ORIGINAL RESEARCH

Cross-sectional and longitudinal associations between urinary zinc and lung function among urban adults in China

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ABSTRACT

Background Exposure to zinc was suggested to be associated with pulmonary damage, but whether zinc exposure affects lung function remains unclear.

Objectives To quantify the association between urinary zinc and lung function and explore the potential mechanisms.

Methods Urinary zinc and lung function were measured in 3917 adults from the Wuhan-Zhuhai cohort and were repeated after 3 years of follow-up. Indicators of systemic inflammation (C reactive protein), lung epithelium integrity (club cell secretory protein-16) and oxidative damage (8-hydroxy-2′-deoxyguanosine and 8-isoprostane) were measured at baseline. Linear mixed models were used to estimate the exposure–response relationship between urinary zinc and lung function. Mediation analyses were conducted to assess mediating roles of inflammation and oxidative damage in above relationships.

Results Each 1-unit increase in log-transformed urinary zinc values was associated with a 35.72 mL decrease in forced vital capacity (FVC) and a 24.89 mL decrease in forced expiratory volume in 1 s (FEV1) in the baseline analyses. In the follow-up analyses, there was a negative association between urinary zinc and FVC among participants with persistent high urinary zinc levels, with an estimated change of −93.31 mL (95% CI −178.47 to −8.14). Furthermore, urinary zinc was positively associated with restrictive ventilatory impairment. The mediation analyses suggested that C reactive protein mediated 8.62% and 8.71% of the associations of urinary zinc with FVC and FEV1, respectively.

Conclusion Urinary zinc was negatively associated with lung function, and the systemic inflammation may be one of the underlying mechanisms.

Key messages

What is the key question?

► What is the association between zinc exposure and lung function decline in the urban population?

What is the bottom line?

► We found that urinary zinc was both cross-sectionally and longitudinally associated with lung function reduction in an urban Chinese adult population, and plasma C reaction protein mediated this association.

Why read on?

► The findings add to understanding of the association between zinc exposure and lung function decline and the underlying mechanisms.

INTRODUCTION

Zinc is a metal element commonly found in the earth's crust. Anthropogenic activities including mining and metallurgy involving zinc, coal combustion and vehicle emissions can introduce large amounts of zinc into the environment.[1] China as the biggest zinc producer, the biggest coal user and the fastest growing vehicle market worldwide, has added to a huge environmental zinc burden.[2–4] As a result, zinc has become one of the most abundant metal pollutants in the air, soil and water in China.[5–7]

Zinc is necessary for humans within a certain concentration range; however, excess zinc can be toxic. Current publications suggest that excess zinc is associated with neurological injury, oral injury, renal dysfunction and liver dysfunction.[8 9] Moreover, excess zinc can cause lung damage through injuring the epithelial airway barrier, promoting airway remodelling, inducing mitochondrial dysfunction, and increasing inflammatory cytokines.[10–12] Several epidemiological studies have investigated the association between zinc and lung health. Occupational exposure to zinc oxide or zinc chloride has been linked to hypersensitivity pneumonitis and progressive diffuse lung injury among smelting workers.[13 14] The PIAMA Birth Cohort Study has reported an increased asthma incidence associated with particulate matter (PM_{10})-bound zinc among schoolchildren.[15] Serum zinc has been reported to be associated with wheezing in a case–control study.[16] Few studies have focused on the association between fine $PM_{2.5}$-bound zinc and lung function, and the results are inconsistent. A cross-over study found a negative association between $PM_{2.5}$-bound zinc and lung function among 59 subjects.[17] However, a repeated-measure study found no statistically significant association between $PM_{2.5}$-bound zinc and lung function among 60 truck drivers and 60 office workers.[18] Lung function is an important parameter in the

evaluation and diagnosis of airway dysfunction, particularly asthma and chronic obstructive pulmonary disease, but there has been limited research on the relationship between zinc exposure and lung function as well as the underlying mechanisms.

Internal zinc levels may vary dramatically among individuals because zinc can enter the body in various ways including air inhalation, food consumption and drinking water. Blood, hair and urinary zinc are reliable biomarkers of internal zinc load in humans and reflect zinc exposure from inhalation, diet and drinking.[19] Urinary zinc is widely used in epidemiological studies of large sample size, because sample collection is relatively simple and non-invasive.[20] Since the biological half-life of zinc is about 1 year, repeated measurements can better reflect zinc load in the body.

Therefore, we developed the present study with 3917 participants from the Wuhan-Zhuhai cohort in China. Urinary zinc and lung function were determined at baseline and repeated at follow-up after 3 years. We further determined C reactive protein (CRP) as a biomarker for systemic inflammation, club cell secretory protein-16 (CC16) for lung epithelium integrity and 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostane for oxidative damage. Our objective was to investigate the cross-sectional and longitudinal associations between urinary zinc and lung function. We further investigated the mediating roles of inflammation and oxidative damage in the association between urinary zinc and lung function.

