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Running title: outdoor air pollution, exhaled 8-isoprostanes and asthma.

Take home message: Exhaled 8-isoprostanes concentration is associated with both outdoor air pollution and current asthma in adults.

Plain language summary: Associations between outdoor air pollution and asthma in adults are still scarce, and the underlying biological mechanisms are poorly understood. Among adults, we studied the associations between 1) long-term exposure to outdoor air pollution and current asthma 2) exhaled 8-isoprostanes, a biomarker related to oxidative stress and current asthma, and 3) outdoor air pollution and exhaled 8-isoprostanes. We found for the first time associations between long-term exposures to outdoor air pollution estimated at individual level, exhaled 8-iso concentration and current asthma. Traffic intensity and O_3 exposure increased significantly the risk of current asthma. Exhaled 8-iso concentration was positively and significantly associated with current asthma. Among participants without asthma, exhaled 8-iso concentration increased significantly with $PM_{2.5}$ exposure and decreased with O_3 and $O_{3\text{-summer}}$ exposures. Results add new insights of a potential role of oxidative stress in the associations between outdoor air pollution and asthma in adults.

ABSTRACT

Associations between outdoor air pollution and asthma in adults are still scarce, and the underlying biological mechanisms are poorly understood.

To study the associations between 1) long-term exposure to outdoor air pollution and current asthma 2) exhaled 8-isoprostanes, a biomarker related to oxidative stress and current asthma, and 3) outdoor air pollution and exhaled 8-isoprostanes.

Cross-sectional analyses were conducted in 608 adults (39% with current asthma) from the first follow-up of the French case-control and family study on asthma (EGEA). The NO_2, NOx, PM_{10}, $PM_{2.5}$, road traffic, and O_3 were assessed by ESCAPE FP7 and IFEN. Models took account city and familial dependence.

The risk of current asthma increased with traffic intensity (adjusted (a)OR=1.09 95%CI: [1.00, 1.18] per 5,000 vehicles/day), with O_3 exposure (aOR=2.04 [1.27, 3.29] per 10 $\mu g/m^3$), and with exhaled 8-iso concentration (aOR=1.50 [1.06, 2.12] per 1 pg/mL). Among participants without asthma, exhaled 8-iso concentration increased with $PM_{2.5}$ exposure (adjusted (a)β=0.23 95 CI [0.005, 0.46] per 5 $\mu g/m^3$) and decreased with O_3 and $O_{3\text{-summer}}$ exposures (aβ=-0.20 [-0.39, -0.01], aβ=-0.52 [-0.77, -0.26] per 10 $\mu g/m^3$, respectively).

Results add new insights of a potential role of oxidative stress in the associations between outdoor air pollution and asthma in adults.

Introduction

According to the World Health Organization, outdoor air pollution caused 3.7 million deaths in the world in 2012, and 9 % of the total mortality in France was recently attributed to particulate matter with a diameter ≤ 2.5 microns ($PM_{2.5}$) [1]. The most studied pollutants in relation with health effects are nitrogen dioxide (NO_2), ozone (O_3) and particulate matter (PM_{10} and $PM_{2.5}$). Evidence of the impact of outdoor air pollution on respiratory health is increasing [2] [3], and the associations between exposure to outdoor air pollution and asthma have been largely studied in children [4]. However, studies on associations between long-term exposure to outdoor air pollution and asthma among adults are still scarce [5].

One biological mechanism proposed to partly explain the association between outdoor air pollution and asthma is oxidative stress [6]. Oxidative stress is an imbalance between the increase to reactive oxygen species (ROS) and antioxidant response [7]. Among the biological markers related to oxidative stress, 8-isoprostanes (8-iso) are known as stable and specific products of lipid peroxidation [8]. Interestingly, 8-iso can be measured non-invasively in exhaled breath condensate (EBC), a fluid close to the lungs [7]. An increase of 8-iso concentration along with a decline of antioxidant defense can induce tissue damages and can contribute to pathophysiological changes as those seen in asthma [9, 10]. 8-iso are known to provoke airway hyperresponsiveness, to increase the production of mucus and to promote contraction of smooth muscles [10] [11]. In a recent systematic review, 8-iso concentration was found higher in adults with severe asthma than in those with mild-to-moderate asthma [12]. To date, epidemiological studies on the associations between long-term exposure to outdoor air pollution and asthma including the measurement of 8-iso in adults are lacking.

In the present paper, among adults from the Epidemiological study of the Genetic and Environmental factors of Asthma (EGEA), we first studied the associations between long-term exposure to outdoor air pollution (NO_2, NOx, PM_{10}, $PM_{2.5}$, traffic load, traffic intensity,

O_3 and $O_{3\text{-summer}}$) and current asthma. Second, we studied the associations between 8-iso measured in EBC with both long-term exposure to outdoor air pollution and current asthma.

Methods

Study population

EGEA is a cohort study based on an initial group of asthma cases recruited in chest clinics from 5 French cities (1991-1995) along with their first-degree relatives, and a group of controls (https://egeanet.vjf.inserm.fr/). Child controls were recruited in surgery hospitals, and adult controls in electoral rolls and from surgery hospitals or check-up centers [13]. The protocol and descriptive characteristics have been described previously [13, 14], and inclusion criteria used to define asthmatic cases and controls were described in the online supplementary material. EGEA collection is certified ISO 9001 [15]. Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris). All participants signed a written informed consent.

The present analyses included 608 adults (≥ 16 years old) in the framework of the first follow-up (EGEA2, 2003-2007), with available data on current asthma, outdoor air pollution assessed by the European Study of Cohorts for Air Pollution Effects (ESCAPE) and exhaled 8-iso concentration (figure 1). Among the 608 participants, 5 had no pollution data from the French Institute for the Environment (IFEN) assessment. No significant differences were found between the participants included and those not included (n=963, see online supplementary table E1).

