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Association of urinary 3-phenoxybenzoic acid level with pulmonary function reduction in an urban elderly population with repeated measures data☆

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ABSTRACT

Pyrethroids are a class of man-made insecticides associated with various adverse health outcomes including respiratory problems. However, there were limited evidences on the relation between 3-phenoxybenzoic acid (3-PBA) as a metabolite of pyrethroids and pulmonary function, particularly among elderly population who have declining pulmonary function. Therefore, we collected urine samples and performed pulmonary function test (PFT) repeatedly in a total of 559 Korean elderly living in Seoul as an urban area. After measurement of urinary 3-PBA levels, cross-sectional relations of visit-to-visit variation in 3-PBA level on visit-to-visit variation in PFT parameters were evaluated using linear mixed effect models and generalized additive mixed models after adjustment for age, sex, body mass index, smoking status, education, visit episode, and phthalate metabolite levels. The Korean elderly were highly exposed to pyrethroids with 30.2% of elderly people with 3-PBA level over reference value derived on the 95th percentile of representative samples (2 ng/mL). Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}) as PFT parameters showed significant reductions by an increase of 3-PBA level (FEV_1, $\beta = -1.48$, p-value < 0.01; FVC, $\beta = -1.14$, p-value < 0.01; and FEF_{25-75}, $\beta = -1.11$, p-value $= 0.03$). The negative associations of 3-PBA level with FEV_1, FVC, and FEF_{25-75} were found only for females (FEV_1, $\beta = -1.64$, p-value < 0.01; FVC, $\beta = -1.47$, p-value < 0.01; and FEF_{25-75}, $\beta = -1.06$, p-value $= 0.07$), but not for males. However, the longitudinal effect of 3-PBA level on the trajectory of FEV_1, FVC, and FEF_{25-75} declines in females was not found. Community-level exposure to pyrethroids was associated with pulmonary function reduction in elderly population, indicating that more stringent control of pyrethroids is necessary to protect the elderly who have declining pulmonary function.

1. Introduction

Pyrethroids are a class of man-made insecticides that are globally used to control various insect pests, including those found at urban area and agricultural crops (Saillenfait et al., 2015; United States Environment Protection Agency (US EPA), 2013). Pyrethroid insecticides were reported to increase adverse health outcomes including developmental, male reproductive, and respiratory problems, even from pyrethroid exposures at environmental levels (Moretto, 1991; Saillenfait et al., 2015). The US EPA has reported that only 1% of pesticides including pyrethroids used every year in North America can reach its target while 99% of pesticides enter the environment without any production of intended benefits (Gavrilescu, 2005; USEPA, 2011). In South Korea, pyrethroids are among the most frequently used insecticides and pesticide consumption volume in Korea ranked the second highest among 19

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countries surveyed in 2010 (Korea Occupational Safety & Health Agency, 2001; Korean Statistical Information Service, 2017; National Institute of Environmental Research, 2006). Therefore, evaluating pyrethroid exposure levels in Korean general population might be important for healthcare of Korean population. Since the general population are exposed to pyrethroids via various exposure routes including foods and mosquito repellents, pyrethroid exposures have been assessed by measuring internal dose such as urinary levels of 3-phenoxybenzoic acid (3-PBA) as a metabolite of pyrethroids.

Pulmonary function reduction is an important respiratory health problem in the general population, particularly in the elderly who have declining pulmonary function. It indicates impaired respiratory health with potentially shorter life expectancy (Parker and Thorslund, 2007; Schunemann et al., 2000). Elderly people more than 60 years is continuously increasing worldwide, with an expectancy to be nearly doubled from 12% to 22% between 2015 and 2050 (World Health Organization, 2018). Korea is also rapidly ageing and was expected to become a super-aged society by 2026 (Korea National Statistical Office, 2005). Since pulmonary function is continuously declining in elderly people, pulmonary function reduction in elderly could be an important health issue in Korea as well as worldwide where elderly population is increasing. Moreover, pyrethroid insecticides were reported to play a role as airway inhaled environmental toxicants (Damalas and Eleftherohorinos, 2011). Previous epidemiologic studies have shown that respiratory health problems could be induced by pyrethroid exposures. A case study for asthmatic patients has reported pulmonary function reduction in asthmatic patients exposed to a commonly used aerosol insect killer containing pyrethroids at home (Newton and Breslin, 1983). Respiratory health problems by pesticide exposures have also been found in occupationally-exposed adult farmers and in 5–16 years of children exposed to pesticides (Hoppin et al., 2006; Salameh et al., 2003). Although there are potential links between exposure to pesticides and respiratory health problems, epidemiological study on the direct relationship between pyrethroid exposures and pulmonary function is limited, particularly among general elderly population. Moreover, because previous epidemiological studies for the relations between pyrethroid exposures and pulmonary function were cross-sectionally designed, within-subject changes could not be considered (Hoppin et al., 2006; Newton and Breslin, 1983; Salameh et al., 2003). Thus a longitudinal panel design which could make each subject to be used as a control of one's own with repeated measurements is required to account for within-subject changes of urinary 3-PBA level and pulmonary function.

