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The effect of varying fatty acid composition in a High-Fat Meal and its impact on
postprandial airway inflammation

Breanna L. Davidson

A thesis submitted to the Graduate Faculty of
JAMES MADISON UNIVERSITY

In

Partial Fulfillments of the Requirements

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Master of Science

Department of Kinesiology

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Abstract

The Western Diet is typically high in saturated fats (SF) or omega-6 polyunsaturated fatty acids (O6FA) with insufficient amounts of omega-3 polyunsaturated fatty acids (O3FA). When chronic, this diet has been associated with an increased risk of respiratory diseases. **PURPOSE:** To examine the effect of varying the fatty acid composition of an acute High-Fat Meal (HFM) on postprandial airway inflammation. **METHODS:** Fifteen individuals [6 M, 9 F; body mass index (BMI) = 25.3 ± 6.6 kg/m²] consumed three HFM smoothies separated by at least 48 hours. The three smoothies were high in SF, O6FA, and O3FA and were standardized to 12 kcal/kg body weight, 63% total fat, and 0.72 g/kg sugar. Airway inflammation was measured using exhaled nitric oxide (eNO), airway function was measured using pulmonary function tests, airway resistance was measured using impulse oscillometry (iOS), and blood triglycerides (TG) and glucose were collected at baseline, 2h and 4h postprandially. **RESULTS:** There was no difference in eNO across time in any condition or between conditions ($p > 0.05$). FEV₁ was increased from baseline to 2h postprandially in the O6FA ($p = 0.038$) and SF-HFM ($p < 0.001$). O6FA was 2.7% higher O6FA compared to that of SF at 4h postprandially ($p = 0.04$). TG increased from baseline to 2h in all conditions ($p < 0.001$) and continued to trend upwards in the SF-HFM and toward baseline in the PUFA HFMs. **CONCLUSION:** An acute HFM did not elicit an airway inflammatory response for any condition. Different fatty acid compositions do not appear to impact eNO during the postprandial period.

Chapter I

The effect of varying fatty acid composition in a High-Fat Meal and its impact on postprandial airway inflammation

Westernized Diet

Poor dietary habits have a significant role in increasing the risk of lifestyle related diseases (1–3). Specifically, the Westernized diet is characterized as being nutrient-poor and calorically dense with high levels of saturated fats or omega-6 polyunsaturated fatty acids (O6FA) with insufficient amounts of omega-3 polyunsaturated fatty acids (O3FA). In addition, Westernized Meals typically consists of excess red and processed meats, dairy products, and refined carbohydrates with an inadequate intake of fish, fruits, vegetables, and whole grains. Specifically, the Western diet is deficient in the amount of O3FA with a current ratio of 20:1 O6FA to O3FA (4). It is important to note that O3FA and O6FA are essential fatty acids and are important for normal proper physiological processes, appetite, and metabolism (5). However, when meals are high in saturated fats or disproportionate in the amount of O6FA to O3FA, this leads to an increased risk of obesity, cardiovascular, metabolic, and respiratory diseases when consumed chronically (6, 7).

Saturated Fat High-Fat Meal and Airway Inflammation

Literature has suggested that meals high in saturated fat can lead to an increase in exhaled nitric oxide (eNO), a validated measure of airway inflammation (8–13). Specifically, Rosenkranz, et al. found that a 80% of subjects experienced an average of 19% increase in eNO following a HFM, who with no observed changes in overall pulmonary function (8). Similarly, Ade and colleagues examined the effect of a HFM on eNO in healthy adults either following 3-weeks of O3FA supplementation or placebo. Their control group experienced an increase in eNO approximately 20% compared to the

experimental group (14). While epidemiological research has shown an association between dietary fat consumption and systemic and pulmonary inflammation, the underlying mechanism linking the two remains unclear. It was originally proposed that the increase in proinflammatory cytokines in systemic circulation mediated an inflammatory response in the airway endothelium (15, 16). However, the observed time courses of airway and systemic inflammation do not support this theory (8, 11, 12, 14, 17). A study by Kurti, et al. found that 8-isoprostane, a marker of oxidative stress that is associated with asthma development, increases systemically following a HFM but did not change in the airway (18). Airway inflammation has been observed to peak at approximately 2 hours and return towards baseline levels at 4 hours postprandially (8, 11, 12, 14, 17, 18). This suggests two different mechanisms through which systemic and airway inflammation increase postprandially. Despite the mechanisms uncertainty, it has been observed that chronic levels of increased fat intake have been associated with increased airway inflammation, which can lead to airway hyperresponsiveness and remodeling of the bronchial tubes, characteristics of asthma (19–21).

The specific fat composition of a HFM is thought to alter the airway inflammation response. Meals with high in polyunsaturated fatty acids (PUFAs), as opposed to meals high in saturated fat, may have a less negative impact on health by either decreasing or completely mitigating the eNO responses following an HFM, depending on the type of PUFA. The proportion of O3FA:O6FA consumed is thought to play a role in the inflammatory response during the postprandial period following a HFM, altering the development of disease (4). Therefore, replacement of saturated fat with balanced proportions of O3FA:O6FA in a HFM and in regular diet in general should decrease

inflammation occurring postprandially, thereby decreasing the risk of chronic respiratory disease.

O6 Polyunsaturated Fatty Acids and Inflammation

O6FA are essential PUFAs not naturally produced by the human body. O6FA comes from animal-based food products including certain meats, flower oils, dairy products, and eggs. When consumed in moderation, O6FA can be important for cell membrane structure, assisting with blood pressure regulation, and inflammation. However O6FA, when disproportionate with O3FA, can lead to an increased risk for obesity and inflammatory diseases, such as asthma (4, 5, 22).

Linoleic acid is the most common PUFA in most western diets and can be desaturated to other forms of O6FA such as arachidonic acid (23). Arachidonic acid is a precursor to eicosanoids, specifically prostaglandins, thromboxanes, and leukotrienes and are pro-inflammatory when derived through this pathway (24, 25). Specifically, arachidonic acid upregulates the production of prostaglandin D₂ (PGD₂), thromboxane A₂ (TXA₂), and leukotriene B₄ (LTB₄) through the cyclooxygenase and lipoxygenase pathways. When these mediators are present in high concentrations, they act to increase bronchoconstriction (26), vasoconstriction, and vascular permeability and platelet aggregation, respectively, (23, 27) while promoting airway inflammation. This initiates a positive feedback loop, increasing bronchoconstriction (25). In addition, these inflammatory mediators transcriptionally upregulate inducible nitric oxide synthase (iNOS) to stimulate an increased release of nitric oxide, a potent bronchodilator, as a way to reduce bronchoconstriction (28, 29). Nitric oxide originates in the airway epithelium due

to an upregulation of iNOS and is observed through exhaled gas, making it a marker airway inflammation (30). High concentrations of nitric oxide inhibit the production of iNOS in the epithelial cells of the lungs until the amount of inflammation is reduced and then return to baseline levels (29, 31). While the optimal ratio of O3FA:O6FA is yet to be determined, intake of uneven concentrations of O6FA to O3FA leads to an increased production of PGD₂, TXA₂, and LTB₄ due to natural competition for the same enzymes. This elicits a proinflammatory physiological state, with increases in vasoconstriction, platelet aggregation, and bronchoconstriction (4, 28). If consumption of O6FA to O3FA is closer to 1:1, O3FA has a higher affinity to be metabolized by the shared enzymes (24) which allow the protective effects of O3FA to better counteract negative inflammatory effects of O6FA than are shown when they are consumed in drastically uneven proportions.

