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Title: Personal exposure to fine particulate matter, lung function and serum club cell secretory protein (Clara)

Running head: PM_{2.5}, lung function and CC16

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24 **Abbreviation list**

25 PM_{2.5} = particulate matters with aerodynamic diameters less than 2.5 µm; SD =
26 standard deviation; IQR = interquartile range; LBAB, lung blood-air barrier; LME = linear
27 mixed effect; BMI = body mass index; CV = coefficients of variation; CI = confidence
28 interval; FEV₁ = forced expiratory volume in 1 sec; FVC = forced vital capacity; PEF =
29 peak expiratory flow; FEF₂₅₋₇₅ = forced expiratory flow at 25-75% of the pulmonary
30 volume; CC16 = Clara cell protein; LME = linear mixed-effect.

ABSTRACT (232 words)

Background: The underlying mechanisms about the association between ambient fine particulate matter (PM_{2.5}) and lung function were unclear yet. Few epidemiological studies have evaluated the potential mediating effects of serum club cell secretory protein (Clara) (CC16), a biomarker of pulmonary epithelium integrity.

Methods: We enrolled 36 healthy, nonsmoking college students for a panel study in Shanghai, China from December 17, 2014 to July 11, 2015. We measured personal and real-time exposure to PM_{2.5} for 72-hour preceding each of four rounds of health examinations, including lung function test and serum CC16 measurement. We used linear mixed-effect models to examine the health effects over various lag times. Furthermore, we analyzed the mediating effect of CC16 on this association.

Results: Average PM_{2.5} exposure ranged from 40 to 52 µg/m³ across different lag periods. PM_{2.5} exposure was negatively associated with lung function and positively associated with serum CC16 concentration. For instance, an interquartile range increase in 0-6 h average exposure to PM_{2.5} was significantly associated with a 1.11% [95% confidence interval (CI): 0.50, 1.74] reduction in 1-sec forced expiratory volume and a 4.14% (95% CI: 0.86, 7.41) increase in serum CC16. Increased serum CC16 contributed 3.9%~40.0% of the association between PM_{2.5} and impaired lung function.

Conclusions: Acute exposure to PM_{2.5} was associated with decreased lung function and increased circulating CC16, which may be an important mediator in the effects on lung function injury induced by PM_{2.5}.

- 53 **Keywords:** Personal exposure; fine particulate matter; lung function; Clara cell protein;
- 54 mediation effect

Text (4499 words)

1. Introduction

Lung function is an objective measure of respiratory health. Airborne particulate matter with an aerodynamic diameter of 2.5 μm or less ($\text{PM}_{2.5}$) can travel deeply into the respiratory tract and reach the lung. A substantial body of environmental epidemiological evidence has shown that ambient exposure to $\text{PM}_{2.5}$, over the long-term (years) and short-term (hours to days), is associated with a range of adverse pulmonary events (Fuentes et al., 2015; Gauderman et al., 2004; Kariisa et al., 2015; Lee et al., 2007; Moshhammer et al., 2006; Mu et al., 2014; Ni et al., 2016; Rice et al., 2016; Zhao et al., 2015). However, there are two major limitations on these studies. First, the subjects of most these studies are restricted to vulnerable populations, such as children (Moshhammer et al., 2006; Rice et al., 2016), adolescents (Fuentes et al., 2015; Gauderman et al., 2004), elderly people (Lee et al., 2007), patients with pulmonary diseases, (i.e., chronic obstructive pulmonary disease) (Kariisa et al., 2015; Ni et al., 2016) or population with high occupational exposure (i.e., traffic policemen) (Zhao et al., 2015). However, studies focusing on young adults who are the major labor force are extremely limited. Second, most of these studies relied on outdoor fixed-site monitoring stations to assess the exposure levels of each participant, leading to considerable measurement errors. There is a dearth of studies exploring the effects of $\text{PM}_{2.5}$ on lung function based on personal exposure monitoring (Trenga et al., 2006).

