Environmental Science & Technology

pubs.acs.org/est

Article

Nitrated Polycyclic Aromatic Hydrocarbons and Arachidonic Acid Metabolisms Relevant to Cardiovascular Pathophysiology: Findings from a Panel Study in Healthy Adults

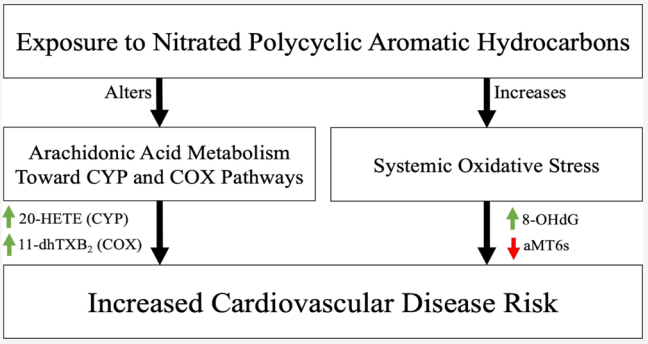
Linchen He, Yan Lin, Drew Day, Yanbo Teng, Xiangtian Wang, Xing Lucy Liu, Erik Yan, Jicheng Gong, Jian Qin, Xiaoli Wang, Jianbang Xiang, Jinhan Mo, Yinping Zhang, and Junfeng Jim Zhang*

Cite This: *Environ. Sci. Technol.* 2021, 55, 3867−3875

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Concerns on nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment have mainly arisen from their mutagenic and carcinogenic effects. The objective of this study is to investigate whether nitro-PAH exposures are associated with biomarkers of cardiovascular pathophysiology. In a panel study design, urines and blood samples were collected up to four times with a 2-week interval from 89 healthy adults. We measured 1-naphthylamine, 2-naphthylamine, 9-aminophenanthrene, 2-aminofluorene, and 1-aminopyrene as biomarkers of nitro-PAH exposures. We measured three urinary metabolites of arachidonic acid (AA) including 20-hydroxyeicosatetraenoic acid (20-HETE) from the cytochrome P450 (CYP) pathway, 8-isoprostane from the nonenzymatic pathway, and 11-dehydro-thromboxane B2 (11-dhTXB$_2$) from the cyclooxygenase (COX) pathway. Urinary malondialdehyde, 8-hydroxy-2′-deoxyguanosine (8-OHdG), and 6-sulfatoxymelatonin (aMT6s) were measured to reflect systemic oxidative stress. Plasma concentrations of the soluble P-selectin and von Willebrand factor (vWF) were measured as biomarkers of platelet activation and endothelial dysfunction. We found that increased urinary concentrations of amino-PAHs were significantly associated with increased 20-HETE, 11-dhTXB$_2$, and 8-OHdG and with decreased 8-isoprostane and aMT6s. Increased amino-PAHs were positively associated with P-selectin and vWF, respectively. These results suggest that exposure to nitro-PAHs increases systemic oxidative stress and alters AA metabolism toward CYP and COX pathways, leading to an increased cardiovascular disease risk.



1. INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are derivatives of PAHs containing at least one nitro functional group on the benzene ring of a PAH.[1] Nitro-PAHs can be produced by the incomplete combustion of organic materials or by the reactions between parent-PAHs and the hydroxyl radical during daytime and the nitrate radical during nighttime.[1−8] Sources included emissions from smoking, vehicles, certain types of food processing, and heating.[9] Compared to parent-PAHs, nitro-PAHs in general have higher potencies for carcinogenicity and mutagenicity.[10,11] It has been recently reported that nitro-PAHs could induce cardiac toxicity in a zebrafish model.[12] However, evidence in humans is lacking concerning whether exposure to nitro-PAHs could induce adverse cardiovascular effects and what are the underlying biological mechanisms.

It has been previously reported that zebrafish exposed to two nitro-PAHs, 1,3-dinitropyrene and 1,8-dinitropyrene, displayed circulatory malfunction and pericardial edema.[12] The authors also reported that exposure to nitro-PAHs altered the expression of several genes related to cytochrome P450 (CYP) metabolism pathways, CYP enzymatic activity, and oxidative stress in zebrafish.[12] CYP enzymes and systemic oxidative stress have been widely reported to play crucial roles in maintaining vascular physiology and cardiovascular homeostasis.[13,14] The dysregulation of CYP enzymes, CYP-dependent metabolites, and redox homeostasis could lead to the onset and progression of many cardiovascular diseases, including hypertension, atherosclerosis, myocardial infarction, and atrial fibrillation.[14,15]

The oxidative metabolism of polyunsaturated fatty acids, such as arachidonic acid (AA), plays a vital role in modulating vascular tone, thrombosis, and oxidative stress.[16,17] For example, CYP enzymes of the 4A and 4F families are involved

Received: December 2, 2020
Revised: January 24, 2021
Accepted: February 10, 2021
Published: February 23, 2021

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

in the metabolism of AA, producing 20-hydroxyeicosatetraenoic acid (20-HETE), a bioactive lipid mediator regulating vascular and cardiac functions implicated in the pathogenesis of cardiovascular disease.[18,19] Moreover, the upregulation of redox homeostasis could affect the metabolism of AA through the nonenzymatic pathway to produce isoprostanes, including 8-isoprostane, that modulate vascular and airway smooth muscle tone.[20] In addition, AA can be metabolized through the cyclooxygenase (COX) pathway producing immunomodulatory prostaglandins and the prothrombotic and vasoconstrictive thromboxane A2 (TXA_2) and its inactive metabolite 11-dehydro-thromboxane B2 (11-dhTXB_2).[21] TXA_2 and other molecules, including P-selectin and von Willebrand factor (vWF), play important roles in platelet activation, aggregation, and coagulation,[22,23] which are critical in various cardiovascular diseases, including ischemic heart disease and stroke.[24] Given evidence that exposure to nitro-PAHs affects the activity of CYP enzymes and induces oxidative stress, we hypothesized that exposure to nitro-PAHs may alter the oxidative metabolism of AA.

To test this hypothesis, we analyzed the stored urine samples collected from a previous study that originally investigated the cardiorespiratory effects of indoor air quality intervention in healthy adults.[25,26] Five urinary amino-PAHs that have been previously used as biomarkers of nitro-PAH exposures were measured: 1-naphthylamine (1-AN), 2-naphthylamine (2-AN), 9-aminophenanthrene (9-APhe), 2-aminofluorene (2-AF), and 1-aminopyrene (1-AP).[2,27] Three urinary AA metabolites pertinent to three different AA metabolism pathways were measured: 20-HETE produced via the CYP pathway; 8-isoprostane produced via nonenzymatic pathways; and 11-dhTXB_2, an inactive metabolite of TXA_2, produced via the COX pathway. In addition, three urinary biomarkers related to systemic oxidative stress were measured: malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and 6-sulfatoxymelatonin (aMT6s).[28−30] We also measured P-selectin and vWF in plasma as biomarkers of platelet activation and endothelial dysfunction. We aim to explore the associations of the urinary amino-PAHs with (1) urinary AA metabolites, (2) biomarkers of systemic oxidative stress, and (3) biomarkers of platelet activation and endothelial dysfunction.

