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Elevated inflammatory Lp-PLA2 and IL-6 link e-waste Pb toxicity to cardiovascular risk factors in preschool children☆

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ABSTRACT

Cardiovascular toxicity of lead (Pb) manifests primarily as an effect on blood pressure and eventual increased risk of atherosclerosis and cardiovascular events. Therefore, we investigated vascular inflammatory biomarkers and cardiovascular effects of Pb-exposed children. A total of 590 children (3–7 years old) were recruited from Guiyu (n = 337), an electronic waste (e-waste)-exposed group, and Haojiang (n = 253), a reference group, from November to December 2016. We measured child blood Pb levels (BPbs), and systolic and diastolic blood pressure. Pulse pressure was calculated for the latter two. Serum biomarkers including lipid profiles and inflammatory cytokines, and plasma lipoprotein-associated phospholipase A2 (Lp-PLA2) were detected. Unadjusted regression analysis illustrated that higher ln-transformed BPb associated with lower systolic blood pressure and pulse pressure. After adjustment for various confounders, the relational degree of lnBPb and blood pressure measures became slightly attenuated or not significant. Elevated BPb was associated with higher Lp-PLA2, interleukin (IL)-6, triglycerides (TG) and lower high-density lipoprotein (HDL). Lp-PLA2 remained inversely associated with pulse pressure and HDL, but positively with ratios of total cholesterol to HDL (Tc/HDL) and low-density lipoprotein to HDL (LDL/HDL). IL-6 was associated negatively with systolic blood pressure, pulse pressure and HDL, and positively associated with TG, Tc/HDL and LDL/HDL. The mediation effect of biomarkers on the association of BPb with pulse pressure was insignificant except for Lp-PLA2. Available data supports the conclusion that e-waste-exposed children with higher BPbs and concomitant abnormal measures of cardiovascular physiology have an augmented prevalence of vascular inflammation, as well as lipid disorder.

1. Introduction

Environmental lead (Pb) exposure constitutes a major public health problem. Though a concerted effort to reduce Pb exposure has been attempted, e.g. the banning of tetraethyl Pb as a vehicle fuel additive (Li et al., 2014), Pb is still the most prominent and widespread environmental contaminant in Guiyu, a typical electronic waste (e-waste) recycling area in southeast China, with a nearly 30-year history of unregulated e-waste disposal (Chen et al., 2011; Lin et al., 2017; Xu et al., 2015a,b). Many electronic components (e.g. cathode ray tube screens, batteries, and resistors) and printed circuit boards are comprised of nearly 10–20% Pb, which is released into the environment by informal manual dismantling processes involving physical and chemical methods (Kaya, 2016). Although Guiyu is a site of heavy metal and organic pollutant co-exposure, local children are primarily exposed to Pb because Pb is present widely in the air, soil, water, sediment and plants (Song and Li, 2014b). Our previous investigations revealed that Pb levels in soil and dust samples are respectively 2.32-times and 4.10-times higher than nearby reference areas (Yekeen et al., 2016). Pb concentrations

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in plants along Guiyu roadsides reach up to 18.74 mg/kg (Alabi et al., 2012). In our recent study, Pb levels as high as 152.96 ng/m^3 in $PM_{2.5}$ have been observed in Guiyu (Zeng et al., 2016). Furthermore, Pb levels in the riverine surface water and sediment of Guiyu are higher than nearby reference areas (Guo et al., 2009). Our earlier studies on Guiyu residents have shown elevated placental Pb levels (median 301.43 ng/g, range 6.51–3465.16 ng/g), and elevated child blood Pb levels (BPbs) (mean 15.30 μg/dL, range 4.40–32.67 μg/dL), throughout more than a decade of Pb testing (Dai et al., 2017; Guo et al., 2010; Huo et al., 2007). Pb exposure derives from Pb-contained dust and fumes, and Pb-polluted hands, food, water, toys and clothing (Bi et al., 2015). Children are particularly susceptible to Pb, whose toxicity to physical development, hematopoietic system development, immunoregulatory response and neural activity have been shown (Liu et al., 2014; Yang et al., 2013; Zhang et al., 2016). Pb exposure has been identified as an important factor contributing to the development and severity of cardiovascular disease (CVD), such as blood pressure dysregulation, disordered lipid metabolism, and atherogenesis in adults (Cosselman et al., 2015). However, Pb-associated cardiovascular toxicity is insufficiently examined in e-waste-exposed children. Therefore, this study was designed to assess the adverse effects of Pb on cardiovascular risk factors in e-waste-exposed children.

