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Assessment of the association of exposure to polycyclic aromatic hydrocarbons, oxidative stress, and inflammation: A cross-sectional study in Augsburg, Germany

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ABSTRACT

Background: Exposure to polycyclic aromatic hydrocarbons (PAHs) has been linked to acute and chronic health effects through the suggested pathways of oxidative stress and inflammation. However, evidence is still limited. We aimed to investigate jointly the relationship of PAHs, oxidative stress, and inflammation.

Methods: We measured 13 biomarkers of PAH exposure (n = 6: hydroxylated polycyclic aromatic hydrocarbons, [OH-PAHs]), oxidative stress (n = 6: malondialdehyde (MDA); 8-hydroxy-2′-deoxyguanosine (8-OHdG); and 4 representatives of the compound class of $F_{2\alpha}$-isoprostanes) in urine, and inflammation (n = 1: high-sensitivity C-reactive protein, [hs-CRP]) in serum from 400 participants at the second follow-up (2013/2014) of the German KORA survey S4. Multiple linear regression models were applied to investigate the interplay between biomarkers.

Results: Concentrations of biomarkers varied according to sex, age, smoking status, season, and a history of obesity, diabetes, or chronic kidney disease. All OH-PAHs were significantly and positively associated with oxidative stress biomarkers. An interquartile range (IQR) increase in sum OH-PAHs was associated with a 13.3% (95% CI: 9.9%, 16.9%) increase in MDA, a 6.5% (95% CI: 3.5%, 9.6%) increase in 8-OHdG, and an 8.4% (95% CI: 6.6%, 11.3%) increase in sum $F_{2\alpha}$-isoprostanes. Associations were more pronounced between OH-PAHs and $F_{2\alpha}$-isoprostanes but also between OH-PAHs and 8-OHdG for participants with potential underlying systemic inflammation (hs-CRP ≥ 3 mg/L). We observed no association between OH-PAHs and hs-CRP levels. While 8-OHdG was significantly positively associated with hs-CRP (13.7% [95% CI: 2.2%, 26.5%] per IQR increase in 8-OHdG), $F_{2\alpha}$-isoprostanes and MDA indicated only a positive or null association, respectively.

Conclusion: The results of this cross-sectional study suggest, at a population level, that exposure to PAHs is associated with oxidative stress even in a low exposure setting. Oxidative stress markers, but not PAHs, were associated with inflammation. Individual risk factors were important contributors to these processes and should be

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considered in future studies. Further longitudinal studies are necessary to investigate the causal chain of the associations.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are non-polar, semi-volatile, organic pollutants composed of several aromatic rings (Keyte et al., 2013). They are ubiquitous and hazardous pollutants which are generated during incomplete combustion of organic materials. Traffic emissions, domestic combustion, and industry practices are suggested to be the main anthropogenic sources of PAHs in urban areas (Kamal et al., 2015).

A large proportion of anthropogenically and naturally generated PAHs is occurring and transported in the air, and here – depending on physico-chemical parameters – bound to particles or in the gas phase (Kraus et al., 2011; Li et al., 2021; Liu et al., 2019). Worldwide, strong efforts are performed to control and decrease the ambient PAH-concentrations by creating limiting regulations and applying new technologies for anthropogenic processes such as heating, industrial processes and traffic. This is on the one hand reflected by relatively low PAH concentrations in our study region as previously reported (Li et al., 2018). On the other hand, depending on the current meteorological conditions (season in general but also specific events), PAHs levels can be significantly increased, either nationwide or only at specific sites (Fuchte et al., 2022). Additionally, it is expected that climate change has an impact on PAH-levels (Garrido et al., 2014) which is not yet fully understood. PAHs are one of the major groups of ambient pollutants that cause severe health effects and have been investigated for decades. Lipophilic PAHs can be absorbed via dermal, respiratory, or ingestion routes (Andersen et al., 2018; VanRooij et al., 1993). Short-term exposure to PAHs can cause acute health effects such as eye and skin irritation, headache, nausea, and vomiting. They can also induce inflammatory processes (Al-Delaimy et al., 2014). Long-term exposure to PAHs can lead to chronic health issues, such as chronic obstructive pulmonary disease, diabetes, and cardiovascular diseases (Alshaarawy et al., 2016; Cao et al., 2020; Yang et al., 2017). PAHs were also related to oxidative stress, genotoxicity, and carcinogenicity in both in vitro and in vivo studies (Danielsen et al., 2011; Kumar et al., 2020; Lan et al., 2004; Lu et al., 2016; McCarrick et al., 2019).

Individuals are always exposed to complex mixtures of low molecular weight, medium molecular weight and high molecular weight PAHs, and with current analytical methods it is impossible to comprehensively reflect the ways of metabolic transformation and excretion of all PAHs after their absorption in the organism. The use of biomarkers allows the assessment of the individual, internal PAH burden, and the determination of monohydroxylated PAHs (OH-PAHs) in urine have been previously used for this purpose (Aquilina et al., 2010; Ifegwu and Anyakora, 2016; Mesquita et al., 2014; Urbancova et al., 2016). In this study, we determined 1-OH-pyrene, the main urinary metabolite of pyrene, and five urinary isomeric OH-phenanthrenes originating from phenanthrene. Pyrene and phenanthrene are medium molecular weight PAHs, which are both abundant in typical environmental PAH-mixtures together with further, especially higher molecular weight PAHs. In contrast to those higher molecular weight PAHs, the OH-PAH metabolites of pyrene and phenanthrene can be reliably determined in low volume urine samples, and their concentrations – especially 1-hydroxypyrene concentrations - can be used for estimating the individual exposure to PAHs.