2. METHODS

2.1. Study Subjects. The study design has been previously described in detail.[25] Briefly, 89 healthy office workers, 22−52 years old, were recruited from a company campus in Changsha City, Hunan Province, China. The participants are free from major self-reported chronic diseases. In addition, participants with abnormal blood lipid levels or markers of liver, kidney, or other metabolic dysfunctions were excluded. From December 1st, 2014 to January 31st, 2015, early morning (7−9 a.m.) urine and blood samples were collected up to four times, with a 2-week interval. They were measured for a set of pathophysiologic biomarkers including the ones described below. All the urine and blood aliquots were stored at −80 and −30 °C, respectively, before analysis. No subjects reported with chronic diseases and self-reported current smokers were included. The study protocol was approved by the Ethics Committee of Shanghai First People's Hospital and the Duke University Campus Institutional Review Board.

2.2. Measurements of Biomarkers. The present study simultaneously analyzed urinary 1-AN, 2-AN, 9-APhe, 2-AF, 1-AP, and cotinine using a modified method described in a previous publication.[2] Briefly, 2 mL of urine samples was incubated with 2 mL of 0.1 M acetate buffer (pH = 5, Acros Organics, U.S.A.) and 20 μL of β-glucuronidase-arylsulfatase (Roche, U.S.A.) at 37 °C for 16 h. The hydrolyzed urine samples were adjusted to pH > 10 with the addition of 500 μL of 10 M sodium hydroxide (Sigma-Aldrich, U.S.A.) and extracted with 6 mL of ethyl acetate (LiChrosolv, U.S.A.). After mixing on a shaker for 60 min, the samples were centrifuged at 3500 rpm for 30 min, followed by a condensation procedure using nitrogen. The residues were reconstituted using 200 μL of H_2O and acetonitrile (1:1, Fisher Chemical, U.S.A.), and 20 μL of reconstitution solution was injected into a high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) system (TSQ Quantum Access Max, Thermo Fisher Scientific, U.S.A.). A Sonoma 3 μm C18 (2) column (100 Å 100 × 2.1 mm, ES Industries, U.S.A.) was used along with a mobile phase that was composed of 99.9% water with 0.1% formic acid (Fisher Chemical, Czech Republic) and 99.9% acetonitrile with 0.1% formic acid at a flow rate of 0.2 mL/min. The HPLC-detailed mobile phase gradient is shown in Table S1, and the detailed parameters used for the HPLC-MS/MS system and method performance for each analyte are shown in Table S2. The total amino-PAH concentration (Σamino-PAH) was calculated as the sum of all the individual amino-PAH concentrations.

Urinary 20-HETE, 11-dhTXB_2, and 8-isoprostane were simultaneously analyzed using a previously reported HPLC-MS/MS method.[31] Urinary MDA, a stable product of lipid peroxidation, was measured as a biomarker of oxidative stress using a previously reported HPLC method.[28] 8-OHdG, as a product of DNA oxidation, was measured as another biomarker of systemic oxidative stress. Urinary aMT6s was measured as a surrogate of circulating melatonin, which is a potent antioxidant and can stimulate antioxidant enzymes.[32−34] Urinary 8-OHdG and aMT6s were simultaneously measured using a previously published HPLC-MS/MS method, involving a liquid−liquid extraction using methanol (20%) at urine pH about 7.[35] Urinary specific gravity was measured and used to adjust for the urine dilution factor. Soluble P-selectin and vWF, biomarkers of platelet activation and endothelial dysfunction, respectively, were measured in plasma using an enzyme-linked immunosorbent assay method (R&D system for P-selectin and RayBiotech for vWF, U.S.A).[25]

2.3. Air Pollutant Exposure Assessment. We continuously monitored the indoor fine particulate matter ($PM_{2.5}$), using a nephelometer (SidePak AM510; TSI Inc), and ozone (O_3), using a UV absorption monitor (model 205; 2B Tech), in the dormitory rooms and offices where participants were working and living on the campus. Ambient $PM_{2.5}$, O_3, nitrogen dioxide (NO_2), sulfur dioxide (SO_2), relative humidity, and temperature were obtained from a local government station (~4.5 km from the company campus). Indoor/outdoor (I/O) ratios of 0.8 for $PM_{2.5}$ and 0.35 for O_3 were used to estimate indoor pollutant concentrations in unknown microenvironments (other than company offices and dorms) according to the previous literature in lightly sealed buildings.[36−39] We used I/O ratios of 0.8 for NO_2 and 0.5 for SO_2 for all indoor microenvironments based on the previous findings on similar physical characteristics of the buildings.[40−43] These measured and estimated pollutant concentrations were coupled with the detailed time-activity data

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

collected from each participant to calculate personal air pollutant exposure averaged over 24 h prior to each visit. The method of the exposure assessment and the relationships between personal air pollution exposures and the current health outcomes have been previously published.[25,31,44,45] In this study, air pollutant exposures were controlled for potential confounding effects in the sensitivity analysis.

2.4. Statistical Analysis. Because the raw data of all the amino-PAHs and outcome variables had right-skewed distributions, they were natural logarithm-transformed before statistical analysis. The repeated measure correlations among urinary amino-PAHs and cotinine were examined.

Linear mixed-effect regression (LMER) models were used to assess the relationships of the urinary amino-PAHs with three AA metabolites and biomarkers of oxidative stress, platelet activation, and endothelial dysfunction, respectively. In this set of models, the concentration of each outcome variable was the dependent variable, and each of the amino-PAHs was the independent variable. We controlled for fixed-effect covariates that have been previously reported to potentially affect cardiovascular health. These included sex, age, body mass index (BMI), urinary cotinine concentration, upper respiratory infection status, and 24 h average ambient temperature and relative humidity. In addition to these covariates, urinary aMT6s was included as a covariate to adjust for the antioxidative level in all models in which urinary MDA, 8-OHdG, and 8-isoprostane concentrations were one of the dependent variables. Subject ID was treated as a random intercept. From the model output, the percent change and 95% confidence interval (CI) in an outcome variable associated with a 10% increase in urinary amino-PAH concentration were calculated.

Four sensitivity analyses were conducted in the present study. First, we conducted a sensitivity analysis by re-examining the aforementioned relationships excluding the measurements from subjects who had reported an upper respiratory infection during the 1 week prior to a clinical visit. Second, these relationships were re-examined excluding the measurements from subjects who had a urinary concentration of cotinine higher than 50 ng/mL, which has been reported as the cut point for active smokers.[46] Third, concerning air pollution exposure might confound the relationships between nitro-PAH exposures and the health outcomes, we further added personal exposure to $PM_{2.5}$, O_3, NO_2, and SO_2 as fixed-effect covariates in the models. Fourth, we tested whether the associations examined in the main analyses are modified by sex.

All statistical analyses were conducted using R software (version 4.0.2) with *rmcorr*, *lme4*, and *lmeTest* packages.[47−49] A *p*-value of 0.05 was used as the cut point for statistical significance. Detailed model results and *p*-values adjusted for multiple testing using the Benjamini−Hochberg method are shown in Table S4. A detailed description of equations for the statistical models is provided in the Supporting Information.

3. RESULTS

3.1. Participant Characteristics. The characteristics of the participants are summarized in Table 1. Of the 89 participants, 64 (71.9%) were male. The mean ± standard deviation (SD) age and BMI of the participants were 31.7 ± 7.8 years and 22.3 ± 2.7 kg/m^2, respectively. Of the 323 measurements obtained from all participants, 36 (11%) of them reported respiratory infection in the week prior to biospecimen collection.

