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Fish Oil Blunts Lung Function Decrements Induced by Acute Exposure to Ozone in Young Healthy Adults: A Randomized Trial

Hoa Chen

Haiyan Tong

Wan Shen
Bowling Green State University, wanshen@bgsu.edu

Tracey S. Montilla

Martin W. Case

See next page for additional authors

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Fish oil blunts lung function decrements induced by acute exposure to ozone in young healthy adults: A randomized trial

Hao Chen [a], Haiyan Tong [b], Wan Shen [a,c], Tracey S. Montilla [b], Martin W. Case [b], Martha A. Almond [d], Heather B. Wells [d,e], Neil E. Alexis [d,e], David B. Peden [d,e], Ana G. Rappold [b], David Diaz-Sanchez [b], Robert B. Devlin [b], Philip A. Bromberg [d,f], James M. Samet [b,*]

[a] Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States
[b] Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Chapel Hill, NC, United States
[c] Department of Public and Allied Health, Bowling Green State University, Bowling Green, OH, United States
[d] Center for Environmental Medicine, Asthma and Lung Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
[e] Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
[f] Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

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ABSTRACT

Background: Over one-third of the U.S. population is exposed to unsafe levels of ozone (O_3). Dietary supplementation with fish oil (FO) or olive oil (OO) has shown protection against other air pollutants. This study evaluates potential cardiopulmonary benefits of FO or OO supplementation against acute O_3 exposure in young healthy adults.

Methods: Forty-three participants (26 ± 4 years old; 47% female) were randomized to receive 3 g/day of FO, 3 g/day OO, or no supplementation (CTL) for 4 weeks prior to undergoing 2-hour exposures to filtered air and 300 ppb O_3 with intermittent exercise on two consecutive days. Outcome measurements included spirometry, sputum neutrophil percentage, blood markers of inflammation, tissue injury and coagulation, vascular function, and heart rate variability. The effects of dietary supplementation and O_3 on these outcomes were evaluated with linear mixed-effect models.

Results: Compared with filtered air, O_3 exposure decreased FVC, FEV_1, and FEV_1/FVC immediately post exposure regardless of supplementation status. Relative to that in the CTL group, the lung function response to O_3 exposure in the FO group was blunted, as evidenced by O_3-induced decreases in FEV_1 (Normalized CTL −0.40 ± 0.34 L, Normalized FO −0.21 ± 0.27 L) and FEV_1/FVC (Normalized CTL −4.67 ± 5.0 %, Normalized FO −1.4 ± 3.18 %) values that were on average 48% and 70% smaller, respectively. Inflammatory responses measured in the sputum immediately post O_3 exposure were not different among the three supplementation groups. Systolic blood pressure elevations 20-h post O_3 exposure were blunted by OO supplementation.

Conclusion: FO supplementation appears to offer protective effects against lung function decrements caused by acute O_3 exposure in healthy adults.

1. Introduction

Tropospheric ozone (O_3) is a major constituent of photochemical smog that is formed by the reaction of sunlight, nitrogen oxides and volatile organic compounds from vehicular and industrial emissions (World Health Organization, 2018). Despite some improvements over the last 40 years (EPA, 2021), more than 120 million Americans continue to live in areas that experience O_3 levels that exceed the National Ambient Air Quality Standard of 70 ppb (American Lung Association, 2021).

Epidemiological studies have shown that exposure to ambient O_3 levels is associated with elevated cardiopulmonary morbidity and mortality (Ji et al., 2011; Lim et al., 2019a; Yin et al., 2017). Controlled human exposure studies have shown that inhalation of O_3 can acutely

* Corresponding author at: Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, U.S. Environmental Protection Agency, 104 Mason Farm Rd, Chapel Hill, NC 27514, United States.
E-mail address: Samet.James@epa.gov (J.M. Samet).

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induce dose-dependent pulmonary function decrements and a transient infiltration of polymorphonuclear neutrophils (PMN) into the airway (Arjomandi et al. 2018; Balmes et al., 1996; EPA, 2013; Samet et al., 2001). Ozone-induced pulmonary effects are believed to be primarily mediated by stimulation of intraepithelial nociceptive vagal C-fibers via activation of transient receptor potential (TRP) A1 cation channels, while the neutrophilic airway inflammation involves activation of NFκB and generation of pro-inflammatory mediators in the surface macrophages and epithelial cells (Bromberg, 2016).

Although acute ozone-induced pulmonary effects have been well documented, more research is warranted to investigate potential mitigation strategies. Since O_3 induces oxidative stress, supplementation with antioxidant nutraceuticals has been proposed as a potential intervention against O_3 toxicity (Samet et al. 2001; Tong 2016). A previous chamber exposure study of adults showed that 2-week supplementation with an antioxidant mixture (250 mg of vitamin C, 50 IU of α-tocopherol, and 12 oz of vegetable cocktail) alleviated ozone-induced reductions in forced vital capacity (FVC) and forced expiratory volume at the end of the first second (FEV_1), but did not modify the increases in airway neutrophils and interleukin-6 (IL-6) (Samet et al., 2001). Unsaturated fatty acids are another possible antioxidant option to mitigate O_3 toxicity because of the reductive potential of their carbon–carbon double bonds. Fish oil (FO) from marine sources is rich in omega-3 polyunsaturated fatty acids (PUFA), particularly in eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), each with multiple carbon–carbon double bonds (Albert et al. 2013). In addition, EPA and DHA are precursors of specialized pro-resolving mediators (SPM) that promote resolution of inflammation and a return to homeostasis (Serhan and Levy, 2018). Olive oil (OO) is a principal component of the Mediterranean diet that is high in oleic acid (18:1), an omega-9 monounsaturated fatty acid. Oleic acid and polyphenols in OO have antioxidant properties that can offer cardiovascular benefits (Moreno-Luna et al., 2012; Perona et al., 2006). Previous studies showed that dietary supplementation with FO or OO can reduce adverse health impacts of exposure to air pollutants, especially to fine particulate matter ($PM_{2.5}$) (Lin et al., 2019; Tong et al., 2012; Tong et al., 2015). Snow and colleagues have found vasoprotective effects and alleviation of cardiac dysfunction by FO or OO enriched diets in rats exposed to 800 ppb O_3 (Snow et al., 2018; Tong et al., 2020). However, no study has specifically investigated the potential health benefits of FO or OO against controlled O_3 exposure in humans.