CVD is a major burden on society, and has been developing at younger ages (Danaei et al., 2014). CVD risk factors involve any measurable quality that may be related to an increased probability of developing future CVD (Poreba et al., 2011). Recent epidemiologic evidence identifies Pb hazard in cardiovascular outcomes, such as stroke, coronary heart disease and peripheral arterial disease, where functional mechanisms of oxidative stress play a vital role (Lamas et al., 2016; Navas-Acien et al., 2007; Solenkova et al., 2014). Furthermore, Pb causally promotes CVD in animal studies, and impairs cardiac and vascular function *in vivo* and *in vitro*, where activation of signaling pathways and inflammatory proteins are possible underlying mechanisms (Fioresi et al., 2013; Simoes et al., 2015; Wildemann et al., 2016). Persistent vascular inflammation may initiate the development of atherosclerotic plaques (Widlansky and Gutterman, 2011). The cardiovascular toxicity of Pb manifests primarily as an effect on blood pressure, and eventually as an increased risk of atherosclerosis and cardiovascular events (Prokopowicz et al., 2017). Pb exposure promotes generation of superoxide and hydrogen peroxide in human vascular smooth muscle and endothelial cells (Ni et al., 2004), and endothelial metabolic dysfunction might impede the transport and metabolism of lipids (Eelen et al., 2015). Ratios of total cholesterol to high-density lipoprotein cholesterol (Tc/HDL) and low-density lipoprotein to HDL (LDL/HDL) are better predictors of CVD than LDL or Tc alone. Based on above theories, we examined potential cardiovascular risk factors of Pb-exposed children examining for disordered regulation of blood pressure and lipid profiles.

During the pathophysiological progression of CVD, biomarkers of endothelial inflammation are mechanistically related to endothelial dysfunction and atherosclerotic risk (Peng et al., 2013). Lipoprotein-associated phospholipase A2 (Lp-PLA2), as a vascular-specific inflammatory biomarker, is a pro-inflammatory enzyme that has been implicated in oxidative damage, cytokine release, vascular dysfunction, and lipid metabolism disorders, characteristic of atherosclerotic progression (Ragab et al., 2015). Mainly expressed in monocytes, neutrophils, macrophages and activated platelets, Lp-PLA2 hydrolyzes oxidized phospholipids to yield pro-inflammatory and pro-atherogenic products (e.g. oxidized fatty acids and lyso-phosphatidylcholine) (Chae et al., 2011; Sakka et al., 2015). These products stimulate the expression of endothelial adhesion molecules and cytokines, and recruit monocytes, which are then activated and transformed into macrophages and apoptotic foam cells, ultimately facilitating fatty streak formation and atherosclerosis (Ikonomidis et al., 2014; Li et al., 2017; Oei et al., 2005; Rosenson and Stafforini, 2012). Ambient pollution exerts adverse effects on Lp-PLA2 (Bruske et al., 2011), possibly through oxidative stress-mediated up-regulation of Lp-PLA2 to accelerate atherosclerotic progression (De Keyzer et al., 2009; Wu et al., 2004). Environmental pollutants induce mitochondrial oxidative injury, activate autophagy, and increase the production of inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and IL-6], especially in susceptible populations (Ruckerl et al., 2014; Zhang et al., 2017). Additionally, the generation of reactive oxygen species (ROS) can stimulate the expression of cytokine cascades through NF-κB-induced transcriptional events (Zhang et al., 2009). IL-1β contributes directly to atherosclerotic plaque development via increased production and decreased clearance of lipid metabolites (McCarty and Frishman, 2014). IL-6 is up-regulated in response to ROS and vascular injury, and highly representative of vascular inflammation, in which the NF-κB-IL-6 signal pathway plays a central role (Brasier, 2010). Previous studies show serum IL-6 is a significant predictor of cardiovascular mortality (Eder et al., 2009; Su et al., 2013). IL-8 acts as a mediator of angiogenesis that could contribute to atherosclerotic plaque formation (Koch et al., 1992). TNF-α up-regulates the expression of arginase in endothelial cells that favors endothelial dysfunction, and increases the transcytosis of lipoproteins across endothelium to accelerate the pathogenesis and progression of atherosclerosis (Zhang et al., 2014). Taken collectively, current evidence highlights the pivotal roles of Lp-PLA2 and cytokines in mediating vascular inflammation, the earliest steps to atherosclerosis.

Available epidemiological research has scarcely explored the pro-inflammatory role of cytokines and Lp-PLA2 in the relationships between BPb and CVD risk factors in susceptible children. The present study aims to investigate preschool children recruited from an e-waste recycling area and the reference area, to evaluate the effects of BPb on CVD risk factors, including blood pressure and lipid profiles. We hypothesize that the capacity of Pb to impair vascular structure will exacerbate endothelial inflammation, perturb blood pressure and reduce the ability to clear lipids, ultimately raising atherosclerotic risk.

2. Materials and methods

2.1. Study population

A total of 590 children (3–7 years old) from Guiyu (n = 337), an e-waste exposed group, and Haojiang (n = 253), a reference group located 31.6 km to the east of Guiyu, were recruited from November to December 2016. The both groups exhibited similar ethnicity, cultural background and population. A questionnaire, on general demographic characteristics, residential environment, child lifestyle and diet habits, and both parent and child medical or disease histories, was administered to children's parents or guardians. All children were screened at entry and enrolled in the study if they were free of any known medical conditions or infectious diseases or CVD. All procedures involving human subjects were approved by the Human Ethics Committee of Shantou University Medical College, China. All participants' guardians provided signed informed consent prior to enrollment. As described previously, fasting venous blood was collected from volunteers (Zhang et al., 2016). Serum was used for lipid profile detection. The rest of the serum supernatant, plasma and whole blood were aliquoted and stored at −80 °C until analysis.

2.2. Exposure measurements

Whole blood Pb was measured over the course of the study period by graphite furnace atomic absorption spectrophotometry (Jena Zeenit 650, Germany). The technical specifications and validity of this instrument are described in detail elsewhere (Guo et al., 2010).

