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The effects of short-term fish oil supplementation on pulmonary function and airway inflammation following a high fat meal

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We are submitting an original article titled: *The effects of short-term fish oil supplementation on pulmonary function and airway inflammation following a high fat meal*. We state that this manuscript is not under consideration elsewhere and that the research reported will not be submitted for publication elsewhere until a final decision is made as to the acceptability of the manuscript. There is no financial or other relationship that influenced the outcome of this paper. In addition, this manuscript represents original work without fabrication, fraud or plagiarism and has been read and approved by all authors.

ABSTRACT

Many environmental and dietary influences can cause immune cells to produce biological mediators that increase airway inflammation. A high-fat meal (HFM) is one stimulus that increases airway inflammation in healthy individuals. Supplementation with omega-3 fatty acids can reduce inflammation systemically and may be beneficial to the airways. **Purpose:** To determine if omega-3 fatty acid supplementation via fish oil would mitigate the airway inflammatory response induced by a single HFM. **Methods:** Seventeen non-asthmatic men (22 ± 2 yrs.) were supplemented with $3000 \text{ mg} \times \text{day}^{-1}$ fish oil or a placebo for 3 wks. Fractional exhaled nitric oxide (F_{ENO} ; a marker of airway inflammation), impulse oscillometry (a measure of respiratory impedance), pulmonary function, and triglycerides were measured prior to and two hours following a HFM. **Results:** Following a HFM, triglycerides increased in both fish oil and placebo groups compared to pre-HFM ($\sim 59\%$ and $\sim 49\%$ respectively, $p < 0.05$). The percent increase in F_{ENO} was greater in the placebo group compared to the fish oil group ($25.7 \pm 16.7\%$ vs. $-1.99 \pm 10.5\%$ respectively, $p < 0.05$). A significant correlation was observed between blood triglycerides and F_{ENO} in the placebo group ($r = 0.61$; $p < 0.05$), but not the fish oil group ($p = 0.21$). **Conclusion:** A single HFM increases airway inflammation and omega-3 fatty acid supplementation via fish oil protects against HFM associated changes in airway health.

INTRODUCTION

Systemic and localized inflammation is triggered by physical injury, microorganisms, chemical toxins, and hypersensitivity (Sherwood 2001). These biological responses are associated with increased production and release of inflammatory mediators from the blood plasma, white blood cells, mast cells, and endothelium all of which contribute to a cascade of additional inflammatory processes (Sherwood 2001). In addition, long-term exposure to inflammation is linked with an increased risk of several disorders, including atherosclerosis, rheumatoid arthritis, inflammatory bowel syndrome, asthma, and adult respiratory distress syndrome (Calder 2003; Libby et al. 2002).

One recently discovered and often overlooked inflammatory trigger is the nutritional content of our diet. Van Oostrom et al. (2003) was one of the first to demonstrate that a single high-fat meal (HFM) containing 40% weight/volume fat emulsion increased monocyte and neutrophil count, suggesting onset of an inflammatory response. This type of single meal was also used to reveal an association between high blood triglycerides and glucose levels and the production of pro-inflammatory cytokines and oxidative stress (Eriksson et al. 2001; van Oostrom et al. 2003). In addition, the recent findings of Rosenkranz et al. (2010) in our laboratory demonstrated for the first time that a HFM significantly increased both blood triglycerides and airway inflammation in healthy men and women. Thus it becomes evident that some component of dietary fat intake elicits an inflammatory response that can be observed both systemically and within the pulmonary system.