Respiratory phenotypes

At EGEA2, the participants with ever asthma answered positively to at least one of the two following questions "*Have you ever had attacks of breathlessness at rest with wheezing?*" or "*Have you ever had asthma attacks?*", or were recruited as asthmatic cases at EGEA1.

Among participants with ever asthma, "*current asthma*" was defined by a report of respiratory symptoms (wheeze, nocturnal chest tightness, attacks of breathlessness following strenuous activity, at rest or at night time), or asthma attacks or use of inhaled and/or oral medicines because of breathing problems in the past twelve months [16] (see the online supplementary material for more details). Only participants with current asthma were included in the analyses because the phenotype "current asthma" which reflects the recent activity of the disease was more relevant than the phenotype "ever asthma" to study the associations between long-term exposure to outdoor air pollution, biological markers and asthma.

Exposure assessment

Thanks to ESCAPE and IFEN assessments, outdoor air pollution exposures (NO_2, NOx, PM, O_3 and road traffic) were assigned to each participant's residential address.

Annual air pollution levels of NO_2 and particulate matter (PM) were derived from ESCAPE standardized models (www.escapeproject.eu/manuals/). Briefly, the ESCAPE monitoring campaigns took place between 2009 and 2010, including 40 measurement sites for NO_2 and NOx in Paris, Lyon, Grenoble and Marseille, and 20 PM measurement sites in Paris and Grenoble. Land-use regression (LUR) models were developed and two indicators of road traffic were also calculated. Traffic intensity on nearest road was defined as the number of motor vehicles circulating per day on nearest road to participant's home, and was expressed in vehicles per day. Total traffic load was defined as the traffic load in all major roads based around a buffer of 100 meters from the participant's home, and was expressed by traffic intensity multiplied by road length. The back-extrapolation is used to transfer the recent or

current LUR models to earlier years. In our study, the estimation of outdoor air pollution by ESCAPE took place after EGEA2, and accordingly we also analyzed the back-extrapolated pollution estimates in order to get a better temporality between outdoor air pollution with current asthma and EBC collection. Back-extrapolated pollution data were available for NO_2, NOx in all cities, and for PM_{10} in Paris.

In order to supplement the ESCAPE data set, we used O_3 and $O_{3\text{-summer}}$ exposures from the IFEN (see the online supplementary material for more details). The O_3 estimate was the yearly mean of ozone level in 2004 for each participant at the residential address, and derived from a geo-statistical model as previously described [17]. The exposure of $O_{3\text{-summer}}$ was assessed from the monthly means from April to September.

Measurement of exhaled 8-isoprostanes

Exhaled Breath Condensate (EBC) was collected at EGEA2 between 2003 and 2006 with an RTubeTM (Respiratory Research Inc., Charlottesville, VA, USA) according to a standardized method, as described previously [18]. Samples were immediately stored at -80°C. Exhaled 8-iso concentration was measured5.3 to 9.4 years after EBC collection, with a specific enzyme immunoassay (EIA) kit (8-isoprostanes EIA kit Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. Approximately 30% of exhaled 8-iso concentration was below the Limit of Detection (< LD, see the online supplementary material for more details).

Statistical analyses

The level of outdoor air pollution was described by city. Due to its skewed distribution, the exhaled 8-iso concentration was log-transformed.

Associations between outdoor air pollution and current asthma, and associations between

exhaled 8-iso concentration and current asthma were studied with logistic models. In order to study the association between outdoor air pollution and exhaled 8-iso concentration independently of asthma, linear regression models were first performed among participants without asthma and sensitivity analyses were conducted by: a) using back-extrapolated pollution estimates, b) studying the associations by city, and c) using bi-pollutant models ($PM_{2.5}$ and O_3 or $O_{3\text{-summer}}$). Analyses with back-extrapolated pollution estimates were performed to get a better temporality between pollution and EBC collection. We also studied the associations between outdoor air pollution and exhaled 8-iso concentration in all participants, and among participants with current asthma.

All estimates were adjusted for age, sex, smoking habits. Further adjustments were conducted for body mass index (BMI), socio-professional category (SPC), and use of cleaning products which is an indicator of exposure to indoor pollution. In order to study only the road traffic effect, estimates of associations between road traffic and current asthma or exhaled 8-iso concentration were also adjusted for background NO_2. As the aim was to study the effect of long-term exposure to outdoor air pollution, we performed sensitivity analyses by excluding participants who lived less than one year at the same address (N=46).

In order to take into account the city-effect on outdoor air pollution levels, we performed meta-analyses including city-specific analyses from the Harvard School of Public Health's macro program (https://www.hsph.harvard.edu/donna-spiegelman/software/metaanal), and pooled analyses with random effect on city.

The above models have taken into account random effects on familial dependence. All the results are expressed for an increase of 1 pg/mL of exhaled 8-iso concentration, an increase of 20 $\mu g/m^3$ of NOx, 10 $\mu g/m^3$ of NO_2, PM_{10}, O_3 and $O_{3\text{-summer}}$ and 5 $\mu g/m^3$ of $PM_{2.5}$. For traffic measures, the results of traffic load are expressed for four million vehicles × m/day in major roads within a 100-m buffer, and the results of traffic intensity are expressed for an increase

of 5,000 vehicles/day on major road. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA).

