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

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EFFECTS OF AN ACUTE BOUT OF MODERATE INTENSITY EXERCISE ON POSTPRANDIAL
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Abstract

A high-fat meal (HFM) induces an increase in blood lipids (postprandial lipemia; PPL), systemic inflammation, and acute airway inflammation. While acute exercise has been shown to have anti-inflammatory and lipid lowering effects, it is unknown whether exercise prior to a HFM will translate to reduced airway inflammation post-HFM. Our purpose was to determine the effects of an acute bout of exercise on airway inflammation post-HFM and to identify whether any protective effect of exercise on airway inflammation was associated with a reduction in PPL or systemic inflammation. In a randomized cross-over study, 12 healthy 18-29 year old men (23.0 ± 3.2 yrs, 178.9 ± 5.5 cm 78.5 ± 11.7 kg) consumed a HFM (1 g fat/1 kg body weight) 12 hours following exercise (EX; 60 min at 60% $\dot{V}O_{2\max}$) or without exercise (CON). Fractional exhaled nitric oxide (FE_{NO} ; measure of airway inflammation), triglycerides (TG) and inflammatory markers (high-sensitivity C-reactive protein (hsCRP), tumor-necrosis factor- α (TNF- α), interleukin-6 (IL-6)) were measured while fasted, two hours and four hours post-HFM. FE_{NO} increased over time (2 HR: CON, $p=0.001$; EX, $p=0.002$, but not by condition ($p=0.991$)). TG significantly increased two and four hours post-HFM ($p<0.001$), but was not significant between conditions ($p=0.256$). Inflammatory markers did not significantly increase by time or condition ($p>0.05$). There were no relationships between FE_{NO} and TG or systemic inflammatory markers for any time point or condition ($p>0.05$). In summary, an acute bout of moderate intensity exercise performed 12 hours prior to a HFM did not change postprandial airway inflammation or lipemia in healthy, 18-29 year old men.

Keywords: inflammation, exercise, pulmonary physiology, dyslipidemia, exercise metabolism

Introduction

High-fat diets are known to be a risk factor for cardiovascular disease, and even a single high-fat meal (HFM) increases circulating TG (post-prandial lipemia; PPL) and circulating pro-inflammatory cytokines. This post-prandial inflammation is a shared risk factor for several chronic diseases, including obesity and asthma. These diseases are commonly expressed in the same individuals, and late-onset asthma commonly experienced in obese individuals, may be due to the effect of high-fat diets on the airways. Even a single HFM has been shown to increase inflammation in the airways (Rosenkranz et al., 2010; Wood et al., 2011, Ade et al., 2014; Kurti et al., 2015), and not just systemically. Recent research has reported a transient increase (~20%) in airway inflammation two hours following the consumption of a single high fat meal (HFM) in non-asthmatic subjects (Rosenkranz et al. 2010). This finding has been confirmed four hours post-HFM in healthy non-asthmatics (Kurti et al. 2015) and asthmatics (Wood et al., 2011). Although the mechanisms behind increases in airway inflammation post-HFM are not completely understood, several mechanisms may increase exhaled nitric oxide. The mechanisms that may be involved are both TLR4-dependent and TLR4-independent (Teng et al., 2014). In TLR4-dependent pathways, lipopolysaccharide (LPS) from the HFM has been shown to increase TLR4 mRNA via a nuclear factor-kappa B (NFkB) driven cascade (Wood et al., 2011), and increase neutrophil influx into the airways (Kurti et al., 2015). However the activation of NFkB from high-fat diets may occur due to TLR4-independent pathways as well (Zhang et al., 2005). Sugita and colleagues (2002) showed that in rats injected with LPS, inducible nitric oxide synthase expression was increased. Dietary fats may increase reactive oxygen species (ROS), therefore increasing transcription factors NFkB and I κ B kinase β (IKK β) (Kim et al., 2010) which upregulate iNOS, contributing to the increase in exhaled nitric oxide (Ricciardolo et al., 2004). The inducible NO isoform may become activated in the airways, which may contribute to the increase in exhaled nitric oxide (Gaston et al. 1994).