In this chamber study, we assessed the acute respiratory and

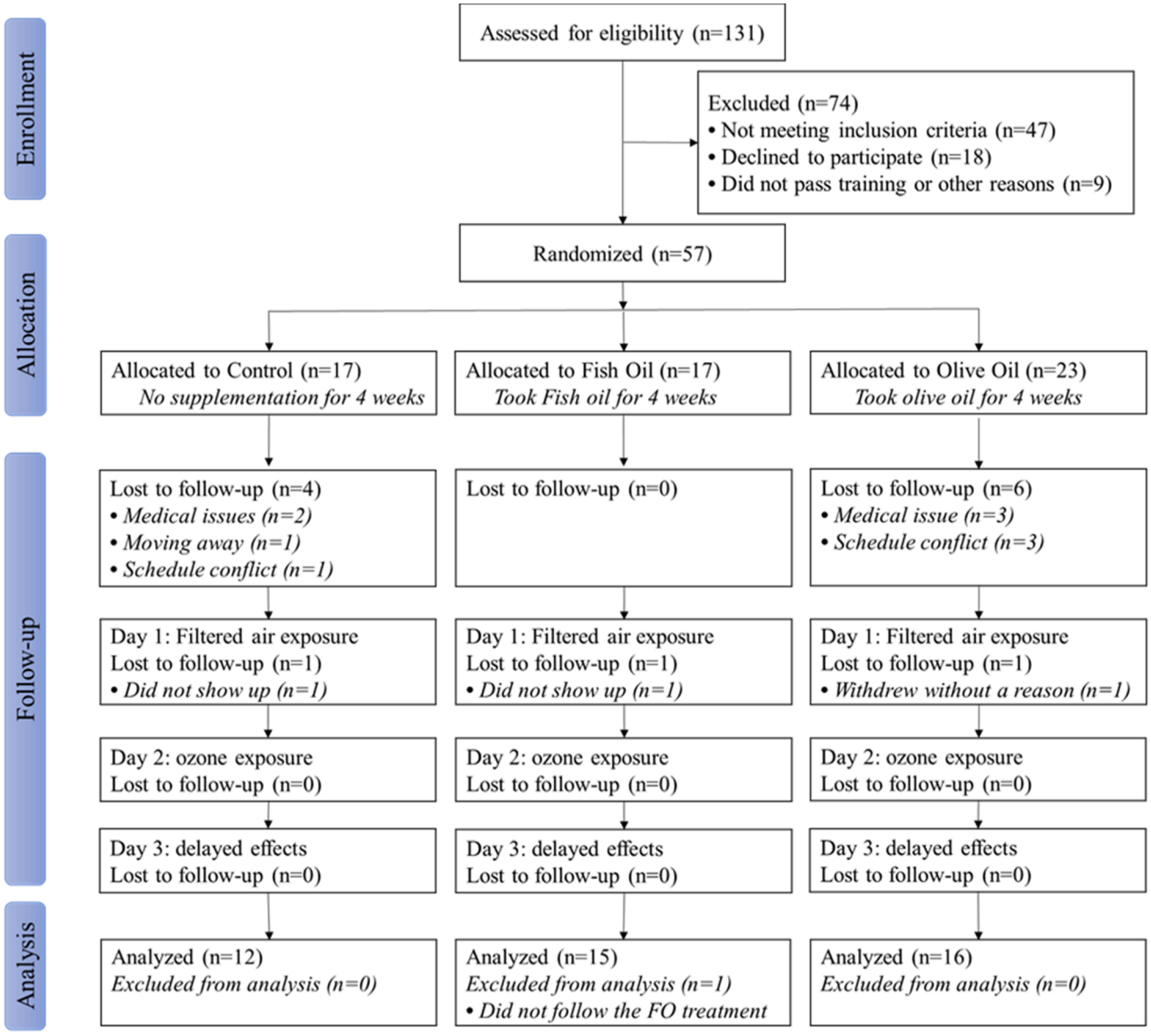

Fig. 1. CONSORT flow diagram for the study.

cardiovascular effects of controlled exposure of young healthy adults to 300 ppb O_3, and investigated whether dietary supplementation with FO or OO can mitigate them. We report that a 4-week regimen of FO supplementation may significantly blunt lung function decrements induced by an acute exposure to O_3.

2. Methods

2.1. Study participants

As shown in Fig. 1, healthy participants, residing in the Research Triangle Area of Central North Carolina, U.S., were recruited to enroll in the study based on the following recruiting criteria: 18–35 years old; body mass index (BMI) between 19 and 30; normal lung function (FVC, FEV_1, and FEV_1/FVC $\geq$ 80% of predicted values); having no history of cardiovascular disease, uncontrolled hypertension ($\geq$140 systolic, $\geq$ 90 diastolic), pulmonary disease, cancer, diabetes, or active allergy; non-smokers for the past year; not taking omega-3 or omega-9 fatty acid (FA) supplements, antioxidant supplements (e.g. vitamin C, vitamin E, beta-carotene, and selenium), β-adrenergic receptor blockers or anti-inflammatory drugs [e.g. nonsteroidal anti-inflammatory drugs (NSAIDs)]. Omega-3 index (OmegaQuant, Sioux Falls, SD), a measurement of EPA and DHA in erythrocyte cell membrane (Harris and Polreis 2016), was used for confirmation of low background levels of omega-3 FA intake. Participants whose omega-3 index was 5% or lower were eligible. Enrolled participants were trained to perform moderate exercise (minute ventilation target was approximately 20 L/min/m^2 body surface area) on the ergometer (stationary bicycle or treadmill). Those who could not complete the exercise without exceeding age-defined heart rate limits due to their physical conditioning were disqualified. More inclusion and exclusion criteria are available in Supplemental Methods.

This study was conducted in the U.S. Environmental Protection Agency Human Studies Facility on the campus of University of North Carolina at Chapel Hill between December 2017 and March 2020. Written informed consent was given by all participants prior to enrollment. This protocol was reviewed and approved by the Institutional Review Board of the University of North Carolina at Chapel Hill (protocol 15–2960) and the U.S. Environmental Protection Agency Human Subjects Review Office, conducted under FDA IND 71,475 and listed in ClinicalTrials.gov (NCT03395119).

2.2. Study design

The first stage of the study was a randomized, double-blind dietary supplementation period (Fig. 2A). Specifically, eligible participants were randomly assigned to receive 3 g/day of FO, 3 g/day of OO, or no supplements (CTL) for 4 weeks. FO supplements consisted of 3 pills of commercially available 1-gram enteric-coated soft-gels (gelatin capsules) formulated to deliver approximately 684 mg omega-3 PUFA (410 mg EPA and 274 mg DHA) as ethyl ester (Pharmavite, LLC, San Fernando, CA). OO supplements consisted of three pills of 1 g of USDA organic certified, cold-pressed, extra virgin olive oil with 56–85% oleic acid content (Arista Industries, Inc., Wilton, CT). All enrolled participants were instructed to avoid foods rich in omega-3 or omega-9 fatty acids and refrain from using antioxidant supplements, β-adrenergic receptor blockers, or anti-inflammatory drugs for 6 weeks.