Results

The mean age of the 608 adults was 43 years, 47% were males, 39% had current asthma, 21% were current smokers, and 37 % were overweight (BMI ≥25 kg/m^2) (table 1). Participants with current asthma were younger, more often men, current smokers and unemployed, and had more often a secondary education level than those without asthma (table 1). Participants with current asthma had also a lower FEV_1, higher airway hyperresponsiveness, higher allergic sensitization, higher IgE level, and higher exhaled 8-iso concentration than those without asthma.

The characteristics of participants were heterogeneous across cities (see the online supplementary table E2 and the online supplementary material for more details).

The pollutant and traffic levels were the highest in Paris, except for the PM_{10} level (online supplementary figures E1 and E2). The PM_{10} and $PM_{2.5}$ levels were above the values recommended by the WHO. The O_3 and $O_{3\text{-summer}}$ levels were the highest in Marseille (online supplementary figure E3). Positive and significant correlations were found between NO_2, NOx, PM_{10}, and $PM_{2.5}$ (correlation coefficient (r) $0.47 \leq r \leq 0.95$, all $P<0.002$) whereas O_3 and $O_{3\text{-summer}}$ levels were negatively correlated with all pollutants ($-0.50 \leq r \leq -0.15$, all $P<0.002$) (data not shown). The NO_2 level was significantly higher in managers and technicians, and $O_{3\text{-summer}}$ level was significantly higher in manual workers (data not shown).

The exhaled 8-iso concentration (25^{th};75^{th} percentiles) was 3.16 (1.40;7.69) pg/mL in all participants and 3.97 (1.85;9.10) pg/mL among those with current asthma (table 1), and was 7 times higher in Paris than in other cities, was higher in women and decreased with age (online supplementary tables E2 and E3). No significant association was found between exhaled 8-iso

concentration and smoking expressed as current smoking habits, quantity of tobacco, or number of pack-years (all P>0.80, data not shown). No inter-plate variability was observed (data not shown) and no association was found between storage time and exhaled 8-iso concentration (regression coefficient=0.02, P=0.39).

Associations between outdoor air pollution and current asthma

Associations between outdoor air pollution and current asthma were not significantly heterogeneous between cities (Q test, p-value>0.08). In pooled analyses, the risk of current asthma increased significantly with traffic intensity (adjusted (a)OR=1.09 95%CI [1.00, 1.18]) and with O_3 exposure (aOR=2.04 [1.27, 3.29], table 2) whatever the adjustment. The results were similar after excluding participants who lived less than one year at the same address. Back-extrapolated exposure estimates gave similar results (online supplementary table E4).

Associations between exhaled 8-iso concentration and current asthma

A positive and significant association was found between exhaled 8-iso concentration expressed as a continuous variable and current asthma (aOR=1.50 [1.06, 2.12], figure 2). No significant association was found when exhaled 8-iso concentration was expressed as <LD or >LD. Overall, the risk of current asthma increased significantly with exhaled 8-iso concentration expressed as < LD, >LD and ≤median, >LD and >median (trend p-value=0.05). Exhaled 8-iso concentration was unrelated to duration of asthma (years), age of asthma onset expressed continuously (years) or in classes (all P>0.40). Further, no significant association was found between exhaled 8-iso concentration with FEV_1 % predicted continuously or in classes (all P>0.60) or allergic sensitization (P=0.12), (data not shown).

Associations between outdoor air pollution and exhaled 8-iso concentration among participants without asthma

Associations between outdoor air pollution and exhaled 8-iso concentration were not heterogeneous between cities (Q test, p-values>0.20). In pooled analyses, exhaled 8-iso concentration increased significantly with $PM_{2.5}$ exposure (adjusted (a)β=0.23 [0.005, 0.46], and decreased with O_3 and $O_{3\text{-summer}}$ exposures (aβ=-0.20 [-0.39,-0.01] and aβ=-0.52 [-0.77,-0.26] whatever the adjustment, table 3). The results were similar after excluding participants who lived less than one year at the same address. Analyses performed with back-extrapolated data also gave similar results (online supplemental table E5). Furthermore, exhaled 8-iso concentration decreased significantly with O_3 and $O_{3\text{-summer}}$ exposures in Paris (aβ=-0.22 [-0.42,-0.02] and aβ=-0.53 [-0.90,-0.16], online supplementary tables E6). After excluding participants from Paris (n=111), no significant associations were observed between O_3 and $O_{3\text{-summer}}$ and exhaled 8-iso concentration (online supplementary table E7). In models adjusted for both $PM_{2.5}$ and O_3, similar result was found only between $O_{3\text{-summer}}$ exposure and exhaled 8-iso concentration (aβ=-0.59 [-0.71,-0.47], online supplementary table E8). In models adjusted for both NO_2 and O_3, the negative associations between O_3 and $O_{3\text{-summer}}$ and exhaled 8-iso concentration remained statistically significant (data not shown). In all participants, only $O_{3\text{-summer}}$ exposure was negatively and significantly associated with exhaled 8-iso concentration (aβ=-0.33 [-0.55,-0.11], data not shown). No association was found between outdoor air pollution and exhaled 8-iso concentration in participants with current asthma (data not shown).

Discussion

For the first time in adults, we found associations between long-term exposures to outdoor air pollution estimated at individual level, exhaled 8-iso concentration and current asthma.

Traffic intensity and O_3 exposure increased significantly the risk of current asthma. Exhaled 8-iso concentration was positively and significantly associated with current asthma. Among participants without asthma, exhaled 8-iso concentration increased significantly with $PM_{2.5}$ exposure and decreased with O_3 and $O_{3\text{-summer}}$ exposures.

