Dietary Supplementation with Olive Oil or Fish Oil and Vascular Effects of Concentrated Ambient Particulate Matter Exposure in Human Volunteers

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BACKGROUND: Exposure to ambient particulate matter (PM) induces endothelial dysfunction, a risk factor for cardiovascular disease. Olive oil (OO) and fish oil (FO) supplements have beneficial effects on endothelial function.

OBJECTIVE: In this study we evaluated the potential efficacy of OO and FO in mitigating endothelial dysfunction and disruption of hemostasis caused by exposure to particulate matter (PM).

METHODS AND RESULTS: Forty-two participants (58 ± 1 years of age) received either 3 g/day of OO or FO, or no supplements (naive) for 4 weeks prior to undergoing 2-hr exposures to filtered air and concentrated ambient particulate matter (CAP; mean, 253 ± 16 μg/m3). Endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery preexposure, immediately postexposure, and 20 hr postexposure. Levels of endothelin-1 and markers of fibrinolysis and inflammation were also measured. The FMD was significantly lower after CAP exposure in the naive (–19.4%; 95% CI: –36.4, –2.3 per 100 μg/m3 CAP relative to baseline; p = 0.03) and FO groups (–13.7%; 95% CI: –24.5, –2.9; p = 0.01), but not in the OO group (–7.6%; 95% CI: –21.5, 6.3; p = 0.27). Tissue plasminogen activator levels were significantly increased immediately after (11.6%; 95% CI: 0.8, 22.2; p = 0.04) and 20 hr after CAP exposure in the OO group. Endothelin-1 levels were significantly increased 20 hr after CAP exposure in the naive group only (17.1%; 95% CI: 2.2, 32.0; p = 0.03).

CONCLUSIONS: Short-term exposure to CAP induced vascular endothelial dysfunction. OO supplementation attenuated CAP-induced reduction of FMD and changes in blood markers associated with vasoconstriction and inflammation, suggesting that OO supplementation may be an efficacious intervention to protect against vascular effects of exposure to PM.

CITATION: Tong H, Rappold AG, Caughey M, Hinderliter AL, Bassett M, Montilla T, Case MW, Berntsen J, Bromberg PA, Cascio WE, Diaz-Sanchez D, Devlin RB, Samet JM. 2015. Dietary supplementation with olive oil or fish oil and vascular effects of concentrated ambient particulate matter exposure in human volunteers. Environ Health Perspect 123:1173–1179; http://dx.doi.org/10.1289/ehp.1408988

Introduction

Epidemiologic studies have demonstrated an association between exposure to ambient particulate matter (PM) at concentrations currently found in major metropolitan areas and a broad range of adverse cardiovascular outcomes (U.S. EPA 2009). A recent scientific statement from the American Heart Association concluded that short-term elevations in PM concentrations are capable of triggering acute coronary syndrome and stroke, worsening heart failure, and provoking arrhythmias among individuals with preexisting heart disease (Brook et al. 2010).

Ambient PM is a complex and variable mixture of particles of varying size that are classified into coarse (PM2.5–10; from 2.5 μm to 10 μm in aerodynamic diameter), fine (PM2.5, < 2.5 μm), and ultrafine (PM0.1; < 0.1 μm) particles. In the present study, research volunteers were exposed to PM2.5 particles concentrated from the air (CAP) in Chapel Hill, North Carolina. Short-term controlled human exposure to PM2.5 increases blood pressure (BP) and impairs endothelial function (Brook et al. 2009) in young healthy adults. Controlled exposure to diesel exhaust, a component of ambient PM, has been shown to affect fibrinolysis (Mills et al. 2005). A recent epidemiologic study has associated long-term exposure to PM with decreased endothelial function (Krishnan et al. 2012). Moreover, PM2.5 exposure in the Chapel Hill airshed was associated with immediate endothelial dysfunction in diabetic patients (Schneider et al. 2008). In addition, several controlled human exposure studies have suggested that air pollution exposure increases BP during (Brook et al. 2009; Urich et al. 2005) and immediately after (Mills et al. 2005) PM exposure, and ambient PM2.5 levels have been associated with higher BP in epidemiologic studies (Auchincloss et al. 2008). However, the mechanisms of these vascular effects associated with exposure to ambient PM are still not well understood.