Recent research has shown dietary supplementation may be protective against increases in post-prandial airway inflammation (Ade et al. 2014), due to a lower sensitivity to a HFM stimulus. Along with adaptations occurring with chronic exercise training, an acute bout of exercise also increases antioxidant enzyme capability (Kramer and Goodyear 2007). However, unlike fish oil or anti-oxidant supplementation, exercise is also known to reduce PPL and also stimulates an anti-inflammatory cascade of cytokines, thereby potentially further lowering the sensitivity to the HFM stimulus and possibly attenuating the inflammatory response to a HFM. It is well established that a single bout of exercise can attenuate PPL (Freese et al. 2014; Gill and Hardman 2003). This attenuation is

generally thought to be an acute response, 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 HFM has been shown to be effective in reducing PPL (Zhang et al. 1998). Additionally, an acute bout of exercise stimulates a unique anti-inflammatory cascade of cytokines beginning with an increase in IL-6 from the contracting muscle (Petersen and Pedersen 2005). In addition to acute effects from exercise, chronic physical activity contributes to an overall anti-inflammatory environment systemically, as well as in the airways. Lowder et al., 2010 showed that there were increased baseline levels of the anti-inflammatory cytokine IL-10 following a training intervention in mice. Additionally Rodriguez-Mendez (2011) showed a decreased baseline eosinophilic inflammation and decreased FE_{NO} after a training intervention in humans. Although PPL and airway inflammation have not been consistently associated (Rosenkranz et al., 2010; Ade et al., 2014; Kurti et al. 2015), both independent risk factors for disease are modifiable with acute exercise. Therefore an acute bout of exercise prior to a HFM may reduce TG, systemic and airway inflammation post-prandially due to lipid modifying, and anti-inflammatory properties.

With both lipid lowering and systemic anti-inflammatory effects, exercise performed before a HFM may provide a protective effect against post-prandial airway inflammation. Therefore, the purpose of this study was to investigate the effect of a single continuous bout of aerobic exercise prior to a HFM on the airway inflammatory response. Additionally, we were interested in identifying whether changes in airway inflammation were associated with changes in PPL or systemic inflammation. We hypothesized that an acute bout of aerobic exercise 12 hours prior to a HFM would protect against subsequent airway inflammation in healthy men and further, would be associated with changes in PPL and systemic inflammation.

Materials and Methods

Subjects

Twelve 18 to 29 year old 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. Verbal and written consent was obtained from all subjects. The

study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University, and conformed to the Declaration of Helsinki.

Experimental Design

Subjects visited the laboratory on three separate occasions. On the first visit, height, weight, and body composition via dual-energy x-ray absorptiometry (DXA) were measured (Preliminary measures). Subjects then performed a $\dot{V}O_2$ max test on an electronically braked cycle ergometer to determine the appropriate intensity for sub-maximal exercise in the experimental condition. On two subsequent visits (day 1 or 2), 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). The experimental design is displayed in Figure 1.

Preliminary Measures

On the subjects' first visit to the laboratory, height and weight were recorded on a standard physician scale and body composition was measured via dual-energy x-ray absorptiometry (v5.6, GE Lunar Corp., Milwaukee, WI, USA). Subjects then completed an incremental test to exhaustion to determine maximal aerobic capacity.

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 $\dot{V}O_2$ max. After resting for 15 min, $\dot{V}O_2$ max was verified (Poole et al. 2008) by setting the work rate at 105% of the work rate corresponding to $\dot{V}O_2$ max. Subjects pedaled at 60-80 rpm until exhaustion. If subjects failed to pedal for at least two minutes, or did not achieve a $\dot{V}O_2$ within the range of $10 \text{ ml } O_2 \text{ W}^{-1}$, validation criteria were not met and a repeated full test was scheduled for a later date. Ventilatory threshold (VT) was estimated by three blinded researchers using the V-slope

method (Beaver et al. 1986) to determine if subjects exercised above or below their threshold and whether this influenced their results.

