Exposure to volatile organic compounds and loss of pulmonary function in the elderly

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ABSTRACT: Volatile organic compounds (VOCs) are reported to cause adverse effects on pulmonary function in occupationally exposed workers. However, evidence is lacking on the effect in the general population. We hypothesised that VOCs impair pulmonary function through enhancing oxidative stress, especially in the elderly population.

A longitudinal panel study of 154 elderly people was performed in South Korea. Repeated spirometric tests were performed up to eight times on different days for each subject. We also measured urinary concentrations of metabolites of the VOC and markers of oxidative stress (malondialdehyde and 8-oxo-2′-deoxyguanosine) on the same day of spirometric tests. A mixed linear regression model was used to evaluate the association among the VOC metabolites, oxidative stress markers and spirometric tests.

We found that the urinary levels of hippuric acid and methylhippuric acid, which are metabolites of toluene and xylene, respectively, were significantly associated with reduction of forced expiratory volume in 1 s (FEV_1), FEV_1/forced vital capacity (FVC), and forced expiratory flow at 25–75% of FVC. We also found significant associations between the metabolites of VOCs and the markers of oxidative stress. In addition, the oxidative stress markers were associated with pulmonary function parameters.

This study suggests that exposure to toluene and xylene exert a harmful effect on pulmonary function by exacerbating oxidative stress in elderly people.

KEYWORDS: Elderly, oxidative stress, pulmonary function, volatile organic compounds

There is a growing concern over health problems related to ambient air pollution. Volatile organic compounds (VOCs) are one of the major ambient air pollutants and are emitted from diverse chemical sources in common daily use. Exposure to VOCs may cause upper and lower respiratory symptoms and contribute to the worsening of asthma. The mechanisms of VOC toxicity, however, have yet to be established, even though some experimental evidence supports oxidative stress having a role [1–3].

Decline of pulmonary function has become one of the most important issues regarding health effects of air pollution because it not only reflects the individual respiratory health status, but predicts cardiovascular morbidity and mortality in the general population [4–6].

Although the diminution of pulmonary function by exposure to ozone and particulate air pollutants has been previously reported [7–9], it is not clear whether indoor or outdoor exposure to

VOCs in ambient air contribute to the reduction of pulmonary function. Although a recent cross-sectional study suggests an association between exposure to VOCs and diminished lung function [10], evidence of a causal link is still lacking.

Therefore, we conducted a longitudinal panel study with repeated measurements to evaluate the effects of VOCs on pulmonary function and the involvement of oxidative stress in the pathogenesis in the elderly, who are known to be especially susceptible to air pollution [11–13].

METHODS

Study subjects
The study population consisted of 161 elderly people aged >60 yrs at six day care facilities within the three different geographical regions of Seoul (three facilities), Cheongju (one facility) and Gangnung (two facilities) in South Korea, which represent a metropolitan area, an industrialised

city and a rural area, respectively. The elderly returned to their home environment when they were not at the day care facilities.

Repeated spirometric tests were performed up to eight times on different days for each subject. We also measured urinary concentrations of metabolites of the VOC and markers of oxidative stress on the same day as spirometric tests. However, three refused participation and four performed spirometric testing only once. Therefore, we included 154 subjects who had performed spirometric testing twice or more in the analysis.

At the first visit, information such as demographic factors, medical history, dietary habits, smoking and alcohol consumption was obtained by a questionnaire. The first voided urines were collected for biomarker measurements at each visit day.

Written informed consent was obtained from every subject, and the study was approved by the institutional review board of the Seoul National University College of Medicine, Seoul, South Korea.

Spirometric measurements

Spirometric testing was conducted four times in the warm season (June to September) and four times in the cold season (October to December) according to 2005 European Respiratory Society/American Thoracic Society recommendations. All tests were performed at the same hour in the morning by one trained technician, and a Microlab® (SensorMedics, Yorba Linda, CA, USA) spirometer was used.

The spirometric tests used in our analyses were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1 as a percentage of FVC (FEV_1/FVC), and forced expiratory flow at 25–75% of FVC ($FEF_{25-75\%}$).