Emergent evidence suggests that omega-6 fatty acids are a primary mechanism mediating the inflammatory response observed with high fat diets (Galland 2010). Furthermore, regular intake of a pro-inflammatory diet, primarily one including omega-6 fatty acids, is often associated with increases in the prevalence of pulmonary distress. The typical western diet often contains disproportionately high omega-6 content relative to the omega-3 fatty acids (Simopoulos 2002; Simopoulos 2008). As such, individuals from populations traditionally with this type of diet have a high percentage of the omega-6 fatty acid arachidonic acid and very low fractions of the omega-3 fatty acid eicosapentaenoic acid (EPA) within their inflammatory cells (Gibney and Hunter 1993; Sperling et al. 1993). However, dietary supplementation with omega-3 fatty acids increases the fraction of omega-3s EPA and docosahexaenoic acid (DHA) within the plasma membrane of cells (De Caterina et al. 1994; Kew et al. 2004; Meydani et al. 1993; Mickleborough et al. 2006; Mickleborough et al. 2003; Sperling et al. 1993) which subsequently produces less biologically active inflammatory mediators (Calder 2003; Obata et al. 1999).

The omega-3 fatty acids EPA and DHA can be derived from a variety of sources including marine or fish oil (Whelan and Rust 2006). As such significant associations between fish intake and lung function suggest that omega-3 fatty acids may have a beneficial influence on pulmonary physiology (Laerum et al. 2007; Schwartz and Weiss 1994). Mickleborough and colleagues have demonstrated the protective effects of fish oil on exercise induced bronchoconstriction in asthmatic populations (Mickleborough et al. 2006; Mickleborough et al. 2003). In addition, Tecklenburg-Lund et al. (2010) reduced airway inflammation in asthmatic subjects following a

3wk period of fish oil supplementation (Tecklenburg-Lund et al. 2010). However, to date, it is unknown if fish oil supplementation for a similar 3 wk period will mitigate the airway inflammatory response elicited by a single HFM.

The rationale for the present study was that because omega-3 fatty acids, and by extension fish oil, has a systemic anti-inflammatory action, airway inflammation following a HFM would be reduced. It was hypothesized that (i) a single HFM would increase post-prandial airway inflammation, (ii) a linear relationship would exist between blood triglycerides and airway inflammation, and (iii) that 3 wks fish oil supplementation would prevent the HFM post-prandial increase in airway inflammation.

MATERIALS AND METHODS

Subjects

Seventeen men (age 22 ± 2 yrs (mean \pm SD)) completed the experiments. All subjects had no history of cardiovascular, pulmonary, or metabolic disease and were non-smokers as determined from medical history questionnaire. Of the subjects none were regularly using anti-oxidant supplements or anti-inflammatory drugs. Subjects were excluded if using bronchial dilators, lipid lowering medication, or fish oil supplements. Subject's dietary habits were not directly monitored prior to or during the study period, but were continuously instructed to not change their diet or physical activity habits. 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

This study was conducted as a randomized, single blinded trial. Following pre-trial testing (i.e., baseline), each subject was randomly assigned to three weeks of either a fish oil supplementation condition or a placebo condition. Subjects in the placebo condition continued their normal diet plus three similar appearing capsules containing room air. In the fish oil condition; subjects continued their normal diet plus three capsules per day containing 1000 mg of fish oil each (containing 180 mg EPA and 120 mg DHA; Spring Valley Omega-3 Fish Oil, Springfield MO). The reported EPA and DHA contents by the manufacture were not verified prior to the study but have been previously reported elsewhere (Harris 2004; Juturu 2008). Subjects were instructed to take the pills each day of the experimental period and were asked not to eat fish during the course of the study and were contacted regularly to inquire about any adverse reactions and to monitor compliance. Subjects visited the laboratory after an overnight fast (12 h) and abstaining from exercise (24 h) following the 3 wk supplementation period (Pre-HFM) and 2 h following the consumption of a HFM (Post-HFM). At each session, resting measurements of pulmonary function, airway impedance, fractional exhaled nitric oxide, and blood triglycerides were made.

The “over-the-counter” HFM used in the present study was identical to that previously employed by Rosenkranz et al. (2010). 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 4.5 g saturated fat (39.8-56.9 g) per serving, 30 mg cholesterol (339.7-485.5 mg) per serving, and 16 g of carbohydrate (106.7-152.5 g) per serving. The meal was ingested within 20 min.