Day 1 – Exercise or rest and standardization of diet

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 fasting measurements for the experimental trial. For the experimental trial, subjects reported to the laboratory 12 hours prior to the time they would come in for their day 2 visit. On their exercise test (between 5 pm and 9 pm), 12 hours before fasting measurements subjects performed one hour of cycling at 60% of $\dot{V}O_2\text{max}$ (Magkos et al., 2007). 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.

Day 2- Return to laboratory after rest or exercise session

Upon reporting to the lab (between 5 am and 9 am), a battery of fasting blood analyses and pulmonary tests were performed. These are described in detail below. After completion of fasting testing, subjects consumed the HFM. The HFM was identical in the CON and EX trial. In EX, the meal was consumed 12 hours after the start of the exercise bout. Measures identical to those performed while fasted were repeated at 2- and 4-h postprandially.

High Fat Meal

The HFM used in the present study was identical to that previously employed by Rosenkranz et al. (2010) and Ade et al. (2014). The meal consisted of a fat dose equivalent to 1 g fat per kg bodyweight and was composed of ice cream (Edy's Grand Vanilla) and whipping cream (Reddi wip original). Ice cream serving size was measured as ml of ice cream = bodyweight in kg $\times$ 4.0625. Whipping cream was measured as mL of whipped cream = bodyweight in kg $\times$ 1.5. The nutritional makeup of the HFM was 7 g of total fat, with 4 g from saturated fat per serving. An additional 3 grams were from polyunsaturated and monounsaturated fat. There were 140 calories per serving, and 16 grams of carbohydrate with 13 grams from sugar per serving. There were an additional 2 grams of protein and 30 mg cholesterol per serving. The meal was ingested within a 20 min timeframe from the first bite.

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 (FVC), forced expiratory flow in 1-s (FEV_1), forced expiratory flow at 25-75% of vital capacity (FEF_{25-75})) (SensorMedics 229 Metabolic Cart, Sensor Medics Corp., Yorba Linda, CA), fractional exhaled nitric oxide tests (FE_{NO}) as a marker of airway inflammation via chemilluminescence (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 American Thoracic Society guidelines (Pellegrino et al. 2005). FE_{NO} tests were performed in triplicate with average values used in analysis.

Blood Sampling and Biochemical Assays

CRP, $TNF-\alpha$, IL-6, blood lipid profiles, and blood glucose were determined while fasted and 2- and 4-h after the consumption of the HFM in all conditions via serial blood draws using an intravenous catheter. 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 3 ml was extracted in order to clear any saline from the sampling line. At each time point, 5 ml blood samples were collected in a disposable syringe. 40 μ l 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 $-60^{\circ}C$.

TC, HDL, LDL, TG, and blood glucose were analyzed using a Cholestech LDX analyzer (Alere San Diego Inc., San Diego, CA). Commercial ELISAs were used to measure IL-6 and $TNF-\alpha$ (Cayman Chemical Company, Ann Arbor, MI) and high-sensitivity C-reactive protein (hsCRP; BQ Kits, Inc., San Diego, CA). Absorbance was read using a microplate reader (Synergy HT Multi-Mode Microplate Reader, Biotek Instruments Inc., Winooski, VT). Intra-assay coefficients of variation for $TNF-\alpha$ were 6.6%, 2.9% for CRP, and 5.06% for IL-6. For each assay, standards were plated in duplicate and samples were plated singly.

Statistical Analysis

Data analyses were conducted using SPSS v22.0 Statistics (IBM Corporation, Armonk, NY). Data were checked for normality, and if parametric assumptions were not met, data were log10 transformed prior to analysis. Data are expressed as mean $\pm$ SD. Two-way repeated measures ANOVA with time (fasted, 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 incremental area under the curve (iAUC) responses.