Table 1. Characteristics of Study Participants

participant characteristics	value
age, mean ± SD [range] (year)	31.7 ± 7.8 [22−52]
male, no. (%)	64 (71.9%)
BMI, kg/m^2, mean ± SD [range]	22.3 ± 2.7 [15.9−29.4]

3.2. Urinary Amino-PAHs and Personal Air Pollutant Exposures. The urinary concentration of amino-PAHs and 24 h average personal exposure to $PM_{2.5}$, O_3, NO_2, and SO_2 are shown in Table 2. Among the five urinary amino-PAHs, 2-AF has the highest median concentration, and 9-APhe has the lowest median concentration. The repeated measure correlations among amino-PAHs are summarized in Table S3. The results show that all the amino-PAHs were positively correlated.

Table 2. Concentrations of Urinary Amino-PAHs and Cotinine and Personal Exposures to $PM_{2.5}$, O_3, NO_2, and SO_2 Averaged over the 24 h Prior to Biospecimen Collection

	mean ± SD	median (IQR)	range
Urinary Amino-PAHs and Cotinine (ng/mL)[a]			
1-AN	8.6 ± 49.3	4.1 (8.4)	0.025−865
2-AN	5.4 ± 7.1	3.6 (7.6)	0.039−56.6
9-APhe	8.1 ± 16.1	0.35 (10.1)	0.089−127
2-AF	23.3 ± 25.6	19.3 (31.7)	0.21−139
1-AP	10.0 ± 7.6	7.6 (5.7)	2.5−59.8
total amino-PAHs (Σamino-PAH)	55.5 ± 75.3	38.5 (45.8)	3.0−988
cotinine	13.5 ± 13.0	10.5 (13.5)	0.038−103
Air Pollution Exposure			
$PM_{2.5}$ (μg/m^3)	38.5 ± 30.4	26.8 (43.1)	2.0−153
O_3 (ppb)	6.8 ± 4.4	4.5 (7.3)	1.7−19.3
NO_2 (ppb)	23.6 ± 6.8	23.5 (8.6)	12.4−39.4
SO_2 (ppb)	6.3 ± 1.7	6.1 (3.1)	3.6−10.6

[a]Concentrations of urinary amino-PAHs are adjusted by specific gravity.

Table 3. Concentrations of Biomarkers

	mean ± SD	median (IQR)	range
Urinary AA Metabolites[a]			
20-HETE (ng/mL)	0.71 ± 0.92	0.50 (0.68)	0.017−7.5
8-isoprostane (ng/mL)	4.6 ± 12.3	1.8 (3.3)	0.17−188
11-dhTXB$_2$ (ng/mL)	1.5 ± 1.2	1.3 (1.1)	0.065−14.2
Systemic Oxidative Stress[a]			
urinary MDA (ng/mL)	88.3 ± 38.4	79.7 (39.7)	7.0−323
urinary 8-OHdG (ng/mL)	4.9 ± 3.0	4.2 (3.4)	0.78−19.4
Melatonin[a]			
urinary aMT6s (ng/mL)	16.6 ± 14.5	12.5 (12.3)	0.34−86.0
Platelet Activation and Endothelial Dysfunction			
plasma soluble P-selectin (pg/mL)	24.3 ± 7.5	22.9 (9.3)	10.9−60.4
plasma vWF (μg/mL)	32.3 ± 15.7	30.1 (20.5)	3.5−82.8

[a]Concentrations of urinary 20-HETE, 8-isoprostane, 11-dhTXB$_2$, MDA, 8-OHdG, and aMT6s are adjusted by specific gravity.

3.3. Associations between Amino-PAHs and AA Metabolites. Using the amino-PAH concentrations and biomarker concentrations longitudinally measured in each of the participants, we examined the relationships between

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

amino-PAHs and three AA metabolites. The results are shown in Figure 1. We found that a 10% increase in each of 1-AN, 2-AN, 9-APhe, 2-AF, 1-AP, and Σamino-PAH was associated with an increase in 20-HETE by 0.7% (0.1 to 1.3%), 0.7% (0.1 to 1.4%), 0.5% (−0.2 to 1.1%), 0.6% (−0.1 to 1.3%), 11.5% (8.9 to 14.2%), and 2.9% (1.4 to 4.3%), respectively. The results show that 10% increases in 1-AP and Σamino-PAH were associated with increases in 11-dhTXB$_2$ by 1.8% (0.4 to 3.3%) and 0.5% (−0.2 to 1.3%), respectively. Moreover, a 10% increase in each of 1-AN, 2-AN, 2-AF, and Σamino-PAH was associated with changes in 8-isoprostane by −0.7% (−1.2 to −0.1%), −0.8% (−1.5 to −0.2%), −0.6% (−1.2 to 0.01%), and −1.5% (−2.8 to −0.1%).

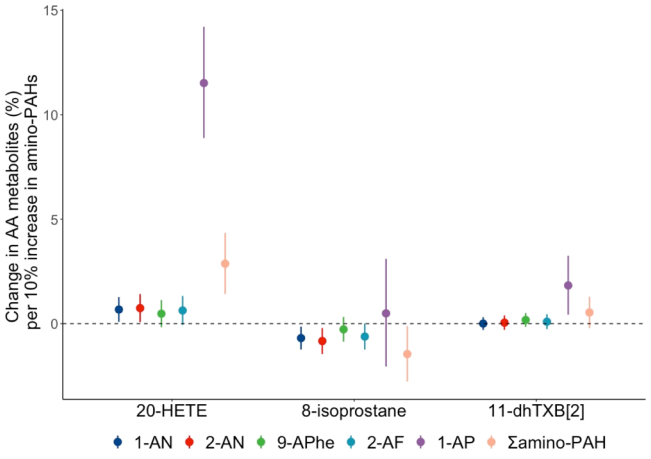


Figure 1. Estimated mean percent change and 95% CIs in AA metabolites associated with a 10% increase in amino-PAHs.

3.4. Associations between Amino-PAHs and Biomarkers of Systemic Oxidative Stress. Figure 2 shows the relationships of amino-PAHs with urinary MDA, 8-OHdG, and aMT6s. We found that a 10% increase in each of 1-AN, 2-AN, 9-APhe, 2-AF, 1-AP, and Σamino-PAH was associated with increases in 8-OHdG by 0.5% (0.2 to 0.8%), 0.6% (0.3 to 0.9%), 0.5% (0.2 to 0.8%), 0.3% (−0.1 to 0.6%), 6.1% (4.9 to 7.2%), and 1.6% (0.9 to 2.2%), respectively. In addition, a 10% increase in each of 1-AN, 2-AN, 1-AP, and Σamino-PAH was associated with changes in the aMT6s level by −0.3% (−0.6 to −0.1%), −0.4% (−0.7 to −0.1%), −1.0% (−2.3 to 0.3%), and −0.6% (−1.2 to 0.1%), respectively. However, we did not find a significant association between any of the amino-PAHs and MDA.

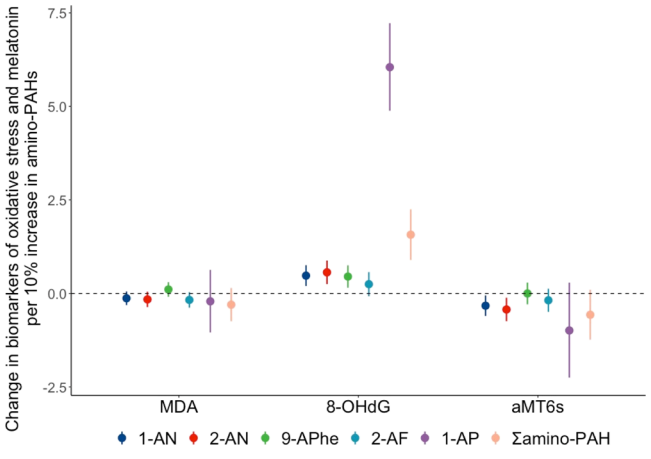


Figure 2. Estimated mean percent change and 95% CIs in biomarkers of systemic oxidative stress associated with a 10% increase in amino-PAHs.

