Lung Function and Short-Term Ambient Air Pollution Exposure
Differential Impacts of Omega-3 and Omega-6 Fatty Acids

Haiyan Tong[1], Siqi Zhang[2], Wan Shen[3,4], Hao Chen[3], Claudia Salazar[1], Alexandra Schneider[2], Ana G. Rappold[1], David Diaz-Sanchez[1], Robert B. Devlin[1], and James M. Samet[1]

[1]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, North Carolina; [2]Institute of Epidemiology, Helmholtz München, Neuherberg, Germany; [3]Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee; and [4]Food and Nutrition Program, Department of Public and Allied Health, Bowling Green State University, Bowling Green, Ohio

ORCID IDs: 0000-0003-3666-2033 (H.C.); 0000-0002-7696-0900 (A.G.R.).

Abstract

Rationale: Exposure to air pollution is associated with adverse respiratory effects. Polyunsaturated omega 3 (n-3) fatty acids (FAs) appear to attenuate the health effects of air pollution.

Objectives: This panel study evaluated whether n-3 FA intake and blood levels of polyunsaturated omega 6 (n-6) FAs can modulate the associations between respiratory effects and short-term exposure to ambient air pollution in healthy adults.

Methods: Sixty-two healthy adults were enrolled into either high or low n-3 FA groups on the basis of n-3 FA intake and erythrocyte n-3 FA concentrations. Low and high n-6 FA groups were dichotomized on the basis of blood n-6 FA levels. Participants underwent three to five testing sessions separated by at least 7 days. At each session, the forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and plasma markers of inflammation (IL-6 [interleukin-6]) and oxidative stress (ox-LDL [oxidized low-density lipoprotein]) were measured. Associations between the ambient ozone and fine particulate matter (PM) (PM with an aerodynamic diameter <2.5 μm [$PM_{2.5}$]) levels and the lung function and blood markers were assessed by using mixed-effect models stratified by FA levels.

Results: Average levels of ozone (40.8 ± 11.1 ppb) and $PM_{2.5}$ (10.2 ± 4.1 μg/m^3) were below national ambient air quality standards during the study period. FVC was positively associated with ozone at a lag of 0 days (lag0) in the high n-3 FA group, whereas the association was null in the low n-3 FA group (for an interquartile range increase in ozone of 1.8% [95% confidence interval (CI): 0.5% to 3.2%] vs. 0.0% [95% CI: −1.4% to 1.5%]); however, the association shifted to being negative at lag4 (−1.9% [95% CI: −3.2 to −0.5] vs. 0.2% [95% CI: −1.2% to 1.5%]) and lag5 (−1.2% [95% CI: −2.4% to 0.0%] vs. 0.9% [95% CI: −0.4% to 2.3%]). A similar pattern was observed in the low n-6 FA group compared with the high n-6 FA group (lag0: 1.7% [95% CI: 0.3% to 3.0%] vs. 0.5% [95% CI: −0.9% to 2.0%] and lag4: −1.4% [95% CI: −2.8% to 0.0%] vs. −0.5% [95% CI: −1.8% to 0.9%]). The associations between FEV_1 and ozone and between FVC and $PM_{2.5}$ also followed a similar pattern. Elevated ozone levels were associated with an immediate decrease in ox-LDL in the high n-3 FA group at lag0 (−12.3% [95% CI: −24.8% to 0.1%]), whereas there was no change in the low n-3 FA group (−7.5% [95% CI: −21.4% to 6.5%]) and there was a delayed increase in IL-6 in the high n-3 FA group compared with the low n-3 FA group (lag4: 66.9% [95% CI: 27.9% to 106.0%] vs. 8.9% [95% CI: −31.8% to 49.6%], lag5: 58.2% [95% CI: 22.4% to 94.1%] vs. −7.4% [95% CI: −48.8% to 34.0%], and lag6: 45.8% [95% CI: 8.7% to 82.9%] vs. −8.5% [95% CI: −49.7% to 32.6%]).

Conclusions: We observed lag-dependent associations between short-term ambient air pollutants and lung function that were differentially modulated by n-3 and n-6 FAs, suggesting that n-3 and n-6 FAs counteract the respiratory response to low levels of ambient air pollution in healthy adults.

Clinical trial registered with clinicaltrials.gov (NCT 02921048)

Keywords: lung function; air pollution; diet; oxidative stress; inflammation

(Received in original form July 1, 2021; accepted in final form November 19, 2021)

Supported by the U.S. Environmental Protection Agency Intramural Research Program. The research described in this article has been reviewed by the Center for Public Health and Environmental Assessment, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does the mention of trade names of commercial products constitute endorsement or recommendation for use.

Author Contributions: Drafting the work: H.T. and S.Z. All authors have substantial contributions to the conception or the design of the work, including the acquisition, analysis, and interpretation of data; critical revision and final approval of the version to be published; and agreement to be accountable for all aspects of the work.

Ann Am Thorac Soc Vol 19, No 4, pp 583–593, Apr 2022

DOI: 10.1513/AnnalsATS.202107-767OC
Internet address: www.atsjournals.org

Air pollution is a leading risk factor for morbidity and mortality around the world (1). Ambient ozone (O_3) and particulate matter (PM) have been associated with cardiopulmonary mortality and morbidity, decrements in lung function, and respiratory and systemic inflammation (2–11).

Implementation of air quality controls has reduced air pollution and improved human health. Interventional strategies at an individual level, including personal exposure reduction, behavioral change, and nutraceutical and pharmacological approaches, can further alleviate the adverse health effects of air pollution (6, 7). Among these interventional approaches, dietary supplementation with polyunsaturated omega 3 (n-3) fatty acids (FAs) has been shown to reduce PM-induced cardiovascular effects (12–16). However, research addressing the effects of n-3 FAs on the respiratory responses to air pollution is sparse. A cohort study showed that higher n-3 FA intake was associated with reduced asthmatic symptoms and reduced symptom severity in response to indoor fine PM (PM with an aerodynamic diameter $\leq$2.5 μm [$PM_{2.5}$]), whereas higher polyunsaturated omega 6 (n-6) FA intake amplified these health effects in children with asthma (17). Fish oil has been demonstrated to ameliorate $PM_{2.5}$-induced lung injury and inflammation in an animal model (18). However, another animal study found that fish oil increased pulmonary injury and inflammation in O_3-exposed rats (19). These studies assessed indoor air pollution or used animal models; thus, the role of FAs on low levels of ambient air pollution–induced respiratory effects in humans needs investigation.

Two main essential n-3 FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are important nutrients for human health through their antiinflammatory and antioxidant properties (20, 21). Western diets are typically deficient in n-3 FAs and abundant in n-6 FAs (22), resulting in a high n-6/n-3 ratio that is believed to promote the pathogenesis of cardiopulmonary and systemic inflammatory disease, whereas increased levels of n-3 FAs (i.e., a low n-6/n-3 ratio) has been shown to inhibit these processes (17, 22, 23). The present panel study was aimed at determining the modulating effects of n-3 and n-6 FAs on the association between respiratory responses and short-term ambient air pollution in healthy adults.

