Arachidonic acid metabolism and inflammatory biomarkers associated with exposure to polycyclic aromatic hydrocarbons

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Abstract

Exposure to polycyclic aromatic hydrocarbons (PAHs) has been associated with systemic inflammation, yet what mechanisms regulate PAHs' inflammatory effects are less understood. This study evaluated the change of arachidonic acid (ARA) metabolites and inflammatory biomarkers in response to increased exposure to PAHs among 26 non-smoking healthy travelers from Los Angeles to Beijing. Traveling from Los Angeles to Beijing significantly increased urinary metabolites of dibenzofuran (800%), fluorene (568%), phenanthrene (277%), and pyrene (176%), accompanied with increased C-reactive protein, fibrinogen, IL-8, and IL-10, and decreased MCP-1, sCD40L, and sCD62P levels in the blood. Meanwhile, the travel increased the levels of ARA lipoxygenase metabolites that were positively associated with a panel of pro-inflammatory biomarkers. Concentrations of cytochrome P450 metabolite were also increased in Beijing and were negatively associated with sCD62P levels. In contrast, concentrations of ARA cyclooxygenase metabolites were decreased in Beijing and were negatively associated with anti-inflammatory IL-10 levels. Changes in both inflammatory biomarkers and ARA metabolites were reversed 4–7 weeks after participants returned to Los Angeles and were associated with urinary PAH metabolites, but not with other exposures such as secondhand smoke, stress, or diet. These results suggested possible roles of ARA metabolic alteration in PAHs-associated inflammatory effects.

1. Introduction

Atherosclerosis is the primary cause of ischemic heart disease and stroke (Hansson, 2005). Hallmarks of early atherogenesis, such as subendothelial accumulation of foam cells, are linked to chronic inflammatory states (Hansson, 2005). A diversity of inflammatory markers, including acute-phase reactants, cytokines, chemokines, and soluble adhesion molecules, contribute to the pathogenesis of atherosclerosis in animals, and predict the risk of cardiovascular diseases among general populations (Vasan, 2006; Thomas and Lip, 2017; Roberts, 2004). Circulating concentrations of these inflammatory biomarkers increase after exposure to air pollution (Feng et al., 2021; Liu et al., 2017), the effects of which are at least partially attributed to the polycyclic aromatic hydrocarbons (PAHs) components (Jiang et al., 2019, 2021; Delfino et al., 2010). This is considered as one of the key mechanisms by which air pollution contributes to the development of cardiovascular
diseases.

Although the inflammatory effects of PAHs-rich air pollution have been extensively studied, marked response heterogeneity has been documented across individuals with different susceptibility, and time of exposures. For example, previous studies have reported stronger inflammatory responses to short-term urban air pollution exposures among individuals with prediabetes and chronic obstructive pulmonary diseases as compared with healthy controls (Han et al., 2019; Chen et al., 2021). Other studies have shown that the pro-inflammatory response to urban air pollution may be inhibited or even become anti-inflammatory after longer exposures (Li et al., 2016; Lin et al., 2011). A substantial number of studies have examined only the short-term inflammatory effects of air pollution. While these studies have enlightened the underlying mechanism by which air pollution triggered acute ischemic cardiovascular events among susceptible population (Brook et al., 2010), the inflammatory responses to prolonged air pollution exposures are less understood, which may differ from those after short-term exposure and could accumulate over years and contribute to the development of atherosclerosis even among healthy young adults (Bevan et al., 2020).

Systemic inflammation is linked to the oxidative modification of lipids, including polyunsaturated fatty acids (PUFAs) such as arachidonic acid (ARA), catalyzed by lipoxygenase (LOX), cyclooxygenase (COX), and cytochrome P450 (CYP). Previous in vivo studies in hyperlipidemic mice have shown that the disruption of LOX and COX or the overexpression of CYP diminished atherosclerosis (Liu et al., 2016; Cyrus et al., 1999; Burleigh et al., 2005). The pro-atherogenic effects of LOX and COX pathways might be at least partially due to the synthesis of acute-phase reactants including CRP, fibrinogen, and von Willebrand factor (vWF) (Lin et al., 2019a). Exposures to non-air pollution factors (e.g., secondhand smoke, stress, and diets) were also estimated with a biomarker approach in our previous studies (Lin et al., 2019a).