Therefore, in the present study, we measured 3-PBA levels in urines repeatedly collected from Korean elderly and then evaluated the relationship between the 3-PBA levels and repeatedly tested pulmonary function. Furthermore, we evaluated intra- and inter-individual variations of 3-PBA levels and derived exceedance rate for existing limit values including the reference guides or reference value derived on the 95th percentile of representative samples (RV_{95}) (Aylward et al., 2018; Schulz et al., 2007).

2. Materials and methods

2.1. Study participants

The Korean Elderly Environmental Panel (KEEP)-II study was conducted as a subsequent study of KEEP-I to further investigate relationships between environmental exposures and health outcomes of the elderly aged sixty years and older. The KEEP-II recruited 1254 elderly people living in Asan as a rural area (695 elderly) and Seoul as an urban area (559 elderly) between December 2012 and April 2015 and surveyed three times at approximately 1-year interval. We collected urine samples every survey for KEEP-II. However, since pulmonary function test (PFT) was performed at every visit only for subjects living in Seoul, only 559 elderly subjects were finally analyzed to assess the relationships between 3-PBA level and PFT parameters. During the follow-up period, 164 (29.3%), 151 (27.0%), and 244 (43.7%) participants visited the elderly welfare center 1, 2, and 3 times, respectively, providing a total of 1195 urines with 1167 PFTs. Demographic information including lifestyles and residence area was collected from each participant by two trained interviewers using a structured questionnaire. All participants don't have any history for occupational exposure to pyrethroids. The present study was approved by the Institutional Review Board at Seoul National University Hospital, Republic of Korea (approval number, H-1209-006-424). All study participants provided written informed consent before the study started.

2.2. Measurement of 3-PBA levels

A total of 1195 urinary samples were collected from 559 participants, directly stored on ice, and on the same day transported to the Green Cross laboratories where urines were stored at −20 °C until analyzed. The 3-PBA level was measured by a previously reported procedure with some modifications (Schettgen et al., 2002). In brief, 3 mL of urine was mixed with 600 μL of 37% HCl and heated at 90 °C for 1 h. After cooling the samples on ice, acidic urine samples were sequentially extracted with 3 mL of *n*-hexane twice and with 1.2 mL of 0.1 M NaOH once. They were then re-extracted with 600 μL of 37% HCl and 1.8 of *n*-hexane once more. After extracted solution was dried in a nitrogen stream, 90 μL of toluene and 18 μL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide were added into the dried sample and then heated at 70 °C for 45 min. After cooling the sample on ice, 2 μL of sample was injected into gas chromatography mass spectrometer (Clarus 680T, Perkin Elmer, Waltham, MA, USA) to measure 3-PBA level. For quality control and quality assurance, the measurement method accuracy was calculated with commercial urine sample purchased from G-EQUAS (G-EQUAS 52 round). The difference between theoretical and observed concentrations was found within appropriate range proposed for commercial urine with $R^2 > 0.995$. The limit of detection (LOD) of 3-PBA level was 0.014 ng/mL for the 1st survey, 0.013 ng/mL for the 2nd survey, and 0.010 ng/mL for the 3rd survey.

2.3. Spirometric measurements

PFT was repeatedly performed for each individual at the same morning hour (just after urine collection) on each visit day by one trained technician supporting two community elderly welfare centers in Seoul. Pulmonary function was tested using a Viasys Microlab® portable spirometer (MicroMedical Ltd., Rochester, Kent, UK) according to the guideline recommended by the 2005 European Respiratory Society/American Thoracic Society (Miller et al., 2005). Spirometric measurements used in our analyses included forced expiratory volume in 1 second (FEV_1, L), forced vital capacity (FVC, L), FEV_1 as a percentage of FVC (FEV_1/FVC, %), and forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}, L/second). Since our subjects were elderly people who have declining pulmonary function, we used PFT parameter scores compared with normal values predicted based on PFT parameter scores of Korean representative samples with same age to ours, which were collected by the Korean National Health Examination Survey on 2001 (Choi et al., 2005).

2.4. Statistical analyses

Concentration of 3-PBA under the LOD was assigned as a default value of LOD level divided by square root of 2, because our dataset for 3-PBA had relatively few data below the detection limit (Hornung and Reed, 1990). Since each individual had repeated 3-PBA levels, means of repeated 3-PBA levels were used to analyze the trend of variability considering intra- and inter-individual variations of 3-PBA levels. Also the variability trend was compared between high- and low-level exposure groups divided by median values for individual mean 3-PBA level (1.26 ng/mL (natural log transformed, 0.23)). To evaluate how much portion intra (within)- or inter (between)-individual variation of 3-PBA levels could explain among total variation (intra-individual variation plus inter-individual variation), intra-class correlation (ICC) defined as the ratio of inter-individual variation to total variation was estimated using a linear mixed effect model with previously reported equation (Kim et al., 2018a). The ICC value was also calculated in high-level exposure group and in low-level exposure group separately. We also derived exceedance rate for existing limit values such as reference guide I (1.7 ng/mL), reference guide II (87 ng/mL), and RV_{95} (2 ng/mL) (Aylward et al., 2018; Schulz et al., 2007). Our 3-PBA levels were also compared with those reported in previous studies.