Methods

Study Subjects

Participants in this study were healthy adults aged 25–55 years who had been nonsmokers for at least 1 year; had no history of cardiopulmonary disease, diabetes, hypercholesterolemia, or active allergy; and were not receiving antiinflammatory medications or antioxidants. Participants were recruited from an area in close proximity to the U.S. Environmental Protection Agency Human Studies Facility (HSF) in North Carolina. All study participants gave informed consent. The institutional review board at the University of North Carolina–Chapel Hill and the U.S. Environmental Protection Agency approved the study.

As described previously (24), participants were enrolled into either a high or a low n-3 FA group on the basis of habitual n-3 FA intake and the erythrocyte n-3 index determined from the total FA content in blood (OmegaQuant) (25). High n-3 FA intake was defined as $\geq$3 g/wk of EPA + DHA for at least 6 months from seafood, fish oil, or other n-3 FA supplements, whereas low n-3 FA intake was set at $\leq$0.5 g/wk. An n-3 index of $\leq$4% or $\geq$5.5% was used for classification into a low versus high n-3 FA status (24). Potential participants with an n-3 FA intake between 0.5 and 3 g/wk and an n-3 index between 4% and 5.5% were disqualified. On the basis of the blood FA profile, low and high n-6 FA groups were dichotomized by below- and above-median levels of n-6 FAs from the enrolled participants.

Study Design

This panel study was conducted between October 2016 and September 2019 and was registered with clinicaltrials.gov (NCT 02921048). Participants were tested during three to five repeat sessions with at least a 7-day interval between subject visits to the HSF. Venous blood collection and lung function were measured at each session. To minimize periodic effects, each participant was always studied around the same time on the same day of the week.

Lung function was measured by using spirometry, which was performed on a 10.2-L dry seal digital spirometer (SensorMedics). The largest value from at least three sets of qualified data was selected for the forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as per American Thoracic Society guidelines (26). Lung function in all participants was measured by using one dedicated spirometer and was measured by the same technician to minimize variability. A portion of venous blood samples was stored at −80°C before biomarker analysis. Plasma levels of IL-6 (interleukin-6) and ox-LDL (oxidized low-density lipoprotein) were measured by using commercial kits.

Air Pollution and Meteorological Measurements

Hourly $PM_{2.5}$ and O_3 concentrations for the study period were obtained from a central air monitoring station (Millbrook) located approximately 44 km (27 miles) from the HSF. We calculated 24-hour average $PM_{2.5}$ and daily maximum 8-hour O_3 concentrations from hourly air pollutant data between 9:00 A.M. and 8:00 A.M. (the next day). The calculated $PM_{2.5}$ and O_3 concentrations were valid when at least 18 hourly values were available for the 24-hour period; otherwise, the exposure concentrations were considered to be missing. For days with missing air pollutant concentrations, we used data from a different central monitoring station (Durham Armory) located approximately 18 km (11 miles) from the HSF. Hourly air

Availability of data and materials: The data sets used during the current study are available from the corresponding author on reasonable request.

Correspondence and requests for reprints should be addressed to Haiyan Tong, M.D., Ph.D., Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, U.S. Environmental Protection Agency, 104 Mason Farm Road, Chapel Hill, NC 27514. E-mail: tong.haiyan@epa.gov.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

temperature, relative humidity, and nitrogen dioxide (NO_2) were also obtained from the Millbrook central monitoring station. We calculated the 24-hour average of meteorological variables and NO_2 concentrations by using the same method as that used for $PM_{2.5}$. Exposure levels on the current and previous 6 days (lag of 0 d [lag0] to lag6) were assigned to each measurement.

Statistical Analysis

We applied linear mixed-effect models (random intercepts for participants) with an interaction term between the air pollutant and FA groups to investigate associations between air pollution and the FVC, FEV_1, IL-6, and ox-LDL in each group and assess the between-group differences in effect estimates. Participants were dichotomized into low and high n-3 FA groups according to the screening process at enrollment, and the dichotomization of other FA groups was based on the blood FA levels below or above the median value. The models were adjusted for individual characteristics, including age, sex, race, marital status, education, and body mass index. We incorporated a penalized spline of time with 8 *df*/yr to control for long-term and seasonal trends and an indicator of the day of the week. Air temperature was adjusted by including separate terms of low and high temperatures (27). For low temperatures, we fitted a penalized spline of the average temperature on the current and previous 6 days (lag0–lag6) for days with a lag0–lag6 temperature lower than the median value; for high temperatures, we fitted a penalized spline of the average temperature on the current and previous day (lag0–lag1) for days with a lag0–lag1 temperature above the median value. In addition, we controlled for relative humidity by using a penalized spline of the lag0–lag6 relative humidity. The *df* of splines of temperature and relative humidity were chosen by using the generalized cross-validation criterion and were detailed in Table E1 in the online supplement. If the *df* selected by generalized cross-validation criterion equaled 1, a linear term of the meteorological variable was included in the model. Linear terms for daily O_3 and $PM_{2.5}$ were incorporated into the interaction with groups separately. For this study, we investigated the immediate effects of air pollution on the current day (lag0), the single-day lagged effects of 1–6 days (lag1–lag6), and the cumulative effects of 7 days (7-d moving average from lag0 to lag6 [lag06]).

Furthermore, we conducted several sensitivity analyses to test the robustness of the results. First, we built two-pollutant models by adding $PM_{2.5}$ and NO_2 separately to the model of O_3 and adding O_3 and NO_2 separately to the model of $PM_{2.5}$; two pollutants in the same model were of the same lag. Second, we changed the *df* of the spline of time trend to 7 *df*/yr. Third, we specified the autocorrelation structure of repeated measurements in the mixed-effect models as first-order autoregressive and spatial linear autocorrelation structures.

Effect estimates are presented as percent changes from the mean of the measured outcome with 95% confidence intervals (CIs) per each interquartile range (IQR) increase in O_3 and $PM_{2.5}$. All analyses were performed by using R (version 3.6.2, R Foundation for Statistical Computing) and the packages "gamm4" and "mgcv." Statistical significance was set at a two-sided $P < 0.05$ for the air pollution effects and a two-sided $P < 0.1$ for the interaction with FA groups.

Results

Participant Characteristics

There were no significant differences in individual characteristics between the high and low n-3 FA groups (Table 1). Fifty-six participants completed five sessions, three completed four, and the remaining three subjects attended three sessions. The numbers of outcome measurements across all sessions were 164 and 137 for the high and low n-3 FA groups, respectively (*see* Table E2 in the online supplement).

Ambient Air Pollution and Meteorological Measurements

Most 24-hour average $PM_{2.5}$ (98.6%) and maximum 8-hour O_3 (98.8%) concentrations during the study period were below the U.S. National Ambient Air Quality Standard for $PM_{2.5}$ and O_3 (35 μg/m^3 for $PM_{2.5}$ [24-h] and 70 ppb for O_3 [8-h], respectively) (Table 2). The average concentrations of $PM_{2.5}$ and O_3 were 10.2 ± 4.1 μg/m^3 and 40.8 ± 11.1 ppb, respectively. There was no significant difference between the air pollution levels to which the high and low n-3 FA groups were exposed (Table E3). The concentrations of O_3 and $PM_{2.5}$ were weakly correlated ($r = 0.16$).

