



Passive smoking and urinary oxidative biomarkers: A pilot study of healthy travelers from Los Angeles to Beijing

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ABSTRACT

There is a great heterogeneity in smoking prevalence and tobacco control policy across different countries. However, it is unknown whether this heterogeneity could cause increased passive smoking and adverse health effects among international travelers. In this pilot study, we collected 190 urine samples from 26 Los Angeles residents before (LA-before), during (Beijing), and after (LA-after) a 10-week visit to Beijing to measure biomarkers of passive smoking (cotinine), exposure to polycyclic aromatic hydrocarbons (OH-PAHs), and oxidative stress (malondialdehyde, 8-isoprostane, and uric acid). The geometric mean concentrations of urinary cotinine were 0.14, 1.52, and 0.22 $\mu\text{g/g}$ creatinine in LA-before, Beijing, and LA-after, respectively. Likewise, OH-PAH levels were significantly higher in Beijing as compared to LA-before or LA-after, in association with the urinary cotinine levels. One-fold increase in urinary cotinine levels was associated with 10.1% (95% CI: 5.53–14.8%), 8.75% (95% CI: 2.33–15.6%), and 25.4% (95% CI: 13.1–39.1%) increases in urinary levels of malondialdehyde, 8-isoprostane, and uric acid, respectively. OH-PAHs mediated 9.1–23.3% of the pro-oxidative effects associated with passive smoking. Taken together, our findings indicate that traveling to a city with higher smoking prevalence may increase passive smoking exposure, in association with pro-oxidative effects partially mediated by PAHs.

1. Introduction

Active smoking caused 6.4 million deaths worldwide in 2015 with cardiovascular diseases as the primary cause (GBD 2015 Tobacco Collaborators, 2017). Passive smoking, which refers to involuntary exposures to tobacco smoke that were either exhaled by smokers (secondhand smoke, SHS) or adhered to the surfaces of clothing, hair, furnishings, and dust (thirdhand smoke, THS) (Sleiman et al., 2010), is thought to induce cardiovascular effects nearly as large as active smoking (Barnoya and Glantz, 2005), and likely to impact adversely a greater portion of the population beyond smokers. It has been estimated that in 2004, more than 1/3 of global population was exposed to SHS at home or workplaces, which caused 603,000 deaths; 379,000 of which

were due to ischemic heart disease (Öberg et al., 2011). Remarkably, substantial heterogeneity in SHS exposures across countries has been documented, with higher exposures in developing countries (Centers for Disease Control and Prevention (CDC), 2007; Öberg et al., 2011; Warren et al., 2011), likely due to higher smoking prevalence and the lack of regulatory policies (King et al., 2013; Thomas et al., 2008). It is less understood, however, whether this heterogeneity could lead to increased exposures to SHS, and subsequent adverse health effects among international travelers who usually spend short time at their destinations.

Previous studies have shown that even brief (minutes to hours) passive smoking could markedly perturb the cardiovascular system with increased oxidative stress as one of the underlying mechanisms

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(Barnoya and Glantz, 2005), which is largely attributed to its content of a large number of toxic redox active components. To date, at least 90 toxic components have been identified in SHS with biological half-lives ranging from milliseconds (free radicals) to years (cadmium) (Brewer et al., 2017; Talhout et al., 2011), some of which could react with gaseous pollutants, leading to the formation of even more toxic products in THS (Sleiman et al., 2010). Of note, polycyclic aromatic hydrocarbons (PAHs), a group of toxic byproducts of incomplete combustions, are of high abundance in tobacco smoke and thought to exert significant effects on the health of passive smokers. However, concrete evidence is lacking to what extent the adverse health effects of passive smoking could be attributable to PAHs exposure.

The United States (U.S.) and China are among the world's most populous countries, with marked differences in the exposure to tobacco smoke. The number of residences traveling between both countries increased rapidly in recent years, reaching 1.31 and 2.59 million in the U.S and China in 2015, respectively (Yearbook of Tourism Statistics, 2018). In our previous studies, we have identified a marked increase in personal exposures to PAHs among healthy young adults who traveled from Los Angeles to Beijing (Lin et al., 2016, 2019, 2021), a city with higher smoking prevalence, population density, and air pollution levels (Los Angeles County Department of Public Health, 2010; Zhang et al., 2016). Furthermore, we observed significant increases in the circulating levels of a panel of oxidative biomarkers, after the participants spent 6–8 weeks in Beijing, which reversed almost completely 4–7 weeks after the participants returned to Los Angeles, and were positively associated with urinary levels of PAHs metabolites (Lin et al., 2019; Lu et al., 2021). Importantly, typical oxidative stress markers have been associated with all major cardiovascular diseases (Daiber et al., 2021). However, our panel of lipid oxidation products in the blood failed to associate with urinary cotinine levels, suggesting that the observed effects were likely due to other sources of PAHs such as air pollution (Lin et al., 2019; Lu et al., 2021) or that other biomarkers would be required to reflect pro-oxidative effects potentially induced by SHS. Therefore, the health effect of increased passive smoking exposures during the travel remained largely unknown.

In the current study, we studied this group of travelers and focused on urinary oxidative biomarkers such as 8-isoprostane, malondialdehyde (MDA) and uric acid, which were measured in 190 urine samples, repeatedly collected from 26 healthy nonsmoking participants. This study aims to determine (i) whether changes in passive smoking exposures during the travel were associated with urinary biomarkers of oxidative stress; and (ii) to what extent the oxidative effect of passive smoking exposure was mediated by PAHs exposure.