The controlled chamber exposures were conducted in the second stage of the study (Fig. 2B and 2C). After the 4-week supplementation, blood fatty acids profile and omega-3 index was assessed again to check

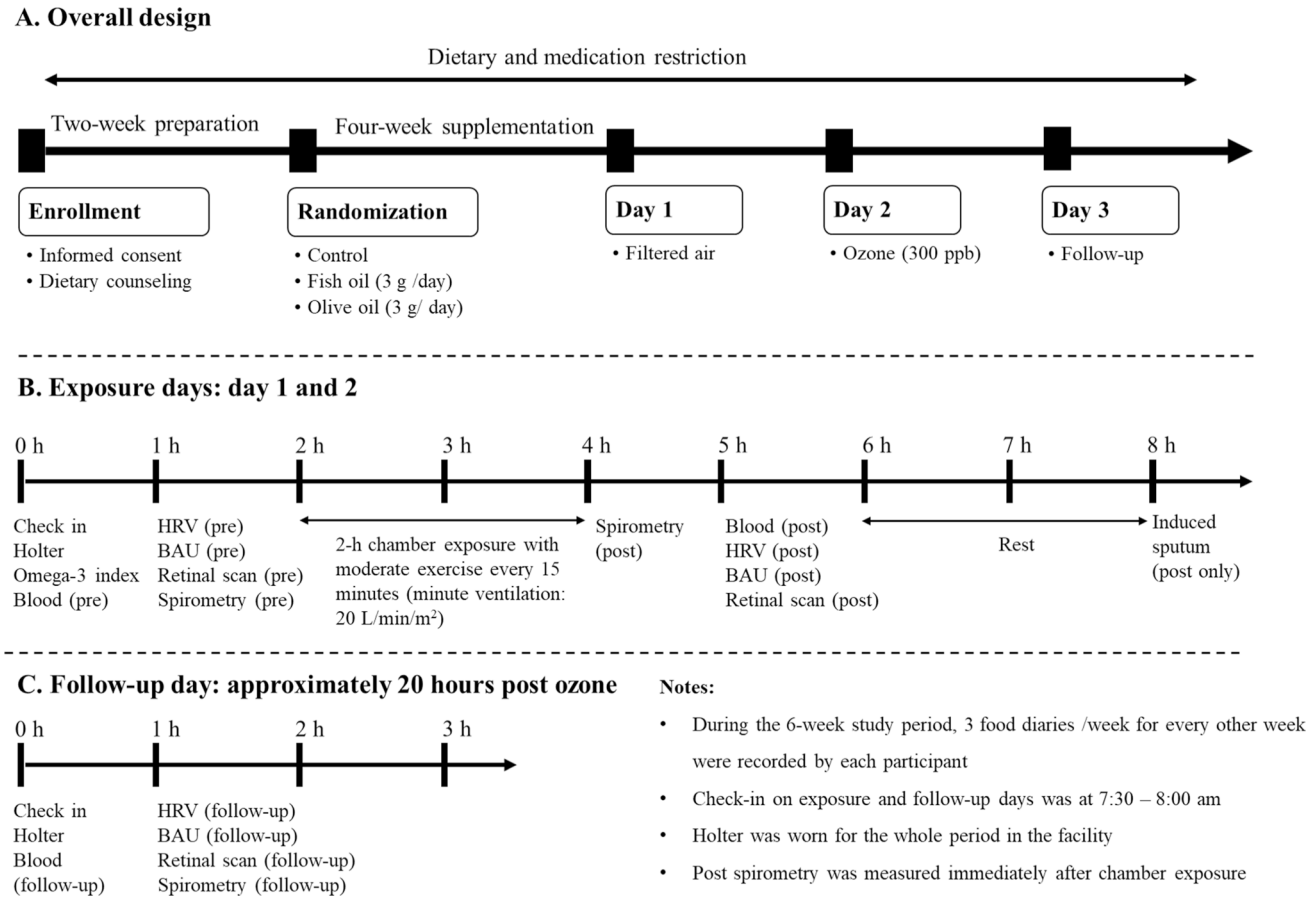

Fig. 2. Schematic representation of the study design. Overall study design (A) included both supplementation and exposure stages. Detailed tests / activities are listed in order they were performed for both exposure days (B) and the follow-up day (C). BAU: brachial artery ultrasound; HRV: heart rate variability.

if the dietary supplementation was successful. Participants were exposed for 2 h to filtered air (a sham consisting of 100 ppb O_3 was introduced for the first 5 min of the exposure) on the first day and to O_3 (mean concentration 300 ± 30 ppb) on the second day while exercising intermittently to a targeted ventilation (V_E) rate at 20 L/min/m^2 on an ergometer every other 15 min. Based on the O_3 levels employed in previous studies (Balmes et al. 1996; Devlin et al. 2012; Frampton et al. 2017; Samet et al. 2001), we chose 300 ppb as the O_3 exposure concentration in this study to induce transient decrements in lung function and self-limiting inflammatory responses in the airways of healthy human volunteers. The exposures were conducted on the same days of the week for all participants. Venous blood, retinal images, brachial artery ultrasound, spirometry, and Holter monitor heart rate variability were assessed before (pre), immediately after (post) the exposure, and on the follow-up day (approximately 20-h post O_3 exposure). Induced sputum samples were collected approximately 4-h following each exposure. Besides spirometry and induce sputum, more details in other parameter methods are available in Supplemental Methods.

2.3. Spirometry

Participants inhaled completely and then exhaled rapidly and completely via a mouthpiece connected by a tube into a spirometer (Vmax, Viasys Healthcare System, Yorba Linda, CA). From this maneuver, FVC, FEV_1 and the ratio FEV_1/FVC were derived. Three tests were performed and the best FVC value of the three were reported according to the guidelines of American Thoracic Society (Miller et al. 2005).

2.4. Induced sputum

Induced sputum samples were collected according to previously published methods (Hernandez et al. 2010). Briefly, participants inhaled increasing concentrations (3%, 4%, and 5%) of hypertonic saline for 7 min each. Manually selected sputum plugs were weighed and treated with 0.1% dithiothreitol for cell and mucus dispersion. Following centrifugation, differential cell counts were analyzed based on 400 cells, and expressed as a percentage of total non-squamous epithelial cells. Sputum samples contained a minimum of 120,000 total cells for analysis, a differential cell count containing<40% squamous epithelial cells, and cell viability of at least 50%, thus minimizing variability in cell recovery and squamous epithelial cell contamination.