Participants with asthma included in the present analyses were mostly recruited in chest clinics as asthma cases, with a careful procedure set up to include true asthmatics using standardized and validated questionnaires. Others were recruited as first-degree relatives of asthmatic cases based on answers to questions on asthma diagnosis. This leads to a group of asthmatics with a wide range of disease expression. In our cross-sectional analyses, cause and consequence cannot be disentangled. It was not possible to study the associations between outdoor air pollution and exhaled 8-iso concentration with the incidence of asthma because only 30 new cases of asthma were reported at EGEA2. But, there is clearly a need for further research to confirm the associations and to clarify its causal underpinnings. Regarding exposure assessment, LUR models are well-adapted to take into account the spatial variation of NO_2, $PM_{2.5}$ [19] and NOx [20], and ESCAPE resolution is accurate to estimate the exposure to markers of road traffic which have a spatial heterogeneity. In addition, IFEN resolution is larger than ESCAPE but suitable for O_3 and $O_{3\text{-summer}}$ which are homogeneous over long distances [21]. We acknowledge that a weakness of our study is the non-compliance of the temporality because outdoor air pollution was estimated by ESCAPE between 2009 and 2010 whereas the collection of EBC and phenotype "current asthma" took place at EGEA2 between 2003 and 2007. To get a better temporality in our analyses, we used the back-extrapolated pollution estimates which were back-extrapolated at participants' residential address at EGEA2, and found similar results. Both back-extrapolated and non-back-extrapolated estimates were highly and significantly correlated in our study (correlation coefficients$\geq$0.98, $P<0.001$) as previously reported by Beelen *et al*. in the same ESCAPE

project and for a longer period [22]. Previously in ESCAPE study, associations between NO_2 back-extrapolated estimates and asthma incidence were similar to those with non-back-extrapolated estimates [23]. We aimed to study the impact of long-term exposure to outdoor air pollution, and therefore conducted sensitivity analyses by excluding participants who lived less one year at the same residential address that did not change our conclusions. We cannot exclude that some non-differential misclassification of pollution exposure may have occurred because the time-activity patterns of participants were not available in our analyses, but in this case, it would led to bias towards the null. Furthermore, IFEN resolution can better represented daily participant's exposures to O_3 and $O_{3\text{-summer}}$, at least for those whom work was close to home. We could not take into account all indoor environmental factors; however, we found similar results after further adjustment for domestic exposure to cleaning products. Furthermore, the adjustment for the socioeconomic position potentially associated with pollutant exposures [24] and asthma gave also similar results. The EGEA study is a case-control and family study. Participants from the same family share genetic background, and also socio-economic and lifestyle factors, that could be associated with the exposure to outdoor air pollution or the asthma risk. We therefore took into account familial dependence through random effects in mixed models. Sub-groups analyses suffer from a lack of power but, as best as possible, we used the most suitable statistical models. Finally, the EIA method has been preferred for dosing exhaled 8-iso concentration rather the GCMS method because the former is better adapted to analyze a larger number of samples as in our study.

We found that traffic intensity and O_3 exposure increased the risk of current asthma. Our results add evidence of the impact of long-term exposure to outdoor air pollution on asthma in adults. Our results are partly in accordance with those of a recent study showing that traffic exposure but not NO_2 exposure, assessed by satellite-based LUR model at residential addresses, was positively associated with current asthma in 1367 adults [25]. We also found a

positive association between O_3 exposure and current asthma. To our knowledge, the literature assessing outdoor air pollution effect on asthma focused mainly on other asthma phenotypes such as asthma onset, asthma severity, or asthma control. A study conducted in California has reported that long-term exposure to O_3 was associated with development of asthma in adult males [26]. Previously in the EGEA study, long-term exposure to O_3 estimated by IFEN was associated with asthma severity [17] and with uncontrolled asthma [27]. Recently, a cohort showed that asthmatic adults exposed to O_3 had a greater risk to develop asthma-chronic obstructive pulmonary disease [28]. Interestingly, various asthma phenotypes were studied such as asthma-onset which reflects the initiation of the disease, and severity and control of asthma which are linked to manifestations of the disease. Asthma reflects both ever asthma and current asthma, and the participants with ever asthma had not necessarily a current asthma. In the EGEA study, the phenotype "current asthma" was defined by the report of respiratory symptoms or asthma attacks or use of inhaled and/or oral medicines because of breathing problems in the past twelve months. To study the associations between long-term exposure to outdoor air pollution, biological markers and asthma, the phenotype "current asthma" was more relevant than the phenotype "ever asthma" because it reflects the recent activity of the disease. Overall, all these findings add evidence of associations between outdoor air pollution and asthma in adults, whatever the studied phenotypes.

This study adds new insights into a potential role of oxidative stress in the associations between long-term exposure to outdoor air pollution and asthma in adults. We reported a significant association between exhaled 8-iso concentration and current asthma after adjustment for age, sex, smoking habits and body mass index. We investigated whether other asthma characteristics could explain this association, but we did not found any significant association between exhaled 8-iso concentration with duration of asthma, age of asthma onset,

lung function, or allergic sensitization. Our results added new evidence to the previous associations reported in the literature with asthma severity and asthma control [12]. We found for the first time that exhaled 8-iso concentration was positively associated with $PM_{2.5}$ exposure in participants without asthma. Unfortunately, we did not have back-extrapolated data for $PM_{2.5}$. In France, $PM_{2.5}$ level has overall decreased between 2002 and 2012 and the association between $PM_{2.5}$ exposure and exhaled 8-iso concentration may be underestimated [29]. The literature mainly focused on short-term exposure to outdoor air pollution and on other biological compartments. In fact, short-term exposure to $PM_{2.5}$ was previously found to be positively associated with EBC 8-iso concentration among healthy adolescents [30], and with 8-iso concentration in urine among adults [31]. We also found counterintuitive negative associations between O_3 and $O_{3\text{-summer}}$ exposures and exhaled 8-iso concentration. Our results are not in accordance with those of a previous study showing that O_3 exposure assigned to residential location was associated with higher plasma 8-iso concentration in 120 healthy students [32]. The inconsistency with our findings may be partially attributed to differences in the study design, in the biological compartment, and in the spatial resolution which was less accurate than in our study. We showed that O_3 exposure and exhaled 8-iso concentration increased the risk of current asthma, and we did not expect negative associations between O_3 and $O_{3\text{-summer}}$ exposures and exhaled 8-iso concentration. We investigated more thoroughly why these associations were negative. The analyses conducted by city showed that Paris heavily weighted the negative associations between O_3 and $O_{3\text{-summer}}$ exposures and exhaled 8-iso concentration. Previously, a study has also reported a negative association between short-term exposure to O_3 and exhaled 8-iso in New Yorker's adolescents [33]. Another explanation is that O_3 is a secondary pollutant mainly produces through complex chemical reactions from NO_2. In our study, O_3 and NO_2 levels were highly and negatively correlated, and NO_2 exposure was also positively associated with exhaled 8-iso concentration but not significantly.

One hypothesis is likely that the positive association between NO_2 and exhaled 8-iso partly explained the negative associations observed for O_3 and $O_{3\text{-summer}}$. Overall, the specific effects of pollutants are hard to disentangle even in bi-pollutant models given the strong correlations between pollutants. It is interesting to note that participants from Paris had particular characteristics as an exhaled 8-iso concentration 7 times higher, were exposed to higher levels of road traffic, NO_2 and PM, and to lower levels of O_3 and $O_{3\text{-summer}}$ than in other cities, suggesting that Parisians may have particular characteristics that could partly explain the negative associations between ozone and exhaled 8-iso concentration. O_3 and $O_{3\text{-summer}}$ were negatively correlated with $PM_{2.5}$, but the negative association between $O_{3\text{-summer}}$ exposure and exhaled 8-iso concentration remained significant in a bi-pollutant model. Previously in EGEA adults, O_3 assessed by IFEN was also found to be negatively associated with the EBC total nitrites/nitrates level, a biological marker of nitrosative stress [34]. In the literature, complex interplays between nitrosative and oxidative stress pathways have been reported [35], including a reciprocal regulation. An alternative explanation is that our results were likely due to other factors that are not included in the present analyses. In all participants, only a negative and significant association between $O_{3\text{-summer}}$ exposure and exhaled 8-iso concentration was found, and no association was found in participants with current asthma. In a directed acyclic graph (DAG), the covariate "current asthma" may be a collider, which makes the interpretation of our results even more difficult in a context of cross-sectional analyses [36].

Several biological mechanisms by which outdoor air pollution may be associated with asthma have been suggested in previous reviews [37, 38]. Outdoor air pollution exposure may increase oxidative stress in airways through the production of reactive oxygen species and local inflammation [9]. The PM can be supports for allergens and their small size gives them a large surface related to oxidative potential [39]. To disentangle the complexity of the

associations between asthma and oxidative stress, and 8-iso in particular, there is a need for longitudinal epidemiological studies. Overall, our results add new insights of a potential role of oxidative stress in the association between long-term exposure to outdoor air pollution and asthma in adults. To better understand the underlying biological pathways between outdoor air pollution and asthma, future epidemiological studies should use individual portable sensors in order to improve the pollution assessment, study the different windows of exposure and try to collect multiple exposures in order to identify exposure profiles through clustering methods.

Conclusions

We found that traffic intensity, O_3 exposure and exhaled 8-iso concentration increased the risk of current asthma, and that among participants without asthma exhaled 8-iso concentration increased with $PM_{2.5}$ exposure, and decreased with O_3 and $O_{3\text{-summer}}$ exposures. Exhaled 8-iso seems to be an interesting oxidative stress-related biomarker adapted to epidemiological studies. Overall, our study adds new insights in the associations between long-term exposure to outdoor air pollution and asthma in adults, and suggests that oxidative stress may partly explained such associations. Longitudinal studies with larger samples are now needed to confirm such results.

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Table 1. Characteristics of adult participants according to asthma status.

Characteristics	In all participants n=608	Participants without asthma n=368	Participants with current asthma n=240	p-value
Age (years), mean ± SD	42.5 ± 17.2	45.5 ± 26.3	37.9 ± 17.5	**<0.0001**[+]
Sex, Men, n (%)	288 (47.4)	160 (43.5)	128 (53.3)	**0.02**[+]
Smoking habits, n (%)				
Never smokers	312 (51.3)	189 (51.4)	123 (51.3)	
Ex-smokers	168 (27.6)	113 (30.7)	55 (22.9)	**0.02**[+]
Current smokers	128 (21.1)	66 (17.9)	62 (25.8)	
BMI (kg/m²), n (%)				
<20	70 (11.5)	38 (10.3)	32 (13.3)	
[20-25[	316 (52.0)	193 (52.4)	123 (51.3)	0.62
[25-30[	165 (27.1)	104 (28.3)	61 (25.4)	
>=30	57 (9.4)	33 (9.0)	24 (10.0)	
Socio-professional category, n (%)	n=605	n=366	n=239	
Unemployed	72 (11.9)	29 (7.9)	43 (18.0)	
Manager	212 (35.0)	138 (37.7)	74 (31.0)	**0.001**[+]
Technician	248 (41.0)	149 (40.7)	99 (41.4)	
Manual worker	73 (12.1)	50 (13.7)	23 (9.6)	
Number of years at the same residential address, mean ± SD (min;max)	n=607 12.4 ± 10.7 (0;48)	13.0 ±10.9 (0;48)	n=239 11.4 ± 10.4 (0;46)	***0.07***[+]
Asthma, n (%) Current	240 (39.5)	/	/	
Duration of asthma (years), mean ± SD (min;max)	/	/	n=229 15.9 ± 11.6 (0;59.6)	
Age of asthma onset (years)			n=228	
⩽4	/	/	80 (35.1)	
]4–16]			79 (34.7)	
>16			69 (30.3)	
FEV1 % predicted, mean ± SD	103 ± 18.9	108 ± 17.3	95.2 ± 18.5	**<0.0001**[+]
Allergic sensitization[¶], n (%)	n=602 327 (54.3)	(n=363) 137 (37.7)	n=239 190 (79.5)	**<0.0001**[+]
Exhaled 8-iso concentration, pg/mL, GM (q1;q3)	3.16 (1.41;7.69)	2.69 (1.14;6.79)	3.97 (1.85;9.10)	**0.004**[+]
Exhaled 8-iso concentration >LD, n (%)	431 (70.9)	253 (68.8)	178 (74.2)	0.15