Experimental Measurements

Pulmonary function was assessed via standard measurements of peak expiratory flow (PEF), forced expiratory flow in 1-sec (FEV₁), forced expiratory flow at 25-75% of vital capacity (FEF₂₅₋₇₅), and forced vital capacity (FVC) (SensorMedics 229 Metabolic Cart, SensorMedics Corp, Yorba Linda, CA, USA) according to the manufactures guidelines and the American Thoracic Society criteria of pulmonary function testing. As such, the highest values from the three repeated trials were recorded. Airway impedance was measured using a commercially available airway oscillometry system (IOS, Jaeger, Germany) (Vink Pediat Pulmon 2003) according to the manufactures guidelines. This system measures impedance (Z) of the total respiratory system, from which calculations of resistance (R) and reactance (X) can be made. In the present study Z, R, and X were measured across a frequency range of 5-35 Hz. The averages of three repeated trials were recorded. Airway inflammation was evaluated using measurements of fractional exhaled nitric oxide (F_{ENO}) via chemiluminescence (Sievers Nitric Oxide Analyzer 280, Sievers Instruments Inc, Boulder, CO, USA) in accordance to the manufactures guidelines and the American Thoracic Society recommendations. F_{ENO} provides reproducible and non-invasive assessment of inflammation in the lower-respiratory tract (Gabbay et al. 1998). Briefly,

while wearing a nose piece to prevent contributions of nasal NO, subjects were asked to inhale orally to total lung capacity and then immediately perform a constant and controlled exhalation flow rate to vital capacity into a rapid NO analyzer. The averages of three repeated trials, all within 5% of the mean value, were recorded. A minimum of 30 seconds of relaxed breathing was given between trials. Measurements of total cholesterol and triglycerides were determined from a finger-stick blood sample and analyzed via an automated lipid analyzer (Cholestech, LDX). Measurements of respiratory impedance and blood lipids were not completed in 4 subjects due to equipment unavailability (2, fish oil; 2, placebo). Subjects were in a seated position for all experimental measurements.

Statistical Analysis

Data are given as mean \pm SD except when otherwise stated. The impact of fish oil supplementation (or placebo) and a HFM post-prandial response were tested by two-way repeated measures ANOVA with a Newman-Keuls test as a post hoc analysis test. Linear correlation analysis was used to assess the relationship between blood triglycerides and airway inflammation across individuals in the placebo and fish oil groups. Significance was set at $p < 0.05$ for all analyses.

RESULTS

Baseline Characteristics

General subject characteristics and pulmonary function measurements at baseline are shown in Table 1. Stature and body mass were not significantly different between fish oil and placebo conditions. At baseline standard pulmonary function measurements, F_{ENO} , respiratory impedance, and blood lipids were not significantly different between experimental groups. However, baseline F_{ENO} varied by approximately 30 ppb across subjects and may reflect the varied caloric and nutritional dietary habits in college aged men.

Standard pulmonary function and Impedance

Following a HFM, triglycerides significantly increased in both fish oil and placebo groups compared to pre-HFM (59% and 49% respectively). Standard pulmonary function test and respiratory impedance test values pre and post-HFM are shown in Table 2. Pre-HFM values of PEF, FEF_{25-75} , and FEV_1 were not different between experimental groups. In addition, these variables were unchanged ($p>0.05$) post-HFM compared to pre in both groups. Similar to standard pulmonary function variables, measurements of respiratory impedance were similar between experimental groups pre-HFM and were unchanged post-HFM.

Exhaled nitric oxide

Figure 1 illustrates the individual and group mean change in F_{ENO} before and after HFM. In the placebo group, post-HFM F_{ENO} significantly increased in 9 of 9 subjects by approximately 4 ppb (range: 0.6-11.2) compared to pre-HFM. In the fish oil group, post-HFM F_{ENO} did not appreciably change compared to pre-HFM ($p>0.05$). In this group, 2 of 8 subjects demonstrated a

less than 1.0 ppb elevation in F_{ENO} compared to pre-HFM. The percent change in F_{ENO} from pre-HFM was significantly less in the fish oil group compared to the placebo group ($-1.99 \pm 3.97\%$ versus $25.7 \pm 35.93\%$ respectively). Figure 2 illustrates the significant association between blood triglycerides and F_{ENO} in the placebo group pre and post-HFM ($r=0.61$, $p<0.05$). However, the regression relating triglycerides and F_{ENO} for the fish oil group was not significant ($r=0.39$, $p=0.21$).

