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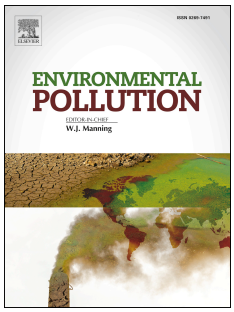

Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts

Tianyu Zhao, Iana Markevych, Marie Standl, Tamara Schikowski, Dietrich Berdel, Sibylle Koletzko, Rudolf A. Jörres, Dennis Nowak, Joachim Heinrich

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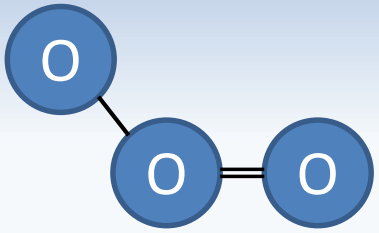
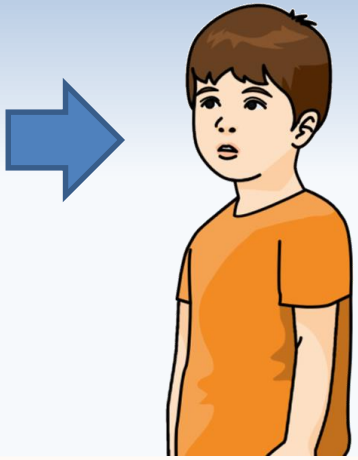


short-term exposure to ambient ozone



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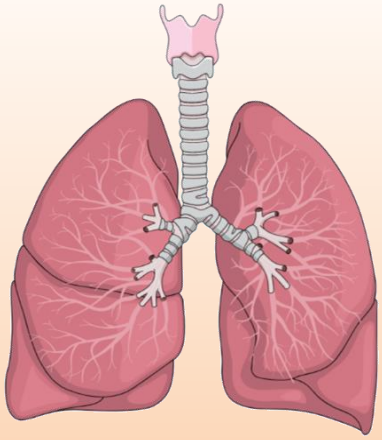

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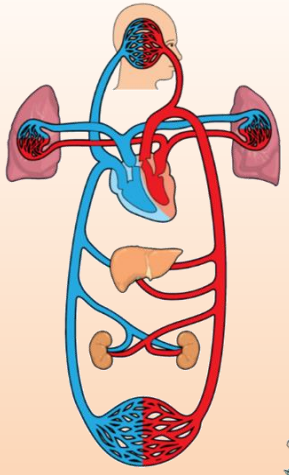
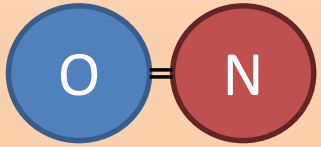
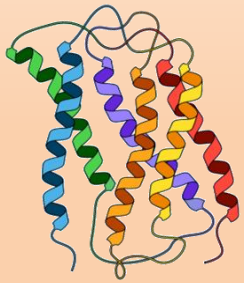
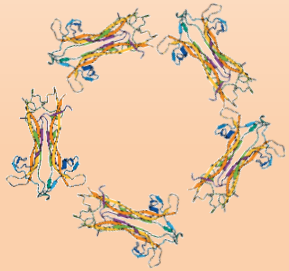




fractional exhaled nitric oxide



interleukin-6



high-sensitivity C-reactive protein

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Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts

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Abstract

Background: While exposure to ambient particulate matter (PM) and nitrogen dioxide (NO_2) is thought to be associated with diseases via inflammatory response, the association between ozone exposure and inflammation has been less investigated.

Aim: We analyzed associations between short-term exposure to ozone, an oxidative pollutant, and three inflammatory biomarkers among children and adolescents.

Methods: These cross-sectional analyses were based on two follow-ups of the GINIplus and LISA German birth cohorts. We included 1330 10-year-old and 1591 15-year-old participants. Fractional exhaled nitric oxide (FeNO) and high-sensitivity - C reactive protein (hs-CRP) were available for both age groups while interleukin (IL)-6 was measured at 10 years only. Maximum 8-hour averages of ozone and daily average concentrations of NO_2 and PM with an aerodynamic diameter < 10 µm (PM_{10}) were adopted from two background monitoring stations 0 (same day), 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling. To assess associations, we utilized linear regression models for FeNO, and logistic regressions for IL-6 and hs-CRP, adjusting for potential covariates and co-pollutants NO_2 and PM_{10}.

Results: We found that short-term ozone exposure was robustly associated with higher FeNO in adolescents at 15-year-old, but not at age 10. No consistent associations were observed between ozone and IL-6 in children aged 10 years. The relationship between hs-CRP levels and ozone was J-shaped. Relatively low ozone concentration (e.g., < 120 µg/m³) were associated with reduced hs-CRP levels, while high concentrations (e.g., ≥ 120 µg/m³) tended to be associated with elevated levels for both 10- and 15-year-old participants.

Conclusions: Our study demonstrates significant associations between short-term ozone exposure and FeNO at 15 years of age and a J-shaped relationship between ozone and hs-CRP. The finding indicates that high ozone exposure may favor inflammatory responses in adolescents, especially regarding airway inflammation.

Keywords

Epidemiology; Air pollution; Nitric oxide; Interleukin-6; C-reactive protein; Dose-response relationship

Capsule

Exposure to higher levels of ambient ozone in adolescents was associated linearly with higher FeNO, and J-shaped with hs-CRP, but not with IL-6.

Abbreviations

BMI, body mass index

CI, confidence interval

FeNO, fractional exhaled nitric oxide

GAM, generalized additive model

GINIplus, German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development

hs-CRP, high sensitivity-C reactive protein

IL, interleukin

IQR, interquartile range

LISA, influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany

MARS, multivariate adaptive regression splines

NO, nitric oxide

NO_2, nitrogen dioxide

NOS, nitric oxide synthases

OR, odds ratio

PM, particulate matter

PM_{10}, particulate matter with an aerodynamic diameter < 10 µm

ppb, parts per billion

SD, standard deviation

UBA, Umweltbundesamt (German Environment Agency)

1. Introduction

Increasing evidence suggests associations between ambient air pollution, especially particulate matter (PM) and nitric oxides, and the onset of adverse health conditions (Buoli et al., 2018; Guan et al., 2016; Hassoun et al., 2019; Rajagopalan et al., 2018). However, ozone, as a major photochemical pollutant and a powerful oxidant, has not equally attracted research attention. Results from recent epidemiological studies indicated that ozone might not only affect the respiratory system (Nhung et al., 2017; Zu et al., 2018), but also influence the cardio-cerebrovascular (Shah et al., 2013; Shah et al., 2015), central nervous system (Croze and Zimmer, 2018; Kasdagli et al., 2019) or mental health (Zhao et al., 2018).

Although the picture remains vague, oxidative stress and inflammation are postulated as mechanisms linking air pollutants exposure with health effect outcomes. Exposure to PM, nitrogen dioxide (NO_2) or ozone has been associated with inflammatory response in animal studies (Ji et al., 2015; Martin et al., 2013; Mishra et al., 2016; Wang et al., 2015; Wilson et al., 2010; Yoshizaki et al., 2017), and in epidemiological studies (Delfino et al., 2010; Liu et al., 2014; Mirowsky et al., 2017; Perret et al., 2017; Ruckerl et al., 2016; Shi et al., 2016). Nevertheless, the majority of epidemiological studies on ozone (e.g., Barraza-Villarreal et al., 2008; Lee et al., 2018; Liu et al., 2009) either had relatively small sample sizes or addressed potentially susceptible population groups, such as the elderly or patients, who are partially predisposed because of risk factors (e.g., age, lifestyle, smoking, diet) or morbidities, yielding overall limited and heterogeneous results. In comparison, studies in general populations, particularly at a young age, appear critical to assess whether ambient ozone exposure can cause local or systemic inflammation at an early stage of life possibly favoring the development of diseases.

The present study aimed to investigate associations between short-term ozone exposure and three inflammatory biomarkers among 10- and 15-year-old children and adolescents residing in two German areas. The markers were fractional exhaled nitric oxide (FeNO), as a noninvasive marker of respiratory inflammation, and interleukin (IL)-6 as well as high sensitivity - C reactive protein (hs-CRP), as systemic markers.