2.3. Physiological parameters

To ascertain that all measurements in preschool children were accurate and consistent, trained staff obtained measurements of participant weight (without shoes and in light clothing) to the nearest 0.1 kg, and height to 0.1 cm; these values were used to calculate the body mass index (BMI). Blood pressure was assessed by trained staff using a manual mercury sphygmomanometer. In an empty and quiet house, participants were individually seated with their backs supported, feet on the floor, and the arm supported in a horizontal position, with the cuff at the level of the heart. Three measurements were taken at intervals of at least 1 min, with a 15-mininute rest before obtaining the first reading; the mean of three measurements was used to analysis.

2.4. Biomarker measurements

Concentrations of serum TG, Tc, LDL, and HDL were determined using a Toshiba TBA-40FR Automatic Biochemical Analyzer (Toshiba Medical System Corporation, Japan). For the characterization of lipid metabolism, TG, Tc, LDL, HDL, ratios of Tc/HDL and LDL/HDL were evaluated as the cardiovascular risk factors.

Plasma Lp-PLA2 determination was performed using an enzyme-linked immuno sorbent assay kit according to the manufacturers' instructions (R&D Systems Inc., USA). Detection of multiple cytokine in serum was accomplished with a ProcartaPlex Human Cytokine & Chemokine Panel 1A (eBioscience, USA), all according to the manufacturers' instructions. A Luminex 200 analyzer (Luminex, USA) was used to generate standard curves and calculate sample results.

2.5. Statistical analysis

All data are expressed, as the median [interquartile range (IQR)] for skewed distribution, or mean ± standard deviation (SD) for normal distribution and percentage for enumeration data. The Mann-Whitney test, independent-sample *t*-test and chi-square test were used appropriately. In order to define the correlation of dependent and independent variables, we simplified the analysis into four different associations: Pb exposure and BPb influences, BPb and CVD risk factors, inflammatory biomarkers and CVD risk factors, as well as BPb and inflammatory biomarkers. Simultaneously, BPb was log-transformed using the natural logarithm (ln-transformed BPb). To probe the mediation effects of inflammatory agents on the associations between BPb and CVD risk factors, multiple mediator analyses adjusted for covariates were conducted as previously outlined (Preacher and Hayes, 2008). Confounders based on the previous literature consisted of: a) sources of Pb exposure and risk factors of CVD: outdoor activities, family member smoking, parent education and diet (including cooking oil, picky eating, sweetmeat consumption, salted products, vegetable and fruit consumption, dairy products, bean products, marine products); b) causes of high BPb and risk factors of CVD: age, gender, BMI, and family history of diseases (including hypertension, diabetes, obesity) (Pastorelli et al., 2012; Payne, 2012). Spearman correlation analyses were applied to explore the links of BPb with inflammatory markers. All analyses were performed using Stata 12.0 (STATA Corp LP, College Station, TX, USA), SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad, San Diego, CA). A $P < 0.05$ was considered significant.

3. Results

3.1. Descriptive characteristics of the study population

Basic characteristics as well as data of BPbs are provided in Table 1 and Supplemental Material Table S1. E-waste-exposed children possessed a lower BMI than the reference group (15.06 ± 1.28 kg/m^2 vs. 15.67 ± 1.68 kg/m^2, $P < 0.001$), though the mean age and gender distribution in both groups were similar (both $P > 0.05$). The median concentration of BPb in the exposed group was 7.14 μg/dL, which was significantly higher than 3.91 μg/dL in the reference group ($P < 0.001$). Of the analyzed samples, 84.4% from the exposed group exceeded the U.S. CDC recommended limit of 5 μg Pb/dL (Betts, 2012), whereas 23.5% exceeded the limit in the reference group ($P < 0.001$).

As shown in Figs. 1 and 2, the exposed group exhibited lower systolic blood pressure (SBP) and pulse pressure (PP) (median 87.33 mmHg and 32.67 mmHg) compared to the reference group (median 90.67 mmHg and 36.00 mmHg) (both $P < 0.01$). Diastolic blood pressure (DBP) was not significant between groups ($P > 0.05$). TG level was higher in the exposed group than that in the reference group (0.85 ± 0.36 mmol/L vs. 0.65 ± 0.21 mmol/L), whereas HDL was clearly lower (1.33 ± 0.26 mmol/L vs. 1.50 ± 0.30 mmol/L) (both $P < 0.01$). Statistically differences were not found between groups for LDL and Tc levels (both $P > 0.05$). Despite this, after controlling for HDL, the ratios of LDL/HDL and Tc/HDL demonstrated significant increases in the exposed group, compared with these in the reference group (1.76 ± 0.53 vs. 1.57 ± 0.47, 3.07 ± 0.65 vs. 2.78 ± 0.52, respectively, both $P < 0.01$).

The exposed group displayed 14.62% more plasma Lp-PLA2 compared to the reference group (93.29 ± 36.36 ng/mL vs. 79.65 ± 30.59 ng/mL, $P < 0.01$) (Fig. 3). Table 2 shows that children in the exposed group had higher median level compared to these in the reference group, for IL-6 (10.00 pg/mL vs. 1.61 pg/mL), IL-8 (2.38 pg/mL vs. 1.59 pg/mL) and TNF-α (2.36 pg/mL vs. 1.86 pg/mL) (all $P < 0.05$). IL-1β, DBP, Tc, and LDL did not differ between the two groups (all $P > 0.05$). Therefore, further analysis for these variables was not performed, and they were included only in the descriptive statistics.