Relationships were determined via linear regression. Significance was set at $p < 0.05$ for all analyses.

Results

Subjects

Subject characteristics are shown in Table 1. All subjects were non-obese ($BMI < 30$). The average $\dot{V}O_{2max}$ was $46.9 \pm 7.9 \text{ mL kg}^{-1} \text{ min}^{-1}$. During the exercise bout, mean $\dot{V}O_2$ was $26.7 \pm 3.8 \text{ mL kg}^{-1} \text{ min}^{-1}$ ($57.4 \pm 6.1\%$ of $\dot{V}O_{2max}$) and $623 \pm 74 \text{ kcal}$ of energy were expended. Six subjects exercised below their ventilatory threshold (VT) and six exercised above VT but the groups did not show a different response in main outcome variables based on VT.

Exhaled Nitric Oxide

Mean and individual FE_{NO} responses are shown in Figure 2. FE_{NO} while fasted was not different between CON and EX conditions ($p=0.853$). FE_{NO} increased $\sim 18\%$ two hours after consuming the HFM in both conditions ($p < 0.05$). In each condition, 11 of the 12 subjects increased FE_{NO} 2 hours postprandially (CON: $p=0.001$; EX: $p=0.002$). Four hours postprandially, FE_{NO} was not significantly different from baseline values in EX ($p=0.145$) and remained elevated in CON ($p=0.043$). However there was no significant difference in FE_{NO} between conditions (0.991).

Blood Analytes

Mean blood analyte values for each condition and time point are shown in Table 2. EX blood lipid profiles were significantly higher in EX compared to CON ($p=0.011$). TG response is shown in Figure 3A. Individual responses are shown in Figures 3B and 3C. In both conditions, TG were significantly elevated at 2- and 4 h postprandially ($p < 0.001$). Triglycerides did not differ between conditions ($p=0.256$), however there was a significant interaction between time and condition ($p=0.016$). There were no significant differences in incremental triglyceride iAUC ($p=0.075$) however the 95% CI's show EX triglycerides were lower than CON (EX: 51.6219-113.3781 mg/dL; CON: 81.435-171.398 mg/dL) (Figure 4). LDL did not significantly change in either condition (CON: $p=0.306$; EX: $p=0.208$). CON and EX values did not differ from each other ($p > 0.05$). Blood glucose was significantly lower than baseline at two hours post-HFM in both conditions ($p=0.005$). There were no changes in TC or HDL across time for either condition ($p > 0.05$). In the exercise condition, changes in blood lipids were not related

to energy expended in the bout at baseline to two hours ($p=0.444$) or four hours ($p=0.202$). There were no significant relationships between changes in blood lipids and FE_{NO} from baseline to two or four hours ($p=0.911$ and $p=0.472$, respectively) or glucose and FE_{NO} (2 hr, $p=0.596$; 4 hr, $p=0.663$), respectively).

Inflammatory Markers

Mean values for hsCRP, $TNF-\alpha$, and IL-6 are shown in Table 2. Inflammatory markers hsCRP and IL-6 were normally distributed, while $TNF-\alpha$ was not normally distributed and log-transformed prior to analysis. Mean fasting hsCRP was not different between conditions ($p=0.194$). All subjects were within the healthy range of hsCRP ($<3.0 \text{ mg L}^{-1}$) for the CON condition. Only one subject fell outside of this range in the EX condition (hsCRP= 3.37 mg L^{-1}). There were no changes ($p>0.05$) in hsCRP between time points or conditions (see Figure 5A).

Mean fasting concentration of $TNF-\alpha$ in the CON and EX conditions were not different from each other ($p=0.859$). Mean IL-6 concentrations while fasted were not significantly different ($p=0.796$). There was no change in concentration of IL-6 or $TNF-\alpha$ over time or condition (see Figures 5B and 5C; $p>0.05$). There were no significant relationships between inflammatory markers and FE_{NO} or TG (all p values >0.05).

