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Original Article

Long-term exposure to diesel engine exhaust induced lung function decline in a cross sectional study

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Abstract: To clarify the effects of lung function following exposure to diesel engine exhaust (DEE), we recruited 137 diesel engine testing workers exposed to DEE and 127 non-DEE-exposed workers as study subjects. We performed lung function tests and measured cytokinesis-block micronucleus (CBMN) cytome index and levels of urinary polycyclic aromatic hydrocarbons (PAHs) metabolites. There was a significant decrease of forced expiratory volume in 1 second (FEV_1), ratio of forced expiratory volume in 1 second to forced vital capacity (FEV_1/ FVC), maximal mid expiratory flow curve (MMF), forced expiratory flow at 50% of FVC ($FEF_{50\%}$), and forced expiratory flow at 75% of FVC ($FEF_{75\%}$) in the DEE-exposed workers than non-DEE-exposed workers (all $p<0.05$). Among all study subjects, the decreases of $FEF_{75\%}$ were associated with the increasing levels of PAHs metabolites ($p<0.05$), and there were negative correlations between FEV_1, FEV_1/FVC, MMF, $FEF_{50\%}$, and $FEF_{75\%}$ with CBMN cytome index (all $p<0.05$). Our results show that long-term exposure to DEE can induce lung function decline which shows mainly obstructive changes and influence of small airways function. The decreased lung function is associated with internal dosage of DEE exposure, and accompany with the increasing CBMN cytome index.

Key words: Diesel engine exhaust, Lung function, The cytokinesis-block micronucleus cytome index, Urinary mono-hydroxylated polycyclic aromatic hydrocarbons, Long-term exposure

Introduction

Diesel engine exhaust (DEE) is a common air pollutant resulting from incomplete combustion of diesel fuel. DEE is a complex mixture comprising of gases and particulate matter absorbed with mutagenic and carcinogenic organic matters. Polycyclic aromatic hydrocarbons (PAHs) and nitroarenes are presented within both gas and particle phases of DEE. Moreover, DEE is classified as a Group I carcinogen by the International Agency for Research on Cancer[1)] , and is a prominent source of particulate matter <2.5 μm in aerodynamic diameter ($PM_{2.5}$) in rural and urban areas. For the general population, traffic emission can be major sources of exposure to DEE. Occupational exposure to DEE through use of diesel-powered equipment predominantly occurred in industries including mining, construction, and transportation. The potential health effects of ambient DEE exposure are of great interest both

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for general and occupational population. Epidemiological studies have shown associations between DEE exposure and several respiratory disorders including airway inflammation[2], allergic respiratory disease[3], chronic obstructive pulmonary disease (COPD)[4, 5], and lung cancer[6–8]. One cross sectional investigation showed an increased risk for decline in FEV_1 in tunnel workers exposed to DEE[9]. Miners of two salt mines were investigated to find that there were dose-response relationships between occupational exposure to potash, DEE and nitrogen oxides and lung function[10]. Another cohort study has also reported that railroad workers exposed to DEE was associated with increased mortality from COPD[4]. Although DEE is ordinary in both urban and rural areas, it is usually very difficult to pick out the effects from other innumerous fuel combustion exposures which coexist in ambient air[11]. Therefore, the inadequate control of potential confounding exposures and lack of quantification of exposure assessment remains hindered the interpretation of occupational and environmental epidemiological studies of DEE[12]. Human controlled-exposure studies have reported mixed findings with respect to the effects of DEE on lung function, including non-significant effects on lung volumes and statistically significant effects on specific airway resistance[13–16]. Although controlled exposure studies have the advantage of excluding the effects of confounding exposures, it also have some weaknesses, such as small sample size and inability to study the chronic effects of exposure compared with observational epidemiology[12].

In the current study, all subjects in the DEE-exposed group had inspected heavy-duty diesel engines for at least one year in a diesel engine manufacturing plant, and there was no other major exposure source except DEE in the workplace. We performed the lung function tests and also evaluated the levels of the metabolites of PAHs in urine samples as internal dosage. In our previous study, we found that the micronucleus (MN), nucleoplasmic bridge (NPB), and nuclear bud (NBUD) frequencies in peripheral blood lymphocytes (PBLs) were higher in the DEE-exposed populations compared with unexposed populations[17]. The CBMN cytome assay is one of the most commonly used methods for measuring chromosomal damage[18]. Considering that inflammation played a significant role in both driving lung function decline and inducing DNA damage and chromosomal instability[19, 20]. The CBMN cytome index was calculated to further explore the correlation between lung function and chromosomal damage associated with inflammation. We found the effects on lung function of long-term exposure to pure DEE and the association of urinary PAHs metabolites with lung function accompanying with increasing of chromosomal damage.

Subjects and Methods

Study population and sample collection

We recruited 264 male workers had been employed for at least one year. The DEE-exposed workers, who tested heavy-duty diesel engines in engine assemble workshop of diesel engine manufacturing plant, were recruited as the DEE-exposed group (n = 137). The workers with no work-related exposure to DEE and other toxicants, who operated and inspected the electric powered water pumps in the water supply plant, were recruited as the control group (n = 127). Standardized occupational questionnaires on demographic characteristics, smoking status, alcohol consumption, working years, recent personal medical events (previous 6 months), and personal medical history were administrated to the workers by an occupational physician. Subjects with a history of tuberculosis, thoracic or abdominal surgery, cancer, recent fever and/or inflammation and those who had been exposed to X-ray within three months were excluded. Individuals who had smoked ≥100 cigarettes in their lifetime were considered smokers; those who still smoked at the time of the interview were defined as current smokers; others were treated as former smokers. Finally, 4 ml venous blood and 50 ml urine were obtained from each subject at the end of shift after at least 4 consecutive working days.

The study was approved by the Research Ethics Committee of National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, and written informed consent was obtained from all study participants.

Lung function tests

Lung function tests were performed using a portable calibrated vitalograph spirometer (CHESTAC-8800, Japan) in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) standards[21]. Technicians performing the lung function tests are certified and have completed the standardized spirometry training course of China and responsible for all lung function testing. Calibration was done with a 3-l calibration syringe at least twice a day before and after the spirometric measurements, according to the guidelines of the manufacturer. Subjects within 1 month of a myocardial infarction were excluded. Testing was performed in the sitting position. Height and weight were measured for each subject with a stadiometer

and a digital scale. Lung function tests included vital capacity (VC, l), forced vital capacity (FVC, l), forced expiratory volume in 1 second (FEV_1, l), maximal mid expiratory flow curve (MMF, l/s), peak expiratory flow (PEF, l/s), forced expiratory flow at 25% of FVC ($FEF_{25\%}$, l/s), forced expiratory flow at 50% of FVC ($FEF_{50\%}$, l/s) , and forced expiratory flow at 75% of FVC ($FEF_{75\%}$, l/s). In addition, FEV_1 as a percentage of FVC was calculated. Lung function values were also expressed as percent of predicted using appropriate equations as follows: $(27.63 - 0.112 \times age) \times height/1{,}000$ for FVC and VC, $(34.4 \times height - 33 \times age - 1{,}000)/1{,}000$ for FEV_1, $(51 \times height/2.54 + 2{,}954 - 46 \times age)/1{,}000$ for MMF, $0.057 \times height - 0.024 \times age + 0.225$ for PEF, $0.03555 \times height - 0.01987 \times age + 2.72554$ for $FEF_{25\%}$, $0.02569 \times height - 0.03049 \times age + 2.40337$ for $FEF_{50\%}$, and $0.01411 \times height - 0.04142 \times age + 1.98361$ for $FEF_{75\%}$. Units are years for age and centimeter for height.

Exposure assessment

Airborne $PM_{2.5}$, elemental carbon (EC), and PAHs monitoring

Details of the airborne $PM_{2.5}$, EC, and PAHs exposure have been described previously[17]. Briefly, the airborne samples were collected from the water supply plant and diesel engine manufacturing plant, respectively. We measured $PM_{2.5}$ in the working environment gravimetrically by a micro-balance. The concentrations of EC in collected PMs were analyzed by thermal optical analysis based on National Institute for Occupational Safety and Health (NIOSH) method 5,040[22]. Quantitative chemical analysis of 16 PAHs from collected PMs were performed by high performance liquid chromatography-mass spectrometry (HPLC) with fluorescence detectors according to the Occupational Safety and Health Administration method 58[23].

Determination of urinary mono-hydroxylated PAHs (OH-PAHs)

The urine samples were tested for six OH-PAHs, including 1-hydroxynaphthalene (1-OHNa), 2-hydroxynaphthalene (2-OHNa), 2-hydroxyfluorene (2-OHFlu), 2-hydroxyphenanthrene (2-OHPh), 9-hydroxyphenanthrene (9-OHPh), and 1-hydroxypyrene (1-OHP) using an HPLC-MS/MS method as described previously[17, 24]. The testing procedure involved enzymatic hydrolysis of urine, liquid-liquid extraction, evaporation under nitrogen to constant volume, and analysis using HPLC-MS /MS. The urinary OH-PAHs were quantified by internal standard calibration curve. Urinary creatinine-correction was applied to the data. Creatinine was determined in all urine samples by Jaffe's colorimetric method. The urinary OH-PAHs concentrations were expressed as micrograms per gram of creatinine (μg/g). Limits of detection (LOD) for six urinary OH-PAHs were in the range of 0.1 – 0.5 μg/l, and we replaced the results below the LOD with $LOD/\sqrt{2}$.

Cytokinesis-block micronucleus (CBMN) cytome assay

Detailed method of the CBMN assay has been reported before[17]. Briefly, the CBMN assay was carried out with fresh heparin-anticoagulated whole blood according to the standardized protocol developed by Fenech *et al*.[25]. Two duplicative slides for each subject were prepared. A total of 2,000 binucleated cells with well-preserved cytoplasm were examined in each slide microscopically to determine MN, NPB, and NBUD frequencies according to the scoring criteria[26].

Statistical analysis

All statistical analyses were performed using SPSS 11.0 software. Normal distribution test was examined using the one-sample K-S test. Natural logarithmic (ln) transformation was applied to urinary OH-PAHs to satisfy the normal distribution. Total OH-PAHs levels were calculated by summing urinary levels of six OH-PAHs. Student *t*-test was used to compare means of quantitative data (age, height, weight, BMI, urinary OH-PAHs concentrations, and lung function parameters), and chi-square test was used to compare the frequencies of qualitative data (current smokers and alcohol users) between the DEE-exposed and control groups. The associations of DEE exposure with lung function were further examined using the multivariate linear regression with adjustment for age, height, weight, smoking status and alcohol use. Because smoking was an important confounding factor, we further repeated the analysis by smoking status. Next, the associations of lung function with categorized DEE exposure duration were examined using the multivariate linear regression. The urinary total OH-PAHs metabolites concentrations in all study subjects further stratified by exposure groups. We applied multivariate linear regression to examine the tendency of lung function with the urinary total OH-PAHs levels with adjustment for age, height, weight, smoking status and alcohol use. The statistically significant level was $p < 0.05$. Associations between lung function values and CBMN cytome index in all study subjects were analyzed using Spearman's rank correlation coefficient.

Table 1. The characteristics of subjects in the non-DEE-exposed and DEE-exposed workers

Variable	Non-DEE-exposed workers (n=127)	DEE-exposed workers (n=137)	*p*-value
Age (yr, mean±SD)*	31.91±11.15	31.99±8.60	0.948
Height (cm, mean±SD)*	171.12±5.79	171.49±5.41	0.591
Weight (kg, mean±SD)*	69.57±13.94	72.29±10.49	0.073
BMI (kg/m^2, mean±SD)*	23.74±4.43	24.58±3.40	0.084
Current smokers, yes/no (% yes)#	61/66 (48.0)	81/56 (59.1)	0.084
Alcohol use, yes/no (% yes)#	83/44 (65.4)	89/48 (65.0)	1.000
DEE exposure years (yr, median(Q1–Q3))	—	8.50 (5.40–9.60)	—

DEE: diesel engine exhaust; BMI: body mass index. **t*-test was used to compare values from both groups. #Chi square test was used to compare values from both groups. Differences were considered significant when $p<0.05$.

Results

General characteristics of study subjects

Characteristics of the study population are shown in Table 1. The distribution according to age, height, weight, BMI, current smoking habits, and alcohol use were similar between the DEE-exposed workers and non-DEE-exposed workers (all $p>0.05$). The DEE-exposed years (median, Q1–Q3) of DEE-exposed group were (8.50, 5.40–9.60) years. Participants in the DEE-exposed group had significantly higher exposures levels to $PM_{2.5}$, EC, and total PAHs than those in the control group (all $p<0.001$). Results of measurements were described in detail by Zhang *et al.*[17]. The DEE-exposed group and control group were exposed to $PM_{2.5}$ of 267.45 μg/m^3 and 91.88 μg/m^3, respectively. The geometric means of the EC level and total PAHs were 11.81 μg/m^3, 0.03 μg/m^3 for workers in the control group and 113.69 μg/m^3, 4.76 μg/m^3 for workers in the DEE-exposed group (all $p<0.001$), respectively. Compared with the control group, six urinary OH-PAHs (including 1-OHNa, 2-OHNa, 2-OHFlu, 2-OHPh, 9-OHPh, and 1-OHP) and total OH-PAHs were significantly higher in the DEE-exposed groups (all $p<0.001$). The concentrations (μg/g creatinine, median, 5%–95%) of 1-OHNa, 2-OHNa, 2-OHFlu, 2-OHPh, 9-OHPh, 1-OHP, and total OH-PAHs were 0.85 (0.11–4.73), 1.28 (0.20–7.48) , 0.61(0.12–1.79), 0.23 (0.09–1.37), 0.40 (0.08–1.95), 0.75 (0.08–3.13), and 4.68 (0.96–16.75) in the control group, and 1.67 (0.35–9.12), 3.20 (0.45–15.33), 1.61 (0.51–4.08), 1.45 (0.48–3.85), 1.13 (0.30–3.62), 2.30 (0.62–6.27), and 12.96 (4.47–32.33) in the DEE-exposed group.

DEE exposure induced decreased lung function of workers

Table 2 presents the VC, FVC, FEV_1, FEV_1/FVC, MMF, PEF, $FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$ of the two groups. The FEV_1, FEV_1/FVC, MMF, $FEF_{50\%}$, and $FEF_{75\%}$ observed in the DEE-exposed workers were significantly lower than in non-DEE-exposed workers (all $p<0.05$). After adjusting for important confounders, including age, weight, height, smoking, and drinking habit, significant associations were still present between reduction in most parameters of lung function and DEE exposure (Table 2). Further analyses by controlling the false discovery rate using Benjamini-Hochberg method showed similar results (data not shown)[27]. Predicted percentages of VC, FVC, FEV_1, MMF, PEF, $FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$ are presented in Table s1. As shown, most parameters of pulmonary function such as predicted percentages of FEV_1, MMF, $FEF_{50\%}$,

Table 2. The lung function indexes of non-DEE-exposed and DEE-exposed workers (mean ±SD)

The lung function indexes	Non-DEE-exposed Workers (n=127)	DEE-exposed workers (n=137)	p-$_{crude}$*	p-$_{adjust}$#
VC (l)	4.52±0.69	4.50±0.60	0.797	0.530
FVC (l)	4.45±0.74	4.41±0.59	0.565	0.383
FEV_1 (l)	3.93±0.64	3.78±0.53	0.043	0.007
FEV_1/FVC	0.89±0.06	0.86±0.05	<0.001	0.001
MMF (l/s)	4.71±1.09	4.25±0.94	<0.001	<0.001
PEF(l/s)	8.28±1.44	8.22±1.43	0.760	0.671
$FEF_{25\%}$ (l/s)	7.78±1.47	7.47±1.29	0.065	0.058
$FEF_{50\%}$ (l/s)	5.42±1.35	4.89±1.20	0.001	0.001
$FEF_{75\%}$ (l/s)	2.49±0.81	2.16±0.61	<0.001	<0.001

VC: vital capacity, FVC: forced vital capacity, FEV_1: forced expiratory volume in 1second, MMF: maximal mid expiratory flow curve, PEF: peak expiratory flow, $FEF_{25\%}$: forced expiratory flow at 25% of FVC, $FEF_{50\%}$: forced expiratory flow at 50 % FVC, and $FEF_{75\%}$: forced expiratory flow at 75% of FVC, OH-PAHs: mono-hydroxylated polycyclic aromatic hydrocarbons. **t*-test was used to compare values from both groups. #Multiple regression analysis of age, height, weight, smoking status, alcohol use, and DEE exposure on different lung function variables.

Table 3. The lung function indexes grouped by urinay total OH-PAHs level in all study subjects (mean±SD)

The lung function indexes	Urinay total OH-PAHs (μg/g creatinine)			
	T1<5.90 (n=88)	T2 5.90–12.44 (n=88)	T3 > 12.44 (n=88)	p-$_{trend}$*
VC (l)	4.69±0.67	4.43±0.62	4.43±0.61	0.214
FVC (l)	4.63±0.72	4.34±0.63	4.31±0.60	0.290
FEV_1 (l)	4.08±0.61	3.75±0.57	3.74±0.53	0.066
FEV_1/FVC	0.88±0.06	0.86±0.05	0.87±0.05	0.131
MMF (l/s)	4.79±1.07	4.27±1.03	4.36±0.95	0.066
PEF (l/s)	8.32±1.33	8.24±1.42	8.19±1.56	0.309
$FEF_{25\%}$ (l/s)	7.79±1.36	7.60±1.38	7.47±1.42	0.062
$FEF_{50\%}$ (l/s)	5.48±1.29	4.91±1.33	5.04±1.23	0.109
$FEF_{75\%}$ (l/s)	2.59±0.82	2.18±0.66	2.20±0.63	0.048

See table 2 for abbreviations. *Multiple regression analysis of age, height, weight, smoking status, alcohol use, and tertiles of urinay total OH-PAHs on different lung function variables.

and $FEF_{75\%}$were significantly lower in the DEE-exposed workers than in non-DEE-exposed workers (all $p<0.05$). Table 2 shows that the levels of FEV_1 were lower in the DEE-exposed population relative to controls (3.93±0.64 l, 3.78±0.53 l, respectively). The levels of FEV_1/FVC were 0.89±0.06 in the control group and 0.86±0.05 in the DEE-exposed group. The DEE-exposed workers had significant lower levels of MMF, $FEF_{50\%}$, and $FEF_{75\%}$ (4.71±1.09 l/s, 5.42±1.35 l/s, and 2.49±0.81 l/s, respectively) than the non-DEE-exposed workers (4.25±0.94 l/s, 4.89±1.20 l/s, and 2.16±0.61 l/s, respectively) . Similar decreases in the DEE-exposed workers compared to non-DEE-exposed workers were observed for the FVC, PEF, and $FEF_{25\%}$, but these results were not significant ($p>0.05$). The values of VC were similar in the two groups ($p>0.05$).

Furthermore, the association between duration of DEE exposure and lung function with adjustment for age, weight, height, smoking, and drinking habit were examined. The values of FEV_1, FEV_1/FVC, MMF, $FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$ were significantly lower in the DEE-exposed workers for 0–4, 4–8, and >8 years compared with non-DEE-exposed workers, and these lung function decreases were DEE exposure duration dependent (Table s2).

Pulmonary function peaks at the age of 20 in human development. Therefore, we assessed the association between DEE exposure and lung function restricted in workers older than 20 years old. Similarly, the values of FEV_1, FEV_1/FVC, MMF, $FEF_{50\%}$, and $FEF_{75\%}$ observed in the DEE-exposed workers were significantly lower than in non-DEE exposed workers when this analysis was restricted in workers older than 20 year old (all $p<0.05$) (Table s3). In the DEE-exposed workers, we also observed that the decreased VC, FVC, FEV_1, and $FEF_{75\%}$ were related with the increasing of age at start of DEE exposure (all $p<0.05$), and the Spearman's rank correlation coefficient were −0.274 ($p=0.001$), −0.313 ($p<0.001$), −0.313 ($p<0.001$), and −0.226 ($p=0.008$), respectively. Furthermore, we have noticed that age was correlated with age at start of DEE exposure (r=0.716, $p<0.001$). Therefore, the observed association between age at start of DEE exposure and age could significantly contribute to the significant association between age at start of DEE exposure and lung function.

Decrease of lung function associated with increase of internal dosage of DEE exposure

To explore the relationship between DEE exposure levels and levels of lung function, we divided the study population into three groups according to internal dosage of DEE exposure. The lung function indexes grouped according to urinary total OH-PAHs concentration are shown in Table 3. A relationship between lung function indexes and urinary total OH-PAHs levels was found among all study subjects. Multivariate linear regression analyses revealed that elevated urinary total OH-PAHs levels were significantly associated with a decrease of $FEF_{75\%}$ ($p_{trend}=0.048$) in all study subjects after adjusting for age, height, weight, smoking status, and alcohol use. The relationship between elevated urinary total OH-PAHs and the decrease of FEV_1, MMF, and $FEF_{25\%}$ was borderline significant ($p_{trend}=0.066$, $p_{trend}=0.066$, and $p_{trend}=0.062$, respectively). There were no significant differences for VC, FVC, $FEF_{50\%}$, and PEF in three tertiles of urinary total OH-PAHs groups in all study subjects (all $p>0.05$). However, when analyzed separately in the DEE-exposed group and the control group, the associations of elevated tertiles of urinary total OH-PAHs with the lung function indexes generally became

Table 4. The lung function indexes stratified by smoking status in all study subjects, as well as in the non-DEE-exposed and DEE-exposed workers (mean±SD)

The lung function indexes	All study subjects (n=264)			DEE-exposed workers (n=137)			non-DEE-exposed workers (n=127)		
	Non-smokers (n=122)	Smokers (n=142)	p-$_{adjust}$*	Non-smokers (n=56)	Smokers (n=81)	p-$_{adjust}$*	Non-smokers (n=66)	Smokers (n=61)	p-$_{adjust}$*
VC (l)	4.60±0.62	4.44±0.65	0.515	4.54±0.54	4.48±0.64	0.605	4.66±0.69	4.38±0.67	0.621
FVC (l)	4.55±0.68	4.32±0.64	0.409	4.43±0.52	4.39±0.63	0.763	4.65±0.78	4.24±0.64	0.563
FEV_1 (l)	3.97±0.59	3.75±0.57	0.373	3.81±0.46	3.77±0.57	0.810	4.12±0.65	3.73±0.57	0.642
FEV_1/FVC	0.88±0.06	0.87±0.05	0.830	0.86±0.05	0.86±0.05	0.989	0.89±0.06	0.88±0.05	0.812
MMF (l/s)	4.60±1.07	4.36±1.00	0.425	4.27±0.95	4.23±0.94	0.947	4.88±1.10	4.54±1.06	0.775
PEF (l/s)	8.22±1.35	8.28±1.51	0.885	8.17±1.33	8.26±1.51	0.694	8.26±1.38	8.30±1.52	0.245
$FEF_{25\%}$ (l/s)	7.63±1.33	7.61±1.44	0.768	7.41±1.25	7.51±1.33	0.579	7.81±1.37	7.75±1.57	0.212
$FEF_{50\%}$ (l/s)	5.27±1.33	5.04±1.27	0.380	4.91±1.26	4.88±1.16	0.941	5.57±1.32	5.25±1.37	0.641
$FEF_{75\%}$ (l/s)	2.44±0.76	2.22±0.69	0.549	2.22±0.64	2.13±0.59	0.491	2.63±0.81	2.35±0.78	0.440

See table 2 for abbreviations. *Multiple regression analysis of age, height, weight, and alcohol use on different lung function variables.

insignificant (Table s4).

Relations of smoking status with the lung function indexes

When further stratified by smoking status, all of lung function indexes (adjusted for age, height, weight, and alcohol use) did not differ significantly between current smokers and non-smokers among both DEE-exposed group and control group (Table 4).

Associations of CBMN cytome index with the lung function

The results of the MN, NPB, and NBUD frequencies between DEE-exposed and control workers have been reported before[17]. The CBMN cytome index was calculated by integrating MN, NPB, and NBUD frequencies, and subjects with missing data for any of MN, NPB, or NBUD frequencies were excluded from the analysis. The results of CBMN cytome index were described and we found that CBMN cytome index of the DEE-exposed workers was significantly higher than that in non-DEE-exposed workers (13.86‰ *vs.* 4.94‰, $p<0.001$)[17]. We then analyzed whether there were associations between the lung function indexes and CBMN cytome index. In whole study subjects, there were negative correlations between lung function index (VC, FVC, FEV_1, FEV_1/FVC, MMF, $FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$) and CBMN cytome index. The decreased FEV_1, FEV_1/FVC, MMF, $FEF_{50\%}$, and $FEF_{75\%}$ were related with increasing of CBMN cytome index (all $p<0.05$) (Fig. 1), and the Spearman's rank correlation coefficient were −0.143 ($p=0.038$), −0.203 ($p=0.003$), −0.217 ($p=0.002$), −0.190 ($p=0.006$) , and −0.234 ($p=0.001$), respectively (Fig. 1). The VC, FVC and $FEF_{25\%}$ were also decreased with increasing of CBMN cytome index, but these relations were not statistically significant (all $p>0.05$).

