**Ambient ozone exposure and depressive symptoms in adolescents:**

**Results of the GINIplus and LISA birth cohorts**

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**Abstract**

**Background:** Depression has been associated with air pollution, as reported by animal and epidemiological studies. However, the relationship between ozone exposure and depression, especially among adolescents, is scarcely investigated.

**Objectives:** The study aimed to analyze associations between ozone exposure and depressive symptoms among German adolescents.

**Methods:** The analyses were based on 2827 adolescents aged 15 from Munich and Wesel areas of the GINIplus and LISA birth cohorts. The depressive symptoms were assessed by the Depression Screener for Teenagers (DesTeen). Long-term ozone exposure was estimated by optimal interpolation techniques and assigned to home addresses. Nitrogen dioxide (NO2) and particulate matter with an aerodynamic diameter < 10 µm (PM10) were assessed by land use regression models. For short-term exposure, maximum 8-hour averages of ozone and daily average concentrations of NO2 andPM10 from the background monitoring sites 0 (same day), 1, 2, 3, and 7 days prior to depressive symptoms assessment were adopted. The cross-sectional analyses were conducted by adjusted logistic regression models controlling for residuals of NO2 and PM10, and covariates identified by a directed acyclic graph.

**Results:** The prevalence of depressive symptoms ranged from 10.9 % to 13.8 % depending on regions. Overall, long- and short-term exposure to ozone were not statistically significantly associated with depressive symptoms. However, subgroup analysis showed inconsistent significant protective associations for short-term exposure to ozone lag 0 day (same day) and depressive symptoms in Wesel (OR = 0.76, 95 % CI: (0.59, 0.98)), but not in Munich (OR = 1.00, 95 % CI: (0.83, 1.21)).

**Conclusions:** Our study does not support the hypothesis that ambient ozone exposure might increase the prevalence of depressive symptoms in German adolescents. Nevertheless, due to a lack of similar studies, these results need to be replicated in other samples.

**Keywords**

Ozone; Air pollution; Depression, Teenager; Epidemiology;

**Highlights**

* Ozone exposure was suggested to cause depression but the evidence is insufficient
* This study was the first on ozone and depressive symptoms in adolescent
* No associations were found between ozone and depressive symptoms in adolescents

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**Abbreviations**

CI, confidence interval;

DAG, directed acyclic graph;

DesTeen, Depression Screener for Teenagers;

ESCAPE, European Study of Cohorts for Air Pollution Effects;

GAM, generalized additive model;

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

IQR, interquartile range;

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

LUR, land use regression;

NO2, nitrogen dioxide;

OR, odds ratio;

PM10, particulate matter with an aerodynamic diameter < 10 µm;

ppb, parts per billion;

SES, socio-economic status

UBA, Umweltbundesamt (German Environment Agency);

WHO, World Health Organization;

1. **Introduction**

It is estimated that the proportion of the global population with depression was 4.4 % in 2015 and that the number of people living with depression between 2005 and 2015 increased by 18.4 % (WHO (World Health Organization), 2017). Given such a high prevalence, depression accounts for a significant proportion of the global burden of disease, leading to over 50 million Years Lived with Disability in 2015 (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). Substantially, to scale up effective treatment for depression over the period 2016-2030, the global investment would be 51.9 billion US dollars (Chisholm et al., 2016).

Depressive symptoms include sad, empty, hopeless or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual capacity of functioning (American Psychiatric Association, 2013). It does not only decrease quality of life (Ruo et al., 2003), but is also associated with several other adverse health outcomes, including other psychiatric (Fergusson and Woodward, 2002) and somatic diseases (Glymour et al., 2010; Hung et al., 2014; O'Neill et al., 2014; Whooley et al., 2008), self-injury (Giletta et al., 2012), and suicide (Dong et al., 2018).

As depression is a complex disorder, its determinants include genetic predisposition (Carver et al., 2011; Milne et al., 2009), as well as neuropsychological and pathophysiological changes (Werner and Covenas, 2010), and socio-economic factors (Arias-de la Torre et al., 2018; Dulaney et al., 2018). Specifically, several recent studies have reported associations between ambient air pollution and depression (Cho et al., 2014; Kim et al., 2016; Pun et al., 2017; Vert et al., 2017). However, the published studies have mainly considered particulate matter (PM) and other gaseous pollutants, such as nitrogen dioxide (NO2). As summarized by our recent systematic review (Zhao et al., 2018), only a few epidemiological studies (Kioumourtzoglou et al., 2017; Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2016; Szyszkowicz et al., 2009; Wang et al., 2014) investigated whether such a powerful oxidant and an important air pollutant as ozone - via pathway like dysregulation of inflammatory cytokines (de Prado Bert et al., 2018)- can also increase risk of depression, and found the current evidence is inconclusive.

Depression occurs in children and adolescents as well, and is assumed to start in early childhood (Ignacio et al., 2014). For adolescents aged between 15 and 19 years old, the global prevalence of depressive disorder is about 4.5 % in females and 3 % in males (WHO, 2017). However, a higher prevalence is also considered. For example, the reported prevalence in German children aged 7 - 10 years was 10.7 % and in children aged 11 - 17 years it was 11.1 % (Ravens-Sieberer et al., 2008). Mojtabai et al. (2016) reported the prevalence of major depressive episodes in American adolescents was 11.3 % in 2014.