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 ($p=.001$). Additionally, within the EX condition, FEV_1/FVC was higher at 4 h compared to 2 h ($p=.025$); however, there were no differences in FEV_1 or FVC individually within or between conditions ($p>0.05$). There were no changes R_{central} or R_{periph} between condition ($p=0.981$) or time points (CON: $p=0.371$; EX: $p=0.272$). There were no significant relationships between pulmonary function and FE_{NO} or TG (all p -values >0.05).

Discussion

Major Findings

The purpose of this study was to investigate the effect of a single continuous bout of aerobic exercise on post-prandial airway inflammation. Our data suggest that an acute bout of moderate intensity exercise twelve hours prior to consumption of a HFM, was not effective in reducing airway inflammation post-HFM, nor were there

associations between changes in PPL and changes in systemic or airway inflammation. Despite this lack of a protective effect for airway inflammation, it is important to consider the potential mechanism by which a single HFM increases airway inflammation, particularly considering that there were no associations between changes in PPL and airway inflammation at any time point.

Postprandial airway inflammation- possible mechanisms

Previous research has shown that a HFM leads to a significant increase in airway inflammation (exhaled nitric oxide; FE_{NO}) two hours postprandially (Ade et al. 2014; Rosenkranz et al. 2010). The fasting FE_{NO} values of our subjects ($\sim 36 \pm 23.3$ ppb) were considerably higher compared to previously reported FE_{NO} values for healthy, non-asthmatic, normal weight men (~ 17 -20 ppb) (Ade et al. 2014; Rosenkranz et al. 2010; Wood et al. 2011). Still, these values were in normal healthy ranges as defined by the American Thoracic Society guidelines. Nevertheless, despite the slightly elevated values while fasted, a HFM appears to provide a sufficient stimulus to increase airway inflammation during the postprandial period. Although the mechanisms explaining the increase in FE_{NO} in the postprandial period are not completely understood, they are likely to be due to both TLR4-dependent and TLR4-independent pathways (Teng et al., 2014). These pathways converge and lead to an increase in NADPH-oxidases, including NF κ B and IKK β (Zhang et al., 2005). Therefore high-fat diets may increase NF κ B activity (Zhang et al., 2005), may lead to the downstream activation of inducible nitric oxide synthase (iNOS) (Sugita et al., 2002), and subsequent increase in FE_{NO} (Gaston et al., 1994). Additionally, competition for anti-oxidant enzymes after a HFM may inhibit the reduction of NO, resulting in increased FE_{NO} in the absence of increased iNOS activity (Bonini et al., 2014). Still, to fully understand the pathways involved, validation with sputum cell counts or directly measured iNOS activity would be needed. In recent work from our laboratory, Kurti et al. (2015) reported the FE_{NO} response following a high-fat meal was significant as a quadratic function, and was not significant linearly, indicating an increase at two hours followed by a subsequent return to fasting levels by four hours. The time course is supported by findings at four hours post-HFM from Wood et al. (2011). Kurti and colleagues also reported the increases in FE_{NO} were not associated with the change in eosinophils or neutrophils. These findings are likely due to study design where, exercise performed post-HFM may have impacted the neutrophilia in the post-prandial period. Still, the percent increase in FE_{NO} relative to values while fasted in the current study ($\sim 18\%$) was in agreement with several

previous studies (Ade et al., 2014, Rosenkranz et al., 2010), and we are confident the increase in FE_{NO} is indicative of cellular stress in the airway subsequent to a HFM.