2. Material and methods

2.1. Study population

The study populations originated from two population-based German birth cohorts “German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development” (GINIplus) and “influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany” (LISA). Both cohorts recruited healthy newborns with a full gestational age (≥ 37 weeks) and a normal birth weight (> 2500 g) from 1995 to 1999. For the GINIplus cohort, 2949 participants from Munich and 3042 participants from Wesel were enrolled in two different arms. The intervention arm, investigating associations between the development of allergy and different hydrolyzed formulas given in the first four months of life, selected participants with at least one atopic parent or sibling. The observation arm selected participants without a family history of allergies or a consent about participating in the intervention from a legal guardian. For the LISA cohort, 1464 participants were recruited from Munich and 348 from Wesel, 976

from Leipzig and 306 from Bad Honnef. All of the subjects had physical examinations including FeNO measurement and blood sampling between the year 2005 to 2009 for the 10-year, and 2010 to 2014 for 15-year follow-ups. Ethical approval of GINIplus and LISA was acquired from the local ethics committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia), and written informed consent was obtained from the legal guardians of participants as well as from the participants themselves. Details on the two cohorts can be acquired elsewhere (Heinrich et al., 2002; von Berg et al., 2010; Zutavern et al., 2006).

We primarily restricted this analysis to participants with complete information on exposure and outcome from the follow-ups at 10 and 15 years residing in Munich and Wesel. Subjects with self-reported infections during the week before the FeNO measurement or blood sampling (863 participants) were excluded (Figure S1). The data from the two cohorts were pooled and stratified by area as we did for previous analyses (Liu et al., 2014; Zhao et al., 2019).

2.2. Measurements of inflammatory biomarkers

2.2.1. Measurements of FeNO

FeNO was measured at both 10- and 15-year follow-ups using the device NIOX MINO® (Aerocrine) in accordance with guidelines (Maestrelli et al., 2007). Before FeNO measurements, participants refrained from eating or drinking for at least one hour, from having nitrite-rich food intake (e.g., green vegetables or fruits, and smoked meats) for at least four hours, and from taking any anti-asthmatic or anti-inflammatory medication for at least four hours. While in a standing position, the participants were asked to inhale nitric oxide (NO)-free air quickly to total lung capacity through the mouthpiece of the NIOX MINO® and then exhale slowly and evenly for at least 6 seconds through the mouthpiece at a flow rate of 50 ± 5 mL/s. A nose clip was used to avoid nasal inspiration. The device automatically controlled the quality of the FeNO measurement, and repeated tests were taken until a value of acceptable quality was displayed (Liu et al., 2014).

2.2.2. Measurements of IL-6 and hs-CRP

During both 10- and 15-year follow-up visits, the venous blood was sampled into serum separator tubes and centrifuged. The serum was stored at -80 °C. Concentrations of IL-6 were measured in the serum of the 10-year-olds only by flow cytometry using a cytometric bead array (BD™ CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) as previously described (Herberth et al., 2009). Concentrations of hs-CRP were determined in the serum of both the 10- and 15-year-olds using the Tina-quant® CRP (latex) high-sensitive assay (Roche, Mannheim, Germany) in one single lab, according to the standard method described in the in manufacturer's instruction (Harris et al., 2017).

2.3. Assessment of ambient ozone, and other pollutants

Data on ozone, NO_2, and PM with an aerodynamic diameter < 10 µm (PM_{10}) of the Munich and Wesel areas were obtained from the German Environment Agency (Umweltbundesamt, labeled as UBA, https://www.umweltbundesamt.de/en), were measured by background monitoring stations, which can present the typical air quality in the city (UBA, 2017),

following standard methods: ozone was measured by ultraviolet photometry, NO_2 by chemiluminescence and PM_{10} by the gravimetric measurement method. One monitoring station is about 9 km northeast of the center of Munich (Johanneskirchen), and one is approximately 2 km northeast of the center of Wesel (Feldmark) (Fuertes et al., 2015; Zhao et al., 2019).

Because ozone concentrations are highly variable, we computed a "maximum of the daily maximum 8-hour average concentration (µg/m³)" as recommended by the UBA (UBA, 2013), which has been used in our previous study (Zhao et al., 2019). We initially calculated a moving 8-hour (7 hours before the hour of interest and the hour itself) average concentration for each hour of the day and subsequently identified the maximum of 8-hour average for each day. In terms of NO_2 and PM_{10}, we adopted 24-hour daily average concentrations (µg/m³).

We utilized a broad time frame for this study. For ozone exposure, the maximum of the daily maximum 8-hour average concentration (µg/m³) was selected over day 0 (same day), and the period between day 0 and the time points of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling (lag 0 day to lag 0-14 days). Regarding the average values of the daily concentrations (µg/m³), the same time frame of lag 0-day to lag 0-14 days was used for NO_2 and PM_{10}.

2.4. Covariates

Based on our published studies on inflammatory biomarkers in GINIplus and LISA cohorts (Liu et al., 2014; Yang et al., 2019), we considered a number of covariates for the present study apart from co-pollutants. These included basic information on area (Munich, Wesel) and study (GINIplus observation, GINIplus intervention, and LISA), as well as participants related factors such as sex (female, male), exact age at each follow-up visit (days expressed in years), body mass index (BMI, kg/m²), onset of puberty (for the 10-year follow-up, based on hormone measurements (Harris et al., 2017): estradiol > 18.4 pmol/L in females; testosterone > 0.09 nmol/L in males; for the 15-year follow-up, based on questionnaire (Petersen et al., 1988): prepubertal, early pubertal, midpubertal, late pubertal, postpubertal), secondhand smoke exposure at home (never, likely never, or ever from birth until 10 or 15 years), time spent in front of a screen (e.g., computer, television; high defined as ≥ 1 hour/day in summer or ≥ 2 hours/day in winter), time spent outside (high defined as ≥ 4 hour/day in summer or ≥ 2 hour/day in winter), physical activity level (low, medium and high were defined as moderate physical activity < 7 h per week, moderate physical activity ≥ 7 h and < 10.5 h per week, moderate physical activity ≥ 10.5h per week, alternatively vigorous physical activity ≥ 3.5 h per week, respectively (Janssen, 2007)), current asthmatic status (as ever doctor-diagnosed asthma from three years onwards and use of asthma medication in the last 12 months, or asthma symptoms in the last 12 months). We also considered factors related to the FeNO measurement or blood sampling: season (warm: April to October; cold: November to March), day time (8:00-11:00, 11.01-14:00, 14:01-19:00), fasting state (yes, no). Family-related factors were involved: maternal smoking during pregnancy (yes/no), maternal age at birth (≤ 30 years, 30-35 years, > 35 years), parental education (based on the highest number of years of school education reported by either parent; low, medium and high were respectively defined as < 10 years, = 10 years, and > 10 years), single-parent family status (yes, no) and net equivalent household income (area-specific tertiles).

Additionally, for the 15-year follow-up, data about smoking (as ever smoking), alcohol consumption (as ever drinking), and medication (as ever taking any medication during the last seven days), were available.

2.5. Statistical analysis

The Chi-square test and Student's t-test were adopted to examine the differences between the selected analytic samples and the original population, as well as the differences between the two analytic samples from Munich and Wesel. The Wilcoxon test was used to examine the differences between pollutants. We also calculated Spearman correlation coefficients to assess correlations between different pollutant metrics.

The concentrations of FeNO were log (ln)-transformed to normalize their distributions. No outliners, as defined as more than quadplex standard deviations (SD) from the mean, were detected. The majority of concentrations of hs-CRP and IL-6 were below the detection limit of the instruments, and no outlier was identified under the definitions hs-CRP > 1mg/dL and IL-6 > 20 pg/mL, respectively. We thus categorized the concentrations of these two systemic biomarkers into two levels. IL-6 was categorized with reference to the minimal detectable concentration (limit of detection, 1.5 pg/mL): undetectable, IL-6 $\leqslant$1.5 pg/mL; detectable, IL-6 > 1.5 pg/mL. Likewise, hs-CRP was categorized, based on the limit of quantification, as following: undetectable, hs-CRP < detection limit (0.020 mg/dL at 10 years and 0.016 mg/dL at 15 years due to modified assays); detectable, hs-CRP $\geqslant$ detection limit.