2. Methods

Study Participants. The study is based on a summer research program between Peking University (PKU) and University of California, Los Angeles (UCLA), in which students from UCLA visit PKU and stay there for 10 weeks in each summer. All participants in this study ($n = 26$) were healthy non-smoking adults recruited in 2014 or 2015 with details described previously (Lin et al., 2019). Demographic information and written informed consent were obtained from all participants with study purpose and risk explained. The study was performed in accordance with guidelines and approval of the Institutional Review Board of both UCLA (Protocol# IRB17-001812) and PKU (Protocol# IRB00001052-14037).

Sample Collection. For each participant, up to nine urine samples were collected before the travel (LA-before), in Beijing (2–10 weeks after arrival in Beijing), and 4–10 weeks after returning to Los Angeles (LA-after). Because PAHs and nicotine are metabolized and excreted rapidly with a half-life less than one day (Benowitz, 1996; St.Helen et al., 2012), each urine was collected at least one week after the previous collection. Each urine collection was coupled with a questionnaire survey that included time spent near smokers in the past three days, and various

activities related to PAHs exposure in the past three days, such as (i) cooking behaviors (time for cooking and barbecuing), (ii) dietary intakes of food rich in PAHs (consumption of barbecue or baked food), and (iii) traffic-related behaviors (driving hours, public transportation usage, and time spent near heavy traffic). All the urine samples were collected using 90-mL polypropylene specimen containers in the morning after fasting for at least 8 h. The samples were aliquoted to 15-mL polypropylene centrifuge tubes and were transported through flight (~12 h) with dry ice. All the urine samples were stored at -20 Celsius and kept away from light until laboratory analysis.

Laboratory Analysis. We used a gas chromatograph and mass spectrometer (GC-MS; Agilent 7890A-5975C) with an isotope dilution method to measure the concentration of cotinine and 11 hydroxylated PAHs in the urine including 1- and 2-hydroxylated naphthalenes (OH-NAPs), 2-hydroxylated dibenzofuran (2-OH-DBF), 2- and 3-hydroxylated fluorenes (OH-FLUs), 1-, 2-, 3-, 4- and 9-hydroxylated phenanthrenes (OH-PHEs), and 1-hydroxylated pyrene (1-OH-PYR). To determine urinary cotinine, 0.5 mL urine was spiked with deuterium-labeled (+/–)cotinine and alkalized with 0.5 mL NaOH (4M)/KCl (1M) solution. The aliquant was then extracted with 2 mL dichloromethane four times and concentrated under nitrogen flow for instrumental analysis (Lin et al., 2019). To determine urinary OH-PAHs, after spiked with deuterium- or ^{13}C - labeled hydroxylated PAHs (i.e., $^{13}\text{C}_6$ -2-OH-NAP, $^{13}\text{C}_6$ -3-OH-FLU, $^{13}\text{C}_6$ -3-OH-PHE and d_9 -1-OH-PYR) as surrogate standards, 2 mL of urine was incubated with β -glucuronidase-sulfatase (*Helix pomatia*, Sigma-Aldrich, St. Louis, MO), followed by liquid-liquid extraction, diazomethane derivation, purification by silica column chromatography and instrumental analysis (Lin et al., 2016, 2021). Two OH-NAPs and two OH-PHEs (i.e., 3-OH-PHE and 9-OH-PHE) could not be distinctly separated in chromatograms; therefore, their summed concentrations were reported as Σ OH-NAPs and Σ 3+9-OH-PHEs, respectively. The concentration of 2-OH-DBF was corrected by $^{13}\text{C}_6$ -3-OH-FLU, while other analytes were corrected by corresponding surrogate standards. After correction, the recovery of all analytes in spiked samples was $99.6 \pm 7.6\%$ (range 85.2–110.7%), and the relative standard deviation was $7.6 \pm 4.3\%$ (range 2.6–15.7%). The limit of quantification (LOQ) of different OH-PAHs ranged from 7.5 pg/mL to 28.5 pg/mL with detailed information listed in Table S1. All the OH-PAHs were detected in at least 97.4% of the urine samples (Table S1). Blank samples were prepared for each eight urine samples, and negligible contamination was observed for all analytes. Concentrations of metabolites from the same PAHs were summed as an exposure indicator for the precursor compound.

Urinary creatinine was measured by a spectrophotometer under a wavelength of 510 nm based on the Jaffe reaction (Toora and Rajagopal, 2002). Urinary MDA concentrations were measured by a high-performance liquid chromatograph (HPLC; Waters2695) coupled with a UV detector under a wavelength of 532 nm, after the reaction with 2-thiobarbituric acid (TBA) (Lin et al., 2016). Urinary concentrations of 8-isoprostane and uric acid were determined based on the same methods that we have previously used for blood testing (Lin et al., 2019; Lu et al., 2021). Briefly, urinary 8-isoprostane was measured with a commercial ELISA kit (R&D, Detroit, MI) according to the manufacturer's instructions. Each urine sample was analyzed in duplicate. Urinary uric acid was measured with a metabolomic approach, and the results were confirmed with the authentic standard. Briefly, urine samples were thawed on ice, followed by centrifugation by 10,000 rpm at 4°C for 10 min. 50 μL supernatant was transferred to a new tube, added with 50 μL methanol and vortexed for 1 min. After centrifugation by 10,000 rpm at 4°C for 10 min, 80 μL supernatant was transferred into the sample vial and diluted with 40 μL water. Samples were analyzed by high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS, 1290II-6550; Agilent) with a 10-cm Zorbax Eclipse C18 column (2.1 mm i.d., 1.8 μm particle size; Agilent) and electrospray ionization in negative mode. Urinary levels of cotinine, OH-PAHs, MDA, and 8-isoprostane were normalized by

creatinine levels, to reduce interferences of diluted/concentrated spot urine samples.

