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Exposure to Polycyclic Aromatic Hydrocarbons Associated with Traffic Exhaust: The Increase of Lipid Peroxidation and Reduction of Antioxidant Capacity

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ABSTRACT

This study was carried out to examine the association between exposure to polycyclic aromatic hydrocarbon with traffic exhaust and biomarkers of lipid peroxidation and antioxidant levels among highway toll station workers. We conducted a cross-sectional study of 47 female highway toll station workers exposed to traffic exhausts and 27 female classroom trainees as a reference group. Exposure assessment was based on a biomarker of polycyclic aromatic hydrocarbon exposure, urinary 1-hydroxypyrene-glucuronide (1-OHPG). Urinary isoprostane was assayed as a biomarker of lipid peroxidation, and plasma antioxidative capacity of lipid-soluble substances (ACL) and water-soluble substances (ACW) was measured. The median concentration of urinary isoprostane was higher among the exposed non-smokers (4.63 ng/mL) compared with the reference non-smokers (3.52 ng/mL, difference: 0.91, 95% CI –0.15 to 1.98) (Wilcoxon rank-sum test: p = 0.04). The median concentration of ACW among non-smoking exposed subjects (37.9 μg/mL Trolox equivalent) was lower than that of the reference non-smokers (86.3 μg/mL). Adjusting for confounding effects by linear regression, a change in log(isoprostane) concentration was significantly related to a unit change in log(1-OHPG) (regression coefficient [ß], β = 0.14, 95% CI 0.07 to 0.21). Exposure to polycyclic aromatic hydrocarbon is associated with increased lipid peroxidation and reduced antioxidative capacity in toll station workers.

Keywords: Air pollution; 1-hydroxypyrene-glucuronide; Isoprostane; Antioxidant capacity.

Abbreviations:
PM: particulate matter
1-OHPG: 1-hydroxypyrene-glucuronide
NO: nitric oxide
PAHs: polycyclic aromatic hydrocarbons
ROS: reactive oxygen species
8-OHdG: 8-hydroxydeoxyguanosine
1-OHP: 1-hydroxypyrene
ACL: antioxidative capacity of lipid-soluble substances
ACW: antioxidative capacity of water-soluble substances
TAC: Total antioxidant capacity

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INTRODUCTION

A large body of epidemiologic research provides evidence that short-term exposure to air pollution is associated with increased cardiovascular mortality and morbidity (Donaldson *et al.* 2001; Dockery, 2001; Dominici *et al.*, 2006). There is limited insight into the mechanisms through which exposure to air pollution may influence the respiratory and cardiovascular systems (Pope *et al.*, 2004; Liu and Meng, 2005). Raupach and colleagues (2006) proposed a model linking second-hand smoke to acute coronary syndromes. This model may also have some relevance to traffic-related air pollution, which contains some of the same gaseous and particulate combustion products. According to this model, oxidants in tobacco smoke and traffic exhausts, as well as free radicals released endogenously from activated neutrophils, play a central role in the causal pathway leading first to vascular inflammation and then to platelet

activation, endothelial dysfunction and indirectly to negative myocardial oxygen balance and to acute coronary syndromes. Squadrito and colleagues (2007) suggested that semiquinone radicals, present in fine particles ($PM_{2.5}$), undergo redox cycling, thereby reducing oxygen and generating reactive oxygen species (ROS). These ROS cause oxidative stress at the site of deposition and produce deleterious effects in the lung.

Previous studies have demonstrated increased oxidative stress in subjects exposed to environmental air pollution (Singh *et al.*, 2008; Svecova *et al.*, 2009) through the assessment of oxidative damage to DNA, lipids, or proteins. Occupational exposure to combustion products, including polycyclic aromatic hydrocarbons (PAHs) have been associated with increased oxidative stress biomarkers in some, but not all, studies (Rossner *et al.*, 2008; Marie *et al.*, 2009). ROS may be responsible for oxidative changes in lipids (Liu and Meng 2005). For example, free radicals may induce peroxidation of arachidonic acid generating F_2-isoprostanes (Cracowski *et al.*, 2002). Urinary or plasma F_2-isoprostanes have been found to be related to smoking (Dietrich *et al.*, 2002) and exposure to environmental tobacco smoke (Dietrich *et al.*, 2003), and are thought to be useful markers of lipid peroxidation.

Both extracellular and intracellular compartments of the lung participate in antioxidant functions. It has been proposed that composition and quantity of antioxidants may serve as an early sign of adverse effects of air pollution (Kelly, 2004). We hypothesize that plasma antioxidant capacity is influenced by exposure to traffic exhaust.

We recently studied toll workers exposed to high levels of $PM_{2.5}$ from traffic exhausts and found elevated levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and plasma NO compared with unexposed subjects (Lai *et al.*, 2005). We also measured urinary 1-hydroxypyrene-glucuronide (1-OHPG) as a biomarker of PAH exposure and found a statistically significant association between the biomarker of exposure (1-OHPG) and oxidative DNA damage (8-OHdG) (Lai *et al.*, 2004a). The metabolite of pyrene in human urine can be measured as 1-hydroxypyrene (1-OHP) after deconjugation of the glucuronide with beta-glucuronidase or directly as 1-hydroxypyrene-glucuronide (1-OHPG) without deconjugation. Since 1-OHPG is approximately 5-fold more fluorescent than 1-OHP, it may provide a more sensitive biomarker for assessing exposure to pyrene in mixtures of PAHs (Strickland *et al.*, 1994; Strickland *et al.*, 1996). Furthermore, the relationship between total PAHs and pyrene was highly correlated in urban environment (Tsai *et al.*, 2003).

The overall objective of the present study was to assess the relation between exposure to high levels of traffic exhausts and the occurrence of lipid peroxidation. We assessed exposure to polycyclic aromatic hydrocarbon with traffic exhaust by measuring urinary 1-OHPG. Urinary isoprostane was used as a measure of lipid peroxidation. We examined the relation between exposure to traffic exhausts and general antioxidant capacity measured as plasma antioxidant capacity of both water and lipid-soluble substances.

METHODS

Study Population

This study was approved by the institutional review board at the Tri-Service General Hospital, Taipei, Taiwan. All the participants signed informed consent prior to study enrolment.

We carried out a cross-sectional study among female toll station workers with occupational exposure to traffic exhausts and a reference group of female workers who were in classroom training to become toll station workers. We recruited all toll station workers in a highway toll station in the Taipei metropolitan area, which had the highest traffic density among all toll stations in Taiwan. All the toll workers work in three shifts. There are 20 tollbooths, 10 used to collect the toll of traffic flow from Taipei City to Taipei County (from north to south), and the remaining 10 booths collect toll from the opposite traffic flow. Out of the 10 booths in both directions, 2 to 3 were designed for bus and truck traffic, whereas the other 7 to 8 booths were for cars and vans. These lanes are divided into those using prepaid tickets and those using cash payment. All the toll workers work in three shifts: morning (from 8:00 AM to 4:00 PM), evening (from 4:00 PM to 00:00 AM) and night shift (from 00:00 AM to 8:00 AM). The number of open booths depends on the traffic flow. During the data collection period, there were 19 (40.4%), 15 (31.9%) and 13 (27.7%) toll workers selected as morning, evening, and night shift of toll collection work, respectively. The exposed group included 47 full-time toll station workers. The reference group consisted of 27 classroom trainees (see Lai *et al.*, 2004a, for additional details). All subjects were healthy without any history of cancer, stroke, diabetes or ischemic heart disease.

Exposure Assessment

Exposure assessment was based on job description/ location and urinary biomarker levels. Job location was expressed as a dichotomous variable based on the type of work: toll collection or office work. The biomarker of exposure to traffic exhaust was urinary concentration of 1-hydroxypyrene-glucuronide (1-OHPG) after the working shift of the first day of the work week from both the exposure and reference groups.

Outcomes of Interest

The primary outcome of interest was lipid peroxidation. We used the concentration of isoprostane in the urine after the working shift as the biomarker of effect of exposure to traffic exhausts on lipids. The secondary outcome was general antioxidative capacity measured as plasma antioxidative capacity of lipid- (ACL) and water-soluble substances (ACW) after the working shift.

Data Collection

In the beginning of the study, we distributed a self-administered questionnaire to the participants, which inquired

about personal characteristics, such as age, education, smoking habits, mode of transportation to work, a history of diseases, consumption of broiled, grilled and barbecued food, use of cooking fuel, cooking practice, use of incense, candle and mosquito coil, vitamin intake habit and use of personal protective equipment. Workers were asked to collect a post-shift urine sample in a container. The urine samples were collected in brown polyethylene 500 mL containers and labelled with subject identification number, date, and time. The samples were transported in a cooler. Venous blood was also collected after the working shift of the first day of the work week drawn into Vacutainers (Becton Dickinson, Rutherford, New Jersey) containing sodium heparin, centrifuged at 4°C for 10 minutes at 1,200 × g, protect from light, and stored at –80°C. All urinary biomarkers were analyzed within 6 months of sample collection.

Analysis of Urinary 1-OHPG

Urine samples were divided into several small volume aliquots and stored at –80°C freezer to minimize the effect of freeze-thaw on the stability of specimens. Urine samples (2 mL) were treated with 0.1 N HCl (90°C) to hydrolyse acid-labile metabolites, as described (Strickland *et al.*, 1994). The hydrolysed samples were loaded onto Sep-Pak C18 cartridges (Waters) and washed with methanol (30% in water). The relatively non-polar metabolites were eluted with methanol (80% in water; 4 mL) and the volume was reduced to 0.5 mL by a centrifugal and vacuum evaporator (Eyela CVE-100, Tokyo, Japan). The concentrated samples were diluted to 4 mL with 15 mM phosphate buffered saline (PBS). Immunoaffinity columns were prepared using poly-prep columns (0.8 × 4 cm) filled with CNBr-activated Sepharose 4B (0.8 mL) coupled with monoclonal antibody 8E11, which recognizes several PAH-DNA adducts and metabolites. Monoclonal antibody 8E11 was obtained from Trevigen, Inc; Gaithersburg, MD, USA. It was originally produced against benzo[a]pyrene-diolepoxide-modifed DNA, and has been shown to recognize 1-OHPG (Strickland *et al.*, 1994). After washing the columns two times with 4 mL of 15 mM PBS, samples in phosphate-buffered saline were loaded on columns and bound material was eluted with 2 mL of 40% methanol. Eluted fractions were quantified by synchronous fluorescence spectroscopy (SFS) using a Perkin-Elmer LS 50B luminescence spectrometer. The excitation-emission monochromators were driven synchronously with a wavelength difference of 34 nm. 1-OHPG, purchased from National Cancer Institute (NCI) Chemical Carcinogen Repository (MRI, Kansas City, MO, USA), produces a characteristic fluorescence emission maximum at 381 nm (347nm excitation). Fluorescence intensity was used to quantify 1-OHPG, as described (Strickland *et al.*, 1994; Kang *et al.*, 1995). The recovery of the assay was 80%. The coefficient of variation of the assay was 8–10% during the period of sample analysis. The limit of detection was 0.06 pmol/mL as determined by the concentration of the standard at which the signal-to-noise ratio was 3. The urinary 1-OHPG concentrations were normalized to urine creatinine. Creatinine was determined spectrophotometrically (Perkin-Elmer Lambda 5 model) with a commercial kit (Boehringer, Mannheim, Germany) based on Jaff's basic picrate method.

Analysis of Urinary Isoprostane

The quantification of 15-F_{2t}-isoprostane in urine, the product of free radical-mediated peroxidation of lipoproteins, was determined by a competitive enzyme-linked immunoassay (ELISA) using Urinary isoprostane kit (Oxford Biomedical Research, Oxford, Ohio, USA) in accordance with the manufacturer's recommendations. Briefly, the urine sample was mixed with an enhancing reagent that essentially eliminated interferences due to non-specific binding, and the 15-F_{2t}-isoprostane in the samples or standards competes with 15-F_{2t}-isoprostane conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-F_{2t}-isoprostane coated on the microplate. The HRP activity resulted in colour development when substrate was added, with the intensity of the colour proportional to the amount of 15-F_{2t}-isoprostane bound and inversely proportional to the amount of 15-F_{2t}-isoprostane unconjugated in the samples or standards. 3N sulfuric acid was added to each well to stop the HRP-catalyzed colour development and absorption at 450 nm was measured with a computer-controlled ELISA reader (MRXII, Dynex Technologies, VA, USA). The lower limit of reliable detection was suggested as 0.2 ng/mL. The lowest value of 15-F_{2t}-isoprostane detected by ELISA in our study was 1.3 ng/mL. Oxford Biomedical reports the following cross-reactivity with other isoprostanes: 4.1% for 9α, 11β-prostaglandin F2α, 3.0% for 13,14-dihydro-15-keto-PGF2α, and < 0.01% for prostaglandin F2α, 6-keto-prostogaldin F1α, prostpglandin E2, prostpglandin D2, and arachidonic acid. The coefficient of variation of the assay was 8–10% during the period of sample analysis.

Photoluminescence Measurements

Measurement of ACW (Antioxidative Capacity of Water-soluble substances) and measurements of ACL (Antioxidative Capacity of Lipid-soluble substances) in plasma were performed using commercial system and kits. The photochemiluminescence (PCL) assay, based on the methodology of Popov and Lewin (1994, 1999), was used to measure the antioxidant activity of plasma with a Photochem® instrument (Analytik Jena AG, Jena, Germany) against superoxide anion radicals generated from luminol, a photosensitizer, when exposed to UV light. The antioxidant activity of plasma was measured using both ACW and ACL kits provided by the manufacturer designed to measure the antioxidant activity of hydrophilic and lipophillic compounds, respectively. Lag time (s) for the ACW assay, obtained from the PCLsoft® control and analysis software was used as the radical-scavenging activity and the antioxidant capacity estimated by comparison with a Trolox standard curve and expressed as μm Trolox sample. The antioxidant index was obtained by dividing the antioxidant capacity by lag time multiplied by 1000 (i.e., antioxidant activity/lag time × 1000). Antioxidant capacity using the ACL kit was monitored for 180 seconds and expressed as μg/mL Trolox sample. ACW kit was monitored for 500 seconds and expressed as μg/mL Trolox sample. Plasma samples were microfuged (5 min at 1,000 × g) prior to analysis. The coefficient of variation of the

assay was within 8% during the period of sample analysis.

Statistical Methods

First, we compared the distributions of isoprostane, ACW and ACL concentrations between the exposed and the reference groups. The concentrations of above biomarkers were not normally distributed (Shapiro-Wilk W test for normal data $p < 0.0001$, $p < 0.01$ and $p < 0.001$, respectively) and therefore we assessed the difference in concentrations using a non-parametric Wilcoxon rank-sum test. Then we elaborated the relation of isoprostane to 1-OHPG, using plots and linear regression analysis adjusting for potential confounders such as group, current smoking habit, current alcohol consumption habit, use of motorcycle as main transportation, frequent exposure to burning incense, and antioxidant capacity. The covariates used in the analyses included: group (exposed/reference group), current smoking habit (yes/no), current alcohol consumption habit (two times per month) (yes/no), use of motorcycle as main transportation (yes/no), frequent exposure to incense (yes/no). Since the creatinine-corrected urinary level was the dependent variable in the multivariate analyses, independent variables may have been unrelated to the chemical concentration itself, but related to the urinary creatinine concentration. In the multivariate regression analyses, we used the creatinine-unadjusted urinary chemical level as the dependent variable to determine significant predictors of exposure to that chemical. All the analyses were performed with the STATA 8 statistical package.

RESULTS

Study Population

The characteristics of the study population were compared between the exposed and the reference workers (Table 1). The mean age was 25.8 years (SD 5.5) among the exposed and 27.0 years (SD 4.7) in the reference workers. The exposed subjects had less often smokers (11% vs. 30%), less frequently alcohol drinking (0% vs. 11.1%), used a motorcycle less often for transportation, and were less frequently exposed to incense burning compared with the reference group.

Comparison of Urinary Isoprostane between Exposed and Reference Groups

The percentage of smokers was greater in the reference group (30%, n = 8) than in the exposed group (11%, n = 5). To eliminate the smoking effect, a stratified analysis was performed.

The median concentration of urinary isoprostane was 5.15 ng/mL (25^{th} percentile 4.00, 75^{th} percentile 5.32) among the exposed smokers and 4.16 ng/mL (25^{th} percentile 3.86, 75^{th} percentile 4.36) in the reference smokers. The median concentration of isoprostane was 4.63 ng/mL (25^{th} percentile 3.48, 75^{th} percentile 5.23) among the exposed non-smokers and 3.52 ng/mL (25^{th} percentile 3.14, 75^{th} percentile 4.32) in the reference non-smokers with a difference of 0.91 (95% CI –0.15 to 1.98) (Wilcoxon rank-sum test: $p = 0.04$).

When isoprostane data from smokers and non-smokers was analysed together, the median concentration for the exposed workers was 4.64 ng/mL (25^{th} percentile 3.48, 75^{th} percentile 5.32) and for the unexposed workers 3.66 ng/mL (25^{th} percentile 3.16, 75^{th} percentile 4.35) (Wilcoxon rank-sum test: $p = 0.02$) as shown in Table 2.

Table 1. Characteristics of the study population.

Characteristic	Exposed		Reference		Total		p value*
	N	%	N	%	N	%	
Number of subject	47	63.5	27	36.5	74	100	
Age[a] (years)	25.8 ± 5.5		27.0 ± 4.7		26.3 ± 5.3		0.32
Current smoking habits							
Smokers	5	10.6	8	29.6	13	17.6	0.03
Nonsmokers	42	89.4	19	70.4	61	82.4	
Alcohol drinking (> 2 times/month)							
Yes	0	0	3	11.1	3	4.1	0.02
No	47	100	24	88.9	71	95.1	
Transportation							
Motorcyclist	6	12.8	16	59.3	22	29.7	< 0.001
Others	41	87.2	11	40.7	52	70.3	
Frequent exposure to incense							
Yes	8	17.0	16	59.3	24	32.4	< 0.001
No	39	83.0	11	40.7	50	67.6	
Vitamin intake habits							0.67
Yes	25	53.2	13	48.1	38	51.4	
No	22	46.8	14	51.9	36	48.6	
BMI(kg/m^2)[a]	21.2 ± 3.2		21.3 ± 4.1		21.3 ± 3.5		0.47

Abbreviation: BMI, Body Mass Index.

[a] Values shown are mean± standard deviation (SD), comparison between the exposed and reference groups by t-test;

* Other categorical data, comparison between the exposed and reference groups by Chi-square test.

Table 2. Concentration of urinary 1-OHPG, urinary isoprostane, and plasma antioxidant capacity measured in exposed and reference subjects by smoking status.

Biomarkers	Exposed Group		Reference Group		p-value*
	N	Median (interquartile range)	N	Median (interquartile range)	
Exposure					
1-OHPG (nmol/mL)					
Smoker	5	1.96(1.20 to 2.15)	8	1.84(1.39 to 2.07)	1.00
Non-smokers	42	1.32(0.77 to 1.93)	19	0.93(0.32 to 1.29)	0.03
Subtotal	47	1.37 (0.77 to 1.98)	27	1.27(0.64 to 1.77)	0.26
p-value†		0.45		0.002	
1-OHPG (μmol/mol creatinine)					
Smoker	5	0.13(0.11 to 0.23)	8	0.16(0.13 to 0.23)	0.69
Non-smokers	42	0.11(0.04 to 0.17)	19	0.07(0.05 to 0.10)	0.01
Subtotal	47	0.11(0.06 to 0.17)	27	0.09(0.06 to 0.13)	0.63
p-value†		0.35		0.003	
Effect					
Isoprostane (ng/mL)					
Smoker	5	5.15(4.00 to 5.32)	8	4.16(3.86 to 4.36)	0.24
Non-smokers	42	4.63(3.48 to 5.23)	19	3.52(3.14 to 4.32)	0.04
Subtotal	47	4.64(3.48 to 5.32)	27	3.66(3.16 to 4.35)	0.02
p-value†		0.51		0.14	
Isoprostane (μg/g creatinine)					
Smoker	5	3.69(3.26 to 4.39)	8	3.02(2.89 to 3.71)	0.11
Non-smokers	42	3.00(2.63 to 4.17)	19	2.75(2.13 to 3.57)	0.10
Subtotal	47	3.04(2.71 to 4.26)	27	2.88(2.62 to 3.67)	0.11
p-value†		0.18		0.14	
Total Antioxidant capacity					
Smoker	5	45.5(44.8 to 62.8)	8	55.5(38.6 to 77.9)	0.77
Non-smokers	41	49.4(34.4 to 72.9)	19	92.7(70.4 to 103.1)	0.005
Subtotal	46	49.1(34.4 to 72.9)	27	76.6(41.0 to 101.2)	0.01
p-value†		0.93		0.11	
ACL Antioxidant capacity					
Smoker	5	9.9(9.2 to 13.2)	8	5.0(2.1 to 10.2)	0.19
Non-smokers	41	6.2(3.4 to14.2)	19	6.4(3.3 to 10.4)	0.76
Subtotal	46	7.9(4.0 to 14.3)	27	6.4(3.0 to 10.4)	0.31
p-value†		0.41		0.63	
ACW Antioxidant capacity					
Smoker	5	35.6(31.5 to 53.6)	8	51.2(33.8 to 75.1)	0.56
Non-smokers	41	37.9(26.9 to 63.6)	19	86.3(58.2 to 92.7)	0.004
Subtotal	46	47.8(26.9 to 63.6)	27	71.6(37.5 to 92.6)	0.006
p-value†		0.85		0.20	

Abbreviation: 1-OHPG, 1-hydroxypyrene-glucuronide

ACW, Antioxidative Capacity of Water-soluble substances (μg/mL Trolox equivalent), ACL, Antioxidative Capacity of Lipid-soluble substances (μg/mL Trolox equivalent); *Comparison between the exposed and reference groups by non-parametric Wilcoxon rank-sum test; †Comparison between the smokers and non-smokers.

Comparison of Plasma ACL and ACW Between Exposed and Reference Groups

As shown in Table 2, the concentrations of ACL were similar between the exposed and the reference groups, but ACW concentrations were lower among the exposed.

The median concentration of ACW was 37.9 μg/mL Trolox equivalent (25th percentile 26.9, 75th percentile 63.6) among the exposed non-smokers and 86.3 μg/mL Trolox equivalent (25th percentile 58.2, 75th percentile 92.7) in the reference non-smokers. The difference was not likely to be explained by chance (Wilcoxon rank-sum test: p = 0.004). We fitted a linear regression model for ACW adjusting for the covariates in Table 1. The adjusted difference in the mean plasma ACW concentration was –23.2 (95% CI –44.2 to –2.2, p = 0.03). The median concentration of plasma ACL and ACW were 9.9 and 35.6 μg/mL Trolox equivalent among the exposed smokers and 5.0 and 51.2 μg/mL Trolox equivalent in the reference smokers, respectively.

Relations of Urinary 1-OHPG to Urinary Isoprostane

There was a significant linear relation between isoprostane and 1-OHPG, as shown in Fig. 1. In linear regression

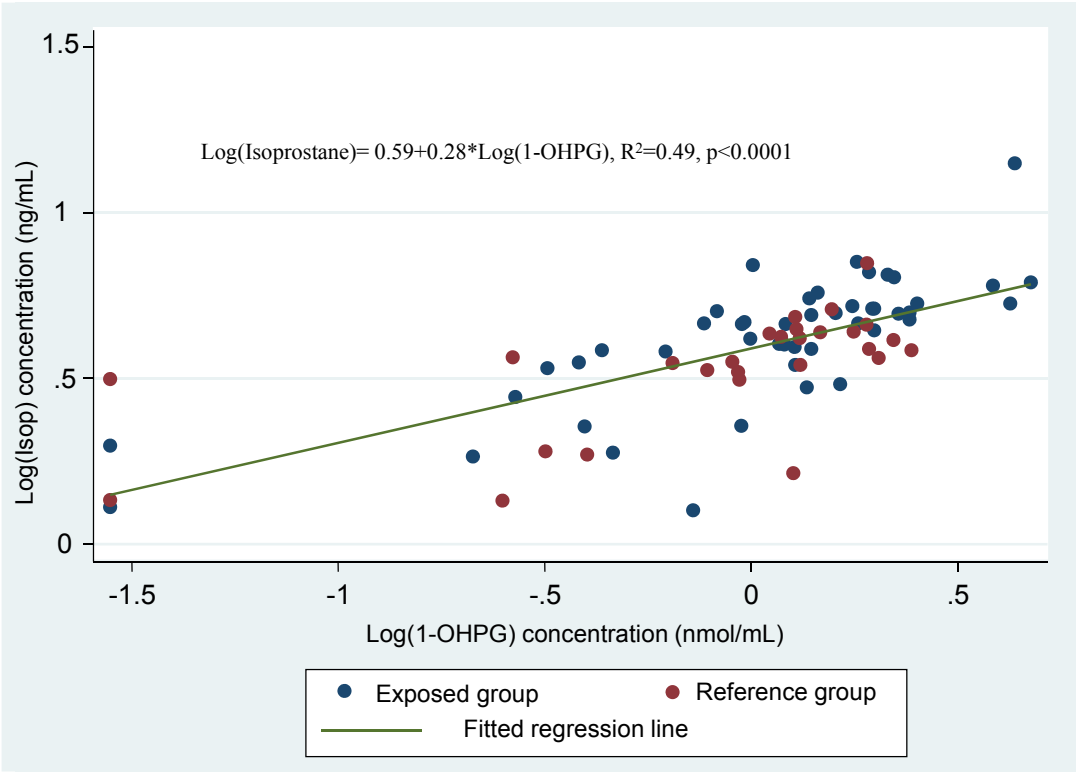

Fig. 1. Relationship between urinary 1-OHPG nmol/mL and isoprostane ng/mL among all study subjects.

adjusting for group, current smoking habit, current alcohol drinking habit, use of motorcycle as main transportation, frequent exposure to burning incense, and antioxidant capacity confounding variables, a change in log(isoprostane) was statistically significantly related to a unit change in log(1-OHPG) (β = 0.14, 95% CI 0.07 to 0.21). Adjustment for confounding did not change substantially on the association (Table 3).

DISCUSSION

The results of the present study indicate an increased amount of lipid peroxidation, measured as urinary isoprostane, in subjects working under conditions of potential oxidative stress due to exposure to polycyclic aromatic hydrocarbons related to traffic exhausts compared with a reference group of women without occupational exposure. Non-smoking female toll workers exposed to polycyclic aromatic hydrocarbons had a 32% higher concentration of urinary isoprostane (ng/mL) compared with non-smoking female classroom trainees, and a 47% lower level of total antioxidant capacity compared with the same reference group. There was a statistically significant linear relation between the log-transformed urinary PAH exposure biomarker (1-OHPG) and the urinary measure of lipid peroxidation (isoprostane). This relation did not change substantially when adjusting for determinants of 1-OHPG such as cigarette smoking and use of motorcycle in transportation.

To enhance the comparability of the two subject groups, we selected a reference group from women who were in training to become toll station workers, but not yet exposed to high levels of traffic exhausts at work. There were, however, some differences in potential determinants of urinary isoprostane. There were more smokers among the classroom trainees (Pearson $X^2 = 4.27$, p = 0.039) chosen for the reference group compared with the toll workers, and smoking is thought to influence urinary isoprostane concentration. Restricting the analyses to non-smokers eliminated the influence of smoking. It is known that besides traffic emissions, personal diet and background exposures to ambient environments and indoor pollutants sources, such as burning incense, and mosquito coils, cooking might affect the worker's urinary 1-OHPG level. But in this study the effect of above factors had been collected and adjusted. None of the workers reported consumption of broiled, grilled and barbecued food 24 hr prior to urine collection. We also applied linear regression analysis to adjust for other potential confounders. The traffic lane for the studied work place was selected for each participant from a rotating schedule. Thus the occupational exposure level was allocated independently from personal characteristics or other determinants of isoprostane and antioxidant capacity levels. The occupational exposure during the studied working days could have been, by chance, related to other factors influencing urinary isoprostane and antioxidant capacity levels; therefore we also fitted multivariate linear regression models with all the potential confounders. There was a statistically significant linear relation between the log-transformed urinary PAH exposure biomarker (1-OHPG) and the urinary measure of lipid peroxidation (isoprostane).

Table 3. Relationship between urinary isoprostane concentration and urinary 1-OHPG in univariate regression of log (Isoprostane) (ng/mL) by log(1-OHPG) (nmol/mL) and a multivariate regression model with potential confounders (N = 74).

Regression	Independent variable	Dependent variable: log(Isoprostane) (ng/mL)			
		B	(SE β)[a]	t	95% CI
Univariate	Log(1-OHPG) (nmol/mL)	0.28	0.03	8.3***	0.22 to 0.35
Multivariate	Log(1-OHPG) (nmol/mL)	0.14	0.04	4.00***	0.07 to 0.21
	Exposed vs. Reference group	0.07	0.04	2.02*	0.006 to 0.145
	Current smoking habit (smoker/ non-smoker)	0.018	0.04	0.43	–0.064 to 0.099
	Current alcohol drinking habit (Drinker/non-drinker)	0.046	0.08	0.61	–0.11 to 0.20
	Transportation (motorcyclist/ others)	0.01	0.03	0.23	–0.06 to 0.08
	Frequent exposure to incense (yes/no)	0.02	0.03	0.68	–0.04 to 0.09
	ACL antioxidant capacity (μg/mL Trolox equivalent)	–0.001	0.002	–0.46	–0.005 to 0.003
	ACW Antioxidant capacity (μg/mL Trolox equivalent)	0.0002	0.0005	0.58	–0.0007 to 0.001
	Creatinine (mg/dL)	0.002	0.0002	5.98***	0.001 to 0.002

[a] SE β = standard error of β coefficient.
*p < 0.05
***p < 0.001

To our knowledge this is the first study to provide evidence that exposure to traffic-related air pollution causes oxidative stress through lipid peroxidation. Several previous studies have applied urinary isoprostane as biomarkers of oxidative stress in general. The formation of isoprostane is related to personal factors (age) (Dohi *et al.*, 2007), occupational exposure (welding fume) (Han *et al.*, 2005), life style (vitamin C, vitamin E supplementation (Huang *et al.*, 2002), smoking, (Dietrich *et al.*, 2002; Dietrich *et al.*, 2003), vegetable and fruit intake (Thompson *et al.*, 2005), and alcohol consumption (Hartman *et al.*, 2005), and occurrence of chronic diseases (asthma, atherosclerosis, ischemia-reperfusion, cardiovascular disease (Cracowski, 2004), systemic lupus erythematosus, inflammatory rheumatic disease, diabetes mellitus (Cracowski *et al.*, 2002), and breast cancer (Rossner *et al.*, 2006). Il'yasova *et al.* (2004) indicated that ELISA measurements overestimates F2-Isoprostane levels than the GC/MS measurements. Although the ELISA is less accurate than the GC/MC method, it is important to note that levels determined by ELISA correlates well with their GC/MS method (r = 0.51 [p < 0.01]). It would be of interest to use more advanced methods of analysis for Isoprostane in these subjects in the future. In the multivariate regression, creatinine was a significant determinant for urinary isoprostane concentration. Adjustment for creatinine is a common way when assessing the concentration and determinants of urinary metabolites. The levels of creatinine reflects liver and renal function, muscle activity and correspondingly age in a complex manner. The effect of creatinine can also be explained by the fact that it is an indicator for urine dilution.

Based on our previous measurements in the Taipei toll station (Lai *et al.*, 2004b), the workers were exposed to very high levels of particulate matter. The median 8-hour concentrations of $PM_{2.5}$ were from 215 to 346 μg/m^3 in the bus and truck lanes, and from 87 to 130 μg/m^3 in the car lanes, while the reference group was exposed to 50 to 70 μg/m^3.

Fine particles from traffic exhausts are known to contain PAHs. The intermediate metabolites of PAH having electrophilic properties can covalently attach to intracellular macromolecules, leading to the generation of ROS (Casillas *et al.*, 1999). The presence of the isoprostane in urine represents the lipid peroxidation of phospholipids that occurs in situ. There are several advantages of urinary 15-F_{2t}-isoprostane measurements. First, this urinary product is not formed ex vivo by autooxidation in urine. Second, a morning void urine sample represents the daily isoprostane excretion in humans, since urinary F_2-isoprostane does not show significant diurnal variation (Richelle *et al.*, 1999). Third, measurement of F_2-isoprostane in urine is a non-invasive method to assess oxidant status.

Out study showed there was a difference in total antioxidant and ACW antioxidant, but not in ACL antioxidant capacity between exposed and reference group. The explanation could be that ascorbic acid (vitamin C), a water soluble vitamin, present as ascorbate in most biological settings, is considered the most important antioxidant in extracellular fluids. Vitamin C is oxidized first as ascorbyl radical and then to dehydroascobate and in the process scavenges free radicals and prevents radical-induced damage of lipoproteins and other macromolecules. Study shown ascorbate was far more effective in inhibiting lipid peroxidation initiated by a peroxyl radical initator than other plasma components, such as urate, bilrubin, and alpha-tocopherol (Sies *et al.*, 1992; Chavez *et al.*, 2007).

Because of difficulty in measuring each antioxidant component separately and interactions among antioxidant

molecules, we assessed the total antioxidant capacity of plasma. The antioxidant capacity within biological systems depends on several sources of antioxidants: 1) enzymes, for example, superoxide dismutase, gutathion peroxidase, catalase, and heme oxygenase (Rahman *et al.*, 2006); 2) large molecules, such as albumin, ferritin (Aycicek *et al.*, 2005); 3) small molecules, such as ascorbic acid, glutathione, tocopheral, carotenoid, phenols, cysteine (Cys) (Moriarty-Craige *et al.*, 2005; Opara and Rockway, 2006); and 4) some hormones, such as melatonin (Tan *et al.*, 2002). Thus cautious interpretation of these data is warranted, since the plasma might represent an extra-cellular compartment. Albumin, uric acid, bilirubin and ascorbic acid are major antioxidant components of plasma. In addition, albumin might play a major role of the total antioxidant capacity of plasma (Aycicek *et al.*, 2005). For example, Haegele *et al.* (2000) found an inverse relationship between plasma xanthophyll carotenoids and 8-OHdG concentration in lymphocyte DNA and urinary 8-epiprostaglandin F2α. Though we have taken into account the vitamin intake, food intake could be another possible confounder to control. Previous studies suggest that PM interacts with biological systems through direct generation of reactive oxygen species (ROS) from the surface of particles, organic chemicals, transition metals or other agitated processes in bodies and further contributes to the oxidative stress process (Gonzalez-Flecha, 2004; Risom *et al.*, 2005). It needs be pointed out that exposure to ozone, nitrogen dioxide, and transition metals may be associated with lipid peroxidation. These might be considered as a limitation of this study. This study applied a cross-sectional design and collected post-shift urine samples only. Genetic difference in PAH-metabolizing enzymes and lipid peroxidation repair in the study population could affect the estimated effects. Sampling at the beginning and end of the working week is justified when the half life of biomarker of exposure is longer than 5 h because of possible accumulation, which is the case with urinary 1-OHPG as a biomarker of exposure to traffic exhausts. In addition, occupational exposures may vary both within workers over time and between workers in the same job. A repeated measurement design in the future work is needed (1) to track the degree of change in biomarker levels over time; (2) to measure the changes of biomarkers and outcomes at the individual level; (3) to describe the mean trajectories and within-individual correlation of biomarkers (Munoz *et al.*, 1997).

CONCLUSION

Our results are consistent with the hypothesis that exposure to traffic-related air pollution increases oxidative stress. Oxidative stress due to air pollution exposure could partly explain the associations between daily levels of particulate matter and adverse cardiovascular health effects. Exposure to traffic exhausts increases the urinary concentration of isoprostane suggesting that it might be a useful biomarker of oxidative stress resulting from vehicle exhaust exposure. Exposure to traffic exhausts may also reduce antioxidant capacity. The component of antioxidant capacity that is directly responsible for antioxidant protection merits further investigation. Further studies are also needed to elaborate the within subject relation between exposure to traffic exhausts and isopostane concentration, and to clarify the influence of dietary intake of antioxidants on the relation with isoprostane.

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