Endothelial dysfunction is a critical early event in the development of atherosclerosis (Ross 1993). Endothelial dysfunction is defined as an imbalance between vasodilating and vasoconstricting substances produced by and acting on the endothelium. Endothelial dysfunction results in elevated expression of chemokines, cytokines, and adhesion molecules that promote smooth muscle cell growth, platelet and leukocyte adhesion, thrombosis, and vascular inflammation. Endothelial dysfunction induced by air pollution exposure has been implicated as an important mechanism in the development of cardiovascular diseases (Brook et al. 2010; Krishnan et al. 2013). Inflammation and oxidative stress mediate the adverse cardiovascular effects of air pollution exposure (Brook et al. 2010). Dietary supplements such as olive oil (OO) and fish oil (FO) have been shown to have antioxidant and anti-inflammatory effects (Moreno and Mitjavila 2003) that might offer protection against air pollution exposure. Consumption of OO, a principal component of the Mediterranean diet, has been shown to improve endothelial function and blood lipid profile (Zern and Fernandez 2005), decrease platelet aggregation (Delgado-Lista et al. 2010).

We thank J. Carter, M. Schmitt, and J. Wood for their excellent clinical and technical assistance in the execution of this study. We thank Pharmavite LLC (Mission Hills, CA) for providing the fish oil and olive oil supplements used in this study.

The U.S. Environmental Protection Agency Intramural Research Program supported this research. This project is supported in part by National Institutes of Health grant RR00946 to the UNC-Chapel Hill Clinical and Translational Research Center. J.H.B. is employed by TRC Environmental Corporation.

The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication. The contents of this article should not be construed to represent agency policy nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Supplemental Material is available online (http://dx.doi.org/10.1289/ehp.1408988).

Advance Publication: 1 November 2015.
et al. 2011), and reduce vascular inflammation (Esposito et al. 2004). In particular, some components of OO, such as polyphenols (Moreno-Luna et al. 2012; Zern and Fernandez 2005) and oleic acid (Perona et al. 2006), have been shown to have beneficial effects on endothelial function. Therefore, OO supplementation is a potential candidate for use as a protective intervention against the adverse vascular effects of PM exposure. Dietary supplementation with FO has been shown to improve endothelial function in patients who smoke tobacco or who have elevated insulin, glucose, or triglyceride levels (Duda et al. 2009a; Saravanan et al. 2010). However, the effects of OO and FO on vascular responses to air pollution exposure have not been examined specifically.

In the present study, we assessed the acute effects of controlled exposure to ambient air PM$_{2.5}$ on vascular endothelial function, blood pressure, and fibrinolysis and inflammation in healthy middle-aged human volunteers, and evaluated the efficacy of a 4-week regimen of dietary supplementation with OO or FO as a means to mitigate vascular effects induced by PM exposure.

**Methods**

**Study participants.** Forty-two participants ranging in age from 50 to 72 years (mean 58 ± 1 years) were enrolled in the study. None had a history of heart disease, uncontrolled hypertension, pulmonary disease, diabetes mellitus, hypercholesterolemia, or active allergy, and none had smoked for the past year. Participants were not taking n-3 or n-9 fatty acid (FA) supplements, anti-inflammatory drugs [e.g., nonsteroidal anti-inflammatory drugs (NSAIDs)], or antioxidant supplements (e.g., beta-carotene, selenium, vitamin C, and vitamin E). All participants were instructed to avoid food sources of n-3 and n-9 FA for 6 weeks and refrain from using any NSAIDs for 2 weeks prior to each exposure. They were also asked to abstain from alcohol and caffeine and adhere to a low-fat diet for 24 hr before exposures. The Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill and the U.S. Environmental Protection Agency approved the study protocol, recruitment materials, and consent forms. All study participants gave informed consent and received monetary compensation for their participation.