Urinary 3-PBA levels were creatinine-adjusted for their urinary dilution correction and then naturally log-transformed for their normality to evaluate the relation between 3-PBA level and PFT parameters. Since the present study had repeated measurements for both 3-PBA level and PFT parameters for each individual, we evaluated the cross-sectional effects of visit-to-visit variation in 3-PBA level on visit-to-visit variation in PFT parameters using a linear mixed effect model. We also evaluated the longitudinal effect of 3-PBA level on the trajectory of pulmonary function decline after adding the interaction between 3-PBA level and age in the model. In these models, we treated subject as a random effect and adjusted for age, sex (male or female), body mass index (BMI, kg/m^2), smoking status (current smoker, ex-smoker, or non-smoker), education (<high school graduate, high school graduate, or ≥ college graduate), visit episode (1st, 2nd, or 3rd), and creatinine-adjusted molar sum of phthalate metabolites which was reported to be associated with pulmonary function reduction (Kim et al., 2018b). Smoking status was defined depending on responses for questions 'Did you smoke 20 packs and more?' and 'Are you currently smoking?'. Subjects who answered 'Yes' to above two questions were defined as current smokers, subjects who smoked 20 packs and more, but were not currently smoking were defined as ex-smokers, and subjects who did not smoke 20 packs and more were defined as non-smokers. The creatinine-adjusted molar sum of phthalate metabolites (nmol/g-creatinine) was calculated by a summation of average molar concentration (nmol/L) of mono-(2-ethyl-5-hydrohexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and molar concentration (nmol/L) of mono-n-butyl phthalate (MnBP) divided by creatinine level (g/L), where molar concentration (nmol/L) for each metabolite was calculated using the following equation: [urinary metabolite level (ng/mL) × 1000] ÷ molecular weight of metabolite (294.34 g/mol for MEHHP, 292.331 g/mol for MEOHP, and 222.24 g/mol for MnBP). To confirm the robustness of the cross-sectional and longitudinal relations between 3-PBA level and pulmonary function, we conducted several sensitivity analyses. Because subjects with a history of abdomen or chest surgery before PFT could be hard to conduct PFT and thus it could affect the relations between 3-PBA level and PFT parameters, we evaluated the relations between 3-PBA level and PFT parameters after excluding cases with a history of abdomen or chest surgery within 1 year before PFT (n = 15). We also evaluated the relations between 3-PBA level and PFT parameters after removing the visit episode or creatinine-adjusted molar sum of phthalate metabolites covariate in the model, and after adding chronic obstructive pulmonary disease (COPD) status, asthma status, and alcohol consumption covariates in the model. Since in many cases non-linear relationship was found between environmental exposures and health outcomes, we also evaluated the non-linear relationships between 3-PBA level and PFT parameters using a generalized additive mixed model (GAMM) after adjustment for same covariates used in the linear mixed effect model. In addition, relationships among PFT parameters were evaluated using Pearson correlation.

For all statistical analyses, we used R version 3.4.3 (The Comprehensive R Archive Network: http://cran.r-project.org) and SAS version 9.4 Enterprise (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Basic characteristics of study participants at the first visit

Our study participants included 117 males and 442 females (Table 1). Mean age of our subjects was 74.1 years ranged from 60 to 94 years. Most participants were non-smokers (86.6%) and low-educated (72.8% for education < high school graduate) (Table 1). FEV_1 and FVC compared with predicted normal values were 100.2% and 101.4%, respectively. However, FEF_{25-75} was found to be very low (69.9%) compared to predicted normal values (Table 1). Because male participants showed lower baseline FEV_1 and FVC scores (below 100%) compared with normal values with higher proportion of current- and ex-smokers compared with female participants, baseline PFT parameters among non-smokers by sex were also examined (see online Supplementary Table S1). However, we did not find any significant difference of PFT parameters between total males and non-smoker males, although FEV_1, FVC, and FEF_{25-75} scores in non-smoker males were a little higher compared with those in current- and ex-smoker males.

3.2. 3-PBA levels and comparison with previous studies

Distribution of urinary 3-PBA levels was shown in Table 2. A

Table 1
Baseline characteristics of study participants.

Characteristic	Male	Female	Total
No. of participants (%)	117 (20.9)	442 (79.1)	559 (100)
Mean age, year (min-max)	74.8 (60–94)	73.9 (60–92)	74.1 (60–94)
Height (cm), mean ± SE	163.2 ± 0.6	151.2 ± 0.2	153.7 ± 0.3
Weight (Kg), mean ± SE	64.7 ± 0.8	56.4 ± 0.4	58.1 ± 0.4
BMI (kg/m^2), no. (%)			
≥25	42 (35.9)	188 (42.5)	230 (41.1)
23 ~ < 25	38 (32.5)	120 (27.2)	158 (28.3)
18.5 ~ < 23	36 (30.8)	129 (29.2)	165 (29.5)
<18.5	1 (0.8)	5 (1.1)	6 (1.1)
Smoking status, no. (%)			
Current smoker	11 (9.4)	4 (0.9)	15 (2.7)
Ex-smoker	52 (44.4)	8 (1.8)	60 (10.7)
Non-smoker	54 (46.2)	430 (97.3)	484 (86.6)
Education, no. (%)			
< High school graduate	54 (46.2)	353 (79.8)	407 (72.8)
High school graduate	31 (26.5)	56 (12.7)	87 (15.6)
≥ College graduate	32 (27.3)	33 (7.5)	65 (11.6)
PFT (%)[a], mean ± SE			
FEV_1	90.6 ± 1.8	102.8 ± 1.1	100.2 ± 1.0
FVC	92.8 ± 1.5	103.7 ± 1.0	101.4 ± 0.8
FEV_1/FVC	74.6 ± 0.9	80.8 ± 0.4	79.5 ± 0.4
FEF_{25-75}	68.6 ± 2.7	70.2 ± 1.2	69.9 ± 1.1

[a] PFT parameter scores (%) compared with normal values predicted based on PFT parameter scores of Korean representative samples with same age to ours.

Table 2
Distribution of 3-PBA levels (ng/mL) by sex and survey.

Survey	Subjects	n	LOD	n < LOD (%)	GM ± SE	Selected percentiles					
						10th	25th	50th	75th	90th	95th
All surveys	Males and females	1195		1 (0.1)	1.25 ± 0.04	0.28	0.56	1.30	2.76	5.22	7.97
	Males	235		1 (0.4)	1.22 ± 0.09	0.27	0.61	1.36	2.42	4.83	8.45
	Females	960		0 (0)	1.26 ± 0.05	0.28	0.56	1.27	2.88	5.27	7.86
1st	Males and females	399	0.014	0 (0)	1.06 ± 0.06	0.27	0.54	1.09	2.00	3.86	6.07
	Males	86	0.014	0 (0)	1.11 ± 0.13	0.26	0.70	1.20	1.86	3.67	5.95
	Females	313	0.014	0 (0)	1.04 ± 0.06	0.28	0.52	0.99	2.03	3.86	6.07
2nd	Males and females	399	0.013	1 (0.3)	2.32 ± 0.12	0.66	1.27	2.45	4.49	8.08	10.49
	Males	74	0.013	1 (1.4)	2.21 ± 0.32	0.58	1.39	2.31	4.71	8.45	11.90
	Females	325	0.013	0 (0)	2.34 ± 0.13	0.68	1.26	2.48	4.44	7.90	9.84
3rd	Males and females	397	0.010	0 (0)	0.81 ± 0.04	0.21	0.38	0.79	1.65	3.27	4.82
	Males	75	0.010	0 (0)	0.75 ± 0.09	0.21	0.39	0.86	1.47	2.61	3.26
	Females	322	0.010	0 (0)	0.82 ± 0.05	0.21	0.38	0.78	1.72	3.50	5.30

total of 1194 (99.9%) urine samples among 1195 urines were detected over LOD. Geometric mean (GM) and standard error (SE) of 3-PBA levels for all urine samples were 1.25 ng/mL and 0.04 ng/mL, respectively (Table 2). When we compared urinary 3-PBA levels between males and females, 3-PBA levels were similar between males and females (GM, 1.22 ng/mL for males and 1.26 ng/mL for females, p-value = 0.66) (Table 2). However, urinary 3-PBA level was different among surveys (GM, 1.06 ng/mL for the 1st survey, 2.32 ng/mL for the 2nd survey, and 0.81 ng/mL for the 3rd survey, p-value < 0.01) (Table 2). Creatinine-adjusted 3-PBA level was also compared by sex and survey. Creatinine-adjusted 3-PBA level was significantly different between males and females (GM, 1.12 ng/mg-creatinine for males and 1.60 ng/mg-creatinine for females, p-value < 0.01) and among surveys (GM, 1.38 ng/mg-creatinine for the 1st survey, 2.52 ng/mg-creatinine for the 2nd survey, and 0.95 ng/mg-creatinine for the 3rd survey, p-value < 0.01) (see online Supplementary Table S2).

The 3-PBA levels in our elderly were compared with those in non-occupationally exposed adults of previous studies (see online Supplementary Table S3). Korean adults including elderly had the highest 3-PBA level compared to adults in other countries regardless of adjustment for creatinine level (GM of 3-PBA levels: 1.25–1.47 ng/mL in Korean adults *vs.* 0.20–0.97 ng/mL in other countries), although the KoNEHS showed slightly higher 3-PBA level than the present study (see online Supplementary Table S3) (Park et al., 2016).

3.3. Variability of repeated 3-PBA levels and exceedance rate for limit values

In our evaluation for intra- and inter-individual variations of 3-PBA levels, ICC of repeated 3-PBA levels was generally high in the total population ($ICC_{total} = 0.76$). However, intra-individual variation of 3-PBA levels in the high-level exposure group was smaller than that in the low-level exposure group, with a big difference in ICC values between high- and low-level exposure groups ($ICC_{high} = 0.78$ *vs.* $ICC_{low} = 0$) (Fig. 1).

A cross-sectional biomonitoring may have limitation to identify proportion of general elderly at risk, particularly for exposure to pollutants with short metabolic half-life. Therefore, we evaluated the proportion of general elderly at risk using individual mean of repeated 3-PBA levels. When we derived exceedance rate for limit values such as reference guides or RV_{95}, the number of elderly people with mean 3-PBA level over reference guides or RV_{95} was 205 (36.7%) for reference guide I (1.7 ng/mL), 169 (30.2%) for RV_{95} (2 ng/mL), and 1 (0.2%) for reference guide II (87 ng/mL) (Fig. 1).

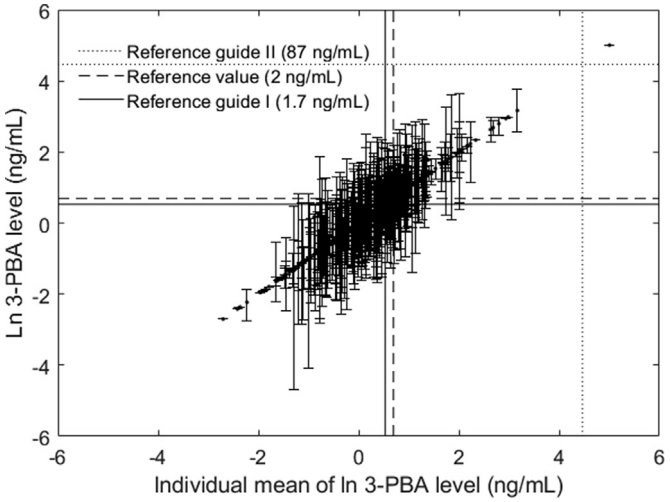

Fig. 1. Variability by individual mean 3-PBA level. Both axes were expressed in natural log scale of 3-PBA (ng/mL). Vertical lines represent individual ranges – minimum and maximum of individual 3-PBA levels.

3.4. Relations between 3-PBA level and PFT parameters

FEV_1, FVC, and FEF_{25-75} showed significant reductions by an increase of 3-PBA level, while FEV_1/FVC was not associated with 3-PBA level (FEV_1, $\beta = -1.48$, p-value < 0.01; FVC, $\beta = -1.14$, p-value < 0.01; FEV_1/FVC, $\beta = 0.08$, p-value = 0.69; and FEF_{25-75}, $\beta = -1.11$, p-value = 0.03) (Table 3). However, the significant effects of 3-PBA level on FEV_1, FVC, and FEF_{25-75} were found only for females (FEV_1, $\beta = -1.64$, p-value < 0.01; FVC, $\beta = -1.47$, p-value < 0.01; and FEF_{25-75}, $\beta = -1.06$, p-value = 0.07), but not for males (FEV_1, $\beta = -0.77$, p-value = 0.40; FVC, $\beta = 0.18$, p-value = 0.80; and FEF_{25-75}, $\beta = -1.25$, p-value = 0.31) (Table 3). In the evaluation for impact of 3-PBA level on the trajectory of pulmonary function decline, we did not find any significant interaction of 3-PBA level with age on FEV_1 and FVC declines in females with estimation impossible for FEF_{25-75}, even though we found the interaction between 3-PBA level and age on FVC of males (p-value = 0.03) (Table 3). Several sensitivity analyses were conducted to confirm the robustness of the cross-sectional and longitudinal relations between 3-PBA level and pulmonary function (see online Supplementary Tables S4–S7). However, the trend for the cross-sectional and longitudinal relations between 3-PBA level and pulmonary function was not changed after exclusion of cases with a history of abdomen or chest surgery, after deletion of visit episode

Table 3
Relations of naturally log-transformed 3-PBA level (ng/mg-creatinine) for cross-sectional effect and the interaction between 3-PBA level and age for longitudinal effect with PFT parameters (%).

		Cross-sectional effect of 3-PBA				Longitudinal effect of 3-PBA			
			95% CI				95% CI		
Outcome	No. of observations	β	Lower	Upper	*p*-Value	β	Lower	Upper	*p*-Value
Total participants									
FEV_1	1167	−1.48	−2.19	−0.78	<0.01	−0.10	−0.24	0.03	0.13
FVC	1167	−1.14	−1.82	−0.46	<0.01	−0.06	−0.19	0.07	0.35
FEV_1/FVC	1167	0.08	−0.31	0.46	0.69	−0.03	−0.10	0.04	0.38
FEF_{25-75}	1167	−1.11	−2.14	−0.09	0.03	−0.13	−0.32	0.06	0.19
Male participants									
FEV_1	228	−0.77	−2.59	1.05	0.40	−0.26	−0.60	0.07	0.12
FVC	228	0.18	−1.23	1.59	0.80	−0.30	−0.56	−0.04	0.03
FEV_1/FVC	228	−0.02	−0.89	0.86	0.97	0.02	−0.14	0.18	0.81
FEF_{25-75}	228	−1.25	−3.68	1.17	0.31	−0.02	−0.47	0.44	0.94
Female participants									
FEV_1	939	−1.64	−2.41	−0.87	<0.01	−0.08	−0.23	0.07	0.30
FVC	939	−1.47	−2.25	−0.69	<0.01	−0.01	−0.16	0.14	0.90
FEV_1/FVC	939	0.10	−0.33	0.52	0.66	−0.02	−0.10	0.06	0.57
FEF_{25-75}	939	−1.06	−2.20	0.08	0.07	–	–	–	–