2.5. Statistics

Differences in subject characteristics and descriptive data were analyzed using one-way ANOVA and Tukey's tests for continuous variables and Chi-square tests for categorical variables. Relative change values were reported for fatty acid levels of erythrocyte cell membrane (fold change: FO or OO / CTL) and for lung function parameters (percent change from pre-exposure or percent change from CTL). To control for day-to-day variability, all endpoints, except for immune cell count in the induced sputum, were normalized by subtracting the values measured before exposure from the immediately post and 20-hr post values (post – pre, follow-up – pre). SAS 9.4 software (Cary, NC) was used for statistical analysis. To assess changes in biological end points between the two exposures and among the CTL, FO and OO groups, we used a two-factor (exposure and supplementation status) mixed effects model on non-and log-transformed data with a participant-specific random intercept. Pairwise comparisons were adjusted using the Tukey-Kramer's tests and $p < 0.05$ was considered significant.

3. Results

3.1. Participant Characteristics

The study was concluded earlier than scheduled due to the COVID-19 pandemic. We screened a total of 131 volunteers for their eligibility to participate in this study and 57 were enrolled in the study. There were 17, 17, and 23 participants assigned to CTL, FO, and OO groups, respectively, but 5, 2, and 7, respectively, were further excluded because they either did not complete the study or did not follow the study instructions (Fig. 1). Final statistical analysis included 43 participants: 12, 15, and 16 participants are in the CTL, FO, and OO group, respectively (Fig. 1). At baseline, there were no statistically significant differences in age, body mass index (BMI), omega-3 index, blood pressure, heart rate, and lung function parameters between the groups (Table 1). At the time of study, all participants were non-smokers (one ex-smoker), and none were taking statins, angiotensin converting enzyme inhibitors, or β-adrenergic receptor blockers. Descriptive statistics of all assessed parameters are summarized in Supplemental Table 1 and 2.

3.2. Blood fatty acid levels

Before dietary supplementation, there were no statistically significant differences in the composition percentages of fatty acids in peripheral blood among the three groups (Table 2). After the 4-week supplementation, the levels of EPA were elevated approximately 6-fold (0.3% vs. 1.9%), docosapentaenoic acid (DPA) 1.2-fold (1.2% vs. 1.4%), DHA 1.5-fold (2.1% vs. 3.2%), total omega-3 fatty acids 1.8-fold (3.9% vs. 6.9%), and the total omega-3 index 1.8-fold higher in the FO group than those in the CTL (4.0% vs. 7.1%) (Table 2). In addition, arachidonic acid (AA), an omega-6 fatty acid, as well as total omega-6 fatty acids and omega-6/omega-3 ratio were all significantly lower in the FO group than those in the CTL group. Though not statistically significant, oleic acid in the OO group trended higher than in the CTL and FO groups.

3.3. Lung function

As shown in Fig. 3, exposure to ozone induced statistically significant decreases in lung function immediately after exposure, regardless of supplementation status. Specifically, immediately following O_3 exposure, the mean decrease in FVC was 5.9% (4.76L vs. 4.49L) in the CTL group, 3.4% (4.66L vs. 4.50L) in the FO group, and 2.3% (5.13L vs.

Table 1
Characteristics of the study participants at baseline.

Characteristics (n = 43)	CTL (n = 12)	FO (n = 15)	OO (n = 16)
Sex (male/female) (# of participants)	6/6	7/8	10/6
Age (years)	24.2 ± 4.5	26.6 ± 3.9	26.8 ± 3.9
Race/Ethnicity (# of participants)			
African-American	0	3	0
Asian	2	2	1
Caucasian	8	10	15
Hispanic	2	0	0
Non-smokers (# of participants)	12	15	16 *
BMI (kg/m^2)	24.4 ± 3.2	25.7 ± 3.1	25.5 ± 2.6
SBP (mmHg)	114.3 ± 6.8	117.5 ± 8.7	114.8 ± 10.4
DBP (mmHg)	75.9 ± 7.0	73.0 ± 7.1	69.5 ± 4.8
Heart rate (bpm)	61.8 ± 9.6	60.7 ± 12.4	59.8 ± 10.2
FVC (L)	4.8 ± 0.9	4.7 ± 0.7	5.1 ± 0.9
FEV_1 (L)	3.9 ± 0.7	3.8 ± 0.6	4.1 ± 0.7
FEV_1/FVC (%)	81.7 ± 7.4	80.9 ± 6.2	79.9 ± 5.1
Omega-3 index before supplementation (%)	4.2 ± 0.5	4.2 ± 0.5	4.1 ± 0.6

Data are presented as number of participants or mean ± SD. BMI, body mass index; CTL: control; DBP, diastolic blood pressure; FEV_1, forced expiratory volume at the end of the first second; FVC, forced vital capacity; FO: fish oil; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OO: olive oil; SBP, systolic blood pressure.
* 1 ex-smoker.

Table 2
Fatty acid composition and omega-3 index in red blood cell membrane before and after 4-week supplementation with FO or OO.

Fatty acid	CTL (n = 12)		FO (n = 15)		OO (n = 16)	
	Baseline	After	Baseline	After	Baseline	After
C16:0 palmitic acid (%)	21.1 ± 1.4	21 ± 0.9	20.8 ± 1.2	21.5 ± 1.2	20.9 ± 2.3	20.8 ± 1.1
C18:1 oleic acid (%)	18.2 ± 3.0	16.8 ± 1.9	18.2 ± 2.2	17.2 ± 2.0	19.1 ± 3.7	17.9 ± 2.0
C18:2 linoleic acid (LA)† (%)	25 ± 2.2	24.7 ± 1.8	24.7 ± 2.0	23.5 ± 1.7	24.4 ± 2.4	25.7 ± 1.9
C18:3 linolenic acid (ALA)‡ (%)	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
C20:4 arachidonic acid (AA)† (%)	11.2 ± 1.5	12.1 ± 1.5	11.5 ± 1.7	10.7 ± 1.6 *	10.8 ± 1.9	10.9 ± 1.1
C20:5 eicosapentaenoic acid (EPA)‡ (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	1.9 ± 0.9 *	0.3 ± 0.1	0.3 ± 0.1
C22:5 docosapentaenoic acid (DPA)‡ (%)	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.4 ± 0.2 *	1.1 ± 0.2	1.0 ± 0.2
C22:6 docosahexaenoic acid (DHA)‡ (%)	2.1 ± 0.4	2.1 ± 0.2	2.2 ± 0.5	3.2 ± 0.6 *	2.1 ± 0.4	2.0 ± 0.4
Total omega-3 fatty acids (%)	4.1 ± 0.4	3.9 ± 0.3	4.1 ± 0.5	6.9 ± 1.4 *	4 ± 0.5	3.8 ± 0.5
Total omega-6 fatty acids (%)	40.5 ± 2.3	41.2 ± 2.2	40.5 ± 1.5	37.8 ± 1.8 *	39.5 ± 3.0	40.9 ± 1.9
Omega-6 / Omega-3 ratio	10 ± 0.9	9.7 ± 3.3	9.9 ± 1.2	5.7 ± 1.3 *	10.1 ± 1.1	10.9 ± 1.3
Omega-3 index (%)	4.2 ± 0.5	4.0 ± 0.2	4.2 ± 0.5	7.1 ± 1.6 *	4.1 ± 0.6	4.0 ± 0.4