Urinary levels of VOC metabolites

On the same day of spirometric tests, tt-muconic acid, hippuric acid, mandelic acid and methylhippuric acid were measured as biomarkers for exposure to benzene, toluene, ethylbenzene and xylene, respectively. To determine the tt-muconic acid levels, 200 μL of urine were mixed with 800 μL of mobile phase (1% acetic acid and methanol, 10:1, v/v) and filtered through a 0.45-μm membrane filter. Absorbance of the filtered solution was measured by HPLC-UV at 259 nm using a Shiseido MF C8 SG80 5 μm (4.6 × 150 mm) column (Tokyo, Japan). To determine the hippuric acid, mandelic acid and methylhippuric acid levels, 200 μL of urine were mixed with 2 mL of distilled water and filtered through a 0.45-μm membrane filter. Absorbance of the filtered solution was measured by HPLC-UV at 225 nm using Waters Atlantis C18 5 μm (4.6 × 150 mm) column (Milford, MA, USA). The mobile phase was 0.55% n-tetrabutylammonium bromide/0.15% KH_2PO_4 and methanol (60:40 v/v).

Urinary levels of oxidative stress biomarkers

For evaluation of oxidative stress, urinary malondialdehyde (MDA) was determined by the measurement of MDA-thiobarbituric acid (TBA) adducts. Aliquots of the upper layer of the centrifuged urine samples were mixed with phosphoric acid and TBA reagent in an iced methanol glass tube, and were boiled for 60 min. After the mixture was boiled, it was cooled in ice water for 5 min and centrifuged after adding methanol.

The absorbance was measured by an HPLC system equipped with a SP930D solvent delivery pump and UV730 absorbance detector (Youngjin company, Seoul, South Korea) on a Nova-Pak C18 column (150 × 3.9 nm) (Waters) at 532 nm with a mobile phase composed of 50 nM KH_2PO_4 (pH 6.8) and methanol in a ratio of 58:42 v/v [14].

Urinary 8-oxo-2′-deoxyguanosine (8-OHdG) levels were quantitatively determined using a competitive *in vitro* ELISA (JICA, Fukuroi, Japan). 50 μL of primary monoclonal antibody and 50 μL of sample or standard were added to microtitre plates, which had been pre-coated with 8-OHdG. The plates were sealed tightly and incubated at 37°C for 1 h, and then washed with 250 μL PBS. 100 μL of horseradish peroxidase-conjugated secondary antibody were then added to each well, incubated and washed. 100 μL of enzyme substrate was then added to each well. Reactions were terminated by adding 100 μL 1 N phosphoric acid. Absorbance readings were taken 3 min after termination using a spectrophotometer (ELX808; Bio-Tek, Winooski, VT, USA) at 450 nm [14].

Ambient levels of VOC

We measured indoor levels of benzene, toluene, ethylbenzene and xylene (once at two places) and outdoor levels (at one place) at the same time for each facility during the study period to compare ambient levels of the VOC with the levels of other reports. The sampling equipment used to collect the VOC was placed 1.5 m above floor level indoors and at ground level outdoors at the day care facilities. Air samples were collected by passing air through adsorbent tubes (Tenax, Eighty Four, PA, USA). The tubes were connected with air sampler (MP-100 H; Sibata Scientific Technology Ltd, Tokyo, Japan) for absorption of the VOC. The sampling rate was 100 mL·min^{-1} for 8 h (09.00 h to 17.00 h) and the samples were analysed by GC/MSD (6890N, Agilent Technologies, Santa Clara, CA, USA) in a capillary column (60 m length, 0.25 mm inner diameter and 0.25 μm film thickness) using Thermal Desorption System (Gerstel GmbH and Co. KG, Mülheim, Germany).

Measurements of potential confounders

Urinary cotinine concentrations

We measured urinary concentrations of cotinine because tobacco exposure was regarded as a confounder and cotinine is widely considered to be the best marker for monitoring tobacco exposure in either actively or passively exposed individuals. The urine samples were stored at -20°C and analysed in batches. Urinary cotinine level was measured using a microplate enzyme immunoassay (UMAX, Dallas, TX, USA). The microplate was coated with an anti-cotinine antibody and cotinine concentration was determined by a competitive reaction with an enzyme-bound and non-enzyme-bound antigens. Reactions were detected at 450 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Personal exposure to PM2.5 and nitrogen dioxide

We measured personal exposure to PM2.5 (particles with a 50% cut-off aerodynamic diameter of 2.5 μm) in participating subjects. The particles were sampled around 24 h for each visit with a personal environmental monitor PM2.5 cyclon (SKC Inc., Washington, PA, USA) containing a pump (Mine Safety

Appliances company, Pittsburgh, PA, USA). The air flow rate ($2 L·min^{-1}$) of the pump was adjusted before each sampling. The $PM_{2.5}$ cyclon was attached with a pin between the chest and head, and the pump was placed in a small bag, which was carried by the subjects during measurement. The $PM_{2.5}$ cyclon and the pump were kept near the bedside during sleep. The 37 mm, 2.0 μm Teflon filters (SKC, Eighty Four, PA, USA) were weighed before and after sampling using a microbalance and the concentration of ambient particulate matter was calculated considering air flow and measurement time.

Personal nitrogen dioxide (NO_2) exposures were also measured simultaneously with $PM_{2.5}$. Polyester housed badge-type NO_2 passive samplers (Envors Co., Daejon, South Korea) were used for the study. One of the investigators gave instructions on how to wear and care for the samplers. Unsealed passive sampling badges were attached with a pin between the chest and head, and were sealed the following day. Wearers were also instructed to keep the badges near the bedside when they slept. The collected samplers were sealed with a gas-tight polyester lid and kept in a zippered plastic bag before and after sampling until analysis. The ambient NO_2 concentration was calculated by using a NO_2 standard solution curve assuming a given sampling rate, *i.e.* $16.9 mL·min^{-1}$, which was supplied by the manufacturer.

Temperature and humidity
Data for outdoor temperature and relative humidity were obtained from the Korea Meteorological Administration, Seoul, South Korea.

Statistical analysis
We calculated the means and distribution percentiles for urinary concentrations of biomarkers. For concentrations below the limits of detection (LOD), a value equal to the LOD divided by two was used in the statistical analysis. We assessed associations between urinary metabolites of VOC, oxidative stress markers and pulmonary function parameters. Linear mixed-effect models were used to estimate the VOC exposure effects on the pulmonary function test (PFT) parameters or the oxidative stress markers, controlling for temperature, humidity, cotinine levels, dietary habits, alcohol consumption and individual characteristics. Personal exposures to $PM_{2.5}$ and NO_2 were also controlled in the statistical models. Because the distributions of biomarker concentrations were skewed, we used log-transformed data for these measurements in the linear mixed models. We treated age, sex, height, weight, date, temperature, relative humidity, cotinine levels, $PM_{2.5}$ and NO_2 exposure levels, dietary habits, alcohol consumption and biomarker concentrations as fixed effects. Each participant was treated as a random effect in the models. Because we had similar results regardless of adjustment for urine creatinine, we report the association results using non-adjusted ones for urinary levels of VOC metabolites and oxidative stress markers.

RESULTS
Participant characteristics and biochemical analyses
The participant mean age was 73.5 yrs old and 63.0% were female. Of a possible total of eight visits, mean number of visits was 6.8 visits per person. The number of persons whose level of urinary cotinine was over $300 μg·L^{-1}$ was 22 (14.3%) (table 1).

The indoor levels (n=12) of benzene, toluene, ethylbenzene and xylene were $5.96±4.08$, $11.18±10.49$, $4.55±1.78$ and $5.24±1.35 μg·m^{-3}$, respectively. The outdoor levels (n=6) were $4.31±3.00$, $6.20±8.71$, $3.10±1.56$ and $2.84±1.49 μg·m^{-3}$, respectively. The results of measurements for urinary markers of VOC metabolites, NO_2, $PM_{2.5}$, outdoor temperature and relative humidity are shown in table 2. The percentage of measurements that were lower than limit of detection (% <LOD) are also shown. Among the measured biomarkers, the % <LOD of tt-muconic acid was >50%.

Effects of VOC metabolites on lung function parameters
The levels of hippuric acid were negatively associated with FEV_1 and $FEF_{25–75\%}$, and methylhippuric acid levels were negatively associated with FEV_1 and FEV_1/FVC (table 3). However, tt-muconic acid and mandelic acid were not significantly associated with either PFT parameters.

To estimate effects of VOC metabolites on lung function, we calculated the estimated values of lung function parameters assuming average values of age, sex, height, weight, temperature, humidity, cotinine level, $PM_{2.5}$ and NO_2 levels, dietary habits and alcohol consumption. As shown in figure 1, FEV_1 is decreased by 21.5 mL (1.2%), FEV_1/FVC by 0.5% (0.6%), $FEF_{25–75\%}$ by $74.6 mL·s^{-1}$ (3.6%) when the value of hippuric acid is at its 90th percentile compared to its 10th percentile. FEV_1 decreases by 18.2 mL (1.0%), FEV_1/FVC by 0.7% (0.7%), and $FEF_{25–75\%}$ by $40.5 mL·s^{-1}$ (1.9%) when methylhippuric acid is at its 90th compared to its 10th percentile. (fig. 1).

Associations among VOC metabolites, oxidative stress and lung function
Except for the tt-muconic acid, all of the other VOC metabolites were significantly associated with urinary MDA levels after adjustment for age, sex, height, weight, cotinine levels, $PM_{2.5}$ and NO_2 levels, dietary habits, alcohol consumption, temperature and humidity. All of the measured VOC metabolites were also significantly associated with urinary 8-OHdG (table 4).

TABLE 1	Characteristics of participants and their pulmonary function		
	Male	**Female**	**Total**
Participants n	57	97	154
Visits per person n	6.9	6.8	6.8
Age yrs	73.4 (64–88)	73.5 (60–91)	73.5 (60–91)
Height cm	163.2±5.5	149.4±5.8	154.5±8.7
Weight kg	63.7±8.7	55.7±8.2	58.7±9.2
FVC L	2.33±0.55	1.81±0.44	2.01±0.55
FEV_1 L	1.95±0.52	1.62±0.41	1.75±0.49
FEV_1/FVC %	0.84±0.09	0.90±0.06	0.87±0.08
$FEF_{25–75\%}$ $L·s^{-1}$	2.12±0.93	2.02±0.79	2.07±0.85
Cotinine $≥300 μg·L^{-1}$	18 (31.6)	4 (4.1)	22 (14.3)

Data are presented as mean±SD, mean age (range) or n (%), unless otherwise stated. FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC.

TABLE 2 Urinary concentrations and distributions of the volatile organic compound (VOC) metabolites, nitrogen dioxide, particles with a 50% cut-off aerodynamic diameter of 2.5 μm (PM2.5), outdoor temperature and relative humidity

VOC metabolites	Measurements n	Mean ± SD	LOD	<LOD %	10 pct	25 pct	50 pct	75 pct	95 pct
Muconic acid mg·L^{-1}	988	0.08 ± 0.10	0.014	79.96	0.05	0.05	0.05	0.05	0.27
Hippuric acid mg·mL^{-1}	984	0.53 ± 0.65	0.020	0.30	0.06	0.14	0.32	0.65	1.84
Mandelic acid mg·mL^{-1}	984	0.08 ± 0.11	0.016	41.67	0.08	0.08	0.04	0.11	0.27
Methylhippuric acid mg·mL^{-1}	984	0.10 ± 0.19	0.004	25.81	0.00	0.00	0.02	0.08	0.47
NO$_2$ ppb	1033	21.68 ± 11.46	0.30	0.0	8.71	12.36	19.70	28.80	43.30
PM2.5 μg·m^{-3}	1021	33.38 ± 22.45			8.87	17.00	28.81	43.59	78.31
Outdoor temperature °C	1037	16.79 ± 8.91			4.5	7.2	19.9	24.9	27.6
Outdoor relative humidity %	1037	68.47 ± 16.78			42.3	58.3	72.6	80.9	90.8

LOD: limit of detection; pct: percentile.

When we analysed the relationships between the oxidative stress biomarkers and lung function parameters, we found that urinary MDA levels were negatively associated with FEV1 and urinary 8-OHdG levels were negatively associated with FEV1/FVC and FEF25–75%. However, FVC was not associated with either the MDA or 8-OHdG levels. Figure 2 shows the possible pathway for the effects of toluene and xylene exposure on lung function through oxidative stress.

Association between indoor levels of VOCs and their metabolites

When we analysed the relationship between indoor VOC levels at the day care facilities and VOC metabolites in urine, we found that urinary hippuric acid and mandelic acid levels were significantly associated with indoor toluene levels (regression coefficient β 0.009; p=0.003) and ethylbenzene levels (β 0.061; p<0.001) while there was no significant association of urinary tt-muconic acid (β -0.001; p=0.589) and methylhippuric acid (β 0.003; p=0.683) with indoor levels of benzene and xylene, respectively, after regression analysis controlling for age, sex, height, weight, date, temperature, relative humidity, cotinine levels, PM2.5 and NO$_2$ exposure levels, dietary habits and alcohol consumption.

DISCUSSION

Our study demonstrates that exposure to toluene and xylene may exert harmful effects on lung function and that oxidative stress could be involved in the pathogenesis in the elderly population. Most of the reported investigations into the health effects of air pollution have been targeted at outdoor air pollutants, such as ozone and particulate matter [7, 8, 15, 16]. The importance of indoor air quality, however, is progressively increasing since people spend more time indoors than outdoors. Although VOCs also exist in the outdoor environment, the concentrations of VOCs are two- to five-fold higher indoors than outdoors, so they are generally regarded as ''indoor'' air pollutants [17, 18].

In this study, we found a significant association between urinary levels of hippuric acid and methylhippuric acid and decreased lung function. These are metabolites of toluene and xylene, respectively, which are widely used in our daily necessities, such as paints, varnishes, rubber and disinfectants. Although benzoic acid from dietary intake is known to be one of the main sources of urinary hippuric acid, significant association between ambient levels of toluene and urinary hippuric acid has been reported [19]. More than 90% of xylene is known to be metabolised to methylhippuric acid, so urinary levels of methylhippuric acid can be used as the total body burden of xylene [20].

TABLE 3 Estimated regression coefficients of urinary concentrations of the volatile organic compund (VOC) metabolites on lung function parameters

VOC metabolites	FEV1 mL		FEV1/FVC %		FEF25–75% mL·s^{-1}	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Muconic acid mg·L^{-1}	34.84 ± 44.41	0.433	-1.40 ± 1.55	0.367	-61.57 ± 114.16	0.590
Hippuric acid mg·mL^{-1}	-18.23 ± 8.52	0.033	-0.47 ± 0.29	0.107	-65.00 ± 21.81	0.003
Methylhippuric acid mg·mL^{-1}	-65.70 ± 30.41	0.031	-2.44 ± 1.00	0.015	-145.85 ± 76.46	0.057
Mandelic acid mg·mL^{-1}	-15.10 ± 45.69	0.741	-1.03 ± 1.58	0.514	-67.67 ± 117.24	0.564

Data are presented as mean ± SE, unless otherwise stated. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF25–75%: forced expiratory flow at 25–75% of FVC. p-values are from mixed regression analysis adjusted for visit number, age, sex, height, weight, outdoor temperature, outdoor relative humidity, diet, cotinine, alcohol consumption, levels of nitrogen dioxide and particles with a 50% cut-off aerodynamic diameter of 2.5 μm.
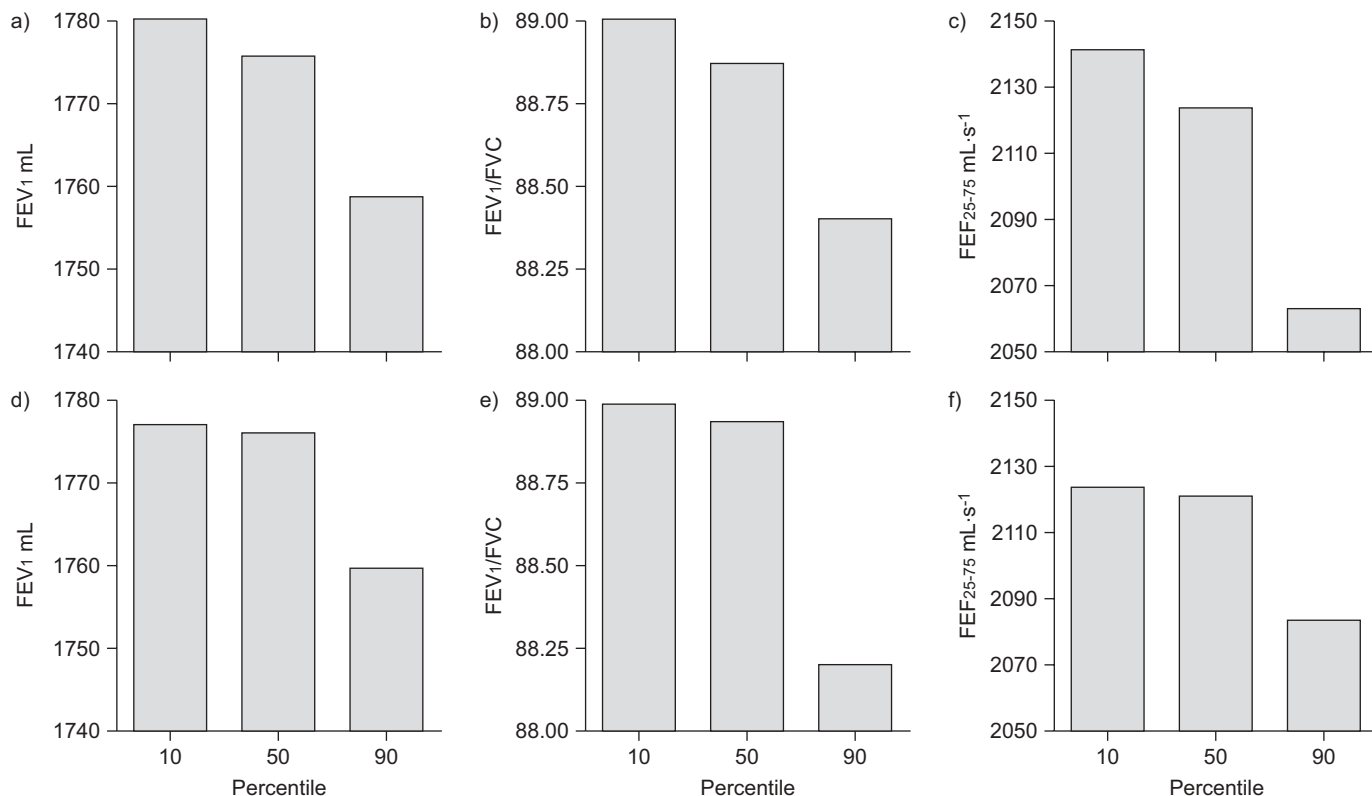
FIGURE 1. Estimated effects of urinary concentrations of a–c) hippuric acid and d–f) methylhippuric acid on lung function parameters. Each level of three pulmonary function parameters at the 10, 50 and 90th percentile of urinary levels of hippuric acid and methylhippuric acid were estimated. Mean values of age, sex, height, weight, outdoor temperature, outdoor relative humidity, area, cotinine and alcohol consumption were used for estimation. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF25–75%: forced expiratory flow at 25–75% of FVC.

Muconic acid and mandelic acid, which were supposed to represent exposure to benzene and ethylbenzene, respectively, did not exhibit an association with lung function parameters. However, this could be due to the concentration under the

TABLE 4 Estimated regression coefficients of urinary concentrations of the volatile organic compound (VOC) metabolites on markers of oxidative stress

VOC metabolites	MDA $\mu mol \cdot L^{-1}$		8-OHdG $ng \cdot mL^{-1}$	
	Coefficient	p-value	Coefficient	p-value
Muconic acid $mg \cdot L^{-1}$	0.54 ± 0.36	0.132	8.23 ± 2.51	0.001
Hippuric acid $mg \cdot mL^{-1}$	0.51 ± 0.06	<0.001	3.92 ± 0.42	<0.001
Methylhippuric acid $mg \cdot mL^{-1}$	0.84 ± 0.21	<0.001	8.20 ± 1.54	<0.001
Mandelic acid $mg \cdot mL^{-1}$	2.20 ± 0.32	<0.001	13.77 ± 2.34	<0.001

Data are presented as mean±SE, unless otherwise stated. MDA: malondialdehyde; 8-OHdG: 8-oxo-2′-deoxyguanosine. p-values are from mixed regression analysis adjusted for visit number, age, sex, height, weight, outdoor temperature, outdoor relative humidity, diet, cotinine, alcohol consumption, nitrogen dioxide and particles with a 50% cut-off aerodynamic diameter of 2.5 μm levels.
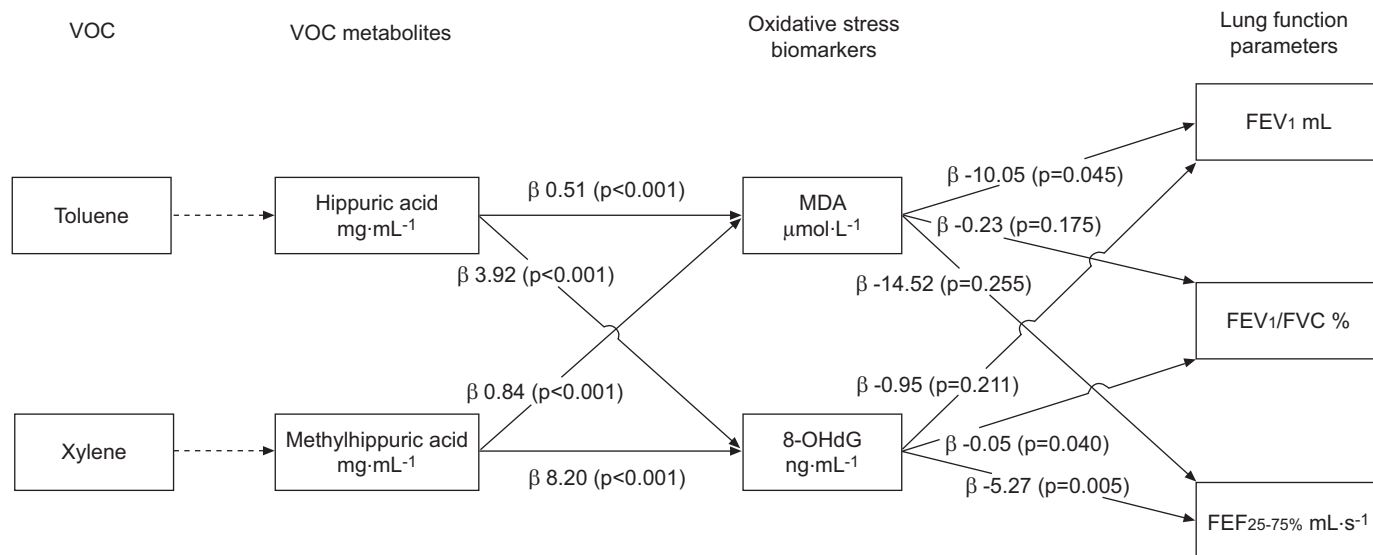
limit of detection (80% and 42%) rather than a true non-association. As shown in professional painters or in sick building syndrome, exposure to an unusually high concentration of VOCs can result in respiratory effects [21, 22]. Our panel consisted of elderly people without occupational exposure, so this study suggests that even ordinary exposure to VOCs can affect lung function, at least in the elderly population.