**Study design.** All exposures were conducted in the U.S. Environment Protection Agency Human Studies Facility (HSF) on the medical campus of the University of North Carolina at Chapel Hill as described previously (Tong et al. 2012). At the end of 28 days of dietary supplementation, each participant was exposed first to filtered air and then returned to the HSF the next morning to undergo exposure to CAP. The participants were blinded to the exposure. The exposures were conducted at the same time of the day and on the same 2 days of the week. Participants were exposed for 2 hr through a face mask in an exposure chamber in which temperature and humidity were controlled. They remained at rest in a seated position throughout the exposure.

The following procedures were performed on each participant beginning at approximately 0800 hours (2 hr before chamber exposure to filtered air): Venous blood was collected, BP was recorded, and the diameter and flow-mediated dilatation (FMD) of the brachial artery were measured by ultrasound. The same procedures were repeated immediately after the 2-hr filtered-air exposure (Post) and again the next morning at approximately 0800 hours (Follow-up). These latter measurements also served as the preexposure values for the CAP exposure. At approximately 1000 hours on the second day, participants were exposed to CAP for 2 hr. As with the filtered-air exposure, post and follow-up measurements were obtained immediately after CAP exposure and beginning at 0800 hours the next morning.

**Dietary supplementation.** As described previously (Tong et al. 2012), all participants were asked to restrict their intake of foods containing n-3 and n-9 FA for the 2-week period prior to and during the 4-week dietary supplementation period. Thirty participants received 3 g/day (three 1-g capsules daily) of OO, 16 participants received 3 g/day (three 1-g capsules daily) of marine-derived n-3 FA (FO), and 13 participants were not supplemented (naive) for 28 days prior to the filtered-air exposure day. The participants were supplemented with OO or FO up to and including the days of exposure. FO and OO assignments were made using a randomized, double-blinded study design. Participants were also asked to keep 3-day food records during the second and fourth weeks of the supplementation period to assess compliance with the dietary restrictions.

**Brachial artery ultrasound.** As described previously (Guthikonda et al. 2007; Schneider et al. 2008), FMD was measured by brachial artery ultrasound in the University of North Carolina Hospitals Clinical Translational Research Center (CTRC) using a 12.5-MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine (Philips), or on-site at the U.S. Environmental Protection Agency HSF using a 15-MHz imaging probe interfaced with an Acuson Sequoia 512 ultrasound machine (Siemens). The participants were transported from the HSF to the UNC-CTRC by an E85-fueled vehicle. Baseline images of the right brachial artery were captured at end diastole. The FMD was assessed after reactive hyperemia induced by inflating a pneumatic tourniquet applied distal to the antecebular fossa to a suprasystolic pressure for 5 min (Guthikonda et al. 2007).
Hyperemic images were captured for 90 sec following cuff deflation. Brachial arterial diameter (BAD) was measured at baseline and at maximum dilation with customized software that utilizes edge-detection technology (Vascular Research Tools, version 5.9.0; Medical Imaging Applications).

**BP measurements.** BP was measured using a validated ambulatory BP monitor (Oscar-2; SunTech Medical) at 15-min intervals during 2 hr of exposure and at 30-min intervals before and after filtered-air and CAP exposure. A single reading at each time point was acquired and used in the analysis.

**Measurement of endothelin-1 and markers of fibrinolysis and inflammation.** Venous blood was obtained 2 hr before, immediately after, and 20 hr after each exposure. Anticoagulated plasma samples were stored at −80°C until assayed in our laboratory using commercially available ELISA kits to quantify levels of fibrinolysis markers including tissue-type plasminogen activator (tPA) and plasminogen (Enzyme Research Laboratories); D-dimer and von Willebrand factor (vWF) (Diagnostica Stago); plasminogen activator inhibitor-1 (PAI-1) (DakoCytomation); inflammatory markers including fibrinogen (Diasorin), interleukin (IL)-6, IL-8, tumor necrosis factor alpha (TNFα), and C-reactive protein (CRP) (Meso Scale Discovery); vascular inflammatory markers including vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) (Meso Scale Discovery); and von Willebrand factor (vWf) (Diagnostica Stago).