Data are presented as mean ± SD. * statistical difference from the control (CTL) after 4-week dietary supplementation, $p < 0.05$. Less-abundant fatty acids that did not differ among the groups are not shown. † omega-6 fatty acid. ‡ omega-3 fatty acid. FO: fish oil; OO: olive oil.

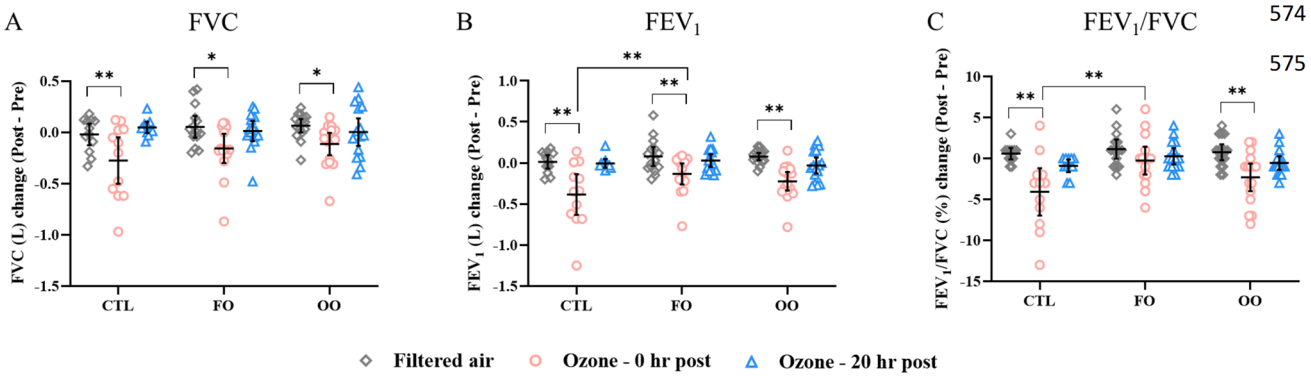

Fig. 3. Changes in spirometric measurements for participants in control (CTL), fish oil (FO), and olive oil (OO) groups. Shown are differences (Post – Pre) in FVC (A), FEV_1 (B), and FEV_1/FVC (C) on filtered air day, ozone day, and the follow-up day. Bars with whiskers indicate means with their respective 95% confidence interval. * $p < 0.05$, ** $p < 0.01$, statistically significant between the two conditions. FEV_1: forced expiratory volume at the end of the first second; FVC: forced vital capacity.

5.02L) in the OO group, compared with pre-O_3 exposure values; the mean FEV_1 was reduced by 10.2% (3.81L vs. 3.42L) in the CTL, 3.5% (3.69L vs. 3.55L) in the FO, and 5.5% (4.03L vs. 3.81L) in the OO participants; decreases in FEV_1/FVC ratio followed a similar pattern, 5.0% (80.83% vs. 76.75%), 0.3% (79.6% vs. 79.33%), and 3.0% (78.75% vs. 76.44%) reductions in the CTL, FO, and OO groups, respectively (Supplemental Table 1). The normalized FEV_1 ($p = 0.005$) and FEV_1/FVC ($p = 0.0004$) in the FO group were significantly higher than those in the CTL immediately post O_3 exposure (Fig. 3B and 3C). Similarly, the normalized FEV_1 in the OO group was borderline higher ($p = 0.096$) than that in the CTL immediately post O_3 exposure (Fig. 3B). In terms of the magnitude of the protective effect of the FO intervention, the O_3-induced loss of FEV_1/FVC ratio observed in the CTL group was significantly blunted by 70% (-4.67% vs. −1.40%, $p = 0.01$) (Table 3). While not statistically significant, the O_3-induced reduction in the FVC and FEV_1 in the CTL group was ameliorated by 19% (-0.26L vs. −0.21L, $p = 0.89$) and 48% (-0.40L vs. −0.21L, $p = 0.11$) by FO supplementation, respectively (Table 3). OO supplementation offer a non-statistically significant, 34% protection against O_3-induced loss in the FEV_1/FVC ratio (-4.67% vs. −3.06%, $p = 0.31$) (Table 3). Ozone-induced decrements in lung function were no longer observable on the follow-up day (20-hour post O_3 exposure). Analysis of log-transformed values generally showed similar results in support of protective effects of FO and OO (Supplemental Table 4).

One participant in the CTL group showed a stronger response to O_3, with lung function values that were 3 standard deviations from the mean. Specifically, this subject had a 22% drop in FVC (pre-O_3: 4.47 L, post-O_3: 3.5 L), a 34% decrease in FEV_1 (pre-O_3: 3.71 L, post-O_3: 2.46 L), and a 16% decrement in FEV_1/FVC (pre-O_3: 83%, post-O_3: 70%) immediately post O_3. The protective effect of FO supplementation remained after exclusion of this subject, showing that the O_3-induced decreases in normalized FEV_1 and FEV_1/FVC values were blunted by 34% ($p = 0.056$) and 62% ($p = 0.005$) in the FO group, respectively (Supplemental Fig. 1, Supplemental Table 3). No significant protective effects against O_3-induced lung function decrements were observable in the OO group after removing the outlier.

Table 3
Changes in spirometric measurements for participants in CTL, FO and OO supplementation groups shown as the differences in FVC, FEV1, and FEV1/FVC following ozone exposure corrected for the differences following filtered air exposure.