Oxidative stress is an important pathway linking exposure to ambient pollution and acute and chronic diseases (Peters et al., 2021). The induction of a disease process begins with the generation of oxidative stress in the organism. Once the pollutants are absorbed, the formation of reactive oxygen species (ROS) such as peroxides, superoxides, hydroxyl radicals, and singlet oxygen can be initiated (Apel and Hirt, 2004; Tao et al., 2003). These species can attack and modify adjacent macromolecules such as proteins, DNA, and lipids in vivo (Risom et al., 2005). Humans have protective mechanisms against ROS, such as antioxidants, or the activation of specific enzymatic processes to remove ROS species and maintain the oxidative stress balance. If this subtle balance is disturbed (Droge, 2002), oxidative stress and the resulting attack on macromolecules can lead to acute and chronic diseases of the respiratory, cardiovascular, or immunological systems (McCord, 1993; Michael et al., 2013; Miller, 2020; Taverne et al., 2013).

Due to the high reactivity of ROS species, direct quantification is critical. Some by-products or end products of oxidative stress that are excreted through faeces or urine can be quantified. We selected the established biomarkers malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and the compound class of $F_{2\alpha}$-isoprostanes. MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes reflect the amount of damaged double bonds of polyunsaturated fatty acids (PUFAs) (Ayala et al., 2014; Yoon et al., 2012), damaged DNA (Evans et al., 2010; Valavanidis et al., 2009), and damaged membrane phospholipids (Galano et al., 2017; Milne et al., 2008; Morrow and Roberts, 1996), respectively. Using a combination of oxidative stress biomarkers increases reliability when assessing individual oxidative stress levels (Zhu et al., 2021).

Inflammation is another possible pathway for air pollution-initiated health effects. Previous epidemiological studies have reported that long-term exposure to ambient pollutants is associated with increased serum levels of C-reactive protein (CRP), a well-known marker for inflammation (Everett et al., 2010; Hennig et al., 2014; Ostro et al., 2014; Pilz et al., 2018). Toxicological studies have led to similar observations—exposure to air pollution induces inflammatory responses such as increased CRP concentrations in human blood (Chuang et al., 2007). Systemic inflammation is induced by ambient air pollution via the production of cytokines such as tumor necrosis factor-α and interleukin-8 (Pope et al., 2016). Previous studies also indicated that PAH exposure is positively associated with oxidative stress and inflammation (Clark et al., 2012; Everett et al., 2010; Farzan et al., 2016; Ferguson et al., 2017; Gerlofs-Nijland et al., 2009; Lu et al., 2016; Vattanasit et al., 2014).

Although many studies have investigated the associations between OH-PAHs and oxidative stress or between OH-PAHs and inflammation or OH-PAHs and lifestyle factors and health characteristics, only two epidemiological studies have jointly considered the interplay, however both being limited by small and/or highly selected populations (Ferguson et al., 2017; Zhang et al., 2020). To fill this gap, we conducted this cross-sectional study among the general adult population and examined if (1) the concentration of OH-PAHs, oxidative stress markers, and high-sensitivity CRP (hs-CRP) varied among subgroups; (2) OH-PAHs were associated with oxidative stress, including potential effect modification by underlying systemic inflammation because large amount of ROS could be generated during the inflammatory process and disturb the balance (Fialkow et al., 2007); (3) OH-PAHs were associated with hs-CRP and (4) the selected oxidative stress markers were similarly associated with hs-CRP.

2. Methods

2.1. Study population

We included a selected subgroup of 400 subjects at different stages of impaired glucose metabolism without prior cardiovascular disease who participated in 2013/2014 in the second follow-up (FF4) of the baseline KORA (Cooperative Health Research in the region of Augsburg) S4 study (1999–2001, N = 4261) (Bamberg et al., 2017). Participants were invited to the study center in Augsburg, where they an-

swered a computer-assisted personal interview and completed a self-administered questionnaire. All individuals were physically examined, and urine and blood samples were collected. The general KORA study design, sampling method, and data collection have been described in detail by Holle et al. (2005). All participants provided written informed consent to participate in the study which was approved by the ethics committee of the Bavarian Medical Association.

2.2. Urinary biomarker measurements

For each participant, a spot urine sample was collected. For further processing, the samples were stored at −80 °C in a central storage unit. All urinary biomarkers were analysed in our lab in 2015/16 using previously established liquid chromatography (LC)- based methods. OH-PAHs (1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 3-hydroxyphenanthrene; 4-OH-Phe, 4-hydroxyphenanthrene; 9-OH-Phe, 9-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene) were measured on an Ultimate 3000 HPLC system with an RF 2000 fluorescence detector (Thermo Scientific, Dreieich, Germany) (Lintelmann et al., 2018). MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes (2,3-dinor-8-iso-$PGF_{2\alpha}$, 8-iso-15(R)-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, and ±5-$iPF_{2\alpha}$) were determined with LC-mass spectrometry, using a triple quadrupole mass spectrometer API-4000 (AB Sciex, Darmstadt, Germany) equipped with an electrospray ion source (ESI) (Wu et al., 2017). The sum concentrations of OH-PAHs (1-OH-Phe, 2-OH-Phe, and 3-OH-Phe) (Hou et al., 2019; Lu et al., 2016) and the sum concentration of $F_{2\alpha}$ -isoprostanes (Montuschi et al., 2004) were calculated. OH-PAHs, biomarkers of oxidative stress, and creatinine concentrations which were below the limit of detection (LOD) were set to half of the concentration of the LOD ($N_{1\text{–OH-Phe}} = 8$, $N_{2\text{–OH-Phe}} = 19$, $N_{3\text{–OH-Phe}} = 10$, $N_{4\text{–OH-phe}} = 152$, $N_{9\text{–OH-Phe}} = 260$, $N_{1\text{–OH-Pyr}} = 14$, $N_{MDA} = 0$, $N_{8\text{-OHdG}} = 0$, $N_{2,3\text{-dinor-8-iso-PGF2}\alpha} = 2$, $N_{8\text{-iso-15(R)-PGF2}\alpha} = 4$, $N_{8\text{-iso-PGF2}\alpha} = 6$, $N_{\pm 5\text{-iPF2}\alpha} = 1$, $N_{creatinine} = 0$). Since more than 20% of 4-OH-Phe concentrations and 9-OH-Phe concentrations were below the LOD, we did not consider 4-OH-Phe and 9-OH-Phe in later data analysis. Creatinine was quantified on an HP 1100 LC system with an ultraviolet detector (Agilent, St. Clara, CA, USA) (Wu et al., 2017). The concentrations of urinary biomarkers were normalised to creatinine concentrations.

2.3. Individual characteristics and clinical parameters

Fasting venous blood samples were collected during the study participants' visits and stored at 4 °C until further processing. Hs-CRP concentrations in serum samples were assayed by latex-enhanced immunonephelometry on a BN II platform (Siemens Healthcare Diagnostics Product GmbH, Marburg, Germany) with an intra-assay coefficient variation of 2.13% (Pilz et al., 2018). Serum creatinine concentrations were determined using an automated Jaffé method (Technicon, SMAC autoanalyzer; Tarrytown, New York, USA) (Aumann et al., 2015). As a marker for kidney disease, we calculated the estimated glomerular filtration rate (eGFR) (mL/min per 1.73 m^2) using the 2009 CKD-EPI creatinine equation (Levey et al., 2009). Body mass index (BMI) was calculated as the weight divided by height (squared). Type 2 diabetes was validated either by an oral glucose tolerance test, a previous diagnosis, or a current intake of glucose-lowering agents. Pre-diabetes was defined as impaired fasting glucose and/or impaired glucose tolerance. Hypertension was defined as blood pressure >140/90 mmHg or treatment of known hypertension (WHO, 1999).

2.4. Ambient air pollution

Long-term ambient air pollution exposure was estimated using land use regression models for all KORA participants' residential addresses (Wolf et al., 2017). Ambient concentrations of particulate matter smaller than 2.5 μm in aerodynamic diameter ($PM_{2.5}$) and nitrogen dioxide (NO_2) were measured at 20 and 40 sites, respectively, between March 2014 and April 2015, and temporally adjusted for discontinuous site measurements. The annual average concentrations were then modelled using linear regression incorporating predictors such as traffic, land use, population, and building density. Details of the estimation can be found in our previous publication (Wolf et al., 2017).

2.5. Statistical analysis

Spearman correlation coefficients were calculated to explore the relationships of urinary biomarkers, individual characteristics, and clinical parameters.

We performed Kruskal-Wallis tests to compare biomarker concentrations across the subgroups. Obesity was defined as BMI ≥30 kg/m^2, potential underlying inflammation was defined by the hs-CRP concentration ≥3 mg/L, and potential renal impairment as eGFR <90 mL/min/1.73 m^2 (Inker et al., 2012). Seasons were defined as: spring: March to May; summer: June to August; autumn: September to November; winter: December to February.

We used multiple linear regression models to investigate the associations between i) OH-PAHs and oxidative stress, ii) OH-PAHs and hs-CRP, and iii) oxidative stress and hs-CRP. The normalised concentrations of urinary biomarkers and the concentration of hs-CRP were log-transformed to approximate normal distribution of the residuals and to stabilise the variance. In the base model, we included age, sex, smoking, and season as potential confounders, as suggested in a previous study (Yang et al., 2015). The time trend was included to adjust for potential fluctuations during the study period. The smoothing parameter for the trend was chosen by optimising the generalised cross-validation criteria (Wood, 2006). In an extended model, we additionally adjusted for obesity, diabetes, and potential renal impairment. We further included an interaction term to investigate the potential effect modification by underlying systemic inflammation. To evaluate the robustness of our results, we altered our base model adjustment by (1) removing season or (2) additionally adjusting for annual mean concentrations of ambient $PM_{2.5}$ and NO_2 exposure (Spearman correlation 0.71). All statistical analyses were performed using the R Statistical Software (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). A two-sided P value < 0.05 was considered to be statistically significant. All effect estimates are presented as percent change of the geometric mean of the biomarkers with corresponding 95% confidence intervals for an interquartile range increase in exposure concentration.

3. Results

3.1. Study population

From the original group of 400 participants, 18 subjects were excluded due to missing values in the main outcomes (OH-PAHs: N = 7; MDA: N = 8; 8-OHdG: N = 9; $F_{2\alpha}$-isoprostanes: N = 9; creatinine: N = 6; extremely low creatinine concentration: N = 1; hs-CRP: N = 4). The mean age of the participants was 56 years (Table 1). Overall, 220 (57%) participants were male, 78 (20%) were smokers, and 163 (43%) reported a smoking history. The geometric mean concentration of hs-CRP was 1.31 mg/L. In total, 80 participants (21%) showed potential underlying inflammation (hs-CRP ≥ 3 mg/L). Overall, 53 participants were diagnosed with diabetes (14%), and 156 participants were diagnosed with pre-diabetes (41%).