DISCUSSION

The primary findings of the present study are that omega-3 fatty acid supplementation has a post-prandial anti-inflammatory effect within the pulmonary system and that a significant relationship exists between blood triglycerides and airway inflammation. These findings are consistent with our first and second hypotheses that a single HFM increases airway inflammation and that its increase is associated with triglyceride levels. The third hypothesis was also well supported by the data. Three weeks of moderate omega-3 fatty acid supplementation via fish oil mitigated the airway inflammatory response following an “over-the-counter” HFM. Taken together these observations increase our understanding of the relationship between dietary fat intake and provide new insight into the anti-inflammatory effects of fish oil within the pulmonary system.

The findings of the present study extend those reported in previous investigations in our laboratory. Using F_{ENO} as a biological marker of airway inflammation, Rosenkranz et al. (2010) determined that a single HFM increases airway inflammation in non-asthmatic subjects. The present study both confirms these findings and extends them by demonstrating a significant

linear relationship between blood triglycerides and F_{ENO} . In addition, the present study demonstrates that this relationship is abolished when subjects are supplemented with dietary fish oil. These findings advocate that fish oil supplementation has positive implications in healthy individuals under dietary induced airway inflammation.

Many food substances induce inflammatory responses following consumption. High fat intake (particularly omega-6 fatty acids) is one such substance that can cause an inflammatory reaction and since the typical Western diet contains an omega-6/omega-3 ratio greater than 15/1 many individuals may be exposed to a pro-inflammatory diet (Simopoulos 2008; Simopoulos 2002). van Oostrom et al. (2004) has recently demonstrated that a single meal consisting of fresh cream elicits a mild inflammatory response (van Oostrom et al. 2004). Likewise, increases in triglycerides and glucose have been shown to be paralleled by the increased production of pro-inflammatory cytokines and oxidative stress (Eriksson et al. 2001; van Oostrom et al. 2003).

In adults and children, high margarine intake, a substance containing high amounts of omega-6 fatty acids, has been associated with an increased likelihood of pulmonary distress (Foliaki et al. 2008; Nagel and Linseisen 2005). Similarly, Rosenkranz et al. (2010) and the current study's placebo group demonstrate that a single HFM increases airway inflammation, but does not impact lung volumes or airway reactivity. While the mechanism defining the relationship between fat intake and airway inflammation is not well defined, one potential mediator may be the fatty acid content of inflammatory cells within the pulmonary system. A Western diet containing a high omega-6/omega-3 fatty acid ratio increases the percentage of arachidonic acid in the membrane of inflammatory cells and is linked to an increased incidence of asthma (Black

and Sharpe 1997; Simopoulos 2002; Simopoulos 2008). When present in high amounts arachidonic acid becomes the primary substrate used in the synthesis of bioactive mediators and cytokines (Calder 2001). Therefore, the HFM induced acute inflammatory response reported by Rosenkranz et al. (2010), Van Oostrom et al. (2004) and that of the placebo group in the current study may be the result of high arachidonic acid content in the pulmonary system. However, these conclusions are only speculative as the present study did not measure changes in the fatty acid content of systemic inflammatory or pulmonary cells.

Blood lipoprotein properties may also contribute to the HFM induced inflammation. Nichols et al. (2006) demonstrated that a high saturated fat meal decreases the anti-inflammatory properties of high-density lipoproteins (HDL) (Nicholls et al. 2006). Similarly, Patel et al. (2009) acutely induced hypertriglyceridaemia causing the formation of triglyceride-enriched HDL, which reduced HDL's anti-inflammatory properties (Patel et al. 2009). In the present study, the significant relationship between triglycerides and airway inflammation reveals that blood lipoproteins may explain in part the negative impact of high-fat intake on airway health.