3.2. Pb exposure and factors influencing BPbs

Multiple linear regression analysis was applied to explore whether there were specific factors connected to ln-transformed BPb in preschool children (Table S2). By adjusting for potential covariates, age, gender and BMI, the regression model showed that the ln-transformed BPb was positively associated with drinking dairy products, child local residence, and house ventilation ($\beta = 0.229$, $\beta = 0.185$, and $\beta = 0.155$, respectively, all $P < 0.05$), showing that the frequency of dairy product consumption dominates the determination of elevated Pb levels in children. However, drinking bean products, distance from residence to road, and paternal education level were negatively correlated with higher ln-transformed BPb ($\beta = -0.220$, $\beta = -0.233$, and $\beta = -0.177$, respectively, all $P < 0.05$), among which greater distance from residence to road contributed much more to lower ln-transformed BPb. In conclusion, child hygiene and dietary habits, and residence environment are the main factors influencing BPbs.

Table 1
Basic characteristics of the study population.

Characteristic	Reference group (n = 253)	Exposed group (n = 337)	*P*
Child age (years)	4.40 ± 1.04	4.52 ± 0.86	0.141[a]
BMI (kg/m^2)	15.67 ± 1.68	15.06 ± 1.28	<0.001[a]
Blood Pb level (μg/dL)	3.91 (3.11, 4.87)	7.14 (5.67, 8.82)	<0.001[c]
≥5 μg/dL [n (%)]	47 (23.5)	200 (84.4)	<0.001[b]

Pb, lead; BMI, body mass index. $P < 0.05$ was considered statistically significant.
[a] Mean ± standard deviation, analyzed by the independent-sample *t*-test.
[b] n (%), analyzed by the χ^2-test.
[c] Median (interquartile range), analyzed by the Mann-Whitney test.

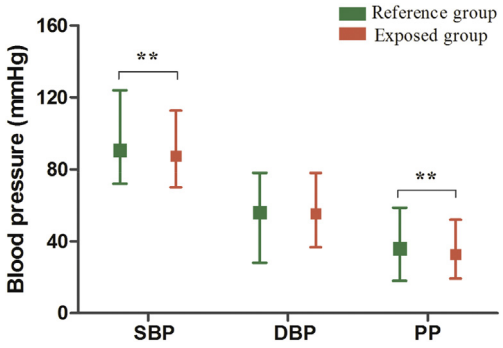

Fig. 1. Blood pressure parameters in children. Exposed group: n = 256. Reference group: n = 212. Results are presented as the median (interquartile range), analyzed by Mann-Whitney test. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure. $^{**}P < 0.01$.

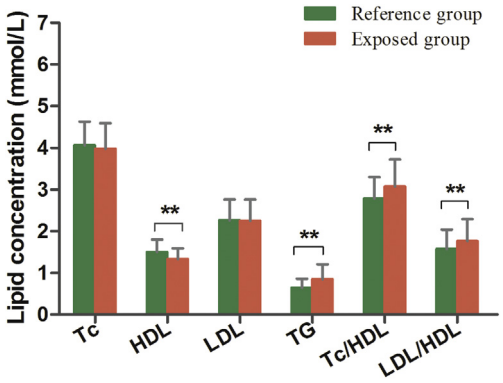

Fig. 2. Serum lipid parameters in children. Exposed group: n = 235; reference group: n = 201. Results are presented as the mean ± standard deviation, analyzed by the independent-sample *t*-test. Tc, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; Tc/HDL, ratio of Tc to HDL; LDL/HDL, ratio of LDL to HDL. $^{**}P < 0.01$.

3.3. Pb exposure and cardiovascular risk factors

With the purpose of understanding the association between BPb and blood pressure parameters, we examined different linear regression models (Table 3). Unadjusted regression analysis illustrated that higher ln-transformed BPb was associated with lower SBP [B (95% CI) = −2.88 (−4.47, −1.29)] and PP [-3.30 (−4.60, −2.01)] (both $P < 0.01$). Further adjustment for gender, age, and BMI, relational degree attenuated lightly (both $P < 0.05$). However, after adjustment for child outdoor activities, diet, family history of diseases, family member smoking, and parent education, these significant relationships were not observed (both $P > 0.05$). In brief, higher BPb plays a role in abnormal measures of cardiovascular physiology.

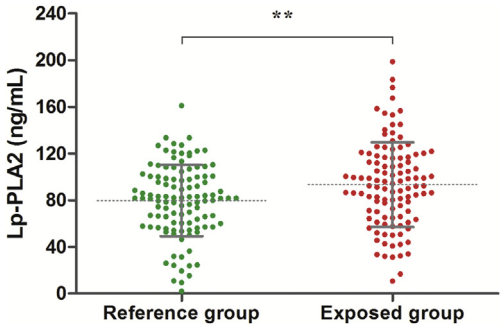

Fig. 3. Lipoprotein-associated phospholipase A2 (Lp-PLA2) concentrations in child plasma. Exposed group: n = 110. Reference group: n = 110. Results are presented as mean ± standard deviation, analyzed by the independent-sample *t*-test. $^{**}P < 0.01$.