The underlying mechanism regarding impaired lung function by $\text{PM}_{2.5}$ is unclear (Paulin and Hansel 2016). Altered integrity and/or increased permeability of the lung

blood-air barrier (LBAB) were associated with pulmonary function injury (Brauner et al., 2009). Short-term exposure to air contaminants, i.e., ambient ozone and particles, can result in lung epithelium hyper-permeability (Broeckaert et al., 1999; Provost et al., 2014), which might lead to acute lung dysfunction. In considering biomarkers for evaluating the permeability and/or the cellular integrity of the pulmonary epithelium, club cell secretory protein (CC16), formerly known as Clara cell protein (Winckelmann and Noack., 2010), is a highly plausible candidate (Broeckaert et al., 2000a; Broeckaert and Bernard 2000). CC16 is a small protein secreted mainly by non-ciliated bronchiolar Clara cells in the respiratory epithelium of bronchioles (Lakind et al., 2007). Although Clara cells are unique to the lung, this specific protein is measurable in circulation by diffusing into plasma across the LBAB (Lakind et al., 2007; Hermans and Bernard 1999).

We here designed a panel study of healthy nonsmokers carried out in Shanghai to evaluate the short-term effect of personal PM_{2.5} exposure on lung function and serum CC16. We also examined whether the LBAB integrity, here assessed by serum CC16, plays a role in the decreased lung function caused by PM_{2.5}.

2. Material and Methods

2.1 Study population and design

Data were collected from 36 healthy college students from Fudan University and East China University of Science and Technology, Shanghai, China. The specific information about the study design has been reported previously (Wang et al., 2016).

101 This longitudinal panel study involved repeated measurements at 4 time
102 points (Wang et al., 2016). Each measurement entailed 72 hours of continuous
103 monitoring. To expand the temporal variations in exposure to $PM_{2.5}$, we
104 scheduled follow-ups in various days of the week in each round, with about two-
105 week interval between each round. Fieldwork was carried out between
106 December 17, 2014 and July 11, 2015.

107 All participants were nonsmokers and all of them provided written informed
108 consent before enrollment. The Institutional Review Board of the School of Public
109 Health, Fudan University approved the study protocol (IRB#2014-TYSQ-09-1).

110 ***2.2 Individual $PM_{2.5}$ exposure monitoring***

111 We used MicroPEM monitors (v3.2 MicroPEM, RTI, USA) to record the real-
112 time $PM_{2.5}$ concentrations and HOBO data loggers (Onset Computer Corporation,
113 Pocasset, Massachusetts) to simultaneously monitor personal exposure
114 temperature and relative humidity. Exposure data were recorded once every half
115 hour.

116 The participants were required to come to Fudan University on Day 1
117 between 10:00 am and 11:00 am to wear a vest with MicroPEM and HOBO and
118 start their monitoring devices. On Day 4, between 10:00 am and 11:00 am, the
119 subjects were required to turn in their monitoring devices. All participants were
120 asked to wear the vests continuously throughout the 72 hours, except when
121 sleeping, taking a shower or engaging in strenuous sports activities, during which
122 times, vest was to be hung at the level of the breathing zone near the site of the
123 activity.

2.3 Pulmonary function measurement

On Day 4 when the subjects turned in their vests, they were required to carry out a series of physiological and biological measurements that included spirometry and phlebotomy. Spirometry was performed using a Fleisch pneumotachograph type spirometer (KoKo™, nSpire Longmont Co, USA). Before the health examination, the spirometer was calibrated by a Calibration Pump (SN: 95290967, Calibration Syringe 3.0 L, CareFusion, Germany)

The same trained lung function technician performed the testing to reduce the surveyor error. Lung function was measured three to eight times at intervals of 3 minutes, and the error of every two measurements of each parameter was required to be within 5% before being recorded as a valid data point. Only the spirograms meeting international standards were accepted at the time of testing (Renzetti 1979).