Since there was only a partial overlap of analytic samples and other differences in data across 10- and 15-year follow-ups, particularly the pubertal development, we analyzed associations between short-term ozone and inflammatory markers for each age group separately. The presence of linearity in the associations between the ozone metrics and inflammatory biomarkers was tested by generalized additive models (GAMs, Hastie and Tibshirani, 1986). The relationship between ln-transformed FeNO and ozone did not deviate from linearity, thereby ozone entered the GAMs as a linear term and fitted linear regression models for analyzing FeNO. Similarly, logistic regression with ozone as a linear term was adopted for IL-6, given the linearity of their relationships. However, ozone and hs-CRP showed a nonlinear exposure-response function (Figures S2 and S3). Therefore, we primarily stratified ozone exposure into “low” and “high” concentrations and treated ozone as a linear term in both. Two different cut-offs were used: first, 120 $\mu g/m^3$ as the maximum daily 8-hour mean concentration as a target value for the protection of human health recommended by the UBA (UBA, 2013) and second, 110 $\mu g/m^3$ as an average value of each lag’s hinge point as calculated by multivariate adaptive regression splines (MARS (Hastie et al., 2009), they were utilized for identifying the optimal hinge points for interpreting the non-linear associations between ozone and hs-CRP). Furthermore, ozone was additionally modeled using thin plate regression splines in GAMs.

The main model was determined after selecting confounders among the aforementioned covariates (subsection 2.4.). A confounder was defined as a correlate related to both the exposure and the outcome (VanderWeele and Shpitser, 2011). Based on this, our main adjustment set contained exact age at each follow-up, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, and net equivalent household income. The set additionally included the two basic design variables

area and study. To separate potential associations with ozone from those of other air pollutants, we also adjusted the models for the residuals of NO_2 and PM_{10}: we regressed each of the NO_2 and PM_{10} variables on each of the ozone metrics and derived model residuals, which were afterward included into the models (Yang et al., 2018; Zhao et al., 2019). We also present models with an adjustment for all covariates mentioned in subsection 2.4. (fully adjusted model). In addition, we built two models for sensitivity analyses considering the main adjustment set: (1) excluding participants with current asthma; (2) excluding 15-year-old participants who ever smoked, consumed alcohol, or took any medication in the last seven days. All the analyses were conducted for Munich and Wesel separately, and for the combined study populations from two areas. We further specifically analyzed the interaction between area (Munich versus Wesel) and ozone in the children aged 10 years by adding an interaction term in the main model.

The results of our analyses are presented as back ln-transformed percent changes for FeNO, and odds ratios (ORs) for IL-6 and hs-CRP, with 95% confidence intervals (CIs) scaled by specific interquartile range (IQR) increase in ozone. R 3.5.2 (R Core Team, 2018) was utilized. GAMs were fitted by *gam* function from the *mgcv* package (Wood, 2011). MARS were fitted by *earth* function from the *earth* package (Milborrow et al., 2019). We considered the significant level as 0.05 in our analyses.

3. Results

3.1. Characteristics of participants

Our analytic samples included 1330 participants aged 10 years and 1591 participants aged 15 years (Figure S1, Table 1). We found that the GINIplus intervention children were more likely to be included in our analytic samples, and the children of parents with high education. The results were in line with our previous findings (Markevych et al., 2019; Zhao et al., 2019).

Table 1

Almost all characteristics differed between participants from Munich and Wesel. Specifically, children from Munich were more likely to have a lower BMI, to spend less time outside, to have less physical activity, to be not exposed to passive smoking at home, and to have parents with higher education levels. However, for the 10 years old children, the difference on the season of the FeNO measurement or blood sampling was not statistically significant between Munich and Wesel; for the 15 years old adolescents, the data disruptions of pubertal development and alcohol consumption state were similar.

The children aged 10 years from Munich had a higher level of FeNO compared with the participants from Wesel. Nevertheless, the children from the two areas had similar levels of IL-6 and hs-CRP. Around 80% of 10-year-old children had a low IL-6 level, and more than 50% had a low hs-CRP level. Regarding the adolescents aged 15 years, the levels of FeNO and hs-CRP were higher among the participants from Munich.

3.2. Characteristics of ozone and other air pollutants

According to Table 2, lag 0-14 days averages of the daily maximum 8-hour ozone concentrations were 69.73 µg/m³ in Munich and 69.85 µg/m³ in Wesel at 10 years, while the numbers were 73.28 and 68.48 µg/m³ at 15 years (detailed concentrations for each lag are listed in Tables S1 and S2). Though the difference between the two areas was not significant at 10 years, Munich had a higher concentration of ozone than Wesel at 15 years. Besides, considering both 10 and 15 years, ozone concentrations were higher at 15 years than they were at 10 years. The NO_2 levels in Munich were higher than in Wesel, while Wesel was more polluted by PM_{10} than Munich.

Table 2

Additionally, NO_2 and PM_{10} were strongly positively correlated with each other. PM_{10} was only weakly correlated with ozone, while the correlation between NO_2 and ozone was moderately negative (Figures S4).

3.3. Associations between ozone and inflammatory biomarkers

The results of associations between short-term ambient ozone and inflammatory biomarkers are separately presented for FeNO, IL-6, and hs-CRP in Tables 3 to 6, Tables S3 to S13, and Figures S5 to S6. Due to missing values in air pollution data for part of the days, the number of participants varied across different lags.

3.3.1. Ozone and FeNO

We observed significant positive associations between ozone and FeNO in adolescents aged 15 years (Table 3), with stronger effects for the shorter lags, and the most significant effect for the combined population was lag 0-2 days (percent change = 7.78, 95% CI: (2.76, 13.05)). No consistent associations were found in 10-year-old subjects. Additionally, there was no significant interaction between area and ozone, although the direction of effect was opposite in the two areas for the 10-year follow-up (Tables 3 and S3).

Table 3

Similar associations were observed in models adjusted for all variables (Table S4). In addition, the models in which asthmatic patients were excluded showed similar effects, indicating that the observed effect estimates were not restricted to asthmatics (Table S5). After excluding smokers, and those who reported consumed alcohol or took medication, the positive associations remained, but the effect estimates were slightly reduced compared to the main models (Table S6).

3.3.2. Ozone and IL-6

We found no significant associations between ozone and IL-6 in children aged 10 years in neither area nor in the combined populations (Table 4). Likewise, the fully adjusted models indicated no association (Table S7). Excluding the currently asthmatic participant did not change the results (Table S8).

Table 4

3.3.3. Ozone and hs-CRP

Overall, the relationship between hs-CRP levels and ozone was J-shaped (Figures S2, S3, S5, and S6). The results stratified by ozone level < 120 versus ≥ 120 µg/m³ are shown in Tables 5 and 6. We identified that a reduced hs-CRP level was correlated with ozone exposure for the subgroup below 120 µg/m³ (German standard), especially for the combined populations of Munich and Wesel, and in adolescents in Wesel aged 15 years. In the subgroup with high ozone concentration, no such effects were observed neither in the children nor in the adolescents (Tables 5 and 6).

Table 5

Table 6

The results from the mains model and the fully adjusted models were similar as well (Table S9 and S10). When asthmatic patients were excluded, the formally protective effect for the ozone subgroup below 120 µg/m³ remained, and the effect estimates for the high ozone subgroup did not change substantially (Table S11). When adolescents smoked, consumed alcohol, and those with medication intakes were dropped, the formally protective effects for the ozone subgroup below 120 µg/m³ were slightly attenuated (Table S12).

As an additional subanalysis, we used a cutoff of 110 µg/m^3 ozone because it was a hinge point according to the results of MARS analyses. The results based on this cutoff are presented in Table S13. Comparing to the cutoff of 120 µg/m^3, we could find an attenuated formally protective effect estimate in the relatively lower ozone concentration subgroup, and an increased estimate pointing towards adverse effects in the high ozone subgroup.

Generally, the results from GAMs (Figures S5 - S6) supported the results from our subgroup-approach and indicated that medium and low-level ozone might either be not associated with hs-CRP or be associated with the reduced hs-CRP level, while high-level ozone could be associated with the elevated hs-CRP level.