Effect size and 95% confidence interval (CI) were obtained after adjustment for age, sex, body mass index, smoking status, education, visit episode, and creatinine-adjusted molar sum of phthalate metabolites. The relation of interaction between 3-PBA level and age with FEF_{25-75} in females could not be evaluated because of infinite likelihood.

or creatinine-adjusted molar sum of phthalate metabolites covariate in the model, and after addition of COPD status, asthma status, and alcohol consumption covariates in the model (see online Supplementary Tables S4–S7).

To evaluate the non-linear relationship between 3-PBA level and PFT parameters, we conducted penalized regression splines using GAMM (Fig. 2). A weak non-linear relationship was found for FVC and FEF_{25-75} in female participants, showing a weak U-shape change of FEF_{25-75} by 3-PBA increase with marginal significance and smaller decrease or no change of FVC by 3-PBA increase in the lower end of exposure with a potential threshold level above which a strong decrease of FVC by 3-PBA increase was found with statistical significance (FVC, p-value = 0.01; and FEF_{25-75}, p-value = 0.095) (Fig. 2). However, the relation between 3-PBA level and FEV_1 remained linear (p-value < 0.01 in females; and p-value < 0.01 in total population) (Fig. 2).

We further analyzed the correlations among PFT parameters to determine how much PFT parameters were related with each other and found that those parameters were significantly related with each other (Pearson correlation coefficients ranging from 0.38 to 0.87, all p-values < 0.01) except for non-significant relation between FVC and FEV_1/FVC (see online Supplementary Table S8).

4. Discussion

In the present study, we repeatedly measured urinary 3-PBA level and PFT parameters in Korean elderly and evaluated the cross-sectional and longitudinal relations between 3-PBA level and pulmonary function. We also evaluated intra- and inter-individual variations of 3-PBA levels and derived exceedance rates for reference guides I and II and RV_{95}.

For pyrethroids with short metabolic half-life < 12 h, it is difficult to identify proportion of population at risk using cross-sectional biomonitoring approach. It is even more challenging for this approach to estimate pyrethroid exposures at relatively low-doses in the general population. For these reasons, a longitudinal study repeatedly measuring 3-PBA levels in general population may be critical for assessing general exposure to pyrethroids. In the present study, we evaluated intra- and inter-individual variations of urinary 3-PBA levels and derived exceedance rates for reference guides I and II and RV_{95} using 3-PBA levels repeatedly measured in general elderly population with a longitudinal panel study design. When we evaluated the pattern of intra- and inter-individual variations in urinary 3-PBA level, intra-individual variation of urinary 3-PBA in the high-level exposure group was smaller than that in the low-level exposure group. In our study, we used original values for 3-PBA (ng/mL), not log-transformed values, for ICC evaluation, because we did not adjust for other variables in the model. However, when we calculated the ICC values using naturally log-transformed 3-PBA levels, naturally log-transformed creatinine adjusted 3-PBA levels, and creatinine adjusted 3-PBA levels, ICC value of urinary 3-PBA levels in high-level exposure group was still bigger than ICC value in low-level exposure group in all above 3-PBA data, even though all ICC values calculated using the three 3-PBA data got smaller than those for 3-PBA levels, not log-transformed values (data not shown here). Based on ICC values in total and high- and low-level exposure groups, urinary 3-PBA variation in total population was mainly attributed to intra-individual variations in low-level exposure group with exposure difference between high- and low-level exposure groups. Moreover, considering our high exceedance rates for reference guide I and RV_{95} with our high 3-PBA levels compared to other studies, Korean elderly were highly exposed to pyrethroids. Furthermore, significantly higher 3-PBA level in females of our study was consistent with those of the Korean National Environmental Health Survey (KoNEHS) conducted in 2012 (Korean Statistical Information Service, 2013). The KoNEHS, a national study for Koreans, revealed a higher creatinine-adjusted 3-PBA level in females than in males (GM of 1.52 ng/mg-creatinine with an inter-quartile range (IQR) of 0.80–2.83 in males; and GM of 2.23 ng/mg-creatinine with an IQR of 1.16–4.28 in females) (Korean Statistical Information Service, 2013). Higher 3-PBA level in females of our study and KoNEHS indicates that female adults have higher level of exposure to pyrethroids than male adults in non-occupationally exposed Korean population. In addition, the difference in 3-PBA level among surveys of our study might be due to temporal exposure to insecticides in daily use such as mosquito repellant and contaminated food intake, because we recruited general elderly people without occupational exposure to pyrethroids.

Although occupational and general exposure to pyrethroids in asthmatic patients or in children have been reported to be related to pulmonary function reduction (Damalas and Eleftherohorinos,

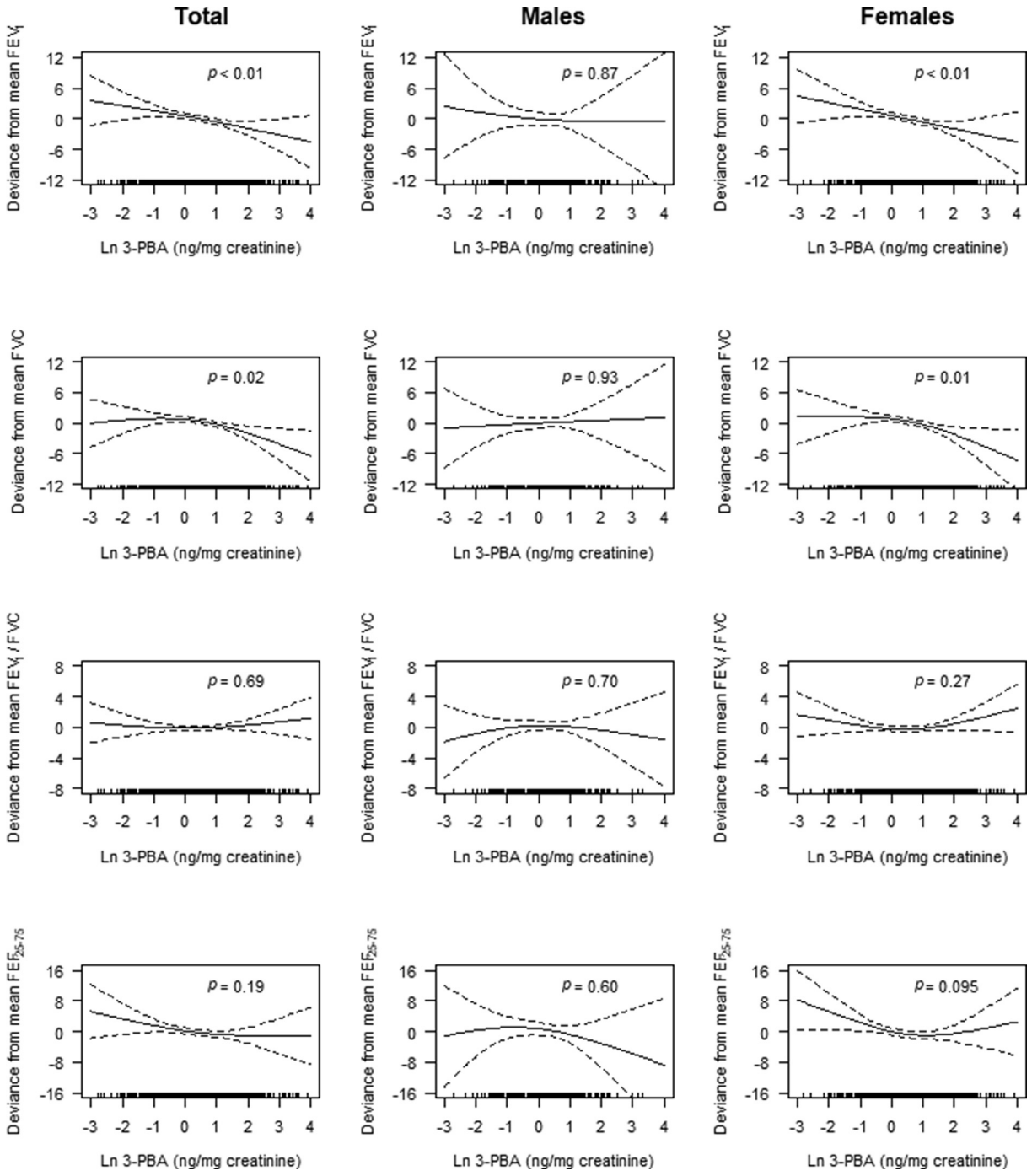

Fig. 2. A penalized regression spline of 3-PBA (ng/mg-creatinine) on PFT parameters (%) obtained using GAMM.

2011; Newton and Breslin, 1983; Schneider et al., 2004), these data is difficult to be generalized to elderly who have declining pulmonary function. Therefore, we evaluated the relationships between 3-PBA levels and PFT parameter scores in elderly population without any occupational exposure. Because baseline PFT parameters were lower in males compared with females and 3-PBA level was generally higher in females compared with males, we evaluated the relationships between 3-PBA levels and PFT parameter scores by sex. In the evaluation by sex, cross-sectionally significant inverse associations of 3-PBA level with FEV_1, FVC, and FEF_{25-75} were found only in female elderly, with regression coefficient for FEF_{25-75} showing similar magnitude and same negative direction in males (even though it was not significant) compared with that in females. The association trend still remained after several sensitivity analyses. However, a weak U-shape change of FEF_{25-75} by 3-PBA increase found in our female participants should be further examined because this shape (apparent decrease of FEF_{25-75} by 3-PBA increase in lower end of exposure, but smaller increase or no change of FEF_{25-75} by 3-PBA increase in upper end of exposure) was not general considering for prevalent relationships between potentially harmful exposures and pulmonary function with a tendency for opposite shape of the relationship in male participants although confidence interval (CI) was too broad in both females and males. Because males had higher proportion of current- and ex-

smokers which could related with lower PFT parameter scores compared with females, we also examined PFT parameter scores among non-smoking males and females, but did not find any significant difference of PFT parameter scores after excluding current- and ex-smokers. Furthermore, we did not find any other trend of associations between 3-PBA level and PFT parameter scores among non-smoking males (data not shown here). In addition, we did not find any longitudinal effect of 3-PBA level on the trajectory of FEV_1, FVC, and FEF_{25-75} declines in females, indicating that longer follow-up period could be needed to pick up accelerated decline considering for pulmonary function slowly declining by aging. Moreover, the relations between 3-PBA level and PFT parameter scores should be further evaluated for elderly living in rural area because our study targeted only elderly people living an urban area and the results obtained from our urban study could be different with those from a rural study.

Only one epidemiological study targeting Canadian general population has reported direct relation between 3-PBA level and PFT parameters (Ye et al., 2016). This representative national study in Canada (Canadian Health Measures Survey, CHMS) showed that one unit increase in naturally log-transformed sum of molar concentration (nmol/g-creatinine) of five pyrethroid metabolites (3-PBA, 4-fluoro-3-PBA, cis-cypermethric acid, trans-cypermethric acid, and 1s-cis-decamethrinic acid) reduced 17.4 mL of FEV_1 in 6–11 years of children and 37.1 mL of FVC in 12–19 years of adolescents while it enlarged 0.3% of FEV_1/FVC in 20–79 years of adults after adjustment for age, sex, height, weight, and ethnicity (Ye et al., 2016). However, we could not directly compare our results with CHMS results because exposure (molar sum of 5 pyrethroid metabolites), PFT parameters (not considering aging), and covariates controlled in the Canadian study were different from ours. In addition, the CHMS was cross-sectionally conducted without considering intra-individual variation.

Ray and Fry (2006) presented that pyrethroids interference with voltage-gated sodium/chloride channels may induce repeated or prolonged polarization or depolarization of neuron cells. The overstimulation of airway neurons by these neurotoxic insecticides could result in exaggerated contractile responses in airway smooth muscles of human and Guinea pig models (Fryer and Jacoby, 1998; Minette and Barnes, 1988; Souhrada and Souhrada, 1989; Souhrada et al., 1988). These previous results account for a potential of pyrethroids reducing pulmonary function observed in the present study as well as in the CHMS. Furthermore, two recent *in vitro* studies have shown that pyrethroids and their metabolite, 3-PBA, possess endocrine disrupting properties with antagonistic or agonistic activity observed in estrogen-, androgen-, or thyroid hormone-receptor mediated reporter gene assays of human and rat models (Du et al., 2010; Sun et al., 2014). Different effect of 3-PBA on PFT parameters by sex in our study might be due to sex difference in endocrine disrupting properties of pyrethroids or 3-PBA. In addition, based on the facts that normal or increased FEV_1/FVC with decreased FVC may indicate restrictive pattern while reduced FEF_{25-75} may mean small airway impairment (Escárcega et al., 2016; Godfrey and Jankowich, 2016; Marseglia et al., 2007; Sperandio et al., 2016), the little higher FEV_1/FVC with FVC and FEF_{25-75} reductions of our study supports that pyrethroid exposures might be related to restrictive and small airway pulmonary function impairments of elderly population. Furthermore, baseline FEF_{25-75} of our elderly population was relatively very low compared with predicted normal value. Because normal value we compared our data to means the values obtained from elderly with healthy pulmonary function, it could not represent pulmonary function obtained from general elderly who have declining pulmonary function.

Our study had several strengths. We used longitudinal panel design with repeated measurements of covariates which may vary over time. Since this study design could make each subject to be used as a control of one's own, we could evaluate short-term effect of pyrethroid exposures on pulmonary function as a rapidly changing covariate in elderly people. Moreover, our study is the first study that evaluates relationships between urinary 3-PBA level and PFT parameters in elderly population. But, our study also had some limitations. Our study population was not representative because we targeted only elderly people living in Seoul as an urban area, although our 3-PBA level was comparable with those measured in adults including the elderly in the KoNEHS. Also, we did not control other environmental pollutants which could have potential for associations with pulmonary function, although we adjusted for phthalate exposure levels in our models because of inverse associations between phthalate metabolites and pulmonary function found in our previous study (Kim et al., 2018b). Therefore, other exposure factors affecting pulmonary function should be further explored in the future.

5. Conclusion

In summary, community-level exposure to pyrethroids was associated with pulmonary function reduction in urban elderly population, particularly among the female elderly, although the longitudinal effect of 3-PBA level on the trajectory of pulmonary function decline was not found. Based on the fact that the Korean elderly were generally exposed to high levels of pyrethroids, further investigations on exposure sources of pyrethroids in Korean elderly and on metabolism and action mechanisms of pyrethroids and 3-PBA are needed. Moreover, considering that our target was the elderly who have declining pulmonary function, urinary 3-PBA levels should be tightly regulated for protecting respiratory health of elderly population.

Conflicts of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.078.

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