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Antioxidant alterations link polycyclic aromatic hydrocarbons to blood pressure in children

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HIGHLIGHTS

- PAH exposure is associated with lower blood pressure in children.
- Oxidative stress and further antioxidant decrease play roles in PAH-related blood pressure decrease.
- The roles of antioxidants in blood pressure are influenced by the level of PAH exposure.
- SOD and GPx are effective biomarkers of abnormal vascular function resulting from PAH exposure in healthy children.

GRAPHICAL ABSTRACT

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ABSTRACT

Exposure to polycyclic aromatic hydrocarbons (PAHs) is associated with changes in blood pressure. However, the association is controversial in different studies, and antioxidants' roles involved in it remain unclear. To investigate the associations among PAH exposure, blood pressure, and antioxidant concentrations, we recruited 403 children (2–7 years old), of which 203 were from Guiyu, an e-waste-recycling area (exposed group), and 200 were from Haojiang, a nearby non-e-waste area (reference group). Levels of blood pressure, plasma vitamin E, serum superoxide dismutase (SOD), serum glutathione peroxidase (GPx), and eight urinary hydroxylated PAHs (OH-PAHs) were measured. Compared with Haojiang children, Guiyu children had higher urinary OH-PAH concentrations but lower systolic pressure, pulse pressure, serum SOD concentration, and serum GPx concentration (all $P < 0.05$). PAH exposure was associated with lower systolic pressure, pulse pressure, SOD (adjusted $\beta = -0.091, -0.104$ and -0.154 , respectively, all $P < 0.05$, in all children), GPx (adjusted $\beta_{\sum 7\text{LMW-OH-PAHs-T3}} = -0.332$, only in Haojiang children) and vitamin E (adjusted $\text{OR}_{\sum 7\text{LMW-OH-PAHs}} = 0.838$, 95% CI: 0.706, 0.995, only in Guiyu children). Serum SOD and GPx were associated with higher blood pressure ($\beta_{\text{SOD-T2}}$ for diastolic pressure = 0.215 in all children, $\beta_{\text{SOD-T3}}$ for systolic pressure = 0.193 in all children, $\beta_{\text{SOD-T3}}$ for pulse pressure = 0.281 in high- $\sum 8\text{OH-PAHs}$ children, $\beta_{\text{GPx-T2}} = 0.283$ and $\beta_{\text{GPx-T3}} = 0.289$ for diastolic pressure in Haojiang children, all $P < 0.05$). Interactions between PAHs and vitamin E were associated with lower systolic pressure and pulse pressure; simple effects of vitamin E to raise systolic pressure and pulse pressure were only

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significant in low- $\sum_8\text{OH-PAHs}$ children. Our results indicate that PAH exposure, especially at high levels, and further antioxidant-decrease are potential risk factors for blood-pressure decrease in children; vascular function of PAH-exposed children may be impaired, manifesting as disordered blood pressure.

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1. Introduction

Cardiovascular disease (CVD) is the leading noncommunicable disease, with nearly half of the 36 million deaths due to noncommunicable diseases being caused by CVDs (World Health Organization et al., 2011). According to the latest WHO report, the annual number of deaths from CVD was 17.5 million in 2012 (World Health Organization, 2016). Blood pressure is important in CVD diagnosis. High blood pressure plays a prominent role in the development of CVD due to atherosclerosis, whereas low blood pressure may also predict cardiovascular disease risk in subpopulations, such as preterm infants and hemodialysis patients (Dempsey, 2017; Mrowka and Reuter, 2016). The regulation of blood pressure is complex because a number of feedback mechanisms are involved in the regulation of arterial blood pressure (Mrowka and Reuter, 2016). Among them, oxidative stress resulting from environmental pollution, including polycyclic aromatic hydrocarbons (PAHs), is involved in alterations in blood pressure and further CVD risk (Alshaarawy et al., 2016; Hu et al., 2018; Korashy and El-Kadi, 2006; Mikhed et al., 2015; van Rossem et al., 2015).

PAHs are a series of carcinogenic organic-chemicals deposited in soil, sediment, peat, coal, crude oil, and other high-molecular weight fuels, which are released into the environment naturally and artificially by incomplete combustion of organic materials (Guo et al., 2011; Kim et al., 2013; World Health Organization, 2017). Important sources of daily PAH release include exhaust gas emission, heating, cooking, and tobacco smoking, and industrial activities involving coke ovens, blast furnaces, and electronic waste recycling. PAHs then enter the human body through the respiratory tract, digestive tract and skin to cause adverse health outcomes (Karimi et al., 2015; Kim et al., 2013; WHO Regional Office for Europe, 2000). Large-scale epidemiologic studies have found that urinary hydroxylated PAHs (OH-PAHs) are positively associated with risk for cardiovascular diseases, including diagnosed atherosclerotic cardiovascular disease, coronary heart disease, angina pectoris, heart attack and stroke (Alshaarawy et al., 2016; Hu et al., 2018; Yin et al., 2018). Associations between PAH exposure and blood pressure, a marker for cardiovascular disease, have been inconsistent, with positive, negative and no correlation all being found between PAH exposure and blood pressure, along with underlying mechanisms including impaired autonomic nervous systems and altered circadian rhythms due to inflammation, and obesity (Gentner and Weber, 2011; Jacobs et al., 2012; Sancini et al., 2014; Trasande et al., 2015; Wang et al., 2016; Yin et al., 2018).