Therefore, it is of public relevance to investigate whether exposure to higher ozone levels can increase depression risk already in childhood and early adolescence. To our knowledge, there is no such research so far. The present study aimed to investigate the association between long- and short-term ambient ozone exposure and depressive symptoms in 15-year old adolescents residing in two German areas.

1. **Materials and Methods**
   1. Study population

Data were obtained from the two ongoing 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 only healthy newborns with a normal birth weight (> 2500 g) at a full term (gestational age ≥ 37 weeks). Briefly, from 1995 to 1998, 5991 children from Munich (N = 2949) and Wesel (N = 3042) were recruited into the GINIplus cohort. There are two different arms in this cohort. The intervention arm allocated participants with at least one atopic parent or sibling to investigate the effect of different hydrolyzed formulas during the first four months of life on later allergy development. The observation study arm included participants who had a negative family history of allergies or whose parents did not consent to participate in the intervention study. LISA recruited 3094 participants from Munich (N = 1464) and Wesel (N = 348), as well as Leipzig (N = 976) and Bad Honnef (N = 306) between 1997 and 1999. Both cohorts were approved by the local ethics committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia) and written consent was obtained from participants’ legal guardians. More details on these two cohorts can be found elsewhere (Heinrich et al., 2002; von Berg et al., 2010; Zutavern et al., 2006).

The present study is restricted to the inhabitants of the cities of Munich and Wesel and their surroundings from the time of recruitment until the time of the 15-year follow-ups because data on NO2 and PM10 could not be assigned to the residents of the Leipzig and Bad Honnef areas. The data from GINIplus and LISA were pooled as the cohorts have very similar design at the later follow-ups, and as this strategy has been widely adopted for our previous analyses (e.g., Fuertes et al., 2016; Markevych et al., 2014). The participants with incomplete outcome, exposure and covariate data were excluded, as well as those who lived in the study area for less than one year (Figure S1), given the time frame of long-term ozone exposure.

* 1. Ambient ozone, NO2, and PM10 exposure
     1. Long-term exposure assessment

Long-term ambient ozone exposure was estimated at residential addresses at 15 years by using data from the German Environment Agency (Umweltbundesamt, labeled as UBA). The UBA-derived ozone estimates (Flemming et al., 2004; Stern and Flemming, 2004) were modelled at a resolution of two square kilometers for each year from 2005 onwards for Germany using optimal interpolation technique implemented in REM-CALGRID, as well as the measured ozone data from 150 German monitoring stations and meteorological data. Annual average concentrations (µg/m³) of ozone, and “number of days per year with a maximum 8-hour average concentration exceeding 120 µg/m³” (days/year, as target value for the protection of human health, according to https://www.umweltbundesamt.de/en/topics/air/ozone) were both used. Ozone estimates a year prior to depression symptoms assessment were assigned.

As air pollution is a complex mixture, we considered several of these co-pollutants in our analyses. Annual average concentrations (µg/m3) of NO2 and PM with an aerodynamic diameter < 10 µm (PM10) at 15-year residential addresses were estimated by area-specific land use regression (LUR) models originally developed within the “European Study of Cohorts for Air Pollution Effects” (ESCAPE, www.escapeproject.eu) (Beelen et al., 2013; Cyrys et al., 2012; Eeftens et al., 2012a; Eeftens et al., 2012b). Briefly, NO2 and PM10 were monitored at 20 and 40 air measuring stations, respectively, for three two-week measurement periods between October 2008 and November 2009 in both the Munich and Wesel study areas. The pollutant annual averages at measurement sites were calculated as averages of these three measurements and adjusted for temporal variation derived from a yearly-operating background measuring station. Land use, population and traffic predictor variables were used to generate area-specific LUR models to estimate pollution at each residential address (model explained variance (R2) of the models ranged from 0.78 to 0.97).

Assignment of air pollution estimates to geocoded residential addresses was done in ArcGIS Geographical Information System (GIS) (version 10.4, ESRI, Redlands, CA).

* + 1. Short-term exposure assessment

The short-term ozone data were derived from UBA as well. The concentrations were obtained from a background monitoring site (www.env-it.de/stationen/public/station.do) in Munich which is approximately 9 km northeast of city center (Johanneskirchen) and a site which is approximately 2 km northeast the center of Wesel (Feldmark) (Fuertes et al., 2015).

For ozone, according to its high within- and across-day variability, as well as recommendation from the UBA (https://www.umweltbundesamt.de/en/topics/air/ozone), we calculated concentration (µg/m³) of moving 8-hour average for every hour (7 hour before and of the hour of interest) and thereby identified a maximum of 8-hour average for every day. The maximum of the daily maximum 8-hour average concentration was selected over 0 (same day), and 1, 2, 3, and 7 days prior to the depressive symptoms assessment (lags 0 to 7 days). Since all of the published prior studies that reported the significant short-term ozone effects may detect the associations within 7 days (reviewed by Zhao et al., 2018), our selected time frames enabled us to detect the possible association between short-term ozone and depressive symptoms.