Dicussion

In this cross-sectional study, we investigated the effects of DEE on lung function of DEE-exposed workers. Two main findings of the study were presented among the chronic DEE purely exposed workers. First, decreases of lung function in workers were associated with long-term exposure to DEE. The decreases of FEV_1/FVC and FEV_1 may indicate airflow obstruction, the decreased levels of $FEF_{50\%}$, $FEF_{75\%}$ and MEF may primarily reflect the impact of DEE exposure on function of small airways. Second, DEE induced decrease of lung function was associated with the internal dosage of DEE exposure, and accompany with the increasing CBMN cytome index which is used to assess the impact of DEE exposure on genomic instability.

In present study, we found FEV_1 and FEV_1/FVC were significantly lower in the DEE-exposed workers compared with the controls. Our results may indicate an association between long-term DEE exposure and airflow obstruction, in terms of a reduction in the ratio of FEV_1 to FVC or increased odds of $FEV_1/FVC<0.7$, as the associations with the decreases of ratio of FEV_1 to FVC and FEV_1 were statistically significant. This is also supported by the studies that diesel exhaust induced inflammatory in the airways, as well as airways obstruction[28]. Lotz *et al.*[10] aslo reported a decrease of FEV_1 in a longitudinal study of salt miners. In a case-control study, the railroad workers involved in DEE-exposed jobs had higher risk of COPD mortality compared with controls [4]. But in another study, Ulvestad *et al.*[9] observed a significant decrease of %FVC and %FEV_1 in the tunnel workers compared with the reference subjects. Adelroth *et al.*[2] found no significant differences in lung function of miners exposed to dust, diesel exhaust and NO_2 compared with controls (EC concentration of 27

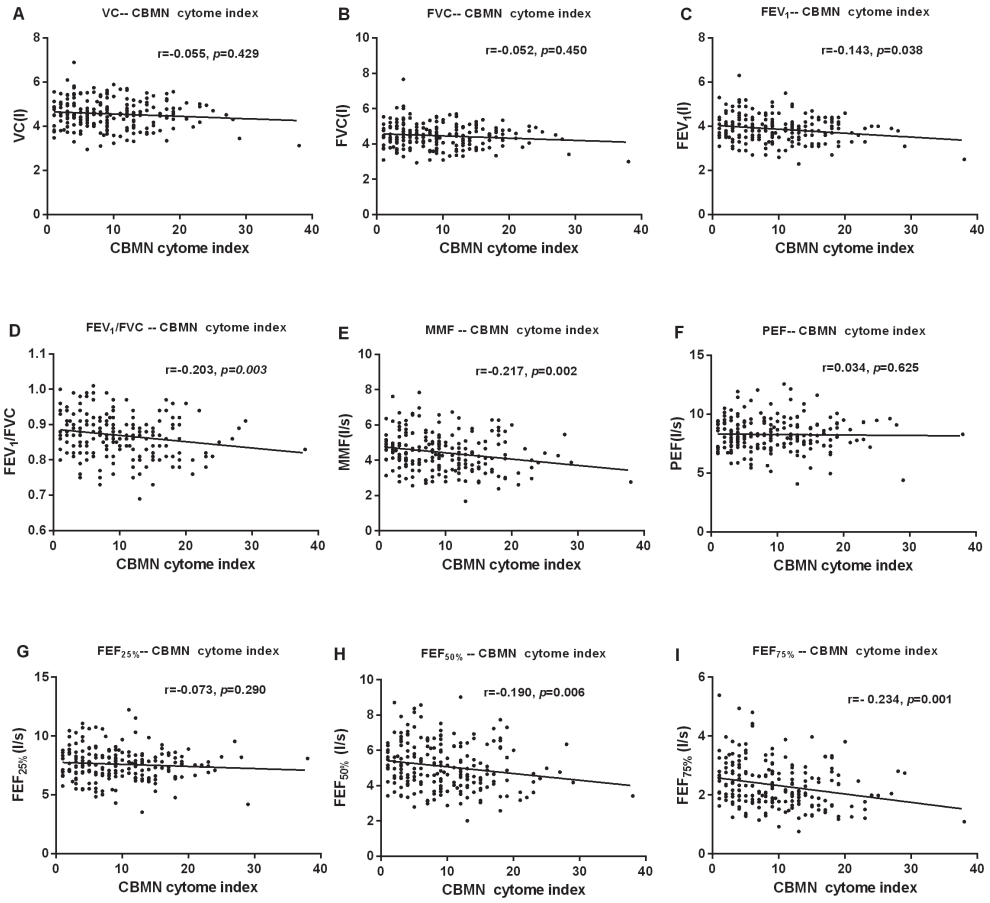

Fig. 1. The correlation of lung function indexes and CBMN cytome index in all study subjects: (A) VC: vital capacity, (B) FVC: forced vital capacity, (C) FEV_1: forced expiratory volume in 1second, (D) FEV_1/FVC, (E) MMF: maximal mid expiratory flow curve, (F) PEF: peak expiratory flow (G) $FEF_{25\%}$: forced expiratory flow at 25% of FVC, (H) $FEF_{50\%}$: forced expiratory flow at 50% FVC, (I) $FEF_{75\%}$: forced expiratory flow at 75% of FVC.

$\mu g/m^3$).Taken the above studies together, the results are inconsistent. A possible explanation for the different effects may be that the lung function changes are related to the composition of the mixed exposure containing DEE and many potentially confounding substances from other combustion sources, such as dust and gasoline engine exhaust. Since some fractions of the mixed exposure are capable of eliciting some of the same health effects as DEE, it is difficult to distinguish the influence of DEE to assess its contribution to the effects of lung function in these studies. Nevertheless it can be assumed that DEE may induce lung function disorders under certain conditions. In addition, the DEE exposure level might be an important factor. The airway effects of exposure to high level of DEE have been explored in controlled human studies[14–16]. Mudway *et al*. (2004)[14] did not find statistically significant effects on FVC and FEV_1 among 25 healthy adults exposed to whole DEE for 2 hours (particulate concentration of 100 $\mu g/m^3$). Consistent with this finding, no significant effects of FVC and FEV_1 were observed in 25 healthy and 15 mildly asthmatic volunteers exposed to diluted whole DEE for 2 hours (particulate concentration of 108 $\mu g/m^3$) [15]. Another study reported that exposure to DEE (300 $\mu g/m^3$) for 2 hours did not induce changes in FEV_1 or FVC of the subject population immediately or after 4 hours (n=4)[16]. These findings are different from our finding that the FEV_1, FEV_1/FVC were significantly reduced in workers long-term exposure to DEE. These short-term experimental studies are only possible to evaluate acute effects at high concentrations, and it is unclear if the results can be generalized to long-term exposure at relatively lower levels, while in real life situations, long-term exposure to DEE or traffic-derived air pollution increases respiratory and cardiovascular morbidity and mortality.

After adjusting for important confounders, including age, weight, height, smoking, and drinking habit, signifi-

cant associations were still present between reduction in most parameters of lung function and DEE exposure. In present study, decline in FEV_1 was paralleled by a decline in FEV_1/ FVC in most subjects, suggesting an obstructive effect of long-term DEE exposure on lung function, as associations with the ratio of FEV_1 to FVC were also statistically significant lower. Our results are similar to previous air pollution studies where exposure to PM was associated with deficits in lung function[29–31]. In Mexico City, reduced rates of lung function growth have been reported in children living in parts of the city with higher particle levels[29]. W James Gauderman *et al.*[30] reported that children who lived within 500 m of a freeway had substantial deficits in 8-year growth of FEV_1 and MEF, compared with children who lived at least 1,500 m from a freeway. But the findings from the limited investigations on long-term air pollution and lung function in adults have been mixed, with some studies observing obstructive patterns and others observing restrictive patterns of lung function decline. The longitudinal SAPALDIA study found that reductions in PM_{10} over an 11-year period were associated with a slower decline in FEV_1 and the ratio of FEV_1 to FVC, but not in FVC[32], suggesting an obstructive pattern of the effect of particulate pollution on lung function decline. However, the Framingham Heart Study[33] found that long-term exposure to traffic and $PM_{2.5}$ in healthy adults was associated with lower FEV_1 and FVC, but associations with FEV_1/FVC ratio were weak or absent, suggested that they did not find an association between long-term air pollution exposure and airflow obstruction. While the ESCAPE meta-analysis involving five cohorts found significant and similar-magnitude associations of NO_2 and PM_{10} with both FEV_1 and FVC, in a restrictive pattern[34]. In view of these differences between the reported results and our results, an important factor is that the subjects who participated in the present study were solely exposed to DEE. In the process of testing of diesel engine, there are no other major exposure sources except the diesel engines. While the subjects who participated in the air pollution studies were exposed to a complex mixture mainly consisting of $PM_{2.5}$ or PM_{10} and other chemicals presented in the gaseous and/or particle phase. The DEE exposure level might be an another important factor. In our study, the $PM_{2.5}$ level (267.45 $\mu g/m^3$) in the DEE-exposed workers was significantly higher than in non-DEE-exposed workers ($p<0.001$), while it is often relatively low in the air pollution studies. In the studies of long-term effects of traffic and particulate air pollution on adult lung function is still emerging. We also found that long-term exposure to DEE was associated with decreased levels of $FEF_{50\%}$, $FEF_{75\%}$ and MEF which primarily reflect the impact of DEE exposure on function of small airways. The earliest change associated with airflow obstruction in small airways is reflected in a proportionally greater reduction in the instantaneous flow measured after 75% of the FVC being exhaled ($FEF_{75\%}$) or in mean expiratory flow between 25% and 75% of FVC.

Another contribution of our study is the evaluation of lung function related to the internal exposure of DEE. Previous studies of DEE mostly have often assessed individual exposures by monitoring airborne $PM_{2.5}$, EC, and PAHs other than internal markers. PAHs and particles which are thought to be of great environmental significance have been documented as major and important components of DEE. Particle phase of DEE is a complex mixture of organic and inorganic compounds including PAHs absorbed onto carbonaceous material. Internal exposure biomarkers represent the absorbed dose of a chemical and integrate all microenvironments and routes of exposure. For now, there are no appropriate biomarkers of exposure for particles in DEE. Urinary OH-PAHs have been used as biomarkers to assess recent exposure to PAHs. Measurement of the urinary OH-PAHs, as internal markers, may be an important way of assessing exposure to DEE. 1-OHP, which is the most frequently measured OH-PAHs biomarker for human exposure to PAHs[35], does not provide a complete assessment of human exposure to PAH mixtures because of the different molecular sizes, shapes, and rates of metabolism of different PAHs[36]. Ideally, multiple PAH metabolites should be used as biomarkers to better understand the extent of exposure to PAHs [37]. Therefore, we measured six urinary OH-PAHs [pyrene metabolite: 1-OHP; naphthalene metabolites: 1-OHNa, 2-OHNa; fluorene metabolites: 2-OHFlu; phenanthrene metabolites: 2-OHPh, 9-OHPh] by HPLC-MS/MS and used the total OH-PAHs to assess exposure to DEE. In this study, multivariate linear regression analyses revealed that elevated level of urinary total OH-PAHs was significantly associated with a decrease in $FEF_{75\%}$ in all study subjects after adjusting for age, height, weight, smoking status, and alcohol use. The relationship between elevated urinary total OH-PAHs and the decrease of FEV_1, MMF, and $FEF_{25\%}$ was borderline significant. However, when analyzed separately in the DEE-exposed group and the contol group, the associations of elevated tertiles of urinary total OH-PAHs with the lung function indexes generally became insignificant. This finding suggests that the significant associations of urinary total OH-PAHs with lung function indexes among all the study subjects might result from the presence of consistently lower

OH-PAHs levels and lung function indexes in non-DEE-exposed workers than in the DEE-exposed workers.

We observed an association between the CBMN cytome index and the lung function parameters in all study subjects. The CBMN cytome index was used to evaluate the impact of DEE exposure on genomic instability more comprehensively. And our previous study showed that the CBMN cytome index was significantly higher in the DEE-exposed workers, indicating increased genomic instability in the DEE-exposed workers compared to non-DEE-exposed workers[17]. In this study, we found that the CBMN cytome index was related with several lung function parameters. On this basis, we inferred that the genetic damage detected in the PBLs could reflect, to some extent, the corresponding damage in the lung. Chronic inflammation often results in tissue damage, an increased mutation rate and genomic instability. The inflammation played an important role in both DNA damage and chromosomal instability[19]. The biological mechanisms of the association between DEE exposure and lung function are not fully elucidated. Lung inflammation has been shown after controlled exposure to DEE[38]. Oxidative stress, which induces the release of reactive oxygen species to cause tissue injury, is a key pathway for pulmonary diseases. And animal studies suggest that long-term DEE exposure result in pulmonary inflammation, oxidative stress, and pulmonary remodeling[39]. As lung function decline is also closely associated with inflammation, this may also explain at least part of the association between lung function and chromosomal instability. Taken together, the findings strengthen the link between pulmonary function injury and cancer risk.

Inhaled cigarette smoke is the main confounder in lung function studies. To control the potential confounding effect of smoking on the associations of DEE exposure with lung function, we adjusted for smoking status in the multiple linear regression analyses. Besides, lung function parameters were compared between current and non-current smokers in the DEE-exposed workers and in non-DEE-exposed workers, respectively. Surprisingly, the non-significant trend was for a higher level of $FEF_{25\%}$ and PMF of smokers than non-smokers in the DEE-exposed workers. One possible explanation is that the relative small effect of smoking on lung function was covered up by the fairly strong effect of caused by DEE. There were similar non-significant trend for a higher level of PMF of smokers than non-smokers in non-DEE-exposed workers. It is possible that the sample size of the control group became smaller after the subjects were stratified to smokers and non-smokers, so that significant change could not be detected. Our results is not in line with that tobacco smoking increases the rate of lung function decline[40, 41], and such a confounding effect of smoking needs further clarification.

Our study has some strength that deserve comments. First, in the present study, the subjects were exposed to DEE in testing workshop. In the testing workshop, there are no other particles and chemicals except for DEE, existing in the occupational environment. Therefore, the subjects who participated in the present study were solely exposed to DEE. Second, the mortality risk of particle exposures is much larger for chronic than acute exposures. Hence, understanding how long-term DEE exposure impacts lung function in adults is critical in determining whether this is a possible pathway explaining the mortality and morbidity associated with air pollution.

However, several limitations should be noted for our study. First, due to relatively short half-lives of PAHs, the information provided by biomonitoring of urinary OH-PAHs is limited to recent exposure, monitoring of a single spot urine sample represents only short-term exposure estimate and the repeated urine metabolite measurements or definitive biomarkers reflecting chronic exposure is needed to fully elucidate this association. Second, this was a cross-sectional study, therefore longitudinal follow-up cohort research is required to provide further clarification about whether the lung function decline exist between the DEE-exposed workers and non-DEE-exposed workers, as well as in smokers and non-smokers. Third, we could not rule out residual and unmeasured confounders including other exposures, although we adjusted for a wide range of confounding factors. Therefore, we recruited controls from the water plant were from the same city as the non-DEE-exposed workers, therefore, all the subjects were exposed to the same level of urban air pollution in their spare time.

Conclusion

In summary, our study finds that long-term exposure to DEE could induce decrease in lung function which shows mainly obstructive changes of airways and influences of small airways function, and the decreased lung function was negatively associated with internal dosage of DEE exposure. Additionally, our study suggests that the decreased lung function was accompanied with increasing of CBMN cytome index which is used to evaluate the impact of DEE exposure on genomic instability. However, further researches are needed to confirm these findings in prospective studies and elucidate the possible mechanisms

of these associations.

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Supplement tables:

Table s1. Percentage predicted lung function indexes of non-DEE-exposed and DEE-exposed workers (mean±SD)

Percentage predicted lung function indexes	Non-DEE-exposed Workers (n=127)	DEE-exposed workers (n=137)	p-$_{crude}$*	p-$_{adjust}$#
% VC (%)	109.50±12.89	109.01±11.97	0.749	0.791
% FVC (%)	107.65±13.81	106.59±11.59	0.498	0.596
% FEV_1 (%)	102.33±11.69	98.63±11.94	0.012	0.012
FEV_1/FVC(%)	88.50±5.57	86.01±5.29	<0.001	<0.001
% MMF (%)	98.60±23.03	87.00±20.34	<0.001	<0.001
% PEF (%)	90.52±15.93	89.63±15.11	0.640	0.635
% $FEF_{25\%}$ (%)	95.27±18.13	91.24±15.61	0.053	0.061
% $FEF_{50\%}$ (%)	92.81±22.23	83.87±20.26	0.001	0.001
% $FEF_{75\%}$ (%)	80.84±23.51	70.86±20.55	<0.001	<0.001

%VC : percent predicted vital capacity, %FVC: percent predicted forced vital capacity, %FEV_1: percent predicted forced expiratory volume in 1 second, %MMF: percent predicted maximal mid expiratory flow curve, %PEF: percent predicted peak expiratory flow, %$FEF_{25\%}$: percent predicted forced expiratory flow at 25% of FVC, %$FEF_{50\%}$: percent predicted forced expiratory flow at 50 % FVC, and %$FEF_{75\%}$: percent predicted forced expiratory flow at 75% of FVC. *t-test was used to compare values from both groups. #Multiple regression analysis of smoking status, alcohol use, and DEE exposure on different percentage predicted lung function variables.

Table s2. The lung function indexes grouped by DEE exposure duration in all study subjects (mean±SD)

The lung function indexes	DEE exposure duration (yr)				
	0 (n=127)	0–4 (n=23)	4–8 (n=42)	>8 (n=72)	p-$_{trend}$*
VC (l)	4.52±0.69	4.81±0.60	4.61±0.60	4.34±0.56	0.510
FVC (l)	4.45±0.74	4.75±0.58	4.50±0.58	4.24±0.53	0.427
FEV_1 (l)	3.93±0.64	4.12±0.51	3.83±0.54	3.65±0.47	0.015
FEV_1/FVC	0.89±0.06	0.87±0.06	0.85±0.05	0.86±0.05	0.002
MMF (l/s)	4.71±1.09	4.54±0.94	4.18±0.93	4.20±0.94	0.001
PEF (l/s)	8.28±1.44	8.78±1.67	8.22±1.25	8.05±1.42	0.272
$FEF_{25\%}$ (l/s)	7.78±1.47	7.83±1.70	7.50±1.23	7.33±1.17	0.024
$FEF_{50\%}$ (l/s)	5.42±1.35	5.19±1.13	4.78±1.14	4.86±1.26	0.004
$FEF_{75\%}$ (l/s)	2.49±0.81	2.42±0.62	2.15±0.63	2.09±0.59	0.001

See table 2 for abbreviations. *Multiple regression analysis of age, height, weight, smoking status, alcohol use, and DEE exposure duration on different lung function variables in all study subjects.

Table s3. The lung function indexes in the non-DEE-exposed and DEE-exposed workers older than 20 years old (mean±SD)

The lung function indexes	Non-DEE-exposed Workers (n=111)	DEE-exposed workers (n=137)	p-$_{crude}$*	p-$_{adjust}$#
VC (l)	4.49±0.71	4.50±0.60	0.865	0.276
FVC (l)	4.38±0.74	4.41±0.59	0.750	0.408
FEV_1 (l)	3.86±0.64	3.78±0.53	0.287	0.012
FEV_1/FVC	0.88±0.06	0.86±0.05	0.001	0.001
MMF (l/s)	4.65±1.09	4.25±0.94	0.002	0.001
PEF(l/s)	8.29±1.41	8.22±1.43	0.730	0.616
$FEF_{25\%}$ (l/s)	7.77±1.45	7.47±1.29	0.086	0.075
$FEF_{50\%}$ (l/s)	5.37±1.36	4.89±1.20	0.004	0.002
$FEF_{75\%}$ (l/s)	2.44±0.82	2.16±0.61	0.003	<0.001

See table 2 for abbreviations. #Multiple regression analysis of age, height, weight, smoking status, alcohol use, and DEE exposure on different lung function variables in the non-DEE-exposed and DEE-exposed workers older than 20 years old.

Table s4. The lung function indexes grouped by tertiles of urinay total OH-PAHs level in the non-DEE-exposed and DEE-exposed workers (mean ±SD)

The lung function indexes	Urinay total OH-PAHs (μg/g creatinine)			p-$_{trend}$*
DEE-exposed workers (n=137)	T1<9.98 (n=45)	T2 9.98–16.40 (n=47)	T3 >16.40 (n=45)	
VC (l)	4.47±0.61	4.60±0.58	4.43±0.62	0.445
FVC (l)	4.38±0.58	4.50±0.54	4.33±0.64	0.418
FEV_1 (l)	3.76±0.55	3.88±0.46	3.71±0.56	0.587
FEV_1/FVC	0.86±0.05	0.86±0.06	0.86±0.05	0.795
MMF (l/s)	4.21±0.98	4.36±0.96	4.18±0.89	0.734
PEF (l/s)	8.28±1.45	8.32±1.44	8.06±1.42	0.611
$FEF_{25\%}$ (l/s)	7.58±1.34	7.52±1.34	7.30±1.21	0.407
$FEF_{50\%}$ (l/s)	4.88±1.23	4.96±1.19	4.83±1.21	0.864
$FEF_{75\%}$ (l/s)	2.14±0.65	2.26±0.63	2.08±0.56	0.917
non-DEE-exposed workers (n=127)	T1<2.96 (n=42)	T2 2.96–7.60 (n=43)	T3 > 7.60 (n=42)	
VC (l)	4.82±0.61	4.49±0.70	4.27±0.66	0.355
FVC (l)	4.86±0.72	4.36±0.71	4.14±0.63	0.344
FEV_1 (l)	4.26±0.61	3.86±0.61	3.67±0.57	0.757
FEV_1/FVC	0.88±0.06	0.89±0.06	0.89±0.05	0.205
MMF (l/s)	4.93±1.05	4.57±1.13	4.64±1.09	0.210
PEF (l/s)	8.31±1.36	8.23±1.33	8.30±1.65	0.477
$FEF_{25\%}$ (l/s)	7.78±1.38	7.68±1.42	7.89±1.62	0.849
$FEF_{50\%}$ (l/s)	5.61±1.26	5.14±1.36	5.51±1.41	0.087
$FEF_{75\%}$ (l/s)	2.72±0.87	2.46±0.78	2.30±0.72	0.578

See table 2 for abbreviations. *Multiple regression analysis of age, height, weight, smoking status, alcohol use, and tertiles of urinay total OH-PAHs level on different lung function variables.

Table s5. Multiple regression analysis of age, height, weight, smoking status, alcohol use and DEE exposure on different lung function variables

Variables	VC		FVC		FEV_1		FEV_1/FVC		MMF		PEF		$FEF_{25\%}$		$FEF_{50\%}$		$FEF_{75\%}$	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value	β (95% CI)	*p*-value
Age (years)	−0.022 (−0.028–−0.015)	<0.001	−0.027 (−0.034–0.020)	<0.001	−0.025 (−0.031–−0.019)	<0.001	0.000 (−0.001–0.000)	0.269	−0.025 (−0.038–−0.011)	<0.001	0.001 (−0.019–0.021)	0.934	−0.005 (−0.025–0.014)	0.580	−0.021 (−0.038–−0.004)	0.018	−0.023 (−0.032–−0.014)	<0.001
Height (cm)	0.063 (0.052–0.074)	<0.001	0.058 (0.046–0.070)	<0.001	0.045 (0.035–0.056)	<0.001	−0.001 (−0.002–0.000)	0.075	0.025 (0.001–0.048)	0.040	0.054 (0.020–0.088)	0.002	0.021 (−0.012–0.054)	0.207	0.030 (0.000–0.059)	0.053	0.022 (0.006–0.038)	0.006
Weight (kg)	−0.001 (−0.006–0.004)	0.662	−0.004 (−0.009–0.002)	0.171	−0.004 (−0.008–0.001)	0.150	0.000 (−0.001–0.001)	0.808	0.004 (−0.007–0.014)	0.518	0.007 (−0.009–0.022)	0.393	0.010 (−0.006–0.025)	0.218	0.004 (−0.009–0.018)	0.536	−0.003 (−0.010–0.004)	0.437
Smoking status	−0.037 (−0.162–0.088)	0.561	−0.048 (−0.177–0.081)	0.467	−0.036 (−0.152–0.080)	0.542	0.001 (−0.013–0.016)	0.876	−0.054 (−0.312–0.204)	0.681	−0.019 (−0.393–0.356)	0.922	−0.017 (−0.382–0.348)	0.926	−0.090 (−0.420–−0.240)	0.591	−0.018 (−0.193–0.158)	0.844
Alcohol use	0.008 (−0.053–0.068)	0.796	0.015 (−0.047–0.078)	0.633	−0.003 (−0.059–0.053)	0.923	−0.004 (−0.011–0.003)	0.267	−0.026 (−0.151–0.099)	0.685	0.020 (−0.161–0.202)	0.824	−0.021 (−0.197–0.156)	0.815	−0.001 (−0.160–0.159)	0.995	−0.022 (−0.107–0.063)	0.612
DEE exposure	−0.037 (−0.152–0.079)	0.530	−0.053 (−0.173–0.066)	0.383	−0.147 (−0.255–−0.040)	0.007	−0.024 (−0.037–−0.011)	0.001	−0.459 (−0.697–−0.220)	<0.001	−0.075 (−0.421–0.271)	0.671	−0.325 (−0.663–0.012)	0.058	−0.513 (−0.818–−0.208)	0.001	−0.322 (−0.484–−0.159)	<0.001