The lung function parameters examined in this study were the conventional spirometric indices used to detect impairment: FEV₁, which measures the maximum volume that can be exhaled within 1 sec; FVC, which measures the total volume exhaled after a maximum inspiration; FEV₁/FVC; peak expiratory flow (PEF), which tests the airflow through the bronchi and thus the degree of obstruction in the airways; forced expiratory flow at 25%-75% of the pulmonary volume (FEF₂₅₋₇₅), which is also used to reflect the obstruction in small airways.

2.4 CC16 detection

We collected blood samples from participants between 10:00 and 11:00 am on day 4 of each round. Blood samples were incubated at 37 °C for 10 min,

centrifuged at 4000 rpm for 10 min, transferred to 1 ml microtubes (Nunc, Naperville, IL, USA), and transferred to -80 °C for storage. All of these procedures were completed within 1 hour after blood collection to minimize *in vitro* changes in CC16 levels.

We measured CC16 using enzyme-linked immunosorbent assays according to the manufacturer's instructions (R&D, Human Uteroglobin Quantikine ELISA Kit, USA).

2.5 Statistical analysis

Because there were repeated measures for all participants, we used linear mixed effect (LME) models to estimate the effect of PM_{2.5} on lung function and CC16. This linear mixed model, by including a random effect for subject, allows each subject to serve as his/her control over time and also accounts for correlations among multiple repeated measurements collected per person (Verbeke and Molenberghs 2009). Lung function and CC16 level were natural log-transformed to better approximate a normal distribution among residuals.

Personal exposure levels (PM_{2.5}, temperature and relative humidity) were averaged over predetermined time intervals, including disjoint periods preceding the time of the health examination, that is, single lag intervals of 0-6 hour (h), 7-24 h, 25-48 h and 49-72 h. The integrated time-specific PM_{2.5} was incorporated in the model as a fixed-effect predictor. Other potential fixed-effect covariates included age, sex, body mass index (BMI), temperature, relative humidity, day of the week and experimental plate for CC16. We did not adjust for time trends for

possible seasonal effects because all four visits for each subject were completed within two months (Wang et al., 2016).

We conducted mediation analyses to evaluate and quantify the contribution of circulating CC16 levels in the process of lung function injury induced by PM_{2.5}. This approach decomposed the total observed effect of PM_{2.5} exposure on lung function into a direct observed effect and a causal mediation effect that goes through CC16 (Peng et al., 2016).

All analyses were conducted with R software (version 2.15.3, R development Core Team) using the “lme4” package for LEM models and “mediation” package for mediation analysis. Statistical tests were two-sided, with $P \leq 0.05$ considered statistically significant.

3. Results

3.1 Data description

A total of 14 males and 22 females, with a mean average age of 24 years, were enrolled in this study. BMI varied from 16.2 to 31.3 kg/m², with an average of 21.2 kg/m². As one participant missed the last visit, we obtained data for 143 repetitions of the subjects.

Table 1 provides the descriptive statistics of personal exposure to PM_{2.5}, temperature and relative humidity. The average exposure to PM_{2.5} ranged from 40 to 52 µg/m³ across the lag periods. There was substantial intra- and inter-individual variation in PM_{2.5} exposure levels, with the coefficients of variation (CV) ranging from 0.68 to 1.15 (data not shown). The personal data on temperature

and humidity also differed within and between the subjects, with the CV ranging from 0.19 to 0.24 for temperature and from 0.18 to 0.23 for humidity, respectively (data not shown). Table 2 shows the descriptive statistics for lung function and CC16. Serum CC16 levels ranged from 12.59 ng/mL to 51.55 ng/mL, with a mean of 23.90 ng/mL.