**Statistical analysis.** To evaluate changes between CAP and filtered-air exposures within participants, as well as differences between the OO, FO, and naive groups, we used a two-factor (supplement and CAP concentration) mixed-effects model with a participant-specific random intercept. Changes within individuals were evaluated at two time points separately: immediately after and approximately 20 hr after exposure to CAP and filtered air, denoted “Post” and “Follow-up,” respectively. Prior to the analysis, we normalized all Post and Follow-up outcomes to their preexposure values (Post/Pre, Follow-up/Pre) to control for day-to-day variability. The exposure variable PM was treated as continuous, and changes between CAP and filtered-air exposures were expressed as percent point differences per 100-μg/m³ increase in CAP concentration relative to baseline, with the associated 95% confidence interval (CI). To test differences in baselines between the three groups and differences between exposures we used a two-factor mixed-effects model [exposure = (before filtered air, before CAPs), group = (OO, FO, naive)].

There were more females than males in each dietary supplement group in this study, and the ratio of females to males varied (9:4, 12:4, and 11:2 in OO, FO, and naive groups, respectively). However, the great majority of female participants (29 of 32) were postmenopausal, and only 4 were on hormone replacement therapy (Table 1). To examine the influence of sex on responses to CAP and the outcomes, we conducted sensitivity analyses for selected end points by adjusting for sex as a confounder and by restricting analyses to women only. We did not have sufficient power to evaluate effect modification of associations with CAP, or of differences according to supplement group, by sex. We also examined the sensitivity of results with respect to medication usage by excluding 5 participants taking statins or ACE inhibitors (angiotensin-converting enzyme) inhibitors. R statistical software (version 2.15.0; R Core Team 2012) was used for analysis, and a p-value ≤ 0.05 was considered significant.

**Results**

Table 1 presents characteristics of the research participants. Age, body mass index (BMI), smoking history, medication usage, BP, serum glucose, and lipids did not differ statistically among the groups. Before starting supplement consumption with OO or FO, or no supplements (naive), all participants reported low dietary intakes of foods rich in n-3 and n-9 FA (Tong et al. 2012). Participants showed good compliance with their adherence to the dietary restriction and OO or FO supplementation schedule (Tong et al. 2012).

**Endothelial function.** The average baseline diameter and FMD of brachial artery measured before, immediately after, and 20 hr after CAP exposure are presented in Supplemental Material, Table S2. Compared with the naive group, the mean value of FMD measured prior to the filtered-air exposure was higher in the FO group (7.19 ± 0.88 vs. 6.57 ± 0.65; p = 0.08) when analyzed using a two-factor mixed-effects linear model for differences in baselines between exposures (filtered air, CAP) and supplemental groups (OO, FO, naive). There were no significant differences among the prefiltered-air-exposure diameter mean values in the OO, FO, and naive groups. The mean percent difference in FMD and baseline BAD per 100-μg/m³ increase in CAP concentration immediately after and 20 hr after CAP exposure relative to filtered-air control is shown in Table 2.
FMD was significantly lower immediately after CAP exposure in the naive group (−19.4% average decrease relative to baseline per 100-μg/m³ increase in CAP concentration; 95% CI: −36.4%, −2.3%; \( p = 0.03 \)) and also in the FO group (−13.7%; 95% CI: −24.5%, −2.9%; \( p = 0.01 \)) (Table 2 and Figure 1). In the OO group, however, exposure to CAP resulted in a smaller nonsignificant decrease in FMD (−7.6%; 95% CI: −21.5%, 6.3%; \( p = 0.27 \)). In the FO group, FMD 20 hr after CAP exposure was still significantly lower than baseline (−21.5%; 95% CI: −37.3%, −5.6%; \( p = 0.01 \)). Differences between the groups were not significant. Reduction in FMD was persistent after adjusting for confounding by sex as well as in the “female-only” analysis (see Supplemental Material, Table S3).

There was also no significant difference in BAD either immediately after or 20 hr after CAP exposure in any of the groups (Figure 1 and Table 2).