In this study, we further assessed serum levels of six inflammatory biomarkers including an anti-inflammatory cytokine interleukin-8 (IL-8) and an anti-inflammatory cytokine (IL-10), monocyte chemoattractant protein-1 (MCP-1), soluble adhesion molecules of CD40-ligand (sCD40L), P-selectin (sCD62P), and intercellular adhesion molecule 1 (sICAM-1) using cytometric bead array as per the manufacturer’s instructions. Briefly, serum samples were centrifuged (3,000, 5 min, 4 °C) and the supernatants were incubated (1 h) with capture beads followed by incubation (2 h, room temperature) with peptide-detecting reagents in the dark. The beads were washed and resuspended in 300 μL washing buffer for flow cytometric analysis (FACSVia; BD Biosciences). The acquired data were analyzed using FCAP Array software (BD Biosciences).

We also determined serum levels of ARA metabolites from the COX pathway (6-keto-prostaglandin F1α [6-keto-PGF1α], prostaglandin F2α [PGF2α], and 15-deoxy-prostaglandin J2 [15-deoxy-PGJ2]) and the CYP pathway (5,6-, 8,9-, 11,12-, and 14,15-dihydroxy-eicosatetraenoic acids [DHETrE]) using LC/MS with a previously established method (Barupal et al., 2019). Briefly, after thawing on ice and vigorous mixing, sera (40 μL) were spiked with oxylipin internal standards (40 μL, 50 nM in MeOH), mixture of 1-cyclohexyluriedo-3-dodecanoic acid and 1-phenyl-3-hexadecenoic acid urea as quality control markers (20 μL, 1,000 nM in MeOH), mixture of 1-cyclohexyluriedo-3-dodecanoic acid and 1-phenyl-3-hexadecenoic acid urea as quality control markers (20 μL, 1,000 nM in MeOH), 1:1 mixture of butylated hydroxytoluene (0.2 mg/mL in MeOH), and EDTA (10 μL, 0.2 mg/mL in MeOH) in methanol. Methanol (150 μL) was then added to reach the final volume of 250 μL. The mixture was vigorously mixed (30 s), centrifuged (15,000 rcf, 2 min), and the supernatant was filtered (0.2 μm PVDF filter) and filtrate was dried in a vacuum centrifuge. Residues were resuspended water-/acetoniitrile/isopropanol (10:9:1, v/v/v; 80 μL) and aliquots of the samples (5 μL) were injected onto a reversed phase HPLC column (Waters Acquity BEH C18, 1.7 μm, 2.1 × 100 mm, 60 °C) equilibrated in eluant A (water/acetic acid, 100/0.1, v/v) and eluted (0.25 mL/min) with an increasing concentration of eluant B on a summer student exchange program between University of California Los Angeles (UCLA) and Peking University (PKU, Beijing, China), in which 10–15 students from UCLA visited PKU for ten weeks in the summer of each year (Figure S1). We have previously shown that participants’ exposure to PAHs was markedly higher in Beijing than Los Angeles likely due to the much worse air quality in Beijing (Figure S2). Thus, this program allowed an evaluation in the same participants of the effects of a drastic change in PAHs exposure and the reversibility of the effects after the cessation of exposure. All study participants were recruited from UCLA in 2014 and 2015 as described previously (Lin et al., 2019a). Inclusion criteria were age >18 years and body mass index (BMI) < 30 kg/m². Exclusion criteria included history of smoking, heart disease, metabolic disorders or having symptoms of asthma, kidney disease, blood coagulation disorders, rheumatological diseases or chronic inflammation during the previous six months. For each participant, matched morning urine and blood samples were collected in Los Angeles (LA-before, 1–3 weeks before departure), Beijing (6–8 weeks after arrival) and in Los Angeles again (LA-after, 4–7 weeks after returning). Participants were asked to fast for 8 h before sample collection to diminish dietary effects. Of the 27 students consented to participated in the study, one was excluded due to hypertension. Samples were collected from 20 participants in all three phases and from six participants in only two study phases. The study was approved by the institutional review boards at both UCLA and PKU and informed consent was obtained from participants prior to the study.