Regression analysis was implemented to estimate the impact of BPb on lipid parameters (Table 4). Our findings showed that each *e*-μg/dL increase in BPb was associated with a 0.07-mmol/L decrease in HDL [95% CI = (−0.12, −0.01)] and a 3.10-mmHg decrease [95% CI = (−1.37, −1.82)] in PP (both $P < 0.05$), whereas the TG level was positively correlated with ln-transformed BPb [0.08 (0.02, 0.14), $P < 0.05$]. Nevertheless, the ratios of Tc/HDL and LDL/HDL were not significantly related with ln-transformed BPb (both $P > 0.05$). Specifically, once considering confounders, significant relevance in the results no longer existed. Consequently, adjusted effects will not be considered hereinafter. In summary, higher BPbs link to the disordered lipid profiles.

3.4. Inflammatory biomarkers and cardiovascular risk factors

We investigated whether inflammation adversely influenced cardiovascular health in study population. The effects per IQR increase in Lp-PLA2 (45.20 pg/mL) on CVD risk factors are listed in Table 4. Lp-PLA2 remained inversely associated with PP [B (95% CI) = −2.09 (−3.33, −0.85)], indicating a 2.09-mmHg decrease in PP per IQR increase in Lp-PLA2. Lp-PLA2 was negatively associated with HDL [-0.05 (−0.11, −0.01)]. However, Lp-PLA2 was positively associated with Tc/HDL and LDL/HDL [0.22 (0.12, 0.32) and 0.20 (0.12, 0.28), respectively]. No associations were found with SBP or TG. Taken collectively, vascular inflammatory biomarkers are involved in abnormal blood pressure and lipid profiles.

3.5. Pb exposure and inflammatory biomarkers

We further characterized the association of pro-inflammatory Lp-PLA2 or cytokine concentrations with BPbs (Fig. 4). Results indicated that the greater the BPb, the higher the Lp-PLA2 level. BPb correlated with both Lp-PLA2 and IL-6 ($r_s = 0.20$ and $r_s = 0.59$ respectively, both $P < 0.01$). However, significant associations among Pb levels and IL-1β, IL-8 and TNF-α were lost. To sum up,

Table 2
Levels of inflammatory cytokines in child serum.

	Reference group (n = 80)			Exposed group (n = 80)			*P*
	P_{25}	P_{50}	P_{75}	P_{25}	P_{50}	P_{75}	
IL-1β (pg/mL)	0.49	0.49	0.72	0.17	0.53	0.76	0.890
IL-6 (pg/mL)	1.61	1.61	3.70	10.00	10.00	10.00	<0.001
IL-8 (pg/mL)	1.11	1.59	2.28	1.55	2.38	4.11	0.004
TNF-α (pg/mL)	1.07	1.86	2.67	2.36	2.36	3.96	0.014

Data are presented by the 25th, 50th, and 75th percentile and analyzed by the Mann-Whitney test. IL, interleukin; TNF, tumor necrosis factor. $P < 0.05$ was considered statistically significant.

Table 3
Contributions of lnBPb to blood pressure parameters in children.

LnBPb	SBP		PP	
	B (95% CI)	β	B (95% CI)	β
Model 1	−2.88 (−4.47, −1.29)**	−0.17	−3.30 (−4.60, −2.01)**	−0.24
Model 2	−1.84 (−3.29, −0.40)*	−0.11	−3.10 (−4.37, −1.82)**	−0.22
Model 3	−0.30 (−2.60, 2.00)	−0.02	−2.11 (−4.38, 0.17)	−0.14

Model 1: unadjusted.
Model 2: adjusted for gender, age and BMI.
Model 3: adjusted for gender, age and BMI, outdoor activities, diet (including cooking oil, picky eating, sweetmeat consumption, salted products, vegetable and fruit consumption, dairy products, bean products, marine products), family history of disease (hypertension, diabetes, obesity), family member smoking, parent education.
LnBPb, ln-transformed blood Pb levels; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient.
*$P < 0.05$; **$P < 0.01$.

results demonstrate that the higher BPb, the higher inflammatory biomarker level.

3.6. Multiple mediator model

To examine the effects of mediators, multiple mediator models were examined (Table 5). Bias corrected 95% CIs indicate a significant direct effect [B (95% CI) = −2.93 (−5.46, −0.40)], total indirect effect [-1.28 (−2.93, −0.22)], and indirect effect through Lp-PLA2 are significant [-0.65 (−1.63, −0.09)]. The proportion of Lp-PLA2 mediated effects in the total effect was 15.33%. The 95% CI for contrasts of Lp-PLA2 with IL-8, or IL-6 with IL-8 did not include zero, indicating that Lp-PLA2 is a significantly stronger mediator than the others. However, other parameters of the mediators or contrasts were insignificant (all CIs crossed zero). No mediation effects on the relationship of BPb with SBP or lipid parameters were found presently (data not shown). Collectively, results display that Lp-PLA2 is the exclusive mediator of the association between BPb and measures of cardiovascular physiology.

