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Air pollution and oxidative stress in adults suffering from airway diseases. Insights from the Gene Environment Interactions in Respiratory Diseases (GEIRD) multi-case control study

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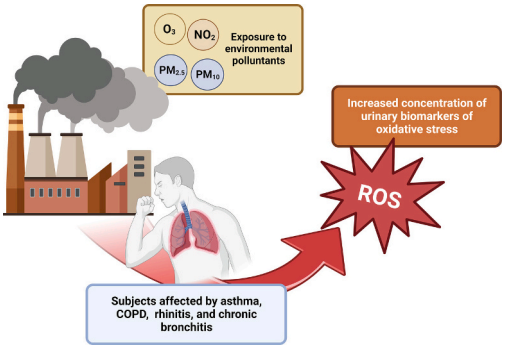
HIGHLIGHTS

- Air pollution increases oxidative stress in people suffering from airway diseases.
- Pollution-related health risk might be currently underrated among susceptible people.
- Three-year exposure to NO_2, PM_{10}, $PM_{2.5}$ is associated with higher 8-iso-prostane and 8-OH-dG.

GRAPHICAL ABSTRACT



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ABSTRACT

Air pollution is a leading risk factor for global mortality and morbidity. Oxidative stress is a key mechanism underlying air-pollution-mediated health effects, especially in the pathogenesis/exacerbation of airway impairments. However, evidence lacks on subgroups at higher risk of developing more severe outcomes in response to air pollution. This multi-centre study aims to evaluate the association between air pollution and oxidative stress in healthy adults and in patients affected by airway diseases from the Italian GEIRD (Gene Environment

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COPD
Rhinitis
Chronic bronchitis

Interactions in Respiratory Diseases) multi-case control study. Overall, 1841 adults (49 % females, 20–83 years) were included from four Italian centres: Pavia, Sassari, Turin, and Verona. Following a 2-stage screening process, we identified 1273 cases of asthma, chronic bronchitis, rhinitis, or COPD and 568 controls. Systemic oxidative stress was quantified by urinary 8-isoprostane and 8-OH-dG. Individual residential exposures to NO_2, PM_{10}, $PM_{2.5}$, and O_3 were derived using an innovative five-stage machine-learning-based approach. Linear mixed regression models tested the association between oxidative stress biomarkers and air pollution tertiles, adjusting by age, sex, BMI, smoking, education and season, with recruiting centres as random intercept. Only cases exhibited higher levels of log-transformed 8-isoprostane and 8-OH-dG in association with NO_2 (β: 0.30 95 % CI: 0.08–0.52 and 0.20 95 % CI: 0.03–0.37), PM_{10} (0.34 95 % CI: 0.12–0.55 and 0.21 95 % CI: 0.05–0.37) and $PM_{2.5}$ (0.27 95 % CI: 0.09–0.49 and 0.18 95 % CI: 0.02–0.34) as compared to the first tertile of exposure. No significant associations were observed for summer O_3. Our findings suggest that exposure to air pollution may increase systemic oxidative stress levels in people suffering from airway diseases. This introduces a potential novel approach available for future epidemiological studies and Public Health for effective prevention strategies oriented at the quantification of early biological effects in susceptible people, whose additional risk level might be currently underrated. Air-pollution-mediated exacerbations, driven by oxidative stress, still deserve our attention.

1. Introduction

Despite the great strides being taken to tackle ambient air pollution, it still represents a leading cause of global mortality and morbidity. This "silent killer" affects both developed and low-middle-income countries, where exposure to particulate matter (PM) and gaseous pollutants, above the recommended levels, is still worrisomely common (Landrigan et al., 2018; Schraufnagel et al., 2019), although no safe levels exist. According to the Global Burden of Diseases (GBD) report, in 2019 air pollution was liable of 6.67 million premature deaths (95 % Uncertainty interval from 5.90 to 7.49) and over 210 million disability-adjusted life years "DALYs" (95 % Uncertainty interval from 189 to 240) (Abbafati et al., 2020). Currently, air pollution ranks as a leading risk factor for many communicable and non-communicable diseases, being able to affect almost all systems of the human body (Thurston et al., 2017). Exposure to air pollution has been consistently associated with cardiovascular, neurovascular, endocrine and respiratory diseases (Bloemsma et al., 2016; Brook et al., 2010; Gowers et al., 2012; Shah et al., 2015).

Alongside other non-communicable diseases, chronic respiratory diseases still pose a substantial concern contributing, in 2017, to 7 % of deaths globally (Yan et al., 2022). Asthma and Chronic Obstructive Pulmonary Diseases (COPD) are the two most prevalent respiratory diseases worldwide with a prevalence of 3.6 % and 3.9 %, respectively (Soriano et al., 2020). In addition to tobacco smoke and occupational exposures, ambient air pollution represents a major risk for the development or exacerbation of respiratory diseases (Gruzieva et al., 2022). Depending on their aerodynamic diameter, air pollutants can differentially reach the respiratory tree regions, where they may induce diverse biological responses or toxicological effects. A physiological response against the exposure to pollutant agents is the production of free radicals like the Reactive Oxygen Species (ROS). However, an overproduction of ROS may lead to pathophysiologic consequences mainly driven by oxidative stress, an imbalance between anti-oxidant and pro-oxidant species (Sies, 2015) able to damage macromolecules and tissues. Exposure to air pollution may cause oxidative stress by triggering ROS overproduction or by overwhelming or depleting the antioxidant defence system (Okeleji et al., 2021). Owing their position and large surface, lungs are further equipped with an extracellular antioxidant defence system, the lining fluid, counteracting undue oxidations of epithelial cells (Kelly, 2003). However, once established, oxidative stress may lead to the development or exacerbation of many respiratory diseases (Chatkin et al., 2022). Airways oxidative stress and inflammation, epithelium structural rearrangement, changes in responsiveness, reduced clearance and impaired host defence against infections are some pathophysiological pathways underlying the adverse effect of air pollution on the respiratory health (Chatkin et al., 2022).