METHODS

Study population

The participants were from the Wuhan-Zhuhai cohort, which has been described previously.[21] A stratified, cluster sampling approach was used to select two urban communities in each city. A total of 4812 residents aged 18–80 years, and who had been living in the sampling communities for ≥5 years were recruited into the cohort in 2012. Participants were followed up after 3 years. Questionnaire investigations and physical examinations including lung function measurements were conducted at baseline and during the follow-up. Early-morning urine samples and fasting venous blood samples were collected for all participants. After excluding individuals with missing information on lung function test (n=106), or with missing information on urinary zinc or urinary creatinine measurement (n=790), data from 3917 participants were analysed in the cross-sectional study. We excluded individuals without a lung function test or a urinary zinc measurement or covariate measurements during the 3-year follow-up, and those lost to follow-up; consequently, 1946 participants were included in longitudinal analyses. The selection process for participants is shown in online supplementary figure 1. The comparison of baseline basic characteristics between the total study population and the participants with 3-year follow-up is shown in online supplementary table 1.

All participants in this study signed a written informed consent.

Lung function test

Lung function tests were conducted using electronic spirometers (Chestgraph HI-101, CHEST Ltd., Tokyo, Japan). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were obtained and recorded according to the American Thoracic Society recommendations.[22] Specific quality controls were performed as follows: (1) daily calibration checks of spirometers before testing, and immediate calibration check after replacing any detector; (2) no air leakage, obstructed mouthpiece, early termination or cut-off of expiration, or cough during the first second of exhalation; (3) extrapolated volume for each test was limited to 5% of FVC or 0.15 L; (4) a minimum exhalation time of 6 s and an expiratory plateau in the volume–time curve and (5) reproducible tests with three acceptable flow–volume curves. Restrictive ventilatory impairment was defined as FVC <80% predicted and FEV1/FVC ≥70%, and obstructive ventilatory impairment was defined as FEV1/FVC <70%.

Biological sample measurement

Morning spot urine samples were collected, divided into clean conical polyethylene tubes, and stored at −20°C until analysis. Urinary zinc levels were measured using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700X series, Agilent Technologies, Santa Clara, California, USA) as previously described.[23] Briefly, 3 mL of each urine sample was mixed with 15 µL of 65% HNO_3 and stored at 4°C overnight. The preprocessed samples were digested and injected into the ICP-MS for determination. We used standard reference material and spiked pooled urine as quality controls. The limit of quantification (LOQ) for urinary zinc was 0.0003 µg/L, and all samples in the present study exceeded the LOQ. Valid urinary zinc concentrations were calibrated by urinary creatinine (Cr) levels and presented as μg/mmol Cr.

Urinary 8-OHdG, urinary 8-isoprostane, plasma CRP and plasma CC16 levels were measured.

$PM_{2.5}$-bound zinc measurement

A subgroup of 240 participants (120 in Wuhan and 120 in Zhuhai) aged 40–60 years were selected from the cohort for evaluation of $PM_{2.5}$ levels. Personal 24 hours $PM_{2.5}$ were sampled and $PM_{2.5}$-bound zinc levels were measured among 178 participants. The 178 participants were of younger age and included less females, less smokers, less drinkers and similar urinary zinc levels, compared with the total study population (data not shown).

Covariate assessment

Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, with participants wearing light indoor clothing. Information regarding demographics, heart disease, physical exercise, cigarette smoking amount, passive smoking amount, alcohol drinking amount, food frequency, traffic exposure time, cooking meals at home and occupational dust exposure was collected from the questionnaires.

More details regarding the measurements of urinary 8-OHdG, urinary 8-isoprostane, plasma CRP, plasma CC16, and $PM_{2.5}$-bound zinc, the evaluation of dietary zinc intake, and the calculations of covariates are shown in the online supplementary file.

Statistical analysis

Concentrations of urinary zinc, urinary 8-OHdG, urinary 8-isoprostane, plasma CRP and plasma CC16 were log-transformed because of skewed distributions.

Linear mixed models with community as a random effect were used to estimate the changes (95% CIs) of FVC and FEV1 associated with continuous or categorical (compared with the first quartile) urinary zinc, to estimate the associations between urinary zinc and mediators, and to estimate the associations between mediators and lung function.

To further evaluate the associations of urinary zinc with FVC and FEV1 at different physical status, stratified analyses by age (<55/≥55 years), gender (male/female) and smoking status (ever/never) in separated linear mixed models were conducted.

The modification effect of each stratification variable in the association between urinary zinc and FVC or FEV1 was estimated by including a product of urinary zinc and the stratification variable in the linear mixed model in the total population.

Considering the possible changes in individual urinary zinc levels over time, we estimated the lung function changes over 3 years associated with different urinary zinc levels by dividing participants into four groups: persistent low (urinary zinc in the first quartile at baseline and follow-up); persistent moderate (urinary zinc in the second and third quartiles at baseline and follow-up); persistent high (urinary zinc in the fourth quartile at baseline and follow-up); and inconsistent zinc group, in which urinary zinc was in different quartiles between baseline and follow-up. We evaluated changes in lung function after 3 years in the persistent moderate and persistent high group, compared with the persistent low group.

Logistic regression models and COX regression models were used to calculate the OR and the HR of obstructive or restrictive ventilatory impairment associated with urinary zinc, respectively.

Mediation analyses were performed to assess the roles of CRP, CC16, 8-OHdG and 8-isoprostane in the associations between urinary zinc and FVC, as well as FEV1. Controlled direct effect, natural direct effect, natural indirect effect (NIE), total effect and proportion mediated were computed. The specific processes of mediation analyses are shown in the online supplementary file.