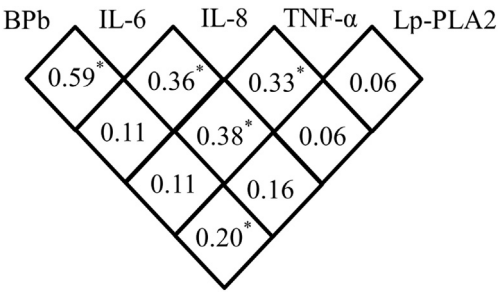

Fig. 4. Spearman rank correlations (r_s) of BPbs and inflammatory biomarkers. BPb, blood Pb levels; IL, interleukin; TNF, tumor necrosis factor; Lp-PLA2, lipoprotein-associated phospholipase A2. *$P < 0.05$.

Table 5
Summary of the mediating effect of Lp-PLA2 on the relationship between LnBPb and PP.

Model [a]	Product of coefficients		Bootstrapping Bias-corrected 95% CI		Percent of mediated effect
	B	SE	Lower	Upper	
Direct effect	−2.93*	1.28	−5.46	−0.40	–
Indirect effects					
Total	−1.28*	0.68	−2.93	−0.22	30.42
Lp-PLA2	−0.65*	0.38	−1.63	−0.09	15.33
IL-6	−1.10	0.70	−2.66	0.08	26.17
IL-8	0.47	0.44	−0.12	1.70	−11.08
Contrasts					
Lp-PLA2 vs. IL-6	0.46	0.79	−1.02	2.09	–
Lp-PLA2 vs. IL-8	−1.11*	0.61	−2.72	−0.22	–
IL-6 vs. IL-8	−1.57*	1.01	−4.19	−0.08	–

LnBPb, ln-transformed blood lead level; PP, pulse pressure; Lp-PLA2, lipoprotein-associated phospholipase A2; IL, interleukin B, unstandardized coefficient; SE, standard error; CI, confidence interval.
*$P < 0.05$.
[a] All regression analyses are controlled for age, gender and body mass index (BMI); 5000 bootstrap samples; n = 141.

Table 4
Contributions of lnBPb and Lp-PLA2 to cardiovascular risk factors in children.

	PP	HDL	TG	Tc/HDL	LDL/HDL
	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
LnBPb	−3.10 (−1.37, −1.82)**	−0.07 (−0.12, −0.01)*	0.08 (0.02, 0.14)*	0.09 (−0.02, 0.20)	0.05 (−0.04, 0.15)
Lp-PLA2[a]	−2.09 (−3.33, −0.85)**	−0.05 (−0.11, −0.01)**	0.02 (−0.03, 0.08)	0.22 (0.12, 0.32)**	0.20 (0.12, 0.28)**

Analysis was adjusted for age, gender and body mass index (BMI).
LnBPb, ln-transformed blood Pb levels; Lp-PLA2, lipoprotein-associated phospholipase A2; IQR, interquartile range; PP, pulse pressure; Tc, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; Tc/HDL, ratio of Tc to HDL; LDL/HDL, ratio of LDL to HDL; B, unstandardized coefficient; CI, confidence interval.
*$P < 0.05$; **$P < 0.01$.
[a] The effects of per Lp-PLA2 IQR increase (45.20 pg/mL) on outcome variables.

4. Discussion

In this study, BPb remained detrimentally associated with SBP, PP, HDL, TG, Tc/HDL and LDL/HDL in preschool children. Lp-PLA2 corresponded adversely with PP, Tc/HDL and LDL/HDL. IL-6 was linked to all outcome variables. BPb was positively associated with Lp-PLA2 and IL-6, but the relationship with IL-1β, IL-8 or TNF-α was not significant. In addition, the mediation effect of Lp-PLA2 was significant on Pb-associated abnormal measures of cardiovascular physiology. Our study comprehensively evaluates the involvement of vascular inflammation in relationships between elevated BPb and CVD risk factors in preschool children.

4.1. Pb exposure and factors influencing BPb

Pb from e-waste recycling not only pollutes the workshop vicinity, but the entire community as well. Pb measured in whole blood is the major biomarker of Pb exposure. Guiyu children continue to suffer from higher BPb (Dai et al., 2017; Huo et al., 2007). Our findings show that Guiyu child BPb is 7.14 μg/dL, while 3.91 μg/dL in the reference group. By contrast, the overall mean BPb has been 4.94 μg/dL in children (0–6 years old) from Jinan communities in northern China, and 0.84 μg/dL in the U.S. children (1–5 years old) (Tsoi et al., 2016; Zhao et al., 2013). By blaming frequent hand-mouth activities and inadequate excretion capability, Guiyu children are more vulnerable to toxic substances than adults (Heacock et al., 2016). Consequently, we explored multifarious influencing factors by a questionnaire: including child outdoor activities, hygiene and dietary habits, family member smoking, parent education, and residence environment. Our findings indicate that dairy product consumption, child local residence in Guiyu, and poor house ventilation are positively related to higher BPb. However, distance from the residence to a road negatively maintains a predominant role in elevated BPb. More hand-to-mouth and object-to-mouth activities promote Pb absorption and accumulation in the child body (Xue et al., 2010). As a whole, results imply that residence dust, plants and air polluted by e-waste recycling, followed by child hygiene and dietary habits, affect child BPb outcomes (Zahran et al., 2013).