Statistical Analysis. Analytes not detected in urine samples were substituted with 1/2 limit of quantification (LOQ) for statistical analysis. Logarithmic transformation was performed for biomarkers with a right-skewed distribution. Depending on the distribution of variables, Student's t-test or Mann-Whitney U were used to investigate the difference between two variables, and Pearson or Spearman correlation tests were used to test associations between two variables when the control of intra-individual variability was not necessary. Otherwise, we built different mixed effects models with random intercept at the participant level as follows. First, we modeled biomarker concentrations as a function of phases as categorical variables to study the changes of biomarker during the travel. Second, we modeled urinary OH-PAH levels as functions of cotinine, year, questionnaire variables, and the interactions of city with cotinine and year, to study the effects of passive smoking on PAHs exposure while controlling other factors. Third, we tested the associations between exposures and health biomarkers in mixed effects models with random intercept at both participant and phase levels.

If significant associations were observed between urinary levels of cotinine, OH-PAHs, and markers of oxidative stress (i.e., MDA, 8-isoprostane, and uric acid), additional mediation analyses were performed to examine whether OH-PAHs mediate the pro-oxidative effects of cotinine. Both mediator and outcome models were linear mixed-effects models with random intercept at the participant level and fixed effects of phases as categorical variables. We used quasi-Bayesian approximation with a simulation of 5000 iterations to estimate the total effects, average direct effects (ADE, the average direct effect of cotinine on the outcome), and average causal mediation effects (ACME, the average causal mediation effects of cotinine on the outcome attributable to OH-PAHs) (Tingley et al., 2014). Alpha was set at 0.05, and all tests were two-tailed. Data were analyzed with the software R with *lme4*, *lmeTest*, and *mediation* packages (www.r-project.org).

3. Results

Demographic characteristics. The mean (standard deviation) age and body mass index of the 26 study participants (14 females and 12 males) were 23.8 (± 5.6) years and 21.6 (± 2.4) kg/m², respectively (Table S2). All participants were healthy young non-smokers and most of them were Asian. No participants were recorded with any heart disease, metabolic disorder, kidney disease, blood coagulation disorders, rheumatological disease or chronic inflammation. Of the 26 participants, 22 provided urine samples in all three phases while four provided urine samples only in two phases with urine samples absent in either LA-before ($n = 3$) or LA-after ($n = 1$).

Passive smoking exposures. The urinary cotinine was detected in all samples collected in Beijing but only in 80.9% of those in Los Angeles. The geometric mean concentration of urinary cotinine was 1.52 (IQR: 0.88–2.25) $\mu\text{g/g}$ creatinine in Beijing, significantly higher ($p < 0.001$) than that in LA-before (geometric mean: 0.14; IQR: 0.06–0.24 $\mu\text{g/g}$

creatinine) or LA-after (geometric mean: 0.22; IQR: 0.09–0.40 $\mu\text{g/g}$ creatinine; Table 1). This trend was consistent in both 2014 (Fig. 1A) and 2015 (Fig. 1B). Correspondingly, 49.0% of urine samples in Beijing were collected after participants had spent time near smokers in the past three days, markedly higher than the frequencies in LA-before (3.0%) or LA-after (3.3%). All this data indicates elevated passive smoking exposures in Beijing.

To our surprise, the cotinine concentration in urine collected from participants who reported to spend time near smokers in Beijing ($n = 47$) was comparable with those who did not ($n = 49$, $p = 0.54$; Fig. S1). Consistently, we observed no associations between urinary cotinine concentrations and self-reported time near smokers after controlling for phase, year, and other activities ($p = 0.83$; Table S3).

Associations between passive smoking and PAHs exposures. Similar to the changes in urinary cotinine levels, traveling from Los Angeles to Beijing led to significant increases in the urinary concentrations of all OH-PAHs except for $\Sigma\text{OH-NAPs}$ ($p < 0.001$; Table 1), which reversed, at least partially, after participants returned to Los Angeles. Of note, the concentrations of most OH-PAHs and cotinine in LA-after were significantly higher as compared with LA-before ($p < 0.05$); however, the differences between LA-before and LA-after were much smaller than the differences between the two cities. These results strongly suggest that participants were subjected to elevated exposures to PAHs in Beijing.

The urinary levels of cotinine were positively associated with the concentrations of $\Sigma\text{OH-PHEs}$ and 1-OH-PYR in the urine ($p < 0.001$), but not with the other OH-PAHs (Table 2). On the other hand, time spent on barbecuing activities and public transportation was positively associated with OH-PAHs ($p < 0.05$), suggesting that behavioral changes related to diet and outdoor air pollution could have also influenced the exposure to PAHs. Since both cotinine concentrations and time spent in public transportation were markedly increased from Los Angeles to Beijing (Table S4), it is likely that both passive smoking and behavioral changes contributed to higher exposure to PAHs in Beijing. Remarkably, the concentrations of 2-OH-DBF, $\Sigma\text{OH-FLUs}$, and $\Sigma\text{OH-PHEs}$ remained significantly higher in Beijing than in LA-before even after adjusting for urinary cotinine concentrations and behavioral changes (Table 2), which likely reflect the effects of the more severe air pollution in Beijing as previously reported (Lin et al., 2019).