The geometric means of the sum OH-PAHs concentration and the concentrations of 1-OH-Phe, 2-OH-Phe, 3-OH-Phe, and 1-OH-Pyr were 0.24, 0.11, 0.05, 0.06, and 0.16 ng/mg creatinine, respectively. The geometric means of the sum of $F_{2\alpha}$-isoprostanes concentration and the concentration of 2,3-dinor-8-iso-$PGF_{2\alpha}$ 8-iso-15(R)-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, and ±5-$iPF_{2\alpha}$ were 3.56, 1.71, 0.44, 0.22, and 1.06 ng/mg creatinine,

Table 1
Descriptive statistics of the study population (N = 400).

Characteristics	Mean ± SD or Total N (%)	Missing N
Personal Characteristics		
Age (years)	56 ± 9.2	
<55	173 (45%)	
≥55, <65	126 (33%)	
≥65	83 (22%)	
Sex (male)	220 (57%)	
Socio-economic & lifestyle characteristics		
BMI (kg/m^2)	28.1 ± 4.8	
Obese (BMI ≥30 kg/m^2)	116 (30.4%)	
Smoking Status		
Non-smoker	141 (37%)	
Ex-smoker	163 (43%)	
Smoker	78 (20%)	
Clinical Characteristics		
hs-CRP (mg/L) (N = 382)	2.39 ± 3.36	4
Potential underlying inflammation (hs-CRP ≥ 3 mg/L)	80 (21%)	4
eGFR (mL/min/1.73 m^2)	89.9 ± 9.3	7
Potential renal impairment (eGFR <90 mL/min/1.73 m^2)	179 (47%)	7
Diabetes Status		
No Diabetes	173 (45%)	
Pre-diabetes	156 (41%)	
Diabetes	53 (14%)	
Hypertension Status		
Hypertension	130 (34%)	
Characteristics of the day of examination		
Season		
Spring (Mar–May)	129 (34%)	
Summer (Jun–Aug)	98 (26%)	
Autumn (Sep–Nov)	71 (18%)	
Winter (Dec–Feb)	84 (22%)	
Urinary biomarkers (ng/mg Creatinine)		
OH-PAHs	0.34 ± 0.39	7
1-OH-Phe	0.16 ± 0.18	7
2-OH-Phe	0.08 ± 0.12	7
3-OH-Phe	0.10 ± 0.14	7
1-OH-Pyr	0.30 ± 0.39	7
MDA	41.76 ± 38.69	8
8-OHdG	3.35 ± 1.93	9
$F_{2\alpha}$-isoprostanes	4.11 ± 2.92	9
2,3-dinor-8-iso-$PGF_{2\alpha}$	2.08 ± 1.63	7
8-iso-15(R)-$PGF_{2\alpha}$	0.52 ± 0.1	7
8-iso-$PGF_{2\alpha}$	0.27 ± 0.23	9
±5-i$PF_{2\alpha}$	1.24 ± 0.99	7
Creatinine (mg/mL)	1.34 ± 0.81	6
Annual mean of individual ambient exposure (μg/m^3)		
$PM_{2.5}$	11.72 ± 1.01	
NO_2	13.62 ± 4.35	

SD, standard deviation; OH-PAHs, sum OH-PAHs concentration; 1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 1-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; $F_{2\alpha}$-isoprostanes, sum $F_{2\alpha}$-isoprostanes concentration; BMI, Body Mass Index; hs-CRP, high sensitivity C-Reactive Protein; eGFR, Estimated Glomerular Filtration Rate from Serum Creatinine and Cystatin C; $PM_{2.5}$, particulate matter smaller than 2.5 μm in aerodynamic diameter; NO_2, nitrogen dioxide.

respectively. The geometric mean concentrations of MDA, 8-OHdG, and creatinine in the study participants were 33.77 ng/mg creatinine, 2.94 ng/mg creatinine and 1.08 mg/mL, respectively. The annual means of individual ambient exposure of $PM_{2.5}$ and NO_2 were 11.72 μg/m^3 and 13.62 μg/m^3, respectively. Supplemental Fig. 1 shows the Spearman correlation coefficients between all pairwise combinations of biomarkers and clinical parameters. Strong correlations were observed within the $F_{2\alpha}$-isoprostane group (0.54–0.90) and the OH-PAH group (0.78–0.93). Therefore, we limited parts of the subsequent analyses to the sum of OH-PAH concentrations and the sum of $F_{2\alpha}$-isoprostanes concentrations as indicators to reduce the number of tests.

3.2. Distribution of biomarker levels across different subgroups

Table 2 shows the median concentrations of biomarkers in the selected subgroups. MDA concentrations were significantly higher in participants with higher age (≥55 years), potential underlying inflammation, potential renal impairment, or in participants who had their examinations in spring/winter. 8-OHdG concentrations were significantly higher in older participants (age ≥55 years). Concentrations of $F_{2\alpha}$-isoprostanes were significantly higher in female participants or participants who visited the study center in autumn/winter. Hs-CRP concentrations were significantly higher in older participants (≥65 years), potential renal impairment, diabetes, obesity, or hypertension.

3.3. Association between OH-PAHs and biomarkers of oxidative stress

All OH-PAHs were significantly positively associated with oxidative stress biomarkers (Fig. 1). For participants with a potential underlying state of inflammation (hs-CRP ≥ 3 mg/L), we observed stronger associations between OH-PAHs and $F_{2\alpha}$-isoprostanes and between OH-PAHs and 8-OHdG, whereas no differences were observed for MDA (Fig. 2). Additional adjustment for obesity, diabetes, and potential renal impairment did not considerably change the estimates.

3.4. Association between OH-PAHs and hs-CRP

We did not observe an association between the sum of OH-PAHs or single OH-PAHs and hs-CRP for both model adjustment sets, although the estimates tended to be slightly higher in the extended covariate model (Fig. 3).

3.5. Association between biomarkers of oxidative stress and hs-CRP

The effect estimates of the three biomarkers of oxidative stress indicated different patterns of association with hs-CRP (Fig. 4). In the base covariate model, MDA was not associated with hs-CRP, $F_{2\alpha}$-isoprostanes indicated a positive association, and 8-OHdG was significantly positively associated. When additionally adjusting for obesity, diabetes, and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant.

3.6. Robustness of multiple linear regression models

All effect estimates remained robust when excluding season from the regression models (Supplemental Tables 1–3) or when additionally adjusting for annual mean concentrations of ambient $PM_{2.5}$ and NO_2.

4. Discussion

4.1. Summary.

In this cross-sectional study of 400 residents of the Augsburg region (Germany) conducted in 2013/2014, we determined biomarkers of PAH exposure, oxidative stress, and inflammation to investigate the interplay between these three groups of biomarkers. (1) The concentrations of OH-PAHs were comparably lower in our study than in other studies. For example, a study carried out among 300 participants from 7 Asian countries reported that the mean concentrations of 2-OH-Phe, 3-OH-Phe, and 1-OH-Pyr ranged between 0.072–0.58 ng/mL, 0.101–0.714 ng/mL, and 0.167–0.667 ng/mL, respectively (Guo et al., 2013) while in our study, the mean concentrations were 0.09, 0.11, and

Table 2
Kruskal-Wallis Test of OH-PAHs, oxidative stress, and inflammation biomarkers for different subsets of participants.

	N	OH-PAHs (ng/mg Creatinine)		MDA (ng/mg Creatinine)		8-OHdG (ng/mg Creatinine)		$F_{2\alpha}$-isoprostanes (ng/mg Creatinine)		hs-CRP (mg/L)	
		Median	p	Median	p	Median	p	Median	p	Median	p
Age											
Age <55	173	0.20	0.11	26.57	<0.01	2.66	<0.01	3.68	0.64	1.13	0.04
Age ≥55, <65	126	0.22		32.77		2.98		3.41		1.12	
Age ≥65	83	0.27		37.26		3.49		3.60		1.48	
Sex											
Female	162	0.21	0.16	29.47	0.19	3.20	0.17	3.77	0.01	1.37	0.08
Male	220	0.24		31.95		2.91		3.39		1.12	
Obesity											
No (BMI <30 kg/m^2)	266	0.24	0.01	30.80	0.69	3.10	0.12	3.61	0.57	0.97	<0.01
Yes (BMI ≥30 kg/m^2)	116	0.20		29.40		2.81		3.62		2.27	
Smoking Status											
Non-smoker	141	0.20	<0.01	32.85	0.20	3.25	0.50	3.77	0.08	1.13	0.50
Ex-smoker	163	0.21		29.38		2.96		3.41		1.15	
Smoker	78	0.30		29.54		2.79		3.64		1.51	
Potential underlying systemic inflammation											
No (hs-CRP < 3 mg/L)	302	0.21	0.04	29.74	0.03	2.96	0.08	3.58	0.55	–	–
Yes (hs-CRP ≥ 3 mg/L)	80	0.26		33.42		3.21		3.67		–	
Potential renal impairment											
No (eGFR ≥90 mL/min/ 1.73 m^2)	199	0.22	0.72	28.45	0.01	2.90	0.04	3.74	0.08	1.10	0.02
Yes (eGFR <90 mL/min/ 1.73 m^2)	179	0.23		33.32		3.18		3.45		1.38	
Type 2 Diabetes											
No	173	0.21	0.46	28.86	0.09	2.92	0.19	3.63	0.58	0.93	<0.01
Pre	156	0.22		31.24		3.06		3.51		1.62	
Yes	53	0.26		34.81		3.18		3.74		1.15	
Hypertension											
No	253	0.21	0.97	29.55	0.30	2.94	0.08	3.68	0.29	1.11	<0.01
Yes	139	0.23		32.18		3.18		3.41		1.41	
Season											
Spring (Mar–May)	129	0.20	<0.01	34.77	<0.01	3.09	0.24	3.68	<0.01	1.11	0.77
Summer (Jun–Aug)	98	0.19		24.35		2.79		2.73		1.15	
Autumn (Sep–Nov)	71	0.21		29.48		3.09		4.03		1.30	
Winter (Dec–Feb)	84	0.33		32.88		3.23		4.01		1.17	

hs-CRP, high sensitivity C-Reactive Protein; eGFR, Estimated Glomerular Filtration Rate from Serum Creatinine and Cystatin C, OH-PAHs, sum OH-PAHs concentration; 1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 1-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; $F_{2\alpha}$-isoprostanes, sum $F_{2\alpha}$-isoprostanes concentration; BMI, Body Mass Index; potential renal impairment, chronic kidney disease.

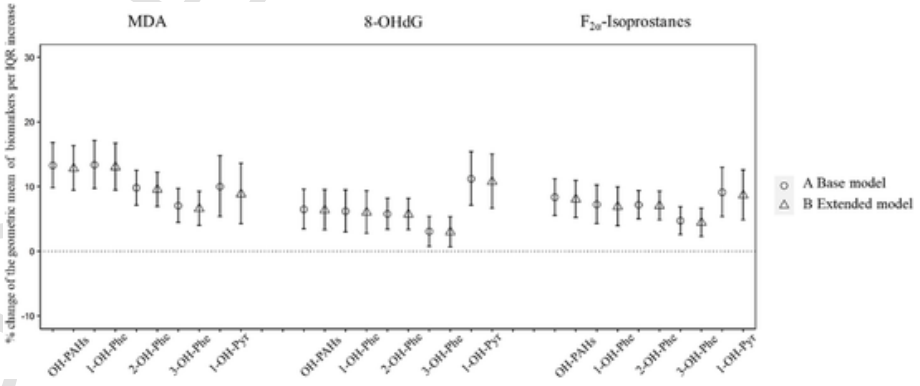

Fig. 1. Percent change in biomarkers of oxidative stress (95% CI) in association with an interquartile range increase in internal exposure biomarkers adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

0.35 ng/ml, respectively (before normalisation by urinary creatinine). The concentrations of the biomarkers of oxidative stress and inflammation were significantly higher in older (MDA, 8-OHdG, and hs-CRP) and obese (hs-CRP) participants, participants with potential underlying inflammation (MDA) and potential renal impairment (MDA, 8-OHdG and hs-CRP), as well as in participants with diagnosed diseases like type 2 diabetes (hs-CRP) or hypertension (hs-CRP), or for participants who had their clinical visit in spring/winter (MDA) or autumn/winter ($F_{2\alpha}$-isoprostanes) compared to their respective counterparts. (2) Positive associations were found between OH-PAHs and biomarkers of oxidative stress, and were more pronounced in participants with potential underlying inflammation. (3) However, no association was observed between OH-PAH and hs-CRP concentrations. (4) Among the three oxidative stress markers, only 8-OHdG was significantly positively associated with hs-CRP, whereas $F_{2\alpha}$-isoprostanes only indicated a positive association, and MDA was not associated at all. When additionally adjusting

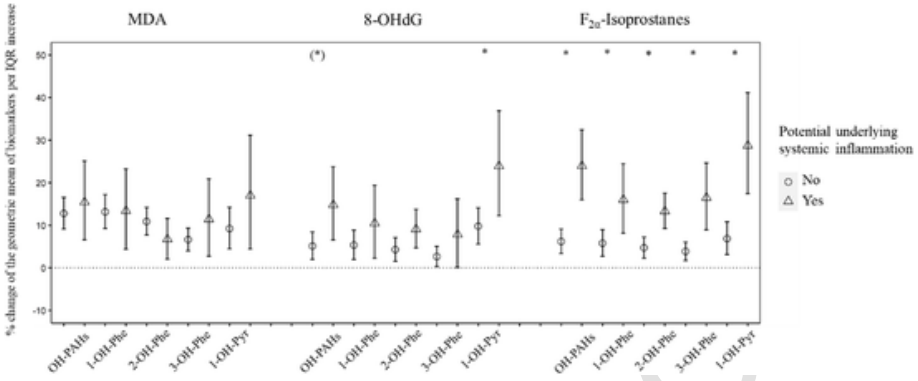

Fig. 2. Effect modification by potential underlying systemic inflammation (hs-CRP ≥ 3 mg/L vs. hs-CRP < 3 mg/L) of the association between OH-PAHs and oxidative stress biomarkers adjusted for age, sex, smoking, trend, and season (Base model). (* p-value of interaction <0.05, (*) p-value of interaction <0.1).

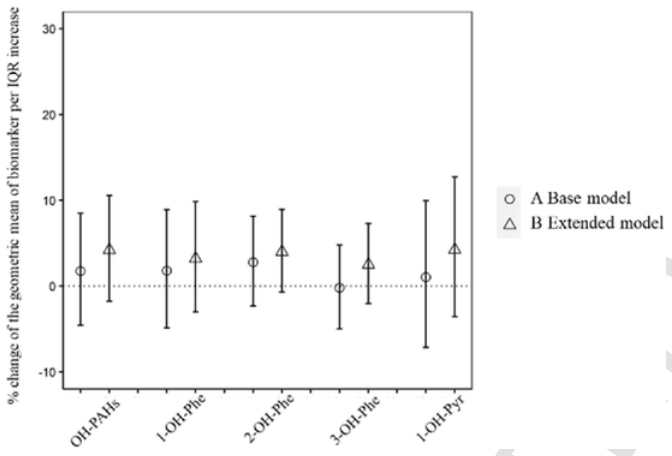

Fig. 3. Percent change in hs-CRP (95% CI) in association with an interquartile range increase in OH-PAHs adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

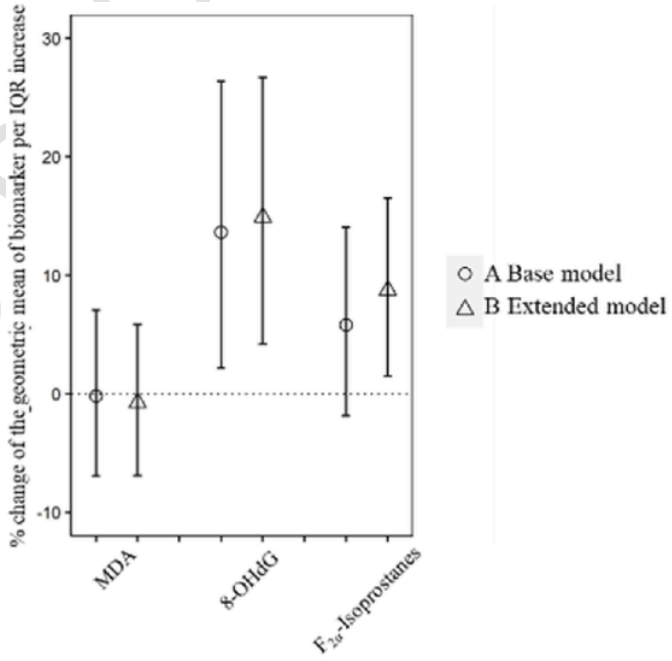

Fig. 4. Percent change in hs-CRP (95% CI) in association with an interquartile range increase in oxidative stress biomarkers adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

for obesity, diabetes, and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant.

4.2. Biomarkers varied across different subgroups

Sex: We observed higher concentrations of $F_{2\alpha}$-isoprostanes in women than in men. A similar study from the U.S. examining 65 participants (19 male, 46 female; 38.6 ± 11.1 years old) also found higher concentration of $F_{2\alpha}$-isoprostanes in females (Ma et al., 2017). Such different concentration levels in males and females may be caused by a decrease in oestrogen levels, which reduces the antioxidative capability in the postmenopausal period (Zaja-Milatovic et al., 2009), or due to the different ratios in content of lean body mass and bone mineral in male and female participants (Ma et al., 2017).

Age: Many studies have reported an association between aging and ROS, suggesting that free radicals play an important role in aging (Finkel and Holbrook, 2000; Guyton et al., 1998; Harman, 1956). In the present study, the biomarkers of ROS damage, MDA and 8-OHdG, showed higher concentrations in the older groups (between 55 and 65 years and older than 65 years) which is consistent with the theory that organisms age because they accumulate oxidative damage generated by ROS.

Smoking: Cigarette smoke contains large amounts of PAHs (Vu et al., 2015), and the group of smokers showed the highest level of PAH metabolites in urine. Similarly, a cohort study among 288 non-smokers and 100 smokers found highly significant differences and dose-response relationships with regard to cigarettes smoked per day for 2- OH-Phe, 3-OH-Phe, 4-OH-Phe, and 1-OH-Pyr (Heudorf and Angerer, 2001). In addition, a cross-sectional study among 4092 participants in China found significant correlations between urinary OH-PAH levels and cigarette smoking (Cao et al., 2020a).

Obesity and chronic diseases: Participants with obesity, potential renal impairment, diabetes, and hypertension showed higher concentrations of hs-CRP, indicating an underlying inflammatory state in these participants. Two studies compared patients at different stages of chronic kidney disease (CKD) with a control group and found higher oxidative stress levels and inflammation in patients with CKD (Karamouzis et al., 2008; Oberg et al., 2004). Accordingly, we observed higher concentrations of MDA, 8-OHdG, and hs-CRP in participants with potential renal impairment, as well as an indication for $F_{2\alpha}$-isoprostanes. Two studies among a cohort of North Indians and a cohort of African Americans found that higher concentrations of hs-CRP were associated with diabetes or, to a lesser degree, insulin resistance (Effoe et al., 2015; Mahajan et al., 2009). A study from Egypt, including 80 participants, reported that hypertension may increase the level of hs-CRP (Abd El Aziz et al., 2019), which matches our observations.

Season: We found significantly higher OH-PAH levels in urine samples collected during winter. We assume that a large part of the internal PAH burden is caused by exposure to PAH-polluted ambient air. Several studies have monitored atmospheric PAHs in Europe and China in recent years and reported similar seasonal variations due to different source contributions between autumn-winter and spring-summer (Albuquerque et al., 2016; Dvorská et al., 2011; Liu et al., 2014; Schnelle-Kreis et al., 2007). Moreover, Li et al. suggested that biomass burning for domestic heating during the heating season (October to March) was the major contributor to atmospheric PAHs in the Augsburg region (Germany) between 2014 and 2015 (Li et al., 2018). This observation was consistent with our results of seasonal variations in OH-PAHs, indicating that ambient PAHs might be an important source of PAH intake. In addition, the levels of oxidative stress markers (MDA and $F_{2\alpha}$-isoprostanes) were significantly higher in samples taken in spring/winter or autumn/winter, which may also be related to increased ambient PAHs pollution. Both short- and long-term studies suggest that higher concentrations of OH-PAHs and increased levels of oxidative stress biomarkers can be detected after exposure to ambient pollutants (Bortey-Sam et al., 2017; Li et al., 2012; Lu et al., 2016; Moller and Loft, 2010; Motorykin et al., 2015; Suzuki et al., 1995).

4.3. Associations between OH-PAHs and oxidative stress and the role of inflammation

Two smaller cohort studies investigated the association between selected oxidative stress markers (MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes) and OH-PAHs in Japan and the United States (Bortey-Sam et al., 2017; Ferguson et al., 2017). Bortey-Sam et al. found significantly positive correlations between the sum of OH-PAHs, 2-OH-naphthalene, 2-3-OH-fluorenes, and MDA, and a positive correlation between 4-OH-Phe and 8-OHdG in urine samples collected from 202 residents of Kumasi, Japan. Ferguson et al. investigated urine samples of 200 pregnant women in the United States and reported that some PAH metabolites were consistently positively associated with urinary oxidative stress markers (8-OHdG and 8-isoprostane) (Ferguson et al., 2017). These findings were confirmed by our study, in which all examined oxidative stress markers showed positive associations with OH-PAHs. It should be mentioned that Ferguson et al. used specific gravity-corrected urinary concentrations in their study. Recent investigations showed that both, creatinine and specific gravity can be used as tools for normalisation or correction and no significant differences considering the results are expected in generally healthy individuals (Sallsten and Barregard, 2021). Although Kuiper et al. recently recommended the specific gravity method (Kuiper et al., 2021), at the time our samples were analysed (2015/16), most other studies were also based on creatinine normalised values.

In our study, the associations between OH-PAHs, 8-OHdG and $F_{2\alpha}$-isoprostanes were more pronounced in participants with potential underlying systemic inflammation, whereas no difference was observed for MDA. This finding indicates that oxidative stress is deteriorated, or is promoted by systemic inflammation. In a review article, Biswas et al. pointed out that inflammation processes can produce reactive species, and an inflammatory status may exaggerate the generation of reactive species (Biswas, 2016). Similarly, other underlying long-term risk factor profiles associated with obesity, diabetes, or CKD, may be responsible for increased levels of oxidative stress, as suggested by our data.

4.4. Associations between OH-PAHs and hs-CRP

A cohort study by Clark et al. using data of 3219 participants aged 20 years and older from the U.S National Health and Nutrition Examination Survey (NHANES) 2001–2004 investigated the relationship between OH-PAHs and inflammatory markers (homocysteine, fibrinogen, white blood cell count), but found no significant differences between high and low levels (75th vs. 25th percentiles) of all PAH metabolites in non-smoking participants (Clark et al., 2012). The study by Ferguson et al. observed positive associations between urinary OH-PAH concentrations and hs-CRP, but not with inflammatory cytokines (IL-1β, IL-6, IL-10, and TNF-α). In this analysis, we observed only slight indications for a positive association between OH-PAHs and hs-CRP levels. Clark et al. did not specify whether they used spot urine or 24 h urine, thus the comparability is limited.

In our previous KORA FF4 study across the full sample of 2252 participants, we observed significant positive associations between long-term exposure to ambient air pollution and hs-CRP (Pilz et al., 2018). In summary, our findings in this relatively low-exposure setting point to only weak associations between OH-PAHs and inflammation.

4.5. Association between oxidative stress and hs-CRP varied for different biomarkers

It has been shown that ROS play an important role in the signalling of inflammatory responses (Peters et al., 2021). In this analysis, 8-OHdG was significantly positively associated with hs-CRP, while MDA showed no association. When additionally adjusting for obesity, diabetes and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant. Although MDA, $F_{2\alpha}$-isoprostanes, and 8-OHdG are all indicators of ROS levels, they are generated from different pathways. While MDA (Ayala et al., 2014; Chen et al., 2011) and $F_{2\alpha}$-isoprostanes (Galano et al., 2017; Milne et al., 2008; Morrow and Roberts, 1996) are generated from non-enzymatic and free radical-mediated oxidation, 8-OHdG is formed enzymatically during DNA impairment and repair (Evans et al., 2010; Valavanidis et al., 2009). Different phases of oxidative stress from tolerance, adaptation, inflammation, and cell death were described by Peters et al. as a continuum (Peters et al., 2021). Therefore, MDA and $F_{2\alpha}$-isoprostanes can be generated through all phases of the continuum, while 8-OHdG is only generated in the later phases, for example, inflammation and cell death when ROS exceed antioxidation (Asanka Sanjeewa et al., 2021; Janovits et al., 2021; Luan et al., 2022; Trettin et al., 2014). Moreover, MDA is highly reactive and very polar. It is generated in the early phase of the exposure but gets cleared very fast (Siu and Draper, 1982; Traverso et al., 2004) whereas $F_{2\alpha}$-isoprostanes are relatively stable (Milne et al., 2008). This might be one explanation why we observed no association between hs-CRP and MDA but an indication for $F_{2\alpha}$-isoprostanes.

4.6. Strengths and limitations

One strength of this study is the selection of various biomarkers of oxidative stress, which were measured in our laboratory using established high-performance analytical methods. Each marker or each group of markers reflects characteristic damage to cellular macromolecules, namely the double bonds of PUFA, DNA, and membrane phospholipids. This allows the investigation of their interplay with respect to the internal PAH burden, which was also determined in our lab, along with the inflammation marker hs-CRP. Another strength of our study is the high number and diversity of individual participants' data available for the sub-cohort of the KORA study. The combination of lab-generated data with comprehensive information of the participants allowed us to perform more comprehensive investigations and better control of confounders than in previous studies.

There were several limitations to this study. First, our study was a cross-sectional study, and each participant was sampled only at a single time point from 2013 to 2014. Second, as a selected subset of participants was included in this analysis, our findings might not be representative for the general population. Third, OH-PAHs and biomarkers of oxidative stress were analysed from urine samples, while hs-CRP was analysed from serum samples. Metabolites in urine are considered as

end products (Ayala et al., 2014; Evans et al., 2010; Morrow and Roberts, 1996) whereas metabolites in serum can function and participate in metabolic processes (Gewurz et al., 1982). However, this could also be considered an opportunity to observe and interpret the data within a larger frame. Fourth, the half-lives of the biomarkers might differ which could be one of the reasons why we did not observe an association between OH-PAHs and hs-CRP (Li et al., 2012; Pepys and Hirschfield, 2003). Finally, only spot urine samples were collected and analysed. However, we applied creatinine normalisation, an effective normalisation method to minimise the differences between the concentrations of OH-PAHs in spot, first-morning, and 24-h urine samples (Li et al., 2010).

5. Conclusion

In this cross-sectional study, we observed associations between exposure to PAHs, oxidative stress, and inflammation, even in a low exposure setting. We found positive associations between OH-PAHs and oxidative stress that were more pronounced in participants with an underlying inflammatory state. Additionally, hs-CRP was positively associated with increased markers of oxidative stress but not directly with PAHs. Individual risk factors were important contributors to these processes and should be considered as potential confounders in future studies. Further longitudinal studies are necessary to investigate the causal chain of the associations.

Declaration of interest

All authors declare that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113993.

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