4. Discussion

4.1. Main study findings

Overall, based on short-term exposure to ozone, we observed positive associations for FeNO among adolescents aged 15 years and no association for FeNO and IL-6 among children at the age of 10 years. Remarkably, a nonlinear J-shaped relationship between ozone and hs-CRP levels was identified, indicating that the below German standard ozone concentrations might be related to the reduced hs-CRP levels, whereas high concentrations tended to be associated with the elevated hs-CRP level in both 10- and 15-year-old participants.

4.2. Interpretations and comparisons with other studies

For the purpose of comparison, we consider a volumetric ozone concentration of 1 ppb equivalent to a gravimetric concentration of 2 µg/m³. The following concentrations and effect estimates were accordingly transformed if needed.

4.2.1. Ozone and FeNO

Catalyzed primarily by the inducible nitric oxide synthase (NOS), NO is formed in the airways when L-arginine oxidizes to L-citrulline (Pijnenburg and De Jongste, 2008). FeNO is recommended by the European Respiratory Society (Horvath et al., 2017) as a marker of Th-2 related airway inflammation and is widely used in studies on respiratory health, especially asthma and allergies.

Several studies investigated ozone exposure versus FeNO, but few of them were conducted among healthy children. Barraza-Villarreal et al. (2008) observed a positive association between ozone exposure and FeNO (per 44 µg/m³ for ozone, 1.23 (95% CI: 0.85, 1.77)) in a longitudinal study of 50 Mexican non-asthmatic children (aged 7.9 to 11.5 years), and the similar positive association in 158 asthmatic children (aged 7.9 – 11.5 years), based on fixed-site monitoring (8-hour moving average concentration ranging from 9.8 to 172.6 µg/m³). Karakatsani et al. (2017) conducted a panel study among 188, 10- to 11-year-old Greek children. The researchers used weekly personal ozone exposure (24-hour average concentration ranged from 4.7 to 10.8 µg/m³; meanwhile the daily concentration at fixed monitor sites ranged from 24.6 to 63.8 µg/m³), and observed that a 10 µg/m³ increase in ozone was associated with an 11.10% (95% CI: 4.23, 18.43) increase in FeNO. Likewise, Nickmilder et al. (2007) also reported a significant increase in FeNO in a panel study with 72 participants aged 6.5 to 15 years, at an ambient 1-hour ozone level of 167 µg/m³ (concentration ranging from 48 to 221 µg/m³).

However, this observed ozone-FeNO association might be sensitive to the range or the level of ozone concentration. Different from the findings mentioned above, based on data from 2240 8- to 9-year-old school children from the USA, Berhane et al. (2011) observed a longer lag structure, as over 1-23 days 8-hour cumulative average values of ozone were associated with higher FeNO levels. The reported ozone concentrations were mainly lower than 120 µg/m³ (detailed numbers were not reported). Moreover, ground on data from 605 children 9 to 13 years old from Turkey, Altug et al. (2014) did not find a significant change in FeNO levels when the weekly ozone concentration ranged from 26.4 to 133.3 µg/m³. It had been hypothesized that there was a threshold effect for the ozone-induced increase in FeNO: Nickmilder et al. (2007) considered the threshold of 135 µg/m³ for 1-hour exposure and of 110 µg/m³ for 8-hour exposure. Even though the different ozone metrics are incomparable across studies, the above two studies (Altug et al., 2014; Berhane et al., 2011) with possibly lower ozone concentrations observed no short-term effects.

Four human exposure studies (Barath et al., 2013; Nightingale et al., 1999; Nightingale et al., 2000; Olin et al., 2001) investigated the effects of a single time high concentration ozone exposure (exposure concentration ranging from 400 to 800 µg/m³, exposure time ranging from 75 minutes to 4 hours) on repeatedly assessed FeNO levels in adults. These studies did not observe that ozone affected FeNO. Thus, they do not support the findings of our

epidemiological study in adolescents. The difference between experimental studies and epidemiological studies might be attributed to characteristics of the participants, in particular age, co-pollutants and the effect of single, relatively short-term exposure.

Overall, studies on ozone exposure versus FeNO in children or adolescents, therefore, have yielded different results. Our study with the null finding in children and positive associations in adolescents adds to the current knowledge, as it has a large sample size, and because we analyzed children and adolescents separately while the other studies mixed them or were conducted only in children. Our results show that even a small difference in age might affect the susceptibility to ozone; thus, this factor should be cautiously considered.

4.2.2. Ozone and IL-6 and hs-CRP

IL-6 and CRP have complex biological effects, being considered as typical biomarkers of systematic inflammation. IL-6 can function as an inflammatory cytokine and an anti-inflammatory myokine; CRP is mainly produced in the liver and secreted into the circulation, in response to IL-6, IL-1 or tumor necrosis factor-α (Del Giudice and Gangestad, 2018).

Studies on ozone exposure versus IL-6 have been rarely conducted in children. The result from a long-term pilot study (Calderon-Garciduenas et al., 2013) included 35 clinically healthy Mexican children (mean age 6.2 years) indicated significantly higher systemic levels of IL-6 after a lifetime exposure to ozone. The observed fourth-highest daily maximum 8-hour average ozone concentrations were 240 µg/m³, 250 µg/m³ and 244 µg/m³ in the year from 2007 to 2009, respectively. The studies performed in asthmatic children generated controversial results. An intervention study performed in Mexico (Sienra-Monge et al., 2004) with 117 (mean age 9.0 years) children with asthma observed increased IL-6 levels in nasal lavage fluid related to ozone exposure (8-hour moving ozone average ranging from 22.2 to 285.0 µg/m³). Liu et al. (2009) studied 182, 9- to 14-year-old asthmatic children in Italy and found that IL-6 in breath condensate was not associated with ozone (3-day average concentration ranging (5^{th} to 95^{th} percentile) from 15 to 42 µg/m³).

In general, the evidence from epidemiological studies regarding IL-6 was inconsistent, possibly due to the diverse study designs, sample sizes, participants' characteristics, and ozone levels. However, results from the human exposure studies showed inconsistency as well. Devlin et al. (1991) found that exposure of 28 volunteers (18 to 35 years of age) to 160 µg/m³ for 6.6 hours was sufficient to initiate an increase of IL-6 in the bronchoalveolar lavage fluid. Similarly, Torres et al. (1997) also observed the positive ozone-associated (440 µg/m³, 4 hours) increase IL-6 in the bronchoalveolar lavage and alveolar lavage fluids among 38 participants age 18 to 40 years. Furthermore, a controlled exposure study (Bennett et al., 2016) with 40 women aged 18 to 35 years found increased plasma IL-6 after exposure to an 800 µg/m³ level, which is a high concentration even among experimental studies, for 2 hours. Nevertheless, under the same exposure condition (800 µg/m³, 2 hours), Fahy (1995) found non-significantly higher IL-6 levels in the induced sputum, based on a small sample of 10 subjects (mean age 30.0 years). Urch et al. (2010) conducted a study with 23 participants aged 21 to 40 years and did not found IL-6 response in the induced sputum nor in blood in relation to 240 µg/m³, 2-hour ozone exposure. Similarly, the result from Arjomandi (2018) was that 240 µg/m³, 3 hours of ozone exposure did not significantly affect the IL-6 in the sputum supernatants. However, Jörres et al. (2000) pointed out that a repeated ozone exposure (400

μg/m³ ozone over 4 hours of intermittent exercise on each of 4 consecutive days) was associated with an increase in IL-6 in bronchoalveolar lavage fluid assessed on the fifth day, as compared to a single-day ozone exposure. It may be assumed that though ozone-induced inflammation might initially occur in the respiratory system, the local or systemic IL-6 levels could not be visible after a single, relatively short-term, low concentration exposure to ozone.