Associations between passive smoking and oxidative biomarkers. We observed significant increases in urinary MDA concentrations in Beijing ($p < 0.001$; Fig. 2A). However, unlike urinary OH-PAHs and cotinine, the increase in MDA concentration in Beijing did not return to baseline levels after the participants returned to Los Angeles (LA-after; Fig. 2A). To our surprise, the concentration of 8-isoprostane did not increase in Beijing but instead, it increased after returning to Los Angeles (LA-after) as compared with levels in LA-before or in Beijing ($p < 0.001$; Fig. 2B). We did not observe significant changes in urinary uric acid concentrations in the study period (Fig. 2C). Of note, the changes of 8-isoprostane and uric acid levels in the urine were markedly different from those in the blood. As reported previously, we found that blood levels of 8-isoprostane and uric acid were higher in

Table 1

Descriptive statistics of urinary exposure biomarkers in Beijing and Los Angeles before (LA-before) and after the trip (LA-after).

	geometric mean (IQR) ^a			p-value for the difference ^b		
	in LA-before ($n = 33$)	in Beijing ($n = 96$)	in LA-after ($n = 61$)	Beijing vs. LA-before	Beijing vs. LA-after	LA-before vs. LA-after
Creatinine	1455 (1184–2256)	1512 (1130–2301)	1446 (997–2272)	0.46	0.38	0.97
Cotinine	0.14 (0.06–0.24)	1.52 (0.88–2.25)	0.22 (0.09–0.40)	<0.001	<0.001	0.038
$\Sigma\text{OH-NAPs}$	2.70 (1.74–3.41)	3.62 (2.42–4.98)	3.48 (1.88–5.58)	0.12	0.97	0.13
2-OH-DBF	0.12 (0.08–0.18)	1.11 (0.83–1.60)	0.14 (0.11–0.22)	<0.001	<0.001	0.30
$\Sigma\text{OH-FLUs}$	0.19 (0.12–0.35)	1.21 (0.88–1.71)	0.28 (0.19–0.34)	<0.001	<0.001	0.007
$\Sigma\text{OH-PHEs}$	0.38 (0.27–0.61)	1.34 (1.01–1.83)	0.51 (0.38–0.70)	<0.001	<0.001	0.007
1-OH-PYR	0.08 (0.04–0.11)	0.20 (0.14–0.29)	0.12 (0.07–0.17)	<0.001	<0.001	0.002

^a Unit: $\mu\text{g/L}$ for creatinine; $\mu\text{g/g}$ creatinine for the others.

^b Tested by mixed-effect model with random intercepts of study participant.

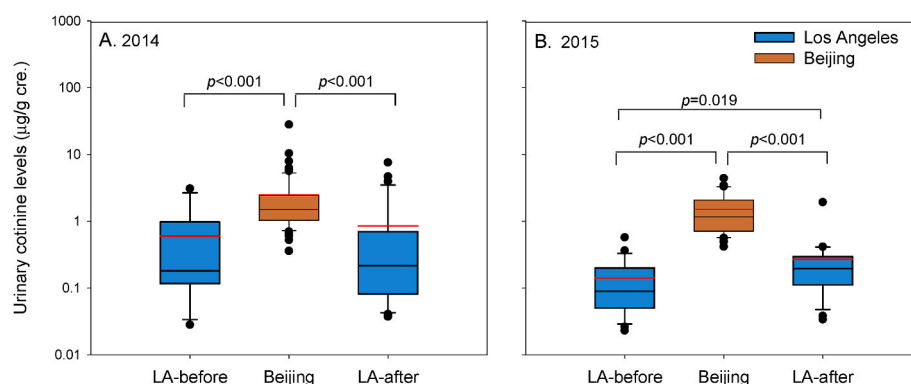


Fig. 1. Urinary cotinine concentrations in LA-before, Beijing, and LA-after in 2014 (panel A) and 2015 (panel B). Blue and orange plots indicate data in Los Angeles and Beijing, respectively; black line - median; red line - mean; box - 25th and 75th percentiles; whiskers - 10th and 90th percentiles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Determinants of logarithmic urinary OH-PAHs concentrations in multivariable mixed-effect model with random intercepts of study participant.^a

Variables	ΣOH-NAP		2-OH-DBF		ΣOH-FLUs		ΣOH-PHE		1-OH-PYR	
	β(SE)	p-value	β(SE)	p-value	β(SE)	p-value	β(SE)	p-value	β(SE)	p-value
Log ₁₀ cotinine	0.12 (0.07)	0.061	−0.01 (0.06)	0.91	0.07 (0.05)	0.21	0.18 (0.05)	<0.001	0.24 (0.06)	<0.001
Log ₁₀ cotinine × City (Beijing = 1)	0.03 (0.11)	0.78	0.14 (0.11)	0.21	0.01 (0.09)	0.88	−0.05 (0.09)	0.61	−0.19 (0.11)	0.078
Beijing vs. LA-before	−0.01 (0.10)	0.93	1.01 (0.10)	<0.001	0.70 (0.08)	<0.001	0.45 (0.08)	<0.001	0.09 (0.10)	0.37
LA-after vs. LA-before	0.05 (0.06)	0.38	0.09 (0.06)	0.14	0.15 (0.05)	0.003	0.12 (0.05)	0.016	0.13 (0.06)	0.031
Year (2015 vs. 2014)	−0.11 (0.10)	0.30	0.08 (0.08)	0.29	0.06 (0.07)	0.40	0.17 (0.06)	0.006	−0.01 (0.09)	0.90
Year × City (Beijing in 2015 = 1)	−0.10 (0.09)	0.25	−0.30 (0.09)	<0.001	−0.21 (0.08)	0.005	−0.14 (0.07)	0.051	−0.04 (0.09)	0.61
Time in cooking (h)	0.02 (0.02)	0.30	−0.01 (0.02)	0.73	−0.03 (0.02)	0.034	−0.00 (0.01)	0.76	−0.02 (0.02)	0.21
Time in barbecuing (h)	0.16 (0.06)	0.006	0.10 (0.06)	0.080	0.11 (0.05)	0.024	0.18 (0.05)	<0.001	0.27 (0.06)	<0.001
Barbecue intake (kg)	−0.18 (0.07)	0.013	−0.00 (0.07)	0.98	−0.01 (0.06)	0.88	−0.13 (0.06)	0.026	−0.16 (0.07)	0.023
Baked food intake (kg)	−0.03 (0.02)	0.22	−0.01 (0.02)	0.68	−0.01 (0.02)	0.41	−0.03 (0.02)	0.073	−0.01 (0.02)	0.57
Driving time (h)	−0.00 (0.01)	0.95	−0.03 (0.01)	0.003	−0.00 (0.01)	0.82	−0.01 (0.01)	0.33	−0.00 (0.01)	0.75
Time near heavy traffic (h)	0.01 (0.02)	0.67	−0.00 (0.02)	0.81	−0.02 (0.01)	0.25	−0.00 (0.01)	0.69	−0.01 (0.02)	0.44
Time in public transportation (h)	0.01 (0.01)	0.68	0.02 (0.01)	0.12	0.04 (0.01)	<0.001	0.01 (0.01)	0.21	0.03 (0.01)	0.006
Time near smokers (h)	−0.00 (0.02)	0.98	−0.01 (0.02)	0.58	−0.01 (0.01)	0.60	−0.01 (0.01)	0.46	0.00 (0.02)	0.82

^a β, SE, and p-value were estimated in full models including all variables.

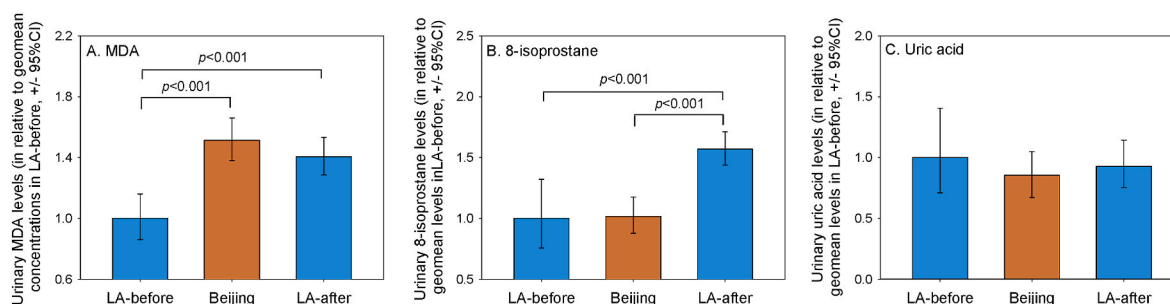


Fig. 2. Urinary concentrations of MDA (panel A), 8-isoprostane (panel B), and uric acid (panel C) in LA-before, Beijing, and LA-after. Blue and orange plots indicate data in Los Angeles and Beijing, respectively. Between-phase differences were tested in mixed-effect models with random intercepts of study participant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Beijing as compared with LA-before or LA-after, while no statistical difference was observed in either biomarker between LA-before and LA-after (Fig. S2) (Lin et al., 2019; Lu et al., 2021).

We found that one-fold increase in urinary cotinine concentrations was associated with 10.1% (95% CI: 5.53–14.8%), 8.75% (95% CI: 2.33–15.6%), and 25.4% (95%CI: 13.1–39.1%) increases in urinary concentrations of MDA, 8-isoprostane, and uric acid, respectively (Fig. 3A–C). Likewise, urinary OH-PAHs concentrations were positively

associated with urinary MDA, 8-isoprostane, and uric acid concentrations as well (Fig. S3). Adjusting for OH-PAHs concentrations attenuated but did not annihilate the association of urinary cotinine with MDA, 8-isoprostane, or uric acid concentrations (Fig. 3A–C) and vice versa (Fig. S3). Given the short half-time of cotinine and PAHs in human body (i.e. several hours) (Benowitz, 1996; St.Helen et al., 2012), these associations suggested oxidative effects of short-term exposures to passive smoking and PAHs. We further performed mediation analysis to

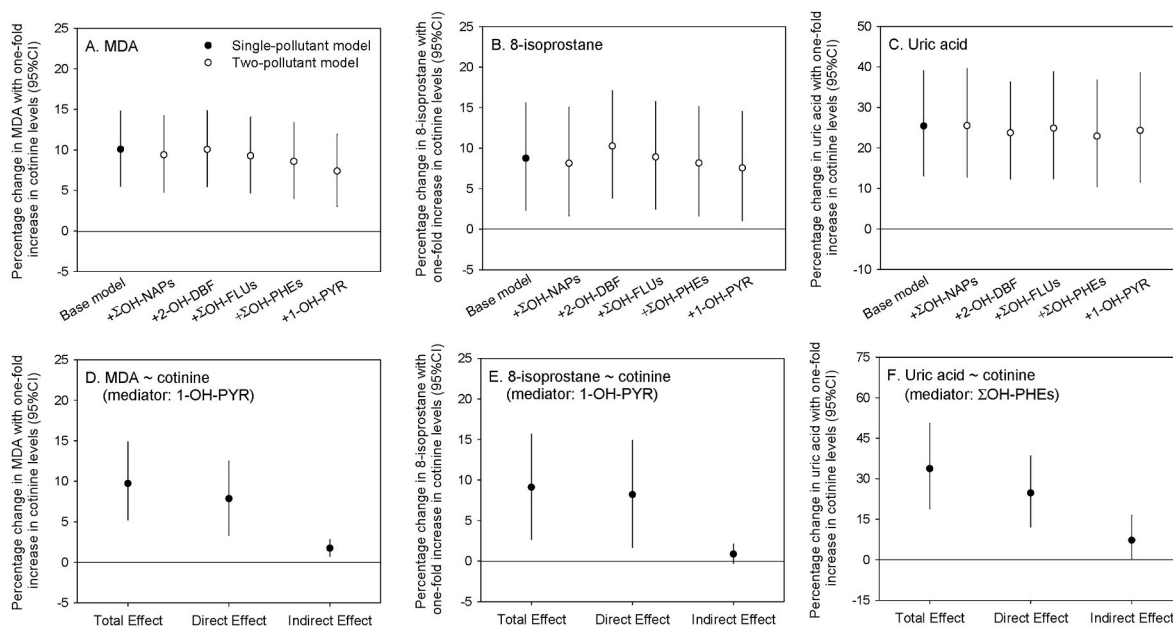


Fig. 3. Changes of urinary MDA (panel A), 8-isoprostane (panel B), and uric acid (panel C) levels in association with one-fold increases in urinary cotinine levels, and the mediating effects of urinary OH-PAH levels (panel D-F). Associations were tested in mixed-effect models with random intercepts of study participant and phase. The mediation analysis was based on mixed-effects models controlled for fixed effects of phases as categorical variables and random intercepts of study participant.

evaluate to what extent the oxidative effects of passive smoking were attributable to PAHs. The results indicated that exposures to PAHs mediated 18.3% (95%CI: 7.28–39.0%), 9.05% (95%CI: 3.53–41.0%), and 23.3% (95%CI: 1.95–48.0%) of passive smoking's effects on MDA, 8-isoprostane, and uric acid levels, respectively (Fig. 3D–F).

Given the significant decreases in exposures, but not urinary levels of oxidative biomarkers from Beijing to LA-after, we explored whether the elevation of oxidative biomarkers in LA-after as compared to LA-before was associated with the accumulation of exposures in Beijing, indicated by the average concentrations of urinary cotinine and OH-PAHs in Beijing. We found that the difference in average 8-isoprostane concentrations between LA-after and LA-before was significantly associated with the average cotinine concentration in Beijing ($p < 0.05$), but not with OH-PAHs concentrations (Fig. S4). These results suggested that the lasting pro-oxidative effects after participants returned to Los Angeles may be related to exposures to passive smoking in Beijing.

4. Discussion

In this study, we followed 26 healthy non-smoking travelers between Los Angeles and Beijing and have found that traveling to the city with higher smoking prevalence resulted in increased exposure to passive smoking, in association with increases in urinary oxidative biomarkers. These results provide the first evidence in support of potentially adverse health effects of passive smoking among a ubiquitous but underappreciated population – international travelers. Our results also indicate that PAHs are important toxic components mediating the pro-oxidative effects of passive smoking.

Increased oxidative stress has been frequently observed in individuals with cardiovascular diseases (Liguori et al., 2018). Blood and urinary biomarkers of oxidative stress have been shown to be partly predictive of future cardiovascular events and mortality in initially healthy subjects, after adjustment for established cardiovascular risk factors (Daiber et al., 2021). In general, oxidative biomarkers have demonstrated good sensitivity in the detection of early effects induced by environmental factors or exposures to toxic agents before the development of cardiovascular pathology (Daiber et al., 2021; Niemann et al., 2017). Compared with blood, urine samples are easy to collect and

have low content of organics and metals, which minimizes the artifactual generation of oxidative products *in-vitro* (Il'yasova et al., 2012). Indeed, we were able to collect multiple urine samples in contrast to only one blood sample from each participant at each phase. We observed increased urinary levels of MDA and cotinine after participants traveled from Los Angeles to Beijing, and increased urinary levels of 8-isoprostane after participants returned to Los Angeles (LA-after), as well as robust associations between passive smoking exposure and urinary levels of MDA, 8-isoprostane, and uric acid, suggesting that increased passive smoking exposure likely contribute to increased oxidative stress among international travelers.

Urinary oxidative biomarkers have been suggested as convenient surrogates of those in the blood as indicators of systemic oxidative stress (Il'yasova et al., 2012); however, we found that changes of oxidative biomarkers in the urine among travelers from Los Angeles to Beijing were different to those in the blood. Thus, while traveling from Los Angeles to Beijing increased the plasma levels of 8-isoprostane by 20.8% and uric acid by 10.5% (Fig. S2) (Lin et al., 2019; Lu et al., 2021), it did not influence the urinary levels of 8-isoprostane or uric acid but only after the individuals returned to Los Angeles in the case of the former. Of note, the increase in blood levels of uric acid in Beijing was accompanied by decreased xanthine levels (Fig. S2) (Lu et al., 2021) and therefore it likely resulted from increased oxidation of xanthine by xanthine oxidase. Uric acid itself is a major free radical scavenger in the blood (Feig et al., 2008) that may counteract non-enzymatic lipid peroxidation through scavenging singlet oxygen, peroxy and hydroxyl radicals (Sautin and Johnson, 2008), probably impeding an increase in the urinary levels of 8-isoprostane in Beijing (Fig. 2B). When plasma levels of uric acid returned to baseline in LA-after (Fig. S2), there was an increase in the urinary 8-isoprostane levels (Fig. 2B). It is possible that the buffering effects of uric acid were insufficient to prevent an increase in urinary MDA, a more terminal product of lipid peroxidation from both enzymatic and non-enzymatic pathways, in Beijing as well as LA-after (Fig. 2A).

We noted that the increase in 8-isoprostane levels in LA-after was consistent across different years and different time points (Fig. S5). Similar results were also observed by a previous study where nine volunteers traveling from Germany to China for 8–42 days exhibited

elevated urinary 8-isoprostane levels four weeks after returning to Germany (Wu et al., 2017). Importantly, the increase in 8-isoprostane levels from LA-before to LA-after in our study was positively associated with the average cotinine level in Beijing (Fig. S4), suggesting that pro-oxidative effects induced by passive smoking may persist several weeks after the exposure cessation. Increases in urinary 8-isoprostane levels have been reported in subjects with coronary artery disease (Schwedhelm et al., 2004) and in association with increased cardiovascular mortality due to coronary artery disease and strokes (Roest et al., 2008). Furthermore, a meta-analysis including 242 studies unveiled increases in blood and/or urinary 8-isoprostane levels for hypertension, chronic heart failure, coronary artery disease and ischemic stroke compared to control groups (van 't Erve et al., 2017). Altogether, this data raises the possibility that participants in our cohort could have had an increased cardiovascular risk due to passive smoking exposures.

Previous animal studies have also shown that tobacco smoke exposure induces oxidative damages not only in the systemic circulation (Van Der Vaart et al., 2004), but also in multiple tissues including kidney (Cigremis et al., 2004; Van Der Vaart et al., 2004), which is possibly attributed to the accumulation of redox-reactive chemicals. While our results suggested PAHs mediating 9.1–23.3% of the pro-oxidative effects of passive smoking, previous studies have shown short biological half-lives of PAHs (i.e., <24 h) (St.Helen et al., 2012). Indeed, the increase in 8-isoprostane levels from LA-before to LA-after was not associated with average OH-PAHs levels in Beijing (Fig. S4). Therefore, the lasting pro-oxidative effects of passive smoking may relate to other redox active chemicals with longer biological half-lives. For example, cadmium, a tobacco smoke component with pro-oxidative effects, has been shown to accumulate in the kidney with a clearance half-life of 25 years (Bernhoft, 2013). Additionally, the slow translocation of cadmium from circulation to organs (half-life = 75–128 days) (Bernhoft, 2013) may also explain why urinary 8-isoprostane was not increased until participants returned to Los Angeles.

Although this study only focused on the pro-oxidative effects of passive smoking, passive smoking may perturb the cardiovascular system through other mechanisms, given its significant contributions to exposures to PAHs, a group of chemicals that have been shown to promote inflammation and subsequent atherosclerosis in animal models (Curfs et al., 2005); and associate with inflammatory biomarkers and the 10-year atherosclerotic cardiovascular risk in epidemiological studies (Delfino et al., 2010; Hu et al., 2018). Among the study participants, exposures to PAHs have also been found to associate with circulating C-reactive protein levels as previously reported (Lin et al., 2019). It is important to note that passive smoking has been thought to be a less important contributor to PAHs exposure than air pollution and diet, especially among population without known smokers in their families or working places (Yu et al., 2015; Zhang et al., 2014). However, we observed significant elevation in urinary cotinine concentrations in Beijing even among participants who reported no proximity to active smokers (Fig. S1), in strong associations with Σ OH-PHEs and 1-OH-PYR levels ($p < 0.001$; Table 2). These results suggest that passive smoking might have been an ignored contributor to PAHs exposures among the non-smokers in Beijing. Indeed, nicotine has been detected in the air of 44 public places tested in a previous study (e.g., hospitals, secondary schools, and restaurants) with a median concentration of $3.0 \mu\text{g}/\text{m}^3$ (Stillman et al., 2007). This level is comparable to that of smoking-permitted workplaces in the U.S. and might cause notable passive smoking exposures among visitors to those places (Hammond, 1999).

In this study, participants' urinary cotinine levels were higher in Beijing than in Los Angeles, likely due to a higher prevalence of smoking (30.2%) (Zhang et al., 2016) as compared with Los Angeles (14.3%) (Los Angeles County Department of Public Health, 2010). To explore whether smoking prevalence could predict the change of passive smoking exposures due to international travels, we compared the urinary cotinine concentrations among travelers in Los Angeles and Beijing with other

non-smokers from 20 countries (Aquilina et al., 2010; Den Hond et al., 2015; Joo et al., 2017; Mori et al., 2011; Tranfo et al., 2017), and explored their relationship with national smoking prevalence (Table S5) (Ng et al., 2014). Remarkably, we found a positive association between urinary cotinine levels and smoking prevalence ($\rho = 0.49$, $p < 0.05$; Fig. 4), supporting the use of smoking prevalence in departure and destination countries to roughly estimate changes of passive smoking exposures during travels.

Effective June 2015, the Beijing government banned smoking in all the indoor areas of public places, including workplaces and public transportation as well as outdoor areas of schools and hospitals. Our data obtained in Beijing, in the summers of 2014 and 2015 reflect passive smoking exposures before and after the ban policy, respectively. Although we observed a trend towards decreased exposures in Beijing from 2014 to 2015 as evidenced by decreased urinary cotinine concentrations (geometric mean in a unit of $\mu\text{g}/\text{g}$ creatinine: 1.68 in 2014 and 1.21 in 2015, respectively), the difference didn't reach statistical significance, and the urinary cotinine concentrations and self-reported time near smokers remained much higher in Beijing than in Los Angeles in 2015. These results suggest that the regulatory policy didn't reduce the passive smoking exposure in this study significantly. However, it is important to note that our study was conducted only 1–3 months after the policy implementation, and future studies are warranted to evaluate the long-term compliance and health benefits of these regulatory policies.