Postprandial lipemia and inflammation

A single bout of moderate exercise performed 12 hours before a high-fat meal has been shown to be effective for attenuating postprandial lipemia (Zhang et al. 1998). Several studies have reported that sedentary men who perform exercise for 30 minutes prior to the HFM show an attenuated PPL response (Murphy, Nevill and Hardman, 2000). Other studies have shown that in contrast to sedentary participants, moderately active individuals need higher energy expenditures to reduce PPL (Brandauer et al., 2013). The 60 min moderate-intensity exercise protocol in the current study did not reduce PPL. Although this duration of exercise has previously been reported to be effective for reducing PPL (Zhang et al., 1998), it is possible the energy expenditure was not high enough given the physical activity habits of our subjects. The review by Katsanos (2004) suggests that an energy expenditure between 600 and 700 kcal may be necessary to see a reduction in PPL for recreationally active individuals. Based on the average body mass of our subjects, the average energy expenditure in the current study was 623 kcal. While not statistically significant, it appeared that with our exercise bout there were reductions in postprandial triglycerides when investigating iAUC differences between CON and EX ($p=0.075$ and 95 CI's: EX: 51.6219-113.3781 mg/dL; CON: 81.435-171.398 mg/dL). Therefore it is possible we would have seen a statistically significant reduction in PPL with a larger sample size, increasing the energy expenditure during the exercise bout, or by including less active subjects. We did not directly assess physical activity, which is a limitation of the study (see limitations). However, all of our subjects reported planned exercise bouts on at least three days per week and $\dot{V}O_2$ values that were slightly higher than those previously reported in our laboratory for recreationally active subjects (Kurti et al., 2015). Still, even with lower postprandial triglycerides in EX compared with CON, there were no significant differences in changes in FE_{NO} with changes in triglycerides. This lack of association between triglycerides and FE_{NO} suggests that the inflammatory processes that occur in the airway epithelium resulting in the increase in FE_{NO} , are independent of systemic inflammation, and are likely due to the mechanisms discussed previously.

Although several studies have assessed the effects of a HFM on inflammatory cytokines, our research is the first to report both systemic and airway inflammation after a HFM utilizing an acute bout of moderate intensity exercise 12 hours prior to a HFM. This study adds to the previous literature, contributing to the conflicting results regarding the

changes in TNF- α and IL-6 post-prandially. While some studies show an increase in TNF- α after a HFM (Nappo et al. 2002), others show a decrease (Blackburn et al. 2006; Payette et al. 2009; Wood et al. 2011). Our data suggest 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 (Blackburn et al. 2006), but not all previous findings (Nappo et al. 2002; Payette et al. 2009; Wood et al. 2011). 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 (Blackburn et al. 2006; Payette et al. 2009). 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. Also, in a recent study by Ghanim et al. (2009), at four hours post-HFM, NFkB-binding activity increased with no increases in C-reactive protein or TNF- α , therefore it is likely the discrepancies in these markers of inflammation are due to the time course in which they are investigated. Also, given that there is large variability in the PPL responses and individual data for inflammatory cytokines, individual differences may often make detecting differences too difficult unless the study has a much larger subject pool. Our primary aim was to determine the post-prandial airway inflammatory response and associations between PPL and inflammation. Given that FE_{NO} peaks early, we did not extend our study beyond the four-hour time point. However it is possible we missed the increase in metabolites due to this restricted time period. hsCRP has a delayed response to whole body changes in inflammation. Typically, alterations in hsCRP are detected 16-24 hours after a stimulus (Castell et al. 1997; Petersen and Pedersen 2005). Notably, due to the delayed response of hsCRP, total systemic inflammatory response to the exercise, not the HFM, was intended to be captured at our sampling time points. In agreement with previous literature, no significant difference in change in hsCRP between CON and EX indicates that there was no overall change in systemic inflammatory status in response to our exercise bout.

Furthermore, our finding that airway inflammation occurred without significant changes in systemic inflammation suggests that the two processes may occur independent of one another. 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 (Busse and Lemanske 2001. Urbano 2007). 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 (FE_{NO}) with the systemic inflammatory environment with markers commonly used for both acute and chronic exercise training interventions.