All models were adjusted for potential risk factors for lung function alteration or zinc exposure including age (years), gender (male/female), height (cm), weight (kg), heart disease (yes/no), physical activity (yes/no), smoking amount (pack-years), passive smoking amount (hours/week-years), alcohol consumption (times/week-years), food frequency (times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day). Two-sided $p<0.05$ was regarded as statistically significant. All statistical analyses were performed using SAS V.9.4 software (SAS) and R V.3.5.1 (R Core Team).

RESULTS

Basic characteristics

The basic characteristics of the 3917 participants (2656 women, 67.8%) with a mean age of 52.5 years are summarised in table 1. The urinary zinc concentrations ranged within 5.58–75.53 µg/mmol Cr (5–95th percentile), with a median concentration of 26.37 µg/mmol Cr. Smoking amount increased across increasing quartiles of urinary zinc ($p<0.05$), and males (14.5±20.8 pack-years) showed a higher smoking amount than females (0.3±2.6 pack-years) with $p<0.001$. The FVC and FEV1 decreased with increasing urinary zinc ($p<0.05$). No statistically significant differences in occupational exposure, passive smoking or alcohol drinking were detected across quartiles of urinary zinc ($p>0.05$).

Cross-sectional association between urinary zinc and lung function

After adjusting for related covariates, each 1-unit increase in log-transformed urinary zinc values was associated with a 35.72 mL decrease in FVC and a 24.89 mL decrease in FEV1. The sensitive analyses by quartiles of urinary zinc indicated that, compared with participants in the first quartile of urinary zinc, those in the fourth quartile showed a 65.72 and 40.23 mL decrease in FVC and FEV1, respectively (table 2).

The cross-sectional association between urinary zinc and restrictive ventilatory impairment was statistically significant, with covariate-adjusted OR (95% CI) of 1.20 (1.07 to 1.34), while the adjusted OR for obstructive ventilatory impairment (0.75, 95% CI 0.50 to 1.13) was statistically non-significant (online supplementary table 2).

Smoking status modified the association between urinary zinc and FEV1 decline (p for modification was 0.030). There was a negative linear association between urinary zinc and FEV1 among non-smokers (–29.20 mL, 95% CI –49.63 to –8.78), but this was not statistically significant among smokers (–23.64 mL, –76.72 to 29.43). Age and gender showed no statistically significant modification effects (table 3).

Longitudinal association between urinary zinc and lung function alteration

Compared with participants with persistent low urinary zinc, FVC in the persistent high group statistically significantly decreased (−93.31 mL, 95% CI −178.47 to −8.14); while FEV1 in the persistent high group decreased (−35.40 mL, 95% CI −99.17 to 28.38) without statistical significance (figure 1).

The longitudinal associations between urinary zinc and restrictive or obstructive ventilatory impairment were statistically non-significant, with the covariate-adjusted HR (95% CI) among participants with persistent high urinary zinc of 1.96 (0.61 to 6.28) and 0.86 (0.24 to 3.05), respectively, compared with the persistent low group (online supplementary table 2).

Mediation effects of oxidative damage and inflammatory response on the association between urinary zinc and lung function

Mediations for the associations of urinary zinc with FVC and FEV1 by plasma CRP were observed, with the NIEs (95% CI) for FVC and FEV1 of −2.80 (−5.49 to −0.10) and −2.04 (−4.05 to −0.04), respectively (table 4). The CRP mediated 8.62% and 8.71% of the associations of urinary zinc with FVC and FEV1, respectively. Urinary 8-OHdG and 8-isoprostane levels increased with elevated urinary zinc (table 1), but there were no statistically significant mediation effects of 8-OHdG or 8-isoprostane on the association between urinary zinc and lung function (table 4).

We further assessed the exposure–mediator and mediator–outcome relationships by CRP. Each 1-unit increase in log-transformed urinary zinc values was associated with a 0.09 pg/mL increase in CRP. Compared with participants in the first quartile of urinary zinc, those in the fourth quartile showed a statistically significant increase in CRP (0.20 pg/mL, 95% CI 0.04 to 0.36). Each 1-unit increase in log-transformed CRP values was associated with a 29.15 and 21.26 mL decline in FVC and FEV1, respectively. Compared with participants in the first quartile of CRP, those in the second, third and fourth quartiles showed 56.57, 93.54, and 134.26 mL decreases in FVC, and 57.85, 64.72, and 113.59 mL decreases in FEV1, respectively (figure 2).

Associations between urinary zinc and potential zinc sources

There were positive associations of urinary zinc with dietary zinc (0.2521, 95% CI 0.0160 to 0.4882) and cigarette smoking amount (0.0035, 95% CI 0.0014 to 0.0057) among smokers; while there was a positive association of urinary zinc with traffic exposure time (0.0003, 95% CI 0.0001 to 0.0007) among non-smokers (table 5). The partial Pearson correlation analysis showed a positive correlation between $PM_{2.5}$-bound zinc and urinary zinc in the panel group ($r=0.21$, $p=0.039$), after adjusting for covariates (online supplementary figure 2).