Outcome	CTL	FO	OO
	Mean (SD)	Mean (SD)	Mean (SD)
ΔΔ FVC (L)			
Immediately post ozone	−0.26 (0.26)	−0.21 (0.25)	−0.18 (0.26)
Follow-up	0.05 (0.17)	−0.04 (0.3)	−0.06 (0.26)
ΔΔ FEV_1 (L)			
Immediately post ozone	−0.40 (0.34)	−0.21 (0.27)	−0.30 (0.22)
Follow-up	−0.03 (0.11)	−0.05 (0.22)	−0.11 (0.19)
ΔΔ FEV_1/FVC (%)			
Immediately post ozone	−4.67 (5.00)	−1.40 (3.18) *	−3.06 (2.49)
Follow-up	−1.45 (1.21)	−0.87 (2.10)	−1.31 (1.74)

Note: Delta-delta values (ΔΔ) for each participant were calculated as: ($Post_{ozone}$ – Pre_{ozone}) – ($Post_{air}$ – Pre_{air}). * $p < 0.05$, statistical difference from control (CTL). FEV_1, forced expiratory volume at the end of the first second; FVC, forced vital capacity; FO: fish oil; OO: olive oil.

3.4. Airway inflammation

As shown in Fig. 4A, O3 caused significant increases in PMN% in the induced sputum samples that were approximately 2–3 times higher than when measured 4-hour post exposure to filtered air. Supplementation with FO or OO did not significantly modify the increase in PMN% observed after O_3 exposure. As expected, the increase in PMN% was accompanied by a decrease in the percent of macrophages in the induced sputum samples (Fig. 4B).

3.5. Systemic inflammation

Compared with filtered air exposure, white blood cell (WBC) numbers in blood were elevated in the OO group 2-hour post O_3 exposure, but not in the CTL or FO group (Fig. 5A). WBC levels significantly decreased on the follow-up day in all supplementation groups relative to those after filtered air exposure (Fig. 5A). Similarly, there were no significant increases in blood neutrophil concentration 2-hour post O_3 exposure, but significant decreases were noted on the follow-up day in all three groups (Fig. 5B). The blood neutrophil concentration in the OO group was significantly higher than that in the FO group 2-hour post O_3 exposure. O_3 exposure induced significant increases in plasma IL-6 concentrations in all three groups, but the elevations were not detected on the follow-up day (Fig. 5C). We did not observe notable changes in other markers of systemic inflammation (Supplemental Fig. 2).

3.6. Blood pressure and triglycerides

Systolic blood pressure (SBP) was not changed immediately post exposure to 300 ppb O_3; however, significant elevations in SBP were observed on the follow-up day in the CTL group. In contrast, there were no increases in SBP 20-hour post O_3 exposure in either FO or OO groups and OO significantly decreased SBP compared with that in the CTL 20-hour post exposure (Fig. 5D). In the CTL group, diastolic blood pressure (DBP) on the follow-up day was significantly higher than that of the air day; however, we did not observe any notable changes in DBP in either FO or OO groups (Fig. 5E). O_3 exposure did not induce any significant changes in blood triglyceride levels. However, the average triglyceride concentrations were lower in the FO group compared with those in both CTL and OO groups, with statistical significance observed immediately post O_3 (Fig. 5F).

We did not find any acute O_3 effects in other blood lipid levels (Supplemental Fig. 3), oxidative stress, injury, or coagulation markers (Supplemental Fig. 4), or vascular function in brachial artery ultrasound and retinal blood vessel measurements (Supplemental Fig. 5). There were no significant changes in heart rate variability or repolarization parameters immediately post exposure (Supplemental Figure 6 and 7). However, SDNN, pNN50, RMSSD, QTc, and heart rate were significantly changed on the follow-up day in all three groups. These effects could be due to circadian rhythm patterns, and we did not observe any modifying effects of FO or OO. Statistical analysis on log-transformed data showed very similar results to all outcome variables (Supplemental Table 1 and Supplemental Table 4).

4. Discussion

In this controlled exposure study of young healthy adults, we investigated the modulatory effects of FO or OO supplementation on the acute respiratory and cardiovascular effects of O_3 exposure. We report that fish oil may offer significant protection against O_3-induced decrements in lung function.

Exposure to ambient O_3 is known to induce acute lung function decrements and increases susceptibility to respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD) (Zhang et al. 2019). Previous controlled exposure studies have established that O_3 causes pulmonary effects including decreased lung function and increased airway neutrophilic inflammation (Arjomandi et al. 2018; Balmes et al. 1996; Frampton et al. 2017; Samet et al. 2001). O_3-induced lung function decrements have been reported as decreased FVC and FEV_1, with no significant change in FEV_1/FVC ratio (Arjomandi et al. 2018; Balmes et al. 1996; Frampton et al. 2017). We similarly observed O_3 exposure effects on FVC and FEV_1 with the addition of decreased FEV_1/FVC ratio. This disparity might be explained by the differences in age of the participants in this study compared to others. For example, the mean participants' age in the MOSES study was 55 years, with an inclusion criterion that specified that the FEV_1/FVC be $\geq$ 65% based on the NHANES III database (Arjomandi et al. 2018). In comparison, the age range of the participants in the present study was 18–35 years and the inclusion criterion for FEV_1/FVC was $\geq$ 80% of predicted values. It is possible that the FEV_1/FVC ratio in younger participants is more likely to be affected by ozone exposure. In this regard, it should be noted that the significant elevation in airway PMN is consistent with those in previous studies (Balmes et al. 2019; Frampton et al. 2017; Samet et al. 2001), demonstrating that the expected pulmonary responses to acute O_3 exposure in young healthy adults in the present study, and providing a suitable experimental setting in which to test the efficacy of FO and OO intervention.

The findings of this study indicate that dietary supplementation with FO and to a lesser degree OO, attenuated O_3 induced lung function effects, as evidenced by a 48% reduction in FEV_1 decrements and a 70%