BMI, body mass index; 8-iso, 8-isoprostanes; GM, geometric mean; q1 and q3, the 25th and the 75th percentiles of the GM; LD, limit of detection of the 8-iso concentration.FEV_1, forced expiratory volume; [¶]: defined by at least one weal ≥ 3 mm to 12 tested allergens; [+]Results in bold represent significant results (p-values≤0.05).

Table 2. Associations between outdoor air pollution and current asthma.

		NO_2	NOx	PM_{10}	$PM_{2.5}$	Total traffic load on major roads in a 100-m buffer of the home[#]	Traffic intensity at the road nearest to a participant's home[#]	O_3	$O_{3\text{-summer}}$
Model 1	n	608	608	437	437	605	**605**	**603**	603
	OR crude (95% CI)	0.99 (0.87,1.15)	1.05 (0.92,1.19)	1.10 (0.65,1.85)	0.89 (0.54,1.45)	1.16 (0.96,1.41)	**1.09 (1.00,1.19)**[¶]	**2.01 (1.26,3.23)**[¶]	1.29 (0.68,2.46)
Model 2	n	608	608	437	437	608	**608**	**603**	603
	OR adjusted (95% CI)	0.99 (0.86,1.14)	1.04 (0.91,1.18)	1.05 (0.62,1.79)	0.82 (0.49,1.36)	1.15 (0.95,1.38)	**1.09 (1.00,1.19)**[¶]	**1.93 (1.21,3.09)**[¶]	1.10 (0.67,1.81)
Model 3	n	605	605	436	436	605	**605**	**600**	600
	OR adjusted (95% CI)	1.00 (0.87,1.16)	1.05 (0.92,1.19)	1.05 (0.61,1.81)	0.84 (0.50,1.39)	1.15 (0.95,1.39)	**1.10 (1.00,1.20)**[¶]	**1.89 (1.19,3.02)**[¶]	1.10 (0.67,1.81)
Model 4	n	603	603	435	435	603	**603**	**598**	598
	OR adjusted (95% CI)	0.98 (0.85,1.14)	1.03 (0.90,1.17)	1.03 (0.59,1.80)	0.82 (0.49,1.39)	1.14 (0.94,1.37)	**1.09 (1.00,1.18)**[¶]	**2.04 (1.27,3.29)**[¶]	1.24 (0.71,2.18)
Model 5	n	557	557	400	400	557	**557**	**553**	553
	OR adjusted (95% CI)	0.99 (0.86,1.16)	1.04 (0.91,1.19)	1.03 (0.57,1.85)	0.89 (0.52,1.53)	1.18 (0.97,1.45)	**1.10 (1.00,1.19)**[¶]	**1.67 (1.06,2.63)**[¶]	1.06 (0.77,1.45)

Data are presented as OR and 95% confidence intervals (95% CI) with the participants without asthma as reference. The logistic models were conducted with random effects on familial dependence (level 2) and city (level 3).
Results are expressed per 20 µg/m^3 increase of NOx exposure, per 10 µg/m^3 increase of NO_2, PM_{10}, O_3 and $O_{3\text{-summer}}$ exposures, per 5 µg/m^3 increase of $PM_{2.5}$ exposure, per 4.000.000 vehicles x m /day increase of total traffic load and per 5.000 vehicles/day increase of traffic intensity.
Model 1: unadjusted; Model 2: adjusted for age, sex, smoking habits and body mass index. Model 3: adjusted for age, sex, smoking habits, body mass index and socio-professional category. Model 4: adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products. Model 5: after excluding participants who lived less than one year at the same address (N=46) and adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products.
[#]Estimates were also adjusted for background NO_2.
[¶]Results in bold represent significant results (p-values≤0.05).

Table 3. Associations between outdoor air pollution and exhaled 8-iso concentration among participants without asthma.