Dietary supplementation with omega-3 fatty acids has a positive influence on inflammatory membrane composition and function. Two omega-3 fatty acids, EPA and DHA can replace arachidonic acid in the plasma membrane of inflammatory cells (Gibney and Hunter 1993; Sperling et al. 1993; Endres et al. 1989; Lee et al. 1985; De Caterina et al. 1994; Meydani et al. 1993; Mickleborough et al. 2006) and competitively inhibit arachidonic acid metabolism (Endres et al. 1989; Lee et al. 1985; Obata et al. 1999). Individuals experimentally supplemented with

fish oil capsules or fed a controlled high-fish diet demonstrate a decreased capacity to produce several inflammatory mediators (Caughey et al. 1996; Endres et al. 1989; Gallai et al. 1995; Kinsella et al. 1990; Lewis et al. 1990; Meydani et al. 1993; Sadeghi et al. 1999).

Dietary fish intake is associated with increased pulmonary function and decreased risk of asthma and asthma-like symptoms (Laerum et al. 2007; Miyamoto et al. 2007; Schwartz and Weiss 1994). Biltagi et al. (2009) demonstrated that 6 weeks of omega-3 fatty acid supplementation in asthmatic children significantly reduced sputum markers of airway inflammation with a concomitant improvement in lung function (Biltagi et al. 2009). Likewise, Mickleborough and colleagues (2003, 2006) determined that 3 weeks of dietary omega-3 fatty acid supplementation decreases the severity of exercise-induced bronchoconstriction (EIB) in both healthy and asthmatic athletes (Mickleborough et al. 2006; Mickleborough et al. 2003). Furthermore, lower pre- and post-exercise inflammatory markers in the omega-3 supplement group compared to placebo diets paralleled the changes in EIB severity. These findings of Biltagi et al (2009) and Mickleborough et al. (2003, 2006), in combination with the present study, demonstrate the broad effectiveness of omega-3 fatty acid supplementation on airway inflammation in the healthy and diseased lung when exposed to inflammatory stress.

Limitations

Previous investigations report an association between airway inflammation and airway hyper-responsiveness in asthmatic patients (Hancox et al. 2007; Porsbjerg et al. 2009; Rasmussen et al.

2009). However, the current study did not evaluate airway hyper-responsiveness, only measurements of resting pulmonary function and airway impedance. Accurate assessment of airway hyper-responsiveness would have required the introduction of a spasmogen, such as Methacholine, saline, or exercise. In addition, dietary habits were not monitored prior to or during the supplementation period. These unmonitored and uncontrolled dietary habits prior to the study period may have contributed to the variability in baseline measurements of F_{ENO} and blood triglycerides reported in Table 1. However, participants were repeatedly instructed throughout the study period not to change their diet and were excluded from participation if they consumed fish oil within 2 months prior to the study. Similarly, the EPA and DHA contents of the fish oil supplementation were not verified prior to the study. Variability of the omega-3 fatty acid content may have contributed to the variability in F_{ENO} responses in the supplement group. The results of this study, therefore, are attributed to the dose of fish oil prescribed and not the specific EPA and DHA contents reported by the manufacture.

Blood triglycerides are increased by the consumption of both fats and carbohydrates. Abbasi et al. (2000) demonstrated that a 2-week low-fat, high-carbohydrate diet increases plasma triglycerides (Abbasi et al. 2000). These data suggest that the HFM used in the current study and that of Rosenkranz et al. (2010) may have been the result of a high carbohydrate intake (16 g carbohydrate per serving). However, Ahuja et al. (2009) reports that a single low-fat, high-carbohydrate meal does not significantly alter blood triglyceride levels (Ahuja et al. 2009). This data suggests that acute consumption of carbohydrates does not contribute to increased blood triglyceride levels and therefore the post-prandial triglyceride levels observed in the current study are attributed primarily to the fat content of the HFM.