Table 1 Basic characteristic of the study population at baseline by quartiles of urinary zinc levels

Variables99	Total (n=3917)	Quartiles of urinary zinc levels (μg/mmol Cr) Q1 (n=979) ≤17.64	Q2 (n=979) 17.65 to 26.37	Q3 (n=979) 26.38 to 38.62	Q4 (n=980) ≥38.63	P value
Female gender (n, %)	2656 (67.8)	741 (75.7)	669 (68.3)	623 (63.6)	623 (63.6)	<0.001*
Age (years, mean±SD)	52.5±12.9	49.8±13.1	51.8±12.7	53.7±12.4	54.8±12.7	<0.001*
Height (cm, mean±SD)	159.2±7.7	158.7±7.4	159.3±7.4	159.3±7.8	159.3±8.2	0.267
Weight (kg, mean±SD)	60.9±10.5	60.4±10.6	60.2±10.3	61.3±10.3	61.6±10.7	0.002*
Smoking amount (pack-years, mean±SD)	4.9±13.7	3.0±9.9	4.7±13.2	5.5±14.7	6.2±16.0	<0.001*
Male†	14.5±20.8	13.3±19.4	13.7±19.6	15.3±21.7	15.7±22.4	0.398
Female†	0.3±2.6	0.1±1.4	0.3±2.8	0.3±3.2	0.3±2.5	0.429
Passive smoking amount (hours/week-years, mean±SD)	50.1±112.0	50.0±106.8	46.6±107.7	50.6±113.0	53.2±120.1	0.216
Alcohol drinking amount (times/week-years, mean±SD)	21.2±71.2	16.2±63.5	23.3±74.2	22.4±72.5	22.8±73.9	0.058
Physical exercise (n, %)	1873 (47.8)	420 (42.9)	465 (47.5)	482 (49.2)	506 (51.6)	0.001*
Food frequency (times/month, mean±SD)						
Grains	86.2±14.1	86.1±14.4	86.7±13.8	85.9±13.9	86.1±14.4	0.456
Fruits and vegetables	57.9±18.3	59.1±17.6	58.4±17.9	56.9±18.3	57.1±19.3	0.030*
Meats	34.6±24.2	33.8±23.7	34.1±24.5	34.9±24.7	35.5±23.9	0.370
Fishes	19.7±20.7	19.9±23.1	20.6±20.2	19.8±20.4	18.5±18.7	0.237
Milk and eggs	19.1±19.2	21.0±25.9	19.8±16.1	18.3±15.8	17.2±17.1	<0.001*
Heart disease (n, %)	912 (23.3)	187 (19.1)	214 (21.9)	242 (24.7)	269 (27.4)	<0.001*
Occupational dust exposure (n, %)	1053 (26.9)	236 (24.1)	268 (27.4)	282 (28.8)	267 (27.2)	0.087
Cooking meals at home (n, %)	2895 (73.9)	713 (72.8)	726 (74.2)	743 (75.9)	713 (72.8)	0.810
Traffic exposure time (minutes/day, mean±SD)	63.3±83.4	59.2±88.5	63.5±83.1	65.4±79.0	65.2±82.5	<0.001*
Log-transformed plasma CRP (pg/mL, mean±SD)	13.27±1.66	13.15±1.60	13.21±1.69	13.35±1.64	13.48±1.68	<0.001*
Log-transformed plasma CC16 (pg/mL, mean±SD)	9.49±1.21	9.55±1.04	9.55±1.08	9.44±1.26	9.38±1.42	0.757
Log-transformed urinary 8-isoprostane (ng/mmol Cr, mean±SD)	4.16±0.84	3.99±0.89	4.11±0.79	4.19±0.82	4.36±0.82	<0.001*
Log-transformed urinary 8-OHdG (umol/mol Cr, mean±SD)	3.94±1.36	3.78±1.35	3.81±1.33	3.94±1.41	4.22±1.32	<0.001*
FVC (mL, mean±SD)	2510.0±669.8	2559.7±662.9	2546.3±656.5	2510.2±696.0	2423.9±663.0	<0.001*
FEV1 (mL, mean±SD)	2193.5±577.0	2232.8±560.1	2221.7±564.0	2181.6±593.8	2137.9±590.0	0.001*

*P<0.05.
†Participants were divided into four groups according to the quartiles of urinary zinc levels among males and females, respectively.
CC16, Clara cell secretory 16-kD protein; CRP, C reactive protein; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

DISCUSSION

In the present study, we identified negative associations between urinary zinc and FVC and FEV1 in an urban adult population. Based on cross-sectional analyses, negative exposure–response relationships of urinary zinc with FVC and FEV1 were detected. In the longitudinal analyses, there was a statistically significant reduction in FVC among participants with persistent high zinc load compared with those of persistent low urinary zinc. Additionally, plasma CRP mediated the associations between urinary zinc and FVC and FEV1.

Few studies have investigated the association between zinc exposure and lung function and the results are inconsistent. Similar to our results, Cakmak *et al* reported that $PM_{2.5}$-bound zinc was associated with diffusion capacity reduction in 59 healthy subjects. The mean $PM_{2.5}$-bound zinc concentrations in their study ranged within 11.1–34.3 ng/m^3,[17] lower than the $PM_{2.5}$-bound zinc in the present study (mean 345.1 ng/m^3). Differing from our results, Pizent *et al* demonstrated increased FVC% and FEV1% associated with elevated serum zinc among 60 white-collar office men but not among 166 white-collar office women.[24] Baccarelli *et al* found no statistically significant association between $PM_{2.5}$-bound zinc (mean 150 ng/m^3) and lung function among 120 workers.[18] The inconsistencies among these previous studies may due to the different evaluation method of zinc exposure, varied zinc exposure levels and the different number of research participants.