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 non-asthmatic subjects by an acute high-fat meal, Wood et al. (2011) reported a single HFM decreases pulmonary function in asthmatics. Overall, evidence from the current study suggests PPL and airway inflammation after the HFM are not associated, which may have implications for the development of both cardiovascular disease and asthma. Chronically high-fat diets are linked to the development of asthma, and the potential mechanisms may impact the airways independent of the systemic effects of high-fat diets. Additionally, diet may affect the airways regardless of physical activity habits. Future work should determine the airway inflammatory response after exercise in insufficiently active individuals, who may be more likely to display a greater attenuation of PPL after an acute bout of exercise, but are also likely to have reduced protection from oxidative stress due to a lower total antioxidant status.

Limitations

Several factors may have influenced our results. First, sampling of blood lipids was only carried out at three time over a 4 hour period. Although plasma TG concentration typically peaks at four hours (Freese et al. 2014; Gill and Hardman 2003), excluding a sampling point at three hours may have hidden TG peaks in subjects with quicker clearance. Additionally, we are limited in detecting differences in $TNF-\alpha$, IL-6, and hsCRP due to analyzing each sample singly. By analyzing in duplicate we may have increased our ability to detect differences by potentially reducing variability. Also, we were not able to provide details of dietary intake in the days leading up to the HFM in both conditions. Subjects kept this log and verified with the investigator that they consumed the exact same meal in both conditions prior to testing. However we do not have records of their meal intake data. It is possible the protein content of the meal influenced the increase in FE_{NO} . This would likely be due to nitrates in the meal or L-Arginine consumption (Kharitonov et al., 1997). However the previous literature reports that a much higher level of L-Arginine and nitrates increase FE_{NO} (Olin et al., 2001) and therefore it is unlikely the protein ingestion in our participants (7.01-10.71 grams of protein with approximately 200.7-286.7 mg

of L-Arginine) was driving the increase in FE_{NO} . Still, it would have been preferable to measure L-Arginine and nitrate levels in our participants. Also, it would have been preferable to objectively measure physical activity via accelerometers, however, all subjects indicated on the medical history questionnaire, that they were active for at least 3 days per week. Our meal also consisted of a substantial proportion of carbohydrate (46%). However, exercise has been shown to be equally as effective for reducing PPL induced by high-carbohydrate, high-fat combination meals as for reducing PPL induced by high-fat only. Also, previous studies from our laboratory that have used the same meal showed a significant increase in PPL and FE_{NO} following the meal (Rosenkranz et al., 2010; Ade et al., 2014) Lastly, in the typical Western diet, high-fat and high-carbohydrate meals are rarely mutually exclusive.

Conclusions

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 exercise prior to a HFM as a possible means to protect against postprandial airway inflammation. Additionally, our study is the first to include systemic markers of inflammation along with measures of airway inflammation and triglycerides in the post-prandial period. Our results provide important insight into the mechanisms involved in postprandial airway inflammation. In our study, there was an increase in airway inflammation in the exercise and control conditions that was not related to the change in triglycerides. The exercise bout lowered PPL, but the decrease was not significant in our group of active subjects. Additionally, our data support previously published work from our laboratory indicating that the severity of airway inflammation following a HFM is uncoupled from the degree of PPL, 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 involved in acute postprandial airway inflammation in order to develop effective interventions and assess the potential deleterious effects of frequent high-fat meals, particularly in those with airway disease.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Table 1 Subject characteristics

Variable	Value	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 (kg m ⁻²)	24.5 ± 2.7	21.4 - 29.7
Body Fat %	14.3 ± 4.5	8.2 - 22.8
VO ₂ max (ml kg ⁻¹ min ⁻¹)	46.9 ± 7.9	30.2 - 59.3
VT (%VO ₂ max)	55.3 ± 8.2	43.1-71.6