The knowledge about associations between ozone exposure and hs-CRP or CRP is currently limited and inconsistent, with data mainly derived from studies in adults. Some positive associations were reported. A panel study (Chuang et al., 2007) with 76 students aged 18 to 25 years reported an increase in hs-CRP in association with an increase in ozone (3-day average concentration ranging from 45.0 to 96.6 μg/m³); but this association disappeared in two-pollutant models. A cross-sectional study (Michikawa et al., 2016) conducted with 2360 participants aged more than 20 years observed positive associations with ozone (mean concentration on the day of blood draw was 69.2 μg/m³). However, most studies found no associations (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Li et al., 2017b; Steinvil et al., 2008). Notably, the majority of the reported associations were formally protective although not statistically significant (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Steinvil et al., 2008). Considering only the ozone levels, the data from short-term studies (Steinvil et al., 2008) and long-term studies (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018) were mainly less than 120 μg/m³ and are comparable with our finding regarding the below German standard ozone concentration condition, where lower ozone levels were associated with the reduced hs-CRP level. While considering the exposure-response relationship, Pilz et al. (2018) reported a non-linear, negative ozone-CRP association with an annual average ozone range of 31.5 to 45.8 μg/m³. Michikawa et al. (2016) adopted logistic regression models and observed positive associations between hs-CRP and ozone, although they found no statistical evidence for a linear trend in the associations. In contrast, other studies adopted linear models but reported no results of linearity test (Chuang et al., 2007; Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Steinvil et al., 2008).

Given that there are no similar studies about ozone and hs-CRP conducted in children or adolescents, we cannot directly compare our results with those of other studies. However, the above-mentioned epidemiological studies support that the ozone concentration below the German standard might be related to the reduced hs-CRP level, underlying the nonlinearity of the response. Our results suggest that the associations in this manner might be highly dose-dependent. Irrespective of whether ozone levels were stratified according to the previously chosen cutoff (120 μg/m³) or statistically identified hinge point (110 μg/m³), the absence of the formally protective effect was consistent for ozone exposures at the relatively lower concentrations. Thereby, the distribution of ozone, especially the "distance" between a specific concentration and the threshold level (e.g., 120 or 110 μg/m³ in the present study) would be critically related to the ozone-induced variation of hs-CRP. The J-shaped, threshold-like or hormesis-like relationship would be important for explaining the association between ozone exposure and hs-CRP level, in accordance with the results by Nickmilder et al. (2007) who reported threshold effects for ozone exposure regarding FeNO, i.e. 135 μg/m³ for 1-hour exposure and 110 μg/m³ for 8-hour exposure.

4.3. Possible mechanisms

The mechanism of ozone-induced variations in inflammatory biomarkers is not clear yet.

Generally, lipid peroxidation is considered to be one of the inducers of ozone-related inflammation; and surface macrophages and epithelial cells are involved in the generation of pro-inflammatory mediators (Bromberg, 2016). Dysfunctions of purine metabolites (Esther et al., 2011) or hormones (Henriquez et al., 2018) might also play a role in response to ozone.

Few animal studies investigated the relationship between ozone exposure and FeNO. Recent data from Niu et al. (2018) indicated that ozone could result in a decrease in *NOS2A* methylation and an increase in inducible NOS expression, suggesting that ozone inhalation may affect DNA methyltransferases. Elevated FeNO levels were also hypothesized to be associated with decreased arginase and elevated arginase-2 methylation (Niu et al., 2018).

Most of the animal or cell studies demonstrated positive associations between ozone exposure and IL-6 (Arsalane et al., 1995; Bhalla et al., 2002; Gonzalez-Guevara et al., 2014; Yu et al., 2002), while few studies investigating CRP had mixed results (Jakubowski et al., 2004; Song et al., 2018). The inconsistent results on IL-6 or CRP across different studies might be additionally attributed to the presence of a threshold effect or hormesis with respect to ozone and inflammation, or attributed to the presence of ozone-induced inflammation in the airways may be more visible.

The evidence regarding ozone versus inflammation is currently scarce, which results in difficulties when interpreting the observed effect, especially the formally protective effect for the below German standard ozone concentration and hs-CRP. However, several animal studies (Chang et al., 2005; Kaya et al., 2017; Wei et al., 2018) and a clinical trial (Niu et al., 2018) reported that ozone therapy (perfusion or injection with ozone or ozone-absorbed liquid) was associated with a reduction in cytokines levels. It has been assumed that the toll-like receptor 4 (Chen et al., 2016) and the nuclear factor - κB pathway (Yu et al., 2016), which mediate the immune responses to lipopolysaccharide, could be suppressed by ozone, accompanied by a reduction of inflammatory cytokines levels. This possible hormetic dose-response relationship of ozone is already observed in different, non-epidemiological studies (Bocci et al., 2011; Martinez-Sanchez et al., 2010).

4.1. Limitations and strengths

Our study has several limitations. The ozone concentrations were measured at a single background monitoring station per area, and the outcome variables IL-6 and hs-CRP had to be dichotomized due to skewed distributions. These factors may have decreased statistical power and affected our results towards the null. Multiple comparison problems stemmed from analyses among several divided groups would be another limitation for statistical power. Further, since the selection by socioeconomic status resulted in initial under-recruitment and in subsequent higher loss to follow-up of participants from families with low socioeconomic status (also reported for other birth cohort studies (Bornehag et al., 2012; MAL-ED Network Investigators, 2017)), the external validity of our study might be limited. In addition, we might have missed some indirect pathways or other possible variables, which may also affect the associations of interest, like temperature (Li et al., 2017a) although we have adjusted the season for the long-term, and the daytime for intraday temperature variance; or humidity (Bind et al., 2014). Finally, a significant limitation is that our analyses were cross-sectional, an approach that cannot confirm the causality of associations. A panel study with repeated measurements of inflammatory markers might have served as a more robust design.

There are also several strengths of the present study. Firstly, we had a relatively large and comparable study sample, especially in terms of the separated age groups (10 and 15 years of age), from two population-based cohorts. Secondly, we adopted a broad time frame (lag 0-day to lag 0-14 days) to detect as many as possible time-dependent associations. Thirdly, a number of data on potentially relevant covariates, including infections, time spent outdoors, physical activity, smoking, drinking, and intake of medication were available. Fourthly, the check for non-linearity which we conducted could be used as a guide for future analysis methods in case of hs-CRP. Finally, we considered two major co-pollutants while analyzing the effects of ozone; thus, we can conclude with more certainty that the observed effects were due to ozone and not to residual confounding by other pollutants.

5. Conclusions

We observed that short-term ambient ozone exposure was associated with elevated levels of FeNO, but not related to systemic levels of IL-6. Moreover, a J-shaped relationship between ozone exposure and systemic hs-CRP was identified. Our findings indicate that acute ozone exposure may cause inflammation, which is most pronounced for airway inflammation in adolescents. No definite conclusion can be drawn currently for systemic inflammation.

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Table 1. Characteristics of study populations