Alteration of blood pressure has been explained by decreased vascular bioavailability of nitric oxide caused by an abnormal production of reactive oxygen species (ROS) that could result from PAH exposure (Liu et al., 2018; Munzel et al., 2010). Among the many processes involved in the pathophysiology of blood-pressure disorder, vascular damage due to oxidative stress (excess bioavailability of ROS) is particularly important: ROS changes the structure and function of target proteins (including angiotensinogen) regulating the functions of endothelial and vascular smooth muscle cells, impairs endothelium-dependent vasodilatation, and promotes endothelial dysfunction, vascular remodeling, and inflammation, leading to vascular damage and further blood-pressure disorder (Higashi et al., 2002; Montezano et al., 2015; Zhou et al., 2010). PAHs are known to be potent oxidant stressors because ROS are normally produced by enzymes, such as cytochrome P450 monooxygenase, peroxidases, and NADPH oxidase, but excessively produced during phase I metabolism of PAHs in humans (Siti et al., 2015; Wang et al., 2019). PAHs alter aortic vasoconstriction via

the activation of ROS and ROS-regulated muscular signaling molecules PKC, MAPK, Rho-kinase, and MLCK (Tzeng et al., 2019). ROS and their health-related impact can be reduced by antioxidants (Siti et al., 2015). A high antioxidant capacity can provide defense against vascular oxidative stress by neutralizing free radicals and scavenging nitric oxide, thereby exerting beneficial effects on vascular function and consequently better blood pressure control (Mikhed et al., 2015). The antioxidant defense system consists of endogenous antioxidants (e.g. albumin, bilirubin, glutathione), exogenous antioxidants (e.g. vitamin E, carotenoids, vitamin C) and antioxidant enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase) that ultimately convert substrates to less reactive forms (Siti et al., 2015). Among them, superoxide dismutase (SOD), glutathione peroxidase (GPx) and vitamin E are commonly used antioxidant biomarkers (Mates et al., 1999). SOD destroys superoxide anion ($\text{O}_2^{\cdot-}$) with remarkably high reaction rates by successive oxidation and reduction of the transition metal ion at the active site, and the SOD family represents the only enzymes able to specifically scavenge $\text{O}_2^{\cdot-}$ (Lei et al., 2016). GPx uses glutathione to catalyze the reduction of hydroperoxides, and it is sensitive to cardiovascular risk factor exposure (Cosic et al., 2016). Vitamin E scavenges lipid peroxyl radicals by donating hydrogen from the phenolic group on the chromanol ring, and plays a long-term antioxidant role because of its lipid-solubility (Jiang, 2014; Mates et al., 1999). Furthermore, vitamin E upregulates the expressions of antioxidant genes including SOD and GPx (Bozaykut et al., 2014).

Guiyu, a town in southeast China, is one of the largest electronic waste (e-waste) recycling areas in the world, receiving 1.6 million tons of e-waste every year (Dai et al., 2020; Guo et al., 2012; Xu et al., 2012). E-waste is irregularly dismantled and open-burned, which releases large amounts of various toxic chemicals, including PAHs, into the environment (Guo et al., 2012; Huo et al., 2007; Wu et al., 2010; Wu et al., 2012). A >40-year history of informal recycling activity in Guiyu has contributed to the elevated levels of PAHs in local soil, air and human biospecimens (Guo et al., 2012; Huo et al., 2019; Xu et al., 2015). E-waste pollution does harm to vulnerable populations (Heacock et al., 2016). Our previous studies found that Guiyu children have pollution-related CVD risks, such as cardiovascular endothelial inflammation and sympathetic disorder (Cong et al., 2018; Lu et al., 2018; Zheng et al., 2019). Although the role of PAH exposure in blood-pressure alteration and CVD risk have been explored in several studies, the results in different studies are controversial and the underlying mechanisms remain uncertain, and the role of oxidative stress/antioxidants in PAH-related blood-pressure alteration is unclear. We hypothesized that oxidative stress and further antioxidant decrease could link different levels of PAH exposure to different degrees of blood-pressure alteration. We evaluated this hypothesis in a cross-sectional study of children from Guiyu and a reference area.

2. Materials and methods

2.1. Study population

A total of 403 healthy children (2–7 years old) were recruited from November to December 2016. Among them, 203 were from Guiyu (exposed group), a well-known e-waste-contaminated area, while the others came from Haojiang (reference group), a reference area located 31.6 km to the east of Guiyu. The detailed information about sampling sites has been described previously (Dai et al., 2019). Informed consent was signed by every participant's guardians. Parents/guardians of all

participants completed questionnaires for collecting general demographic characteristics, including residential environment, dietary habits, family economic and cultural levels, and family medical history. Physiological parameters were measured, including height, weight, head circumference, chest circumference, and blood pressure. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Fasting elbow venous blood was collected by coagulation-promoting plain tubes (for serum) and EDTA anticoagulant plain tubes (for plasma), which were aliquoted and stored at -80°C until analysis. Urine in the morning was collected by BD tubes, and stored at -20°C until analysis. This study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Blood pressure measurement

Systolic pressure and diastolic pressure were taken seated in the physical examination in the morning, with standard mercury sphygmomanometers with 14-cm cuffs. The means of three arm-measurements were used as the systolic pressure and diastolic pressure of the participants, and pulse pressure was calculated as systolic pressure minus diastolic pressure.