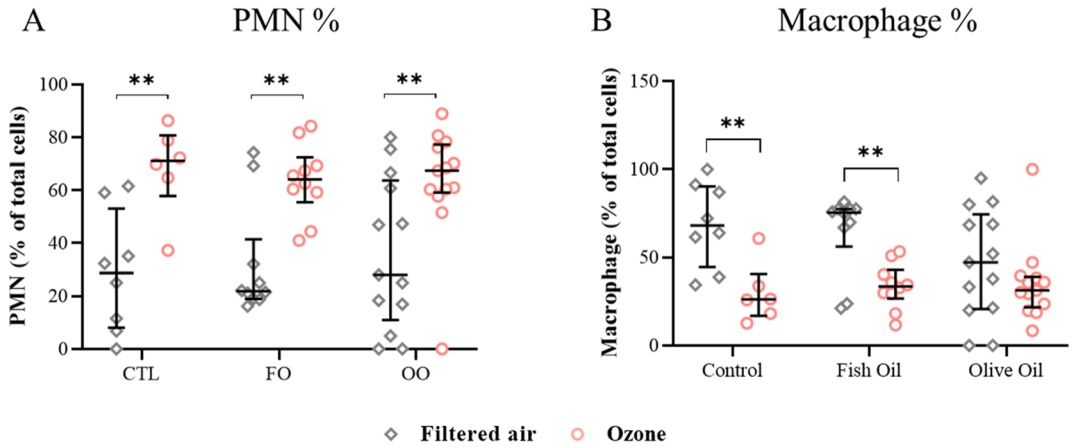

Fig. 4. Percentage of immune cells in the induced sputum in the control (CTL), fish oil (FO), and olive oil (OO) groups. Shown are percentage of total cells in PMN (A) and macrophages (B) after exposure to filtered air or ozone. Bars with whiskers indicate means with their respective 95% confidence interval. ** $p < 0.01$, statistically significant between the two conditions. PMN: polymorphonuclear neutrophils.

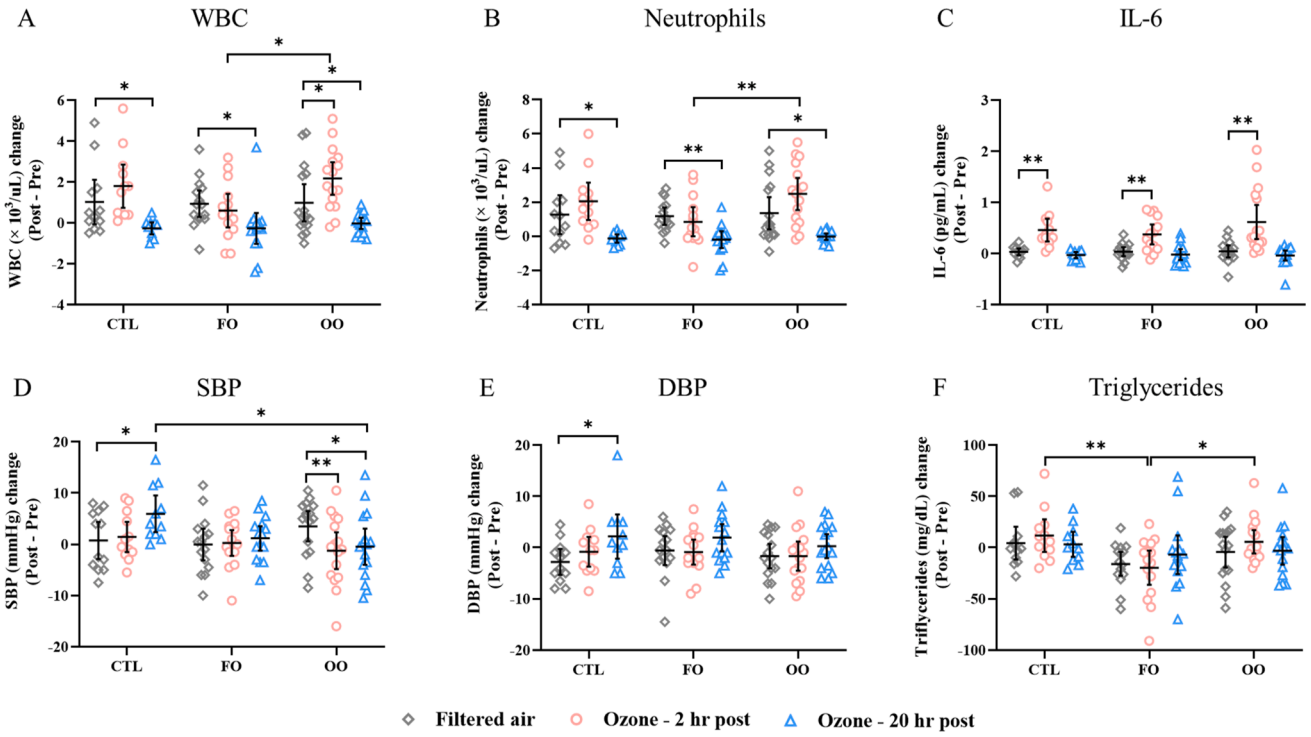

Fig. 5. Changes in systemic inflammation markers, blood pressure, and triglyceride levels for participants in control (CTL), fish oil (FO), and olive oil (OO) groups. Shown are differences (Post – Pre) in blood WBC (A), neutrophils (B), and plasma IL-6 (C), SBP (D), DBP (E), and triglycerides (F) on filtered air day, ozone day, and the follow-up day. Bars with whiskers indicate means with their respective 95% confidence interval. * $p < 0.05$, ** $p < 0.01$, statistically significant between the two conditions. DBP: diastolic blood pressure; IL-6: interleukin 6; SBP: systolic blood pressure; WBC: white blood cells.

smaller decrease of the FEV_1/FVC drop in the FO group compared with those in the CTL. It is noteworthy that these rescue effects of FO were still evident after excluding an outlier in the CTL group. However, this "outlier" participant showing the highest decrements in lung function parameters may be of biological relevance as a sensitive responder to the O_3 exposure. Variability in the sensitivity of lung function to ozone exposure has been documented in other human studies (Frampton et al. 1997). Interestingly, we only observed an "outlier" participant in the CTL group, but not in the FO or OO groups, indicating that FO and OO may have a general normalizing effect on the pulmonary function response to O_3-induced decrements. This speculation is also supported by the observation that the inter-subject variability in the ΔFVC, ΔFEV_1, and ΔFEV_1/FVC immediately post O_3 exposure in the FO and OO group was smaller compared with that in the CTL group. Moreover, although not statistically significant, linear regression analyses show an inverse correlation between blood omega-3 indices and levels of oleic acid and lung function decrements immediately post O_3 exposure (data not shown), suggesting an additional line of evidence in support of beneficial effects of high omega-3 PUFA and OO against O_3 exposure.