3.5. Associations between Amino-PAHs and Biomarkers of Platelet Activation and Endothelial Dysfunction. Figure 3 shows the relationships of amino-PAHs with plasma P-selectin and vWF. The results show that a 10% increase in each of 1-AN, 2-AN, 2-AF, and Σamino-PAH was associated with increases in P-selectin by 0.2% (0.1 to 0.3%), 0.2% (0.1 to 0.3%), 0.1% (0.02 to 0.2%), and 0.3% (0.01 to 0.5%), respectively. In contrast, we did not find a significant association between vWF and any of the amino-PAHs, although the trend of a positive association appeared to be consistent across all the amino-PAHs.

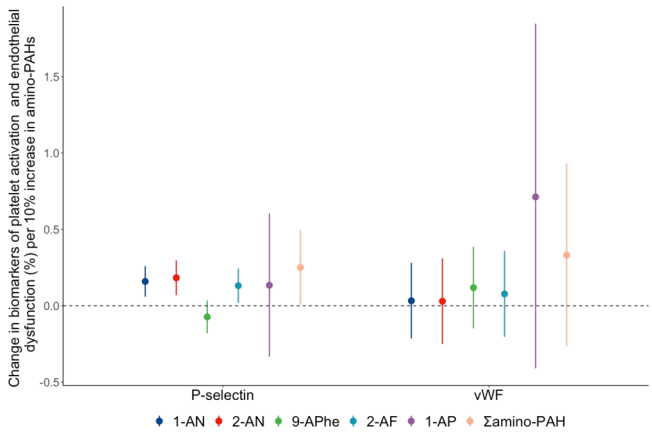


Figure 3. Estimated mean percent change and 95% CIs in biomarkers of platelet activation and endothelial dysfunction associated with a 10% increase in amino-PAHs.

3.6. Sensitivity Analysis. Four sensitivity analyses were conducted in the present study. First, we conducted a sensitivity analysis using a dataset excluding measurements from subjects who reported having an upper respiratory infection 1 week prior to biospecimen collection (36 measurements from 27 subjects). Second, the aforementioned associations were re-examined excluding measurements with urinary cotinine concentrations higher than 50 ng/mL (nine measurements from eight subjects). In these two sub-datasets, we found little differences in amino-PAH associations with all the health outcomes (Figures S1−S6). Third, adding 24 h average personal exposure to $PM_{2.5}$, O_3, NO_2, and SO_2 as fixed-effect covariates in our LMER models did not markedly change the results of the main analysis (Figures S7−S9). However, all significant associations between amino-PAHs; namely, 1-AN, 2-AF, 2-AN, and Σamino-PAH; and P-selectin became attenuated and nonsignificant in this sensitivity analysis. Fourth, the interactions between urinary amino-PAHs and sex were nonsignificant in any of the associations examined in the main analyses (Table S5).

4. DISCUSSION

It has been reported that exposure to nitro-PAHs can induce cardiac toxicity in an animal model;[12] however, human evidence is lacking whether and how nitro-PAHs exposure can adversely affect the cardiovascular system. The main findings of this study are that increased urinary concentrations

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

of amino-PAH (biomarkers of nitro-PAH exposures) were significantly associated with increased urinary 20-HETE, 11-dhTXB$_2$, and 8-OHdG and decreased 8-isoprostane and aMT6s in healthy adults. These associations were not markedly changed after controlling for air pollutant exposures in terms of the effect size and statistical significance (see Figures S7−S9). The results suggest a potential biological mechanism by which exposure to nitro-PAHs can increase oxidative stress and alter the metabolism of AA toward CYP and COX pathways. The increased levels of systemic oxidative stress and the AA metabolites, including 20-HETE and thromboxanes, have been linked to the pathogenesis of cardiovascular diseases.[18,50,51]

This is the first study that examined the relationships between urinary amino-PAHs and AA metabolites in healthy adults. We found that amino-PAHs were significantly and positively associated with 20-HETE, a metabolite of AA formed by CYP enzymes of the 4A and 4F families.[52] The results expand the previous findings of an animal study that nitro-PAH exposures altered the expression of genes related to CYP enzymes of the 1A family by suggesting that it may also alter the activity of CYP enzymes of the 4A and 4F families.[12] Future toxicological studies are needed to further explore the underlying mechanisms. It is worth noting that 20-HETE plays an important role in the regulation of vascular tone and blood pressure.[18] It promotes the endothelial dysfunction and endothelial activation, increases myogenic tone, sensitizes smooth muscle cells, and stimulates smooth muscle cell contractility, migration, and proliferation.[18,19,52] The increase of 20-HETE levels has been associated with a number of cardiovascular diseases such as hypertension, myocardial infarction, and stroke.[18,53]

AA can be metabolized through the COX pathway, which involves two major COX enzymes, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[54] TXA_2 is a metabolite of AA formed only by the COX-1 enzyme, and it is further metabolized into the inactive form 11-dhTXB$_2$. In the present study, we found that increased urinary amino-PAH concentrations were associated with the increased urinary 11-dhTXB$_2$ concentration. The results suggest that exposure to nitro-PAHs may increase the activity of the COX-1 enzyme and/or suppress the activity of COX-2. This suggestion is partially supported by a previous study finding that diesel exhaust particle extract and associated PAHs inhibited the COX-2 activity *in vitro* but do not inhibit the activity of COX-1.[55] In addition, *in vitro* exposure of rabbit platelets to pyrene and anthracene induced production of TXB_2 via a calcium carrier-dependent mechanism, supporting our findings of a particularly strong association between 1-AP and 11-dhTXB$_2$.[56] The current findings call for further investigations on the effects of nitro-PAH exposure on COX enzymes and associated health consequences. TXA_2, as a vasoconstrictor, may be an important link in the mechanism by which nitro-PAH exposures can induce cardiovascular disorders.[57] It may also be a marker of platelet activation and a subsequent procoagulant state,[58,59] which is implicated in the pathophysiology of a variety of cardiovascular disorders.[24] Although we did not observe significant associations with the procoagulation markers of soluble P-selectin and vWF, there were suggestive positive associations that suggest a need to further explore the effects of nitro-PAH exposures on hemostasis.

Several *in vivo* and *in vitro* toxicological studies have reported that exposure to nitro-PAHs can induce reactive oxygen species (ROS).[12,60,61] Specifically, ROS can be produced during the metabolism process of nitro-PAHs, and the increased ROS level may further lead to increased systemic oxidative stress.[1] In addition, according to the current findings, nitro-PAH exposures may alter the metabolism of AA through CYP and COX pathways, during which ROS and/or oxidative modulators can be further produced.[62] These findings are confirmed by the current results that increased urinary concentrations of amino-PAH were associated with increased 8-OHdG and decreased aMT6s, indicating a higher level of DNA oxidative damage and the lower antioxidant level, respectively. These results are also partially supported by a previous study finding significant and positive associations of urinary 8-OHdG with urinary 1-AN and 2-AN in a group of 95 truck terminal workers.[27] In addition, melatonin is a potent antioxidant and can stimulate antioxidant enzymes, and it is a key endogenous factor in limiting oxidative damage.[32,33] Melatonin can also modulate many biological pathways related to the cardiovascular function.[63,64] A lower circulating melatonin level has been associated with a higher risk of cardiovascular disease, including hypertension, congestive heart failure, and coronary heart disease.[65,66] The nitro-PAHs-induced increase in systemic oxidative stress and decrease in circulating melatonin level might be an important mechanism leading to adverse cardiovascular effects.