Conclusion and Relevance

In conclusion, the present study demonstrates for the first time that omega-3 fatty acid supplementation mitigates the airway inflammatory response induced by a single HFM. The key implications of the present study are twofold. First, the current study in parallel with that of Rosenkranz et al. (2010) demonstrate that the healthy airway is sensitive to acute dietary fat intake. This information provides insight into the pulmonary inflammatory response, which is often only studied in clinical populations. Secondly, the results of the current study suggest that only short-term supplementation with a moderate (3000 mg/day) dose of capsulated fish oil is required to attenuate the HFM induced airway inflammatory response. Conversely, the HFM stimulus used in the current study had no influence on resting pulmonary function. These findings suggest that individuals chronically exposed to a high-fat diet may be at an increased risk for pulmonary inflammation, but that short-term dietary supplementation with fish oil may mitigate these responses. Future studies should focus on determining the potential beneficial effects of fish oil supplementation on chronic high-fat consumption and pulmonary health.

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Table 1 Baseline subject characteristics

	Placebo condition	Fish oil condition
Age, years	22 ± 2	23 ± 3
Stature, cm	179 ± 7	176 ± 8
Body mass, kg	78.3 ± 9.44	82.7 ± 11.4
Pulmonary function		
eNO, ppb	20.2 ± 10.7	14.8 ± 5.78
PEF, l s ⁻¹	9.35 ± 2.79	7.93 ± 1.63
FEF 25–75, l s ⁻¹	4.59 ± 0.88	3.64 ± 0.69*
FEV1, l	4.45 ± 0.69	4.05 ± 0.46
FVC, l	5.39 ± 0.78	5.26 ± 0.91
Impulse oscillometry		
Z5, kPa l ⁻¹ s ⁻¹	0.33 ± 0.12	0.36 ± 0.06
R5, kPa l ⁻¹ s ⁻¹	0.31 ± 0.12	0.34 ± 0.06
R20, kPa l ⁻¹ s ⁻¹	0.27 ± 0.08	0.27 ± 0.06
X5, kPa l ⁻¹ s ⁻¹	-0.10 ± 0.04	-0.11 ± 0.02
Resonance freq	11.4 ± 3.30	12.4 ± 3.97
Blood lipids		
TRG, mg dl ⁻¹	76.3 ± 40.6	119.8 ± 65.7
TC, mg dl ⁻¹	156 ± 22.2	177 ± 27.3

Values are mean ± SD

eNO exhaled nitric oxide, *PEF* peak expiratory flow, *FEV25–75* forced expiratory flow between 25 and 75 %, *FEV1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *FEV1/FVC* ratio of forced expiratory volume in 1 s to forced vital capacity, *Z5* total respiratory impedance, *R5* & *R20* resistive component of respiratory impedance at 5 and 20 Hz, respectively, *X5* reactive component of respiratory impedance at 5 Hz, *TRG* triglycerides, *TC* total cholesterol

* Significantly different from Placebo ($p < 0.05$)

Table 2 Pulmonary function tests

	Pre-HFM		Post-HFM	
	Placebo	Fish oil	Placebo	Fish oil
Pulmonary function				
eNO, ppb	18.2 ± 10.0	16.4 ± 8.07	22.0 ± 11.5*	15.7 ± 7.73
PEF, l s ⁻¹	9.25 ± 1.01	8.23 ± 1.53	9.04 ± 1.41	8.81 ± 2.55
FEF 25–75, l s ⁻¹	5.50 ± 3.28	3.87 ± 1.10	4.39 ± 0.96	3.78 ± 0.89
FEV1, l	4.45 ± 0.63	4.03 ± 0.64	4.35 ± 0.61	4.03 ± 0.54
FVC, l	5.43 ± 0.63	5.11 ± 0.84	5.31 ± 0.66	5.22 ± 1.11
Impulse oscillometry				
Z5, kPa l ⁻¹ s ⁻¹	0.32 ± 0.07	0.39 ± 0.16	0.23 ± 0.08	0.37 ± 0.12
R5, kPa l ⁻¹ s ⁻¹	0.31 ± 0.07	0.37 ± 0.15	0.28 ± 0.07	0.34 ± 0.10
R20, kPa l ⁻¹ s ⁻¹	0.26 ± 0.05	0.29 ± 0.08	0.24 ± 0.06	0.26 ± 0.07
X5, kPa l ⁻¹ s ⁻¹	-0.09 ± 0.02	-0.12 ± 0.07	-0.06 ± 0.08	-0.13 ± 0.06
Resonance freq	10.3 ± 2.37	12.6 ± 4.66	9.93 ± 2.16	13.0 ± 4.87
Blood lipids				
TRG, mg dl ⁻¹	74.9 ± 32.5	85.0 ± 27.6	119 ± 44.4*	165 ± 29.2*
TC, mg dl ⁻¹	162 ± 25.4	173 ± 43.7	162 ± 28.5	174 ± 58.1

Values are mean ± SD

Significantly different from Placebo ($p < 0.05$)

eNO exhaled nitric oxide, PEF peak expiratory flow, FEV25–75 forced expiratory flow between 25 and 75 %, FEV1 forced expiratory volume in 1 s, FVC forced vital capacity, FEV1/FVC ratio of forced expiratory volume in 1 s to forced vital capacity, Z5 total respiratory impedance, R5 & R20 resistive component of respiratory impedance at 5 and 20 Hz, respectively, X5 reactive component of respiratory impedance at 5 Hz, TRG triglycerides, TC total cholesterol

* Significantly different from Pre-HFM ($p < 0.05$)

Figure 1

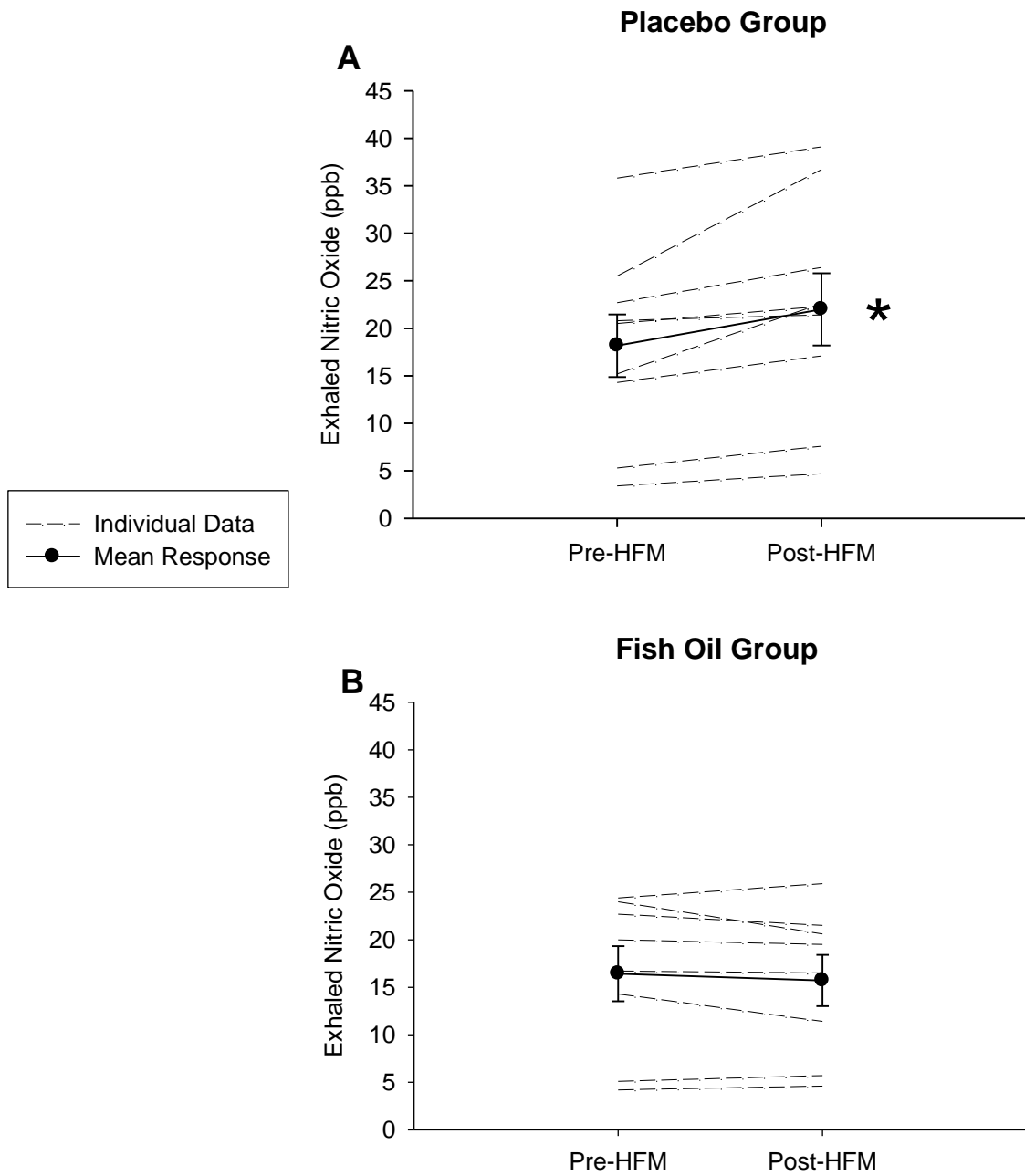