Laboratory analysis. Previously, we have quantified urinary metabolites of polycyclic aromatic hydrocarbon (OH-PAHs), plasma concentrations of ARA and their oxidative metabolites (i.e., 8-isoprostane, 5-, 12- and 15-HETEs), and serum concentrations of acute-phase reactants including CRP, fibrinogen, and von Willebrand factor (vWF) (Lin et al., 2019a). Exposures to non-air pollution factors (e.g., secondhand smoke, stress, and diets) were also estimated with a biomarker approach in our previous studies (Lin et al., 2019a).

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(acetonitrile/isopropanol/acetic acid, 90/10/0.1, v/v/v: min/%B; 0/2, 1/40, 2.5/45, 4.5/50, 10.5/65, 12.5/75, 14.8/85, 14.5/95, 15/25, 16/25). The effluent was passed to an on-line electrospray ionization source attached to triple quadrupole mass spectrometer (SCIEX 6500+–QTRAP) operating in the negative ion multiple reaction monitoring mode with previously optimized settings for the compounds of interest (source temperature 525 °C, electrospray voltage −4.5 kV/+3 kV, curtain gas pressure 35 psi, nebulizer and turbo gas pressure 50 psi), and data were recorded for the following parent ion to fragment ion transitions: 6-keto-PGF1α 369/163, PGF2α 353/193, 15-deoxy-PGJ2 315/271, 5, 6-DiHETrE 337/145, 8,9-DiHETrE 337/127, 11,12-DiHETrE 337/167, 14,15-DiHETrE 337/207. SCIEX Multi-Quant 3.0.2 software was used for quantification (Barupal et al., 2019). Each batch of samples were coupled with a laboratory blank sample and none of the targeted compounds were detected in any of the blank samples. The method reproducibility was 7.7%, estimated based on the relative standard deviation (RSD) of the peak areas of deuterium-labeled 6-keto-PGF1α spiked into the serum before the pretreatment.

Statistical Analysis. For undetected analytes, we assigned a value of half of the detection limit before the statistical analysis. We tabulated the mean (± standard deviation) or geometric mean with the interquartile range (IQR) by phase (i.e., LA-before, Beijing, and LA-after) as appropriate. The difference in biomarker concentrations between phases was evaluated in linear mixed-effects models with random intercepts at the participant level. Multiple comparisons for inflammatory biomarkers (n = 9) and ARA metabolites (n = 12) were adjusted by the Benjamini-Hochberg method to control the overall false discovery rate (FDR) of 5%. We used linear mixed-effects models with random intercepts at both participant and phase levels to examine the associations between inflammatory biomarkers and exposures. The associations of ARA metabolites with inflammatory or exposure biomarkers were also tested with linear mixed-effects models with random intercepts at both participant and phase levels. Different from the models used in our previous study (Lin et al., 2019a), we have additionally controlled the fixed effects of ARA concentrations to attribute the associations to the effects of metabolic pathway instead of metabolite itself. Alpha was set at 0.05, and all tests were two-tailed. Data were analyzed with the statistical software R with lme4, and lmeTest packages (www.r-project.org).