		NO_2	NOx	PM_{10}	$PM_{2.5}$	Total traffic load on major roads in a 100-m buffer of the home[#]	Traffic intensity at the road nearest to a participant's home[#]	O_3	$O_{3\text{-summer}}$
	n	253	253	185	**185**	253	253	**250**	**250**
Model 1	β crude (95% CI)	0.05 (-0.008,0.11)	0.03 (-0.02,0.08)	0.13 (-0.10,0.36)	**0.27 (0.05,0.49)**[¶]	0.07 (-0.04,0.18)	0.03 (-0.02,0.08)	**-0.22 (-0.41,-0.03)**[¶]	**-0.55 (-0.81,-0.28)**[¶]
	p-value	0.08	0.22	0.30	**0.02**	0.18	0.14	**0.02**	**0.001**
	n	253	253	185	**185**	253	253	**250**	**250**
Model 2	β adjusted (95% CI)	0.04 (-0.02,0.10)	0.02 (-0.03,0.07)	0.12 (-0.11,0.36)	**0.25 (0.03,0.46)**[¶]	0.04 (-0.07,0.15)	0.02 (-0.03,0.07)	**-0.20 (-0.38,-0.02)**[¶]	**-0.52 (-0.78,-0.26)**[¶]
	p-value	0.24	0.59	0.33	**0.03**	0.50	0.37	**0.03**	**0.002**
	n	253	253	185	**185**	253	253	**250**	**250**
Model 3	β adjusted (95% CI)	0.03 (-0.03,0.09)	0.01 (-0.05,0.07)	0.11 (-0.12,0.34)	**0.23 (0.005,0.46)**[¶]	0.03 (-0.09,0.15)	0.02 (-0.02,0.06)	**-0.20 (-0.39,-0.01)**[¶]	**-0.52 (-0.77,-0.26)**[¶]
	p-value	0.29	0.63	0.33	**0.04**	0.56	0.35	**0.04**	**0.002**
	n	233	233	172	**172**	233	233	**230**	**230**
Model 4	β adjusted (95% CI)	0.03 (-0.03,0.10)	0.008 (-0.05,0.07)	0.11 (-0.14,0.36)	**0.23 (-0.002,0.46)**[¶]	0.04 (-0.08,0.16)	0.02 (-0.03,0.07)	**-0.23 (-0.43,-0.03)**[¶]	**-0.56 (-0.83,-0.29)**[¶]
	p-value	0.34	0.79	0.39	**0.054**	0.53	0.34	**0.03**	**0.001**

Data are presented as crude β and 95% confidence intervals (95% CI). The linear regression models were conducted with random effects on familial dependence (level 2) and city (level 3). 8-iso concentration was log10 transformed.
Results are expressed per 20 µg/m^3 increase of NOx exposure, per 10 µg/m^3 increase of NO_2, PM_{10}, O_3 and $O_{3\text{-summer}}$ exposures, per 5 µg/m^3 increase of $PM_{2.5}$ exposure, per 4.000.000 vehicles x m /day increase of total traffic load and per 5.000 vehicles/day increase of traffic intensity.
Model 1: unadjusted; Model 2: adjusted for age, sex and smoking habits; Model 3: adjusted for age, sex, smoking habits and cleaning products. Model 4: after excluding participants who lived less than one year at the same address (N=46) and adjusted for age, sex, smoking habits, body mass index, socio-professional category and cleaning products.
[#]Estimates were also adjusted for background NO_2.
[¶]Results in bold represent significant results (p-values≤0.05).

Figure legends

Figure 1. Flow chart of the studied population.
ESCAPE, European Study of Cohorts for Air Pollution Effects; 8-iso, 8-isoprostanes;
LD, limit of detection of the 8-iso concentration; EBC, Exhaled Breath Condensate.

Figure 2. Associations between exhaled 8-iso concentration and current asthma.
8iso, 8-isoprostanes; LD, limit of detection of the 8-iso concentration.
The logistic models were conducted with random effects on familial dependence.
Estimates were adjusted for age, sex, smoking habits and body mass index. 8-iso concentration was log10 transformed. Figures are OR (95% confidence intervals) with participants without asthma as reference.
[#]Result is expressed of an increase for one unit of the concentration in 8-iso.
[¶]The 8-iso concentration below the limit of detection was used as reference category.