Fig. 1 Absolute changes in fractional exhaled nitric oxide pre- and post-HFM in placebo (**a**) and supplement group (**b**). *Dashed lines* represent individual data and *filled circles* represent the group mean. Fractional exhaled nitric oxide (airway inflammation) increased ~4 ppb in the placebo group after HFM vs. ~-1 ppb in the fish oil group. *Asterisks* significantly different than pre-HFM ($p < 0.05$). Data presented as mean \pm SE

Figure 2

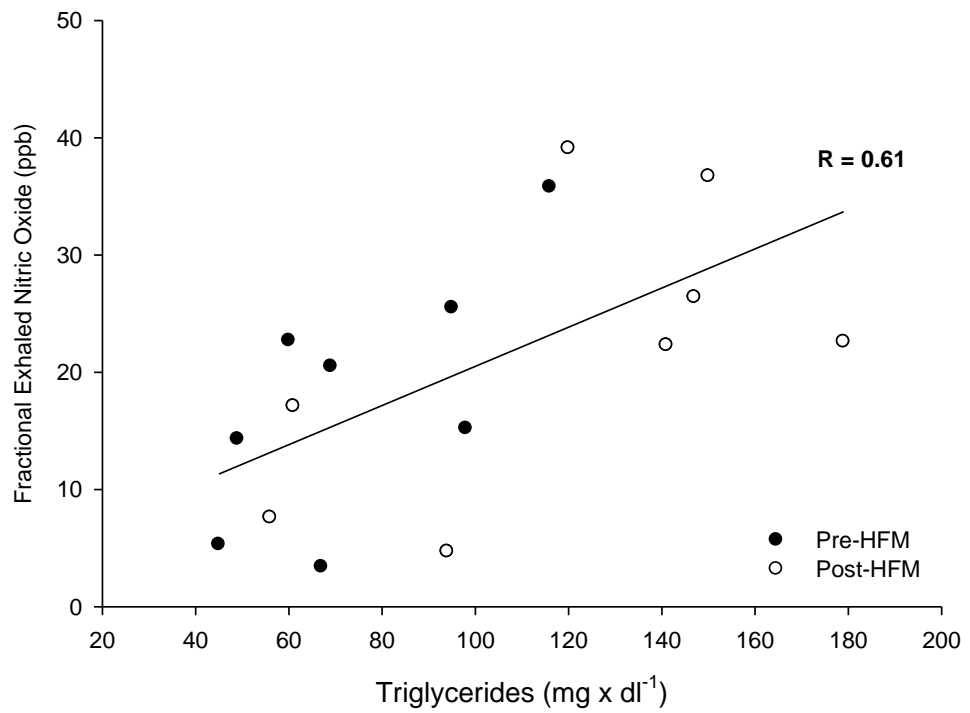


Fig. 2 Relationship between blood triglycerides and fractional exhaled nitric oxide pre and post-HFM ($p < 0.05$)