For NO2 and PM10, we utilized average of the daily concentrations (µg/m³) of 0 (same day), and 1, 2, 3, and 7 days prior to the day that depressive symptoms were evaluated for our analysis (lags 0 to 7 days, same time frames as in the case of ozone).

* 1. Depressive symptoms

Depressive symptoms were assessed by Depression Screener for Teenagers (DesTeen). DesTeen is a specific validated tool for screening of adolescent depression in Germany. It contains 14 items on four-point scale that focus on cognitive and emotional symptoms for assessing the depressive symptoms over the preceding two weeks (Allgaier et al., 2014; Pietsch et al., 2011). This questionnaire was filled by children at the 15-year follow-up. Presence of depressive symptoms was defined as a total score ≥ 12 (Pietsch et al., 2011) and considered as an outcome in associations with both long- and short-term ozone exposures.

* 1. Covariates

Based on our previous analyses on behavioral problems in GINIplus and LISA cohorts in relation to environmental factors (Fuertes et al., 2016; Markevych et al. 2014; Tiesler et al. 2013), other than co-pollutants, potential covariates included: cohort (GINIplus observation, GINIplus intervention and LISA), exact age of a child at the 15-year follow-up, sex of the child, 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), maternal age at birth (≤ 30 years, 30-35 years, > 35 years), net equivalent household income (area-specific tertiles), single parent family status (yes/no), maternal smoking during pregnancy (yes/no), secondhand smoke exposure at home (never, likely never, or ever from birth until 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), and time spent outside (high defined as ≥ 4 hour/ day in summer or ≥ 2 hour/day in winter). Additionally, the Global Severity Index score greater than the 90th percentile based on the Brief Symptom Inventory 18 was used to evaluate parental psychopathology (Derogatis, 2001; Fuertes et al., 2016).

* 1. Statistical analysis

The Chi-square test or Student's t-test was adopted to examine the differences between the selected analytic and the original population, as well as the differences between the two analytic populations from Munich and from Wesel. The Wilcoxon test was used to examine the differences of pollutants between these two areas. We also calculated Spearman correlation coefficients to assess relationships between different pollutant metrics for area and combined participants of two areas.

Individual associations between each of the ozone exposure variables and depressive symptoms at 15 years were assessed by logistic regression models. Ozone was modelled as continuous variables in regression analyses, because the relationship of ozone with depressive symptoms did not show major deviations from linearity in generalized additive models (GAMs) (Hastie and Tibshirani, 1986).The results are presented as odds ratios (ORs) and 95 % confidence intervals (CIs) scaled by specific interquartile range (IQR) increase in ozone values. Furthermore, we also had a model with the DesTeen score as original count data, analyzed by negative binomial regressions. The resulted count ratios and 95 % CIs were scaled by IQR of ozone as well.

To reduce multicollinearity, unnecessary adjustment and over-adjustment, we defined a minimal adjustment set of adjustment variables (Rohrig et al., 2014) from the potential covariates by using a directed acyclic graph (DAG) (Greenland et al., 1999) in DAGitty (Textor et al., 2011; Textor et al., 2016). Thus, our main models were adjusted for income, parental education, parental psychopathology, single parent family status, time spent by a child outside, time spent in front of a screen, exact age, and sex of the child (Figure S2). Since area and cohort are basic design variables, we additionally adopted these two variables in our main models. We also present minimal adjusted models, which considered only area, cohort and sex and models with an adjustment for all covariates mentioned in the subsection 2.4. All the analyses were conducted for Munich and for Wesel separately, as well as for the entire study population. Given the higher prevalence of depression in females (WHO, 2017), we also stratified the analyses by sex of the child.

We adjusted our models for residuals of PM10 and NO2. Briefly, we regressed each of the PM10 and NO2 variables on each of the ozone variables and derived model residuals, which were afterwards included into models in a similar manner to how it was done before (Yang et al., 2018). All analyses were conducted using R 3.4.4 (R Core Team, 2018). GAM models were fitted by *gam* function from the *mgcv* package (Wood, 2011). The negative binomial regression models were fitted by *glm.nb* function from the *MASS* package (Venables and Ripley, 2002).

1. **Results**
   1. Characteristics of participants and pollutants

After the selection of subjects with complete data, 1565 15-year-old participants from Munich and 1262 from Wesel were included in this study (Figure S1). We found that GINIplus intervention children were more likely to be included in our analytic samples (p < 0.001), as well as children of parents with high education (p < 0.001).The characteristics of participants from both study areas are listed in Table 1. The overall prevalence for depressive symptoms was 12.5 %, and it was higher in Munich compared to Wesel (13.8 % *vs* 10.9 %, p = 0.022). The participants from Munich and Wesel differed in nearly all characteristics. Specifically, children from Munich were more likely to have parents with higher education (p = 0.003), older mothers at birth (p < 0.001), to spend less time in front of a screen (p < 0.001), as well as outside (p < 0.001), and to be less exposed to passive smoking at home (p < 0.001).

(Table 1)

The distributions of long- and short-term air pollutants concentrations are presented in Table 2. The median ozone concentrations were 43.3 µg/m³ in Munich and 42.4 µg/m³ in Wesel, respectively. The ozone concentrations in Munich were higher than in Wesel (p < 0.001). However, the number of days with ozone levels exceeding 120 µg/m3 in Munich was slightly less than in Wesel (15 days/year *vs* 16 days /year, p < 0.001). Unlike ozone, the concentrations of NO2 and PM10 were higher in Wesel compared to Munich (p < 0.001).