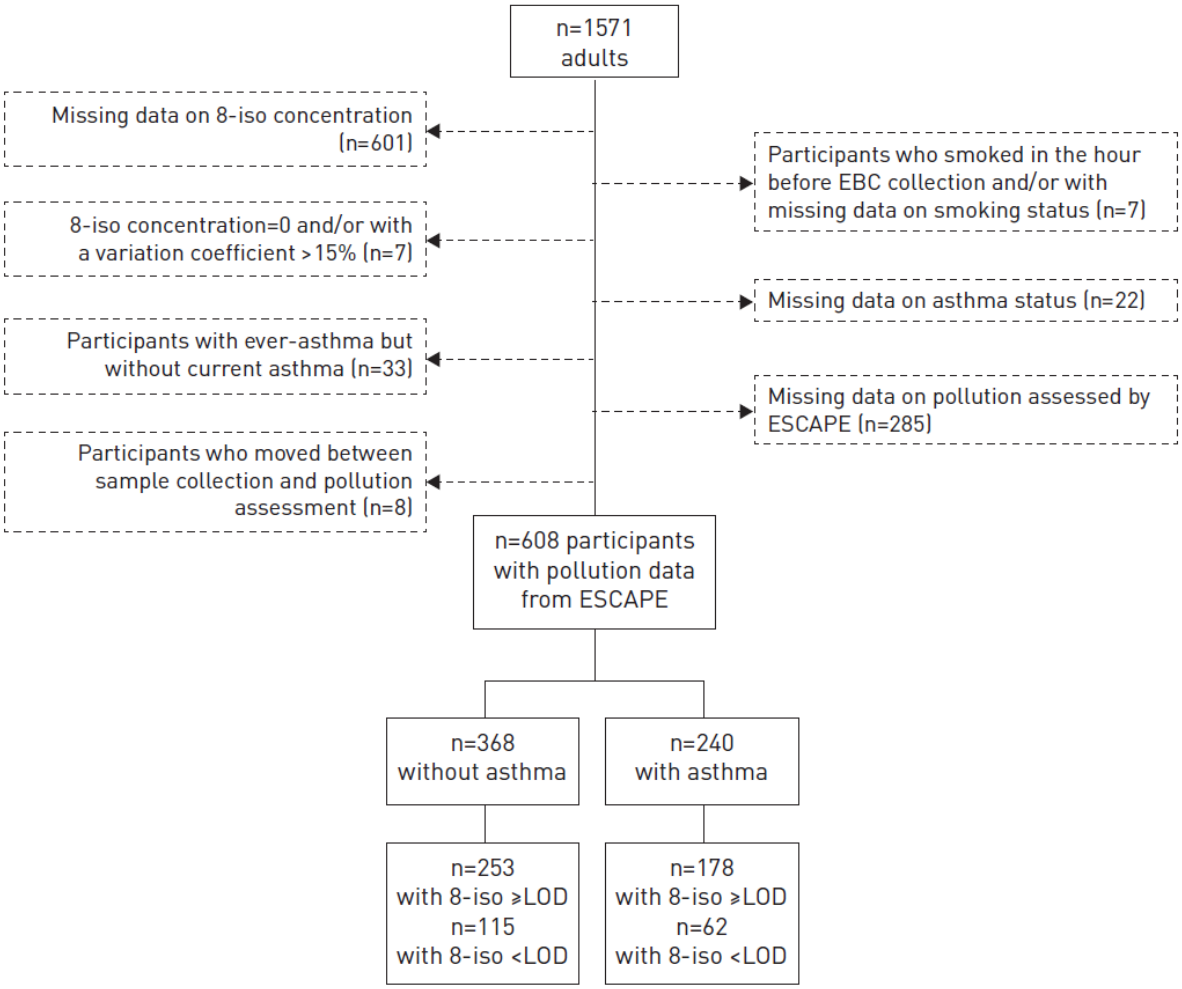
n=1571
adults
Missing data on 8-iso concentration (n=601)
Participants who smoked in the hour before EBC collection and/or with missing data on smoking status (n=7)
8-iso concentration=0 and/or with a variation coefficient >15% (n=7)
Missing data on asthma status (n=22)
Participants with ever-asthma but without current asthma (n=33)
Missing data on pollution assessed by ESCAPE (n=285)
Participants who moved between sample collection and pollution assessment (n=8)
n=608 participants with pollution data from ESCAPE
n=368
without asthma
n=240
with asthma
n=253
with 8-iso ≥LOD
n=115
with 8-iso <LOD
n=178
with 8-iso ≥LOD
n=62
with 8-iso <LOD

Figure 1

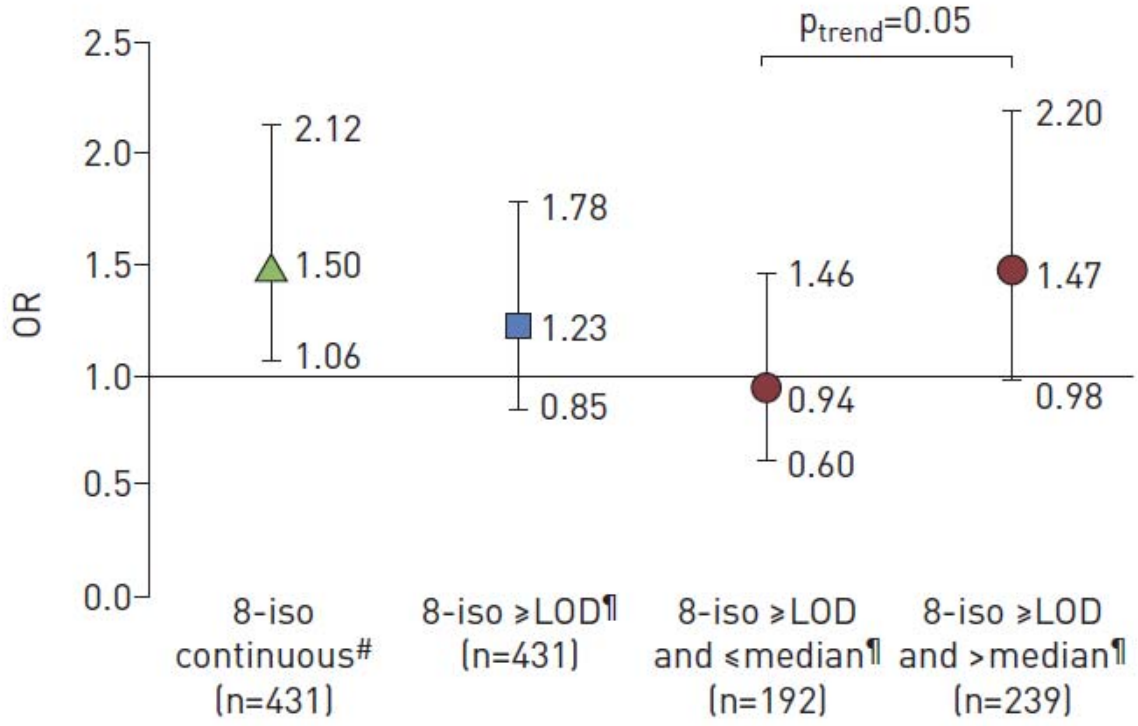
p_{trend}=0.05
OR
2.5
2.0
1.5
1.0
0.5
0.0
2.12
1.50
1.06
1.78
1.23
0.85
1.46
0.94
0.60
2.20
1.47
0.98
8-iso continuous# (n=431)
8-iso ≥LOD¶ (n=431)
8-iso ≥LOD and ≤median¶ (n=192)
8-iso ≥LOD and >median¶ (n=239)

Figure 2