Table 1. Participant characteristics at baseline

Characteristic	All (*N* = 62)	Low Omega 3 Group (*n* = 28)	High Omega 3 Group (*n* = 34)
Age, yr	38 ± 9	37 ± 8	40 ± 9
BMI, kg/m^2	24.6 ± 3.2	24.9 ± 3.3	24.4 ± 3.1
Sex			
Female	39 (62.9)	18 (64.3)	21 (61.8)
Male	23 (37.1)	10 (35.7)	13 (38.2)
Race			
White	45 (72.6)	19 (67.9)	26 (76.5)
African American	14 (22.6)	9 (32.1)	5 (14.7)
Asian	3 (4.8)	0 (0)	3 (8.8)
Marital status			
Single	26 (41.9)	13 (46.4)	13 (38.2)
Married	29 (46.8)	12 (42.9)	17 (50.0)
Separated/divorced	7 (11.3)	3 (10.7)	4 (11.8)
Education			
Graduate degree	25 (40.3)	9 (32.1)	16 (47.1)
College degree	31 (50.0)	16 (57.1)	15 (44.1)
High school/trade school	6 (9.7)	3 (10.7)	3 (8.8)
Smoking history			
Nonsmoker	54 (87.1)	22 (78.6)	32 (94.1)
Ex-smoker	8 (12.9)	6 (21.4)	2 (5.9)
Systolic blood pressure, mm Hg	111.3 ± 9.5	113.0 ± 8.8	109.9 ± 9.9
Diastolic blood pressure, mm Hg	70.4 ± 7.1	71.5 ± 6.7	69.5 ± 7.3

Definition of abbreviations: BMI = body mass index; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; SD = standard deviation.
Values are expressed as either the mean ± SD or the number (%). A low omega 3 intake was defined as ⩽0.5 g/week of EPA + DHA for at least 6 months and an omega 3 index ⩽4%; a high omega 3 intake was defined as ⩾3 g/wk of EPA + DHA for at least 6 months and an omega 3 index ⩾5.5%.

Table 2. Air pollutants concentrations and meteorological measurements during the study period (October 6, 2016, to September 5, 2019)

	Mean ± SD	Range	IQR	Spearman Correlation Coefficient			
				$PM_{2.5}$	O_3	NO_2	Temperature
$PM_{2.5}$, μg/m^3	10.2 ± 4.1	1.8 to 68.0	4.7	—	—	—	—
O_3, ppb)	40.8 ± 11.1	10 to 71	17	0.16	—	—	—
NO_2, ppb	5.3 ± 3.8	0.8 to 24.2	3.8	0.45	−0.13	—	—
Temperature, °C	16.5 ± 8.9	−8.6 to 31.1	15.2	−0.10	0.47	−0.42	—
Relative humidity, %	70.2 ± 15.6	30 to 100	22.2	−0.19	−0.46	−0.21	0.17

Definition of abbreviations: IQR = interquartile range; $PM_{2.5}$ = particulate matter with an aerodynamic diameter ≤2.5 μm; SD = standard deviation.

FA Profile

The mean levels of the n-3 index were 3.96% for the low n-3 FA group and 6.75% for the high n-3 FA group. Compared with those of the low n-3 FA group, the levels of total n-3 FAs, EPA, and DHA in the high n-3 FA group were significantly higher, whereas the levels of total n-6 FAs were significantly lower (Table 3). The n-6/n-3 ratio was significantly lower in the high n-3 group. The n-3 index was positively correlated with the total n-3 FAs, EPA, and DHA and negatively correlated with the n-6 FA concentration and n-6 FA/n-3 FA ratio (Figure E1).

Influence of the n-3 Index on the Association between Ozone and Lung Function

FVC was positively associated with O_3 concentrations at lag0 in the high n-3 FA group compared with the low n-3 FA group (for an IQR increase in O_3 of 1.8% [95% CI: 0.5% to 3.2%] vs. 0.0% [95% CI:−1.4% to 1.5%]; interaction *P* value [$P_{interaction}$] = 0.05). This association shifted to being negative at lag4 (−1.9% [95% CI: −3.2% to −0.5%] vs. 0.2% [95% CI: −1.2% to 1.5%]; $P_{interaction}$ = 0.02) and lag5 (−1.2% [95% CI: −2.4% to 0.0%] vs. 0.9% [95% CI: −0.4% to 2.3%]; $P_{interaction}$ = 0.01) (Figure 1A).

Table 3. Profile of fatty acids in whole blood

Fatty Acid	Low Omega 3 Group (*n* = 28)	High Omega 3 Group (*n* = 34)	*P* Value*
Omega 3 index, %	3.96 ± 0.15	6.75 ± 0.21	<0.001
Total omega 3 fatty acids, %	3.77 ± 0.12	6.58 ± 0.20	<0.001
EPA	0.34 ± 0.01	1.19 ± 0.08	<0.001
DPA	1.06 ± 0.05	1.35 ± 0.05	0.001
DHA	1.94 ± 0.10	3.58 ± 0.13	<0.001
ALA	0.41 ± 0.03	0.46 ± 0.04	0.61
Total omega 6 fatty acids, %	39.96 ± 0.53	36.66 ± 0.46	<0.001
AA	10.58 ± 0.31	10.20 ± 0.28	0.35
LA	25.37 ± 0.69	23.22 ± 0.39	0.03
GLA	0.32 ± 0.03	0.22 ± 0.01	0.001
DGLA	1.49 ± 0.06	1.19 ± 0.04	<0.001
Total saturated fatty acids, %	34.81 ± 0.47	35.66 ± 0.31	0.20
Adrenic acid	1.55 ± 0.11	1.26 ± 0.08	0.002
Palmitic acid	21.03 ± 0.24	20.99 ± 0.24	0.77
Stearic acid	11.44 ± 0.33	12.19 ± 0.19	0.12
Total monounsaturated fatty acids, %	20.65 ± 0.53	20.28 ± 0.49	0.55
Oleic acids	18.93 ± 0.55	18.59 ± 0.47	0.54
Ratio			
AA/EPA	32.68 ± 1.93	10.35 ± 0.95	<0.001
Omega 6/omega 3	10.94 ± 0.44	5.76 ± 0.20	<0.001

Definition of abbreviations: AA = arachidonic acids; ALA = α-LA; DGLA = dihomo-GLA; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; GLA = γ-LA; LA = linoleic acid; SE = standard error.
Values are expressed as mean ± SE. A low omega 3 intake was defined as ≤0.5 g/wk of EPA + DHA for at least 6 months and an omega 3 index ≤4%; a high omega 3 intake was defined as ≥3 g/wk of EPA + DHA for at least 6 months and an omega 3 index ≥5.5%.
**P* values for differences between groups were derived by using Kruskal-Wallis rank sum tests.

Similarly, FEV_1 was positively associated with O_3 at lag0 in the high n-3 FA group versus the low n-3 FA group (2.3% [95% CI: 0.5% to 4.2%] vs. 0.1% [95% CI: −2.0% to 2.2%]; $P_{interaction}$ = 0.09). But the association trended negative at lag3–lag5 in the high n-3 FA group. In contrast, no associations were observed in the low n-3 FA group (Figure 1B).

Influence of the n-3 Index on the Association between $PM_{2.5}$ and Lung Function

The association between FVC and $PM_{2.5}$ followed a similar pattern to that observed for O_3 in the high n-3 FA group, whereas a null association was seen in the low n-3 FA group (Table E4). Specifically, FVC was positively associated with $PM_{2.5}$ concentrations at lag0 (1.1% [95% CI: 0.1% to 2.1%]) and then transitioned to a negative association at lag3 (−1.5% [95% CI: −2.4% to −0.6%] vs. −0.4% [95% CI: −1.4% to 0.6%]) and lag4 (−1.3% [95% CI: −2.1% to −0.4%] vs. 0.3% [95% CI: −0.7% to 1.3%]) (Figure 2A). In the low n-3 FA group, FVC was only positively associated with $PM_{2.5}$ at lag0 (1.1% [95% CI: 0.0% to 2.2%]). The association between FEV_1 and $PM_{2.5}$ shifted from trending positive at lag0 to trending negative at lag4–lag5 in the high n-3 FA group (Figure 2B). No association was observed between FEV_1 and $PM_{2.5}$ in the low n-3 FA group.

Influence of Other FAs on the Association between Air Pollution and Lung Function

Similar to the impact of the n-3 index, the impact of total n-3 FAs, EPA, and DHA on the associations between lung function and O_3 and $PM_{2.5}$ followed a pattern that was similar to those seen in the high and low n-3 FA groups (Table E5). In particular, compared with the null association at low

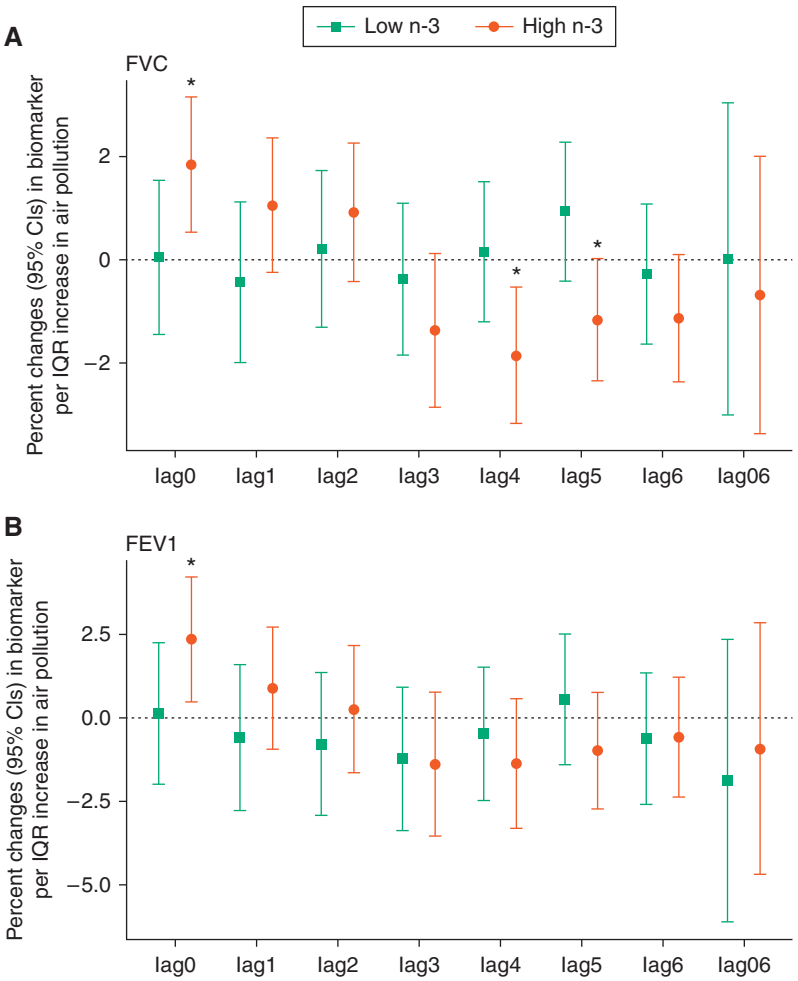

Figure 1. Influence of n-3 index on associations between ambient ozone concentrations and percent changes of (*A*) FVC and (*B*) FEV_1 in healthy adults. Effect estimates (95% CIs) were correlated with per-IQR increases in ozone concentrations and were adjusted for time trend, season, day of the week, temperature, relative humidity, age, sex, race, marital status, education, and body mass index. *Interaction *P* value < 0.1 for the difference in the effect estimate between the high and low n-3 fatty acid groups. CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; lag0 = lag of 0 days; lag06 = 7-day moving average from lag0 to lag6; n-3 = omega 3.

levels of total n-3 FAs, the association between FVC and O_3 was positive at high levels of total n-3 FAs at lag0 (1.8% [95% CI :0.5% to 3.1%] vs. 0.1% [95% CI: −1.4% to 1.6%]) and shifted to being negative at lag4 (−1.7% [95% CI: −3.1% to −0.4%] vs. 0.0% [95% CI: −1.4% to 1.3%]) and lag5 (−1.2% [95% CI: −2.4% to 0.0%] vs. 0.8% [95% CI: −0.5% to 2.1%]) (Figure 3A). FEV_1 was positively associated with O_3 at lag0 at high levels of total n-3 FAs (2.4% [95% CI: 0.5% to 4.2%]) compared with low levels of total n-3 FAs (−0.1% [95% CI: −2.2% to 2.1%]) (Figure 3B).

In contrast, the effects of n-6 FAs on the associations between lung function and air pollutants were opposite to those seen with n-3 FAs (Table E6). The association between FVC and O_3 was positive in the low n-6 FA group but null in the high n-6 FA group at lag0 (1.7% [95% CI: 0.3% to 3.0%] vs. 0.5% [95% CI: −0.9% to 2.0%]) and was negative at lag4 (−1.4% [95% CI: −2.8% to 0.0%] vs. −0.5% [95% CI: −1.8% to 0.9%]) (Figure 3A). The same was true for the association between FEV_1 and O_3, which was positive in the low n-6 FA group at lag0 (2.0% [95% CI: 0.0% to 4.0%]), whereas the association was null in the high n-6 FA group (0.8% [95% CI: −1.2% to 2.8%]) (Figure 3B). As expected, the findings for the n-6/n-3 ratio followed a pattern similar to that observed for the n-6 FAs (Table E6). FVC was positively associated with O_3 at lag0 for the low ratio of n-6 FAs to n-3 FAs, whereas there was no significant association for the high ratio of n-6 FAs to n-3 FAs (1.9% [95% CI: 0.5% to 3.2%] vs. 0.2% [95% CI: −1.3% to 1.6%]; $P_{interaction} = 0.07$) (Figure 4A). The association between FEV_1 and O_3 was positive for the low ratio of n-6 FAs to n-3 FAs at lag0 (2.2% [95% CI: 0.3% to 4.1%]) and then shifted downward at delayed lag days, whereas no significant association for the high ratio of n-6 FAs to n-3 FAs was shown (Figure 4B).

Association between Air Pollution and Oxidative Stress and Inflammation Markers

Ox-LDL was measured as a marker of systemic oxidative stress (28). The association between ox-LDL and O_3 was negative at lag0 (−12.3% [95% CI:−24.8%

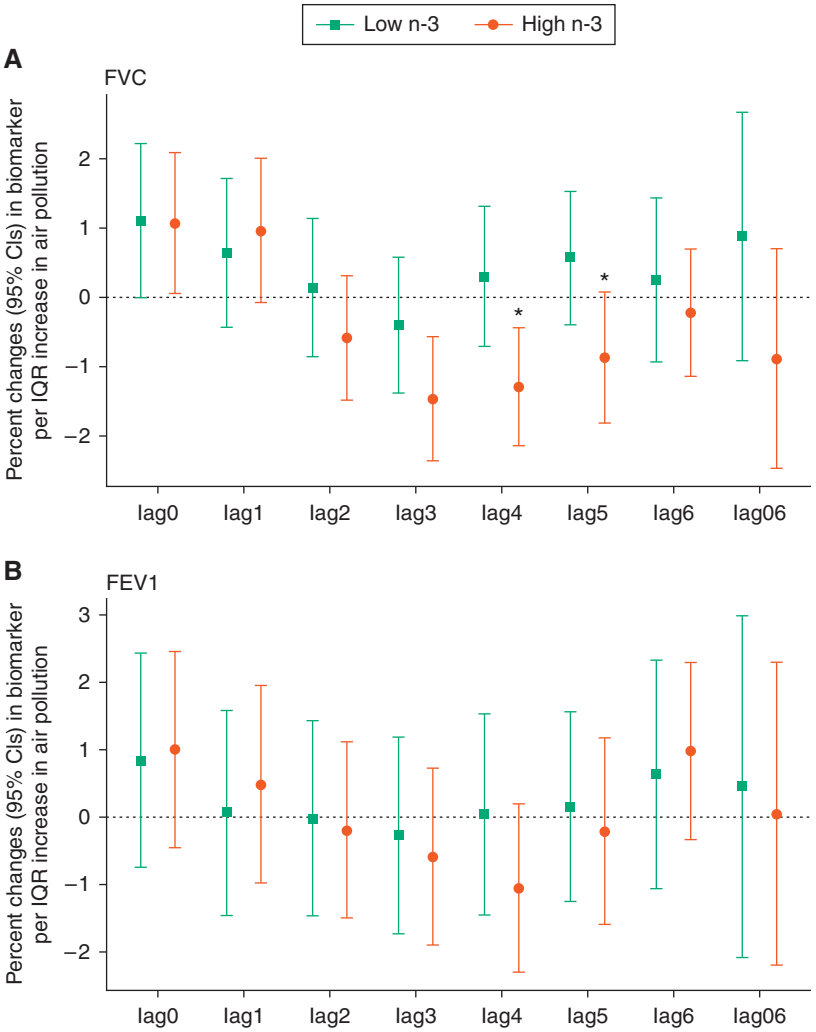

Figure 2. Influence of n-3 index on associations between concentrations of ambient particulate matter with an aerodynamic diameter ≤2.5 μm and the percent changes of (*A*) FVC and (*B*) FEV_1 in healthy adults. Effect estimates (95% CIs) were correlated with per-IQR increases in concentrations of ambient particulate matter with an aerodynamic diameter ≤2.5 μm and were adjusted for time trend, season, day of the week, temperature, relative humidity, age, sex, race, marital status, education, and body mass index. *Interaction *P* value < 0.1 for the difference in the effect estimate between the high and low n-3 fatty acid groups. CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; lag0 = lag of 0 days; lag06 = 7-day moving average from lag0 to lag6; n-3 = omega 3.

to 0.1%]) but was null at lag3–lag6 in the high n-3 FA group (Figure 5A). In the low n-3 FA group, the association between ox-LDL and O_3 was null at lag0 (−7.5% [95% CI: −21.4% to 6.5%]) and then shifted to being positive at lag4 (18.2% [95% CI: 5.4% to 31.0%]) (Figure 5A). There was no significant association between ox-LDL and $PM_{2.5}$ in the high n-3 FA group, but a positive association was seen at lag5 (11.5% [95% CI: 2.1% to 20.9%]) and lag06 (17.3% [95% CI: 0.7% to 34.0%]) in the low n-3 FA group (Table E7).

IL-6, one of the systemic markers of inflammation, was examined in detail. A positive association between IL-6 and O_3 was observed in the high n-3 FA group at lag4 (66.9% [95% CI: 27.9–106.0%]), lag5 (58.2% [95% CI: 22.4–94.1%]), lag6 (45.8% [95% CI: 8.7–82.9%]), and lag06 (117.0% [95% CI: 38.1–195.8%]), but no significant association was seen in the low n-3 FA group (Figure 5B). However, the association was negative at lag2 in the high n-3 FA group between IL-6 and O_3 as well as between IL-6 and $PM_{2.5}$ (Table E7).

The effect estimates generally remained stable in two-pollutant models (Tables E8 and E9) and in models with alternative adjustment for the time trend and autocorrelation structures (Tables E10 and E11).

Discussion

In this panel study, we investigated whether dietary habits with high n-3 FA intake can modulate the health effects from exposure to short-term ambient air pollution by examining the associations between air pollution and respiratory responses in participants with high and low n-3 FA levels. Interestingly, we observed a two-phased pattern of respiratory response in individuals with a high n-3 FA level: an immediate protective effect on the association between lung function and ambient O_3 and $PM_{2.5}$ at lag0, which shifted to a negative association at lag3–lag5. This pattern coincided with

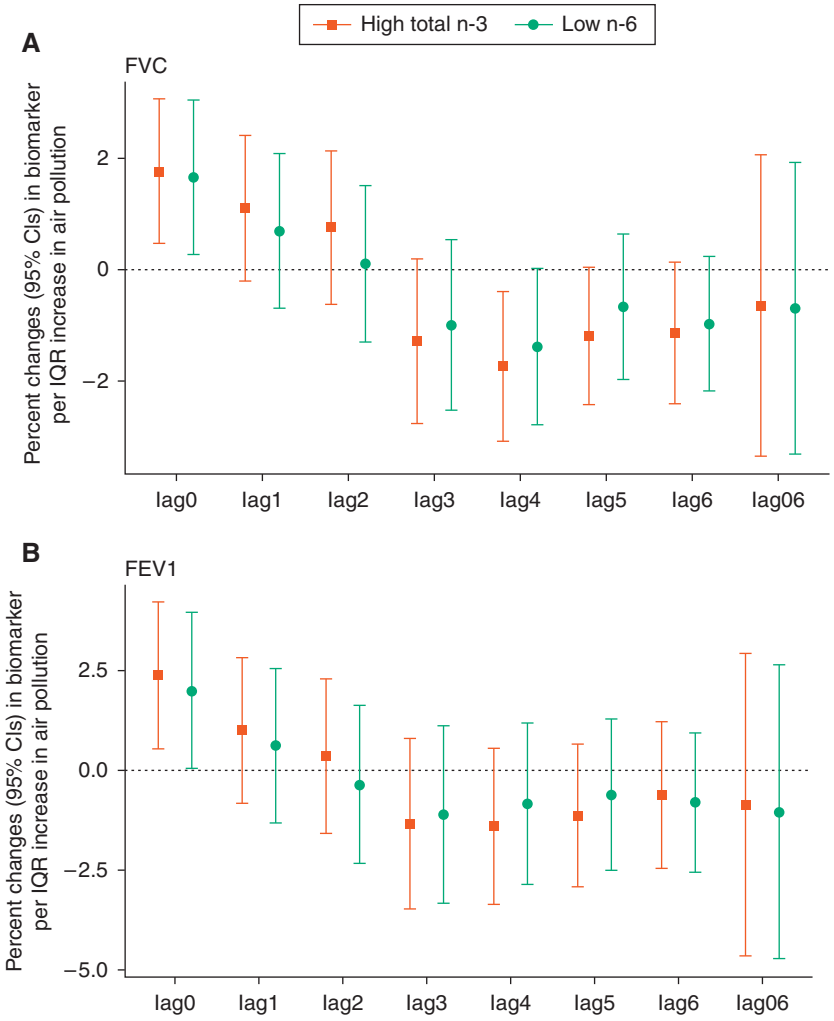

Figure 3. Influence of high total n-3 fatty acids and low n-6 fatty acids on the association between ambient ozone concentrations and the percent changes of (*A*) FVC and (*B*) FEV_1 in healthy adults. Effect estimates (95% CIs) were correlated with per-IQR increases in ozone concentrations and were adjusted for time trend, season, day of the week, temperature, relative humidity, age, sex, race, marital status, education, and body mass index. CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; lag0 = lag of 0 days; lag06 = 7-day moving average from lag0 to lag6; n-3 = omega 3; n-6 = omega 6.

decreased serum lipid oxidation at lag0 and a delayed onset of inflammation. The pattern was also observed in participants with low levels of n-6 FAs, suggesting that n-3 and n-6 FAs counteract the respiratory response to ambient O_3 or $PM_{2.5}$ exposure. To our knowledge, this investigation represents the first evidence of differential modulation of n-3 and n-6 FAs on the association between lung function and short-term exposure to low levels of ambient air pollution over the course of a few days in healthy adults.

The health effects of air pollution exposure are mediated through airway and systemic inflammation and oxidative stress (4, 5). O_3 and $PM_{2.5}$ are oxidants that generate reactive oxygen species and induce oxidative stress, leading to inflammation and cellular injury in the lungs (5). It is proposed that oxidative stress is the first response to air pollution exposure in humans, followed by a more delayed response in other variables (29). In support of this notion, we observed that plasma ox-LDL, a marker of systemic oxidative stress (28), was negatively associated with O_3 levels at lag0 in the high n-3 FA group, whereas the associations of FVC and FEV_1 with O_3 were positive, suggesting that the antioxidant properties of n-3 FAs counteract the initial oxidative stress presented by O_3. However, at subsequent lag days, the pattern of these associations shifted such that the negative association between ox-LDL and O_3 in the high n-3 FA group became null. Furthermore, the association between ox-LDL and O_3 shifted from being null to being positive in the low n-3 FA group. At the same time, the association between FVC and O_3 shifted from being positive to being negative in the high n-3 FA group. In addition, the association between IL-6 with O_3 reverted to being positive in the high n-3 FA group. We also observed similar albeit weaker effects associated with $PM_{2.5}$ exposure. Supporting these clinical observations, a previous animal study demonstrated that 8 weeks of fish oil supplementation worsened the pulmonary response to acute O_3 exposure and increased IL-6 in bronchoalveolar lavage fluid in rats (19). Nonetheless, these biphasic respiratory responses warrant further investigation.

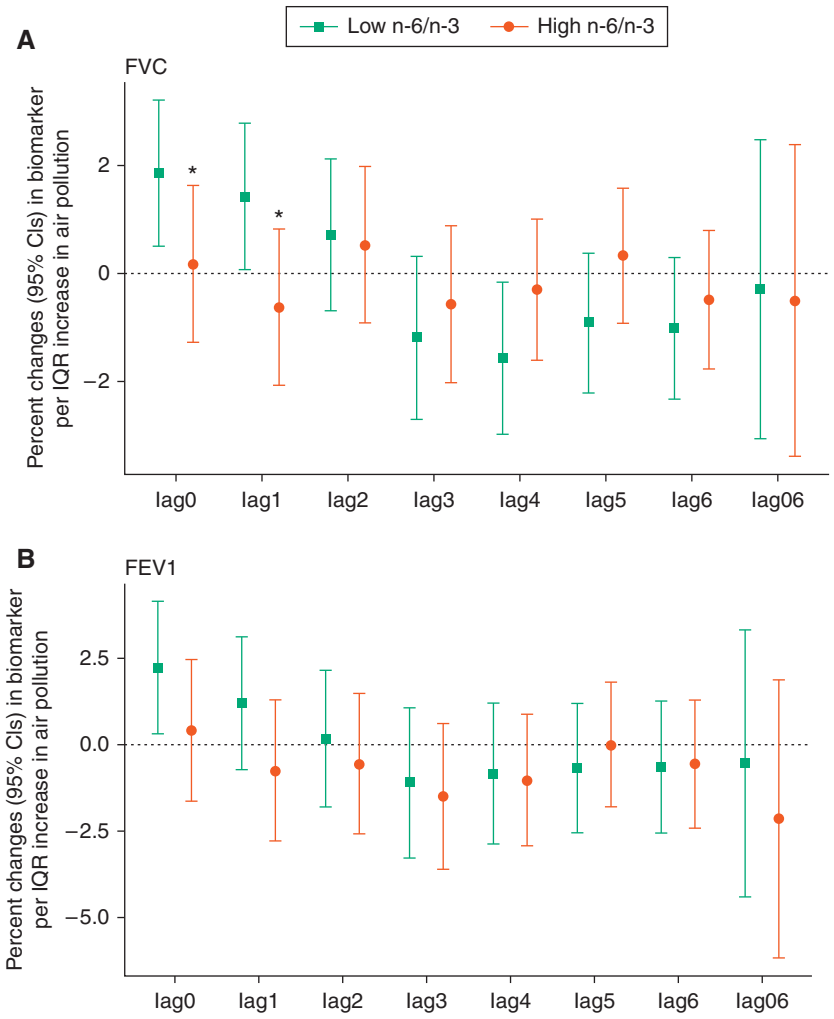

Figure 4. Influence of the ratio of n-6 fatty acids to n-3 fatty acids on the association between ambient ozone concentrations and the percent changes of (*A*) FVC and (*B*) FEV_1 in healthy adults. Effect estimates (95% CIs) were correlated with per-IQR increases in ozone concentrations and were adjusted for time trend, season, day of the week, temperature, relative humidity, age, sex, race, marital status, education, and body mass index. *Interaction *P* value < 0.1 for the difference in the effect estimate between groups. CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; lag0 = lag of 0 days; lag06 = 7-day moving average from lag0 to lag6; n-3 = omega 3; n-6 = omega 6.

The antioxidant effects of n-3 FAs, such as EPA, are attributed to their ability to scavenge reactive oxygen species associated with cellular membranes and lipoproteins by inhibiting free radical propagation through the multiple double bonds (30, 31). However, this antioxidant capacity wanes as the long hydrocarbon chain and double bonds are oxidized, leading to isomerization (31). Furthermore, the oxidation of the multiple double bonds in EPA and DHA (32) can produce highly reactive products such as aldehydes that are known to underlie pathological conditions (33). In support of this, an *in vitro* study has demonstrated that supplementation with EPA and DHA potentiates oxidative stress in response to a subsequent O_3 exposure in human airway epithelial cells (34). Therefore, we propose that a combined mechanism may contribute to the current findings. Initially, at lag0, the reactivity of n-3 FAs may offer protection against ambient O_3 and $PM_{2.5}$ exposure through scavenging action, serving to spare tissue targets from oxidation. However, any initial protection may become an exacerbation at lag3–lag5 through the accumulation of reactive products of the oxidation of n-3 FAs produced by exposure to O_3 and $PM_{2.5}$, leading to increased inflammation and contributing to neurogenic decrements in lung function. These findings imply a potential modulatory role for n-3 FAs in determining human susceptibility to the adverse health effects of air pollution exposure.

Antiinflammatory diets are high in n-3 FAs, whereas proinflammatory diets are associated with n-6 FAs (35). Variations in intake of n-6 and n-3 FAs ultimately determine the spectrum of downstream production of pro- and antiinflammatory mediators derived from these polyunsaturated FAs. It has been suggested that increased systemic inflammation is associated with reduced lung function (36). A clinical study demonstrated that a high intake of n-6 FAs is associated with a significant reduction in FEV_1, particularly among smokers in a population of Dutch adults (37). In the current study, we

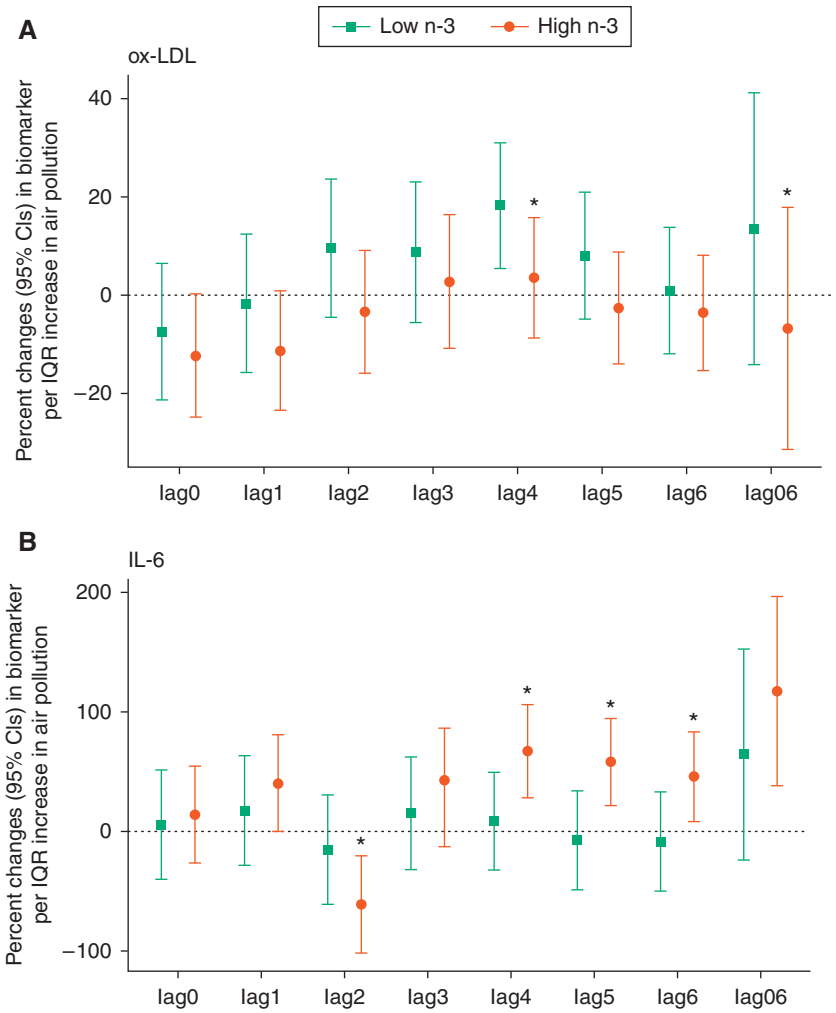

Figure 5. Influence of n-3 index on associations between ambient ozone concentrations and the percent changes of plasma (*A*) ox-LDL (oxidized low-density lipoprotein) and (*B*) IL-6 (interleukin-6) in healthy adults. Effect estimates (95% CIs) were correlated with per-IQR increases in ozone concentrations and were adjusted for time trend, season, day of the week, temperature, relative humidity, age, sex, race, marital status, education, and body mass index. *Interaction *P* value < 0.1 for the difference in the effect estimate between the high and low n-3 fatty acid groups. CI = confidence interval; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IQR = interquartile range; lag0 = lag of 0 days; lag06 = 7-day moving average from lag0 to lag6; n-3 = omega 3.

observed positive associations between FVC and O_3 for low levels of n-6 FAs and a low ratio of n-6 FAs to n-3 FAs at lag0, which resembled the effects of high n-3 FA levels, suggesting that n-3 and n-6 FAs counteract the pulmonary effects in response to ambient O_3 exposure.

There are a few limitations of this study. First, this study used central air monitors rather than personal monitors for air pollution data. This could have possibly introduced nondifferential exposure misclassification and biased the effects toward the null. Second, only the effects of $PM_{2.5}$ and O_3 were considered in this study, although other air pollutants, such as NO_2, may play a role. However, the two-pollutant model with adjustment for NO_2 suggested that the effects of $PM_{2.5}$ and O_3 were independent of NO_2. Finally, although we restricted dietary intake and medication usage during the study period, there could have been residual confounding from other unmeasured lifestyle factors (e.g., exercise, stress).

Conclusions

This study demonstrates that the association between short-term exposure to low levels of ambient air pollution and lung function is influenced by n-3 FAs, showing that the association is protective in the immediate response but transitions to being deleterious in a delayed response in healthy participants. This may be a reflection of the dynamic impact of n-3 FAs on the lung, from acting as antioxidants to acting as substrates for the generation of reactive products that lead to systemic oxidative stress and inflammation in response to ambient air pollution exposure. In addition, n-3 and n-6 FAs differentially modified the association between lung function and short-term ambient air pollution. These findings may have implications for human susceptibility, such that to achieve protection from dietary polyunsaturated FAs, it is essential to have a balanced intake of n-3 and n-6 FAs. The protection provided by n-3 FAs to the respiratory

system may be compromised by exposure to air pollution over time in healthy adults, even at low levels, further emphasizing the need to reduce n-6 FA intake. Given the growing interest in the role of n-3 FAs in the cardiovascular system, it is important to delve further into their role on lung function in the context of the health effects of air pollution exposure. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Drs. Urmila Kodavanti and Neil Alexis for their critical review and valuable suggestions. The authors thank Tracey Montilla, Julie Wood, Martin Case, Lisa Dailey, Joleen Soukup, and Shirley Harder in the Public Health and Integrated Toxicology Division at the U.S. Environmental Protection Agency for their excellent technical support. The authors thank the recruitment service from MPF Federal.