**Blood pressure.** There was no significant difference in BP before filtered-air exposure between the OO, FO, and naive groups (Table 1). There were statistically nonsignificant increases in systolic BP 30 min after CAP exposure in the naive group (+2.5 mmHg per 100-μg/m³ increase in CAP concentration compared with filtered-air exposure; \( p = 0.09 \)) and 60 min after exposure in the FO (+2.7 mmHg; \( p = 0.06 \)) and OO (+2.1 mmHg; \( p = 0.22 \)) groups. Diastolic BP was significantly increased 30 min after exposure in the naive group (+2.1 mmHg per 100-μg/m³ increase in CAP concentration compared with filtered-air exposure; \( p = 0.04 \)) and 60 min after exposure in the FO (+2.1 mmHg; \( p = 0.008 \)) and OO (+2.1 mmHg per 100-μg/m³ increase in CAP concentration compared with filtered-air exposure; \( p = 0.03 \)) groups.

**Blood markers of fibrinolysis.** The average concentrations of blood markers of fibrinolysis at baseline, immediately postexposure, and 20 hr post exposure are presented in Supplemental Material, Table S4. In all three supplement groups, we observed higher levels of plasma PAI-1 in the morning (0800–0900 hours) and lower levels in the afternoon (1300–1400 hours). The observed variation is consistent with previous reports (Paschos and FitzGerald 2010; Rudnicka et al. 2007) and may indicate diurnal variation in levels of plasma PAI-1; however, this study was not designed to further examine this hypothesis. The baseline tPA concentration (i.e., before filtered-air exposure) was significantly lower in the OO group compared with the naive group, and baseline PAI-1 and plasminogen concentrations were significantly lower in the OO and FO groups compared with the naive group (see Supplemental Material, Table S4), possibly due to effects of OO and FO supplementation (Mehta et al. 1988; Pérez-Jiménez et al. 2006).

In the OO group, the average plasma concentration of tPA increased immediately after CAP exposure in the OO group (11.6%; 95% CI: 0.8%, 22.2%; \( p = 0.04 \)), and this increase persisted for 20 hr, at which time there was a 10.9% increase (95% CI: 0.1%, 21.8%; \( p = 0.05 \)) for this group (Table 2 and Figure 2). In addition, plasma D-dimer levels were 11.6% lower (95% CI: −24.5%, −2.9%; \( p = 0.01 \)) (Table 2 and Figure 1).
The association between CAP exposure and endothelial dysfunction induced by PM exposure has been implicated in the development of cardiovascular disease. Specifically, endothelial dysfunction induced by air pollution exposure has been implicated as an important mechanism in the development of cardiovascular diseases (Brook et al. 2010; Krishnan et al. 2013). Mechanistically, oxidative stress and inflammation are two mechanisms that have been associated with the adverse health effects of air pollution exposure. It has been suggested that antioxidant components in certain food groups can limit oxidative damage and thereby protect endothelial function (Moreno and Mitjavila 2003). The results presented here suggest that dietary supplementation with OO may prevent deleterious effects of CAP exposure on vascular function and might, therefore, represent a practical approach to reduce the mortality and morbidity of cardiovascular diseases associated with PM exposure.

There was a smaller CAP-induced reduction in FMD in participants supplemented with OO compared with FO-supplemented and unsupplemented participants in this study, supporting a role for OO in protecting vascular function from PM-induced health effects. The oleic acid in OO has antioxidant and anti-inflammatory properties (Perona et al. 2006), and OO supplementation has been shown to improve endothelial function and lipid profile (Zern and Fernandez 2005) and lower the risk of coronary heart disease events (Estruch et al. 2013; Hertog et al. 1995). Two months of dietary supplementation with OO was previously associated with improved endothelial function and a decrease in BP in a study of young women with mild hypertension (Moreno-Luna et al. 2012). Furthermore, in a clinical trial in healthy young adults, 16 weeks of supplementation with 4 g/day of OO led to an increase in FMD (Singhal et al. 2013). Our finding suggests that 4 weeks of dietary OO supplementation attenuate PM-induced reductions in FMD, possibly due to the effect of OO to protect against the adverse effects of PM exposure on endothelial function.
We also observed in this study that, on average, participants in the OO supplementation group had significant changes in the levels of tPA to CAP exposure, whereas those in FA and naive groups did not. There was a significant increase in plasma levels of tPA immediately after CAP exposure, and this effect persisted 20 hr postexposure in the OO group. tPA is a serine protease that catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for the breakdown of thrombi. In contrast to tPA, D-dimer was significantly reduced 20 hr after CAP exposure in the OO group, although the effect was no longer present after eight weeks of supplementation. These findings provide the basis for a future cohort study focusing on between-group comparisons in order to confirm and further evaluate OO supplementation as a protective intervention against the adverse health effects of PM inhalation.