(Please insert Table 1 here)

(Please insert Table 2 here)

3.2 Individual $PM_{2.5}$ -lung function relationship

There were negative associations between $PM_{2.5}$ levels and lung function of FEV_1 , FVC, FEV_1/FVC and PEF, usually occurring within the first 24 h after exposure (Figure 1). For example, at lag 0-6 h, an interquartile range (IQR 25.9 $\mu g/m^3$) increase in personal exposure to $PM_{2.5}$ was associated with decreases of 1.11% (95%CI: 0.50%, 1.71%) in FEV_1 , 1.00% (95%CI: 0.29%, 1.73%) in FVC, 1.33% (95%CI: 0.65%, 2.01%) in FEV_1/FVC and 1.02% (95%CI: 0.12%, 1.92%) in PEF. However, there were no significant short-term associations between $PM_{2.5}$ levels and FEF_{25-75} at all lag times.

(Please insert Figure 1 here)

3.3 Individual $PM_{2.5}$ -CC16 relationship

$PM_{2.5}$ was positively associated with CC16 within the first 24 h (Figure 2). For example, an IQR increase in personal exposure to $PM_{2.5}$ was associated with increases of 4.14% (95%CI: 0.86%, 7.41%) and 4.10% (95%CI: 0.93%, 7.27%) at lags of 0-6 h and 7-24 h, respectively.

(Please insert Figure 2 here)

3.4 Mediation analysis

We estimated the mediated effect of serum CC16 using lags of 0-6 h and 7-24 h, respectively. As shown in Table 3, we found a significant mediation in the process of “PM_{2.5}→CC16→FEV₁/FVC”, that was, the pulmonary epithelial hyper-permeability mediated 34.4% of the association between PM_{2.5} exposure and FEV₁/FVC at lag 7-24 h (P=0.05). The mediations of other parameters of lung function were positive but not statistically significant (Proportion=3.9%~40.0%, P=0.17~0.86).

(Please insert Table 3 here)

4. Discussion

In the present panel study of young, healthy adults with personal PM_{2.5} monitoring, we found that acute exposure to PM_{2.5} was associated with decrements in lung function parameters. The effect of PM_{2.5} on FVC and PEF occurred within the first 6 h and the effects on FEV₁ and FEV₁/FVC remained for the first 24 h. We did not find a significant association between PM_{2.5} and FEF₂₅₋₇₅.

To the best of our knowledge, this study is the first panel study to report the effects of individual measurements of PM_{2.5} exposure on lung function among a young healthy adult population. The estimated health effects of PM_{2.5} exposure on lung function in our analysis were comparable to previous studies. For example, in a one-day hike in Boston, USA, Korrick et al. found that FVC declined for 0.4% (95%CI: 0.2%, 0.6%) across the IQR (9 µg/m³) for PM_{2.5} among adult hikers (Korrick et al., 1998). Schindler et al. also found that an IQR

(4.5 $\mu\text{g}/\text{m}^3$) increase in $\text{PM}_{2.5}$ was associated with 0.43% (95%CI: 0.09%, 0.78%) and 0.35% (95%CI: 0.04%, 0.66%) decreases in FEV_1 and FVC, respectively, in a Canadian Health Measures Survey (Korrick et al., 1998). Moshhammer et al. studied a panel of schoolchildren in Austria and reported a 0.41% ($P=0.027$) decrease in PEF for an increase of 10 $\mu\text{g}/\text{m}^3$ in $\text{PM}_{2.5}$ after an 8 hours exposure (Moshhammer et al., 2006). The only previous panel study with healthy, non-smoking college students carried out in Wuhan University, China, showed that a 10 $\mu\text{g}/\text{m}^3$ increase of outdoor $\text{PM}_{2.5}$ was associated with 1.54 L/min (95% CI: 0.04, 3.03) decreases in evening PEF on the day after exposure (Zhang et al., 2015). However, another panel study conducted in Beijing, China, did not find a significant association between $\text{PM}_{2.5}$ and FEV_1 or FVC among truck drivers (Baccarelli et al., 2014).

Our study found that serum CC16, a biomarker of LBAB integrity and permeability (Broeckaert et al., 2000a; Hermans et al., 1999), is positively associated with acute exposure to $\text{PM}_{2.5}$ among young healthy adults, occurring 6 h after exposure and lasting for 24 h, which was consistent with previous epidemiological studies (Bernard et al., 1997; Broeckaert et al., 2000a; Burgess et al., 2001). For instance, in Belgium, Bernard et al. conducted an intervention experiment among firefighter and found that the serum CC16 levels elevated by 320% on average after 0.5~1 h exposure to the fire, compared to normal levels.²⁸ Provost et al. found an increase of 0.21 $\mu\text{g}/\text{L}$ (95%CI: 0.10, 0.32) in circulating CC16 with each 5 $\mu\text{g}/\text{m}^3$ elevation in PM_{10} after 1 day of exposure and this upward trend persisted for 1 week among Belgian adolescents (Provost et al.,

2014). Another two studies found urine CC16 also increased after exposure to PM_{2.5}, with a time lag beginning slightly later (24 h) than that observed in blood (Jacquemin et al., 2009; Timonen et al., 2004). However, in a traffic-related health study, Zuurbier et al. did not find the associations between PM_{2.5} exposure and changes in serum CC16 after 2 h commute during the morning rush hour (Zuurbier et al., 2011), which was in line with another traffic-related study assessing the association between PM_{2.5} and pulmonary function, including serum CC16 (Zhao et al., 2015).

It has been suggested that changes in serum CC16 levels may stem from three basic mechanisms: i) decreased secretion in the respiratory tract; ii) increased leakage through the LBAB; or iii) a reduction of renal clearance (Broeckaert et al., 2000b). However, acute increased CC16 levels in circulation induced by air pollutants, including smoking (Bernard et al., 1997) and ozone (Broeckaert et al., 2000a), could be regarded as a peripheral indicator for reflecting the leakage of the LBAB despite a decreased production of CC16 from damaged Clara cells (Brauner et al., 2009; Hermans et al., 1999). Although increased circulating CC16 across the LBAB could be used as a surrogate for indicating epithelium impairment (Broeckaert et al., 2000a; Broeckaert and Bernard 2000), the relationship between PM_{2.5}, the integrity of lung epithelium and early lung function abnormalities has not been well characterized. In this study, we found a significant mediating effect (proportion=34.4%, P=0.05) of CC16 serum levels in the association between PM_{2.5} and FEV₁/FVC at lag 7-24 h. That is to say, 34.4% of the PM_{2.5}-induced lung function decline might be

explained by the hyper-permeability of the LBAB. Our findings suggest a potential involvement of lung epithelium integrity in the acute effects of PM_{2.5} on impaired lung function.

The current study has several strengths. First, compared to previous panel studies, which relied on outdoor fixed air quality monitors, our study based on real-time personal sampling can estimate exposure levels more accurately. Second, the LME models allowed each subject to serve as his/her own control and accounted for correlations among repeated measurements within the subject (Verbeke and Molenberghs 2009). Third, because only healthy, young and non-smoking college students were enrolled, this study avoided bias related to unhealthy lifestyle, chronic diseases, etc.

Nevertheless, our study was also subject to several limitations. First, the relatively small sample size might have limited our capability to detect significant effects. Second, we could not make a causal conclusion about CC16 and lung function because they were simultaneously measured. Third, we did not monitor the personal exposure to gaseous air pollutants (i.e., ozone and nitric oxide), which might have confounded our findings (Blomberg et al., 2003).

5. Conclusion

This study on healthy young adults suggested that acute exposure to PM_{2.5} was significantly associated with lung function impairment and increase in circulating CC16. Our findings also highlighted a possible involvement of the LBAB in the effects of acute injury on lung function by PM_{2.5}. Future work is

needed to elucidate the role of LBAB in the health effects of PM_{2.5} on lung function in a more controlled experimental setting or in animal models.

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Table 1. The descriptive statistics on personal PM_{2.5}, temperature and relative humidity at different averaging periods preceding the health outcomes measurements.

Variables	Lag	Mean	SD	Min	Median	Max	IQR
PM _{2.5} (µg/m ³)	0-6 h	39.69	45.89	1.68	26.81	226.40	25.88
	7-24 h	42.48	41.00	7.83	28.74	191.50	32.93
	0-24 h	42.00	41.18	7.03	28.39	200.30	26.78
	25-48 h	52.28	43.28	8.92	36.42	197.20	48.10
	49-72 h	48.16	32.53	6.10	39.78	249.10	39.21
Temperature (°C)	0-6 h	21.85	5.16	9.88	24.36	28.57	7.79
	7-24 h	22.64	4.50	9.97	24.69	28.54	6.25
	0-24 h	22.44	4.64	10.24	24.60	28.54	6.67
	25-48 h	22.46	4.43	10.76	24.03	29.01	6.06
	49-72 h	22.38	4.33	10.43	23.29	30.85	6.78
Relative humidity (%)	0-6 h	60.36	10.83	36.04	60.78	84.99	15.37
	7-24 h	53.98	11.77	27.54	52.20	74.78	18.93
	0-24 h	55.57	11.25	31.04	54.97	75.96	17.48
	25-48 h	55.88	11.11	25.16	56.74	78.54	17.70
	49-72 h	55.33	12.63	22.71	54.84	81.56	19.64

Abbreviations: PM_{2.5}, particulate matter with an aerodynamic diameter ≤ 2.5 µm; SD, standard deviation; IQR, interquartile range.

443 **Table 2.** Summary statistics of lung function and CC16.

Variables	Mean	SD	Min	Median	Max	IQR
Lung function						
FEV ₁ (L 1 st sec)	3.22	0.51	2.20	3.26	4.33	0.78
FVC (L)	3.76	0.67	2.23	3.68	5.51	0.95
FEV ₁ /FVC	86.37	6.97	66.73	86.43	99.63	9.72
PEF (L/s)	7.10	1.65	4.20	6.62	11.1	2.42
FEF ₂₅₋₇₅ (L/s)	3.47	0.84	1.93	3.41	5.90	1.17
CC16 (ng/mL)	23.90	6.83	12.59	22.71	51.55	7.63

444 Abbreviations: FEV₁, forced expiratory volume in 1 sec; FVC, forced vital capacity;
 445 PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expiratory flow at 25%-75% of the
 446 pulmonary volume; CC16, Clara cell protein.

447 **Table 3.** The proportion of PM_{2.5} on lung function that mediated by CC16.

Lung function	Lag 0-6 h	Lag 7-24 h
	Proportion (%) (P)	Proportion (%) (P)
FEV ₁ (L 1 st sec)	4.91 (0.63)	3.86 (0.54)
FVC (L)	29.7 (0.33)	5.04 (0.69)
FEV ₁ /FVC	40.02 (0.17)	34.4 (0.05)
PEF (L)	9.5 (0.54)	18.4 (0.38)
FEF ₂₅₋₇₅ (L/s)	9.3 (0.47)	14.7 (0.86)

448 Abbreviations: FEV1, forced expiratory volume in 1 sec; FVC, forced vital capacity;
 449 PEF, peak expiratory flow; FEF25-75, forced expiratory flow at 25%-75% of the
 450 pulmonary volume.

FIGURE LEGENDS:

FIGURE 1. Changes in a) FEV₁, b) FVC, c) FEV₁/FVC, d) PEF and e) FEF₂₅₋₇₅ associated with an interquartile range increase of personal PM_{2.5} levels using different lag structures. The X-axis refers to different time windows; the Y-axis refers to the corresponding changes (mean and 95% confidence intervals).

Abbreviation: FEV₁, forced expiratory volume in 1 sec; FVC, forced vital capacity; PEF, peak expiratory flow; FEF₂₅₋₇₅, forced expiratory flow at 25%-75% of the pulmonary volume.

FIGURE 2. Changes in CC16 associated with an interquartile range increase of personal PM_{2.5} levels using different lag structures. The X-axis refers to different time window; the Y-axis refers to the corresponding changes (mean and 95% confidence intervals).

Abbreviation: CC16, Clara cell protein.

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