The association between zinc exposure and lung function is complex because zinc can be both nutritious and toxic, depending on exposure amount. Urinary zinc levels in our study (median 312 μg/L, 5–95th percentile of 85–1040 μg/L) were similar to that among the general population in Hainan Island (371, 126–981 μg/L),[25] but higher than that among Canadians

Table 2 Estimated changes of FVC (mL) and FEV1 (mL) by urinary zinc levels at baseline

	Continuous urinary zinc (μg/mmol Cr)		Categorical urinary zinc (μg/mmol Cr)				
	Estimated change (95% CI)	P value	Q1 (≤17.64)	Q2 (17.65 to 26.37)	Q3 (26.38 to 38.62)	Q4 (≥38.63)	P trend
FVC							
Model 1	−34.89 (−67.36 to −2.43)*	0.035*	Ref	8.69 (−49.74 to 67.14)	2.81 (−56.15 to 61.78)	−69.20 (−128.55 to −9.85)*	0.026*
Model 2	−36.51 (−59.49 to −13.53)*	0.002*	Ref	−12.02 (−52.86 to 28.81)	−16.64 (−58.16 to 24.87)	−72.09 (−114.05 to −30.13)*	0.001*
Model 3	−35.72 (−59.29 to −12.15)*	0.004*	Ref	−13.58 (−55.04 to 27.87)	−18.71 (−61.03 to 23.62)	−65.72 (−108.74 to −22.70)*	0.005*
FEV1							
Model 1	−36.06 (−64.34 to −7.78)*	0.013*	Ref	−0.16 (−51.12 to 50.80)	−25.25 (−76.65 to 26.14)	−61.83 (−113.55 to −10.10)*	0.012*
Model 2	−27.31 (−46.30 to −8.32)*	0.005*	Ref	−9.91 (−43.74 to 23.92)	−28.17 (−62.51 to 6.18)	−46.60 (−81.29 to −11.90)*	0.005*
Model 3	−24.89 (−44.38 to −5.41)*	0.019*	Ref	−11.59 (−45.92 to 22.75)	−28.6 (−63.71 to 6.38)	−40.23 (−75.83 to −4.63)*	0.023*

Model 1: crude model.
Model 2: adjusted for age (continuous, years), gender (male/female), height (continuous, cm), and weight (continuous, kg).
Model 3: adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day).
All models included community (Wuhan/Zhuhai) as a random effect.
*P<0.05.
FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

generally (median 274 μg/L).[20] In the present study, elevated urinary zinc was both cross-sectionally and longitudinally associated with lung function reduction, and with a greater decline in FVC than FEV1. Furthermore, we found that urinary zinc was associated with restrictive ventilatory impairment rather than obstructive ventilatory impairment in cross-sectional analysis, but the underlying mechanisms of such association require more research.

The mechanisms underlying the lung function reduction associated with zinc exposure are unclear. Published literature provides several factors that could contribute to the association. First, systemic inflammatory response might partially explain the association. In vitro, zinc enhances the expression of tumour necrosis factor-α (TNF-α) and interleukin-8 (IL-8) in human airway epithelial cells.[12 26] In vivo, zinc exposure from oropharyngeal aspiration increases the levels of inflammatory cytokines such as IL-4, IL-5, IL-6 and IL-13 in bronchoalveolar lavage fluid in mice.[27] Wu *et al* demonstrated that each quartile increase of $PM_{2.5}$-zinc was associated with a 22% increase in plasma TNF-α levels among adults.[28] Blanc *et al* reported that occupational zinc exposure increased the levels of inflammatory cytokines including IL-6, IL-8 and TNF-α in bronchoalveolar lavage.[29] In our study, as a key biomarker of systemic inflammation, plasma CRP increased with elevated urinary zinc levels and played a mediating role in associations between urinary zinc and lung function decline. Systemic or specific inflammatory response could induce excessive collagen production and deposition, increase goblet cell differentiation and mucus secretion, and promote lung remodelling,[30 31] finally leading to lung function decline. Second, oxidative damage might be another mechanism involved. In vitro, excess zinc exposure has been reported to deplete total reduced glutathione, and increase heme oxygenase-1 mRNA expression in pulmonary cells.[11 12] In vivo, excess dietary zinc can increase 8-OHdG and superoxide levels

Table 3 Estimated changes of lung function (ML) associated with urinary zinc levels at baseline stratified by age, gender and smoking status

	FVC		FEV1	
Stratified variables	Estimated change (95% CI)	P for modification	Estimated change (95% CI)	P for modification
Age		0.498		0.645
<55 years	−37.05 (−71.47 to −2.63)*		−27.72 (−55.52 to 0.07)	
≥55 years	−37.84 (−69.66 to −6.02)*		−26.38 (−53.34 to 0.58)	
Gender		0.541		0.708
Female	−39.18 (−63.65 to −14.71)*		−35.98 (−56.16 to −15.79)*	
Male	−54.74 (−108.92 to −0.56)*		−20.40 (−65.09 to 24.29)	
Smoking status		0.469		0.030*
Non-smoker	−39.89 (−64.65 to −15.14)*		−29.20 (−49.63 to −8.78)*	
Smoker	−34.26 (−98.38 to 29.87)		−23.64 (−76.72 to 29.43)	