However, in the present study, the results show that increasing levels of amino-PAHs were significantly associated with decreasing concentrations of 8-isoprostane. As a metabolite of AA through the nonenzymatic pathway, 8-isoprostane is produced by the interaction of AA with ROS. The current result suggests that nitro-PAH exposures may alter the metabolism of AA toward the CYP and COX pathways, leading to increased 20-HETE and 11-dhTXB$_2$ and decreased 8-isoprostane. In addition, this change in AA metabolism could be a potential explanation for the nonsignificant relationships between urinary concentrations of amino-PAHs and MDA. Specifically, MDA can be produced by the metabolism of AA through a nonenzymatic pathway during lipid peroxidation and through an enzymatic pathway during the biosynthesis of TXA_2 (COX pathway).[67] Therefore, nitro-PAH exposures might have lowered MDA production by suppressing the AA nonenzymatic pathway and increased MDA production through upregulating the COX pathway. These opposite effects might have led to nonsignificant associations between urinary amino-PAHs and MDA. In addition to CYP, COX, and nonenzymatic pathways, AA can be also metabolized though the lipoxygenase (LOX) pathway, which is not measured in this study. It has been previously reported that increased PAH exposures were associated with increased LOX activity in a group of 26 nonsmoking healthy adults.[68] Whether and how nitro-PAHs exposures may affect AA metabolism through the LOX pathway needs to be further investigated.

People can be exposed to nitro-PAHs from different sources.[69,70] For example, it has been reported that 1-nitronaphthalene and 2-nitronaphthalene are largely produced through smoking,[71] while 1-nitropyrene, 2-nitrofluorene, and 9-nitrophenanthrene are mainly found in diesel exhaust.[72−74] In this study, 1-AP has a particularly strong association with 20-HETE, 11-dhTXB$_2$, 8-OHdG, and aMT6s. In addition, 1-AN and 2-AN are significantly associated with 20-HETE, 8-OHdG, and aMT6s. The results confirm previous findings that exposure to diesel emissions and smoking can adversely affect the cardiovascular system.[75,76]

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

Four sensitivity analyses were conducted in this study. First, given that respiratory infection might affect AA metabolism, systemic oxidative stress, and vascular function, a sensitivity analysis was conducted by re-examining the aforementioned relationships when the measurements from subjects who had reported an upper respiratory infection were excluded. Second, concerning active smokers might expose to PAHs and other air pollutants not measured in this study, we conducted another sensitivity analysis to examine the associations between amino-PAHs and the health outcomes excluding measurements from active smokers (urinary cotinine > 50 ng/mL). Third, a copollutant analysis was conducted by controlling for personal exposure to $PM_{2.5}$, O_3, NO_2, and SO_2 as fixed-effect covariates in the models. The results of these sensitivity analyses do not markedly change the observed associations in the main analyses supporting the robustness of our main results. We only found that after controlling for personal air pollutant exposure in the LMER models, the associations between amino-PAHs and P-selectin were changed to be nonsignificant, and the effect sizes were also decreased. The results indicate that air pollution exposure is a potential confounder for these relationships, warranting future studies to further investigate the health effects of nitro-PAH on hemostasis. Fourth, the nonsignificant interaction terms between sex and amino-PAHs suggest that sex may not modify the associations examined in the main analyses (see Table S5). However, this result should be cautiously interpreted, concerning the unbalanced sex distribution (only 25% of the participants were female) in this study of a relatively small sample size.

The strengths of this study include a well-controlled population with little variability in potential confounders, detailed assessment of and adjusting for air pollutant coexposures, and repeated biofluid assessments measuring a variety of relevant biomarkers. Specifically, the collection of multiple urine samples that were assessed for contemporaneous biomarkers is a strength which should reduce the impact of potential exposure misclassification due to the acute nature of nitro-PAH metabolism. Also, these samples were all collected at the same time of the day, reducing the impacts of daily variation patterns in urinary metabolism and nitro-PAH acute exposures. On the other hand, this study has some limitations including a limited sample size, a homogeneous population not necessarily representative of the local region or generalizable to other regions, and limited controlling for potential sources of within-individual variation, such as the alcohol intake and medication usage. Another limitation is that although we were able to control for criteria air pollutant coexposures, other forms of PAH metabolites that could potentially confound our results were not able to be controlled. In addition, we used microenvironmental concentrations and time-activity data to calculate time-weighted personal pollutant exposures. Although this method is less accurate than personal monitoring, it is more accurate than simply using concentrations of ambient pollutants.

In summary, increased urinary concentrations of amino-PAH were associated with increased 20-HETE, $11dhTXB_2$, and 8-OHdG and decreased 8-isoprostane and aMT6s. The results suggested that exposure to nitro-PAHs may increase systemic oxidative stress and alter AA metabolism toward CYP and COX pathways. Given that the oxidative metabolism of AA plays a vital role in modulating systemic oxidative stress, vascular tone, and thrombosis, our findings provide the first evidence in humans suggesting a potential pathophysiologic mechanism by which nitro-PAHs may adversely affect cardiovascular health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c08150.

Description of the HPLC-MS/MS method, statistical analysis, model results, correlations among urinary amino-PAHs and cotinine, and sensitivity analysis results (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Junfeng Jim Zhang − *Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States; Global Health Institute, Duke University, Durham, North Carolina 27708, United States; Duke Kunshan University, Kunshan City, Jiangsu Province 215316, China;* orcid.org/0000-0003-3759-6672; Phone: (919) 681-7782; Email: junfeng.zhang@duke.edu; Fax: (919) 613-8061

Authors

Linchen He − *Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States; Global Health Institute, Duke University, Durham, North Carolina 27708, United States;* orcid.org/0000-0002-3559-9530

Yan Lin − *Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States; Global Health Institute, Duke University, Durham, North Carolina 27708, United States*

Drew Day − *Seattle Children's Research Institute, Seattle, Washington 98121, United States*

Yanbo Teng − *Duke Kunshan University, Kunshan City, Jiangsu Province 215316, China*

Xiangtian Wang − *Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States*

Xing Lucy Liu − *Global Health Institute, Duke University, Durham, North Carolina 27708, United States*

Erik Yan − *Global Health Institute, Duke University, Durham, North Carolina 27708, United States; Duke Kunshan University, Kunshan City, Jiangsu Province 215316, China*

Jicheng Gong − *BIC-ESAT and SKL-ESPC, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China; Center for Environment and Health, Peking University, Beijing 100871, China*

Jian Qin − *Guangxi Medical University, Nanning, Guangxi Province 530021, China*

Xiaoli Wang − *Tianjin University of Technology, Tianjin 300384, China*

Jianbang Xiang − *Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington 98195, United States;* orcid.org/0000-0001-5196-2574

Jinhan Mo − *Department of Building Science, Tsinghua University, Beijing 100084, China; Beijing Key Laboratory of Indoor Air Quality Evaluation and Control, Beijing 100084, China;* orcid.org/0000-0002-3178-6507

Yinping Zhang − *Department of Building Science, Tsinghua University, Beijing 100084, China; Beijing Key Laboratory of*

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

Indoor Air Quality Evaluation and Control, Beijing 100084, China; orcid.org/0000-0001-9175-7890

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.0c08150

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The original sample collection was supported by the National Natural Science Foundation of China (nos. 51420105010 and 51521005). L.H. was supported by a scholarship from the Duke Global Health Institute to conduct the current analysis.

■ REFERENCES

(1) Bandowe, B. A. M.; Meusel, H. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment−a review. *Sci. Total Environ.* **2017**, *581*−*582*, 237−257.

(2) Gong, J.; Zhu, T.; Kipen, H.; Rich, D. Q.; Huang, W.; Lin, W.-T.; Hu, M.; Zhang, J. Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers of exposure to traffic-emitted pollutants. *Environ. Int.* **2015**, *85*, 104−110.

(3) Bamford, H. A.; Baker, J. E. Nitro-polycyclic aromatic hydrocarbon concentrations and sources in urban and suburban atmospheres of the Mid-Atlantic region. *Atmos. Environ.* **2003**, *37*, 2077−2091.

(4) Lin, Y.; Qiu, X.; Ma, Y.; Ma, J.; Zheng, M.; Shao, M. Concentrations and spatial distribution of polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs (NPAHs) in the atmosphere of North China, and the transformation from PAHs to NPAHs. *Environ. Pollut.* **2015**, *196*, 164−170.

(5) Lin, Y.; Ma, Y.; Qiu, X.; Li, R.; Fang, Y.; Wang, J.; Zhu, Y.; Hu, D. Sources, transformation, and health implications of PAHs and their nitrated, hydroxylated, and oxygenated derivatives in PM2. 5 in Beijing. *J. Geophys. Res. Atmos.* **2015**, *120*, 7219−7228.

(6) Lin, Y.; Qiu, X.; Ma, Y.; Wang, J.; Wu, Y.; Zeng, L.; Hu, M.; Zhu, T.; Zhu, Y. A novel approach for apportionment between primary and secondary sources of airborne nitrated polycyclic aromatic hydrocarbons (NPAHs). *Atmos. Environ.* **2016**, *138*, 108−113.

(7) Zhuo, S.; Du, W.; Shen, G.; Li, B.; Liu, J.; Cheng, H.; Xing, B.; Tao, S. Estimating relative contributions of primary and secondary sources of ambient nitrated and oxygenated polycyclic aromatic hydrocarbons. *Atmos. Environ.* **2017**, *159*, 126−134.

(8) Arey, J.; Atkinson, R. *PAHs: An Ecotoxicological Perspective*; John Wiley & Sons: Hoboken, U.S.A., 2003.

(9) Möller, L.; Lax, I.; Eriksson, L. C. Nitrated polycyclic aromatic hydrocarbons: a risk assessment for the urban citizen. *Environ. Health Perspect.* **1993**, *101*, 309−315.

(10) Huang, L.; Chernyak, S. M.; Batterman, S. A. PAHs (polycyclic aromatic hydrocarbons), nitro-PAHs, and hopane and sterane biomarkers in sediments of southern Lake Michigan, USA. *Sci. Total Environ.* **2014**, *487*, 173−186.

(11) Shen, G.; Tao, S.; Wei, S.; Zhang, Y.; Wang, R.; Wang, B.; Li, W.; Shen, H.; Huang, Y.; Chen, Y.; Chen, H.; Yang, Y.; Wang, W.; Wang, X.; Liu, W.; Simonich, S. L. M. Emissions of parent, nitro, and oxygenated polycyclic aromatic hydrocarbons from residential wood combustion in rural China. *Environ. Sci. Technol.* **2012**, *46*, 8123−8130.

(12) Chlebowski, A. C.; Garcia, G. R.; La Du, J. K.; Bisson, W. H.; Truong, L.; Massey Simonich, S. L.; Tanguay, R. L. Mechanistic investigations into the developmental toxicity of nitrated and heterocyclic PAHs. *Toxicol. Sci.* **2017**, *157*, 246−259.

(13) Fleming, I. Cytochrome p450 and vascular homeostasis. *Circ. Res.* **2001**, *89*, 753−762.

(14) Elbekai, R. H.; El-Kadi, A. O. S. Cytochrome P450 enzymes: central players in cardiovascular health and disease. *Pharmacol. Ther.* **2006**, *112*, 564−587.

(15) Hunter, A. L.; Cruz, R. P.; Cheyne, B. M.; McManus, B. M.; Granville, D. J. Cytochrome p450 enzymes and cardiovascular disease. *Can. J. Physiol. Pharmacol.* **2004**, *82*, 1053−1060.

(16) Rao, G. H. R.; White, J. G. Role of arachidonic acid metabolism in human platelet activation and irreversible aggregation. *Am. J. Hematol.* **1985**, *19*, 339−347.

(17) Nakamura, Y.; Kozuka, M.; Naniwa, K.; Takabayashi, S.; Torikai, K.; Hayashi, R.; Sato, T.; Ohigashi, H.; Osawa, T. Arachidonic acid cascade inhibitors modulate phorbol ester-induced oxidative stress in female ICR mouse skin: differential roles of 5-lipoxygenase and cyclooxygenase-2 in leukocyte infiltration and activation. *Free Radic. Biol. Med.* **2003**, *35*, 997−1007.

(18) Rocic, P.; Schwartzman, M. L. 20-HETE in the regulation of vascular and cardiac function. *Pharmacol. Ther.* **2018**, *192*, 74−87.

(19) Gross, G.; Falck, J.; Gross, E.; Isbell, M.; Moore, J.; Nithipatikom, K. Cytochrome P450 and arachidonic acid metabolites: role in myocardial ischemia/reperfusion injury revisited. *Cardiovasc. Res.* **2005**, *68*, 18−25.

(20) Janssen, L. J. Isoprostanes and lung vascular pathology. *Am. J. Respir. Cell Mol. Biol.* **2008**, *39*, 383−389.

(21) Reilly, M.; Fitzgerald, G. Cellular activation by thromboxane A2 and other eicosanoids. *Eur. Heart J.* **1993**, *14*, 88−93.

(22) Tomaiuolo, M.; Brass, L. F.; Stalker, T. J. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. *Interv. Cardiol. Clin.* **2017**, *6*, 1.

(23) Davì, G.; Santilli, F.; Vazzana, N. *Thromboxane Receptors Antagonists And/or Synthase Inhibitors*; Springer: Berlin, Germany, 2012.

(24) Raskob, G. E.; Angchaisuksiri, P.; Blanco, A. N.; Buller, H.; Gallus, A.; Hunt, B. J.; Hylek, E. M.; Kakkar, A.; Konstantinides, S. V.; McCumber, M.; Ozaki, Y.; Wendelboe, A.; Weitz, J. I. Thrombosis. *Arterioscler., Thromb., Vasc. Biol.* **2014**, *34*, 2363−2371.

(25) Day, D. B.; Xiang, J.; Mo, J.; Li, F.; Chung, M.; Gong, J.; Weschler, C. J.; Ohman-Strickland, P. A.; Sundell, J.; Weng, W.; Zhang, Y.; Zhang, J. Association of ozone exposure with cardiorespiratory pathophysiologic mechanisms in healthy adults. *JAMA Intern. Med.* **2017**, *177*, 1344−1353.

(26) Day, D. B.; Xiang, J.; Mo, J.; Clyde, M. A.; Weschler, C. J.; Li, F.; Gong, J.; Chung, M.; Zhang, Y.; Zhang, J. Combined use of an electrostatic precipitator and a high-efficiency particulate air filter in building ventilation systems: Effects on cardiorespiratory health indicators in healthy adults. *Indoor Air* **2018**, *28*, 360−372.

(27) Neophytou, A.; Hart, J.; Chang, Y.; Zhang, J.; Smith, T.; Garshick, E.; Laden, F. Short-term traffic-related exposures and biomarkers of nitro-PAH exposure and oxidative DNA damage. *Toxics* **2014**, *2*, 377−390.

(28) Gong, J.; Zhu, T.; Kipen, H.; Wang, G.; Hu, M.; Ohman-Strickland, P.; Lu, S.-E.; Zhang, L.; Wang, Y.; Zhu, P.; Rich, D. Q.; Diehl, S. R.; Huang, W.; Zhang, J. Malondialdehyde in exhaled breath condensate and urine as a biomarker of air pollution induced oxidative stress. *J. Exposure Sci. Environ. Epidemiol.* **2013**, *23*, 322.

(29) He, L.; Cui, X.; Xia, Q.; Li, F.; Mo, J.; Gong, J.; Zhang, Y.; Zhang, J. Effects of personal air pollutant exposure on oxidative stress: Potential confounding by natural variation in melatonin levels. *Int. J. Hyg. Environ. Health* **2020**, *223*, 116−123.

(30) Basler, M.; Jetter, A.; Fink, D.; Seifert, B.; Kullak-Ublick, G. A.; Trojan, A. Urinary excretion of melatonin and association with breast cancer: meta-analysis and review of the literature. *Breast Care* **2014**, *9*, 182−187.

(31) He, L.; Lin, Y.; Wang, X.; Liu, X.; Wang, Y.; Qin, J.; Wang, X.; Day, D.; Xiang, J.; Mo, J.; Zhang, Y.; Zhang, J. Associations of ozone exposure with urinary metabolites of arachidonic acid. *Environ. Int.* **2020**, *145*, 106154.

(32) Arendt, J. Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. *Rev. Reprod.* **1998**, *3*, 13−22.

(33) Reiter, R. J.; Mayo, J. C.; Tan, D.-X.; Sainz, R. M.; Alatorre-Jimenez, M.; Qin, L. Melatonin as an antioxidant: under promises but over delivers. *J. Pineal Res.* **2016**, *61*, 253−278.

