expression of these mediators via increased dietary proportion of omega-3 fatty acids is thought to lead to a decrease in airway inflammatory response to a stimulant such as a HFM. Because acute exercise was ineffective in reducing the stimulus for airway inflammation (postprandial lipemia) in our study, dietary intervention to increase omega-3 cell membrane content and thus, reduction of the sensitivity of the airways to the stimulus, may be a more effective means of reducing postprandial airway inflammation, at least in active subjects.

It is important to note that although inflammation is often thought of as detrimental to health, it is often an early stimulus necessary for healthy adaptations and functions to occur. For instance, production of NO in the airways in a well-controlled manner is beneficial and

necessary for maintenance of airway health. In response to microbial or viral pathogens or allergens, inflammation and production of NO carries out a protective role by forming NO radicals, S-nitrothiols, or peroxynitrites in the infiltrating host cells or microbes (4). Only when there is an overproduction of NO, typically by induced macrophages, are there damaging effects and cell death. Although our data suggests a well-regulated inflammatory response to an acute HFM, chronically high-fat diets may lead to this damaging overproduction of NO (4).

Limitations

Several factors may have influenced our results. First, sampling of blood lipids was only carried out at two time points that were separated by two hours. Although plasma TG concentration typically peaks at four hours (34, 41), excluding a sampling point at three hours may have hidden TG peaks in subjects with quicker clearance. Additionally, if clearance rates were altered by the exercise intervention, our timing of sampling may have artificially truncated the true peak TG in either condition. This is particularly relevant in an active population as TG clearance via LPL activity is related to lean body mass and has been shown to be quicker in trained subjects.

One possible confounding factor in our study was the administration of IV fluids throughout the sampling period in order to carry our serial venous blood draws without multiple punctures. Subjects acquired approximately 0.5-1L of extra fluid during the course of the sampling period. Assuming the subjects retained this fluid, four hour samples could be diluted. The extent of the effect of an extra liter of IV fluid on plasma sample concentrations of blood lipids and cytokines is unknown. Potentially, our four hour concentrations would be consistently underestimating physiological plasma concentrations had plasma volume been held constant. Future studies may want to account for this introduction of intravenous fluids by measuring

hematocrit at each sample time. Additionally, we are limited in detecting differences in TNF- α, IL-6, and hsCRP due to analyzing each sample singly. By analyzing in duplicate we may increase our ability to detect differences by potentially reducing variability. Next, we did not assess the dietary status of our subjects as subjects were not required to keep food logs. Similarly, endogenous antioxidant status of the subjects was unknown. Given that omega-3 fatty acid intake has been shown to have a significant moderating effect on postprandial airway inflammation (2), depending on the amount of omega-3 fatty acids subjects consumed, this may explain some of the variability in eNO response to the HFM. The macronutrient content of the experimental meal also limits our ability to isolate the high-fat content as the cause of airway inflammation. Our meal also consisted of a substantial proportion of carbohydrate (46%). While it has been shown that a 2-week high carbohydrate diet can increase lipemia (1), an acute high-carbohydrate, low-fat meal has not been shown to have the same effect (3). Furthermore, exercise has been shown to be equally as effective in reducing PPL induced by high-carbohydrate, high-fat meals as in reducing PPL induced by high-fat only. Additionally, in the typical Western diet, high-fat and high-carbohydrate meals are rarely mutually exclusive. Even so, we cannot exclude the possibility of carbohydrate contribution to airway inflammation.

Future Directions

Future research based on the results of our study is worthwhile to expand our understanding of airway inflammation following a HFM and to identify effective interventions. Future studies should focus on deriving the mechanism for increased airway inflammation following a HFM. If the mechanisms of this bronchial sensitivity to macronutrient intake can be identified, designing interventions to promote airway health can be more easily developed. Dose-response for eNO and fat content of a meal should be investigated as well as the effects of

chronically high-fat diets on airway function and inflammation. Although acute exercise was not effective in reducing PPL or eNO in our population, exercise interventions should not be discarded as a possible means to attenuate airway inflammation. Instead consideration should be given to the timing and intensity of exercise and the population studied. For example, exercise bouts performed closer to the consumption of a HFM may provide different stimuli such as airway stretch and increased IL-6 that may provide protect against airway inflammation. Exercise of different intensities may also affect airway inflammation as higher intensity exercise requires increased ventilatory flow rates and larger tidal volumes. Also, a more inactive population may have beneficial effects on airway inflammation from exercise before a high fat meal as exercise in this population is more likely to decrease postprandial lipemia (41). Exercise training effects on postprandial airway inflammation are also worth studying. Chronic exercise leads to an increase in bioavailability of antioxidants (100) which may contribute to attenuation of the postprandial inflammatory response. Other research should examine the effects of combined diet and exercise interventions in order to identify possible additive effects.

Conclusion

A high-fat diet has been implicated in the development of chronic levels of airway inflammation associated with the pathogenesis of asthma and other respiratory ailments. Thus far, very little research has evaluated interventions to prevent acute airway inflammation caused by a HFM. To our knowledge, this study is the first to assess an exercise intervention as a possible means to protect against postprandial airway inflammation. Our results provide important insight on the mechanisms involved in postprandial airway inflammation. Specifically, our data suggests, for the first time, that the severity of airway inflammation following a HFM can be uncoupled from the degree of postprandial lipemia, thus implicating the existence of other

mediating processes in the regulation of post-HFM airway inflammation. Future research should aim to identify the specific mechanisms resulting in acute postprandial airway inflammation in order to develop effective interventions and assess the potential deleterious effects of frequent high fat meals.

Chapter 6 - References

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