Although the results of exposure biomarkers (i.e., cotinine and OH-PAHs) has clearly indicated that traveling from Los Angeles to Beijing may be accompanied by considerable risk in passive smoking exposure, it is important to note that these findings on oxidative biomarkers are only associative due to our limited capacity to control potential confounders in this natural experiment. Even if we have attempted to control the effects of unmeasured exposure on the associations between passive smoking and oxidative biomarker using a random intercept of phase, this approach cannot control the within-city variations of potential confounders and the between-phase difference in oxidative biomarker remained subject to confounding effects. Particularly, although we were able to partially address the confounding effects of combustion-originated air pollution with urinary 2-OH-DBF and Σ OH-FLUs, we cannot control the pro- and/or anti-oxidative effects of dietary factors and psychological stress (Ghezzi et al., 2018; Romieu et al., 2008), which were previously found to change during the travel as well (Lin et al., 2019).

Strengths and Limitations. In this natural experiment, we performed repeated measurements among the same travelers, allowing each participant to serve as his/her own control. The built-in "crossover" feature (i.e., from LA-before to Beijing vs. from Beijing to LA-after) allowed us to control the effects due to travel itself and the exposures during the flights. Thus, the observed effects were most likely attributable to the exposures in each city. Despite these strengths, our study has several limitations. First, the population size in this study is relatively small ($n = 26$). However, in our previous study on the same cohort (Lin et al., 2019), we successfully identified significant increases (false discovery rate <5%) in a panel of oxidative biomarkers in the blood after traveling from Los Angeles to Beijing, suggesting that the sample size of our study would provide enough statistical power to detect significant pro-oxidative effects given the drastic difference in exposures between the two cities. Nevertheless, our results have an exploratory nature and future studies with larger sample size and multiple locations are warranted to confirm the findings of this pilot study. Second, changes in urinary levels of cotinine depend not only on the degree of exposure to passive smoking, as determined in here, but also on the activity of inducible cytochromes P450 responsible for nicotine metabolism (Bramer and Kallungal, 2003), which was not assessed in our study. Third, our study only depicts the scenario of traveling from a less-polluted to a more-polluted city. The health effects of passive smoking exposures during a reversed travel remain unclear. Finally, we

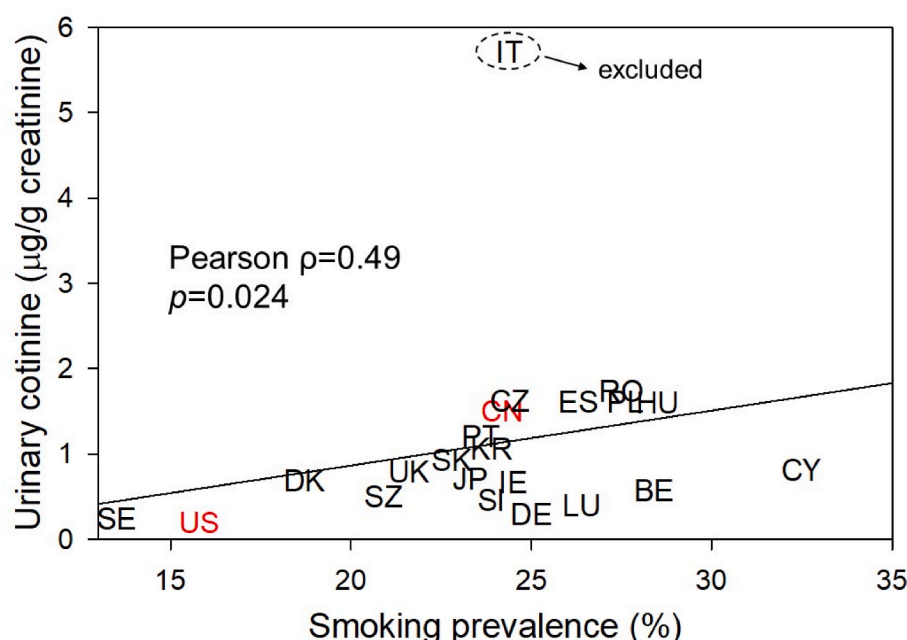


Fig. 4. Correlation between population's geometric mean concentration of cotinine in urines and the corresponding national smoking prevalence in 2012. Detailed information on the population was shown in Table S5. National Smoking prevalence data was obtained from Ng et al. Red symbols indicate data from our study. Abbreviations: US – United States; SE – Sweden; LU – Luxembourg; SI – Slovenia; SZ – Switzerland; BE – Belgium; IE – Ireland; DK – Denmark; JP – Japan; UK – United Kingdom; CY – Cyprus; SK – Slovak Republic; KR – Korea; PT – Portugal; CN – China; HU – Hungary; PL – Poland; ES – Spain; CZ – Czech Republic; RO – Romania; IT – Italy. Data in Italy was considered as an outlier and was removed from the correlation analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

did not measure ambient levels of nicotine in the different microenvironments, hence the location where exposures occurred was unclear. While future studies are warranted to address these limitations, our results are relevant in the design of policies aimed at reducing passive smoking exposures.

5. Conclusions

In this pilot study, we have shown that healthy young adults traveling from Los Angeles to Beijing were inadvertently exposed to elevated levels of tobacco smoke, which led to increased exposure to PAHs in the destination city. Passive smoking exposures were significantly associated with systemic pro-oxidative effects of cardiovascular relevance as indicated by urinary oxidative biomarkers, with 9.1–23.3% of effects mediated by PAHs. Future studies are needed to better understand the reversibility of pro-oxidative effects induced by passive smoking, the impact of regional differences in smoking control on exposures to passive smoking among travelers, and to determine whether these effects influence the risk of developing cardiovascular diseases.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2022.114048>.

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