(34) He, L.; Norris, C.; Cui, X.; Li, Z.; Barkjohn, K. K.; Teng, Y.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M. H. Role of Endogenous Melatonin in Pathophysiologic and Oxidative Stress Responses to Personal Air Pollutant Exposures in Asthmatic Children. *Science of The Total Environment* **2021**, *773*, 145709.
(35) He, L.; Liu, X.; Zhang, J. Simultaneous quantification of urinary 6-sulfatoxymelatonin and 8-hydroxy-2′-deoxyguanosine using liquid chromatography-tandem mass spectrometry. *J. Chromatogr., B* **2018**, *1095*, 119−126.
(36) Chen, C.; Zhao, B. Review of relationship between indoor and outdoor particles: I/O ratio, infiltration factor and penetration factor. *Atmos. Environ.* **2011**, *45*, 275−288.
(37) Weschler, C. J. Ozone in indoor environments: concentration and chemistry. *Indoor Air* **2000**, *10*, 269−288.
(38) He, L.; Cui, X.; Li, Z.; Teng, Y.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M. H.; Zhang, J. J. Malondialdehyde in Nasal Fluid: A Biomarker for Monitoring Asthma Control in Relation to Air Pollution Exposure. *Environ. Sci. Technol.* **2020**, *54*, 11405−11413.
(39) Cui, X.; Li, Z.; Teng, Y.; Barkjohn, K. K.; Norris, C. L.; Fang, L.; Daniel, G. N.; He, L.; Lin, L.; Wang, Q.; Day, D. B.; Zhou, X.; Hong, J.; Gong, J.; Li, F.; Mo, J.; Zhang, Y.; Schauer, J. J.; Black, M. S.; Bergin, M. H.; Zhang, J. Association Between Bedroom Particulate Matter Filtration and Changes in Airway Pathophysiology in Children With Asthma. *AMA Pediatr.* **2020**, *174*, 533−542.
(40) Nazaroff, W. W.; Gadgil, A. J.; Weschler, C. J. *Modeling of Indoor Air Quality and Exposure*; ASTM International: West Conshohocken, U.S.A., 1993.
(41) Yocom, J. E. A Critical Review. *J. Air Pollut. Contr. Assoc.* **1982**, *32*, 500−520.
(42) He, L.; Li, Z.; Teng, Y.; Cui, X.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M. H.; Zhang, J. Associations of personal exposure to air pollutants with airway mechanics in children with asthma. *Environ. Int.* **2020**, *138*, 105647.
(43) Cui, X.; Li, F.; Xiang, J.; Fang, L.; Chung, M. K.; Day, D. B.; Mo, J.; Weschler, C. J.; Gong, J.; He, L.; Zhu, D.; Lu, C.; Han, H.; Zhang, Y.; Zhang, J. Cardiopulmonary effects of overnight indoor air filtration in healthy non-smoking adults: A double-blind randomized crossover study. *Environ. Int.* **2018**, *114*, 27−36.
(44) He, L.; Hu, X.; Gong, J.; Day, D.; Xiang, J.; Mo, J.; Zhang, Y.; Zhang, J. Endogenous melatonin mediation of systemic inflammatory responses to ozone exposure in healthy adults. *Sci. Total Environ.* **2020**, *749*, 141301.
(45) Hu, X.; He, L.; Zhang, J.; Qiu, X.; Zhang, Y.; Mo, J.; Day, D. B.; Xiang, J.; Gong, J. Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure. *Environ. Pollut.* **2020**, *263*, 114503.
(46) Kim, S. Overview of cotinine cutoff values for smoking status classification. *Int. J. Environ. Res. Publ. Health* **2016**, *13*, 1236.
(47) Bates, D.; Maechler, M.; Bolker, B.; Walker, S.; Christensen, R. H. B.; Singmann, H.; Dai, B. *Package 'lme4'*, 2015.
(48) Kuznetsova, A.; Brockhoff, P. B.; Christensen, R. H. B. *Package 'lmertest'*, 2015.
(49) Bakdash, J. Z.; Marusich, L. R. Repeated measures correlation. *Front. Psychol.* **2017**, *8*, 456.
(50) Waldman, M.; Peterson, S. J.; Arad, M.; Hochhauser, E. The role of 20-HETE in cardiovascular diseases and its risk factors. *Prostaglandins Other Lipid Mediators* **2016**, *125*, 108−117.
(51) Smyth, E. M. Thromboxane and the thromboxane receptor in cardiovascular disease. *Clin. Lipidol.* **2010**, *5*, 209−219.
(52) Wu, C.-C.; Gupta, T.; Garcia, V.; Ding, Y.; Schwartzman, M. L. 20-HETE and Blood Pressure Regulation. *Cardiol. Rev.* **2014**, *22*, 1.
(53) Williams, J. M.; Murphy, S.; Burke, M.; Roman, R. J. 20-Hydroxyeicosatetraeonic Acid: A New Target for the Treatment of Hypertension. *J. Cardiovasc. Pharmacol.* **2010**, *56*, 336.
(54) Choi, S.-H.; Aid, S.; Bosetti, F. The distinct roles of cyclooxygenase-1 and-2 in neuroinflammation: implications for translational research. *Trends Pharmacol. Sci.* **2009**, *30*, 174−181.
(55) Rudra-Ganguly, N.; Reddy, S. T.; Korge, P.; Herschman, H. R. Diesel exhaust particle extracts and associated polycyclic aromatic hydrocarbons inhibit Cox-2-dependent prostaglandin synthesis in murine macrophages and fibroblasts. *J. Biol. Chem.* **2002**, *277*, 39259−39265.
(56) Yamazaki, H.; Imamura, E.; Kamei, S.; Yamauchi, A.; Kakiuchi, Y.; Tai, H.-H. Polycyclic aromatic hydrocarbons affect the calcium ionophore induced activation of rabbit platelet. *Chemosphere* **1990**, *21*, 21−28.
(57) Schrör, K. Thromboxane A2 and platelets as mediators of coronary arterial vasoconstriction in myocardial ischaemia. *Eur. Heart J.* **1990**, *11*, 27−34.
(58) Théorêt, J.-F.; Yacoub, D.; Hachem, A.; Gillis, M.-A.; Merhi, Y. P-selectin ligation induces platelet activation and enhances micro-aggregate and thrombus formation. *Thromb. Res.* **2011**, *128*, 243−250.
(59) FitzGerald, G. A. Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. *Am. J. Cardiol.* **1991**, *68*, B11−B15.
(60) Nagy, E.; Adachi, S.; Takamura-Enya, T.; Zeisig, M.; Möller, L. DNA adduct formation and oxidative stress from the carcinogenic urban air pollutant 3-nitrobenzanthrone and its isomer 2-nitro-benzanthrone, in vitro and in vivo. *Mutagenesis* **2007**, *22*, 135−145.
(61) Shang, Y.; Zhou, Q.; Wang, T.; Jiang, Y.; Zhong, Y.; Qian, G.; Zhu, T.; Qiu, X.; An, J. Airborne nitro-PAHs induce Nrf2/ARE defense system against oxidative stress and promote inflammatory process by activating PI3K/Akt pathway in A549 cells. *Toxicol. in Vitro* **2017**, *44*, 66−73.
(62) Speed, N.; Blair, I. A. Cyclooxygenase-and lipoxygenase-mediated DNA damage. *Canc. Metastasis Rev.* **2011**, *30*, 437−447.
(63) Thakor, A. S.; Allison, B. J.; Niu, Y.; Botting, K. J.; Serón-Ferré, M.; Herrera, E. A.; Giussani, D. A. Melatonin modulates the fetal cardiovascular defense response to acute hypoxia. *J. Pineal Res.* **2015**, *59*, 80−90.
(64) Reiter, R. J.; Tan, D.-X.; Paredes, S. D.; Fuentes-Broto, L. Beneficial effects of melatonin in cardiovascular disease. *Ann. Med.* **2010**, *42*, 276−285.
(65) Imenshahidi, M.; Karimi, G.; Hosseinzadeh, H. Effects of melatonin on cardiovascular risk factors and metabolic syndrome: a comprehensive review. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2020**, *393*, 521−536.
(66) Grossman, E. Should melatonin be used to lower blood pressure? *Hypertens. Res.* **2013**, *36*, 682−683.
(67) Ayala, A.; Muñoz, M. F.; Argüelles, S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longevity* **2014**, *2014*, 1.
(68) Lin, Y.; Ramanathan, G.; Zhu, Y.; Yin, F.; Rea, N. D.; Lu, X.; Tseng, C.-H.; Faull, K. F.; Yoon, A. J.; Jerrett, M.; Zhu, T.; Qiu, X.; Araujo, J. A. Pro-Oxidative and Proinflammatory Effects After Traveling From Los Angeles to Beijing: A Biomarker-Based Natural Experiment. *Circulation* **2019**, *140*, 1995−2004.
(69) Chen, Y.; Du, W.; Shen, G.; Zhuo, S.; Zhu, X.; Shen, H.; Huang, Y.; Su, S.; Lin, N.; Pei, L.; Zheng, X.; Wu, J.; Duan, Y.; Wang, X.; Liu, W.; Wong, M.; Tao, S. Household air pollution and personal exposure to nitrated and oxygenated polycyclic aromatics (PAH s) in rural households: Influence of household cooking energies. *Indoor Air* **2017**, *27*, 169−178.
(70) Zhuo, S.; Du, W.; Shen, G.; Wang, R.; Pan, X.; Li, T.; Han, Y.; Li, Y.; Pan, B.; Peng, X.; Cheng, H.; Wang, X.; Shi, G.; Xing, B.; Tao, S. Urban air pollution and health risks of parent and nitrated polycyclic aromatic hydrocarbons in two megacities, southwest China. *Atmos. Environ.* **2017**, *166*, 441−453.
(71) Havey, C. D.; Dane, A. J.; Abbas-Hawks, C.; Voorhees, K. J. Detection of nitro-polycyclic aromatic hydrocarbons in mainstream and sidestream tobacco smoke using electron monochromator-mass spectrometry. *Environ. Chem. Lett.* **2009**, *7*, 331−336.

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875

(72) Toriba, A.; Kitaoka, H.; Dills, R. L.; Mizukami, S.; Tanabe, K.; Takeuchi, N.; Ueno, M.; Kameda, T.; Tang, N.; Hayakawa, K.; Simpson, C. D. Identification and quantification of 1-nitropyrene metabolites in human urine as a proposed biomarker for exposure to diesel exhaust. *Chem. Res. Toxicol.* **2007**, *20*, 999−1007.
(73) Wheelock, K.; Zhang, J.; McConnell, R.; Tang, D.; Volk, H. E.; Wang, Y.; Herbstman, J. B.; Wang, S.; Phillips, D. H.; Camann, D.; Gong, J.; Perera, F. A novel method for source-specific hemoglobin adducts of nitro-polycyclic aromatic hydrocarbons. *Environ. Sci.: Processes Impacts* **2018**, *20*, 780−789.
(74) Jesus, R. d.; Mosca, A. C.; Guarieiro, A. L.; Rocha, G. O. d.; Andrade, J. B. d. In vitro Evaluation of Oxidative Stress Caused by Fine Particles (PM2. 5) Exhausted from Heavy-Duty Vehicles Using Diesel/Biodiesel Blends under Real World Conditions. *J. Braz. Chem. Soc.* **2018**, *29*, 1268−1277.
(75) Sydbom, A.; Blomberg, A.; Parnia, S.; Stenfors, N.; Sandström, T.; Dahlén, S.-E. Health effects of diesel exhaust emissions. *Eur. Respir. J.* **2001**, *17*, 733−746.
(76) Banks, E.; Joshy, G.; Korda, R. J.; Stavreski, B.; Soga, K.; Egger, S.; Day, C.; Clarke, N. E.; Lewington, S.; Lopez, A. D. Tobacco smoking and risk of 36 cardiovascular disease subtypes: fatal and non-fatal outcomes in a large prospective Australian study. *BMC Med.* **2019**, *17*, 128.

https://dx.doi.org/10.1021/acs.est.0c08150
Environ. Sci. Technol. 2021, 55, 3867−3875