4.2. Pb exposure and cardiovascular risk factors

Our findings show that associations of BPb with blood pressure measures were negative or null within the range of detection. BPb in the exposed group was higher but SBP and PP were lower than these in the reference group, and DBP overlapped within two groups. Similarly, a cross-sectional study conducted in the U.S. children (1–10 years old) reveals a small, negative correlation between BPb and SBP, but no significant correlation between BPb and DBP (Selbst et al., 1993). Conversely, a study in Yugoslavia children (5.5 years old) reports that every 10-μg/dL increase in BPb is associated with a 0.5-mmHg increase in SBP [95% CL = (−0.2, 1.3)] and a 0.4-mmHg increase in DBP [95% CL = (−0.1, 0.9)] (Factor-Litvak et al., 1996). Findings suggest that children with higher BPb may be prone to abnormal measures of cardiovascular physiology, namely abnormal blood pressure, signifying impaired function in cardiovascular system. Pb exposure promotes generation of ROS (Guo et al., 2014). ROS-induced vascular smooth muscle and endothelial cell apoptosis accelerate endothelial injury, vascular smooth muscle cell loss, as well as elastin fragmentation, which are conducive to the decrease of peripheral vascular tension (Chistiakov et al., 2015; Ellinsworth, 2015; Yu et al., 2015). We speculate that one of the main causes of the abnormal SBP and PP may be a decrease of peripheral vascular resistance in Guiyu children (Buckley and Ramji, 2015; Lohmeier and Iliescu, 2015). Although fairly minor fluctuations in the levels of blood pressure would not be clinically significant, molecular and cellular effects initiating adaptive or maladaptive responses could potentially provoke future atherosclerotic plaque in a vicious cycle. Macrophages and endothelial cells might endocytose Pb to produce foam cells and endothelium disruption followed by cell apoptosis. In the event of endothelial layer disruption, Pb can reach and accumulate in smooth muscle cells, aggravating inflammation and lipid peroxidation, eventually triggering atherosclerosis (Di et al., 2016). Considering the prevalence of Pb exposure in Guiyu children, even a minor physiological change could increase CVD risk in the future.

What is noteworthy is that co-exposure to heavy metals and organic pollutants occupy a place in the development of CVD (Arrebola et al., 2015; Wildemann et al., 2015). Our research is based on typical environmental conditions for e-waste recycling and toxicant co-exposure, where dioxins, furans, and heavy metals are released and all detectable in the environment and human tissue samples (Awasthi et al., 2016; Song and Li, 2014a; Xu et al., 2015a,b). Essentially, the health of an e-waste-exposed population may be more complicated due to the combined effect of organic pollutants and heavy metals. Regrettably, our present investigation lacks of suitable data on multiple contaminant interactions due to limited volume of blood samples. However, our earlier research indicated Guiyu children are principally threatened by Pb because Pb is a heavy metal of high toxicity and exists diffusely in local air, soil, water, sediment and plants (Song and Li, 2014b). For another, Pb exposure derives from a wealth of sources, including Pb-contained dust or fumes, Pb-contaminated food, water, air, hands or clothing (Bi et al., 2015). Thus, the significance of Pb toxicity to children can never be overemphasized. More or prospective research is warranted to verify the unexpected association of BPb with blood pressure measures and elucidate the role of co-exposure as a modifier. Our findings may be an understanding of the deleterious actions of Pb exposure and is worth consideration in future primary prevention strategies for CVD.

Although Pb toxicity has been widely investigated in adults, its effects on child lipid profiles are poorly elucidated. Results show that concentrations of TG, LDL/HDL ratio, and Tc/HDL ratio are higher, while HDL is lower in Guiyu children. We observed a positive association between BPb and TG, based on an adjusted model. Concurrently, a negative relationship of BPb with HDL exists. Findings of positive relationship between BPb and disordered lipid profiles have been reported (Ademuyiwa et al., 2005; Kamal et al., 2011). Our observations are consistent with animal studies (Alya et al., 2015). Pb exposure promotes generation of ROS in endothelial cells. Endothelial dysfunction could impede the transport and metabolism of lipids (Eelen et al., 2015). Our results suggest the possibility of a lipid disorder, where retention of TG-rich lipoproteins may enter in the intima of arteries, promote expression of endothelial adhesion molecules and macrophage chemotaxis, and aggravate endothelial inflammation (Welty, 2013). Additionally, HDL plays a vital part in anti-atherogenic effects, attenuates inflammation, owns anti-oxidative and antithrombotic properties, and maintains endothelial function (Fisher et al., 2012). Therefore, elevated serum TG and low HDL substantially facilitates fatty streak formation and atherosclerotic progress (Ellulu et al., 2016). We favor that vascular structure and function of Pb-exposed children may be impaired, manifesting as disordered blood pressure and lipid profiles. It is extremely necessary in our next step to assess the incidence of CVD in Guiyu adults and children.

4.3. The role of pro-inflammatory Lp-PLA2

In present study, Lp-PLA2 concentrations are elevated in Guiyu children compared with Haojiang individuals, suggesting that

Guiyu children are more likely to suffer from vascular inflammation. Lp-PLA2 is positively associated with BPb, suggesting an effect of Pb on increased levels of pro-inflammatory and pro-atherogenic markers, similar to the effects of environmental toxicants (e.g. $PM_{2.5}$) (Bruske et al., 2011). Results show Lp-PLA2 is inversely associated with PP and HDL, but positively associated with Tc/HDL and LDL/HDL ratios. However, no associations are found with SBP or TG. Contrarily, a prior study shows that Lp-PLA2 activity is related to higher SBP and DBP in adults (Li et al., 2016). Moreover, Lp-PLA2 exerts a significant mediation effect on the relationship between BPb and PP, demonstrating that vascular inflammation contributes to Pb-associated maladaptive regulation of cardiovascular physiology. Additionally, other markers included in the mediation models do not significantly affect the association of BPb with blood pressure. This indicate that Lp-PLA2 might act as an exclusive mediator for Pb-associated blood pressure alterations in children. Lp-PLA2 is an independent risk factor and predictor for atherosclerosis. Apparently, Pb-induced elevated CVD risk is associated with an elevation of inflammatory markers (Silveira et al., 2014). On the one hand, Pb accumulates in human aortae to increase vascular oxidative stress, trigger inflammation, and damage vascular structure and function (Perlstein et al., 2007). On the other hand, Pb stimulates inflammatory production so as to potentiate injury in cardiovascular physiology (Cakmak et al., 2014). Therefore, our findings accord with the importance of the exposure-endothelial inflammation mechanism. The negative ("protective") effect of lower PP could be due to functional adaption to endothelial injury, or compensatory mechanisms responding to the increases in inflammatory biomarkers triggered by Pb.

Analogously, Rolla et al. summarize that plasma Lp-PLA2 levels positively correlate with Tc and LDL, and negatively associate with HDL (Rolla et al., 2015). Lp-PLA2 functions as a unique mediator of vascular inflammation originating from the endothelial membrane and platelet-activating factor (e.g. phospholipids), participating in lipid peroxidation (Dada et al., 2002). Higher Lp-PLA2 levels impede endothelial growth and repair, and also stimulate cell infiltration, apoptosis and vascular dysfunction (Lavi et al., 2007). Vascular inflammation and lipid disorder are proposed to be conducive to the initiation and promotion of atherosclerosis (Krintus et al., 2014). Our results support studies showing that increased Lp-PLA2 is involved in vascular inflammation and closely related to abnormal vascular physiology, which are a prerequisite of atherosclerosis and heralding an increased risk of CVD. Lp-PLA2 may represent a valuable early biomarker for CVD risk in e-waste-exposed children through increased susceptibility to lipid peroxidation.

4.4. The role of cytokines

Our results indicate that IL-6, IL-8 and TNF-α levels are higher in the e-waste-exposed group, indicative of elevated inflammation. However, IL-6, but not IL-8 or TNF-α, increased with BPb elevation. Increasing levels of IL-6 are associated with all outcome variables. IL-8 is positively associated with PP, while TNF-α is only negatively related to TG levels (Table S3). Our findings are similar to a prior study showing Pb levels are positively associated with IL-6, and TNF-α in taxi drivers (Brucker et al., 2015). Pro-inflammatory cytokines derive from lymphocytes and endothelial cells for immune responses to environmental Pb exposure, acting as upstream of inflammation (Ashok et al., 2015; Cabral et al., 2015; Vattanasit et al., 2014; Xu et al., 2017). IL-6 causes monocyte activation in vascular inflammation (Tieu et al., 2009). IL-8 is a crucial mediator atherosclerotic plaque formation because of its high sensitivity to oxidants (Apostolakis et al., 2009). TNF-α up-regulates the expression of arginase in endothelial cells, which favors endothelial dysfunction (Murdaca et al., 2013; Steyers and Miller, 2014). In total, although IL-6, IL-8, TG and HDL do not significantly mediate such associations between BPb and measures of cardiovascular physiology, increased levels of IL-6 and IL-8 in children with higher BPb suggest that vascular inflammation participates in the responses to environmental heavy metal toxicants. The consequent endothelial dysfunction is a leading player in the initiation and maintenance of atherosclerosis, and may serve as an early marker for future CVD risk of Pb exposure.

4.5. Limitations

Firstly, this cross-sectional study provides an association between Pb and physiological responses, but does not prove the cause and effect, which is indispensable for identifying specific links in future prospective studies. Secondly, each biomarker or outcome used in our study has been related to cardiovascular morbidity or mortality in clinical studies. Nevertheless, the implications of these biomarker changes for cardiovascular risk assessment cannot be estimated due to a lack of data on mortality or morbidity rates. Thirdly, the determination of the impact of multiple contaminants is deficient due to the limited volume of blood samples. This could result in a loss of statistical significance and underestimates of pollutant mediated changes. However, Pb is the most prominent and widespread environmental contaminant in e-waste recycling areas, profoundly disrupting child health. Future studies should focus on the joint influences of various pollutants and trace elements on CVD risk.

5. Conclusion

The increased BPbs observed in e-waste-exposed children are accompanied by elevated Lp-PLA2, IL-6, IL-8 and TNF-α concentrations, disordered lipid parameters, and aberrant blood pressure measures. Available data supports the conclusion that e-waste-exposed children with higher BPb and concomitant abnormal measures of cardiovascular physiology have a high prevalence of vascular inflammation, as well as lipid disorder. The next study will explore the combined effects of heavy metals and organic pollutantswith longer follow-up in larger studies and a wider range of indicators regarding CVD risk factors of Pb toxicity.

Conflicts of interest

The authors declare they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.11.094.

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