Models were adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day), and included community (Wuhan/Zhuhai) as a random effect in models.
*P<0.05.
FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

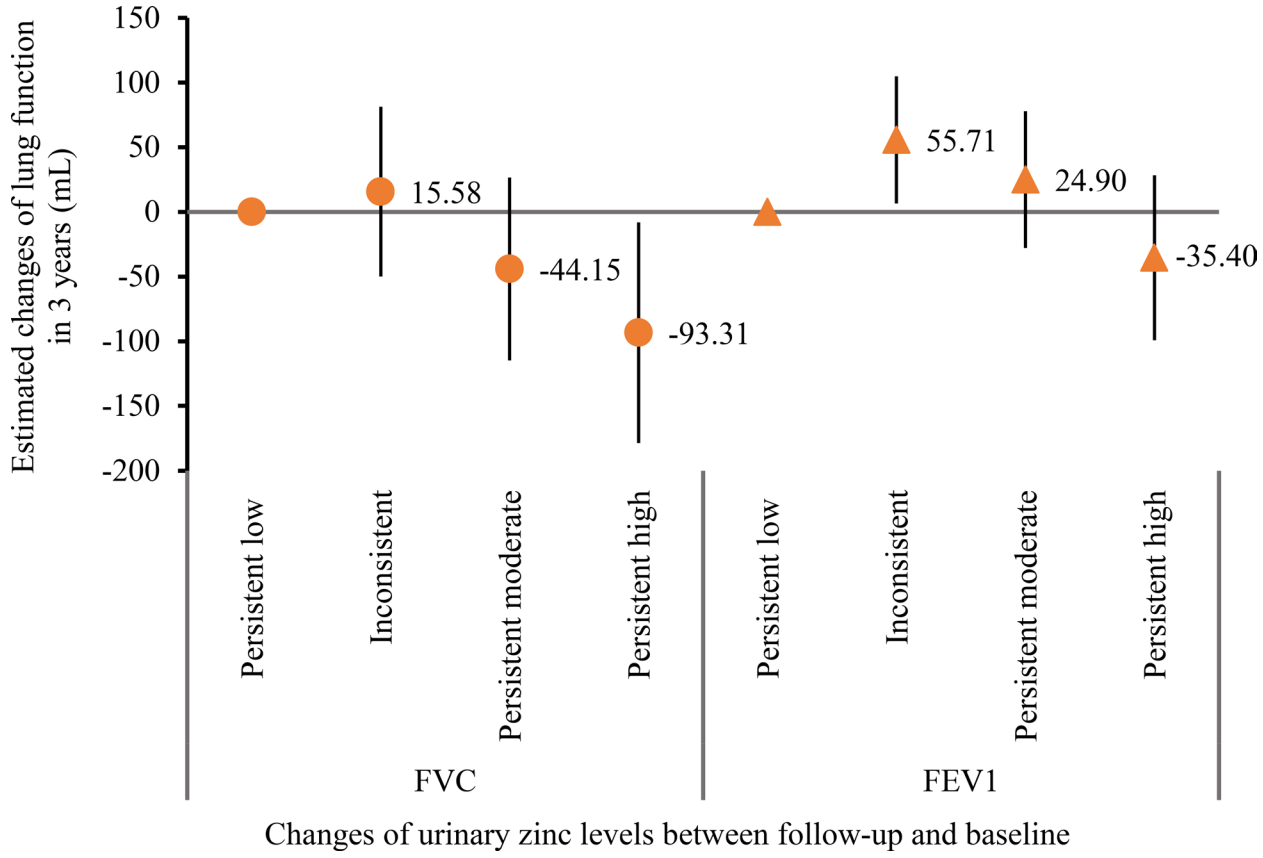

Figure 1 Longitudinal associations between urinary zinc levels and changes of lung function in 3 years (mL), according to changes in urinary zinc levels between baseline and follow-up. Persistent low: urinary zinc baseline in Q1 (≤18.05 µg/mmol Cr) and follow-up in Q1 (≤22.42 µg/mmol Cr). Persistent moderate: urinary zinc baseline in Q2–Q3 (18.06–39.32 µg/mmol Cr) and follow-up in Q2–Q3 (22.43–51.51 µg/mmol Cr). Persistent high: urinary zinc baseline in Q4 (≥39.33 µg/mmol Cr) and follow-up in Q4 (≥51.52 µg/mmol Cr). Inconsistent: urinary zinc was in different quartiles between baseline and follow-up. Models were adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day), and included community (Wuhan/Zhuhai) as a random effect in models. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

in experimental animals.[32] In the present study, we observed increased 8-isoprostane and 8-OHdG levels across increasing quartiles of urinary zinc. However, we did not find a mediating role of urinary 8-isoprostane or 8-OHdG in the association between urinary zinc and lung function, and another oxidative damage pathway may have been involved. Oxidative damage has been reported to directly damage lung tissue, initiate alveolar epithelial cell death, induce respiratory muscle dysfunction and trigger inflammation.[33–35]

Urinary zinc reflects zinc exposure from multiple sources including traffic emission, fuel smoke, cigarette smoke and dietary intake. Traffic emission has been reported as the main source of zinc in atmospheric PM_{10} in London and Barcelona,[36] and the dominant source of zinc in street dust in 53 cities in China.[37] Consistent with previous studies, we found a positive association between urinary zinc and traffic exposure time among non-smokers, and a positive correlation between urinary zinc and personal 24 hours $PM_{2.5}$-bound zinc in the panel group. Cigarette smoke is another important source of zinc exposure. Zinc is the fourth most prominent metal in tobacco, and 13%–21% of tobacco zinc can transfer to cigarette smoke.[38] Smokers have shown higher serum zinc concentrations than non-smokers.[39] In line with previous findings, we detected a positive association between urinary zinc and smoking amount among smokers. Interestingly, a greater decline in lung function was associated with urinary zinc among non-smokers, and smoking status modified the relationship. Other toxicants in tobacco may partially mask the effect of zinc on lung function among smokers.