3. Results

Twenty-six healthy young adults, 23.8 ± 5.6 years of age and body mass indices of 21.6 ± 2.4 kg/m², were involved (Table 1). They had a normal plasma concentration range of total cholesterol, but the high-density lipoprotein cholesterol was lower (<40 mg/dL, Table 1). (Navab et al., 2011) The total and high-density lipoprotein cholesterol concentrations did not change significantly in the study period as reported previously (Lin et al., 2019a).

Exposure to PAHs and Other Factors. Traveling to Beijing significantly increased urinary metabolite concentration of a panel of PAHs, including dibenzo[ghi]perylene (2-OH-DBP, +800%), fluorene (ΣOH-FLUs, +568%), phenanthrene (ΣOH-PHEs, +277%), and pyrene (1-OH-PYR, +176%) (Table 1). As reported previously, the increased concentration of OH-PAHs in fasting morning urine samples in Beijing was mainly driven by increased inhalation exposure to air pollution and secondhand smoke, given comparable intakes of PAHs-rich food between the two cities (Lin et al., 2016, 2019a). Levels of PAH metabolites were decreased towards baseline after participants returned to Los Angeles (Table 1). Traveling to Beijing also changed the levels of cotinine, cortisol, caffeine, theobromine, proline, betaine, and vitamin E, which may reflect changes in passive smoking exposure, travel-related stress, and intake of coffee, chocolate, fruits, and/or antioxidants, respectively (Lin et al., 2019a).

Changes of Inflammatory Biomarkers. We have previously reported significant increases in CRP and fibrinogen levels after traveling from Los Angeles to Beijing (Lin et al., 2019a). In this study, we further observed increased levels of IL-8 (36.3%; 95%CI: 16.5%–59.5%) and IL-10 (45.0%; 95%CI: 1.8%–106%) after the travel to Beijing (Fig. 1). On the other hand, there were significant decreases in circulating levels of MCP-1 (~50.3%; 95%CI: –60.9% to –36.8%), sCD40L (~63.3%; 95% CI: –79.5% to –34.1%), and sCD62P (~31.3%; 95%CI: –44.0% to –15.6%) in Beijing as compared with LA-before (Fig. 1). All these changes reversed towards baseline after participants returned to Los Angeles (Fig. 1).

The changes of CRP, IL-8, sCD62P, and sICAM-1 levels were significantly associated with urinary OH-PAHs concentrations (p < 0.05, Fig. 2). In contrast, levels of CRP, IL-8, or sCD62P were not associated with other exposure biomarkers of non-air pollution factors (Figure S3). We found that the sICAM-1 concentration was significantly associated with theobromine concentrations (p < 0.05), but the associations between sICAM-1 and urinary OH-PAHs concentrations remained robust after adjusting for theobromine concentrations (Figure S4). Although levels of fibrinogen, IL-10, MCP-1, and sCD40L markedly changed when traveling from Los Angeles to Beijing, they were not significantly associated with urinary OH-PAHs concentrations (Fig. 2). Instead, changes of IL-10 and MCP-1 concentrations were significantly associated with the proline betaine level.

Changes of ARA Metabolites. We previously reported significant increases in circulating levels of ARA LOX metabolites (5-, 12-, and 15-HETEs), together with insignificant changes in ARA and its non-enzymatic oxidation product (8-isoprostane) concentrations in Beijing (Lin et al., 2019a). Here we have assessed metabolites derived from the other two ARA pathways (COX and CYP). Traveling from Los Angeles to
Beijing led to significant decreases in the levels of COX metabolites, including 6-keto-PGF1\(\alpha\) (83.8%; 95%CI: 94.0% to 56.4%) and PGF2\(\alpha\) (42.7%; 95%CI: 60.0% to −17.9%, Table 2), which recovered to the baseline levels after returning to Los Angeles. We also found that the level of 5,6-DiHETrE, a metabolite from CYP pathways, was higher in Beijing than in LA-before and LA-after; and the difference between Beijing and LA-after was statistically significant (FDR < 5%, Table 2).