Of the measured parameters of lung function, FVC did not show any association with markers of VOC exposure in contrast to FEV1, FEV1/FVC, and FEF25–75%. This suggests that the primary site of the pulmonary toxicity of VOCs might be the airways, especially the small airways rather than the pulmonary parenchyme.

The estimated changes of FEV1 were 21.5 mL for hippuric acid and 18.2 mL for methylhippuric acid, assuming the level of each metabolite changes to its 90th from the 10th percentile. Even though we assumed extreme circumstances, this is a relatively large change considering the annual loss of FEV1 in healthy nonsmokers in the general population is estimated to be ~30 mL·yr^{-1} [23].

The proposed mechanism of VOC toxicity was oxidative stress [1–3], which is also thought to be the main mechanism of cigarette smoke-induced lung injury, acute exacerbation of chronic obstructive pulmonary disease, and many of the harmful effects of other air pollutants on respiratory epithelium [24–27]. The results of our study support the association

FIGURE 2. Possible pathway for the effects of toluene and xylene exposure on lung function through oxidative stress. VOC: volatile organic compound; MDA: malondialdehyde; 8-OHdG: 8-oxo-2'-deoxyguanosine; FEV1: forced expiratory volume in 1 s; FVC forced vital capacity; FEF25–75%: forced expiratory flow at 25–75% of FVC.

between exposure to VOCs and increased systemic level of oxidative stress. Oxidative stress is also known to be influenced by many other factors, such as dietary intake, alcohol consumption and cigarette smoking, in addition to air pollution [19, 26–29]. In our data, however, significant associations between VOC metabolites and markers of oxidative stress were found even after adjustment for dietary intake, cotinine level, alcohol consumption, and PM2.5 and NO2 levels.

We also found a significant association between markers of oxidative stress and parameters of lung function. This is in line with the current understanding of the relationship of oxidative stress to a reduction in pulmonary function and increased cardiopulmonary mortality [20, 30]. In addition, it supports the hypothesis that VOCs might influence lung function by exacerbating oxidative stress.

The strengths of the present study merit discussion. First, to the best of our knowledge, this is the first study to demonstrate the detrimental effects of toluene and xylene exposure on lung function in the course of ordinary environmental exposure. Secondly, the panel study design with repeated measurements of spirometric tests, urinary metabolites of VOCs and oxidative stress biomarkers up to eight times for each participant, provided a good opportunity to evaluate the short-term effects of changes in VOC exposure over time. Thirdly, by using biomarkers to evaluate personal exposures and outcome, this study demonstrated a possible pathway for the VOC effect, which suggests involvement of oxidative stress.

However, there are also some limitations to this study. We could not show associations between levels of indoor benzene and xylene and their metabolites. Because we only measured indoor levels of VOCs once at two spots in each day care facility, the data for the levels of indoor VOCs are thought to have failed to reflect personal variation of exposure and variation over time in contrast to those of the metabolites. The other reason for discrepancy between levels of indoor benzene and xylene and their metabolites could be that subjects were

exposed to different levels in their home. Even though we adjusted for potential sources of oxidative stress in the statistical analyses, including personal exposure levels to PM2.5 and NO2, concomitant exposure to other pollutants, such as ozone and heavy metals, could have had some impact on lung function. Another concern relates to whether the VOC metabolites reflect true VOC exposure in the ordinary environmental circumstances. Although we controlled possible confounders, such as dietary habits, in the statistical model, we could not prove confidently that the VOC metabolites represent exposure to the chemicals in ambient air very well when the exposure levels are not high. Finally, we did not adjust levels of urinary biomarkers for levels of urine creatinine. All samples used in this analysis, however, were from the first morning urine in order to reduce the problem of dilution.

In conclusion, our findings suggest that exposure to toluene and xylene increase oxidative stress, which results in a deterioration of lung function in the elderly population.

STATEMENT OF INTEREST
None declared.

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