O3 Polyunsaturated Fatty Acids and Inflammation

O3FAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic (EPA), are also essential PUFAs that must be consumed through diet. These are commonly found in fish oils, seed oils, and certain plants. O3FA are anti-inflammatory and may reduce bronchoconstriction in asthmatics (25) or mitigate the airway inflammatory response in healthy adults leading to less bronchoconstriction to occur (14). O3FA counteract O6FA through increased concentrations in PGI₃ which stimulates vasodilation and inhibits platelet activation; TXA₃, a weak platelet aggregator; and LTB₅, a weak inflammation inducer (27). Recent literature also suggests that O3FA upregulate protective lipid mediators, such as protectins, resolvins, and maresins and downregulate other proinflammatory cytokines such as interleukins 1 and 6 (IL-1 and IL-6) (32–36). The attenuation in inflammatory markers further decreases concentrations of platelet activated

factors and PG mediators resulting in decreased iNOS release from airway endothelial cells (37, 38). These reactions decrease the amount of proinflammatory activity following a HFM, minimizing the amount of bronchoconstriction, resulting in a reduced amount of eNO.

Long-term dietary consumption relatively high in O3FA can lead to decreases in inflammatory markers related to cardiovascular disease (33), metabolic syndrome (39), and risk of obesity (5), while also impacting pulmonary function (40). Dietary fatty acid composition, specifically when high in O3FA, has been shown to decrease asthma prevalence (41). In a meta-analysis by Yang et al., fish intake or dietary supplementation with O3FA was inversely associated with asthma incidence in children, however no significant association was found in adults (41). However, Li et al. followed participants of the CARDIA study for 20 years and found that higher intake of O3FA was inversely associated with asthma incidence in adults (42).

Further research on the effect of increasing O3FA consumption on airway inflammation and as a treatment for asthma has been investigated. Ade, et al. found that the eNO response was mitigated during the postprandial period following a HFM in subjects who had undergone a 3-week O3FA supplementation when compared to a group who received a placebo supplement, despite postprandial serum triglyceride levels between both groups being similar at pre- and post-HFM (14). Emelyanov, et al. reported decreased daytime wheezing and airway inflammation in steroid-naïve atopic asthmatics following 8-weeks of O3FA supplementation (43). Research on the short- and long-term effects of O3FA supplementation largely suggest that increasing O3FA dietary intake can lead to profound health benefits.

Prior research on the effect that a single HFM has on airway inflammation have used meals that were high in saturated or Trans fatty acids. Moreover, literature surrounding the topic of a more balanced O6FA to O3FA ratio or higher O3FA intake has been conducted using prolonged dietary intervention with O3FA supplementation. No investigations have been done examining the effect of an acute HFM that is high in O3FA or O6FA on airway inflammation. Therefore, the purpose of this study is to determine whether varying a meal to be high O3FA or O6FA in an acute HFM will alter the airway inflammation response when compared to a meal high in saturated fat. We hypothesize that the airway inflammatory responses following the PUFA HFMs will be less than that following the typical Westernized meal that is high in saturated fats. Additionally, we predict that an the HFM high in O3FA will have an attenuated eNO response when compared to a meal high in O6FA.

Definition of terms

Postprandial period: 4h period immediately following consumption of a HFM

High fat meal: 12 kcal/kg body weight; 63% calories from fat, 0.72 g/kg body weight sugar

Chapter II

Methodology

Participants

Participants will be recruited using posted fliers and word-of-mouth from the James Madison University community and surrounding Harrisonburg-Rockingham area. Fifteen healthy young adults between the ages of 18-35 years will be recruited for this study. Healthy will be defined as without any cardiovascular, metabolic, or respiratory disease diagnosis and not currently taking medications that could affect airway inflammation, blood lipids, or glucose.

Experimental Design

This study will utilize a double-blind randomized crossover experimental design. Participants will be asked to come to the lab on four occasions: the initial visit and three separate HFM sessions. At the initial session, informed consent will be attained. Following consent of participation, familiarization and baseline sampling including pulmonary function tests, exhaled gases and anthropometric measurements will occur. Finally, the procedures for a Food Intake Record consisting of three weekdays and one weekend day will be explained. All Food Intake Records will be analyzed using the Nutrition Data System for Research (NDSR, Minneapolis, MN) and reviewed for quality assurance.

In the subsequent three visits, participants will complete each high-fat meal (HFM) condition, in a randomized order, separated by at least 48 hours. Prior to each condition, they will be asked to abstain from exercise for the 48 hours leading into each meeting. Upon arrival to their first HFM condition, participants will return their 4-day Food Intake Record. They will be asked to replicate their diet from the 24-hours leading into their first

session, for their final two subsequent sessions. This replication will be recorded using a 24-hour Food Intake Record and reviewed prior to the start of each HFM session to ensure compliance and accuracy. Blood lipids, glucose, airway inflammation, and pulmonary function will be assessed at baseline (0), 2, and 4 hours postprandially. Following the final session, participants will be asked to wear a tri-axial Actigraph GTX3+ accelerometer (Actigraph, LLC, Pensacola, FL) for seven consecutive days and complete an international physical activity questionnaire (IPAQ) to assess habitual physical activity level.

HFM Composition

All participants will complete each of the three HFM challenges. For each testing session, participants will be asked to arrive at the lab following a 10-hour fast. Each meal challenge will be a smoothie, standardized to 12 kcal/kg body weight and 0.72 g/kg body weight sugar. Each meal will consist of a total fat content of 63% fat. Fat composition will vary as follows: saturated fat meal (SF-HFM) will contain <0.02% fat from O6FA and O3FA; the high O6FA HFM (O6FA-HFM) will have a ratio of 15:1 O6FA:O3FA with 0.11 g/kg body weight of saturated fat; the high O3FA HFM (O3FA-HFM) will have a ratio of 15:1 O3FA:O6FA and contain 0.11 g/kg body weight saturated fat. Smoothie randomization procedure will be done using a random number generator to select which fatty acid composition to go with each flavor (blueberry, strawberry, or peach). The order in which subjects will receive each condition will also be randomized using the same approach. Each smoothie will be made by a Registered Dietitian, not participating in testing protocols, from the James Madison University Department of Health Professions. Smoothies will be standardized and made within 3 days prior to each session and brought

to the James Madison University Human Performance Lab in Godwin Hall to ensure researchers are blinded to the condition of each trial.

HFM Testing Protocol

At each high-fat smoothie session, at baseline, 2- and 4- hours postprandially, eNO, blood lipids, blood glucose, and pulmonary function tests will be measured. Following baseline measurements, the smoothie will be consumed within a 20 minutes period, with time for the postprandial period beginning upon completion of the smoothie. To control for minimal physical activity, all participants will be asked to either remain in the lab for the duration of the session or walk solely to lecture classes within Godwin Hall during the four-hour study period.

Blood Analyses

Blood lipids and glucose will be obtained using a finger stick to collect the blood and analyzed with a CardioChek Plus Analyzer (PTS Diagnostics, Indianapolis, IN). Each blood glucose measure will require a 5 microliter (μL) blood sample and blood lipids require a 40 μL sample. These measures will be used to assess metabolic responses to each HFM condition.

Exhaled Gases

During each session, airway inflammation will be assessed using a Niox Vero (Circassia AB, Morrisville, NC). This equipment measures eNO and is an easy to understand, noninvasive way to assess airway inflammation. This test requires participants to exhale for approximately 6-10 seconds at a steady rate into the mouthpiece of the eNO analyzer. These tests will be performed according to ATS/ERS guidelines for eNO

assessment (44). At each time point, eNO assessment will be completed twice, then averaged.

Pulmonary Function Tests

Pulmonary function will be assessed using maximum flow-volume loops using Vmax Encore metabolic cart (Vyair Medical, Mettawa, IL). These tests will be performed according to ATS/ERS guidelines for pulmonary function testing (45). Participants will be asked to wear a nose clip and then maximally inhale, forcibly exhale for approximately 6 seconds, then maximally inhale once more into a spirometer to complete the assessment. This will determine each participant's forced expiratory volume in 1-second (FEV₁), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of forced vital capacity ((FEF_{25-75%} of FVC)), and peak expiratory flow (PEF). Measurements will be repeated until three measurements are within a 10% range; these values will be averaged and used for analyses.

Airway resistance will be measured using impulse oscillometry system (iOS) also using Vmax Encore metabolic cart (Vyair Medical, Mettawa, IL). This method requires participants to wear a nose clip and breathe normally on a mouthpiece while the machine sends repeated bursts of air into the subject's mouth, allowing assessment of resistance in the central and peripheral airways. Three measurements within 20% will be averaged and used for analyses.

Anthropometric Measurements

During the initial session, height will be measured with a stadiometer (Charder Model HM 200P, Charder Electronic Co Ltd., Taichung, Taiwan) and weight with a standard physician's scale (Dymo Pelouze model 4040, Newell Brands, Hoboken, NJ). Body

composition will be assessed during the initial visit for each participant via dual x-ray absorptiometry (DEXA) using GE Lunar iDXA (Fairfield, CT). Participants will be asked to wear comfortable clothing and remove all metal (i.e. jewelry, sweatshirts with zippers, shoes, etc.) and lie supine in the center of the table for approximately 10 minutes while the scan occurs. Waist circumference will also be measured using Gulick tape measurer (Creative Health Products, Ann Arbor, MI) at the narrowest part of the waist.

Statistical Analyses

The data will be analyzed for airway inflammation, PFTs, iOS, and blood lipids and glucose using a repeated measures ANOVAs assessing time (hour) by condition (one of the three smoothie conditions). Post hoc testing with a Bonferroni correction will be used if any of the ANOVAs run result in statistical changes. Additional exploratory analyses will be done to determine whether lifestyle factors, such as habitual physical activity or habitual dietary intake, are associated with changes in airway inflammation measures during the postprandial period following a HFM.

Chapter III**Manuscript**

The effect of varying fatty acid composition in a High-Fat Meal and its impact on postprandial airway inflammation

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Abstract

The Western Diet is typically high in saturated fats (SF) or omega-6 polyunsaturated fatty acids (O6FA) with insufficient amounts of omega-3 polyunsaturated fatty acids (O3FA). When chronic, this diet has been associated with an increased risk of respiratory diseases. **PURPOSE:** To examine the effect of varying the fatty acid composition of an acute High-Fat Meal (HFM) on postprandial airway inflammation. **METHODS:** Fifteen individuals [6 M, 9 F; body mass index (BMI) = 25.3 ± 6.6 kg/m²] consumed three HFM smoothies separated by at least 48 hours. The three smoothies were high in SF, O6FA, and O3FA and were standardized to 12 kcal/kg body weight, 63% total fat, and 0.72 g/kg sugar. Airway inflammation was measured using exhaled nitric oxide (eNO), airway function was measured using pulmonary function tests, airway resistance was measured using impulse oscillometry (iOS), and blood triglycerides (TG) and glucose were collected at baseline, 2h and 4h postprandially. **RESULTS:** There was no difference in eNO across time in any condition or between conditions ($p > 0.05$). FEV₁ was increased from baseline to 2h postprandially in the O6FA ($p = 0.038$) and SF-HFM ($p < 0.001$). O6FA was 2.7% higher O6FA compared to that of SF at 4h postprandially ($p = 0.04$). TG increased from baseline to 2h in all conditions ($p < 0.001$) and continued to trend upwards in the SF-HFM and toward baseline in the PUFA HFMs. **CONCLUSION:** An acute HFM did not elicit an airway inflammatory response for any condition. Different fatty acid compositions do not appear to impact eNO during the postprandial period.

Introduction

The Western Diet is characterized by being energy-dense and nutrient-poor (46). When specifically examining fat intake, excess amounts of saturated and omega-6 fatty acids (O6FA) and insufficient amounts of omega-3 (O3FA) are common, resulting in an average O6FA:O3FA ratio of 20:1 (2). While the specifically recommended ratio of O6FA:O3FA has yet to be defined, evidence suggests that lowering the ratio closer to 1:1 could be beneficial for health (4, 21). When a diet with a high O6FA: O3FA ratio is consumed chronically, the imbalance in fatty acids has been associated with an increased risk of cardiovascular, metabolic, and respiratory diseases (6,7). Meals high in fat have also demonstrated increases in exhaled nitric oxide (eNO), a validated measure of airway inflammation (8-13), however, how the specific type and proportion of fat in a high-fat meal (HFM) may alter the airway inflammatory response remains to be elucidated. Rosenkranz, et al. found that 80% of subjects experienced an average increase in eNO by 19% following an acute HFM that was composed primarily of saturated fat (8). While this acute HFM transiently increased airway inflammation, there were no corresponding decreases in pulmonary function through the maximal flow-volume loop (MFVL) which is the gold standard to assess pulmonary function. However, chronic postprandial airway inflammation has been shown to increase airway hyperresponsiveness and remodeling of the bronchial tubes, which are characteristics of asthma (18-20). Acute inflammation can become chronic, thus by limiting the amount of exposure to acute airway inflammation long term risk of damage to the airways is reduced.

O3FA has long been recognized to have anti-inflammatory properties (5, 18, 47). In the airways specifically, subjects who consumed O3FA supplementation for three weeks

exhibited an attenuated eNO response after an acute HFM challenge compared to a control group without O3FA supplementation (14). However, the benefits of O3FA can be diminished by the excess intake of O6FA commonly found in the Westernized Diet (4, 18). While O3FA and O6FA are both essential polyunsaturated fatty acids (PUFAs), they are desaturated using the same enzymes and can create a more or less inflamed physiological state depending on whether O6FA or O3FA is metabolized. Over time, the metabolism of O3FA or O6FA can result in different impacts on both systemic and airway inflammation following a HFM (4, 5). When O6FA is consumed at much higher proportions to O3FA chronically, this can lead to an increased risk for obesity and inflammatory diseases, such as asthma (4, 5, 21).

Despite the understanding that the ratio of O6FA to O3FA can have a significant impact on the inflammatory state, little research has examined the airway response to an acute HFM that is high in O3FA or O6FA. The majority of current research on single HFM includes meals that are high in saturated fat (8, 10, 11, 12, 14). Therefore, the purpose of this study is to determine whether varying the ratio of O3FA vs O6FA in an acute HFM will alter the airway inflammatory response when compared to a meal high in saturated fat. We hypothesize that the airway inflammatory responses following the PUFA HFMs will be less than that following the typical Westernized Diet that is high in saturated fats. Additionally, we predict that the HFM high in O3FA will have an attenuated eNO response when compared to the meal high in O6FA.

Methodology

Participants

Participants were recruited using word-of-mouth from the James Madison University community. Fifteen healthy young adults (6 male, 9 female; ages 21.9 ± 1.5 years) were recruited. All subjects were free of any cardiovascular, metabolic, or respiratory disease diagnosis and were not taking medications that could affect airway inflammation, blood lipids, or glucose. All subjects were asked to discontinue any supplementation with ergogenic aids. The habitual diet was assessed using a 4-day Food Intake Record which was completed following the initial visit and returned at the first HFM session. All Food Intake Records were analyzed using the Nutrition Data System for Research (NDSR, Minneapolis, MN) and reviewed for quality assurance. Habitual PA was assessed via tri-axial Actigraph GTX3+ accelerometer (Actigraph, LLC, Pensacola, FL) worn for seven consecutive days following the final HFM session. Subjects were also asked to complete the International Physical Activity Questionnaire (IPAQ) to assess habitual physical activity level.