Conclusion

In this study we observed that a 2-hr exposure to CAP impaired vascular endothelial function for up to 20 hr, and that dietary supplementation with OO, but not FO, attenuated the FMD reduction induced by CAP exposure. Further, supplementation with OO seemed to alter blood markers associated with fibrinolysis and vasoconstriction in PM-exposed human volunteers. These data, therefore, suggest that OO supplementation should be further examined as a possible intervention to protect against the adverse vascular effects of air pollution exposure.

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In order to avoid potential variations in FA equilibrium times and particle washout periods, this study did not use a standard crossover design involving a randomized exposure to air and CAP. In addition, conclusions derived from the small number of participants included in the study may not be applicable to the population as a whole. Furthermore, the modest sample size and the number of secondary end points measured, which could inflate the significance of the findings, are additional statistical limitations.

It has been hypothesized that FO-mediated enhancement of endothelial function is a potential strategy by which the population can be protected from the adverse effects of PM. Previous studies have demonstrated that FO (EPA and DHA) improve systemic arterial compliance in individuals with dyslipidemia (Nestel et al. 2002) and improve endothelial function in individuals with dyslipidemia, heart failure, and diabetes (Duda et al. 2009b; Saravanan et al. 2010). However, trials of FO supplementation in healthy volunteers have shown inconsistent results (Khan et al. 2003; Singhal et al. 2013). One study found that 8 months of FO supplements improved endothelial function in middle-aged men and women (Khan et al. 2003). By contrast, 16 weeks of DHA supplementation had no effect on indices of endothelial function in a study of healthy young volunteers (Singhal et al. 2013). In general, studies have shown that EPA and DHA levels in blood are not linked to FMD in normal healthy adults (Egert and Stehle 2011; Leeson et al. 2002; Sanders et al. 2011; Saravanan et al. 2010; Singhal et al. 2013). Consistent with these reports, in the present study we did not observe a significant benefit of 4 weeks of FO supplementation on CAP-induced reduction in FMD.

The mechanism by which OO supplementation attenuates CAP-induced vascular effects was not investigated in this study. It is possible that OO supplementation increases the bioavailability of plasma nitrites/nitrates, which in turn improves endothelial function (Moreno-Luna et al. 2012). In the present study, OO supplementation appeared to blunt the CAP-induced reduction in FMD, consistent with an effect of OO on the bioavailability of NO. Another function of OO may activate the fibrinolysis system that protects against thrombosis induced by CAP exposure. However, it is difficult to reconcile this mechanism with the simultaneous decrease in D-dimer level observed in these participants. One possibility is that OO could be decreasing prothrombotic factors that were not measured in this study.

In addition, we observed that plasma levels of endothelin-1 were increased after CAP exposure in the unsupplemented group but not in participants supplemented with OO or FO. Several studies have shown increased plasma endothelin-1 levels after controlled exposure of young healthy volunteers to ultrafine CAP (Samet et al. 2009). The increase in tPA level suggests that the supplementation with OO may activate the fibrinolysis system that protects against thrombosis induced by CAP exposure. However, it is difficult to reconcile this mechanism with the simultaneous decrease in D-dimer level observed in these participants. One possibility is that OO could be decreasing prothrombotic factors that were not measured in this study.
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