References

1 Cohen AJ, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, *et al.* Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet* 2017;389:1907–1918.
2 Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC, *et al.* Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. *Am J Respir Crit Care Med* 2011;183:1215–1221.
3 Orellano P, Reynoso J, Quaranta N, Bardach A, Ciapponi A. Short-term exposure to particulate matter (PM_{10} and $PM_{2.5}$), nitrogen dioxide (NO_2), and ozone (O_3) and all-cause and cause-specific mortality: systematic review and meta-analysis. *Environ Int* 2020;142:105876.
4 Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV, *et al.*; American Heart Association Council on Epidemiology and Prevention, Council on the Kidney in Cardiovascular Disease, and Council on Nutrition, Physical Activity and Metabolism. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation* 2010;121:2331–2378.
5 Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. *Occup Environ Med* 2003;60:612–616.
6 Carlsten C, Salvi S, Wong GWK, Chung KF. Personal strategies to minimise effects of air pollution on respiratory health: advice for providers, patients and the public. *Eur Respir J* 2020;55:1902056.
7 Rajagopalan S, Brauer M, Bhatnagar A, Bhatt DL, Brook JR, Huang W, *et al.*; American Heart Association Council on Lifestyle and Cardiometabolic Health; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Clinical Cardiology; Council on Cardiovascular and Stroke Nursing; and Stroke Council. Personal-level protective actions against particulate matter air pollution exposure: a scientific statement from the American Heart Association. *Circulation* 2020;142:e411–e431.
8 Arjomandi M, Balmes JR, Frampton MW, Bromberg P, Rich DQ, Stark P, *et al.*; MOSES (The Multicenter Ozone Study in Older Subjects). Respiratory responses to ozone exposure. *Am J Respir Crit Care Med* 2018;197:1319–1327.
9 Balmes JR, Arjomandi M, Bromberg PA, Costantini MG, Dagincourt N, Hazucha MJ, *et al.* Ozone effects on blood biomarkers of systemic inflammation, oxidative stress, endothelial function, and thrombosis: the Multicenter Ozone Study in Older Subjects (MOSES). *PLoS One* 2019; 14:e0222601.
10 Frampton MW, Balmes JR, Bromberg PA, Stark P, Arjomandi M, Hazucha MJ, *et al.* Multicenter Ozone Study in Older Subjects (MOSES): Part 1. effects of exposure to low concentrations of ozone on respiratory and cardiovascular outcomes. *Res Rep Health Eff Inst* 2017;(192, Pt 1):1–107.
11 Rich DQ, Frampton MW, Balmes JR, Bromberg PA, Arjomandi M, Hazucha MJ, *et al.* Multicenter Ozone Study in Older Subjects (MOSES): Part 2. effects of personal and ambient concentrations of ozone and other pollutants on cardiovascular and pulmonary function. *Res Rep Health Eff Inst* 2020;(192, Pt 2):1–90.
12 Lin Z, Chen R, Jiang Y, Xia Y, Niu Y, Wang C, *et al.* Cardiovascular benefits of fish-oil supplementation against fine particulate air pollution in China. *J Am Coll Cardiol* 2019;73:2076–2085.
13 Romieu I, Téllez-Rojo MM, Lazo M, Manzano-Patiño A, Cortez-Lugo M, Julien P, *et al.* Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. *Am J Respir Crit Care Med* 2005;172:1534–1540.
14 Tong H, Rappold AG, Diaz-Sanchez D, Steck SE, Berntsen J, Cascio WE, *et al.* Omega-3 fatty acid supplementation appears to attenuate particulate air pollution-induced cardiac effects and lipid changes in healthy middle-aged adults. *Environ Health Perspect* 2012;120: 952–957.
15 Tong H, Rappold AG, Caughey M, Hinderliter AL, Bassett M, Montilla T, *et al.* Dietary supplementation with olive oil or fish oil and vascular effects of concentrated ambient particulate matter exposure in human volunteers. *Environ Health Perspect* 2015;123:1173–1179.
16 Tong H. Dietary and pharmacological intervention to mitigate the cardiopulmonary effects of air pollution toxicity. *Biochim Biophys Acta* 2016;1860:2891–2898.
17 Brigham EP, Woo H, McCormack M, Rice J, Koehler K, Vulcain T, *et al.* Omega-3 and omega-6 intake modifies asthma severity and response to indoor air pollution in children. *Am J Respir Crit Care Med* 2019;199: 1478–1486.
18 Li J, Li H, Li H, Guo W, An Z, Zeng X, *et al.* Amelioration of $PM_{2.5}$-induced lung toxicity in rats by nutritional supplementation with fish oil and vitamin E. *Respir Res* 2019;20:76.
19 Snow SJ, Cheng WY, Henriquez A, Hodge M, Bass V, Nelson GM, *et al.* Ozone-induced vascular contractility and pulmonary injury are differentially impacted by diets enriched with coconut oil, fish oil, and olive oil. *Toxicol Sci* 2018;163:57–69.
20 Delmastro-Greenwood M, Freeman BA, Wendell SG. Redox-dependent anti-inflammatory signaling actions of unsaturated fatty acids. *Annu Rev Physiol* 2014;76:79–105.
21 Lee JH, O'Keefe JH, Lavie CJ, Marchioli R, Harris WS. Omega-3 fatty acids for cardioprotection. *Mayo Clin Proc* 2008;83:324–332.
22 Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002;56:365–379.
23 Wendell SG, Baffi C, Holguin F. Fatty acids, inflammation, and asthma. *J Allergy Clin Immunol* 2014;133:1255–1264.
24 Shen W, Weaver AM, Salazar C, Samet JM, Diaz-Sanchez D, Tong H. Validation of a dietary questionnaire to screen omega-3 fatty acids levels in healthy adults. *Nutrients* 2019;11:1470.
25 Harris WS, Von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? *Prev Med* 2004;39: 212–220.
26 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al.*; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
27 Stafoggia M, Samoli E, Alessandrini E, Cadum E, Ostro B, Berti G, *et al.*; MED-PARTICLES Study Group. Short-term associations between fine and coarse particulate matter and hospitalizations in Southern Europe: results from the MED-PARTICLES project. *Environ Health Perspect* 2013;121:1026–1033.
28 Lund AK, Lucero J, Harman M, Madden MC, McDonald JD, Seagrave JC, *et al.* The oxidized low-density lipoprotein receptor mediates vascular effects of inhaled vehicle emissions. *Am J Respir Crit Care Med* 2011;184:82–91.
29 Rajagopalan S, Al-Kindi SG, Brook RD. Air pollution and cardiovascular disease: JACC state-of-the-art review. *J Am Coll Cardiol* 2018;72: 2054–2070.
30 Mason RP, Jacob RF, Shrivastava S, Sherratt SCR, Chattopadhyay A. Eicosapentaenoic acid reduces membrane fluidity, inhibits cholesterol domain formation, and normalizes bilayer width in

atherosclerotic-like model membranes. *Biochim Biophys Acta* 2016; 1858:3131–3140.
31 Mason RP, Libby P, Bhatt DL. Emerging mechanisms of cardiovascular protection for the omega-3 fatty acid eicosapentaenoic acid. *Arterioscler Thromb Vasc Biol* 2020;40:1135–1147.
32 Porter NA, Caldwell SE, Mills KA. Mechanisms of free radical oxidation of unsaturated lipids. *Lipids* 1995;30:277–290.
33 Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chem Phys Lipids* 2009;157:1–11.
34 Corteselli EM, Gold A, Surratt J, Cui T, Bromberg P, Dailey L, *et al.* Supplementation with omega-3 fatty acids potentiates oxidative stress in human airway epithelial cells exposed to ozone. *Environ Res* 2020; 187:109627.
35 Sears B. Anti-inflammatory Diets. *J Am Coll Nutr* 2015;34:14–21.
36 Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax* 2004;59:574–580.
37 McKeever TM, Lewis SA, Cassano PA, Ocké M, Burney P, Britton J, *et al.* The relation between dietary intake of individual fatty acids, FEV_1 and respiratory disease in Dutch adults. *Thorax* 2008;63:208–214.