With the adjustment of ARA concentrations, we examined associations between ARA metabolites and OH-PAHs to evaluate the effects of PAHs on different ARA metabolic pathways. The concentrations of urinary OH-PAHs were significantly associated with increased levels of 8-isoprostane and 15-HETE, and decreased levels of 6-keto-PGF1\(\alpha\) and PGF2\(\alpha\) (\(p < 0.05\), Fig. 2). Although the levels of 12-HETE and 5,6-DiHETrE were also significantly changed during the travel, they were not associated with urinary OH-PAHs levels. Instead, the level of 12-HETE was negatively associated with cotinine and vitamin E levels (\(p < 0.05\)), while the level of 5,6-DiHETrE was negatively associated with cortisol levels (\(p < 0.05\), Figure S5).

**Relationship Between Inflammatory Biomarkers and ARA Metabolites.** Different ARA metabolic pathways appear to be linked to different inflammatory responses (Fig. 3A). In general, the LOX and COX metabolites were positively associated with pro-inflammatory changes, since the level of CRP, vWF, IL-8, MCP-1, sCD62P, and sICAM-1 were positively associated with LOX metabolites, while the anti-inflammatory IL-10 was negatively associated with 6-keto-PGF1\(\alpha\) levels (Fig. 3A). In contrast, the CYP-derived metabolite 5,6-DiHETrE was negatively associated with sCD62P levels, suggesting an anti-inflammatory effect.

**4. Discussion**

In this natural experiment, we exploited the drastic difference in PAHs exposure between Los Angeles and Beijing, in combination with a...
unique cohort traveling between the two cities within a well-defined timeframe, to study healthy young adults’ inflammatory responses to PAHs exposures and its relationship with ARA metabolism. We found that the pro-inflammatory effects after traveling from Los Angeles to Beijing were accompanied by increased levels of anti-inflammatory cytokines and decreased levels of chemokine and soluble adhesion molecules in healthy young adults, which were differentially associated with ARA metabolites from various pathways. The changes of both inflammatory biomarkers and ARA metabolites were significantly associated with urinary OH-PAHs levels but not with cotinine levels, suggesting the observed effects may be linked to exposure to air pollution.

The development of pro-atherosclerotic vascular diseases involves systemic inflammatory responses (Hansson, 2005) which have been associated with short-term exposures to air pollution in both healthy and susceptible populations (Delfino et al., 2009; Pope et al., 2016). Furthermore, long-term exposure to air pollution is associated with the progression of atherosclerosis with stronger associations among hypertensive and older individuals (Kaufman et al., 2016), while healthy young adults are less susceptible. Here, the pro-inflammatory increase in inflammatory responses to prolonged exposure to air pollution observed effects may be linked to exposure to air pollution.

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adhesion molecules cannot be explained by the changes of other cytokines while the changes of other cytokines might be subsequent given their correlations with IL-8 (Table S1). Indeed, PAHs have been shown to upregulate IL-8 through the interaction with aryl hydrocarbon receptor (Podechard et al., 2008). On the other hand, IL-8 was positively associated with 12-HETE, 15-HETE, and 8-isoprostane. Previous studies have shown that IL-8 upregulates 12/15-LOX in polymorphonuclear leukocytes and vascular smooth muscle cells (Fogh et al., 1992; Natarajan et al., 1997). It was also shown that reactive oxygen species in turn stimulate the production of IL-8 in systemic circulation (DeForge et al., 1992). Thus, these results suggest a close linkage between the pro-inflammatory and pro-oxidative effects of air pollution and/or PAHs, partially through the interaction between IL-8 and ARA LOX pathways.

