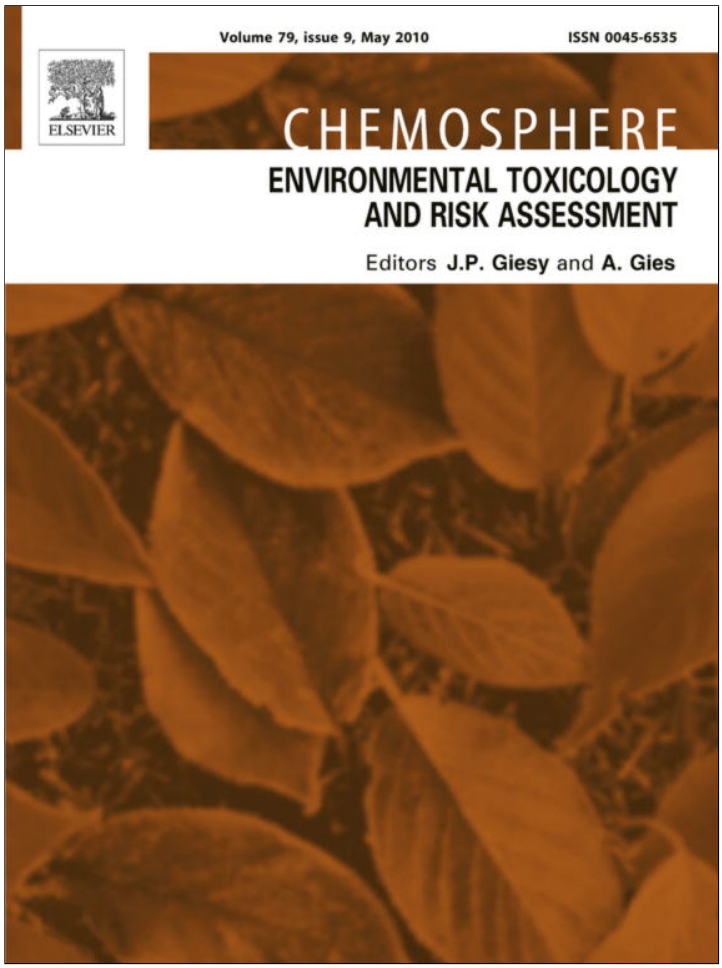

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Increased urinary 8-hydroxy-2′-deoxyguanosine excretion in long-distance bus drivers in Taiwan

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ABSTRACT

Professional bus drivers are exposed to environments containing air pollution and reactive oxygen species (ROS) that can induce cellular oxidative stress and DNA damage. This study investigated environmental factors associated with oxidative DNA damage in a cohort of long-distance bus drivers. In a comparison study, urinary 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of DNA oxidative damage, was examined in 120 male long-distance bus drivers and 58 male office workers in Taiwan. Multivariate logistic regression was used to analyze association between urinary 8-OHdG levels and environmental factors. Bus drivers had higher urinary 8-OHdG levels (adjusted odds ratio (aOR) = 9.4, 95% confidence interval (CI) = 3.5–28.2) compared with office workers. Increased urinary 8-OHdG level was significantly related to cigarette smoking (aOR = 18.0, 95% CI = 7.1–52.1), consumption of energy drinks (aOR = 5.0, 95% CI = 2.1–12.6), and regular exercise (aOR = 3.8, 95% CI = 1.5–10.2). A strong exposure–response relationship was found between urinary 8-OHdG and urinary cotinine ($p < 0.0001$). Among nonsmokers, bus drivers (aOR = 3.9, 95% CI = 1.0–17.7) had higher urinary 8-OHdG than office workers. Among both bus drivers and office workers, those who drank energy drinks (aOR = 3.7, 95% CI = 1.2–12.2) had higher 8-OHdG levels than those who did not drink energy drinks. Adjusted for smoking, levels of 8-OHdG were increased in long-distance bus drivers exposed to traffic exhaust and ingested energy drinks. Future studies should explore what aspects of energy drinks may contribute to increased urinary 8-OHdG.

1. Introduction

Occupational exposure to vehicle exhaust is known to constitute an important occupational health hazard for professional drivers. Exhaust emissions contain large quantities of oxidants, including nitrogen oxides (NO_x) as well as compounds that induce the generation of reactive oxygen species (ROS), such as polycyclic aromatic hydrocarbons (PAHs) and benzo[a]pyrene (B[a]P) (Ng et al., 1998; Park et al., 2006). ROS are oxygen free radicals and reactive oxygen species, produced endogenously but also through exposure to pollutants. Transition metals in ambient particulate matter (PM), such as vanadium, copper, iron, and platinum, are also capable of redox cycling that result in the production of ROS (Pinkerton et al., 2008; Valavanidis et al., 2009). Reactive oxygen, nitrogen species and PM can cause excessive generation of ROS within tissues that can damage macromolecules such as DNA, lipids, and proteins, interfere with cellular DNA repair and contribute to structural and functional damage to DNA (Autrup et al., 1999).

Oxidative stress induced free radical formation is thought to contribute to DNA damage that can lead to many chronic diseases including cancer, cardiovascular disease and aging. The DNA adduct, 8-hydroxy-2′-deoxyguanosine (8-OHdG), has been used as a marker that represents cumulative oxidative DNA damage in animals and humans expose to mutagenic agents such as those found in urban air pollution (Kim et al., 2004). In many studies, 8-OHdG is also used for risk assessment of various cancers and degenerative diseases (Valavanidis et al., 2009).

The biological relevance of 8-OHdG as a mutagen derives from its ability to induce G–T transversions (Shibutani et al., 1991; Klein et al., 1992), that are among the most frequent somatic mutations found in human cancers (Toyokuni et al., 1995; Wiseman and Halliwell, 1996). Thus, 8-OHdG is considered to be both a sensitive biomarker of *in vivo* oxidative DNA damage and a functionally important DNA modification involved in a number of diseases including cancer. Increased evidence has shown malignant cells contain high levels of oxidized DNA lesions (Cooke et al., 2003). Compared to healthy individuals, significantly increased urinary 8-OHdG level was reported in patients with cancer, atherosclerosis, and diabetes (Wu et al., 2004).

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The International Agency for Research on Cancer (IARC) classified diesel engine exhaust as probably carcinogenic (group 2A) and petrol exhaust as possibly carcinogenic (group 2B) to humans (IARC, 1989). National Toxicology Program (NTP) listed diesel exhaust particulate in the report on carcinogen as reasonably anticipated to be a human carcinogen (NTP, 1998). In vehicles, the cabin has recently been recognized as an important microenvironment that can provide personal exposure to many organic compounds (Romieu et al., 2008). The concentration of CO, benzene, toluene and xylenes in the personal air monitor samples measured for car drivers were found to be much higher than in those of cyclists. On busy roads, especially with stationary traffic, the emissions of preceding cars may enter the cabin space leading to increased levels of pollutant exposure (van Wijnen et al., 1995).

Compared to the general population, professional drivers experience significant excess exposure risk to diesel air pollution. Long-term exposure to diesel exhaust in high risk occupations such as for truck drivers, bus drivers, dock drivers, and railroad workers, is associated with a 1.2–1.5-fold increase in the relative risk of lung cancer compared with workers classified as unexposed, or who have been exposed to lower levels or to high levels for shorter periods of time (HEI, 1999). This study in high traffic density Taiwan compared the levels of urinary 8-OHdG between male long-distance bus drivers and male office workers. The role of both environmental and occupational factors on oxidative DNA damage for the highly exposed individuals was investigated. Since smoking contributes to oxidative damage, we also determined urinary cotinine levels for all subjects to adjust for the effects of smoking.

2. Materials and methods

2.1. Study subjects and data collection

Study subjects were randomly selected from two long-distance transportation companies in Taiwan. The exposure group included 150 adult male long-distance bus drivers who drove national expressway and provincial highway routes more than 100 km on work days and had an employment history of greater than one year. All the buses used diesel engine. The comparison group consisted of 75 male office workers in the same transportation companies including executives, officers, administrators and clerks. Participants were interviewed by trained interviewers and completed a questionnaire survey. Urine samples were collected at the bus station after completion of the interview. Informed consent was obtained from each participant. Subjects were excluded if they refused to provide urine samples or did not complete the questionnaire. The response rates were 80% and 77% for exposed and non-exposed group, respectively.

The structured questionnaire had been reviewed by environmental and occupational epidemiology experts. The questionnaire included information on social and demographic characteristics, height, weight, employment history, weekly working hours, and lifestyle choices including exercise frequency, cigarette smoking, betel chewing, and consumption of alcohol, tea, coffee and energy drinks. Bus drivers were interviewed after the working shift in the bus station and the comparison groups were interviewed during work hours in their offices.

2.2. Urinary 8-OHdG measurement

Studies have shown that 8-OHdG levels are not significantly different between the spot urine and the 24-h urine samples, which suggests that the 8-OHdG levels in spot urine samples could be used to estimate systemic oxidative stress throughout the body, simplifying the process of sample collection (Loft et al., 1999). Spot urine samples were collected in 50 mL polypropylene centrifuge tubes refrigerated at 4 °C, transported to the laboratory, and stored at −80 °C prior to analysis. To control for variation due to dilution, urinary creatinine levels were determined by an automated analyzer (Hitachi 7250, Tokyo, Japan) using the Jaffe colorimeter reaction (Nerurkar and Sahasrabudhe, 1960). At the time of analysis, urine samples were thawed at 4 °C and centrifuged at 2000 g for 10–15 min to remove the particulate matter.

Urinary 8-OHdG level was measured using the *OXIS* research™ 8-OHdG ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan) which has high sensitivity and specificity. At room temperature, 50 μL of primary antibody was added to 50 μL aliquot of each sample and standard in microtiter plates pre-coated with 8-OHdG for assay, using the procedure developed by Yin et al. (1995). Urinary 8-OHdG concentration was determined by comparing the absorbance values at 450 nm using a spectrophotometer. The calibration curve was generated by including standards containing 0.5, 2, 8, 20, 80 and 200 ng mL^{-1} 8-OHdG. The concentration in the urinary specimen was expressed as μg g^{-1} creatinine.

2.3. Urinary cotinine measurement

The direct barbituric acid (DBA) assay for urinary cotinine determination is based on the König reaction that requires opening of the pyridine ring of cotinine by reaction with cyanogen chloride to form a glutaconaldehyde derivative, which reacts with barbituric acid to form an orange colored complex (Peach et al., 1985; Ubbink et al., 1993). Measurement of cotinine was standardized against a wide rage (0–50 ng mL^{-1}). The cotinine concentrations were determined by comparing the absorbance values at 545 nm using spectrophotometer, with a calibration curve generated by 0.25, 2.5, 5, 12.5, 25 and 50 ng mL^{-1} cotinine. The concentration in the urinary specimen was expressed as ng mL^{-1}.

2.4. Statistical analysis

Statistical analysis was performed using SAS for Windows version 8.0 (SAS Inc. Carey, NC, USA). Mean (±standard deviations) and median urinary 8-OHdG and cotinine levels were calculated. Cumulative distributions of urinary 8-OHdG were depicted using graphical presentation by study group and covariate. Student's *t*-test and ANOVA were performed to evaluate the differences of urinary 8-OHdG levels by subgroups. Multivariate logistic regression and adjusted odds ratio (aOR) with 95% confidence intervals (CI) were used to determine the association between urinary 8-OHdG level and environmental factors. Because urinary 8-OHdG levels were not normally distributed, median 8-OHdG level (7.3 μg g^{-1} creatinine) of all study subjects was used in multivariate logistic regression analysis. Statistical significance was set at $p < 0.05$ based on a two-sided calculation. By stratifying smoking status, multivariate logistic regression was performed to identify factors that may increase urinary 8-OHdG levels.

3. Results

3.1. Demographical characteristics of study subjects

The average age was not significantly different between bus drivers and office workers (Table 1). Approximate 45% of office workers had more than 13 years education compared to 5% of bus drivers. The average body mass index (BMI) among bus drivers was higher than in office workers (24.8 and 23.8 kg m^{-2}, $p = 0.059$). Prevalence of overweight (BMI > 25.0 kg m^{-2} for population in Taiwan) was 42% among bus drivers compared to 29% among office workers. The average length of employment was shorter among

Table 1
Demographic characteristics of study subjects.

Variables	Bus drivers n = 120 (%)	Office workers n = 58 (%)	p-Value
Age (years)			
20–34	21 (17.5)	19 (32.8)	0.0263
35–49	78 (65.0)	24 (41.2)	
⩾50	21 (17.5)	15 (25.9)	
Mean (years ± SD)	42.0 ± 6.8	40.9 ± 10.7	0.413
Education (years)			
⩽9	39 (32.5)	5 (8.6)	<0.0001
10–12	75 (62.5)	27 (46.6)	
⩾13	6 (5.0)	26 (44.8)	
Marriage			
Single/divorced	24 (20.0)	24 (41.4)	0.003
Married	96 (80.0)	34 (58.6)	
BMI (kg m^{-2})			
⩽24.9	70 (58.3)	41 (70.7)	0.107
⩾25.0	50 (41.7)	17 (29.3)	
Mean (BMI ± SD)	24.8 ± 3.4	23.8 ± 3.0	0.059
Employment history (years)			
⩽10	74 (61.7)	28 (48.3)	0.012
>10	46 (38.3)	30 (51.7)	
Mean (years ± SD)	7.8 ± 7.8	11.0 ± 9.9	0.019
Work hour (hours/week)			
⩽50	54 (45.0)	45 (77.6)	<0.0001
>50	66 (55.0)	13 (22.4)	
Mean (hours ± SD)	58.5 ± 13.9	47.9 ± 7.4	<0.0001

SD = standard deviation.

bus drivers. The average weekly working hours were significantly higher in bus drivers than in office workers (58.5 and 47.9 h, respectively, $p < 0.0001$).

No significant differences were found between bus drivers and office workers with respect to the prevalence of cigarette smoking and consumption of alcohol, coffee and energy drinks (data not shown). Of the 178 subjects, 21.7% of the 120 bus drivers and 3.5% of the 58 office workers were current betel chewers ($p = 0.002$). Tea drinking was more frequent in office workers. More office workers exercised regularly compared to bus drivers (43.1% and 26.7%, respectively, $p = 0.03$).

3.2. Urinary 8-OHdG measurement

The average level of urinary 8-OHdG was significantly higher in the bus drivers (9.5 ± 5.7 μg g^{-1} creatinine) compared to it in office workers (7.3 ± 5.4 μg g^{-1} creatinine, $p = 0.015$) (Table 2). Based on the multivariate regression analysis, the risk of having a urinary 8-OHdG level in excess of 7.3 μg g^{-1} creatinine was significantly higher in bus drivers than in office workers (aOR = 9.4, 95% CI = 3.5–28.2). Higher urinary 8-OHdG was found in subjects 35–49 years of age (aOR = 4.5, 95% CI = 1.0–22.1), smokers (aOR = 18.0, 95% CI = 7.1–52.1), those who drank energy drinks (aOR = 5.0, 95% CI = 2.1–12.6), and those who exercised at least once a week (aOR = 3.8, 95% CI = 1.5–10.2). Although it did not reach statistical significance, subjects who worked more than fifty hours weekly had higher urinary 8-OHdG level. BMI value, betel chewing, and consumption of alcohol, tea or coffee were not found to be associated with increased urinary 8-OHdG level.

Fig. 1 demonstrates that urinary 8-OHdG level was consistently the highest in smoking bus drivers, followed by smoking office workers, non-smoking bus drivers and non-smoking office workers. A strong exposure–response relationship was found between urinary 8-OHdG and urinary cotinine ($p < 0.0001$, Fig. 2). Self-reported passive smoking was not significantly related to the increased urinary level of 8-OHdG (data not shown). Betel nut chewing was not related to increased 8-OHdG when compared to

Table 2
Urinary 8-OHdG level in relation to suspected risk factors by multivariate regression analysis.

Variable	N	8-OHdG (μg g^{-1} creatinine)	aOR	95% CI
Study group				
Office workers	58	7.3 ± 5.4	1.0	
Bus drivers	120	9.5 ± 5.7	9.4	3.5–28.2
Age, years				
20–34	40	8.9 ± 5.1	1.0	
35–49	102	9.2 ± 6.3	4.5	1.0–22.1
⩾50	36	7.4 ± 4.0	2.0	0.6–7.3
BMI, kg m^{-2}				
⩽24.9	111	9.1 ± 5.9	1.0	
⩾25.0	67	8.2 ± 5.1	0.9	0.4–2.1
Cigarette smoking				
No	70	5.9 ± 3.9	1.0	
Yes	108	10.6 ± 5.8	18.0	7.1–52.1
Alcohol drinking				
No	121	8.8 ± 5.9	1.0	
Yes	57	8.7 ± 5.1	0.6	0.2–1.4
Areca chewing				
No	150	8.4 ± 5.7	1.0	
Yes	28	11.0 ± 5.0	0.8	0.2–2.5
Tea				
No	30	9.9 ± 8.0	1.0	
Yes	148	8.6 ± 5.1	1.0	0.3–3.2
Coffee				
No	51	8.2 ± 4.7	1.0	
Yes	127	9.0 ± 6.0	1.3	0.5–3.6
Energy drinks				
No	105	7.9 ± 5.1	1.0	
Yes	73	10.0 ± 6.2	5.0	2.1–12.6
Exercise regularly				
No	121	8.5 ± 5.5	1.0	
Yes	57	9.3 ± 6.0	3.8	1.5–10.2
Employment years				
⩽10	102	9.1 ± 5.8	1.0	
>10	76	8.3 ± 5.4	1.2	0.4–3.3
Weekly work hours				
⩽50	99	8.1 ± 5.1	1.0	
>50	79	9.6 ± 6.1	0.9	0.3–2.2

Values represented by mean ±standard deviation (SD) for 8-OHdG levels, and aOR (adjusted odds ratio), and 95% CI (confidence interval) were measured by comparing with the median 8-OHdG (7.3 μg g^{-1} creatinine) of all study subjects.

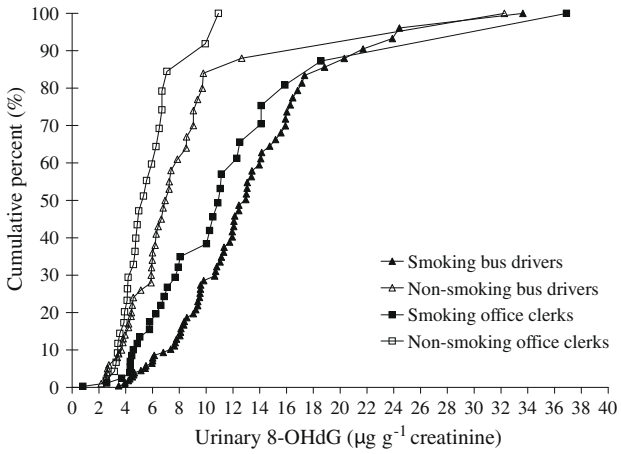

Fig. 1. Cumulative distributions of urinary 8-OHdG by study group and smoking status.

results from nonsmokers and non-betel chewers. But, subjects who smoked and chewed betel nuts had increased urinary 8-OHdG

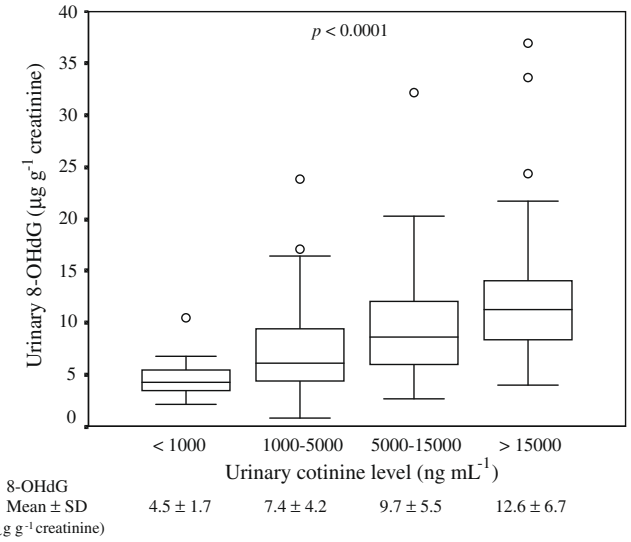

Fig. 2. Average levels of urinary 8-OHdG by urinary cotinine level for all study subjects.

levels compared with subjects who smoked but did not chew betel nuts (aOR = 18.3, 95% CI = 4.9–67.8) (data not shown).

Current smoking increased urinary 8-OHdG by 80% in bus drivers and 73% in office workers (Table 3). Among smokers, bus drivers had higher levels of urinary 8-OHdG compared with office workers (aOR = 3.0, 95% CI = 1.1–9.4). Workers who were employed more than 10 years had higher level of urinary 8-OHdG compared with those who were employed less than 10 years (aOR = 3.6, 95% CI = 1.1–13.4). Among nonsmokers, bus drivers had higher urinary 8-OHdG level compared to office workers (aOR = 3.9, 95% CI = 1.0–17.7) and consumption of energy drinks was related to higher urinary 8-OHdG excretion (aOR = 3.7, 95% CI = 1.2–12.2). Betel chewing was not included in the multivariate regression model among nonsmokers because there were only two betel chewers among nonsmokers.

4. Discussion

This study compared the urinary levels of 8-OHdG between Taiwanese male long-distance drivers and office workers. The results clearly demonstrated that long-distance bus driving, and, in all subjects, cigarette smoking, consumption of energy drinks and regular exercise correlated with higher urinary 8-OHdG excretion. Cigarette smoke contains several well-known ROS that have consistently been documented to increase oxidative stress in human body and urinary 8-OHdG excretion (Pourcelot et al., 1999). Among smokers, urinary 8-OHdG in bus drivers was 25.6% higher than the

Table 3
Urinary 8-OHdG level in relation to suspected risk factors by smoking status by multivariate regression analysis.

Variable	Smokers				Nonsmokers			
	N	8-OHdG (μg g^{-1} creatinine)	aOR	95% CI	N	8-OHdG (μg g^{-1} creatinine)	aOR	95% CI
Study group								
Office workers	32	9.0 ± 5.4	1.0		26	5.2 ± 2.0	1.0	
Bus drivers	76	11.3 ± 5.4	3.0	1.1–9.4	44	6.3 ± 6.7	3.9	1.0–17.7
Age, years								
20–34	27	10.2 ± 5.6	1		13	6.0 ± 1.9	1	
35–49	62	11.1 ± 6.4	1.5	0.3–7.9	40	6.2 ± 4.9	3.3	0.5–28.0
⩾50	19	9.7 ± 4.1	0.7	0.2–2.2	17	5.0 ± 2.1	3.6	0.6–28.2
BMI, kg m^{-2}								
⩽24.9	69	11.0 ± 5.8	1		42	6.0 ± 4.8	1.0	
⩾25.0	39	10.1 ± 5.9	0.5	0.2–1.1	28	5.7 ± 2.1	0.9	0.3–2.5
Alcohol drinking								
No	70	10.9 ± 6.1	1.0		51	6.0 ± 4.3	1.0	
Yes	38	10.2 ± 5.2	0.7	0.3–1.8	19	5.6 ± 2.8	1.2	0.3–4.3
Areca chewing								
No	82	10.5 ± 6.0	1.0					
Yes	26	11.2 ± 5.1	1.1	0.4–3.3				
Tea								
No	18	13.0 ± 8.9	1.0		12	5.1 ± 2.0	1.0	
Yes	90	10.2 ± 4.9	0.5	0.1–1.6	58	6.1 ± 4.2	1.5	0.3–8.2
Coffee								
No	30	10.4 ± 4.8	1		21	4.9 ± 2.0	1.0	
Yes	78	10.7 ± 6.2	1.2	0.4–3.8	49	6.3 ± 4.5	1.2	0.3–5.4
Energy drinks								
No	62	10.1 ± 5.5	1.0		43	4.8 ± 1.9	1.0	
Yes	46	11.4 ± 6.3	2.2	0.9–5.6	27	7.7 ± 5.5	3.7	1.2–12.2
Exercise regularly								
No	74	10.1 ± 5.5	1.0		47	6.0 ± 5.5	1.0	
Yes	34	11.7 ± 6.3	1.9	0.7–5.4	23	5.7 ± 3.1	1.1	0.3–3.7
Employment history, years								
⩽10	71	10.2 ± 5.7	1.0		31	6.6 ± 0.7	1.0	
>10	37	11.4 ± 6.0	3.6	1.1–13.4	39	5.4 ± 0.6	1.2	0.4–4.5
Weekly work hours								
⩽50	51	10.6 ± 5.8	1.0		48	5.4 ± 2.3	1.0	
>50	57	10.7 ± 5.9	0.9	0.3–2.3	22	7.0 ± 6.1	1.3	0.4–4.6

Values represented by mean ±standard deviation (SD) for 8-OHdG levels; aOR (adjusted odds ratio) and 95% CI (confidence interval) were calculated by comparing the median 8-OHdG level ± of smokers (9.9 μg g^{-1} creatinine) to nonsmokers (5.0 μg g^{-1} creatinine).

levels observed in office workers. Among nonsmokers, urinary 8-OHdG in bus drivers was 21.2% higher than in office workers. We suggest that after adjusting for smoking status, traffic exhaust exposure may contribute approximately a 20–25% elevation of urinary 8-OHdG excretion in long-distance bus drivers.

Research has identified oxidative stress as one potential mechanism underlying the toxic effect of air pollutants. A hierarchical oxidative stress model has been proposed to explain the dose-dependent response to air pollutant exposures. Lower exposure leads to formation of ROS activating an antioxidant response leading to synthesis of enzymes related to detoxification, cytoprotective and antioxidant responses. Higher exposures are thought to activate the transcription NF-κB and activator protein-1 response, alter the function of mitochondria or NADPH, and increase expression of pro-inflammatory cytokines (Xiao et al., 2003; Romieu et al., 2008).

Sulfate and ozone, two major traffic-related pollutants in Taiwan, were reported to be associated with inflammation, oxidative stress, blood coagulation and autonomic dysfunction in healthy young humans (Chuang et al., 2007). Linear models indicated a significant exposure–response association between $PM_{2.5}$ exposure and urinary 8-OHdG levels ($p = 0.03$) among workers exposed to fine particulates (Kim et al., 2004). Among female highway toll station workers exposed to high levels of traffic exhaust and possible metabolites of PAHs, the mean urinary 8-OHdG was significantly higher among nonsmokers (13.6 mg g^{-1} creatinine) compared with the unexposed nonsmokers (7.3 mg g^{-1} creatinine) (Lai et al., 2005). Levels of oxidative and nitrosative stress markers (urine 8-oxodeoxyguanosine (8-oxodG) and plasma nitrotyrosine (NT),) in bus drivers were significantly higher than matched controls in Czech Republic (Rossner et al., 2007). In the Copenhagen metropolitan area, urinary 8-OHdG was found to be significantly related to PAH-albumin adducts among non-smoking bus drivers and mail carriers ($p = 0.002$) (Autrup et al., 1999). Adjusting for smoking, workers exposed to benzene from gasoline was reported a significant increase of urinary 8-OHdG excretion (Nilsson et al., 1996).

The excretion of urinary 8-OHdG reflects both the oxidation of DNA and cellular excision repair. Personal physiological factors (age, metabolic rates, disease), lifestyle (smoking, betel nut chewing, exercise, diet), and environmental exposures may also alter the level of urinary 8-OHdG. Agents that increase repair would be expected to increase excretion of 8-OHdG in urine. Smoking is consistently identified as a strong confounder for DNA oxidative damage in epidemiological studies. Our study found a strong positive linear relationship between urinary 8-OHdG and cotinine. Compared to non-smoking community men in Taiwan, the OR of having elevated levels of urinary 8-OHdG were 6.6 (95% CI = 2.1–20.8) for smoking community men, 5.0 (95% CI = 1.7–14.7) for non-smoking taxi drivers and 4.6 (95% CI = 1.4–15.0) for smoking taxi drivers (Chuang et al., 2003). Controlling for confounding factors such as smoking is imperative in epidemiologic studies.

In one published study, a higher risk of elevated levels of urinary 8-OHdG was also observed for betel chewers compared with non-chewers (OR = 1.6; 95% CI = 1.1–3.6) (Chuang et al., 2003). A synergistic effect of smoking and betel quid chewing to oral cancer has been reported. Formation of N-nitrosoproline in the oral cavity in betel and tobacco chewers was higher than in betel quid chewers (Ko et al., 1995). Our study found that compared to nonsmokers and non-betel nut chewers, subjects who smoked and chewed betel nuts had increased urinary 8-OHdG level compared with subjects who smoked but did not chew betel nuts. However, the effect of chewing betel nuts is less than the effect of air pollution exposure after long hours of professional driving.

Urinary 8-OHdG level is affected by increased oxidative DNA damage and decreased repair capacity during aging (Fraga et al., 1990). The mechanisms responsible for the higher urinary 8-OHdG levels observers in our study among subjects aged 35–49 years compared to younger or older subjects requires further study. Sedentary work duties and lack of regular exercise may result in higher BMI among bus drivers. No significant difference of 8-OHdG level by BMI was found, although an inverse linear relationship between BMI and 8-OHdG in leukocyte DNA ($p = 0.002$) was suggested in a study of 51 healthy males (van Zeeland et al., 1999). Less education, long-term driving, and stressful workload may lead to increased prevalence of betel nut chewing for long-distance bus drivers. The average weekly working hours were significantly longer for bus drivers compared to office workers, although both bus drivers and office workers have worked overtime based on maximum workload (44 h $week^{-1}$) by Labor Laws and Regulations in Taiwan.

It was reported that physical laboring, day-night shift work, smoking and low BMI (<21.8 kg m^{-2}) increases urinary 8-OHdG, while moderate physical exercise has an opposite effect (Kasai et al., 2001). Endurance exercise may systemically increase oxidative stress but simultaneously induce antioxidant defense enzyme activities. The basal 8-OHdG levels in leukocyte DNA of physically active subjects were significantly lower compared with the levels of sedentary subjects (Sato et al., 2003). Increases in urinary excretions of 8-OHdG are correlated with training status (hours of exercise/week) of the volunteers (Orhan et al., 2004). In our study, the average urinary 8-OHdG is higher in subjects who exercised regularly than those who did not. Exercise may induce active DNA repair enzymes resulting in excision and excretion of 8-OHdG residues. The current literature is insufficient to conclude at what level of increased oxidative stress the potential benefits may outweigh the risks from exercise (Urso and Clarkson, 2003).

Tea polyphenols, especially catechins, have been shown to alter the production of reactive oxygen species, glutathione metabolism, lipid peroxidation, and protein oxidation *in vitro* (Raza and John, 2008). Adjustment for baseline measurements and confounders, urinary 8-OHdG of healthy smokers decreased significantly after drinking decaffeinated green tea for four months (−30%, $p = 0.002$) (Hakim et al., 2003). One animal experiment has shown that coffee drinking leads to a significant increase in excretion of urinary 8-OHdG on day 130 (1.36, 46.6, and 64.6 ng mg^{-1} creatinine in control, 0.62%, and 1.36% coffee-diet group, respectively, $p < 0.05$) (Sakamoto et al., 2003). However, consumption of coffee may inhibit inflammation and thereby reduce the risk of cardiovascular and other inflammatory diseases in postmenopausal women (Andersen et al., 2006). Our study showed that consumption of tea or coffee did not alter urinary 8-OHdG levels.

Energy drinks contain large doses of caffeine, sugar, and other legal stimulants like vitamins, taurine, guarana, ginseng, and green coffee bean extract chlorogenic acid. Energy drinks increase energy and alertness, boost the heart rate and blood pressure and dehydrate the body. McCusker et al. (2006) reported the caffeine concentration of the ten caffeinated energy drinks ranged from none detected to 141.1 mg/serving. The caffeine content in energy drinks is not currently regulated by the Food and Drug Administration (FDA). Caffeine content for the majority of energy drinks included in the study was higher than the maximum allowed limit for cola beverages (45.3 mg/8.4 oz or 86.4 mg/16 oz) based on FDA reports (Food and Drug Administration, 1987). There is some evidence that caffeine and sugar components of energy drinks could cause serious health problems for those who do not restrict their combined guarana and caffeine intake. There are possible long-term adverse health effect of mixing energy drinks and alcohol (Clauson et al., 2008).

Although coffee has been suggested of having potent antioxidative effect (Natella et al., 2007; Hoelzl et al., 2009), it has been reported that coffee-diet led to a significant increase in excretion of urinary 8-OHdG in rats (Sakamoto et al., 2003). The authors also

demonstrated that chlorogenic acid alone did not increase 8-OHdG formation in human placental DNA, but it dramatically increased 8-OHDG formation in the presence of transition metal irons or H_2O_2. Whether energy drinks, which contain high level of caffeine, and environmental pollutants synergized the oxidative damage and increase 8-OHdG excretion in bus drivers requires further investigation.

Few limitations of the study should be of concern. Data collection was conducted on work days, thus, urinary 8-OHdG level could represent the extent of occupational exposure of drivers. Moreover, there is a possibility that office workers might also be partly exposed to traffic exhaust, although at a much lower amount than professional drivers. Spot urinary 8-OHdG level may reflect oxidative DNA base damage at a certain period of time, but may not be representative of individual long-term oxidative exposure. Because all participants were male adults in our study, the results of this limited study cannot be generalized to female or other vulnerable populations, such as elders and children. The study did not carry out individual exposure assessments that would more accurately quantify and validate types and levels of air pollutants and their contribution to urinary 8-OHdG excretion. We did not investigate the effect of diet or nutrients on oxidative DNA damage. Diet is a major source of antioxidants and it is important to examine whether antioxidant defense mechanisms could be increased by dietary means to protect against adverse health effects of air pollutants. Finally, there are significant aspects related to the analytical challenge, intra-individual variation, other confounding factors and inter-laboratory differences, implying that further work is needed to reach a consensus on the background level of 8-OHdG (Pilger and Rudiger, 2006).

5. Conclusions

This study suggests that long-distance bus driving, cigarette smoking, and energy drink consumption may elevate the excretion of 8-OHdG in urine, a reliable biomarker for oxidative DNA damage. Urinary 8-OHdG detection can be used to assess the oxidative damage of environmental and occupational exposure by comparing 8-OHdG data between a control group and exposure group in epidemiology studies. Reducing workload is necessary to decrease the occupational exposure and health hazards for long-distance bus drivers in Taiwan. Smoking cessation should be considered an important strategy to promote health among both bus drivers and office workers. The increase in urinary 8-OhdG excretion associated with energy drink consumption requires further investigation.

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