Our study has several strengths. First, it was conducted among a large urban population. Second, individual exposure amount of zinc was assessed by urinary zinc concentration which could reflect zinc exposure from different routes. Third, repeated lung function measurements were conducted with a 3-year interval, which allowed us to estimate the long-term effect of zinc on lung function. Fourth, we identified a mediating role of CRP in the association between urinary zinc and lung function, which might contribute to understanding the potential mechanisms.

However, this study also has several limitations. First, single spot urinary samples were used to determine the zinc concentrations. Nevertheless, the interclass correlation coefficient of urinary zinc levels between baseline and follow-up was 0.46 (95% CI 0.40 to 0.51), suggesting that urinary zinc levels in the total population were relatively stable when lifestyle and diet changed little. We further tested the association between urinary zinc and lung function among participants with stable urinary zinc levels, and the results remained unaltered. Second, we failed to collect detailed data on dietary patterns and did not measure food zinc levels, but we estimated food zinc

Table 4 Total and direct effect of urinary zinc on lung function decline and assessment of mediation effect

Mediator	No of values	Additive interaction effect		Mediation effect			
		Estimated changes (95% CI)	P value	Controlled direct effect Natural direct effect	Natural indirect effect	Total effect	Proportion mediated (%)
FVC (mL)							
Plasma CRP	3151	−0.23 (−15.30 to 14.85)	0.976	−29.70 (−55.19 to −4.22)*	−2.80 (−5.49 to −0.10)*	−32.50 (−58.10 to −6.91)*	8.62*
Plasma CC16	3151	−12.70 (−32.45 to 7.06)	0.208	−31.96 (−57.54 to −6.38)*	−0.54 (−1.57 to 0.48)	−32.50 (−58.10 to −6.91)*	–
Urinary 8-isoprostane	3503	2.77 (−21.13 to 29.67)	0.820	−33.64 (−58.75 to −8.54)*	2.14 (−5.45 to 9.73)	−31.50 (−55.43 to −7.57)*	–
Urinary 8-OHdG	3645	1.02 (−15.95 to 17.98)	0.907	−34.35 (−58.40 to −10.30)*	−1.18 (−4.59 to 2.22)	−35.54 (−59.35 to −11.72)*	–
FEV1 (mL)							
Plasma CRP	3151	−2.57 (−15.02 to 9.88)	0.685	−21.37 (−42.39 to −0.34)*	−2.04 (−4.05 to −0.04)*	−23.41 (−44.50 to −2.32)*	8.71*
Plasma CC16	3151	−5.70 (−22.00 to 10.60)	0.493	−22.95 (−44.03 to −1.87)*	−0.47 (−1.34 to 0.41)	−23.42 (−44.50 to −2.33)*	–
Urinary 8-isoprostane	3503	5.21 (−14.60 to 25.03)	0.606	−21.89 (−42.67 to −1.11)*	1.32 (−4.96 to 7.60)	−20.57 (−40.38 to −0.76)*	–
Urinary 8-OHdG	3645	−6.64 (−20.71 to 7.43)	0.355	−24.07 (−44.00 to −4.13)*	0.62 (−2.20 to 3.44)	−23.44 (−43.18 to −3.71)*	–

Models were adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), traffic exposure time (minutes/day), and community (Wuhan/Zhuhai).
*P<0.05.
CC16, Clara cell secretory 16-kD protein; CRP, C reactive protein; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

levels using food frequency combined with food consumption ratio and food zinc content in China. Third, covariates including heart disease, physical activity, smoking, drinking, food frequency, occupational dust exposure, cooking meals at home and traffic exposure time were self-reported with possible recall bias. However, face-to-face investigations were conducted following uniform criteria by trained investigators, and each completed questionnaire was logically checked. Fourth, categorical analyses showed that the negative associations between urinary zinc and lung function were limited to participants in the fourth quartile of urinary zinc and participants with persistent high urinary zinc. The potential reason

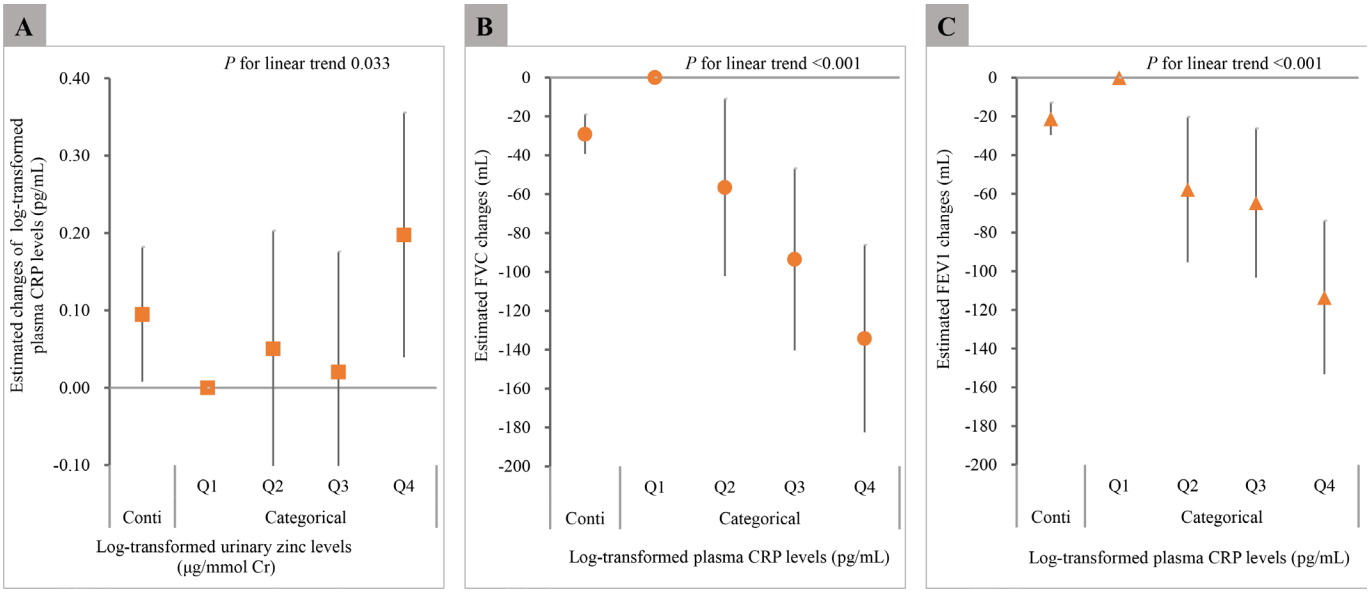

Figure 2 Linear association between log-transformed urinary zinc levels and plasma CRP (A), and associations between plasma CRP levels and FVC (B) and FEV1 (C). Models were adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day) and included community (Wuhan/Zhuhai) as a random effect in models. CRP, C reactive protein; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Table 5 Associations of urinary zinc levels with potential sources of zinc

	Total		Smoker		Non-smoker	
Variables	Estimated changes (95% CI)	P value	Estimated changes (95% CI)	P value	Estimated changes (95% CI)	P value
Dietary source	–0.0104 (–0.1136 to 0.0927)	0.843	0.2521 (0.0160 to 0.4882)*	0.036*	–0.0617 (–0.1770 to 0.0536)	0.294
Smoking amount (pack-years)	0.0030 (0.0012 to 0.0047)*	0.001*	0.0035 (0.0014 to 0.0057)*	0.001*	NA	NA
Passive smoking amount (hours/week-years)	0.0002 (0.0000 to 0.0003)	0.096	0.0003 (0.0000 to 0.0006)	0.079	0.0001 (–0.0002 to 0.0003)	0.515
Alcohol drinking amount (times/week-years)	0.0000 (–0.0004 to 0.0003)	0.800	0.0000 (–0.0004 to 0.0003)	0.830	0.0000 (–0.0005 to 0.0005)	0.981
Cooking meals at home	0.0108 (–0.0390 to 0.0606)	0.670	0.0319 (–0.0512 to 0.1149)	0.452	0.0020 (–0.0592 to 0.0632)	0.949
Traffic exposure time (minutes/day)	0.0002 (–0.0001 to 0.0004)	0.134	–0.0001 (–0.0004 to 0.0003)	0.783	0.0003 (0.0001 to 0.0007)*	0.045*

Models were adjusted for age (continuous, years), gender (male/female), height (continuous, cm), weight (continuous, kg), heart disease (yes/no), physical activity (yes/no), smoking amount (continuous, pack-years), passive smoking amount (continuous, hours/week-years), alcohol consumption (continuous, times/week-years), food frequency (continuous, times/month), occupational dust exposure (yes/no), cooking meals at home (yes/no), and traffic exposure time (minutes/day), and included community (Wuhan/Zhuhai) as a random effect.
*P<0.05.
NA, not applicable.

might be that zinc could injure lung function only if present in excess.

In conclusion, excess urinary zinc exposure was both cross-sectionally and longitudinally associated with lung function decline in an urban Chinese population. The CRP played a mediating role in the association between zinc exposure and lung function reduction.

Contributors All authors meet the criteria of authorship. MZ and WC designed the study. MZ, LX, SY, BW, TS, AT, XW, GM and WC collected the data. MZ and WC performed the statistical analysis, interpreted the results, and drafted the manuscript. LX, SY, BW, TS, AT, XW and GM critically reviewed the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Competing interests None declared.

Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets generated and analysed during the current study are available from the corresponding author on reasonable request (e-mail, wchen@mails.tjmu.edu.cn).

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Correction: *Cross-sectional and longitudinal associations between urinary zinc and lung function among urban adults in China*

Zhou M, Xiao L, Yang S, *et al*. Cross-sectional and longitudinal associations between urinary zinc and lung function among urban adults in China. *Thorax* 2020;75:771–9.

This article has been corrected since it was published online. Tables 2 and 3 were missing some minus symbols and have been amended accordingly.

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