(Table 2)

The short-term ozone exposure ranged widely (Table 2). For example, the daily maximum 8-hour average concentration (7 days prior to the day of depressive symptoms assessment) ranged from 4.2 to 135.2 µg/m³, confirming the notion that single day average of short-term ozone exposure is strongly varying from day to day. The daily maximum 8-hour average concentrations were 76.2 µg/m³ in Munich and 63.0 µg/m³ in Wesel. Similar to long-term exposure, the short-term ozone level in Munich was higher than in Wesel (p < 0.001), while Wesel was more polluted by NO2 and PM10 than Munich (p < 0.001).

Additionally, NO2 and PM10 were positively and strongly correlated with each other considering both long-term exposure and short-term exposure. However they were weakly correlated with ozone in both long- and short-term exposure, except that moderate negative correlations between short-term exposures of ozone and NO2 were detected (Figures S3 and S4) (Figures S3 and S4).

* 1. Long-term ambient ozone exposure and depressive symptoms

Table 3 shows the adjusted ORs for long-term exposure to ozone and depressive symptoms (results from different sensitivity analyses can be found in Supplementary, Tables S1 – S3). No significant associations were found in main analysis (Table 3). This holds true for the two study areas (Munich, Wesel), and for the two types of exposure metrics (annual means, number of days per year exceeding the limit). For example, an OR = 1.08 (95 % CI: (0.92, 1.26)) per IQR increase (2.9 µg/m³) in annual ozone concentration in Munich, and 0.95 (95 % CI: (0.69, 1.32)) per IQR increase (7 days/year) in days with ozone levels exceeding 120 µg/m3 in Wesel. Similarly, no significantly associations were detected in the models with the DesTeen score as original variable (Table 4). There were also no significant associations between long-term exposure to ozone and depressive symptoms in the sensitivity analyses (Supplementary Tables S1 – S3).

(Table3, Table4)

* 1. Short-term ambient ozone exposure and depressive symptoms

Overall, the results of short-term ozone exposure and depressive symptoms were mixed and not significant for the entire study population (Table 3). Due to a lack of exposure data across all days of the year, the numbers of participants varied between analyses (Table 3). Within several subgroup analyses, one statistically significant protective association was detected for Wesel for lag 0 day (same day): an IQR increase (38.5 µg/m³) in ozone concentration decreased the odds of depressive symptoms by 24 % in Wesel (OR = 0.76, 95 % CI: (0.59, 0.98), p = 0.037). This association was also found in two sensitivity analyses: models adjusted for minimal covariates (OR = 0.77, 95 % CI: (0.60, 0.99), p = 0.038, Table S1), and models adjusted for all covariates (OR = 0.74, 95 % CI: (0.57, 0.96), p = 0.024, Table S2). No such association was observed for Munich (Table 3 and Tables S1, S2, and S3). Sex-stratified models also identified the same inverse association with short-term ozone exposure in males from Wesel (for lag 0 day, OR = 0.61, 95 % CI: (0.39, 0.96), p = 0.033, Table S3), but similar decreased odds ratios were not observed for Munich males (Table S3). Additionally, when considered the score of DesTeen as the original count data, there were no significant associations between ozone exposure and depressive symptoms (Table 4)

1. **Discussion**
   1. Main study findings

The overall results of our analyses based on long-term exposure to ozone and depressive symptoms do not support the notion that increased ambient ozone levels increase the prevalence of depressive symptoms in our sample of 15-year-old German adolescents. This finding was robust across different adjustment strategies and statistical approaches, and did not depend on the study area and sex of participant. Even though some statistically significant protective associations were detected for short-term ozone exposure, the results are isolated and inconsistent between the two study areas. Therefore, these results of potential short-term associations should be interpreted with caution.

* 1. Interpretations and comparisons with other studies

According to our results, there were no associations between long-term ozone exposure and depressive symptoms in adolescents. Considering the protective effects for short-term exposure in Wesel, and the sex-specific results for males in Wesel and the inconsistency between Wesel and Munich, we interpret the protective effects as chance findings.

Although there are no published studies in adolescents, the conclusion of our recent systematic review of epidemiological studies (Zhao et al., 2018) - the evidence about ozone exposure and depressive disorder is inconclusive - is in line with the interpretations of this study. More specifically, Wang et al. (2014) analyzed data from a cohort of 732 adults (mean age 78.1 years) in USA. They reported no significant associations between short-term changes in ozone over two weeks preceeding assessement and depressive symptoms. Furthermore, Szyszkowicz et al. (2009) investigated associations between emergency department visits for depression and short-term air pollution in Canada and found also no statistically significant associations for ozone exposure using data from 27,047 emergency department visits. However, some studies have reported associations between ozone and depression outcomes. Szyszkowicz (2007) found an increased risk for daily emergency department visits for depression and 1-day lagged ground level ozone for females during the warm season based on 15,556 patients. Similarily, Szyszkowicz et al. (2016) also reported that ground on 118,602 patients, emergency department visits for depression were associated with ozone between 1 and 7 days prior to emergency department visit among males, as well as between 1 and 5, and 8 days for females. Kioumourtzoglou et al. (2017) used data from a prospective cohort study with 41,844 women (mean age 66.6 years) in the United States investigated the association between air pollution and oneset of depression defined as doctor’s diagnosis or use of antidepression medication. Hazard ratios for both outcomes were reported to be associated with ozone in summer (May to September) ozone. Lim et al. (2012) reported the Korean version of the Geriatric Depression Scale-Short Form scores were positively associated with increases in a 3-day (lag 0-2) moving average of ozone, based on a cohort with 537 participants (mean age 71 years).

There are several potential reasons why our findings might be different from the studies that reported associations of ozone with depression. While our study subjects are adolescents, other studies were mainly conducted in the elderly (Kioumourtzoglou et al., 2017; Lim et al., 2012) who was reported to have a higher prevalence of depressive disorders compared to adolescents (WHO, 2017). Moreover, we used depressive symptoms as outcomes, while other studies used emergency department visits (Szyszkowicz, 2007; Szyszkowicz et al., 2016), which is an acute outcome for patients. Additionally, almost all of the reported direct associations were detected with short-term (i.e. days) exposure (Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2016) instead of long-term (i.e. seasons) exposure (Kioumourtzoglou et al., 2017). The present study appears to be the first study that adopted annual ozone metrics to investigate the association between ozone exposure and the development of depressive symptoms.

A further possible reason to why we failed to uncover positive associations between ozone exposure and depressive symptoms is the low ozone levels in our study. At 1 atmosphere pressure, 25 °C, in case of the long-term exposure, our average concentration was approximately 21.6 parts per billion (ppb) in summer months. For comparison, in a previous study that observed the association of interest, the average long-term concentration of ozone during the summer months was 31.9 ppb (Kioumourtzoglou et al., 2017), which is higher than our ozone levels. Regarding the short-term exposure, apart from the studies conducted in patients which are different from other cohort studies, the reported daily maximum ozone level (metric similar to what we used) associated with depression was 48.1 ppb (Lim et al., 2012), while ours was around 36.4 ppb. On the other hand, the study that observed no associations between ozone and depressive symptoms had generally lower daily ozone level - 23.4 ppb (Wang et al., 2014). Nevertheless, we should be aware that these ozone estimates differed greatly across studies and cannot be compared directly. Additionally, even though there is no definite conclusion about exposure-response relationships between ozone exposure and health (Goodman et al., 2015), the heterogeneous results on ozone and depressive symptoms suggest that the levels of ozone might critically affect the association. If further studies confirm this assumption, it should be considered when revising air quality guidelines for ozone.

* 1. Potential mechanisms

Although our study did not find associations between ozone exposure and depressive symptoms, it is plausible to consider ozone exposure as a potentially contributing risk factor in increasing depression prevalence (Zhao et al., 2018). Ozone exposure can either provoke the production of pro-inflammatory cytokines which may cross the blood-brain barrier (Dantzer and Kelley, 2007; Dunn and Swiergiel, 1998), or increase vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), tumor necrosis factor α (TNF α) and c-Fos expression in some brain regions (Araneda et al., 2008), and thereby affect normal brain function. Ozone was also reported to have an ability to affect the secretion of hormones (Gonzalez-Pina and Paz, 1997) or the metabolism of neurotransmitters (Odermatt and Gumy, 2008; Thomson et al., 2013), resulting in a pathological process of mental disorder. Some animal studies provide evidence for this speculation. Ozone exposure might perturb normal activity/social behavior of mice (Musi et al., 1994). Rat experiments indicated that ozone inhalation elevated hippocampal superoxide accumulation and lipid peroxidation, as well as attenuated the antidepressant effects of imipramine, desipramine and escitalopram (Mokoena et al., 2010; Mokoena et al., 2015); in addition, the indicated compromised central monoamine level was similar to that noted in depression (Mokoena et al., 2015).

* 1. Strengths and limitations

There are several strengths of this study, including two different metrics of ozone data for long-term ozone exposure (annual means and number of days per year), comprehensive time frames (annual average of ozone and lag effects for 0 to 7 days) for long-term and short-term ozone exposure. We could include data from two different areas. Additionally, information on many potential confounders, including air co-pollutants, time spent outdoors and parental psychopathology, were available. Use of such statistical techniques, as DAG and adjustment for residuals of co-pollutants helped us to implement parsimonious and yet minimally biased models. To the best of our knowledge, this is the first study on ambient ozone exposure and depressive symptoms in adolescents.

Our study is not without limitations. Firstly, our analyses were cross-sectional, which cannot infer that the depressive symptoms were caused by ozone exposure. Secondly, we might have neglected some indirect pathways, such as feelings of annoyance from air pollution (Dzhambov et al., 2018b), through which the association of interest might be concealed or cancelled. To better uncover the possible relationships between exposure, outcome, and covariates, including moderation and mediation, more sophisticated techniques like structural equation modeling can be considered in the future studies instead of conventional regression analyses (e.g., Dzhambov et al., 2018a; Dzhambov et al., 2018b). Thirdly, due to selection bias by socio-economic status (SES) that initial under-recruitment and later higher loss to follow-up of participants are from families with low SES, which is also reported by other birth cohorts studies (Bornehag et al., 2012; Jacobsen et al., 2010; MAL-ED Network Investigators, 2017), the external validity of our study is limited, and the generalizability to the general German population of this age is questionable. Fourthly, the depressive symptoms were evaluated by screening questionnaires answered by participants, instead of being clinically diagnosed by medical doctors. Even though the questionnaire-based depressive symptoms prevalence of 12.5 % in our study was similar with the prevalence of 11.1 % in children aged 11-17 years reported in a previous German study which used the Depression Scale for Children (CES-DC) (Ravens-Sieberer et al., 2008) and the prevalence of 11.3 % in the US (Mojtabai et al., 2016), outcome misclassification could be present. Furthermore, the reported depressive symptoms may bias the associations of interest due to recall bias when answering the questionnaire (Kruijshaar et al., 2005). Fifthly, we might neglect other possible variables, like noise exposure (Seidler et al., 2017), or alcohol intake of parents (Pisinger et al., 2016), which may also affect the association. Finally, there is the most important drawback that the relatively coarse spatial resolution of the ozone raster of 2 km limited the precision of the exposure and might have obscured the effect estimates.

1. **Conclusions**

Our study does not support the hypothesis that long- and short-term ambient ozone exposure might increase the prevalence of depressive symptoms in adolescents. However, since no other studies investigated this association in young-aged populations, our results should be interpreted with caution. Further studies with more precise exposure assessment conducted in various populations and conditions are needed.

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Table 1. Characteristics of the study population

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Category | Munich  n (%) | Wesel  n (%) | p-value | All  n (%) |
| Cohort | GINIplus intervention | 477 (30.5) | 442 (35.0) | < 0.001 \* | 919 (32.5) |
|  | GINIplus observation | 510 (32.6) | 688 (54.5) |  | 1198 (42.4) |
|  | LISA | 578 (36.9) | 132 (10.5) |  | 710 (25.1) |
| Age *a* |  | 15.23 ± 0.29 | 15.15 ± 0.32 | < 0.001 \* | 15.20 ± 0.30 |
| Sex | Female | 796 (50.9) | 636 (50.4) | 0.830 | 1432 (50.7) |
|  | Male | 769 (49.1) | 626 (49.6) |  | 1395 (49.3) |
| Parental education *b* | Low (< 10 years) | 147 (9.4) | 403 (31.9) | 0.003 \* | 550 (19.5) |
|  | Medium (= 10 years) | 206 (13.2) | 297 (23.5) |  | 503 (17.8) |
|  | High (> 10 years) | 1212 (77.4) | 562 (44.5) |  | 1774 (62.7) |
| Maternal age at birth | ≤ 30 years | 474 (30.3) | 624 (49.5) | < 0.001 \* | 1098 (38.8) |
|  | > 30 to ≤ 35 years | 749 (47.8) | 505 (40.0) |  | 1254 (44.4) |
|  | > 35 years | 342 (21.9) | 133 (10.5) |  | 475 (16.8) |
| Income (euro/month) *c* | Low | (134, 1560] | (162, 1070] |  | - |
|  | Medium | (1560, 2250] | (1070, 1530] |  | - |
|  | High | (2250, 5130] | (1530, 5130] |  | - |
| Parental psychopathology *d* | Abnormal | 215 (13.7) | 142 (11.3) | 0.055 | 358 (12.7) |
| Single parent family | Yes | 208 (13.3) | 137 (10.9) | 0.056 | 345 (12.2) |
|  | Missing | 61 (3.9) | 45 (3.6) |  | 106 (3.7) |
| Smoking exposure | During pregnancy | 178 (11.4) | 175 (13.9) | 0.053 | 353 (12.5) |
|  | between 0 and 15 years | 445 (28.4) | 671 (53.2) | < 0.001 \* | 1116 (39.5) |
| Time spent outside *e* | High | 148 (9.5) | 319 (25.3) | < 0.001 \* | 467 (16.5) |
|  | Low | 1377 (88.0) | 892 (70.7) |  | 2269 (80.3) |
|  | Missing | 40 (2.6) | 51 (4.0) |  | 91 (3.2) |
| Time in front of a screen *f* | High | 1267 (81.0) | 1105 (87.6) | < 0.001 \* | 2373 (83.9) |
|  | Low | 297 (19.0) | 157 (12.4) |  | 454 (16.1) |
| Depressive symptoms (DesTeen) | Normal | 1349 (86.2) | 1125 (89.1) | 0.022 \* | 2474 (87.5) |
|  | Abnormal | 216 (13.8) | 137 (10.9) |  | 353 (12.5) |
| Total |  | 1565 (55.4) | 1262 (44.6) |  | 2827 (100) |

Note:

1. Mean ± standard deviation
2. According to German education system, calculated as the highest number of years of school education for either parent
3. Net equivalent household income (euro/month), Min and max of area-specific tertiles
4. According to the Global Severity Index score (Derogatis. 2001), subscore is categorized at 90th percentile (Fuertes et al.. 2016)
5. High is defined as ≥ 4 hours per day in summer or ≥ 2 hours in winter
6. High is defined as ≥ 1 hour per day in summer or ≥ 2 hours per day in winter

\* Significant difference was detected between participants from Munich and from Wesel in this variable, p < 0.05

Table 2. Concentrations of ozone and other air pollutants

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Exposure | Area | Air pollutant | Mean | SD | Min | Max | Median | IQR | p-value |
| Long-term | Munich | O3-UBA-annual *a* | 43.1 | 2.9 | 31.0 | 48.4 | 43.3 | 2.9 | - |
|  |  | O3-UBA-number of days *b* | 14.7 | 4.7 | 6.0 | 26.0 | 15.0 | 8.0 | - |
|  |  | NO2 *c* | 19.7 | 5.0 | 11.5 | 58.0 | 18.6 | 6.4 | - |
|  |  | PM10 *c* | 20.0 | 2.3 | 14.8 | 32.0 | 20.4 | 3.0 | - |
|  | Wesel | O3-UBA-annual *a* | 41.1 | 3.3 | 30.4 | 48.4 | 42.4 | 3.2 | - |
|  |  | O3-UBA-number of days *b* | 15.6 | 4.5 | 6.0 | 26.0 | 16.0 | 7.0 | - |
|  |  | NO2 *c* | 23.7 | 3.3 | 11.5 | 59.8 | 21.8 | 6.1 | - |
|  |  | PM10 *c* | 25.5 | 1.3 | 14.8 | 32.7 | 22.5 | 4.9 | - |
|  | All | O3-UBA-annual *a* | 42.2 | 3.2 | 30.4 | 48.4 | 42.4 | 3.2 | p < 0.001 *\** |
|  |  | O3-UBA-number of days *b* | 15.1 | 4.6 | 6.0 | 26.0 | 16.0 | 7.0 | p < 0.001 † |
|  |  | NO2 *c* | 21.5 | 4.7 | 11.5 | 59.8 | 21.8 | 6.1 | p < 0.001 † |
|  |  | PM10 *c* | 22.5 | 3.3 | 14.8 | 32.7 | 22.5 | 4.9 | p < 0.001 † |
| Short-term | Munich | Ozone *d* | 72.1 | 25.3 | 7.9 | 134.8 | 76.2 | 35.6 | - |
|  |  | NO2 *e* | 20.7 | 6.9 | 10.6 | 47.5 | 18.3 | 8.4 | - |
|  |  | PM10 *e* | 16.3 | 8.8 | 3.9 | 62.8 | 14.3 | 8.4 | - |
|  | Wesel | Ozone *d* | 62.7 | 27.7 | 4.2 | 135.2 | 63.0 | 38.5 | - |
|  |  | NO2 *e* | 22.2 | 8.5 | 6.2 | 44.5 | 20.7 | 13.3 | - |
|  |  | PM10 *e* | 23.1 | 9.2 | 10.3 | 52.2 | 20.7 | 10.7 | - |
|  | All | Ozone *d* | 67.9 | 26.8 | 4.2 | 135.2 | 71.3 | 39.7 | p < 0.001 *\** |
|  |  | NO2 *e* | 21.4 | 7.7 | 6.2 | 47.5 | 19.3 | 10.6 | p < 0.001 † |
|  |  | PM10 *e* | 19.3 | 9.6 | 3.9 | 62.8 | 16.9 | 10.9 | p < 0.001 † |

Note:

Abbreviation: IQR, interquartile range; SD, standard deviation

1. Annual average concentration (µg/m3), from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de)
2. Number of days per year with maximum daily 8-h concentration exceeding 120 µg/m3 (days/year), from the UBA
3. Annual average concentration (µg/m3), from the “European Study of Cohorts for Air Pollution Effects” (ESCAPE, www.escapeproject.eu)
4. The maximum 8-hour (7 hour before and the hour of interest) daily average (µg/m3),7 days prior to the depressive symptoms assessment from the background monitor stations, from the UBA
5. Average of the daily concentration (µg/m3), 7 days prior to the depressive symptoms assessment, from the UBA

\* This metric was higher in Munich than in Wesel, p < 0.05

† This metric was higher in Wesel than in Munich, p < 0.05

Table 3. Adjusted associations between ozone exposure and depressive symptoms

(Models adjusted for the DAG-identified covariates)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Exposure | Area | Pollutant | DesTeen |  |
|  |  |  | 15-year (OR, 95%CI) | Participants |
| Long-term | Munich | O3-UBA-annual *a* | 1.08 (0.92, 1.26) | 1565/1565 |
|  |  | O3-UBA-days *b* | 1.07 (0.74, 1.55) | 1565/1565 |
|  | Wesel | O3-UBA-annual *a* | 1.10 (0.91, 1.32) | 1262/1262 |
|  |  | O3-UBA-days *b* | 0.95 (0.69, 1.32) | 1262/1262 |
|  | All | O3-UBA-annual *a* | 1.08 (0.94, 1.23) | 2827/2827 |
|  |  | O3-UBA-days *b* | 1.02 (0.81, 1.28) | 2827/2827 |
| Short-term | Munich | Lag 0 day *c* | 1.00 (0.83, 1.21) | 1524/1565 |
|  |  | Lag 0-1 days *d* | 0.97 (0.81, 1.15) | 1528/1565 |
|  |  | Lag 0-2 days *e* | 1.00 (0.84, 1.20) | 1535/1565 |
|  |  | Lag 0-3 days *f* | 0.99 (0.81, 1.20) | 1544/1565 |
|  |  | Lag 0-7 days *g* | 0.90 (0.74, 1.10) | 1559/1565 |
|  | Wesel | Lag 0 day *c* | 0.76 (0.59, 0.98) \* | 1200/1262 |
|  |  | Lag 0-1 days *d* | 0.86 (0.67, 1.10) | 1201/1262 |
|  |  | Lag 0-2 days *e* | 0.88 (0.69, 1.13) | 1238/1262 |
|  |  | Lag 0-3 days *f* | 0.88 (0.69, 1.13) | 1250/1262 |
|  |  | Lag 0-7 days *g* | 0.95 (0.76, 1.19) | 1262/1262 |
|  | All | Lag 0 day *c* | 0.90 (0.77, 1.06) | 2724/2827 |
|  |  | Lag 0-1 days *d* | 0.92 (0.79, 1.08) | 2729/2827 |
|  |  | Lag 0-2 days *e* | 0.95 (0.82, 1.11) | 2773/2827 |
|  |  | Lag 0-3 days *f* | 0.94 (0.81, 1.10) | 2794/2827 |
|  |  | Lag 0-7 days *g* | 0.92 (0.79, 1.07) | 2821/2827 |

Note:

Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio;

1. ORs and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).

2. All estimates are from logistic regression models adjusted for PM10 and NO2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up and sex of the child, cohort and area (only for the area “all”).

3. Participants, “sample number analyzed/total number analyzed”; missings are due to a lack of exposure data.

1. Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de)
2. Number of days per year with maximum 8-h concentration exceeding 120 µg/m3, from the UBA
3. The maximum of the daily maximum 8-hour average concentration was selected over 0 days (same day) prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
4. The maximum of the daily maximum 8-hour average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
5. The maximum of the daily maximum 8-hour average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
6. The maximum of the daily maximum 8-hour average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
7. The maximum of the daily maximum 8-hour average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA

\* p = 0.037

Table 4. Adjusted associations between long-term ozone exposure and depressive symptoms

(Models with the DesTeen score as count data, adjusted for the DAG-identified covariates)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Exposure | Area | Pollutant | DesTeen |  |
|  |  |  | 15-year  (Count ratio, 95%CI) | Participants |
| Long-term | Munich | O3-UBA-annual *a* | 0.99 (0.97, 1.03) | 1565/1565 |
|  |  | O3-UBA-days *b* | 0.97 (0.89, 1.05) | 1565/1565 |
|  | Wesel | O3-UBA-annual *a* | 1.01 (0.97, 1.05) | 1262/1262 |
|  |  | O3-UBA-days *b* | 0.98 (0.91, 1.05) | 1262/1262 |
|  | All | O3-UBA-annual *a* | 0.99 (0.97, 1.03) | 2827/2827 |
|  |  | O3-UBA-days *b* | 0.98 (0.93, 1.03) | 2827/2827 |
| Short-term | Munich | Lag 0 day *c* | 0.99 (0.95, 1.03) | 1524/1565 |
|  |  | Lag 0-1 days *d* | 0.99 (0.95, 1.03) | 1528/1565 |
|  |  | Lag 0-2 days *e* | 1.00 (0.96, 1.04) | 1535/1565 |
|  |  | Lag 0-3 days *f* | 0.99 (0.96, 1.04) | 1544/1565 |
|  |  | Lag 0-7 days *g* | 0.98 (0.94, 1.02) | 1559/1565 |
|  | Wesel | Lag 0 day *c* | 0.95 (0.90, 1.00) | 1200/1262 |
|  |  | Lag 0-1 days *d* | 0.97 (0.93, 1.03) | 1201/1262 |
|  |  | Lag 0-2 days *e* | 0.98 (0.93, 1.03) | 1238/1262 |
|  |  | Lag 0-3 days *f* | 0.98 (0.93, 1.03) | 1250/1262 |
|  |  | Lag 0-7 days *g* | 0.99 (0.95, 1.05) | 1262/1262 |
|  | All | Lag 0 day *c* | 0.97 (0.93, 1.00) | 2724/2827 |
|  |  | Lag 0-1 days *d* | 0.98 (0.95, 1.02) | 2729/2827 |
|  |  | Lag 0-2 days *e* | 0.99 (0.96, 1.03) | 2773/2827 |
|  |  | Lag 0-3 days *f* | 0.99 (0.96, 1.03) | 2794/2827 |
|  |  | Lag 0-7 days *g* | 0.99 (0.96, 1.02) | 2821/2827 |

Note:

Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio;

1. Count ratios and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).

2. All estimates are from negative binomial regression models adjusted for PM10 and NO2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up and sex of the child, cohort and area (only for the area “all”).

3. Participants, “sample number analyzed/total number analyzed”; missings are due to a lack of exposure data.

1. Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de)
2. Number of days per year with maximum 8-h concentration exceeding 120 µg/m3, from the UBA
3. The maximum of the daily maximum 8-hour average concentration was selected over 0 days (same day) prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
4. The maximum of the daily maximum 8-hour average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
5. The maximum of the daily maximum 8-hour average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
6. The maximum of the daily maximum 8-hour average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA
7. The maximum of the daily maximum 8-hour average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA)