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Plasma Nitrogen Oxides Levels in Taxi Drivers and Community Residents

C. Y. Chuang,[1] J. C. Hung,[1] C. Y. Yang,[2] Y. C. Ma,[1] F. C. Sung[1,3]

[1] Institute of Environmental Health, National Taiwan University College of Public Health, 1 Jen-Ai Road Section 1, Room 1521, Taipei 100, Taiwan
[2] Institute of Public Health, Kaohsiung Medical University, 100 Shi-Chuan First Road, Kaohsiung 807, Taiwan
[3] Institute of Prevention Medicine, National Taiwan University College of Public Health, Taipei 100, Taiwan

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Vehicle exhaust fumes are a main source of air pollution in cities (Bate 1995). In the combustion process, nitrogen in the fuel and the air is converted into nitrogen oxides (NOx), nitric oxide (NO) and nitrogen dioxide (NO_2). The emitted NO is then oxidized to NO_2 in the atmosphere. NO_2 causes morphological and physiological changes in pulmonary epithelium to lung damage, and is concerned to have genotoxic effect (Hayashi et al. 1987; Victorin 1994). NOx may induce DNA damage and mutagenesis, resulting in DNA oxidation by peroxynitrite, deamination of DNA and formation of the N-nitroso compounds (Wink et al. 1991; Stamler 1994; Filep et al. 1997).

The primary metabolites of NOx are nitrite (NO_2^-) and nitrate (NO_3^-). NO_2 absorbed in the lung via inhalation is converted into NO_2^-. After diffusing to the vascular space, NO_2^- interacts with erythrocytes and is oxidized into NO_3^- (Yoshida et al. 1980). In addition, NO is also generated endogenously from L-arginine functioning as a signal in nerve cells, the mediation of vasodilation in endothelial cells and the mediation of cytotoxicity in macrophages (Bredt et al. 1994). However, NO produced in infected and inflamed tissues may induce carcinogenesis (Tamir et al. 1996). The body burden of NOx via intake of food and water is also of concern (Marletta 1988).

This study was designed to determine whether occupational exposure to traffic exhaust increases the burden of pollutants in the body. This paper compares plasma NOx concentrations between taxi drivers and community residents and measures potential associated effects such as the Framingham heart disease risk predictions.

MATERIALS AND METHODS

The study population consisted of 181 male taxi drivers and 92 male community residents as potential participants for, respectively, the study group and the reference group, stratified by smoking status. Taxi drivers were recruited at a taxi rest station in Taipei City. To be eligible for this study, those who were former smokers (n=41) or had less than 3-year experience as taxi drivers (n=45) were excluded from the study group. The reference group consisted of male citizens

Correspondence to: F. C. Sung

residing in a community with the lowest air pollution levels in Taipei metropolitan, according to pollution index available from Taiwan air pollution statistics. Former smokers (n=17) were also excluded from the reference group. With informed consent, a physician interviewed potential subjects with respect to health status, performed blood pressure measurements and obtained blood specimens. Study subjects were asked to complete a self-reported questionnaire on age, height and weight, education, cigarette smoking, and dietary intake. Two post 8-hour fasting venous blood specimens were collected from each person in the morning and transported to our laboratory in boxes refrigerated at 4°C. One sample was collected in non-anticoagulant tubes for measures performed on sera, while the second sample was collected in heparinized vacutainer tubes for measures performed on plasma. Within four hours of sampling, all blood samples were centrifuged at 3,000 rpm for 15 minutes to remove blood cells. The supernatant was transferred to an eppendorf tube and stored at –20°C until analysis.

Serum globulin, total cholesterol, low-density-lipoprotein (LDL)-cholesterol, high-density-lipoprotein (HDL)-cholesterol, and triglyceride were measured directly using a Hitachi 7250 auto-mechanic analyzer. NOx in plasma was measured using a nitric oxide analyzer (Sievers, Boulder, CO, USA). To avoid interfering with analysis, two volumes of 4°C absolute ethanol was added to the thawed plasma sample and centrifuged at 14,000 rpm for 10 minutes to remove protein. A saturated vanadium chloride (VCl_3) solution (1.0 g VCl_3/100 ml 0.1 N HCl) was added in 20 μl deproteinized plasma to reduce NO_2^- and NO_3^- to NO (Archer 1993). NO reacts with ozone and becomes NO_2 in an excited state, decaying to a weak infrared ray above 600 nm, detected by chemiluminescence. The amount of NOx in a sample was determined by intrapolation from 1-100 μ*M* standard solutions of sodium nitrate ($NaNO_3$). The detection limit was 10 n*M*, recovery rate (mean ± SD) was 98.2 ± 5.8 % at a concentration of 50 μ*M* and the reproducibility for the analysis of triplicate samples was within 5.0 % coefficient of variation.

Data analysis compared the average levels and the cumulative distributions of plasma NOx between taxi drivers and community men, by smoking status. One way ANOVA was used to test the difference in clinical characteristics by study group and smoking status. A multiple linear regression was performed to estimate the relationships between plasma NOx level, potential exposures and the Framingham heart disease prediction score estimated by age, blood pressure, levels of total cholesterol (or LDL cholesterol) and HDL cholesterol, smoking status and diabetes mellitus (Wilson et al. 1998). The data analysis was performed using SPSS 10.0 for Windows. A *p*-value less than 5% was considered statistically significant.

RESULTS AND DISCUSSION

Nitrogen oxides are generated by environmental agents and by the endogenous process. Gaseous NO may directly attacks DNA or tissue to form 8-nitroguanine in human lung fibroblast cells, contributing to multistage carcinogenesis (Arimoto et al. 1999). Endogenous NO induced by activating inducible nitric oxide

synthase may partly cause DNA damage resulting in p53 protein accumulation and phosphorylation (Forrester et al. 1996). Cigarette smoking is one NOx exposure source associated with coronary, cerebral and peripheral vascular diseases (Holbrook et al. 1984). Cigarette smoking stimulates endothelium-derived NO production (Roberts et al. 1996) and is associated with an increased in serum NO level (Sarkar et al. 1999), while another study showed that cigarette smoking reduces the content of NOx in plasma (Node et al. 1997). Among taxi drivers, we found the average plasma NOx level was higher for smokers than non-smokers (47.9 ± 9.7 vs. 42.4 ± 6.0 μM, $p = 0.009$) (Figure 1). Among community men, the NOx level was also higher for smokers than for non-smokers (43.1 ± 5.8 vs. 37.7 ± 7.5 μM, $p = 0.034$). Therefore, smoking is causally associated with increased plasma NOx.

NO is also produced during the inflammation process and may play a role in carcinogenesis. Moussa et al. (2000) found the average plasma NOx levels in patients with liver cirrhosis (42.4 ± 26.9 μM) and with hepatocellular carcinoma (49.4 ± 49.1 μM) were significantly higher than those with chronic hepatitis C without cirrhosis (32.3 ± 8.9 μM) and healthy control subjects (35.5 ± 15.1 μM). Except for cancer patients, these average levels were less than those in our study subjects, who were assumed to be healthy. Although NOx can be produced during inflammation, it is also a substance that can be beneficial in endothelium-derived relaxation and nerve transmission. The serum globulin, regarding as inflammation, for taxi drivers in this study was higher than that for community men (Table 1). However, in the multiple regression analysis (Table 2), no significant association was shown between the levels of serum globulin and plasma NOx.

The average plasma NOx concentration, after controlling for smoking, was higher in taxi drivers than in community men ($p = 0.058$). This increment in non-smoking taxi drivers supports the hypothesis that occupational exposure to traffic air pollutants may elevate the level of plasma NOx. The cumulative percentage distributions of plasma NOx for our study groups demonstrated that the gain of NOx in plasma from the taxi driving alone was similar to that from smoking in community men (Figure 2). The distribution for taxi drivers with smoking behavior clearly differed from that of non-smoking community men, suggesting an additional effect of traffic exposure and smoking.

The extracts of cigarette smoke may impair the endothelium-dependent dilation of coronary arteries by increasing superoxide anion-mediated degradation of NO (Murohara et al. 1994). In the present study, the result was consistent with a previous study (Node et al. 1997) in which the mean Framingham heart disease risk prediction score was negatively associated with the plasma NOx at higher risk for smokers. However, the relationship was not statistically significant in the multiple regression (Table 2). Taxi driving and smoking were the only significant factors associated with plasma NOx levels.

Since NOx content in food intake affects the plasma concentration of NOx, all study subjects were asked to fast for at least 8 hours prior to blood specimen collection. We found no relationship between the level of plasma NOx and the

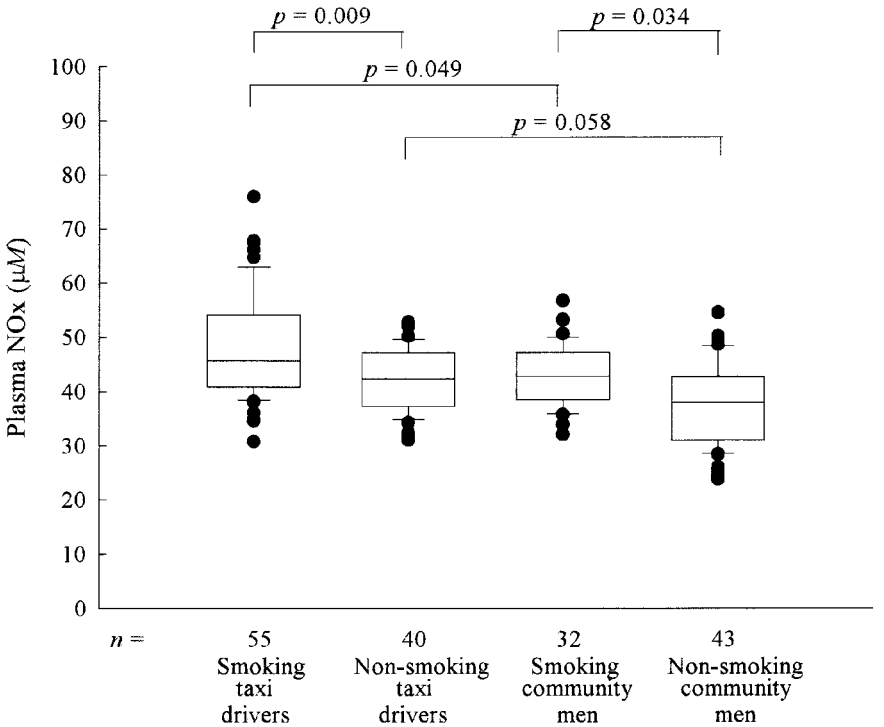

Figure 1. Average plasma NOx level by study group.

Table 1. Clinical characteristics of the study groups.

Variables	Taxi drivers		Community men		p-value
	smoke	non-smoke	smoke	non-smoke	
Numbers	55	40	32	43	
Mean age (yr)	38.9±3.8	40.7±3.8	43.0±5.9	45.2±8.0	<0.001
Systolic blood pressure	119±15	128±20	122±17	127±19	0.035
Diastolic blood pressure	79±12	84±13	82±12	87±14	0.029
Body mass index	25.2±3.0	25.2±3.3	25.4±3.2	25.0±2.7	0.948
Serum globulin	2.9±0.4	3.0±0.3	2.3±0.5	2.5±0.5	<0.001
Serum total cholesterol	207±40	205±38	197±35	206±41	0.715
Serum LDL cholesterol	120±37	134±32	130±30	133±44	0.224
Serum HDL cholesterol	44±11	45±12	40±10	45±13	0.246
Serum triglycerides	216±166	157±150	133±88	144±130	0.022
Diabetes (%)	5.5	2.5	6.3	7.0	0.819
Framingham heart disease risk prediction score (%)	7.9±3.0	5.2±2.3	11.0±7.4	7.8±4.3	<0.001
Comparative risk of the same age	2.4±0.9	1.5±0.6	2.6±1.5	1.7±0.7	<0.001

Values are mean ± standard deviation. Units are mmHg for blood pressure, kg/m^2 for body mass index, g/dL for serum globulin, mg/dL for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

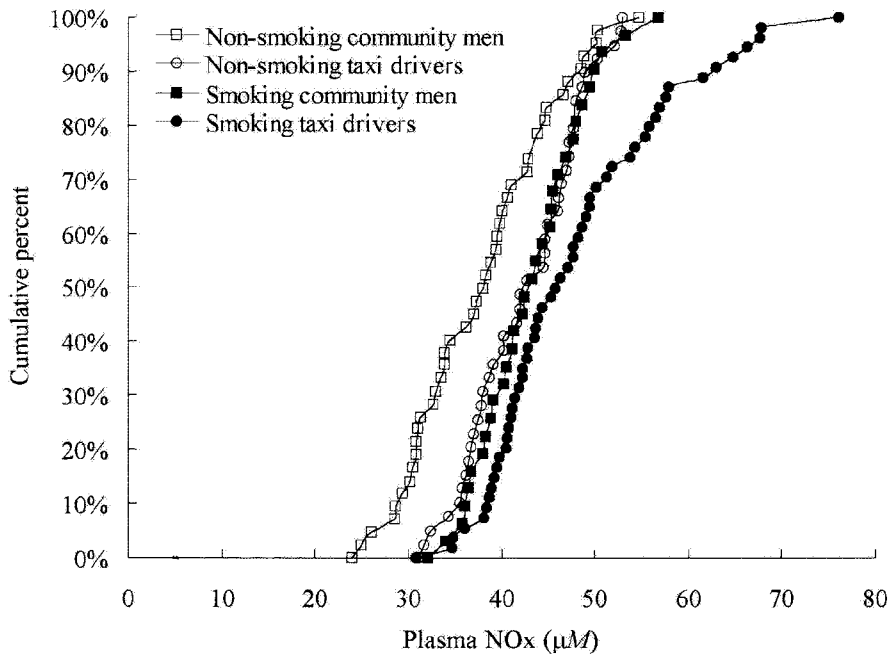

Figure 2. Cumulative percentage distribution of plasma NOx level by study group.

Table 2. Multiple regression analysis for the effect of selected variables on plasma NOx (n = 153, $R^2 = 0.275$, $p < 0.001$).

Variables	Standard regression coefficient	*t*-value	*p*-value
Intercept		4.455	<0.001
Study group[a]	0.280	2.838	0.005
Age (yr)	0.105	1.086	0.279
Education (yr)	-0.038	-0.317	0.752
Smoking status (Y/N)[b]	0.309	3.491	0.001
Passive smoke exposure (Y/N)[b]	0.083	1.064	0.289
Charcoal-broiled foods[c]	0.026	0.325	0.745
Nitrified meats[c]	-0.037	-0.434	0.665
Serum globulin (g/dL)	0.053	0.598	0.551
Mean Framingham heart disease risk prediction score (%)	-0.040	-0.428	0.669

[a]Taxi drivers vs. community men; [b]Y/N: Yes vs. No; [c]≥ 1/week vs. < 1/week.

consumption of charcoal-broiled foods and nitrified meats. Taxi driving and smoking remained as the only significant factors associated with the plasma NOx level in the multivariate regression mode. In conclusion, the present study suggests that smoking and occupational exposure are important contributors to the increment level of plasma NOx for study subjects.

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