2.3. Exposure measurement

The following urinary hydroxylated metabolites of eight PAHs were measured by gas chromatography-mass spectrometry (GC-MS) by Campo's method (Campo et al., 2008): 1-hydroxynaphthalene (1-OHNAp), 2-hydroxynaphthalene (2-OHNAp), 2-hydroxyfluorene (2-OHFlu), 9-hydroxyfluorene (9-OHFlu), 2-hydroxyphenanthrene (2-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe) and 1-hydroxypyrene (1-OHPyr). In brief, urine was treated with β -glucuronidase (CNW Technologies GmbH, Germany) at pH 5 for 12 h, and then extracted three times with n-hexane. After drying under a nitrogen stream, the extractant was silanized by BSTFA + 1%TMCS (REGIS Technologies, America), and then subjected to GC-MS (Agilent 7890A-5975C, Agilent Technologies, America). PAH standards were purchased from AccuStandard (America), Dr. Ehrenstorfer GmbH (Germany), Toronto Research Chemicals (Canada) and Chiron (Norway). Deuterium-labeled 1-hydroxynaphthalene-D7 and 1-hydroxypyrene-D9 (Toronto Research Chemicals, Canada) were used as internal standards. The calibration curves showed excellent linearity (r^2 ranged from 0.995 to 0.999). Relative standard deviation (RSD%) was within 0.71%–4.74%. Recoveries for a surrogate standard ranged from 84.56% to 117.38%. $\sum_8\text{OH-PAHs}$ was defined as the sum of eight congeners in urine, while $\sum_{7\text{LMW}}\text{OH-PAHs}$ was defined as the sum of seven low molecular weight congeners in urine, including 1-OHNAp, 2-OHNAp, 2-OHFlu, 9-OHFlu, 2-OHPhe, 4-OHPhe and 9-OHPhe.

2.4. Measurements of antioxidants

Concentrations of serum SOD and GPx, as well as plasma vitamin E were determined by enzyme-linked immunosorbent assay kits (catalog No. LS-F4233, LS-F27702 and LS-F10662 from LifeSpan BioSciences, Inc., America).

2.5. Statistical analysis

Statistical analyses were conducted using SPSS version 22.0 (IBM Corporation, NJ, USA) and GraphPad Prism 5.0 (GraphPad, San Diego, CA). $P < 0.05$ was considered significant, and all tests were two-tailed. Normally distributed data are described by mean $\pm$ standard deviation (SD), whereas non-normally distributed data are described by median [interquartile range (IQR)]. Children were divided to two groups by their residence [Guiyu (exposed group) vs. Haojiang (reference group)] or by the median of PAH exposure (high PAH exposure group vs. low

PAH exposure group). Independent-sample T, chi-square and non-parametric tests were used to evaluate the differences between groups. Spearman correlation analysis was used to evaluate correlations. Multiple linear regression (B, β , 95% CI and P), logistic regression (ORs and 95% CIs) and tertile regression models were used to estimate the associations between PAH exposure, antioxidants and blood pressure. Non-normally distributed dependent variables were log-transformed to normal distribution to adapt to multiple linear regression, while continuous dependent variables were transformed to ordinal variables, by median or tertiles, to adapt to logistic regression. In regression analyses, missing values were removed. Dummy variables were used in tertile regression: response variables were treated as categorical variables divided into tertiles (T1, T2 and T3), with the lowest tertile serving as the reference group (Ernst et al., 2019). Interactions between PAH exposure and antioxidants, which could influence blood pressure, were evaluated by multivariate analysis of variance (MANOVA).

3. Results

3.1. Demographic characteristics of the study population

The demographic characteristics of Guiyu and Haojiang children are shown in Table 1. Age, gender and family history of hypertension in these two groups had no significant difference. Compared with Haojiang children, Guiyu children had lower weight, chest circumference, BMI, and family socioeconomic level, and a higher rate of passive smoking.

3.2. PAH exposure and blood pressure

Compared with Haojiang children, Guiyu children had higher urinary OH-PAH concentrations, lower systolic pressure and lower pulse pressure (Table 1, Fig. 1). In all children, systolic pressure was negatively correlated with urine 1-OHNAp ($r_s = -0.107$, $P < 0.05$), while pulse pressure was negatively correlated to urine $\sum_8\text{OH-PAHs}$, $\sum_{7\text{LMW}}\text{OH-PAHs}$, 1-OHNAp, 2-OHNAp and 2-OHFlu concentrations ($r_s = -0.109$, -0.112 , -0.113 , -0.117 , -0.104 , respectively; all $P < 0.05$). In all children, each increase in $\sum_{7\text{LMW}}\text{OH-PAHs}$ was associated with a 0.091 decrease in log [systolic pressure] ($B = -0.001$, $\beta = -0.091$, 95% CI for B: -0.001 , 0.000 , $P < 0.05$) and a 0.104 decrease in log [pulse pressure] ($B = -0.002$, $\beta = -0.104$, 95% CI for B: -0.003 , 0.000 , $P < 0.05$), both adjusted for gender, age, BMI, passive smoking, education degrees of parents, monthly average family income, and family history of hypertension.

3.3. PAH exposure and antioxidant concentrations

Compared with Haojiang children, Guiyu children had lower serum SOD ($P < 0.05$), GPx ($P < 0.05$) and plasma vitamin E ($P > 0.05$) (Fig. 2, Table 1). Children with $\sum_8\text{OH-PAHs} >$ median had lower SOD concentrations (70.25 ng/mL) than children with $\sum_8\text{OH-PAHs} <$ median (82.41 ng/mL, $P < 0.05$). In all children, serum SOD concentration was negatively correlated to urinary 1-OHNAp and 2-OHFlu concentrations ($r_s = -0.178$, $P < 0.05$; $r_s = -0.242$, $P < 0.01$, respectively), while neither serum GPx nor plasma vitamin E had a significant correlation with PAH exposure. However, in Guiyu children, there was a positive correlation between $\sum_8\text{OH-PAHs}$ and GPx ($r_s = 0.338$, $P < 0.05$). Regression models indicated that the associations between urinary OH-PAHs and blood antioxidants were different in each group: (1) In all children combined, higher PAH exposure was estimated to be associated with lower log [SOD] (but not GPx or vitamin E) (Table 2). (2) In Guiyu children, higher PAH exposure was estimated to be associated with lower log [SOD] (Table 2), higher log [GPx] (Table 2) and lower vitamin E ($\text{OR}_{\sum_{7\text{LMW}}\text{OH-PAHs}} = 0.838$, 95% CI: 0.706, 0.995, adjusted for gender, age, BMI, passive smoking, cooking oil, soy intake, vitamin E supplementation, education degrees of parents, and monthly average family income). (3) In Haojiang children, the increase in PAH exposure was

Table 1
Demographic characteristics of the study population.

Characteristics	Guiyu		Haojiang		P
	n	Mean $\pm$ SD or median (P25, P75) or N (rate)	n	Mean $\pm$ SD or median (P25, P75) or N (rate)	
Age (years)	202	4.60 $\pm$ 0.83	200	4.50 $\pm$ 0.98	0.243 ^a
Gender [n (%)]	203		200		
Male		113 (55.7%)		116 (58.0%)	0.636 ^b
Female		90 (44.3%)		84 (42.0%)	
Passive smoking [n (%)]	203	154 (75.9%)	200	96 (48.0%)	<0.001 ^b
Household income > 6000 yuan/month [n (%)]	176	47 (26.7%)	195	103 (52.8%)	<0.001 ^b
Father with university education [n (%)]	198	18 (9.1%)	197	102 (51.8%)	<0.001 ^b
Mother with university education [n (%)]	197	29 (14.7%)	199	92 (46.2%)	<0.001 ^b
Family history of hypertension [n (%)]	200	59 (29.5%)	200	53 (26.5%)	0.504 ^b
Height (cm)	201	104.27 (99.81, 109.82)	200	106.10 (100.27, 111.84)	0.067 ^c
Weight (kg)	201	16.18 (14.48, 18.16)	199	17.11 (15.47, 19.51)	0.001 ^c
Head circumference (cm)	201	49.36 $\pm$ 1.70	200	49.55 $\pm$ 1.62	0.254 ^a
Chest circumference (cm)	201	49.85 (48.43, 51.85)	200	50.80 (48.86, 52.75)	0.001 ^c
BMI (kg/m ²)	201	14.93 (14.29, 15.89)	199	15.42 (14.51, 16.49)	0.001 ^c
Urine Σ_8 OH-PAHs (μ g/L)	201	6.438 (5.333, 9.894)	200	5.709 (4.688, 7.321)	<0.001 ^c
Plasma vitamin E (μ mol/L)	73	5.44 (4.44, 6.65)	67	5.80 (4.27, 7.12)	0.867 ^c
Systolic pressure (mm Hg)	200	87.33 (83.33, 92.67)	198	90.67 (86.67, 98.00)	<0.001 ^c
Diastolic pressure (mm Hg)	200	55.33 (50.67, 60.00)	198	56.00 (51.33, 60.00)	0.636 ^c
Pulse pressure (mm Hg)	200	32.67 (28.67, 37.17)	198	36.67 (31.33, 40.75)	<0.001 ^c

^a Independent samples *t*-test.

^b Chi-square test.

^c Independent-samples nonparametric test.

estimated to be associated with a decrease in log [GPx] (but not SOD or vitamin E) (Table 2).

3.4. Antioxidants and blood pressure

In all children together, systolic pressure was lower in the low-SOD group (serum SOD concentration < median) than in the high-SOD group (median systolic pressure 87.67 mm Hg vs. 91.33 mm Hg, $P < 0.01$), while diastolic pressure was lower in the low-GPx group (GPx concentration < median) than in the high-GPx group (median diastolic pressure 53.67 mm Hg vs. 58.00 mm Hg, $P < 0.05$). Compared with children in the lowest SOD tertile (T1), children in the middle tertile (T2) were associated with higher diastolic pressure, while children in the highest SOD tertile (T3) were associated with higher systolic pressure and higher pulse pressure (Fig. 3). Compared with the lowest GPx tertile (T1), the middle GPx tertile (T2) and the highest GPx tertile (T3) were associated with higher diastolic pressure and lower pulse pressure (Fig. 3). Vitamin E had no significant independent relationship with blood pressure. Therefore, only endogenous antioxidant enzymes had the potential to prevent PAH-dependent decreases in blood-pressure.

3.5. Interaction between PAH exposure and antioxidants plays a role in blood pressure

SOD yielded a significant main effect for systolic pressure: SOD was independently associated with higher systolic pressure without significant

interaction with PAH-exposure (Table 3). After stratification by level of Σ_8 OH-PAHs, only in children exposed to high levels of PAHs (Σ_8 OH-PAHs > median), systolic pressure and diastolic pressure were lower in the low-SOD group (SOD < median) than in the high-SOD group (median systolic pressure 87.33 mm Hg vs. 92.00 mm Hg, $P < 0.01$; median diastolic pressure 52.00 mm Hg vs. 58.00 mm Hg, $P < 0.05$). Neither the main effect of GPx nor the GPx-by- Σ_8 OH-PAHs interaction effect on blood pressure was found, but diastolic pressure was lower in the low-GPx group only in children exposed to high levels of PAHs (Σ_8 OH-PAHs > median) (median diastolic pressure 52.33 mm Hg vs. 58.67 mm Hg, $P < 0.05$). Vitamin E yielded nonsignificant main effects on blood pressure, but there were significant vitamin E-by- Σ_8 OH-PAHs interaction effects on systolic pressure and pulse pressure (Table 3). Systolic pressure and pulse pressure were lower in the low-vitamin E group (plasma vitamin E < median) than in the high-vitamin E group, which were only found in children exposed to low levels of PAHs (Σ_8 OH-PAHs < median) (86.00 mm Hg vs. 90.00 mm Hg, $P < 0.01$; 31.33 mm Hg vs. 36.33 mm Hg, $P < 0.05$; and Table 3).

4. Discussion

4.1. PAH exposure and blood pressure

The present study found that PAH-exposure level is higher in the e-waste recycling area than the reference area. This is consistent with our previous studies in Guiyu: compared with the reference population, PAH exposure levels in umbilical cord blood, peripheral venous blood

Fig. 1. Urine OH-PAH concentrations in children. Reference group (Haojiang): $n = 200$. Exposed group (Guiyu): $n = 201$. Data are presented as median (interquartile range). * $P < 0.05$, *** $P < 0.001$, analyzed by Mann-Whitney test.

Fig. 2. Comparison of serum concentrations of SOD and GPx between the two groups. Reference group (Haojiang): $n_{\text{SOD}} = 75$, $n_{\text{GPx}} = 72$. Exposed group (Guiyu): $n_{\text{SOD}} = 74$, $n_{\text{GPx}} = 43$. Data are presented as median (interquartile range). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, analyzed by Mann-Whitney test.

and urine are higher in the exposed population (Cheng et al., 2020; Dai et al., 2019; Guo et al., 2012; Huang et al., 2020; Huo et al., 2019; Xu et al., 2013, 2015; Zeng et al., 2020). Elevated PAH levels in environmental and biological samples are also found in other e-waste recycling areas, including Agbogbloshie (Ghana) and Bui Dau (Vietnam) (Asamoah et al., 2019; Feldt et al., 2014; Hoa et al., 2020). In the pyrolysis and combustion of materials combining e-waste and biomass in a laboratory scale horizontal reactor, PAHs are formed and released, with the dominant PAH being naphthalene (Soler et al., 2018). This is consistent with our present results.

In this study, PAH exposure, as measured by eight urinary OH-PAHs, exhibited associations with decreased systolic pressure and pulse pressure in preschool children. This is consistent with our previous and several other studies. Lu et al. found in the same population that e-waste Pb exposure is negatively associated with systolic and pulse pressure in preschool children (Lu et al., 2018). A negative correlation between 1-hydroxypyrene and diastolic pressure through the autonomic nervous system was found in 374 Italian outdoor workers (Sancini et al., 2014). Given that the aryl hydrocarbon receptor (AhR) agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin has been reported to decrease the inotropic response to adrenergic stimulation in the cardiovascular system, it is possible that PAH, an AhR agonist, could decrease blood pressure by decreasing the inotropic response to adrenergic stimulation in cardiovascular system (Canga et al., 1988). Another mechanism of decreased blood pressure is speculated to involve the homeostatic regulation of cardiac output. Given that Guiyu children had higher heart rates, their stroke volume should be decreased by neurohumoral regulation to meet a stable cardiac output (Cong et al., 2018; Siti et al., 2015). On the contrary, PAH exposure is found to be positively associated with blood pressure in several epidemiologic studies whose study design was quite different from the present study. In these studies, 77.4% (Yin et al., 2018), 100% (Wang et al., 2016) and 100% (Jacobs et al., 2012) of the populations were adults in whom cardiovascular events had

already emerged, and the cardiovascular risk factors were explored retrospectively. In the present study, 100% of the population were healthy preschool children, and the cardiovascular risk factors were explored prospectively. Because of the different hemorheology between children and adults, decreases and increases in blood pressure can both reflect vascular dysfunction. Furthermore, no association between PAH exposure and blood pressure was found in 10- to 14-year-old children proximal to an oil refinery in Saudi Arabia (Trasande et al., 2015), which is different from that of the present study, probably because of the different hereditary susceptibilities and living habits. Inconsistent results in different studies probably result from different populations, blood pressure states (normal, acute hypertension or chronic hypertension), confounding factors and exposure doses. More studies, including large-sample epidemiological research and meta-analysis, are needed to identify the exact impact of PAH exposure on blood pressure.

4.2. PAH exposure and antioxidant decrease

In the present study, higher PAH exposure was estimated to be associated with lower SOD in total children and Guiyu children, as well as decreased vitamin E only in Guiyu children. This suggests that excessive oxidative stress associated with PAH exposure contributes to antioxidant consumption, especially in Guiyu children. This result is consistent with a previous study in another e-waste recycling area located in Longtang, Qingyuan, China, where Lu et al. found that PAH exposure contributed from e-waste recycling activities is associated with oxidative stress in people living near e-waste dismantling sites (Lu et al., 2016). The above associations probably derive from ROS produced during cytochrome P450-mediated metabolism of PAHs in vivo. This explanation is dependent on an animal study which found that benzo(a) pyrene exposure increased liver CYP1A1 and hepatic lipid peroxidation levels, but reduced gastric SOD activity, suggesting that ROS produced by PAH exposure consumes SOD by CYP450-mediated metabolism

Table 2
Linear regression of urine OH-PAHs and blood antioxidants.

Population	Urine OH-PAHs	Log [SOD] β (95% CI)	Log [Gpx] β (95% CI)
Total children	2-OHFlu	-0.154 (-0.229, -0.002)*	-
Guiyu children	Total $\sum_8$ OH-PAHs	-0.099 (-0.008, 0.003)	0.387 (0.011, 0.094)*
	Low $\sum_8$ OH-PAHs (T1)	0	0
	Medium $\sum_8$ OH-PAHs (T2)	-0.350 (-0.134, -0.015)*	0.034 (-0.389, 0.466)
	High $\sum_8$ OH-PAHs (T3)	-0.272 (-0.109, 0.002)	0.465 (0.096, 0.844)*
	Total $\sum_{7\text{LMW}}$ OH-PAHs	-0.102 (-0.008, 0.003)	0.386 (0.011, 0.094)*
	Low $\sum_{7\text{LMW}}$ OH-PAHs (T1)	0	0
	Medium $\sum_{7\text{LMW}}$ OH-PAHs (T2)	-0.327 (-0.129, -0.009)*	0.122 (-0.292, 0.567)
	High $\sum_{7\text{LMW}}$ OH-PAHs (T3)	-0.279 (-0.111, 0.001)	0.524 (0.140, 0.918)*
	Low $\sum_{7\text{LMW}}$ OH-PAHs (T1)	0	0
	Medium $\sum_{7\text{LMW}}$ OH-PAHs (T2)	-	-0.102 (-0.263, 0.118)
	High $\sum_{7\text{LMW}}$ OH-PAHs (T3)	-	-0.332 (-0.487, -0.053)*
Haojiang children			

All were adjusted for gender, age, BMI, passive smoking, education degrees of parents, and monthly average family income. Low (T1), medium (T2) and high (T3) $\sum_8$ OH-PAHs were derived by the tertiles of $\sum_8$ OH-PAHs. Low (T1), medium (T2) and high (T3) $\sum_{7\text{LMW}}$ OH-PAHs were derived by the tertiles of $\sum_{7\text{LMW}}$ OH-PAH. - Model cannot be fitted.

* $P < 0.05$.

Fig. 3. Coefficients (B and 95% CIs) of antioxidants for log [systolic pressure], log [diastolic pressure] and log [pulse pressure]. Results from multiple linear regression models adjusted for gender, age, BMI, passive smoking, education degrees of parents, monthly average family income, and family history of hypertension. Response variables are divided into tertiles (T1, T2 and T3), with the lowest tertile (T1) serving as the reference group. * $P < 0.05$. /Model cannot be fitted. High-PAH children: Children with urinary $\sum_8\text{OH-PAHs} > \text{the median } 5.92 \mu\text{g/L}$. Low-PAH children: Children with urinary $\sum_8\text{OH-PAHs} < \text{the median } 5.92 \mu\text{g/L}$.

(Badary et al., 2003). A reasonable explanation for the associations only found in Gujiyu children instead of Haojiang children is their higher body burden of toxic substances (Song and Li, 2014).

Oxidative stress and antioxidant disorder in the present study is in line with our previous studies. Li et al. found that the concentrations of antioxidant enzymes catalase and glutathione S-transferase omega-1 in the exposed group were lower than in reference umbilical cord tissue samples, suggesting excessive oxidative stress and a disordered antioxidant system in children exposed to e-waste (Li et al., 2018). Oxidative stress associated with e-waste pollution was also found in another e-waste recycling area located in Longtang, Qingyuan (Lu et al., 2017; Zhang et al., 2016). The negative effect of PAH exposure on SOD concentration in our study is consistent with in vitro experiments and epidemiology research, which indicate that PAHs reduce SOD activity by increasing ROS (Awodele et al., 2015; Ke et al., 2018). The effect of PAH exposure on blood vitamin E is not constant in the limited amount

of previous studies. Gawlik et al. found in rats that after exposure to benzo[a]pyrene, plasma lipid peroxidation levels are elevated, while erythrocyte vitamin E levels are reduced, suggesting that ROS produced by PAH exposure can exceed the protective capacity of vitamin E, which is in line with the present study (Gawlik et al., 2012). Contrarily, an elevated level of plasma vitamin E is found in seabirds exposed to PAHs in crude oil, resulting from uncontrolled diets or enhanced oxidative resistance caused by acute exposure (Perez et al., 2010). The increased vitamin E after PAH exposure in other studies could be explained by an adaptive response: the ability of a cell, tissue, or organism to better resist (oxidative) stress damage by prior exposure to a lesser amount of (oxidative) stress, and acute exposure could lead to enhanced oxidation resistance (Crawford and Davies, 1994; Sureda et al., 2018). In the present study, although the concentration of GPx in Gujiyu children was lower than in Haojiang children, PAH exposure was estimated to be associated with higher log [GPx] in Gujiyu children and decreased log

Table 3
Multivariate analysis of variance of blood pressure.

Total children	Factors	Response variables (mean $\pm$ SD)	P
Model 1		Log (systolic pressure)	0.009
Main effect	SOD (1, 2)	1.945 $\pm$ 0.036 vs. 1.967 $\pm$ 0.045	0.005
	$\sum_8\text{OH-PAHs}$ (1, 2)	1.955 $\pm$ 0.040 vs. 1.952 $\pm$ 0.041	0.497
Interaction	SOD (1, 2) by $\sum_8\text{OH-PAHs}$ (1, 2)		0.150
Model 2		Log (systolic pressure)	0.004
Simple effect	SOD (1, 2) within $\sum_8\text{OH-PAHs}$ (1)	1.955 $\pm$ 0.041 vs. 1.964 $\pm$ 0.047	0.297
	SOD (1, 2) within $\sum_8\text{OH-PAHs}$ (2)	1.940 $\pm$ 0.032 vs. 1.970 $\pm$ 0.434	0.002
Model 3		Log (systolic pressure)	0.016
Main effect	Vitamin E (1, 2)	1.949 $\pm$ 0.042 vs. 1.963 $\pm$ 0.040	0.051
	$\sum_8\text{OH-PAHs}$ (1, 2)	1.955 $\pm$ 0.040 vs. 1.952 $\pm$ 0.041	0.813
Interaction	Vitamin E (1, 2) by $\sum_8\text{OH-PAHs}$ (1, 2)		0.011
Model 4		Log (systolic pressure)	0.006
Simple effect	Vitamin E (1, 2) within $\sum_8\text{OH-PAHs}$ (1)	1.940 $\pm$ 0.037 vs. 1.972 $\pm$ 0.044	0.001
	Vitamin E (1, 2) within $\sum_8\text{OH-PAHs}$ (2)	1.957 $\pm$ 0.045 vs. 1.952 $\pm$ 0.034	0.667
Model 5		Log (diastolic pressure)	0.033
Main effect	SOD (1, 2)	1.735 $\pm$ 0.055 vs. 1.751 $\pm$ 0.049	0.117
	$\sum_8\text{OH-PAHs}$ (1, 2)	1.737 $\pm$ 0.062 vs. 1.743 $\pm$ 0.054	0.115
Interaction	SOD (1, 2) by $\sum_8\text{OH-PAHs}$ (1, 2)		0.090
Model 6		Log (diastolic pressure)	0.044
Simple effect	SOD (1, 2) within $\sum_8\text{OH-PAHs}$ (1)	1.752 $\pm$ 0.053 vs. 1.750 $\pm$ 0.051	0.950
	SOD (1, 2) within $\sum_8\text{OH-PAHs}$ (2)	1.723 $\pm$ 0.054 vs. 1.752 $\pm$ 0.048	0.013
Model 7		Log (pulse pressure)	0.043
Main effect	Vitamin E (1, 2)	1.519 $\pm$ 0.098 vs. 1.547 $\pm$ 0.088	0.061
	$\sum_8\text{OH-PAHs}$ (1, 2)	1.541 $\pm$ 0.090 vs. 1.524 $\pm$ 0.089	0.329
Interaction	Vitamin E (1, 2) by $\sum_8\text{OH-PAHs}$ (1, 2)		0.042
Model 8		Log (pulse pressure)	0.027
Simple effect	Vitamin E (1, 2) within $\sum_8\text{OH-PAHs}$ (1)	1.493 $\pm$ 0.103 vs. 1.555 $\pm$ 0.095	0.007
	Vitamin E (1, 2) within $\sum_8\text{OH-PAHs}$ (2)	1.541 $\pm$ 0.090 vs. 1.538 $\pm$ 0.080	0.860

Factors are ordinal variables: values lower than the median are assigned as 1, while values higher than the median are assigned as 2. Response variables are compared between these 2 levels of factors.

[GPx] in Haojiang children in the highest tertile of $\sum_{7\text{LMW}}\text{OH-PAHs}$, suggesting a residence-dependent and dose-dependent association between PAH exposure and GPx. The anomalous positive association between PAH exposure and GPx in Guiyu children and other studies (Guo et al., 2017; Kamal et al., 2015; Li et al., 2013) may result from an adaptive response involving the ability of a cell, tissue, or organism to better resist (oxidative) stress damage by prior exposure to a lesser amount of (oxidative) stress (Crawford and Davies, 1994). The negative effect of PAH exposure on GPx concentration in Haojiang children with high PAH levels ($\sum_{7\text{LMW}}\text{OH-PAHs}$ T3) is in line with animal experiments, which found that PAH exposure from oil-leakage reduced GPx activity in fish liver, and that intraperitoneal injection of BaP significantly repressed the expression of *gpx1* in mice (Lin et al., 2018; Smeltz et al., 2017). Decreased GPx in Haojiang children and other studies could be explained by antioxidant consumption. As a result, we suggest that low-level PAH exposure may stimulate antioxidant activity through an adaptive response, while high-level PAH exposure may consume or down-regulate antioxidants through excessive oxidative stress produced by PAHs.

4.3. Antioxidants and blood pressure

In the present study, SOD and GPx were estimated to reverse the decreasing trend of blood pressure related to PAH exposure after adjusting confounding factors, while vitamin E had no significant association with blood pressure. This suggests that endogenous antioxidant enzymes have a significant potential to revert blood-pressure alteration independently, which was not found for vitamin E, an exogenous antioxidant. It is reasonable to suggest that antioxidants, especially endogenous antioxidant enzymes, could revert blood-pressure disorder by scavenging the ROS associated with PAH exposure. Oxidative stress could disturb blood-pressure regulation by inhibiting baroreceptor activity and disturbing sympathetic activity (Chen et al., 2019; Li et al., 1996). Antioxidant therapy has been suggested in several studies to restore normal baroreflex function by decreasing levels of ROS (Bruno et al., 2012; Garcia et al., 2017; Hatcher et al., 2016). Interestingly, antioxidants play a suppressing role in blood pressure in the hypertensive (Ahmad et al., 2017). A reasonable explanation is that the autonomic regulation of blood pressure in the hypertensive is down-regulated, while the autonomic regulation for blood pressure in Guiyu children (healthy children with relatively low blood pressure) is up-regulated.

4.4. Interaction between PAH exposure and antioxidants in blood-pressure alteration

Although PAHs, SOD, GPx and vitamin E are suggested to influence blood pressure independently in many studies, no study has shown their interactions (Ahmad et al., 2017). In the present study, both the SOD decrease and GPx decrease could independently link PAH exposure to blood-pressure decrease, whereas vitamin E could only link them by the interaction with PAH exposure, suggesting that SOD and GPx could be used as better biomarkers, than vitamin E, of abnormal vascular function resulting from PAH exposure in healthy children. Furthermore, the role of antioxidants in blood pressure could be influenced by PAH-exposure level: SOD and GPx could reverse the decreasing trend of blood pressure, especially in children exposed to high levels of PAHs ($\sum_8\text{OH-PAHs}$ > the median 5.92 $\mu\text{g/L}$), whereas vitamin E could reverse the decreasing trend of blood pressure only in children exposed to low levels of PAHs ($\sum_8\text{OH-PAHs}$ < the median 5.92 $\mu\text{g/L}$). It suggested that the protection by endogenous antioxidant enzymes against PAH exposure is still effective at high exposure levels ($\sum_8\text{OH-PAHs}$ > the median 5.92 $\mu\text{g/L}$), whereas the protection from the exogenous antioxidant vitamin E is only effective at low PAH-exposure levels ($\sum_8\text{OH-PAHs}$ < the median 5.92 $\mu\text{g/L}$). A possible explanation is that a PAH-activated AhR and vitamin E both could upregulate the expression of SOD and GPx through the Keap1-Nrf2-ARE pathway, leading to stronger direct

antioxidant capacity of SOD and GPx than vitamin E alone (Bozaykut et al., 2014; Cho and Kleeberger, 2015; Dietrich, 2016).

4.5. Limitations

First, results in the present study should be interpreted with caution because it is a cross-sectional study, and the subjects were all healthy children with neither hypertension nor hypotension. Second, Guiyu children are exposed to multiple toxic substances, but the present study is focused only on eight OH-PAHs. Our results are still meaningful since a prior large-sample study was unable to find an association of blood pressure with either lead exposure or cadmium exposure in children (Cao et al., 2009; Chen et al., 2006). Although we cannot evaluate all toxic chemicals in limited amounts of urine samples, associations found in the present study are still meaningful because children were stratified by urinary OH-PAH levels in the statistical models. Further studies should evaluate more PAHs and other toxic substances. Third, blood pressure in one morning is difficult to reflect the long-term blood pressure status, so we would like to measure blood pressure on at least three different days in further studies. Blood pressure in both the supine and upright positions will be measured for more information in further studies. Fourth, although animal experiments have proven that PAH exposure induces blood-pressure alterations and oxidative stress in the cardiovascular system, further studies are needed to explain the causality, direction and extent of the associations. Fifth, the complex interaction between genetic factors and PAH exposure in blood-pressure alterations need to be explored in further research.

5. Conclusions

The current study supports the hypothesis that children with elevated exposure levels of PAHs have exacerbated oxidative stress and decreased antioxidant concentrations, which may foretell blood-pressure disorders in e-waste recycling areas. SOD and GPx may be effective biomarkers of abnormal vascular function resulting from PAH exposure in healthy children. The preventive role of antioxidants could be influenced by PAH-exposure levels: SOD and GPx could reverse the decreasing trend of blood pressure, especially in children exposed to high levels of PAHs, whereas vitamin E could reverse the decreasing trend of blood pressure only in children exposed to low levels of PAHs. The current study provides a reference for CVD primary prevention, especially for child exposure to e-waste pollution. To explore the exact role of PAH-related oxidative stress in CVD risk, more probing experimentation of a clinical translational character is needed.

CRediT authorship contribution statement

Qihua Wang: Investigation, Writing - original draft. **Xijin Xu:** Conceptualization, Project administration. **Zhijun Zeng:** Methodology, Data curation, Formal analysis. **Xiangbin Zheng:** Formal analysis, Writing - review & editing. **Kai Ye:** Investigation. **Xia Huo:** Funding acquisition, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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