Previous studies have shown that the increase in IL-8 and CRP after short-term exposure to PAHs-rich air pollution mixture was accompanied with the increase in MCP-1 and adhesion molecules (Feng et al., 2021; Liu et al., 2017). In our study, these inflammatory biomarkers were positively correlated with each other (Table S1). Levels of ARA LOX metabolites were also positively associated with MCP-1 and adhesion molecules (Fig. 3), which is supported by previous animal studies showing the upregulation of MCP-1, CD62P, and ICAM-1 expression by 12/15-LOX overexpression or increased 12/15-LOX metabolites (Wen et al., 2008; Ozeki et al., 1998). However, the actual levels of MCP-1 and sCD62P were significantly lower in Beijing, which is consistent with previous studies reporting increased IL-8 but decreased MCP-1 expression and secretion by peripheral blood mononuclear cells after incubation with PAHs (Fahy et al., 1999). Of note, the decrease in MCP-1 and adhesion molecules cannot be explained by the changes of other inflammatory biomarkers or ARA LOX metabolites as discussed above.

Our results suggest that decreased MCP-1 and sCD62P levels in Beijing may relate to the alteration of ARA CYP and COX pathways. Among the participants, we have observed evidence of COX pathways inhibition, which has also been previously shown to suppress MCP-1 and adhesion molecule expressions in animals (Burleigh et al., 2005; Mitchell et al., 2021). Furthermore, we found borderline associations between ARA COX metabolites and levels of MCP-1 (p = 0.056, Fig. 3B) and (p = 0.071, Fig. 3C). The lack of statistical significance may be due to the small sample size of the study, since we have previously observed significant associations between urinary ARA COX metabolites and circulating sCD62P levels in a panel study of 89 healthy adults (He et al., 2020). On the other hand, participants exhibited increased levels of 5,6-DiHETrE in Beijing, the precursor of which (i.e., epoxyeicosatrienoic acids) has been previously shown to inhibit CD62P expression in human platelets (Krötz et al., 2004). Consistently, there is a negative association between 5,6-DiHETrE and sCD62P levels (Fig. 3).

PAHs-rich air pollution mixture has been shown to upregulate the expression of LOX, COX, and CYP in animal and cell-culture studies (Abbas et al., 2009; Tzeng et al., 2007; Yin et al., 2013). Our previous studies have also shown positive associations between daily variations of urinary ARA COX metabolites and airborne pollutants (He et al., 2020, 2021). However, in the present study, blood levels of ARA COX metabolites were significant decreased in Beijing, which may relate to the strong pro-inflammatory and pro-oxidative effects induced by the travel from Los Angeles to Beijing. On one hand, the travel caused drastic increases in levels of LOX metabolites by 81.2-998% and CYP-derived 5,6-DiHETrE by 27.0% (Table 2), which may have competed for the oxidation of ARA through the COX pathways, leading to decreased biosynthesis of ARA COX metabolites. On the other hand, the level of IL-10 was significantly increased in Beijing, likely in response to the pro-inflammatory effects, and was negatively associated with 6-keto-PGF1α levels (Fig. 3). Because IL-10 has been previously identified as a COX inhibitor (Berg et al., 2001), these results provide another explanation why ARA COX metabolite levels were decreased in Beijing.

Previous studies in hyperlipidemic mice have documented significant anti-atherosclerotic effects of LOX and COX disruption and CYP overexpression (Liu et al., 2016; Cyrus et al., 1999; Burleigh et al.,
In our previous study with a low-density lipoprotein receptor knockout mouse model, ten-week ultrafine particle exposures led to increased LOX and COX but decreased CYP metabolite levels, and ultimately promoted the formation of atherosclerotic plaques (Li et al., 2013). In contrast, we and others have shown that in healthy humans, combustion-originated air pollution exposures were associated with increased levels of LOX and CYP metabolites but decreased levels of COX metabolites (Wang et al., 2021). These results suggested that PAHs-rich air pollution mixture induces pro-inflammatory changes in ARA metabolism (i.e., increased LOX pathways) consistently in both healthy humans and susceptible animals. However, these pro-inflammatory effects were accompanied with anti-inflammatory changes in COX and CYP pathways in health humans, as opposed to those in susceptible animals who developed atherosclerosis. These results suggest a probable role of ARA metabolic pathways in maintaining the hemostatic balance between pro- and anti-inflammatory responses, calling for future studies in humans to examine whether susceptibility factors would perturb ARA metabolism and anti-inflammatory responses to air pollution and/or PAHs, as well as their impacts on the development of cardiovascular diseases.

The significant associations of urinary OH-PAHs with inflammatory biomarkers and ARA metabolites in our study, together with the mechanistic plausibility based on previous animal and cell-cultured studies (Podechard et al., 2008; Fogh et al., 1992; Natarajan et al., 1997; Abbas et al., 2009; Zheng et al., 2007; Yin et al., 2013; Berg et al., 2001), suggested that PAHs and/or the correlated combustion-originated pollutant are probable causative agents of the observed effects in this study. However, it is important to note that the increase in urinary OH-PAHs in Beijing was at least partially resulted from severe air pollution which likely led to increased exposure to other pollutants such as metals and volatile organic compounds (VOCs). Nevertheless, the effect of metals and VOCs pollution on ARA metabolism remains poorly understood. Several studies show that exposures to metals (e.g., cadmium) and VOCs upregulated COX pathways (Fritsch-Decker et al., 2011; Sharma et al., 2020; Kong et al., 2019; Mőgel et al., 2011; Fritsch-Decker et al., 2011; Sharma et al., 2020; Kong et al., 2019; Mőgel et al., 2011), which cannot explain the observation in our study. Limited evidence also suggested a possible effect of metal pollution on the other ARA pathways (Alqahtani et al., 2020; Mai et al., 2016). Thus, we cannot rule out the possibility of potential effects of metals or VOCs which warrants further research.

Limitations. The major study limitation is that we cannot rule out the effects of factors other than PAHs and air pollution (e.g., microbiome) due to the nature of the experiment. Nevertheless, we have adopted multiple approach to decrease the likelihood of potential confounding effects including (1) using a “cross-over” feature (i.e., from LA-before to Beijing vs. from Beijing to LA-after) to control the effects of travel itself and/or the exposures during the flights; (2) collecting biospecimens after at least 8-h fasting to decrease the dietary effects; (3) using a biomarker-approach at personal-level to capture known exposures that may change during travel; and (4) introducing a random intercept of phase in statistical models to account for unknown factors that may change during the travel. Despite these efforts, it is still possible that there were unmeasured exposures that may confound the effects, and therefore our findings should be considered as associative and require further validation.

As another limitation, our study did not measure key pro- or anti-inflammatory ARA metabolites such as leukotrienes and lipoxins. Instead, we measured key ARA metabolites serving as markers of the various pathways. In addition, the presence of other PUFAs should be taken into consideration since they may compete the metabolism of ARA and influence the associated inflammatory responses. For example, a recent randomized trial has shown that dietary fish oil supplementation rich in omega-3 PUFAs prevented the increases in acute-phase reactants, pro-inflammatory cytokines, and soluble adhesion molecules induced by short-term PM$_{2.5}$ exposures (Lin et al., 2019b). Last, our study is also limited by a small number of participants. Although we have observed significant changes in 7 out of 9 inflammatory biomarkers and 6 out of 12 ARA metabolites, future studies with larger sample size are warranted to confirm these findings.

In summary, healthy young Los Angeles travelers, following exposure to higher-levels of PAHs in Beijing for 6–8 weeks, exhibited pro-inflammatory increases in acute-phase reactants and cytokines, as well as anti-inflammatory decreases in chemokine and soluble adhesion molecules. The pro- and anti-inflammatory effects were associated with changes in ARA metabolites from different pathways. Our findings suggest a probable linkage between ARA metabolites and inflammatory effects in healthy young adults under the stress of air pollution and/or PAHs. It is to be examined in the future whether and how ARA metabolic pathways could contribute to the balance between pro- and anti-inflammatory effects induced by air pollution and/or PAHs.

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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Appendix A. Supplementary data

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