It should be noted that although we observed significant protective effects of FO supplementation against acute O_3 exposure, the absolute rescued values in FEV_1 and FEV_1/FVC are relatively small (0.19L and 3.27%) when considering the baseline values. However, for safety reasons, the participants in this study were young, healthy and carefully screened to be at low risk during their participation in this study, thus it is possible that more pronounced protective effects of FO may be observed in a susceptible population. Although not as robust as FO, OO supplementation may also offer protective effects against lung function decrements induced by O_3 exposure. One possible explanation is that the blood oleic acid levels after OO supplementation did not differ from those in the CTL, potentially masking benefits of oleic acid due to high background levels. Consistent with previous studies (Tong et al. 2012; Tong et al. 2015), we found that a 4-week dietary regimen of FO could significantly elevate blood levels of omega-3 PUFA but not oleic acid levels (which showed an increasing trend). This is likely due to the difficulty in controlling oleic acid content in the diet due to the growing presence of oleic acid-rich foods in the American diet (Guasch-Ferre et al. 2020). In addition, although both FO and OO can offer antioxidant properties through their carbon–carbon double bonds, one conjecture regarding the stronger protection of FO than OO may lie behind the multiple double bonds of omega-3 PUFA (EPA and DHA) as compared to the single double bond of oleic acid.

O_3 induced lung function decrements are believed to involve involuntary inhibition of inspiration, rather than bronchoconstriction. The inhibition may be mediated through activation of transient receptor potential (TRP) A1 cation channels by adduction of electrophiles such as 4-oxo-nonenal, formed through ozonation of unsaturated membrane fatty acids, leading to stimulation of intraepithelial nociceptive vagal C-fibers (Bromberg 2016). So how does omega-3 PUFA in the FO offer protection against O_3-induced lung function impairment through the autonomic nervous system? One possibility is that supplementation with FO results in elevated levels of omega-3 PUFA in the airway surface liquid, providing extracellular substrates with which O_3 can react, thereby sparing cellular membranes. On the other hand, ozonation of extracellular omega-3 PUFA generates potent electrophiles that activate pro-inflammatory pathways in the airway macrophages and epithelial cells, leading to a neutrophilic airway inflammation (Bromberg 2016). This might also explain why we observe elevated PMN% in the sputum samples regardless of supplementation status. It is worth noting that prolonged O_3 exposure may produce enough oxidative electrophiles, possibly tipping the scale from benefit to harm of omega-3 PUFA supplementation. We have observed such biphasic moderation of omega-3 PUFA on the association between ambient O_3 exposure and lung function parameters in a recent panel study (Tong et al. 2021).

In the present study, we found that O_3 exposure led to statistically significant reductions in WBC and neutrophil counts 20-hour post O_3 exposure, regardless of the supplementation status. This could be explained by the large influx of systemic neutrophils into the airways

initiated by the pro-inflammatory response to acute O_3 challenge in the lung. In addition, plasma IL-6 levels were significantly elevated 2-h post ozone exposure in all three dietary groups. O_3-induced IL-6 elevation had been reported in the bronchioalveolar lavage fluid but not in the circulation (Balmes et al. 2019; Samet et al. 2001). Nevertheless, IL-6 is a chemoattractant and plays an important role in the transition from a neutrophilic immune response to monocyte influx (Kaplanski et al. 2003). The shift of immune cell profile from granulocytic to monocytic mediated by IL-6 may reflect the fact that acute inflammation caused by O_3 inhalation is transient in nature.

Although previous controlled human exposure studies have mostly reported null effects of O_3 on blood pressure (Barath et al. 2013; Brook et al. 2009; Frampton et al. 2015; Rich et al. 2018), we observed elevated SBP and DBP 20-h post O_3 exposure among unsupplemented participants. Significant changes in blood pressure were not observable in the FO group and, interestingly, there were O_3-associated decreases in SBP in the OO group. The decreased blood pressure levels in participants supplemented with OO is consistent with findings from other studies (Massaro et al. 2020; Moreno-Luna et al. 2012). The findings that elevated blood pressure was only observed in the CTL group, indicate that FO and OO may promote reduced susceptibility to O_3-induced changes in blood pressure. In addition, plasma triglyceride levels in the FO group were significantly lower than those in both CTL and OO groups. This is consistent with the fact that icosapent ethyl, a highly purified ethyl ester of EPA, is currently an FDA-approved medication to treat hypertriglyceridemia (Bhatt et al. 2019).

Mechanisms of air pollution induced health impacts such as oxidative stress and inflammation are possible targets for dietary interventions (Tong 2016). Clinical studies have shown that nutraceuticals with antioxidant and anti-inflammatory properties including vitamin C, vitamin E, and omega-3 polyunsaturated fatty acids, or mixed vegetables may be effective against respiratory and cardiovascular effects caused by exposure to $PM_{2.5}$ or O_3 (Romieu et al. 2002; Samet et al. 2001; Tong et al. 2012; Tong et al. 2015). Observational studies also indicate that a dietary intake of beneficial fats may offer protection against adverse health effects of exposure to ambient air pollutants (Chen et al. 2021; Chen et al. 2022; Hansell et al. 2018; Lim et al. 2019b; Tong et al. 2021). The findings of the current study further reiterate the notion that a diet rich in unsaturated fatty acids may lower individual susceptibility to health impacts of exposure to air pollutants such as O_3.

One limitation of this study is the lack of a standard cross-over design in which the order of the exposures to filtered air and O_3 would be randomized. The fixed order of the exposures to air and O_3 was required to avoid carry over effects of the O_3 exposure which would have necessitated the introduction of a wash-out period during which some participants would have continued taking supplements, reducing the practical feasibility and potentially increasing the likelihood of loss-to-follow-up of the study participants. Another limitation is the relatively smaller-than-planned sample size due to early termination of the study resulting from the COVID-19 pandemic, and the restriction of the study population to young healthy adults, which may underestimate the potential benefits to the general population. However, the results of a retrospective power analysis conducted using the data from this study are consistent with the number of subjects in each group, arguing that the study was powered adequately. Nevertheless, small sample size can render study results prone to type II statistical errors, and it is possible that more pronounced effects would be observed with a larger sample size, especially if it were to include susceptible groups such as asthmatics.

5. Conclusion

The findings of this study show that the effect of acute O_3 exposure on pulmonary function in young healthy adults may be blunted by dietary supplementation with fish oil and olive oil. Dietary supplementation with beneficial oils may represent a safe and effective strategy to offer protection against the respiratory effects of acute exposure to O_3.

Author contributions

Conceptualization: J.M.S., H.T., W.S., M.A.A., N.E.A., D.B.P., D.D.S., and R.B.D.; Investigation: H.C., H.T., W.S., T.S.M., M.W.C., M.A.A., H.B. W., J.M.S.; Formal analysis: H.C., H.T., A.G.R., P.A.B., N.E.A., R.B.D., J. M.S.; Project administration: H.C., A.G.R., J.M.S.; Writing- original draft, Writing - review and editing: H.C., J.M.S.. All authors have contributed significantly to revising the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclaimer

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Availability of data and materials

The data presented in the current study will be made available in ScienceHub.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107407.

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