Values are means ± SD

VT ventilatory threshold *BMI* body mass index

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Table 2: Blood analytes

	CON			EX		
	Fasting	2 h	4 h	Fasting	2 h	4 h
TRG (mg dL ⁻¹)	52 ± 3	97 ± 38*	89 ± 23*	70 ± 20	100 ± 29*	87 ± 16*
TC (mg dL ⁻¹)	143 ± 24	150 ± 25	146 ± 25	145 ± 20	143 ± 19	147 ± 22
HDL (mg dL ⁻¹)	44 ± 4	46 ± 15	47 ± 13	45 ± 11	44 ± 14	45 ± 13
LDL (mg dL ⁻¹)	88 ± 14	84 ± 15	83 ± 19	88 ± 18	79 ± 18	84 ± 19
Glucose (mg dL ⁻¹)	81 ± 7	70 ± 17*	76 ± 7	78 ± 7	70 ± 11*	78 ± 5
IL-6 (pg mL ⁻¹)	29.8 ± 38.0	30.8 ± 35.5	33.4 ± 38.7	34.3 ± 39.5	33.0 ± 38.6	30.6 ± 35.2
TNF-α (pg mL ⁻¹)	6.48 ± 2.13	6.94 ± 2.08	7.75 ± 2.91	6.66 ± 2.62	6.72 ± 2.78	7.44 ± 2.27
CRP (mg L ⁻¹)	0.25 ± 0.32	0.26 ± 0.43	0.27 ± 0.45	0.60 ± 0.96	0.70 ± 0.83	0.74 ± 0.92

Values are means ± 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)

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Table 3: Pulmonary Function Tests

	CON			EX		
	Fasting	2 h	4 h	Fasting	2 h	4 h
FVC (L)	5.89 ± 0.27	5.97 ± 0.96	5.84 ± 0.87	6.00 ± 0.88	5.89 ± 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	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}	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}	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 ± 25.1*	40.1 ± 22.0	37.4 ± 23.0	43.3 ± 23.6*	41.1 ± 23.7

Values are mean ± SD

FVC forced vital capacity *FEV₁* forced expiratory volume in 1 second *FEF₂₅₋₇₅* forced expiratory flow during 25-75% of vital capacity *R_{central}* central resistance of the airways *R_{periph}* peripheral resistance of the airways *FE_{NO}* fractional exhaled nitric oxide.

* Significantly different from baseline (p<0.05)

**Significantly different from 4 h (p<0.05)

† Significantly different from CON (p< 0.05)

Fig. 1. Experimental Design for the control (rest) or experimental (exercise) day. HFM, high-fat meal; PFTs; pulmonary function tests; FE_{NO}, exhaled nitric oxide; Impulse Osc, impulse oscillometry. PFTs, blood analyses, Impulse Osc. were performed in CON and EX at baseline and then 2 hr and 4 hr after HFM. Participants were resting in the CON condition at the equivalent time to the exercise session.

Fig. 2 Changes in group mean for CON (filled circles) and EX (open circles) for FE_{NO} after HFM (A) and individual FE_{NO} responses for CON (B) and EX (C) conditions. Fractional exhaled nitric oxide (airway inflammation) increased ~6.5 ppb from baseline to 2 h in both conditions. *Asterisks* significantly different from baseline ($p<0.05$). Group data presented as mean $\pm$ SE

Fig. 3 Changes in group mean 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 triglycerides from fasted levels was seen at 2- and 4 h in both conditions. *Asterisks* significantly different from fasted levels ($p<0.05$). Group data presented as mean $\pm$ SE

Fig 4. Differences in AUC triglycerides for every subject are displayed by subtracting the EX AUC triglycerides from the CON AUC triglycerides. The difference in AUC triglycerides in the EX was not significant ($p=0.075$)

Fig. 5 Changes in group mean 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. Data presented as mean $\pm$ SE

Figure 1. Experimental Design for Day 1 (exercise or rest)**CONTROL** **EXPERIMENTAL**