Variable	Category	10 years			15 years		
		Munich n (%)	Wesel n (%)	All n (%)	Munich n (%)	Wesel n (%)	All n (%)
Study	GINIplus observation	243 (28.83)	235 (48.25)	478 (35.94)	273 (29.61)	327 (48.88)	600 (37.71)
	GINIplus intervention	330 (39.15)	185 (37.99)	515 (38.72)	341 (36.98)	269 (40.21)	610 (38.34)
	LISA	270 (32.03)	67 (13.76)	337 (25.34)	308 (33.41)	73 (10.91)	381 (23.95)
Age	Mean ± SD	10.03 ± 0.19	10.03 ± 0.10	10.04 ± 0.16	15.20 ± 0.28	15.12 ± 0.30	15.17 ± 0.29
Sex	Female	392 (46.50)	232 (47.64)	624 (46.92)	470 (50.98)	366 (54.71)	836 (52.55)
	Male	451 (53.50)	255 (52.36)	706 (53.08)	452 (49.02)	303 (45.29)	755 (47.45)
BMI	Mean ± SD	16.92 ± 2.10	17.87 ± 2.63	17.27 ± 2.35	20.47 ± 2.95	21.03 ± 3.30	20.12 ± 3.47
Time spent outside [a]	High	119 (14.12)	139 (28.54)	258 (19.40)	82 (8.89)	181 (27.06)	263 (16.53)
	Low	724 (85.88)	348 (71.46)	1072 (80.60)	840 (91.11)	488 (72.94)	1328 (83.47)
Time in front of a screen [b]	High	208 (24.67)	205 (42.09)	413 (31.05)	745 (80.80)	588 (87.89)	1333 (83.78)
	Low	626 (74.26)	281 (57.70)	907 (68.20)	169 (1.88)	77 (11.51)	246 (15.46)
	Missing	9 (1.07)	1 (0.21)	10 (0.75)	8 (0.87)	4 (0.60)	12 (0.75)
Physical activity [c]	High	270 (30.03)	199 (40.86)	469 (35.26)	200 (21.69)	225 (33.63)	425 (26.71)
	Medium	230 (27.28)	123 (25.26)	353 (26.54)	233 (25.27)	175 (26.16)	408 (25.64)
	Low	220 (26.10)	85 (17.45)	305 (22.93)	329 (35.68)	151 (22.57)	480 (30.17)
	Missing	123 (14.59)	80 (16.43)	203 (15.26)	160 (17.35)	118 (17.64)	278 (17.47)
Puberty (10 years) [d]	Yes	421 (49.94)	206 (42.30)	627 (47.14)	-	-	-
	No	412 (48.87)	252 (51.75)	664 (49.92)	-	-	-
	Missing	10 (1.19)	29 (5.95)	39 (2.93)	-	-	-
Puberty (15 years) [e]	Prepubertal	-	-	-	2 (0.22)	3 (0.45)	5 (0.31)
	Early pubertal	-	-	-	19 (2.06)	14 (2.09)	33 (2.07)
	Midpubertal	-	-	-	155 (16.81)	118 (17.64)	273 (17.16)
	Late pubertal	-	-	-	558 (60.82)	385 (57.55)	943 (59.27)
	Postpubertal	-	-	-	76 (8.24)	45 (6.73)	121 (7.61)
	Missing	-	-	-	112 (12.15)	104 (15.55)	216 (13.58)
Parental education [f]	Low (< 10 years)	85 (10.08)	157 (32.24)	242 (18.19)	72 (7.81)	202 (30.19)	274 (17.22)
	Medium (= 10 years)	118 (14.00)	105 (21.56)	223 (16.77)	140 (15.18)	156 (23.32)	296 (18.60)
	High (> 10 years)	638 (75.68)	224 (46.00)	862 (64.81)	709 (76.90)	310 (46.34)	1019 (64.05)
	Missing	2 (0.24)	1 (0.21)	3 (0.23)	1 (0.11)	1 (0.15)	2 (0.13)
Maternal age at birth	≤ 30 years	278 (32.98)	237 (48.67)	515 (38.72)	299 (32.43)	319 (47.68)	618 (38.84)
	> 30 to ≤ 35 years	387 (45.91)	197 (40.45)	584 (43.91)	433 (46.96)	281 (41.00)	714 (44.88)
	> 35 years	178 (21.12)	53 (10.88)	231 (17.37)	190 (20.61)	69 (10.31)	259 (16.28)
Single parent family	Yes	98 (11.63)	32 (6.57)	130 (9.77)	120 (13.02)	84 (12.56)	204 (12.82)
	No	731 (86.71)	452 (92.81)	1183 (88.95)	764 (82.86)	566 (84.60)	1330 (83.60)
	Missing	14 (1.66)	3 (0.62)	17 (1.28)	38 (4.12)	19 (2.84)	57 (3.58)
Smoking exposure	During pregnancy	97 (11.51)	67 (13.76)	164 (12.33)	94 (10.20)	103 (15.40)	197 (12.38)

	between 0 and 10/15 years	259 (30.72)	240 (49.44)	499(37.52)	257 (27.87)	351 (52.47)	608 (38.21)
Income (euro/month) [g]	Low	244 (28.94)	138 (28.34)	382 (28.72)	277 (30.04)	178 (26.61)	455 (28.60)
	Medium	295 (34.99)	155 (31.83)	450 (33.83)	279 (30.26)	199 (29.75)	478 (30.04)
	High	249 (29.54)	153 (31.42)	402 (30.23)	270 (29.28)	208 (31.09)	478 (30.04)
	Missing	55 (6.52)	41 (8.24)	96 (7.22)	96 (10.41)	84 (12.56)	180 (11.31)
Season [h]	Warm	560 (66.43)	341 (70.02)	901 (67.74)	715 (77.55)	485 (72.50)	1200 (75.42)
	Cold	283 (33.57)	146 (29.98)	429 (32.26)	207 (22.45)	184 (27.50)	391 (24.58)
Time	8:00-11:00	305 (36.18)	95 (19.51)	400 (30.08)	415 (45.01)	284 (42.45)	699 (43.93)
	11:01-14:00	118 (14.00)	39 (8.01)	157 (11.80)	172 (18.66)	62 (9.27)	234 (14.71)
	14:01-19:00	420 (49.82)	353 (72.48)	773 (58.12)	335 (36.33)	323 (48.28)	658 (41.36)
Fasting state of blood sample	Yes	192 (22.78)	43 (8.83)	235 (17.67)	95 (10.30)	17 (2.54)	112 (7.04)
	No	651 (77.22)	439 (90.14)	1090 (81.95)	550 (59.65)	350 (52.32)	900 (56.57)
	Missing	0 (0.00)	5 (1.03)	5 (0.38)	277 (30.04)	302 (45.14)	579 (36.39)
Participant smoking	Yes	-	-	-	71 (8.16)	28 (4.19)	99 (6.22)
	No	-	-	-	839 (91.84)	633 (94.62)	1472 (92.52)
	Missing	-	-	-	12 (1.30)	8 (1.20)	20 (1.26)
Participant consumed alcohol	Yes	-	-	-	146 (15.84)	102 (15.25)	248 (15.59)
	No	-	-	-	745 (80.80)	535 (79.97)	1280 (80.45)
	Missing	-	-	-	31 (3.36)	32 (4.78)	63 (3.96)
Medication intake last 7 days	Yes	-	-	-	263 (28.52)	43 (6.43)	306 (19.23)
	No	-	-	-	659 (71.48)	626 (93.57)	1285 (80.77)
Current asthma [i]	Yes	44 (5.22)	33 (6.78)	77 (5.79)	56 (6.07)	42 (6.28)	98 (6.16)
	No	787 (93.36)	447 (91.79)	1234 (92.78)	848 (91.97)	612 (91.48)	1460 (91.77)
	Missing	12 (1.42)	7 (1.44)	19 (1.43)	18 (1.95)	15 (2.24)	33 (2.07)
FeNO	ppb (median; IQR)	13; 11	11; 8	12; 10	18; 12	14; 10	16; 12
hs-CRP [j]	Undetectable	452 (53.62)	244 (50.10)	696 (52.33)	65 (7.05)	221 (33.03)	286 (17.98)
	Detectable	391 (46.38)	243 (49.90)	634 (46.67)	857 (92.95)	448 (66.97)	1305 (82.02)
IL-6 [k]	Undetectable	704 (83.51)	387 (79.47)	1091 (82.03)	-	-	-
	Detectable	139 (16.49)	100 (20.53)	239 (17.97)	-	-	-
Total		843 (63.38)	487 (36.62)	1330 (100.00)	922 (57.95)	669 (42.05)	1591 (100.00)

Note:

Abbreviations: BMI, body mass index; FeNO, fractional concentration of exhaled nitric oxide; IL-6, interleukin-6; hs-CRP, high sensitivity-C reactive protein; SD, standard deviation;

a. High is defined as ≥ 4 hours per day in summer or ≥ 2 hours in winter
b. High is defined as ≥ 1 hour per day in summer or ≥ 2 hours per day in winter
c. Low, moderate physical activity < 7 h per week; medium, moderate physical activity ≥ 7 h and < 10.5 h per week; high, moderate physical activity ≥ 10.5 h per week or vigorous physical activity ≥ 3.5 h per week
d. Puberty onset, females: estradiol > 18.4 pmol/L, males: testosterone > 0.09 nmol/L
e. Puberty stage, according to puberty category scores from Puberty Development Scale (Petrersen et al., 1988)
f. Highest number of years of school education for either parent was calculated, based on the German education system,
g. Net equivalent household income (euro/month), according to area-specific tertiles
h. Warm, April to October; cold, November to March

i. Ever doctor-diagnosed asthma from three years onwards, use of asthma medication in the last 12 months or asthma symptoms last 12 months
j. Due to modified assays, 10 year, detection limit was 0.020 mg/dL; 15 year, detection limit was 0.016 mg/dL
k. IL-6, detection limit was 1.5 pg/mL

Table 2. Averaged concentrations of ozone and other air pollutants for lag 0-14 days