Experimental Design

This study utilized a double-blind randomized crossover experimental design. Participants were asked to come to the lab on four occasions: the initial visit and three separate HFM sessions.

At the initial session, informed consent was performed followed by familiarization and baseline sampling of pulmonary function tests, exhaled gases, and anthropometric measurements.

HFM Session Protocol

In the subsequent three visits, participants completed each HFM condition, in a randomized order, each separated by at least 48 hours. Conditions were randomized using a random number generator. Prior to each condition, they were asked to abstain from exercise for 48 hours and fast for at least 10-hours. At the first HFM condition, participants returned their 4-day Food Intake Record. Before each subsequent HFM session, participants replicated their diet for the 24-hours before their first session. This replication was recorded using a 24-hour Food Intake Record and reviewed prior to the start of each HFM session to ensure compliance. Airway inflammation, pulmonary function, and blood glucose and lipids were assessed at baseline (0), as well as 2 and 4 hours postprandially (Figure 1). Following baseline assessment, the smoothie was consumed within 20-minutes. The postprandial timeline began immediately upon completion of the smoothie. To control for minimal physical activity, all participants had to remain in the lab for the duration of the session or walk solely to lecture classes within Godwin Hall during the 4-hour study period.

HFM Composition

Each HFM condition was associated with a particular flavor of smoothie (strawberry, peach, or blueberry) and was standardized to 12 kcal/kg body weight, 0.72 g/kg body weight sugar, and 63% total fat (Table 1). Each smoothie was made within 3 days prior to each session by a Registered Dietitian Nutritionist from the James Madison University Department of Health Professions who did not participate in testing protocols, ensuring researchers and participants were blind to each condition.

Airway Inflammation

During each session, airway inflammation was assessed using a Niox Vero (Circassia AB, Morrisville, NC). Testing was performed according to ATS/ERS guidelines for eNO assessment (43). Subjects were seated in the same upright in the posture for every assessment with feet flat on the floor without a nose clip. Subjects were asked to inhale to maximum capacity then exhale at a constant flow rate for 6 seconds. At each time point, eNO assessment was completed twice, then averaged.

Pulmonary Function Tests

Pulmonary function was assessed using the MFVL on a Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). These tests were performed according to ATS/ERS guidelines for pulmonary function testing (44). This test was used to determine each participant's forced expiratory volume in 1-second (FEV_1), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of forced vital capacity (($FEF_{25-75\%}$ of FVC)), and peak expiratory flow (PEF). This test was repeated until three measurements within 10% of each other were obtained; these three values were averaged and used for analyses.

Airway resistance was measured using the impulse oscillometry system (iOS) on the Vmax Encore metabolic cart (Vyaire Medical, Mettawa, IL). Subjects were seated upright with their feet flat, neck slightly extended, a noseclip on, and mouth covering a mouthpiece with a tongue depressor. The same posture was performed for each test. Subjects were instructed to keep their cheeks hollow and breathe normally for approximately 20 seconds as resistance and reactance were measured. Three measurements within 20% were averaged and used for analyses.

Blood Analyses

Blood lipids and glucose were obtained using a finger stick to collect the blood and analyzed with a CardioChek Plus Analyzer (PTS Diagnostics, Indianapolis, IN). Each blood glucose measure required 15 microliters (μL) and blood lipids required a 40 μL blood sample. These measures were used to assess metabolic responses to each HFM condition.

Anthropometric Measurements

During the initial session, height was measured with a stadiometer (Charder Model HM 200P, Charder Electronic Co Ltd., Taichung, Taiwan) and weight with a standard physician's scale (Dymo Pelouze model 4040, Newell Brands, Hoboken, NJ). Waist circumference was measured and body composition was assessed via dual x-ray absorptiometry (DEXA) using GE Lunar iDXA (Fairfield, CT).

Statistical Analyses

Data was first checked for normality and adjusted if baseline measurements were not normally distributed. The data was analyzed for airway inflammation, blood lipids, and blood glucose using a repeated measures ANOVA assessing time (3 time points) by condition (3 conditions) using IBM SPSS Statistics v26.0 (IBM Corporation, Armonk, NY). *Post hoc* tests utilized a LSD correction. Significance for all analyses was *set a priori to* $p < 0.05$.

Results

Subject Characteristics

Fifteen college-aged participants (21.9 ± 1.5 years old; 6 M/ 9F) successfully completed the study. Subject characteristics are presented in Table 2. The only difference between sexes was mean body fat percentage (males: $18.4 \pm 6.8\%$; females: $30.8 \pm 10.3\%$; $p=0.022$). The average time spent in moderate to vigorous physical activity (MVPA) per week for all participants was 6.6 ± 2.7 hours with a range of 3.5-10.3 hours per week. The nutritional information for each HFM condition is presented in Table 2.

Airway Inflammation

There was no time by condition interaction, nor main effects for time or condition for eNO ($p>0.05$; Figure 2).

Pulmonary Function Data

There was no significant time by condition effect for any of the iOS airway resistance data ($p>0.05$, Table 3). There was a significant time by condition interaction in the FEV₁ for the O6-HFM and SF-HFM ($p=0.019$, Table 4). FEV₁ significantly increased in the O6FA-HFM ($p=0.038$) and in the SF-HFM ($p<0.001$). There was a significant difference in FEV₁ between the O6FA-HFM and SF-HFM at 4h ($p=0.04$) where it was 2.7% higher in O6FA-HFM compared to SF-HFM.

Biochemical Data

Mean cholesterol, high density lipoproteins (HDL), and calculated low density lipoproteins (LDL) are presented in Table 5. There was a significant time by condition interaction for calculated LDL ($p=0.007$). The time by condition effect for cholesterol approached significance ($p=0.054$).

There was a significant main effect of time for the SF, O6FA, and O3FA-HFM in triglycerides ($p \leq 0.001$; Figure 3). From baseline to 2h triglycerides increased 25.7% in the SF-HFM, 30.4% in the O6FA-HFM, and 30.5% in the O3FA-HFM ($p < 0.001$ for all conditions). From 2 to 4h postprandially, the triglyceride concentrations were significantly lower in the O6FA and O3FA HFM ($p = 0.02$ and 0.016 , respectively) decreasing 22.9 and 16.6%, respectively. Triglycerides trended upwards throughout the duration of the study for the SF-HFM increasing 25.8% from baseline to 2h baseline ($p < 0.001$) and remaining 31.1% higher than baseline at 4h ($p < 0.001$). There was no significant difference between condition at any time point ($p > 0.05$).

When examining glucose, there was a significant effect of condition ($p = 0.0048$) and time ($p < 0.001$; Figure 4). At 2h postprandially glucose was significantly higher in the PUFAs meals than the saturated HFM ($P = 0.049$). There was a significant main effect of time in the high O6FA and O3FA HFMs ($p = 0.001$ and 0.004 , respectively) from baseline to 4h and from 2h to 4h postprandially. At 4h postprandially, plasma glucose levels were significantly lower than baseline for the high O6FA ($p = 0.001$) and O3FA HFMs ($p = 0.006$).

Discussion

Major findings

The present study aimed to examine the effect of varying the fatty acid composition in an acute HFM on airway inflammation. To our knowledge, no studies have been performed examining these effects. We hypothesized that (1) both PUFA HFMs would result in a lower airway inflammatory response compared to that of the SF-HFM and (2) that the O3FA-HFM would result in a further attenuated eNO response compared to that of the O6FA-HFM. Our results do not support these hypotheses; eNO did not differ across condition or time points, indicating that the HFMs in the present study failed to create an airway inflammatory response, regardless of the fatty acid composition. These findings also do not support previous research regarding the effect of a HFM on postprandial airway inflammation (8, 11, 12, 14).

SF-HFM and Airway inflammation

Prior literature has indicated that meals high in saturated fat have consistently led to increases in postprandial airway inflammation (8, 10-12, 14). However, the precise mechanism remains unclear, as the time-courses of airway and systemic inflammation do not support original theories that systemically-circulated proinflammatory cytokines mediated the airway inflammatory response (17). The present study failed to observe any increase in airway inflammation after the SF-HFM, which could potentially be due to a lower amount of saturated fat in SF-HFM. Previous studies used a SF-HFM composition that contained 1 g of fat per kilogram of body weight with 4.5 g per serving coming from saturated fat whereas the present study used slightly less (0.82 g/kg body weight total fat, 0.5 g/kg saturated fat) than that amount (8, 12, 14). This could suggest a threshold effect

of the amount of saturated fat needed to see a response in eNO and should be considered for future research. Additionally, although Rosenkranz did find statistical increases in eNO, the average increases were relatively small (mean change = 3.3 ppb) and were unlikely to be clinically significant (8). Furthermore, the equipment used to assess eNO differed between previously conducted research and the present study, which is another possible explanation for the discrepancy (8, 11, 12, 14).

O6FA and Airway Inflammation

The present study did not find any changes in eNO in response to the O6FA-HFM. Although research has shown an effect of chronic exposure to elevated O6FA, there is little to no research on the acute effects of an O6FA-HFM on airway inflammation. The lack of an increase in postprandial eNO following an O6FA-HFM in the present study could be due to the type of O6FA used in the HFM, linoleic acid (LA). LA is the most common O6FA in the Western diet and can be desaturated into other forms of O6FA, most often into arachidonic acid (AA) (22). AA upregulates proinflammatory eicosanoids (22-24) that lead to increased bronchoconstriction (25), vasoconstriction, vascular permeability and platelet aggregation (22, 26) which in turn promote airway inflammation. In a systematic review by Rett and Whelan, it was determined that neither increasing nor decreasing LA intake in adults consuming a Western Diet had an effect on the amount of AA concentration in plasma phospholipids and thus, did not impact the production of the aforementioned pro-inflammatory mediators (48). This is likely due to the already large amount of LA consumed in the Western Diet, oversaturating the LA to AA pathways. Therefore, it is possible that the present study did not see a change in postprandial eNO due to the subject LA to AA pathway already having been oversaturated with no potential to increase the

negative effect of high O6FA on airway inflammation. The present study also utilized whole meals that included fruit, which led to a moderate antioxidant content. Research by Tenero et al. found that 4 weeks of antioxidant supplementation in children with asthma significantly decreased eNO levels (from 14 ppb to 11 ppb) (49). This could have resulted in an inhibition of oxidation and could result in a decreased amount of postprandial airway inflammation. While the acute effect of antioxidant consumption on airway inflammation has yet to be determined, this suggests a potential protective effect of antioxidants on the airways.

O3FA and Airway Inflammation

Previous literature has indicated an attenuation of airway inflammation following short or long-term O3FA supplementation (14, 42). In the current study, there was no significant difference in the postprandial eNO responses between the O3FA-HFM and either O6FA-HFM and SF-HFM. Therefore, at the doses used in this study, it appears that there is no protective effect of an acute dose of O3FA in preventing airway inflammation.

This is the first study, to the authors' knowledge, to examine the acute impact of high O3FA consumption on airway inflammation. The present study utilized a meal with whole food to mimic a more true-to-life situation and found no significant effect on postprandial eNO. Long-term experimental studies have indicated a protective effect following the supplementation of O3FA (14, 42, 50). For example, Emelyanov et al. found expired hydrogen peroxide levels, another marker of airway inflammation, to be significantly reduced in asthmatics following 8 weeks of O3FA supplementation (42). Ade et al. examined the effect of 3-weeks of fish oil supplementation on eNO following a meal

high in saturated fat in healthy, non-asthmatic subjects and reported eNO was unchanged pre- to post-HFM while the control group still saw ~20% increase in postprandial eNO (14). O3FA decrease the proinflammatory eicosanoids normally upregulated by O6FA while also upregulating protective lipid mediators, which work to inhibit other proinflammatory cytokines (31-35). This may lead to an attenuation in the airway inflammatory response in healthy adults, resulting in a lower postprandial eNO response (14). These mechanisms may still translate to an acute HFM, but would likely require meals with higher levels of fat to detect significant differences.

Limitations

One potential limitation of the present study is that seasonal pollen increases occurred during a portion of data collection. Airway epithelial cells are highly susceptible to environmental allergens or respiratory infections and could result in an allergen-induced airway inflammatory response, thus increasing eNO responses. However, in the present study, eNO was closely monitored to ensure drastically abnormal reactions did not occur. Moreover, if this had occurred, it would have resulted in an exacerbated eNO response, resulting in significant changes between time points or conditions. Due to the lack of contrasts over time or between conditions, it is unlikely that this had an effect. Additionally, previous studies have examined differences in postprandial eNO using chemiluminescence (8, 11, 12, 14). However, Niox analyzers have been found to have a high correlation with chemiluminescence analyzers (0.972; 51) and had good sensitivity and specificity in healthy subjects, therefore the eNO analyzer was unlikely the cause of the discrepancy between previous literature and the results presented in this study. It is also important to

note that it has yet to be determined how long it would take for O3FA or O6FA to produce EPA, DHA, or AA. Therefore, it is possible that if the observed postprandial period were longer, we may have seen an effect. However, HFM challenges that were high total fat and SF have reported eNO peaked at 2h postprandially and trended back towards baseline at 4h postprandially thus validating the time course of the present study.

Conclusion

In summary, our findings indicated no increase in airway inflammation following high fat meal with varying fatty acid compositions (i.e. SF, O6FA, and O3FA). Specific meal composition could have played a role in the absence of airway inflammation after a HFM. Future research should examine a HFM with higher fat content to fully understand the impact of a HFM on postprandial airway inflammation and compare the effects of supplementation versus true foods in the same experiment.

IV. Appendicies

Table 1. Nutritional information for each HFM smoothie.

	O3FA-HFM	O6FA-HFM	SF-HFM
Kcal (g/kg)	12.01	12.02	12.1
Total fat (g/kg)	0.83	0.83	0.82
Sat fat (g/kg)	0.11	0.11	0.5
Omega-6 (g/kg)	0.05	0.2	0.02
Omega-3 (g/kg)	0.4	0	0.01
Sugar (g/kg)	1.02	0.98	1.02
Sat Fat (% of Total Fat)	14.07	1.31	61.28
Omega-6 (% of Total Fat)	6.61	23.33	0.02
Omega-3 (% of Total Fat)	48.11	<0.01	0.02

*Total Fat is 63% of total kcal.

Table 2. Participant characteristics

	<i>N=15 (M=6, F=9)</i>
	Mean ± SD
Age (years)	21.9 ± 1.5
Height (in)	67.9 ± 3.7
Weight (kg)	74.7 ± 14
Waist Circumference (cm)	78.6 ± 10.4
Body Mass Index (kg/m ²)	25.3 ± 5.6
Body Fat (%)	25.8 ± 10.8
Time in MVPA (hours/week)	6.6 ± 2.7

Table 3. Impulse oscillometry system (iOS) data from baseline to 4h postprandially following a HFM high in SF, O6FA, or O3FA.

		O3FA-HFM	O6FA-HFM	SF-HFM	
R5	Baseline	2.9 ± 0.8	3.0 ± 0.8	3.1 ± 0.8	p=0.892
	2h	2.9 ± 0.8	2.9 ± 0.8	3.0 ± 0.9	
	4h	2.9 ± 0.7	2.9 ± 0.8	3.0 ± 0.9	
R20	Baseline	2.7 ± 0.6	2.8 ± 0.7	2.8 ± 0.7	p=0.506
	2h	2.6 ± 0.5	2.7 ± 0.7	2.7 ± 0.6°	
	4h	2.7 ± 0.6	2.7 ± 0.6#	2.7 ± 0.7	
X5	Baseline	-1.0 ± 0.3	-1.0 ± 0.3	-1.0 ± 0.3	p=0.640
	2h	-1.0 ± 0.2	-1.0 ± 0.3	-1.0 ± 0.3	
	4h	-1.0 ± 0.2	-1.0 ± 0.3^	-1.0 ± 0.3	
FRES	Baseline	12.5 ± 3.7	13.0 ± 4.7	13.4 ± 4.6	p=0.343
	2h	11.8 ± 4.8	11.3 ± 2.4	11.3 ± 4.1	
	4h	12.4 ± 4.0	12.0 ± 3.1	12.1 ± 3.1	
AX	Baseline	3.1 ± 1.9	3.4 ± 2.1	3.3 ± 2.2	p=0.293
	2h	3.2 ± 2.1	2.7 ± 1.4	3.1 ± 2.0	
	4h	2.7 ± 1.3	2.9 ± 2.0	3.0 ± 1.9	

R5: central airway resistance; R20: peripheral airway resistance; X5: amount of resistance while breathing in at an impulse frequency of 5 Hz; FRES: resident frequency; AX: area under the curve between 5Hz and Fres.

*indicates significance from baseline to 2h (p<0.05) ^indicates significance from 2h to 4h (p<0.05) #indicates significance between baseline and 4h (p<0.05) †indicates significance between O6FA and SF HFM (p<0.05) ‡indicates significance between O6FA and O3FA HFM (p<0.05) °indicates significance between O3FA and SF HFM (p<0.05)

Table 4. Pulmonary function test (PFT) data from baseline to 4h postprandially following a HFM high in SF, O6FA, or O3FA.

		O3FA-HFM	O6FA-HFM	SF-HFM	
FVC	Baseline	4.6 ± 1.1	4.6 ± 1.1	4.5 ± 1.0	p=0.498
	2h	4.6 ± 1.1	4.5 ± 1.0	4.5 ± 1.0	
	4h	4.6 ± 1.1	4.5 ± 1.0	4.4 ± 1.0	
FEV₁	Baseline	3.7 ± 0.9	3.6 ± 0.8	3.7 ± 0.8	p=0.019
	2h	3.8 ± 0.9	3.7 ± 0.8*	3.7 ± 0.9*	
	4h	3.8 ± 0.9	3.7 ± 0.8 [^] #	3.6 ± 0.8 ⁺	
FEV₁/FVC	Baseline	81.4 ± 5.4	79.7 ± 7.0	81.8 ± 4.5	p=0.218
	2h	81.8 ± 4.5	82.1 ± 5.0*	83.0 ± 4.7*	
	4h	82.4 ± 4.1	82.8 ± 4.8 [#]	83.2 ± 4.5 [#]	
FEF_{25-75%}	Baseline	3.8 ± 1.1	3.6 ± 1.0	3.7 ± 0.9	p= 0.078
	2h	3.9 ± 1.0	3.9 ± 1.0*	3.9 ± 1.0*	
	4h	3.9 ± 1.0	4.0 ± 1.0 [#]	3.9 ± 1.0	
PEF	Baseline	7.6 ± 1.9	7.2 ± 1.8	7.6 ± 1.8	p=0.381
	2h	7.5 ± 1.9	7.6 ± 1.9*	7.5 ± 1.6	
	4h	7.7 ± 2.0	7.6 ± 1.7 [#]	7.7 ± 2.0	

FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; FEV₁/FVC (%): ratio of forced expiratory volume in 1 s to forced vital capacity; FEF_{25-75%}: forced expiratory flow between 25 and 75%; PEF: peak expiratory flow.

*indicates significance from baseline to 2h (p<0.05) [^]indicates significance from 2h to 4h (p<0.05) [#]indicates significance between baseline and 4h (p<0.05) ⁺indicates significance between O6FA and SF HFM (p<0.05) [†]indicates significance between O6FA and O3FA HFM (p<0.05) [°]indicates significance between O3FA and SF HFM (p<0.05)

Table 5. Metabolic markers from baseline to 4h postprandially following HFM either high in O3FA, O6FA, or saturated fat.

		O3FA-HFM	O6FA-HFM	SF-HFM	
Cholesterol	Baseline	141 ± 23.1	151.9 ± 23.7	143 ± 23.2	
	2h	139.8 ± 21.9	146.6 ± 24.2	144.7 ± 22.4	p=0.054
	4h	141.5 ± 21.1	153.5 ± 27.8	145.7 ± 22.7	
HDL	Baseline	51.7 ± 7.1	51.9 ± 8.4	53 ± 9.4	p=0.185
	2h	51.6 ± 8.1	52.6 ± 9.7	52.3 ± 11.4	
	4h	51.9 ± 8.4	51.9 ± 11.1	52.3 ± 9.2	
Calculated LDL	Baseline	71 ± 20.3	80.4 ± 18.9	71.7 ± 16.3	p=0.007
	2h	62.1 ± 19.1*	63.1 ± 21.8*	67.7 ± 17	
	4h	67.1 ± 17.0	77.8 ± 23.6^	66.9 ± 18.7	

*indicates significance from baseline to 2h (p<0.05) ^indicates significance from 2h to 4h (p<0.05) #indicates significance between baseline and 4h (p<0.05) †indicates significance between O6FA and SF HFM (p<0.05) ‡indicates significance between O6FA and O3FA HFM (p<0.05) °indicates significance between O3FA and SF HFM (p<0.05)

Figure 1. The HFM session protocol. Time increments are listed in minutes. Exhaled nitric oxide is listed as eNO. Impulse oscillometry system is listed as iOS. Pulmonary function tests are listed as PFTs.

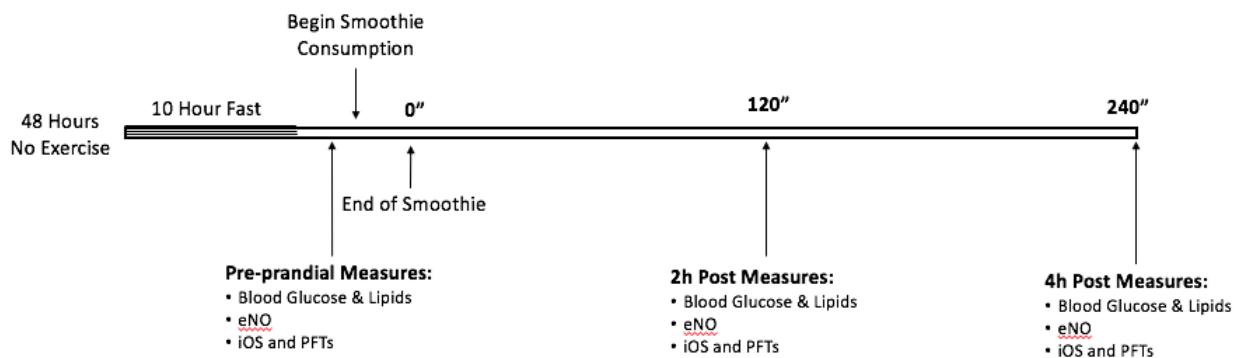


Figure 2. Exhaled nitric oxide (eNO) from baseline to 4h postprandially following meals high in O3FA, O6FA, or saturated fat.

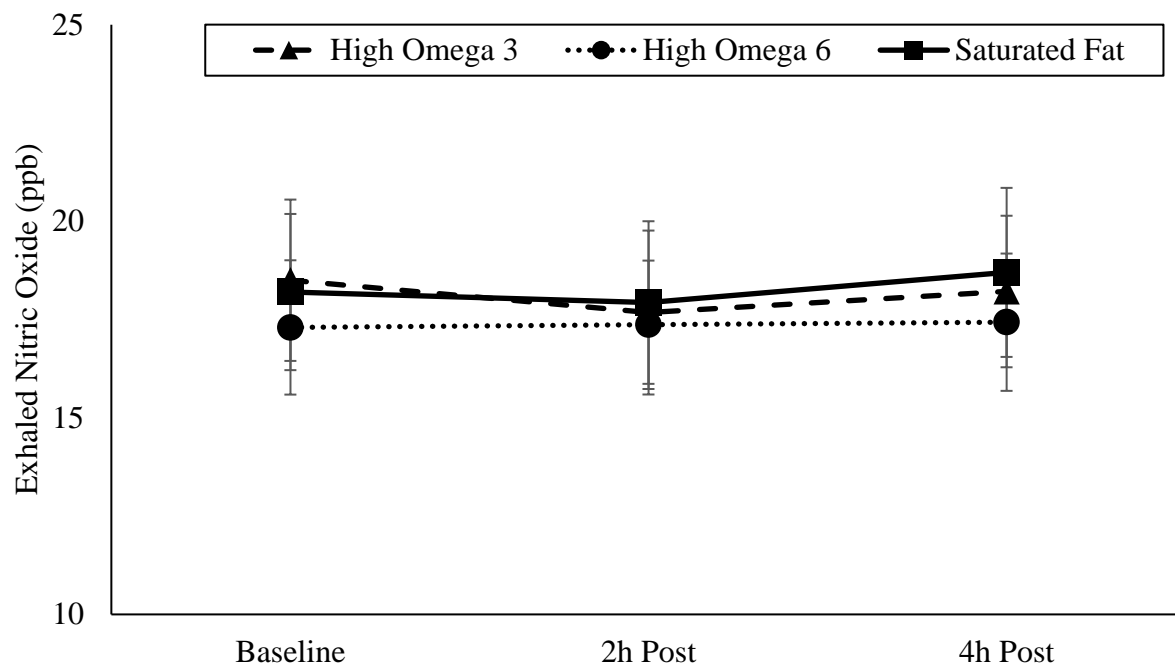
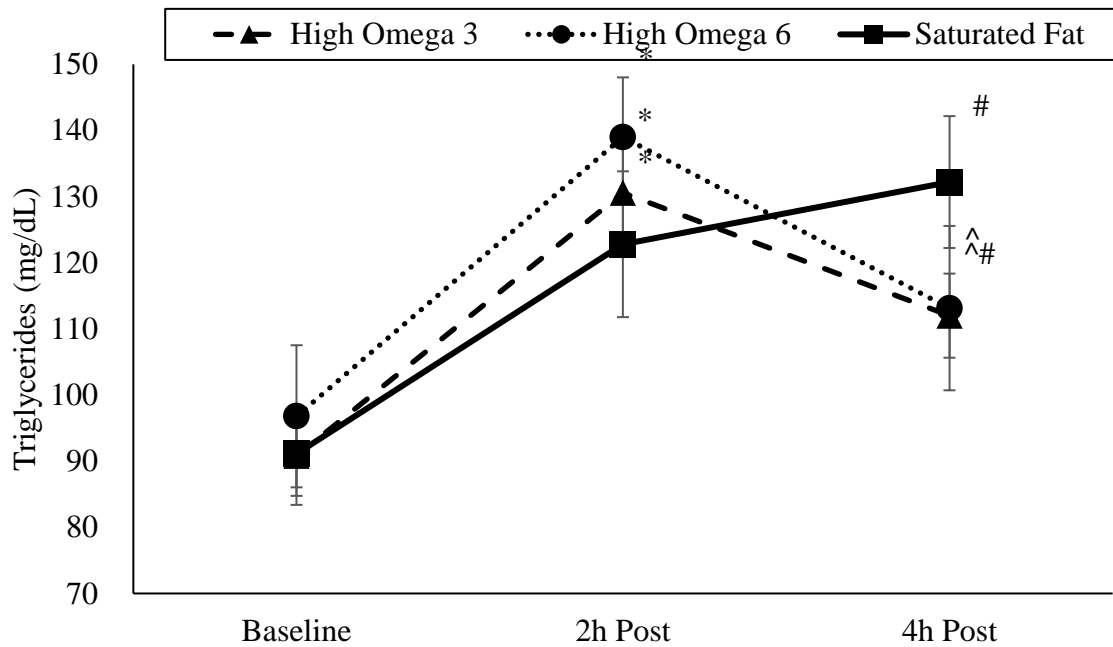
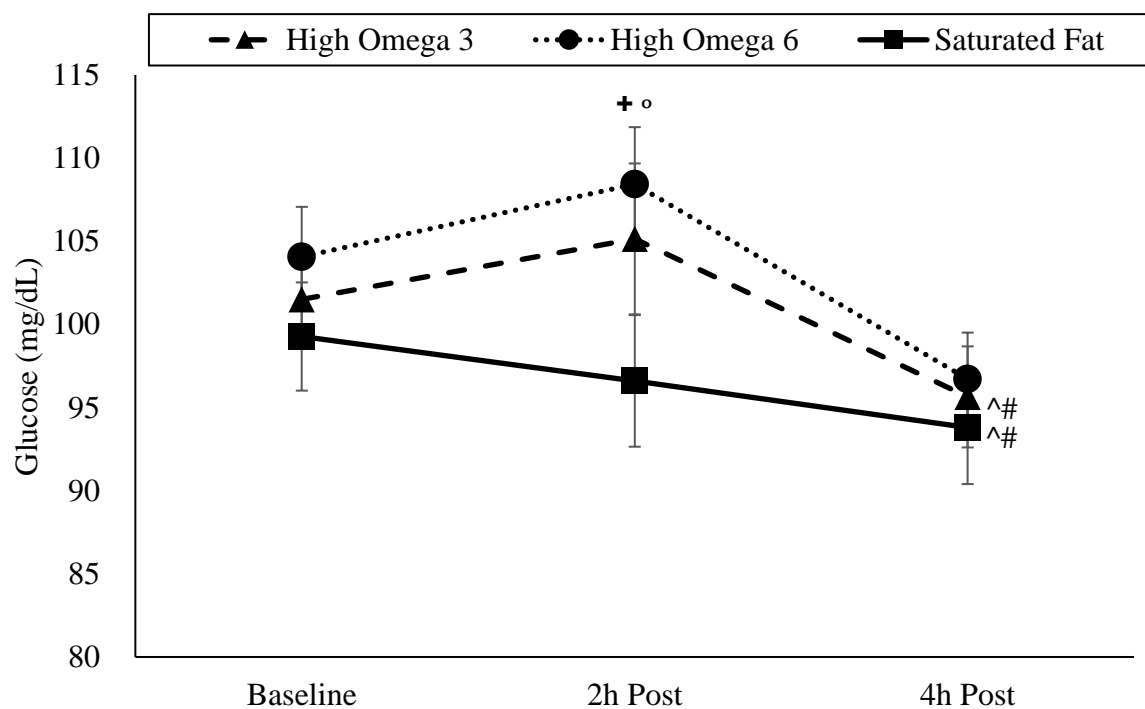


Figure 3. Mean plasma triglyceride response from baseline to 4h postprandially following consumption of HFM either high in O3FA, O6FA, or saturated fat.



*indicates significance from baseline to 2h (p<0.05) ^ indicates significance from 2h to 4h (p<0.05) # indicates significance between baseline and 4h (p<0.05)

Figure 4. Mean plasma glucose response from baseline to 4h postprandially following consumption of HFM that was high in O3FA, O6FA, or saturated fat.



*indicates significance from baseline to 2h ($p < 0.05$) ^indicates significance from 2h to 4h ($p < 0.05$) #indicates significance between baseline and 4h ($p < 0.05$) †indicates significance between O6FA and SF HFM ($p < 0.05$) ‡indicates significance between O6FA and O3FA HFM ($p < 0.05$) °indicates significance between O3FA and SF HFM ($p < 0.05$)

Project Title: *Does Varying the Fatty Acid Composition of a High-Fat Meal Impact Post-Prandial Airway Inflammation, Lipemia, and Glycemia?*

Consent to Participate in Research

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Drs. Stephanie Kurti, Elizabeth Edwards, and Jeremy Akers from James Madison University. To participate, you must be in good health and not diagnosed with any cardiovascular, pulmonary, or metabolic diseases. The purpose of this study is to determine whether varying fatty acid composition impacts how the airways respond to a single high fat meal. Previous research tells us that this inflammatory response is associated with asthma development, particularly if inflammation remains elevated chronically. Therefore we aim to determine whether varying the fatty acid composition of the smoothie can alter the airway inflammatory response to a single high-fat meal. This study will contribute to the knowledge of both practitioners and clinicians, and may provide an important public health message in providing dietary recommendations for those with elevated airway inflammation, particularly asthma. Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Research Procedures

This study consists of a questionnaire, a dietary recall log, a body composition scan, and three high-fat smoothie sessions administered to individual participants in the Human Performance Laboratory at James Madison University. The total time required for participation in the research study is outlined on the following page (time required section). The specific procedures during each visit are included here:

Initial visit: On the first visit to the laboratory, you will be briefed on the study and asked to complete the international physical activity questionnaire. You will also be advised how to accurately complete a 4-day food intake record. If you'd like to see any of these questionnaires prior to consenting, please ask a researcher and we'll be happy to provide one for you.

After completion of the questionnaire, we'll assess your height and weight, and then perform a DEXA scan to assess your body composition scan (how much of your body is fat vs. lean mass). You will then begin the high-fat meal session that you were randomized to.

Meal testing sessions (3 total): Your HFM testing sessions will be after a 10 hour fast. We will do a baseline finger-stick to assess blood lipids and glucose. You will be asked to perform several experimental measurements prior to consumption of a single high-fat meal (peach, blueberry, or strawberry smoothie). Some of the meals may contain dairy and gluten. These same measurements will be performed at baseline (0), 2, and 4 hours post-HFM. These include the following: Pulmonary function testing, exhaled nitric oxide, finger-sticks for glucose and triglycerides.

Time Required

Participation in this study will require ~19-20 hours your time over the course of 4 separate visits. Upon completion of the study, you will receive all of your data. The visits are outlined below:

Baseline Testing: On the first visit to the laboratory, you will complete the required questionnaire, be advised on how to complete the food log, and undergo the body composition scan, This will take approximately an hour.

HFM Sessions: The three remaining HFM sessions will each be ~6 hours in length. During this time, finger-sticks will be performed before, at 2, and 4 hours after the HFM to assess triglycerides and glucose. Exhaled nitric oxide will be measured for assessment of airway inflammation and pulmonary function tests will be done to assess lung function. These tests will also take place at baseline (0), 2, and 4 hours post-HFM.

Risks

Participation in this study does have some risks, although they are small. The risk of any serious event during this study is very small. Possible risks include:

Finger sticks: You may experience discomfort and bruising after the finger-stick.

High fat meal: The high-fat meal protocol is of very short duration, and any changes in blood lipids and glucose are unlikely to create any long-term health problems.

Additionally, this meal is not out of line with meals consumed on a regular basis by a significant portion of the US population.

DEXA: The DEXA scan entails a low dose of radiation equivalent to approximately one transatlantic flight (0.015 mSv= milliseievert). While there is no validated questionnaire to define extensive exposure, radiation exposure is cumulative (200 DEXA scans is equal to the cumulative exposure of living at sea level for a year (3 mSv). DEXA scans carry minimal X-ray exposure. To minimize exposure, the DEXA scan will only be performed once. All body composition assessment will be performed according to the American College of Sports Medicine guidelines for body composition assessment).

Benefits

By participating in this study, you will learn about your current metabolic health (blood lipids and glucose), body composition, chronic diet, weekly physical activity, and lung function values. Your data will be provided to you upon completion of your participation in the study. If an emergency arises and you must dropout, you may still receive your data. Having these tests done in a lab would cost several hundreds of dollars.

Society may benefit from more knowledge about how various fatty acid composition impacts the airway inflammatory response to a high fat meal, which is associated with respiratory disease development (i.e. asthma). Additionally, society will learn whether lifestyle (chronic diet and exercise) may attenuate these deleterious postprandial responses.

Confidentiality

The results of this research will be presented at the American College of Sports Medicine Annual meeting as well as the American Society of Nutrition annual conferences. The results of this project will be coded in such a way that the respondent's identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the JMU researchers. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions about the Study

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Researcher's Name: Dr. Stephanie Kurti
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Department of Kinesiology
 James Madison University

Questions about Your Rights as a Research Subject

Dr. David Cockley
 Chair, Institutional Review Board
 James Madison University
 (540) 568-2834
cocklede@jmu.edu

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

 Name of Participant (Printed)

 Name of Participant (Signed)

 Date

 Name of Researcher (Signed)

 Date

ID _____ AC _____ m0

Activity Monitor Log

As a participant in this program, we ask that you wear your Activity Monitor for one week. Begin wearing the monitor on the **Requested Start Date**. Please try to wear the monitor for seven consecutive days, but if you do need to skip a day for any reason, continue wearing it until you have worn it for a full seven days.

Requested Start Date: / / 18 **When you get up in the morning**

Requested End Date: / / 18 **When you go to bed at night**

Please record the actual dates/times you wore the accelerometer in the table below. Please be as precise as possible in reporting the times you put on and removed the monitor.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
Time of day you put on the unit							
Time of day you took off the unit							
Any time you did not wear the unit? (e.g. naps, bathing)							
Please provide comments about problems that occurred while you were wearing the unit.							

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

Yes

No **→**

Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

No vigorous job-related physical activity

→ ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do

moderate physical activities like carrying light loads **as part of your work**?
Please do not include walking.

_____ **days per week**

No moderate job-related physical activity **→** *Skip to question 6*

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

No job-related walking **→** *Skip to PART 2: TRANSPORTATION*

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

No traveling in a motor vehicle **→** *Skip to question 10*

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

No bicycling from place to place



Skip to question 12

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
 _____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

No walking from place to place



***Skip to PART 3:
 HOUSEWORK, HOUSE
 MAINTENANCE, AND
 CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
 _____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

No vigorous activity in garden or yard



Skip to question 16

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
 _____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

No moderate activity in garden or yard



Skip to question 18

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

No moderate activity inside home



*Skip to PART 4:
RECREATION, SPORT
AND LEISURE-TIME
PHYSICAL ACTIVITY*

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

No walking in leisure time



Skip to question 22

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

No vigorous activity in leisure time



Skip to question 24

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

No moderate activity in leisure time



Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ hours per day

_____ minutes per day

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ hours per day

_____ minutes per day

This is the end of the questionnaire, thank you for participating.

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