Exposure to air pollution has been associated with oxidative stress biomarkers in both animals and humans (Bellisario et al., 2019; Gangwar et al., 2020; Li et al., 2016). Despite the biological plausibility of a higher susceptibility to pollution-associated oxidative stress in people suffering from airway diseases compared to healthy ones (Auerbach and Hernandez, 2012; Liu et al., 2021; MacNee and Donaldson, 2003), available evidence is still scarce or inconclusive. Some authors reported no changes of oxidative stress biomarkers due to air pollution in airway-diseased individuals (Choi et al., 2021; Havet et al., 2018; He et al., 2020; Liu et al., 2009; Patel et al., 2013; Pirozzi et al., 2015), while others observed a positive association (Grady et al., 2018; Liang et al., 2019; Liu et al., 2021; Suresh et al., 2009; Wang et al., 2022). Even less evidence is available about the induction of air pollution-derived oxidative stress among respiratory-diseased adults. Previous literature often refers to paediatric population (e.g. asthmatic children) (Suresh et al., 2009; Vincenzo et al., 2023), whose exposure to air pollution could mainly be associated with the physiopathology of asthma but barely with exacerbations and comorbidities. Notably, after the exposure to environmental *stimuli*, healthy subjects can physiologically counteract ROS and repair ROS-derived damage, while diseased people may undergo significant functional and structural consequences that, in turn, may lead to accumulating ROS and oxidative stress (Jesenak et al., 2017). Besides its role in the pathophysiology of several respiratory diseases, uncontrolled oxidative stress may disrupt the glucocorticoid receptor signalling, leading to the activation of pro-inflammatory pathways in immune cells (Lewis et al., 2021). Moreover, a sustained ROS production enhances the inflammatory response in both asthmatics and COPD patients by activating redox-sensitive transcription factors (Chamitava et al., 2020). Oxidative stress in respiratory diseased people may also interfere with the efficacy of corticosteroid-based therapies by decreasing the activity of histonedeacetylase-2 (HDAC-2) co-repressor (Chamitava et al., 2020).

Altogether, these mechanisms can trigger respiratory symptoms, disease exacerbation (Sierra-Vargas et al., 2023), and the possible onset of inflammatory-related comorbidities, leading – over time - to the worsening of the patients' health *status* and management.

In light of the importance of this topic, we hypothesised that, compared to healthy individuals, susceptible subgroups of the population, like people suffering from airway diseases, may be more prone to air pollution-mediated oxidative stress. Therefore, we aimed at investigating the association between residential exposure to air pollution and systemic oxidative stress in adults. We tested our hypothesis by separately analysing the effect of three-year exposures to air pollution and oxidative stress biomarkers in respiratory-diseased cases and healthy controls. Our study may provide new insights on the effects that air pollution might exert on the redox imbalance in vulnerable people.

2. Material and methods

2.1. Study design and recruiting centres

The present study is based on a secondary analysis of data collected in the frame of the Gene Environment Interaction Respiratory Disease (GEIRD) multicentre, multi-case control study, originally involving adults from seven Italian centres. GEIRD adopted a multi-case control design to compare patients, belonging to a specific respiratory phenotype, to controls without any related diseases among asthma, allergic rhinitis, COPD and chronic bronchitis. Cases and controls were randomly selected from pre-existing population-based cohorts (the Italian Study on Asthma in Young Adults "ISAYA" and the European Community Respiratory Health Survey "ECRHS") (Roberto De Marco et al., 2003; Marinoni, 1995) or randomly sampled from the general population (20–84 years, male/female ratio: 1/1) to guarantee they belonged to the same source population and to minimise a potential dilution bias. Following a two-stage strategy, eligible subjects were first administered a postal screening questionnaire (phase I) to ascertain the presence of respiratory symptoms. Then, they underwent a clinical examination (phase II) including lung function tests, and blood and urine sample collection for the measurement of biomarkers among a set of other tests and questionnaires, as described elsewhere (De Marco et al., 2010). The GEIRD Project was approved by the Ethics Committee of the coordinating centre (Verona) and by the Ethics Committees of all other recruiting centres. All participants provided their written informed consent prior to the study beginning.

Current analyses include GEIRD cases of asthma, allergic rhinitis, COPD and chronic bronchitis and controls from four recruiting centres, namely Pavia, Sassari, Turin, and Verona, based on biological analysis and residential address availability and completeness. Data refer to the second stage of the GEIRD study and include subjects enrolled between the years 2008 and 2014.

2.2. Lung function testing

At clinical stage, all study participants underwent lung function testing according to the American Thoracic Society reproducibility criteria (Crapo et al., 1995). Forced Expiratory Volume in the first second (FEV_1) and Forced Vital Capacity (FVC) were calculated according to the Quanjer's equation (Quanjer et al., 2012) and then expressed as percentage of predicted values and Lower Limit of Normal (LLN) corresponding to the 5th percentile of a healthy, non-smoking population. Subjects with FEV_1/FVC <70 % or <LLN, additionally underwent bronchodilator challenge test by performing the spirometry for a second time following the administration of 400 μg of salbutamol. According to a standardised protocol (Chinn et al., 1997), subjects with FEV_1/FVC >70 % and >LLN underwent the methacholine challenge test, considered positive if, compared to baseline, a 20 % drop in FEV_1 occurred with a provocation dose <1 mg (PD20).

2.3. Definition of cases and controls

The enrolled subjects were grouped in cases and controls according to the following characteristics:

- asthma cases were individuals self-reporting asthma or asthma-like symptoms and one among the following treats: 1) at least one asthma attack during the last year; 2) current use of asthma medications; 3) a pre-bronchodilator FEV_1/FVC <70 % or <LLN; and 4) a positive methacholine challenge test;
- rhinitis cases were subjects reporting lifetime nasal allergies, sneezing, recurrent nasal or eye symptoms either in the presence of dust, pollen and animals or in absence of cold or flu;
- chronic bronchitis cases were subjects self-reporting cough and phlegm over three consecutive months or two consecutive years, with post-bronchodilator FEV_1/FVC >70 % and >LLN;
- COPD cases were patients self-reporting cough and phlegm over three consecutive months or two consecutive years, with a post-bronchodilator airflow limitation of FEV_1/FVC <70 % or <LLN;
- controls were all subjects not falling in the abovementioned definitions and with a pre-bronchodilator FEV_1/FVC > 70 % and >LLN, a FEV_1 > 80 % of predicted values, and a negative methacholine challenge test.

2.4. Oxidative stress biomarkers

During the clinic visit, all subjects provided a spot urine sample that was stored at 4 °C for 24 h, then at −80 °C, until analyses. Urinary 8-iso-prostaglandin F2α (8-isoprostane) and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OH-dG) were assessed by specific Enzyme-Linked Immunosorbent Assays (ELISAs) following the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI, USA and Cosmo Bio LTD, Tokyo Japan, respectively). Both biomarkers indirectly quantify systemic oxidative stress yet reflecting lipid-peroxidation and DNA damage-repair degree, respectively. To account for urine dilution, we indexed oxidative stress biomarkers to creatinine concentration (mg/dL), assessed by a colorimetric assay relying on the Jaffé reaction (Cayman Chemical Ann Arbor, MI, USA). We expressed both biomarkers as ng/mg of creatinine, as previously reported (Bono et al., 2014).

2.5. Exposure to air pollution

Daily mean concentrations of NO_2, PM_{10}, $PM_{2.5}$ and O_3 were estimated using satellite observations and further elaborated by machine learning algorithms in the frame of two previous studies, namely "*Big data in Environmental and occuPational Epidemiology*" BEEP and "*Use of BIG data for the evaluation of the acute and chronic health Effects of air Pollution in the Italian population*" BIGEPI (Silibello et al., 2021; Stafoggia et al., 2017, 2019). Over the period 2013–2015, satellite-based Aerosol Optical Depth (AOD) and PMs data from available monitoring stations were collected accounting for additional spatiotemporal information (e.g. land use characteristics, meteorological parameters, point emission sources, etc.). Daily averages of PM_{10} and $PM_{2.5}$ were predicted using a four-stage model, as described elsewhere (Stafoggia et al., 2019). An integrated approach coupling a Chemical Transport Model (CTM) with machine learning techniques (Silibello et al., 2021) enabled the estimation of daily NO_2 and summer O_3 (April–September). All air pollution estimations have a spatial resolution of 1 km.

Since most of the visits were between 2008 and 2014, we back-extrapolated the 2013–2015 maps of air pollution and assigned to each participant a three-year average exposure (i.e. individual long-term exposures).

2.6. Other covariates and potential confounders or effect modifiers

In the current analysis, the following covariates were considered:

- The recruiting centre (Pavia, Sassari, Turin, and Verona) accounting for to centre-specific unmeasured characteristics;
- Season of sampling collection (spring, summer, autumn and winter) derived from calendar period corresponding to the visit date;
- Age (years) calculated from participants birth date to the visit date;
- Sex (females and males);
- Body Mass Index (BMI) derived from the ratio between weight and squared height (kg/m^2) measured during visits;
- Self-reported smoking habits (never smoker, past smoker i.e. not smoking in the last month, or current smoker);
- Self-reported education level (Low = completed before the age of 16 years) as a proxy of socioeconomic *status*;

- The case/control indicator (0 = control, 1 = COPD, 2 = asthma, 3 = rhinitis and 4 = chronic bronchitis).

2.7. Statistical analysis

Continuous variables are presented as mean (± Standard Deviation, SD), median (± Interquartile Range, IQR) or geometric mean (GM ± 95 % Confidence Intervals, CIs), for normal, skewed and lognormal distributions, respectively. Categorical variables are expressed as absolute (number of observations) and relative (percentage) frequencies. In descriptive analysis, differences between cases and controls were tested using *t*-test or Mann-Whitney *U* test, for continuous variables (i.e. age, BMI, air pollution data and oxidative stress biomarkers) and by χ^2 test for categorical variables (i.e. GEIRD centre, season of sample collection, sex, smoking habits and education level). To check the normality of the variable distributions we used Shapiro-Wilk test and visual inspection (QQ-plot) along with kurtosis and skewness calculations. Oxidative stress biomarker distributions (8-isoprostane and 8-OH-dG) were markedly right-skewed and followed a lognormal distribution, thus they were log-transformed (natural logarithm) to stabilise their variance. To deal with a non-linear relationship between exposures (NO_2, PM_{10}, $PM_{2.5}$ and O_3) and outcomes (8-isoprostane and 8-OH-dG), we categorised the exposure variables into tertiles and assessed the single-pollutant univariate association with log-transformed oxidative stress biomarkers using univariate Linear Mixed Models, one at a time and separately for cases and controls (i.e. crude estimates). Recruiting centres were included as a four-level random intercept, to account for the potential heterogeneity among different centres. Single-pollutant rather than multi-pollutant models were used due to the high correlation among air pollutants (Pearson's r ranging from 0.54 to 0.98) that would have implied high collinearity among variables. Then, we performed linear multivariable models fully adjusted by age, sex, BMI, smoking habits, education level, and season of sample collection. The goodness of fit was checked by verifying the assumptions underlying the models. As sensitivity analysis, we tested the same associations stratifying by individuals having at least one among rhinitis (n = 1119), asthma (n = 613) and chronic bronchitis (n = 211). COPD cases were not considered due to the limited sample size (n = 54).

Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Stata 17.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC) and figures using GraphPad Prism version 9.4.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

3. Results

Overall, 1841 adults aged between 20 and 83 years (49 % females) from four Italian centres (Pavia, Sassari, Turin, and Verona) were included in the present study. Table 1 summarises individuals' main characteristics, according to their *status* of cases or controls, along with their residential exposure to air pollution and urinary oxidative stress biomarkers (8-isoprostane and 8-OH-dG). Among different study centres, Verona recruited the largest number of subjects (n = 1293, 70 %), while Turin the lowest (n = 146, 8 %). Across seasons, clinical visits occurred fairly equally and for 1.5 % of cases, the visit date was not available. Among cases, there were slightly more smokers (23 %) than in controls (17 %), while we observed the opposite concerning past smokers (28 % and 31 %, respectively). Cases and controls were similarly educated, both reporting around 20 % of low-educated individuals. All other characteristics were equitably distributed among cases and controls, with the only exception of age (p = 0.001) and exposure to NO_2 (p = 0.040).

Table 2 reports ranges (minimum-maximum) and medians with IQRs of air pollutants across tertiles, according to the recruiting centre. Depending on locations and air pollutants, subjects exhibited different degrees of exposure being generally more exposed in Turin, with the

Table 1
Main characteristics of individuals included in the analysis according to their case-control *status*.

Characteristics	Controls n = 568 (30.9 %)	Cases n = 1273 (69.2 %)	*p*-Value	Overall n = 1841
GEIRD recruiting centre:			<0.0001[a]	
Pavia	49 (32.9)	100 (67.1)		149
Sassari	61 (24.1)	192 (75.9)		253
Turin	17 (11.6)	129 (88.4)		146
Verona	441 (34.1)	852 (65.9)		1293
Season of biological sample collection:			0.072[a]	
Spring	162 (29.3)	391 (70.1)		553
Summer	115 (34.1)	222 (65.9)		337
Autumn	134 (28.3)	340 (71.7)		474
Winter	157 (34.9)	293 (65.1)		450
Missing	–	27 (2.0)		27
Sex:			0.320[a]	
Females	290 (31.9)	618 (68.1)		908
Smoking habits:			0.013[a]	
Never smoker	295 (32.4)	616 (67.6)		911
Past smoker (not smoking in the last month)	175 (32.7)	361 (67.4)		536
Current smoker	97 (24.7)	295 (75.3)		392
Missing	1 (0.2)	–		1
Education level:			0.205[a]	
Low (completed before the age of 16 years)	116 (33.7)	228 (66.3)		344
Age (years), mean (SD)	49.8 (12.7)	47.6 (12.5)	0.001[b]	48.3 (12.6)
BMI (kg/m^2), mean (SD)*	25.4 (4.43)	25.2 (4.37)	0.460[b]	25.3 (4.39)
Three-year average air pollution (μg/m^3), median (IQR)				
Nitric dioxide (NO_2)	28.4 (23.8–34.4)	29.0 (23.8–34.8)	0.040[c]	29.0 (23.8–34.5)
Particulate matter with a diameter ≤10 μm (PM_{10})	38.9 (36.4–40.6)	39.2 (35.9–40.7)	0.295[c]	39.2 (36.2–40.7)
Particulate matter with a diameter ≤2.5 μm ($PM_{2.5}$)	25.2 (24.0–25.7)	25.2 (23.6–25.7)	0.527[c]	25.2 (23.4–25.7)
Summer ozone (O_3)	71.5 (69.6–73.5)	71.3 (68.7–73.2)	0.438[c]	71.3 (68.9–73.4)
Urinary oxidative stress biomarkers (ng/mg creatinine)†				
8-Isoprostaglandin F2α (8-isoprostane)**	0.67 (0.61–0.75)	0.74 (0.68–0.80)	0.114[c]	0.72 (0.67–0.76)
8-Oxo-7,8-dihydro-20-deoxyguanosine (8-OH-dG)***	3.6 (3.3–3.9)	3.9 (3.7–4.1)	0.205[c]	3.8 (3.6–4.0)

Air pollution exposures are estimated at residential level and expressed as three-year averages over the period 2013–2015.
GEIRD: Gene Environment Interaction Respiratory Disease.
SD: Standard Deviation.
IQR: Interquartile Range.
BMI: Body Mass Index.
† Data are presented as Geometric Mean (95 % CIs: Confidence Intervals).
* n = 1 (0.2 %) missing observations for controls and n = 152 (12 %) missing observations for cases.
** n = 75 (13 %) missing observations for controls and n = 300 (24 %) missing observations for cases.
*** n = 5 (0.8 %) missing observations for controls and n = 15 (1.2 %) missing observations for cases.
[a] Obtained performing the χ^2 test after the exclusion of missing data.
[b] Obtained performing two-tailed *t*-test.
[c] Obtained performing the Mann-Whitney *U* test.

Table 2
Distribution of exposure to air pollution according to the recruiting centres.

Air pollutants (μg/m^3)	GEIRD recruiting centres				
	Pavia n = 149 (8.1 %)	Sassari n = 253 (13.8 %)	Turin n = 146 (7.9 %)	Verona n = 1293 (70.2 %)	Overall n = 1841
NO_2	Range: 8.9–39.6	Range: 4.8–27.4	Range: 16.7–67.4	Range: 9.1–40.3	Range: 4.8–67.4
Tertiles					
1st	23.8 (23.0–24.6)	13.7 (11.2–17.4)	20.8 (18.8–24.4)	21.9 (15.1–24.4)	17.6 (12.5–23.3)
2nd	29.0 (27.0–30.5)	27.4 (27.4–27.4)	27.2 (27.2–27.2)	29.0 (27.3–30.9)	28.9 (27.3–30.9)
3rd	34.4 (33.9–36.0)	–	51.5 (43.7–57.3)	35.9 (34.5–37.3)	35.9 (34.6–39.9)
PM_{10}	Range: 12.0–39.1	Range: 11.7–25.7	Range: 22.4–42.2	Range: 11.2–36.8	Range: 11.2–42.2
Tertiles					
1st	32.7 (30.9–33.0)	18.0 (17.0–18.6)	29.0 (26.2–31.9)	31.9 (28.1–32.7)	21.9 (17.9–32.3)
2nd	34.0 (33.8–34.2)	–	34.1 (33.4–34.2)	33.8 (33.5–34.3)	33.9 (33.5–34.3)
3rd	35.2 (34.8–36.0)	–	37.2 (36.4–38.2)	34.9 (34.6–35.5)	35.2 (34.7–35.7)
$PM_{2.5}$	Range: 8.7–29.0	Range: 7.8–13.9	Range: 16.9–30.9	Range: 9.1–27.9	Range: 7.8–30.9
Tertiles					
1st	24.1 (23.0–24.4)	9.0 (8.6–9.7)	22.9 (20.0–24.3)	23.4 (21.6–24.1)	17.9 (9.1–23.8)
2nd	25.3 (25.0–25.4)	–	25.2 (25.2–25.3)	25.2 (24.9–25.4)	25.2 (24.9–25.4)
3rd	25.9 (25.7–26.0)	–	27.8 (27.0–28.3)	25.9 (25.6–26.2)	26.0 (25.7–26.3)
O_3 tertiles	Range: 66.4–86.4	Range: 65.3–88.2	Range: 61.2–75.6	Range: 62.0–103.4	Range: 61.2–103.4
1st	68.7 (68.4–69.3)	69.3 (68.5–69.8)	66.3 (64.9–68.2)	67.3 (66.9–68.6)	67.7 (66.9–68.9)
2nd	71.1 (70.7–71.3)	70.5 (70.1–71.4)	71.2 (70.7–71.3)	71.4 (70.8–71.9)	71.4 (70.8–71.9)
3rd	72.9 (72.7–73.3)	75.5 (73.2–80.2)	73.6 (73.4–74.2)	74.2 (73.5–77.8)	74.3 (73.5–78.2)

Air pollution exposures are estimated at residential level and expressed as three-year averages during 2013–2015, here presented as Median (Interquartile Range).
GEIRD: Gene Environment Interaction Respiratory Disease.
NO_2: Nitric dioxide.
PM_{10}: Particulate matter with a diameter ≤10 μm.
$PM_{2.5}$: Particulate matter with a diameter ≤2.5 μm.
O_3: Summer (April–September) ozone.

only exception of summer O_3, and less exposed in Sassari. Due to the largely lower air pollution levels in Sassari, none of the individuals recruited at this centre belonged either to the third tertile of NO_2 or to the second and third tertile of both PM_{10} and $PM_{2.5}$.

Both unadjusted and adjusted associations between systemic oxidative stress and tertiles of exposure to air pollution (first tertile as reference category) are presented in Fig. 1, separately for cases and controls. After the adjustment by age, sex, BMI, smoking habits, education level, and season of sample collection, the estimates remained fairly the same. As depicted by Fig. 1, only cases, but not controls,

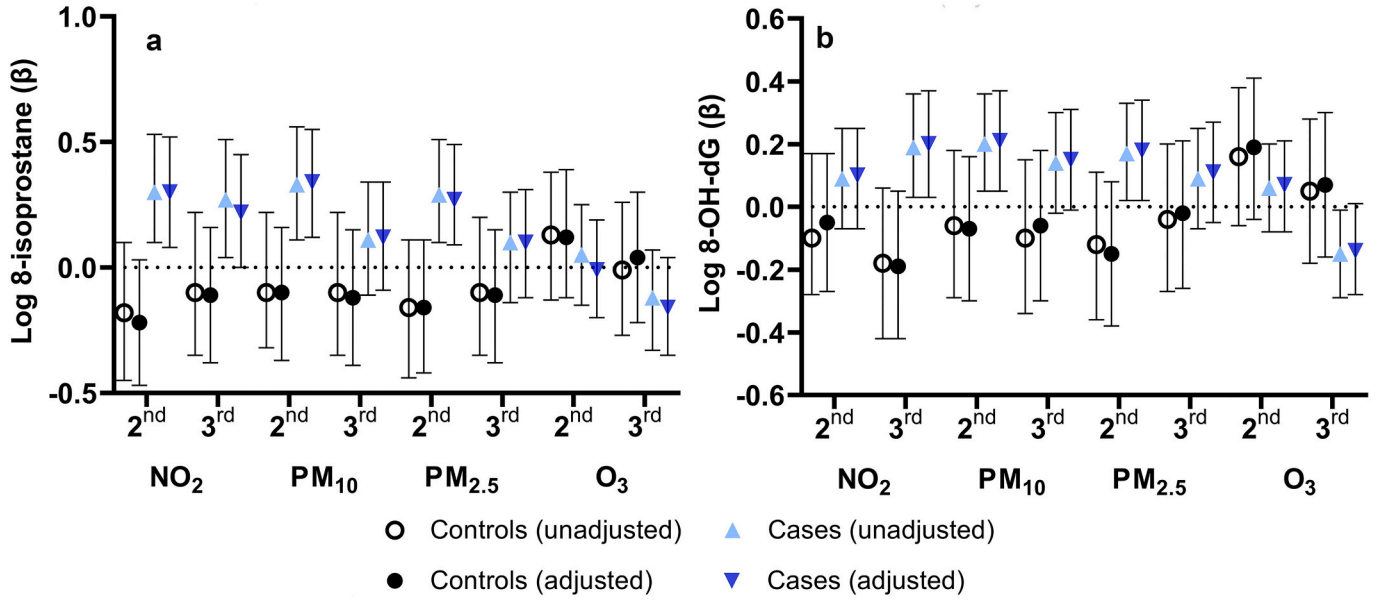

Fig. 1. Associations between systemic 8-isoprostane (a) and 8-OH-dG (b) with three-year average exposure to air pollution, at residential level in cases and controls separately.
Footnote: estimates are derived from single-pollutant univariable and multivariable Linear Mixed Models with recruiting centres as a random intercept. Multivariable models are adjusted by age, sex, Body Mass Index (BMI), smoking habits (past smoker i.e. not smoking in the last month, or current smoker vs never smoker as reference category), education level (low i.e. completed before the age of 16 vs high as reference category), and season of sample. Air pollutants were assessed at residential level over the period 2013–2015 and categorised into tertiles (1st tertile as reference category): NO_2: Nitric dioxide; PM_{10}: Particulate Matter with a diameter ≤10 μm; $PM_{2.5}$: Particulate Matter with a diameter ≤2.5 μm; O_3: Summer (April–September) ozone. Cases are adults affected by asthma, chronic bronchitis, rhinitis or Chronic Obstructive Pulmonary Disease (COPD). (a) Unadjusted and adjusted estimates for cases: n = 973, n = 934, respectively; controls: n = 493, n = 489 respectively. (b) Cases: n = 1258, n = 1219, respectively; for controls: n = 562, n = 558, respectively.

exhibited higher levels of systemic oxidative stress in association with their respective exposure to air pollution. Compared to the lowest exposure level (first tertile), cases belonging to the second tertile of exposure showed an average increase of both urinary biomarkers as follows. Concerning 8-isoprostane (Fig. 1a), we observed a 35 % increase (β: +0.30, 95 % CIs from 0.08 to 0.52, $p = 0.008$) linked to NO_2, 41 % (β: +0.34, 95 % CIs from 0.12 to 0.55, $p = 0.003$) for PM_{10}, and 31 % (β: +0.27, 95 % CIs from 0.09 to 0.49, $p = 0.014$) associated with $PM_{2.5}$. As to 8-OH-dG (Fig. 1b), the extent of the increase was generally slighter but still significant in association with both PM_{10} and $PM_{2.5}$, increasing by 23 % (β: +0.21, 95 % CIs from 0.05 to 0.37, $p = 0.009$) and by 20 % (β: +0.18, 95 % CIs from 0.02 to 0.34, $p = 0.026$), respectively. Furthermore, 8-OH-dG was affected by the highest level (third tertile) of NO_2 exposure, showing an increase of 22 % (β: +0.21, 95 % CIs from 0.03 to 0.37, $p = 0.018$) as compared to the first tertile. A similar trend was observed for 8-isoprostane (β: +0.22, 95 % CIs from 0.00 to 0.45, $p = 0.055$). No significant associations were observed for summer O_3.

Stratified analyses (Fig. 2), yielded similar results as per rhinitis and asthmatic cases. Depending on air pollutants and biomarkers, the associations were weaker or stronger, as follows. In rhinitis cases, 8-isoprostane increased by 28.4 % for both subjects belonging to the second and to the third tertile of exposure to NO_2 compared to the first one (β: +0.25, 95 % CIs from 0.02 to 0.48, $p = 0.03$ and +0.25, 95 % CIs from 0.01 to 0.48, $p = 0.044$, respectively). Being mildly exposed to PM_{10} (second vs first tertile) was associated with higher 8-isoprostane in rhinitis and asthmatic cases (β: +0.30, 95 % CIs from 0.08 to 0.53, p = 0.009 and β: +0.32, 95 % CIs from 0.02 to 0.62, $p = 0.034$, respectively). In asthmatics, also $PM_{2.5}$ (second vs first tertile) was linked to higher systemic lipid peroxidation (β: +0.30, 95 % CIs from 0.01 to 0.59, $p = 0.045$). 8-OH-dG was higher in both rhinitis and asthmatic cases exposed to the third tertile of NO_2 as compared to those exposed to the first one (β: +0.25, 95 % CIs from 0.06 to 0.41, p = 0.008 and +0.26, 95 % CIs from 0.04 to 0.48, $p = 0.023$, respectively). Only in association with PM_{10}, we observed a significant increase of 8-OH-dG in all case groups (β: +0.23, 95 % CIs from 0.06 to 0.39, $p = 0.007$ and +0.25, 95 % CIs from 0.04 to 0.47, $p = 0.022$, and +0.55, 95 % CIs from 0.04 to 1.05, p = 0.034, respectively). In rhinitis cases, $PM_{2.5}$ exposure was linked to a 21 % increase of 8-OH-dG (β: +0.19, 95 % CIs from 0.03 to 0.35, p = 0.023). Unexpectedly, lower levels of both biomarkers were observed for the highest exposure to O_3 in rhinitis and asthmatic cases (β: −0.17, 95 % CIs from −0.32 to −0.02, $p = 0.028$ and β: −0.20, 95 % CIs from −0.39 to 0.00, p = 0.04, respectively).

4. Discussion

We observed an association between three-year average exposures to ambient air pollution and systemic oxidative stress in adults suffering from respiratory diseases, but not in controls. Two different urinary biomarkers of oxidative stress, 8-isoprostane and 8-OH-dG, were higher in association with the exposure to NO_2, PM_{10}, $PM_{2.5}$ but not with summer O_3.

Interestingly, both oxidative stress biomarkers were higher in people belonging to the second tertile of exposure, compared to the first one, but not in those most exposed (third tertile). The only exception pertains to the NO_2-exposure highest level, in response to which 8-OH-dG and 8-isoprostane were strongly and barely associated, respectively. We also found that the same associations remained statistically significant in analysis stratified by different respiratory disease phenotypes, with the sole exception of people suffering from chronic bronchitis. This might be partly due to the relatively limited sample size of cases with chronic bronchitis ($n < 211$, depending on the biomarker). For the same reason, we did not perform the analysis on COPD cases alone ($n = 54$) and generally observed wider CIs indicating less precise estimates and higher instability of the stratified analyses. Surprisingly, people suffering from rhinitis and asthma who belonged to the highest exposure group of O_3, exhibited lower levels of 8-OH-dG than those less exposed.

In single-pollutant models, PM_{10} seemed the main driver of the systemic pro-oxidant effect observed in cases, being related to 41 % average increase of 8-isoprostane and 23 % of 8-OH-dG. This might be due to the different nature of exposure variables encompassing particulate and gaseous polluting agents, as well as primary and secondary pollutants. PMs and NO_2 are mainly primary pollutants because directly released by their respective emission sources, while O_3 is a secondary pollutant formed in the atmosphere as a result of a photochemical reaction catalysed by the sun (Chatkin et al., 2022).

Therefore, depending on both the meteorological conditions and the concentration of primary pollutants, the secondary ones may largely vary over the seasons and show high correlation with primary pollutants.

Depending on the centre, participants of our study were exposed to

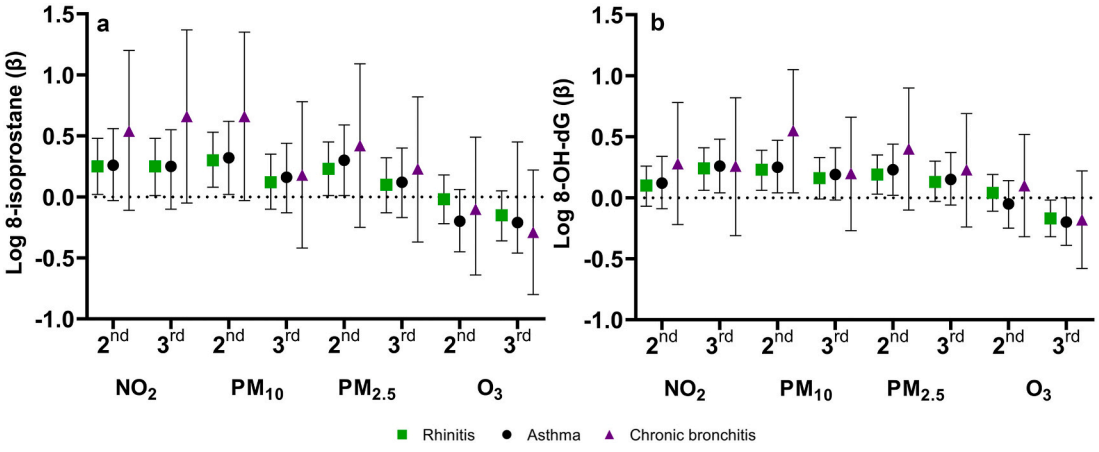

Fig. 2. Associations between systemic 8-isoprostane (a) and 8-OH-dG (b) with three-year average exposure to air pollution, at residential level stratified by people suffering from at least one among rhinitis, asthma and chronic bronchitis.

Footnote: estimates are derived from single-pollutant multivariable Linear Mixed Models with recruiting centres as a random intercept. Models are adjusted by age, sex, Body Mass Index (BMI), smoking habits (past smoker i.e. not smoking in the last month, or current smoker vs never smoker as reference category), education level (low i.e. completed before the age of 16 vs high as reference category), and season of sample. Air pollutants were assessed at residential level over the period 2013–2015 and categorised into tertiles (1st tertile as reference category). NO_2: Nitric dioxide; PM_{10}: Particulate Matter with a diameter ≤10 μm; $PM_{2.5}$: Particulate Matter with a diameter ≤2.5 μm; O_3: Summer (April–September) ozone. (a) Rhinitis: $n = 828$, asthma: $n = 559$, and chronic bronchitis: $n = 162$. (b) Rhinitis: $n = 1086$, asthma: $n = 738$, and chronic bronchitis: $n = 207$.

an average annual level of air pollution complying or not complying the Ambient Air Quality Standards for the European Union (The European Commission, 2008). Individuals from Sassari were exposed to average air pollution concentrations not exceeding the EU standards, while those from Turin showed the opposite condition as to NO_2 and PMs. In Verona and Pavia, only $PM_{2.5}$ concentrations were above the EU standards. Besides the ambient air pollution concentrations, related biological responses also depend on the physical-chemical characteristics of the polluting agents. PMs adsorb organic and inorganic components including polycyclic aromatic hydrocarbons, benzene and metals alongside other immunogenic substances like aeroallergens (Nozza et al., 2021). Consequently, their toxicological effects may include a large variety of biological responses associated with the development of persistent oxidative stress and inflammation (Chatkin et al., 2022) due to both mechanical and chemical actions. Although fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particles pose more concern due to their capacity of reaching the bloodstream and organs, their effects partly overlap with those attributed to coarse particles (PM_{10}). It is worth mentioning that PM_{10} includes all smaller particles, hence by using single-pollutant analysis we cannot exclude we are partly referring to shared effects among differently sized particles. Concerning gaseous pollutants like NO_2, it can easily reach the terminal bronchioles where is then transferred across the blood–gas interface to the bloodstream where systemic effects, including oxidative stress, are alike. In our study, exposure to NO_2 seemed to exert the strongest effect right after the one observed for PM_{10}.

In a framework of a current knowledge of known detrimental respiratory effects due to long-term exposure to air pollution (Marchetti et al., 2023), our findings are partly in line with some authors (Choi et al., 2021; Grady et al., 2018; Havet et al., 2019; Liang et al., 2019; Liu et al., 2021; Suresh et al., 2009; Vincenzo et al., 2023; Wang et al., 2022) reporting that children and adults affected by respiratory diseases are more prone to air pollution-related oxidative stress than healthy individuals. Exposure to polycyclic aromatic hydrocarbons was associated with higher systemic oxidative stress levels yet lower antioxidant biomarkers in asthmatic children (Suresh et al., 2009; Vincenzo et al., 2023) and higher oxidative stress in COPD adults (Liu et al., 2021), both compared to controls. Interestingly, a worse lung function corresponded to more intense lipid peroxidation (Liu et al., 2021). In atopic asthmatic children ($n = 89$) exposure to Benzo(a)pyrene was positively associated with plasmatic 8-isoprostane and urinary 8-OH-dG levels but not in controls or non-atopic asthmatic subjects (Choi et al., 2021). In COPD patients (47–90 years old) urinary 8-OH-dG increased in association with short-term exposure to black carbon (Grady et al., 2018). Liang et al. (2019) observed that asthma can be considered a condition enhancing the susceptibility to air pollution-mediated oxidative stress in a sample of thirty asthmatic commuters. Similarly, exhaled 8-isoprostane increased in COPD patients with a short-term exposure to traffic-related air pollution (Wang et al., 2022). Exposure to O_3 and PM_{10} was associated with higher plasma fluorescent oxidation products (FlOPs) levels in adults suffering from persistent asthma (Havet et al., 2019), while NO_2, and $PM_{2.5}$ were significantly associated with increased lipid peroxidation degree in exhaled breath condensate from asthmatic children. Noteworthy, all the aforementioned studies investigated short-term exposures, with only a very few exceptions, using different study designs, age groups and, seldom, other biological specimens or biomarkers. Hence, we cannot completely compare our results to them in terms of exposures, outcomes and study designs. In some other cases, our findings did not overlap previous literature at all (Havet et al., 2018; He et al., 2020; Liu et al., 2009; Patel et al., 2013; Pirozzi et al., 2015). For instance, exhaled 8-isoprostane significantly increased with $PM_{2.5}$ exposure, and decreased with summer O_3 exposures in healthy adults, but not in asthmatics, although traffic intensity, O_3 exposure and exhaled 8-isoprostane concentration increased the risk of current asthma (Havet et al., 2018). In their study, Havet and colleagues adopted a similar design but quantified oxidative stress at pulmonary level, using the exhaled breath condensate specimen. Interestingly, the authors (Havet et al., 2018) found a similar and unexpected result in relation to summer O_3 as we observed in our analysis and as has also been previously reported by Patel et al. (2013). As previously discussed (Maio et al., 2023; Nuvolone et al., 2018; Stafoggia et al., 2022), this might be due to the single-pollutant approach that is not able to account for the interaction between O_3 and other pollutants. Indeed, protective effects of O_3 are no longer observed when the multipollutant adjustment has been used in other studies (Maio et al., 2023; Nuvolone et al., 2018; Stafoggia et al., 2022).

An intriguing hypothesis that potentially explains the oxidative stress increase in response to medium rather than utmost exposure level in our sample may depend on potential adaptive responses to long-term high exposures. Indeed, as already mentioned, three out of four studied centres (Turin, Pavia, and Verona) are located in the Po Valley, one of the most polluted areas in Europe. This might have implied that people from Pavia, Turin and Verona have been exposed to considerable concentrations of air pollutants over time. In this context, environmental exposures may trigger the activation of a hormetic process resulting in more efficient anti-oxidant and repair cellular systems (Rossnerova et al., 2020). As to the air pollution effect observed in people affected by respiratory diseases but not in controls, we speculate the following reasoning. First, people suffering from airway diseases may be genetically more susceptible presenting specific polymorphisms that lead to a reduced antioxidant capacity (Fuertes et al., 2020). Second, the deposited dose rate of air pollutants may be much higher in people with respiratory diseases, potentially amplifying their biological effects (Liu et al., 2021; Wang et al., 2022). Finally, the antioxidant function of the lining fluid in the lungs of respiratory-diseased subjects may be partly compromised, thus ineffective against ROS insults (Kelly, 2003). To avoid overstating our results, these should be considered as hypotheses which verification deserves further research.

Our study faced some limitations. The measurement of air pollution at residential level may misestimate the real exposure of participants because it did not consider time-activity patterns. However, our approximation is an objective assessment able to elude the exposure misclassification, common in case-control studies. Another source of information bias pertains to all the other instruments used in different centers to assess the variables under investigation (e.g. questionnaires, biological and clinical analyses, etc.). However, in the frame of GEIRD, we used standardised questionnaires, administered by specifically trained operators and standardised protocols across all the recruiting centres. Additionally, all the biological analyses were performed by one laboratory in charge of this duty, to avoid variability across laboratories. Therefore, thanks to standardised protocols and methods, we partly overcome issues related to the information bias. The single-pollutant approach may oversimplify the complexity of air pollution exposure that, in the air we breathe, is a mixture of many pollutants. We hypothesise that our results may overestimate the association between a single pollutant and oxidative stress, as they may represent themselves or surrogates of other correlated pollutants. However, in our study we opted for a trade-off between the real exposure characterisation and the stability of model estimates and standard errors. Indeed, multi-pollutant models yielded imprecise results, due to the high collinearity among the environmental variables (Variance inflation factor > 2.5, data not shown). The main strengths of our study relate to the following aspects. First, our analysis was performed on a relatively large study population from different Italian cities, which potentially extends the external validity of our results. Second, susceptible groups like respiratory-diseased adults were considered in relation to the potential early biological effects due to air pollution, adding important insights on an under-investigated Public Health issue. Third, the exposure assessment has been back-extrapolated starting from the clinical visit date to better represent the temporality between the urine sample collection and air pollution exposure. Fourth, given the lack of literature on this topic, our findings may serve as a piece of knowledge for future research. Finally,

we used a unique exposure model for all the investigated cities and this allows limiting the heterogeneity introduced when different modeling approaches are used in multi-centric studies to evaluate air pollution exposure. The novelty and strengths of our study rely on several aspects, not always addressed in previous research. In the past decades, air pollution exposure assessment often focused on the use of one among the following techniques: ground-based, or single-site air monitoring stations (Brokamp et al., 2019) and biomarkers of exposure. Only recently, innovative approaches and advances in spatiotemporal models let researchers the implementation of advanced machine learning algorithms and satellite-based data to accurately estimate individual-level daily exposures at high spatial resolutions (Brokamp et al., 2019). A notable lack of previous research refers to a relatively small number of epidemiological studies on the long-term effects of air pollution (Tonne, 2017), often affected by a temporal shift between the exposure assessment and the actual year of recruitment (Hoek, 2017). In addition, the evaluation of air-pollution-related early biological effects has not always been performed in large-scale studies or on people suffering from airway disease from the general population. Moreover, while biomarkers of oxidative stress have been extensively studied in patients suffering from COPD, the same cannot be said for people affected by chronic bronchitis or other respiratory diseases (Chamitava et al., 2020) even less in the context of air-pollution-derived effects.

5. Conclusion

In conclusion, our findings suggest that exposure to air pollution may increase systemic oxidative stress levels in people suffering from airway diseases but not in healthy controls. We observed that respiratory-diseased people appear to be more prone to early biological effects in response to air pollution, which reinforces the imperative need of specific air quality standards able to minimise risks for everyone. Effective prevention strategies could consider early biological effects, including oxidative stress, observed in susceptible people, whose additional risk levels might be currently underrated. To confirm our results, further research is needed and it might consider an extension of our approach applied to longitudinal cohort studies, which can provide the highest level of causative evidence. Indeed, in the framework of a raising concern of the possible outcomes of the exposure to airborne pollutants, the implementation of our approach could be useful even in different settings, to better characterise the possible link with various oxidative stress-based chronic diseases. Although in the present study two complementary biomarkers of oxidative stress have been used, is imperative that future works quantify a panel of biomarkers assessed at different time points throughout the study. Additionally, given the wide impact that air pollution still has on the human population, multicentric studies with a greater external validity are warranted, especially those involving people with different air pollution exposures and from low-income countries. Our findings are valuable as part of a key Public Health topic, still evolving and relevant to many stakeholders including 1) healthcare and Public Health professionals, whose understanding of the biological mechanisms behind air pollution exposure is still limited and requires continuous scientific-based advancement; 2) policy decision-makers, whose decisions related to people's health need to be increasingly supported by updated scientific evidence; and 3) people from the general population and vulnerable subjects, whose awareness should be constantly increased as additional and cost-effective health promotion strategy.

CRediT authorship contribution statement

Roberto Bono, Alessandro Marcon and Giulia Squillacioti: Conceptualization; Giulia Squillacioti, Alessandro Marcon, Massimo Stafoggia and Sara Maio: Methodology; Giulia Squillacioti, Massimo Stafoggia: Formal analysis; Pierpaolo Marchetti, Angelo G. Corsico, Pietro Pirina, Giuseppe Verlato, Roberto Bono: Investigation and Data curation; Giulia Squillacioti: Writing - Original draft preparation; Giulia Squillacioti and Federica Ghelli: Visualization; Roberto Bono, Alessandro Marcon: Supervision; Giulia Squillacioti, Valeria Bellisario, Federica Ghelli, Alessandro Marcon, Pierpaolo Marchetti, Angelo G. Corsico, Pietro Pirina, Sara Maio, Massimo Stafoggia, Giuseppe Verlato Roberto Bono: Writing - Reviewing and Editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

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