Area	Air pollutant	Mean	SD	Min	Max	Median	IQR	Mean	SD	Min	Max	Median	IQR
		10 years						15 years					
Munich	Ozone [a]	69.73	27.23	16.41	137.78	77.12	43.04	73.28	24.72	12.12	117.46	79.10	40.27
	NO_2 [b]	29.10	8.27	16.59	73.19	27.57	10.77	20.58	5.74	12.21	40.00	19.14	6.85
	PM_{10} [b]	21.03	9.82	9.09	91.84	18.49	8.76	17.09	5.69	8.24	46.30	16.01	5.47
Wesel	Ozone [a]	69.85	31.31	14.53	160.38	66.30	43.58	68.48	23.31	6.93	118.96	70.92	35.86
	NO_2 [b]	24.72	8.10	10.40	53.25	23.51	11.33	19.88	6.85	8.04	41.84	18.29	10.21
	PM_{10} [b]	24.77	7.73	11.47	68.16	23.25	8.91	21.64	7.29	11.98	45.25	19.31	9.00
All	Ozone [a]	69.78	28.78	14.53	160.38	73.43	43.14	71.26	24.24	6.93	118.96	75.85	38.56
	NO_2 [b]	27.50	8.47	10.40	73.19	25.57	10.51	20.29	6.24	8.04	41.84	19.04	8.05
	PM_{10} [b]	22.40	9.28	9.09	91.84	20.28	9.31	19.00	6.79	8.24	46.30	17.22	7.16

Note:

Abbreviation: SD, standard deviation; IQR, interquartile range

a. The maximum 8-hour (7 hours before and the hour of interest) daily average ($\mu g/m^3$), 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations of UBA. The detailed concentrations for each lag are listed in Tables S1 and S2

b. Average of the daily concentration ($\mu g/m^3$), 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations of UBA. The detailed concentrations for each lag are listed in Tables S1 and S2

Table 3. Adjusted associations between short-term ozone and FeNO at the ages of 10 and 15 years

Area	Pollutant	10 years			15 years		
		Main model (Percent change, 95% CI)	p value	Participants	Main model (Percent change, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	-4.13 (-11.40, 3.72)	0.293	835/843	4.92 (-1.19, 11.40)	0.117	911/922
	Lag 0-1 days [b]	-4.61 (-12.38, 3.85)	0.276	830/843	**7.73 (0.67, 15.28)**	**0.031**	921/922
	Lag 0-2 days [c]	-3.53 (-11.82, 5.53)	0.432	834/843	7.16 (-0.01, 14.85)	0.050	922/922
	Lag 0-3 days [d]	-5.18 (-13.41, 3.83)	0.250	837/843	3.47 (-3.40, 10.83)	0.330	922/922
	Lag 0-5 days [e]	-5.14 (-13.08, 3.53)	0.237	842/843	0.93 (-5.78, 8.12)	0.791	922/922
	Lag 0-7 days [f]	-5.60 (-13.62, 3.16)	0.203	842/843	3.39 (-3.61, 10.90)	0.351	922/922
	Lag 0-10 days [g]	-8.89 (-17.51, 0.62)	0.066	842/843	0.19 (-7.11, 8.07)	0.960	922/922
	Lag 0-14 days [h]	**-10.37 (-19.64 -0.03)**	**0.049**	843/843	-0.27 (-7.90, 7.99)	0.946	922/922
Wesel	Lag 0-day [a]	1.40 (-7.32, 10.94)	0.762	406/487	6.63 (-0.10, 13.82)	0.054	622/669
	Lag 0-1 days [b]	3.00 (-6.44, 13.39)	0.547	397/487	**8.40 (1.20, 16.10)**	**0.021**	626/669
	Lag 0-2 days [c]	4.40 (-5.03, 14.77)	0.373	425/487	**9.68 (2.54, 17.32)**	**0.007**	648/669
	Lag 0-3 days [d]	3.71 (-5.24, 13.50)	0.429	447/487	**9.40 (2.21, 17.09)**	**0.009**	658/669
	Lag 0-5 days [e]	5.41 (-2.91, 14.45)	0.209	469/487	**7.66 (0.70, 15.10)**	**0.030**	669/669
	Lag 0-7 days [f]	6.00 (-2.12, 14.80)	0.152	479/487	6.02 (-0.56, 13.04)	0.074	669/669
	Lag 0-10 days [g]	5.15 (-2.54, 13.44)	0.195	483/487	6.45 (-0.45, 13.83)	0.067	669/669
	Lag 0-14 days [h]	5.30 (-2.44, 13.64)	0.185	484/487	6.34 (-0.47, 13.62)	0.069	669/669
All	Lag 0-day [a]	-2.28 (-7.91, 3.68)	0.445	1241/1330	**5.69 (1.22, 10.34)**	**0.012**	1533/1591
	Lag 0-1 days [b]	-2.17 (-8.21, 4.27)	0.500	1227/1330	**7.34 (2.39, 12.54)**	**0.003**	1547/1591
	Lag 0-2 days [c]	-0.59 (-6.86, 6.10)	0.859	1259/1330	**7.78 (2.76, 13.05)**	**0.002**	1570/1591
	Lag 0-3 days [d]	-1.96 (-8.06, 4.55)	0.547	1284/1330	**6.04 (1.06, 11.25)**	**0.016**	1580/1591
	Lag 0-5 days [e]	-0.80 (-6.63, 5.39)	0.794	1311/1330	4.04 (-0.83, 9.16)	0.105	1591/1591
	Lag 0-7 days [f]	-0.80 (-6.58, 5.34)	0.793	1321/1330	4.82 (-0.08, 9.95)	0.054	1591/1591
	Lag 0-10 days [g]	-1.74 (-7.62, 4.50)	0.575	1325/1330	3.65 (-1.45, 9.01)	0.163	1591/1591
	Lag 0-14 days [h]	-1.83 (-7.91, 4.66)	0.572	1327/1330	3.87 (-1.32, 9.33)	0.147	1591/1591

Note:

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2). Percent change was back transformed from the ln-transformed FeNO
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table 4. Adjusted associations between short-term ozone and IL-6 at the age of 10 years

Area	Pollutant	Main model (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	1.12 (0.84, 1.49)	0.424	835/843
	Lag 0-1 days [b]	1.22 (0.90, 1.65)	0.208	830/843
	Lag 0-2 days [c]	1.14 (0.82, 1.56)	0.437	834/843
	Lag 0-3 days [d]	1.18 (0.85, 1.63)	0.324	837/843
	Lag 0-5 days [e]	1.18 (0.86, 1.61)	0.299	842/843
	Lag 0-7 days [f]	1.31 (0.95, 1.80)	0.096	842/843
	Lag 0-10 days [g]	1.36 (0.95, 1.94)	0.094	842/843
	Lag 0-14 days [h]	1.35 (0.92, 1.99)	0.127	843/843
Wesel	Lag 0-day [a]	1.07 (0.75, 1.52)	0.713	406/487
	Lag 0-1 days [b]	0.93 (0.64, 1.35)	0.689	397/487
	Lag 0-2 days [c]	1.09 (0.77, 1.55)	0.616	425/487
	Lag 0-3 days [d]	1.06 (0.76, 1.48)	0.727	447/487
	Lag 0-5 days [e]	1.09 (0.81, 1.47)	0.576	469/487
	Lag 0-7 days [f]	1.06 (0.79, 1.43)	0.695	479/487
	Lag 0-10 days [g]	1.04 (0.78, 1.38)	0.796	483/487
	Lag 0-14 days [h]	1.06 (0.79, 1.42)	0.686	484/487
All	Lag 0-day [a]	1.12 (0.90, 1.38)	0.310	1241/1330
	Lag 0-1 days [b]	1.13 (0.90, 1.41)	0.303	1227/1330
	Lag 0-2 days [c]	1.14 (0.91, 1.43)	0.265	1259/1330
	Lag 0-3 days [d]	1.14 (0.91, 1.43)	0.240	1284/1330
	Lag 0-5 days [e]	1.15 (0.93, 1.42)	0.199	1311/1330
	Lag 0-7 days [f]	1.18 (0.96, 1.46)	0.121	1321/1330
	Lag 0-10 days [g]	1.16 (0.94, 1.44)	0.173	1325/1330
	Lag 0-14 days [h]	1.17 (0.94, 1.47)	0.162	1327/1330

Note:

Abbreviation: IL-6, interleukin-6; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table 5. Adjusted associations between ozone and hs-CRP at the age of 10 years with ozone stratified by < 120 versus ≥ 120 µg/m³

Area	Pollutant	Main model < 120 µg/m³ (OR, 95% CI)	p value	Participants	Main model ≥ 120 µg/m³ (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.97 (0.92, 1.03)	0.385	775/843	1.03 (0.45, 2.39)	0.938	60/843
	Lag 0-1 days [b]	0.96 (0.91, 1.03)	0.249	785/843	1.61 (0.72, 3.61)	0.253	45/843
	Lag 0-2 days [c]	0.97 (0.91, 1.04)	0.382	775/843	1.79 (0.92, 3.49)	0.092	59/843
	Lag 0-3 days [d]	0.96 (0.90, 1.03)	0.274	771/843	1.65 (0.95, 2.88)	0.083	66/843
	Lag 0-5 days [e]	**0.93 (0.87, 0.99)**	**0.032**	724/843	1.34 (0.98, 1.82)	0.067	118/843
	Lag 0-7 days [f]	**0.91 (0.85, 0.98)**	**0.014**	708/843	1.22 (0.94, 1.58)	0.131	134/843
	Lag 0-10 days [g]	0.94 (0.86, 1.02)	0.122	679/843	1.26 (0.98, 1.62)	0.069	163/843
	Lag 0-14 days [h]	0.93 (0.84, 1.03)	0.146	647/843	1.13 (0.90, 1.42)	0.290	196/843
Wesel	Lag 0-day [a]	1.00 (0.91, 1.09)	0.937	373/487	0.67 (0.36, 1.25)	0.226	33/487
	Lag 0-1 days [b]	0.99 (0.90, 1.09)	0.782	354/487	0.82 (0.57, 1.19)	0.309	43/487
	Lag 0-2 days [c]	0.99 (0.90, 1.09)	0.844	374/487	0.86 (0.62, 1.18)	0.357	51/487
	Lag 0-3 days [d]	0.97 (0.87, 1.08)	0.595	376/487	0.92 (0.68, 1.24)	0.573	71/487
	Lag 0-5 days [e]	0.94 (0.83, 1.05)	0.276	373/487	1.12 (0.91, 1.38)	0.287	96/487
	Lag 0-7 days [f]	0.92 (0.81, 1.04)	0.180	363/487	1.08 (0.88, 1.33)	0.445	116/487
	Lag 0-10 days [g]	0.91 (0.79, 1.04)	0.181	348/487	1.00 (0.82, 1.21)	0.962	135/487
	Lag 0-14 days [h]	0.89 (0.76, 1.03)	0.111	330/487	1.00 (0.82, 1.23)	0.984	154/487
All	Lag 0-day [a]	0.98 (0.93, 1.03)	0.410	1241/1330	1.24 (0.80, 1.92)	0.334	93/1330
	Lag 0-1 days [b]	0.97 (0.92, 1.02)	0.257	1139/1330	0.91 (0.66, 1.25)	0.561	88/1330
	Lag 0-2 days [c]	0.97 (0.92, 1.03)	0.329	1149/1330	1.05 (0.80, 1.39)	0.712	110/1330
	Lag 0-3 days [d]	0.96 80.91, 1.02)	0.155	1147/1330	1.15 (0.91, 1.47)	0.241	137/1330
	Lag 0-5 days [e]	**0.92 (0.87, 0.98)**	**0.007**	1097/1330	**1.22 (1.04, 1.43)**	**0.018**	214/1330
	Lag 0-7 days [f]	**0.91 (0.85, 0.96)**	**0.001**	1071/1330	1.15 (0.98, 1.34)	0.079	250/1330
	Lag 0-10 days [g]	**0.92 (0.86, 0.98)**	**0.012**	1027/1330	1.09 (0.94, 1.26)	0.235	298/1330
	Lag 0-14 days [h]	**0.90 (0.83, 0.98)**	**0.011**	977/1330	1.04 (0.90, 1.20)	0.584	350/1330

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table 6. Adjusted associations between ozone and hs-CRP at the age of 15 years with ozone stratified by < 120 versus ≥ 120 µg/m³

Area	Pollutant	Main model < 120 µg/m³ (OR, 95% CI)	p value	Participants	Main model ≥ 120 µg/m³ (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.98 (0.96, 1.01)	0.294	825/922	0.97 (0.60, 1.56)	0.891	59/922
	Lag 0-1 days [b]	1.01 (0.97, 1.04)	0.742	888/922	1.00 (0.71, 1.41)	0.999	33/922
	Lag 0-2 days [c]	1.01 (0.98, 1.04)	0.574	862/922	0.88 (0.59, 1.31)	0.531	66/922
	Lag 0-3 days [d]	1.01 (0.98, 1.05)	0.499	819/922	0.91 (0.73, 1.14)	0.413	103/922
	Lag 0-5 days [e]	1.00 (0.96, 1.03)	0.875	742/922	1.01 (0.85, 1.20)	0.938	180/922
	Lag 0-7 days [f]	1.00 (0.95, 1.04)	0.827	672/922	0.95 (0.82, 1.10)	0.473	250/922
	Lag 0-10 days [g]	0.98 (0.93, 1.03)	0.403	634/922	0.98 (0.85, 1.15)	0.837	288/922
	Lag 0-14 days [h]	0.98 (0.93, 1.03)	0.403	615/922	0.97 (0.83, 1.14)	0.723	307/922
Wesel	Lag 0-day [a]	0.99 (0.93, 1.05)	0.669	590/669	1.44 (0.88, 2.37)	0.165	32/669
	Lag 0-1 days [b]	**0.93 (0.87, 0.99)**	**0.018**	593/669	1.30 (0.70, 2.39)	0.415	33/669
	Lag 0-2 days [c]	**0.91 (0.85, 0.98)**	**0.011**	582/669	0.99 (0.71, 1.40)	0.976	66/669
	Lag 0-3 days [d]	**0.90 (0.84, 0.97)**	**0.008**	577/669	0.81 (0.63, 1.05)	0.117	81/669
	Lag 0-5 days [e]	**0.89 (0.82, 0.96)**	**0.003**	560/669	0.93 (0.76, 1.14)	0.501	109/669
	Lag 0-7 days [f]	**0.88 (0.81, 0.95)**	**0.002**	542/669	0.96 (0.81, 1.13)	0.591	127/669
	Lag 0-10 days [g]	**0.85 (0.78, 0.94)**	**0.001**	517/669	1.04 (0.89, 1.22)	0.630	152/669
	Lag 0-14 days [h]	0.91 (0.83, 1.01)	0.078	491/669	1.00 (0.87, 1.15)	0.985	178/669
All	Lag 0-day [a]	0.98 (0.95, 1.01)	0.222	1442/1591	1.27 (0.95, 1.69)	0.108	91/1591
	Lag 0-1 days [b]	0.98 (0.95, 1.01)	0.126	1481/1591	1.24 (0.90, 1.71)	0.191	66/1591
	Lag 0-2 days [c]	0.97 (0.94, 1.01)	0.102	1444/1591	1.02 (0.81, 1.29)	0.836	126/1591
	Lag 0-3 days [d]	**0.96 (0.93, 0.99)**	**0.049**	1396/1591	0.94 (0.80, 1.10)	0.417	184/1591
	Lag 0-5 days [e]	**0.95 (0.91, 0.98)**	**0.003**	1302/1591	1.03 (0.91, 1.17)	0.626	289/1591
	Lag 0-7 days [f]	**0.94 (0.90, 0.98)**	**0.003**	1214/1591	1.05 (0.95, 1.16)	0.350	377/1591
	Lag 0-10 days [g]	**0.92 (0.88, 0.96)**	**< 0.001**	1151/1591	1.09 (0.99, 1.21)	0.071	440/1591
	Lag 0-14 days [h]	**0.93 (0.89, 0.98)**	**0.006**	1106/1591	1.06 (0.97, 1.16)	0.228	485/1591

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Highlights

Ozone exposure was associated with FeNO in adolescents aged 15 years, not 10 years

Ozone exposure was not associated with IL-6 in children aged 10 years

Ozone exposure and hs-CRP possibly had a J-shaped exposure-response relationship

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: