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Prenatal and childhood exposure to air pollution and traffic and the risk of liver injury in European children

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Background: Nonalcoholic fatty liver disease is the most prevalent pediatric chronic liver disease. Experimental studies suggest effects of air pollution and traffic exposure on liver injury. We present the first large-scale human study to evaluate associations of prenatal and childhood air pollution and traffic exposure with liver injury.

Methods: Study population included 1,102 children from the Human Early Life Exposome project. Established liver injury biomarkers, including alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and cytokeratin-18, were measured in serum between ages 6–10 years. Air pollutant exposures included nitrogen dioxide, particulate matter <10 μm (PM_{10}), and <2.5 μm. Traffic measures included traffic density on nearest road, traffic load in 100-m buffer, and inverse distance to nearest road. Exposure assignments were made to residential address during pregnancy (prenatal) and residential and school addresses in year preceding follow-up (childhood). Childhood indoor air pollutant exposures were also examined. Generalized additive models were fitted adjusting for confounders. Interactions by sex and overweight/obese status were examined.

Results: Prenatal and childhood exposures to air pollution and traffic were not associated with child liver injury biomarkers. There was a significant interaction between prenatal ambient PM_{10} and overweight/obese status for alanine aminotransferase, with stronger associations among children who were overweight/obese. There was no evidence of interaction with sex.

Conclusion: This study found no evidence for associations between prenatal or childhood air pollution or traffic exposure with liver injury biomarkers in children. Findings suggest PM_{10} associations maybe higher in children who are overweight/obese, consistent with the multiple-hits hypothesis for nonalcoholic fatty liver disease pathogenesis.

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The HELIX data warehouse has been established as an accessible resource for collaborative research involving researchers external to the project. Access to HELIX data is based on approval by the HELIX Project Executive Committee and by the individual cohorts. Further details on the content of the data warehouse (data catalog) and procedures for external access are described on the project website (http://www.projecthelix.eu/index.php/es/data-inventory). The data used in this analysis are not available for replication because specific approvals from HELIX Project Executive Committee and the University of Southern California Institutional Review Board must be obtained to access them.

The authors declare that they have no conflicts of interest with regard to the content of this report.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become an epidemic in the pediatric population and is now one of the most common chronic liver diseases in children,[1,2] currently affecting 3–13% of the general pediatric population in Western countries.[3] In large population studies, the prevalence of elevated serum levels of alanine aminotransferase (ALT), an established biomarker of liver injury and clinical screening tool for pediatric NAFLD, has almost tripled,[4] coinciding with the childhood obesity epidemic of the past several decades. Children with NAFLD may suffer future comorbidities, which can have lifelong effects, including low-bone mineral density, type 2 diabetes, an adverse cardiometabolic risk profile, and development of cardiovascular disease.[1,5] In the next decade, liver complications due to NAFLD are predicted to become the most frequent indication for liver transplantation.[5]

What This Study Adds

Nonalcoholic fatty liver disease has become an epidemic in children and is now one of the most common pediatric chronic liver diseases. We used a population-based multicohort study spanning six European countries to assess whether prenatal or childhood exposure to air pollution or traffic relate to biomarkers of liver injury and suspected nonalcoholic fatty liver disease in children. Results indicate no evidence of elevated liver enzyme levels in association with exposure to air pollution and traffic in either prenatal or postnatal periods. Findings from interaction analyses, however, suggest PM_{10} effect estimates may be higher in children who are overweight or obese.

Emerging experimental evidence indicates that ambient and near-roadway pollution causes liver injury and may contribute to NAFLD development.[6–9] Mice chronically exposed to airborne particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$) developed hepatic steatosis, inflammation, and fibrosis.[8,9] The epidemiologic literature is scant with only two studies having examined this relation in children. A small prospective study of 74 US children of mean age 14 years recruited from an obesity clinic found an association of childhood nitrogen dioxide (NO_2) exposure and residential traffic volume with cytokeratin-18 (CK-18), a biomarker hepatocyte apoptosis.[10] The generalizability of these results is unclear since all study participants were overweight or obese. In a cross-sectional study of 150 newborns maternal residential exposure to particulate matter (PM) was associated with higher levels of liver injury biomarkers, including ALT, aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) in cord blood.[11] Thus, whether prenatal pollution and traffic exposures relate to liver injury later in childhood is uncertain. Given the public health importance of the pediatric NAFLD epidemic, the ubiquity of air pollution and traffic exposure, and the scarcity of human studies, further investigations, including prospective studies, are urgently needed to clarify the effect of pollution and traffic exposure on liver injury and NAFLD risk in children.

We present the first large-scale population-based human study to examine the relationship between early life air pollution exposure and risk of liver injury in childhood. We used data from a well-established population-based multicohort study across six European countries, the Human Early Life Exposome (HELIX) study, to assess whether prenatal or childhood exposure to air pollution or traffic relate to four noninvasive clinical biomarkers of liver injury and suspected NAFLD: ALT, AST, GGT, and CK-18. We examined exposure to outdoor air pollutants, indoor pollutants, and traffic. Finally, we examined the potential for effect heterogeneity by sex and overweight or obesity status, given prior evidence of differential NAFLD prevalence by these factors.[3]

Methods

Study population

The study population was drawn from the HELIX study,[12] a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in six European countries. This includes the Born in Bradford (BiB) study in the United Kingdom,[13] the Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant (EDEN) study in France,[14] the INfancia y Medio Ambiente (INMA) cohort in Spain,[15] the Kaunas cohort (KANC) in Lithuania,[16] the Norwegian Mother, Father, and Child Cohort Study (MoBa),[17] and the RHEA Mother Child Cohort study in Crete, Greece.[18] The full HELIX protocol and database have been described in detail previously.[19] Briefly, a subcohort of 1,301 mothers and their singleton children across the six cohorts (approximately 200 children per cohort) was followed up in 2014–2015 for clinical examination, interview with the mothers, and collection of biologic samples. Data collection was standardized across cohorts and performed by trained staff.

The study population comprised 1,102 (85%) mother-child pairs from the HELIX subcohort, following inclusion criteria regarding availability of data on all four liver enzymes, ALT, AST, GGT, and CK-18. All participating families provided written informed consent. Approval for the HELIX project was obtained from the local ethical committees at each site. Additionally, the current study was approved by the University of Southern California Institutional Review Board.

Air pollution and traffic exposure assessment

Here, we provide a brief description of the assessment methods for exposures included in this study. A detailed description of methods used to assess air pollution and traffic is provided in Tamayo-Uria et al.[20] The following ambient air pollutants were examined: NO_2 and particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$) and <10 µm (PM_{10}). These were assessed for the pregnancy period (averages for each trimester and across entire pregnancy) and in the year before childhood study visit based on both home and school address using land-use regression (LUR) models developed in the context of the European Study of Cohorts for Air Pollution Effects (ESCAPE) project[21–25] or dispersion models,[26] temporally adjusted to measurements made in local background monitoring stations.[20] Site-specific ESCAPE LUR models were used for most cohorts.[20–25] Exposure assessment for $PM_{2.5}$ and PM_{10} in the BiB cohort (United Kingdom) was conducted using the ESCAPE LUR model for London/Oxford, United Kingdom, adjusting for background PM concentration based on air monitoring data from Bradford.[20,27] In the EDEN cohort (France), assessment of $PM_{2.5}$ exposure was conducted based on the European-wide

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ESCAPE LUR model[28] and assessment of NO_2 and PM_{10} (only for pregnancy period) exposure was conducted based on dispersion models.[20,26] Routine background ambient air monitoring stations that were active during the whole study period provided daily background concentration data used for temporal adjustment.[20] The following markers of traffic were examined: inverse distance to nearest road, traffic load on all roads within a 100 m buffer, and traffic density on nearest road. These were assessed for the full pregnancy period and in the year before childhood study visit based on both home and school address and were calculated from road network maps following the ESCAPE protocol,[21,23] applying the land-use regression (LUR) methods and Geographic Information System (GIS) predictor variables used within the ESCAPE project as described in Eeftens et al.[23] We examined the following indoor air pollutants: NO_2, $PM_{2.5}$, benzene, and the sum of benzene, toluene, ethylbenzene, and xylene (BTEX). These were assessed for the year before childhood study visit and were estimated using a prediction model that combined measurements in the homes of a subgroup of children with questionnaire data from the subcohort.[20] As part of a child panel study nested within the HELIX subcohort (all cohorts except MoBA), indoor NO_2, benzene, and TEX (toluene, ethylbenzene, and xylene) were measured in the homes of 157 participants. $PM_{2.5}$ was measured in INMA, BiB, and EDEN. Panel study participants were followed for one week in two seasons. NO_2, benzene, and TEX sampling were conducted over 7 days, and $PM_{2.5}$ sampling was conducted over 24 hours. The last day of the first week of measurements was the same day as the subcohort examination, which included the main HELIX questionnaire.[20]

Biomarkers of liver injury

Collection and laboratory analysis of liver enzyme levels have previously been reported in detail.[29] Identical predefined standardized protocols across all six cohorts were followed to collect and process blood samples. Briefly, at the end of clinical examinations as part of the subcohort follow-up visit blood samples following a median fasting time of 3.3 hours were collected from children into 4 ml silica plastic tubes. Samples were gently inverted 6–7 times, spun down at 2,500 g for 15 minutes at 4°C, and then frozen at −80°C under optimized and standardized procedures. Concentrations (IU/L) of ALT, AST, and GGT in serum were assessed by Biochemistry Laboratory of the Clínica Universidad de Navarra using homogenous enzymatic colorimetric methods on a Colorimetry Cobas 8000 analyzer according to the manufacturer's instructions (Roche Diagnostics GmbH Mannheim). CK-18 in serum was measured by ELISA (M30 Apoptosense® ELISA, PEVIVA) according to the manufacturer´s instructions. All coefficients of variation were less than 3%.

Covariate assessment

Using directed acyclic graph theory,[30] a set of variables considered to be sufficient for confounding adjustment were decided upon *a priori*. The covariates were cohort, maternal age (years), maternal prepregnancy BMI (kg/m^2), maternal education level (low, middle, high), paternal education level (low, middle, high), and maternal active smoking during pregnancy (yes, no). Additionally, data on child's sex (male, female) and age (years) and BMI (kg/m^2) at follow-up visit were assessed. Information on maternal age at birth, maternal prepregnancy BMI, maternal education, paternal education, and smoking status during pregnancy from each study participant was obtained by each cohort during pregnancy or at birth by questionnaire or medical records. Birthdate and newborn sex were obtained at birth. During the follow-up examination, anthropometric data were collected using regularly calibrated instruments. Height was measured with a stadiometer and weight with a digital weight scale, both without shoes and with light clothing. Height and weight measurements were converted to BMI for age-and-sex z-scores using the international WHO reference curves to allow comparison with other studies.[31] Overweight and obese children were defined as those above the age-and sex-specific 85th and 95th percentiles, respectively, as recommended by WHO (http://www.who.int/mediacentre/factsheets/fs311/en/). Maternal alcohol consumption during pregnancy (yes, no) obtained in each cohort during pregnancy or at birth by questionnaire or medical records is used in a sensitivity analysis.

Statistical analyses

Skewed exposure variables were transformed to improve model fit. The following were natural log transformed: ambient NO_2, inverse distance to nearest road, and all indoor pollutants (NO_2, $PM_{2.5}$, benzene, and BTEX). The following were cube root transformed: traffic load on all roads within a 100 m buffer and traffic density on nearest road. Analyses were conducted on transformed variables, but plots are shown with back-transformed values for interpretability. Single imputation of missing data was done using a chained equations method,[32] as described previously in detail.[20] The proportion of missing data was minimal, ranging from 1.2% for maternal age at birth to 4.9% for paternal education. The associations between liver injury biomarkers with air pollution exposures and markers of traffic were estimated separately based on generalized additive models (GAM) using the R package "mgcv."[33] Smooth functions of exposure were fitted using a penalized regression spline[33] to allow for possible nonlinear exposure-response functions. Covariates included in the models were cohort, paternal education level, and maternal age, education level, prepregnancy BMI, and active smoking during pregnancy. Maternal age and prepregnancy BMI were each fitted with a smooth function using penalized regression splines. We also examined associations with categories of exposure based on tertiles using the same modeling approach as described above. We conducted a sensitivity analysis further adjusting for maternal alcohol consumption during pregnancy as a potential confounding variable. We then evaluated possible interaction between exposure and child's sex (male; female) or overweight/obese status at follow-up assessment (dichotomous: not overweight or obese; overweight or obese). Because GAM models indicated linear exposure-response curves for almost all exposure-outcomes, interactions were assessed using an interaction term in models with exposure included as a continuous variable with a linear term. For exposure-outcomes with significant nonlinearity, interactions were additionally assessed using smooth parameterizations that allowed for quantification of interactions.

All hypotheses were tested assuming a 0.05 significance level and a two-sided alternative hypothesis. *P* values from GAM models with smooth terms for exposure were adjusted for multiple comparisons for each biomarker using a method of computing *P* values adjusted for correlated tests (P_{ACT}).[34] It was not possible to use the P_{ACT} adjustment for the *P* values from the categorical or interaction models, so we instead used the false discovery rate (FDR) procedure to adjust these *P* values.[35] All statistical analyses were performed using R 3.5.1.[36]

Results

Characteristics of the study populations

Distribution of child and parental sociodemographic characteristics among 1,102 study participants are shown in Table 1. The median maternal age at participant's birth was 31 years (interquartile range [IQR]: 27.8–34.1 years) with a median age at follow-up in childhood of 8.2 years (IQR: 6.6–9.1 years).

There were slightly more males than females (596 [54.1%] compared with 506 [45.9%], respectively) and most children came from parents of middle to high education. Distribution of liver enzyme concentrations among participants are shown in Table 1. Exposures distribution of ambient (outdoor) air pollutants, markers of traffic, and indoor air pollutants among study participants are shown in Table 2. Median exposure concentrations during pregnancy for ambient NO_2, PM_{10}, and $PM_{2.5}$ were 18.4, 22.5, and 14.9 μg/m^3, respectively.

Associations with liver enzymes

Modeled associations between ambient air pollution exposure during different age windows and ALT are shown in Figure 1, and *P* values unadjusted for multiple comparisons are in eTable 1; http://links.lww.com/EE/A139. No results were statistically significant after adjustment for multiple comparisons. Null associations with ALT were also observed for markers of traffic exposure in utero and in childhood and indoor air pollution exposure during childhood (eFigures 1; http://links.lww.com/EE/A139 and 2; http://links.lww.com/EE/A139; eTable 1; http://links.lww.com/EE/A139). Modeled associations for AST, GGT, and CK-18 with ambient air pollution, markers of traffic, and indoor air pollution are shown in the supplement (eFigures 2–8; http://links.lww.com/EE/A139; http://links.lww.com/EE/A139; eTable 1; http://links.lww.com/EE/A139). No results were statistically significant after adjustment for multiple comparisons. AST was borderline statistically significantly associated with ambient PM_{10} exposure in trimester 1 ($P = 0.055$ after adjustment for multiple comparisons) with a positive association observed for PM_{10} exposures above approximately 30 μg/m^3 (eFigure 3; http://links.lww.com/EE/A139). Null associations were similarly observed for models with tertiles of exposure (eTables 2–5; http://links.lww.com/EE/A139). Additional adjustment for maternal alcohol consumption during pregnancy did not markedly change model results (results not shown).

Interaction with sex and overweight/obese status

Results of interaction models by sex are shown in the supplement (eFigures 9–17; http://links.lww.com/EE/A139). No clear differences by participant's sex were observed in associations for air pollution or traffic exposure with any liver enzyme biomarker. Results of interaction models by overweight/obese status are shown in Figure 2 for ALT with ambient air pollution and in the supplement for the other liver enzyme biomarkers and exposures (eFigures 18–25; http://links.lww.com/EE/A139). The association between prenatal PM_{10} exposure and ALT was statistically significantly different by whether participants were overweight or obese at follow-up assessment ($P = 0.045$ after adjustment for multiple comparisons), with larger positive slopes among those who were overweight or obese ($\beta = 0.120$ per 1 μg/m^3; SE = 0.065) compared with a slightly negative slope among those

Table 1.

Distribution of sociodemographic characteristics and liver injury biomarkers in the study population

N	1,102
Cohort	
MoBa, Norway	270 (24.5)
INMA, Spain	211 (19.1)
EDEN, France	196 (17.8)
KANC, Lithuania	166 (15.1)
RHEA, Greece	165 (15.0)
BiB, United Kingdom	94 (8.5)
Maternal characteristics	
Age at birth, years, median (IQR)	31.0 (27.8–34.1)
Prepregnancy BMI, kg/m^2, median (IQR)	23.5 (21.1–26.7)
Education	
High	505 (45.8)
Middle	434 (39.4)
Low	163 (14.8)
Active smoking during pregnancy	
No	945 (85.8)
Yes	157 (14.2)
Paternal characteristics	
Education	
High	596 (54.1)
Middle	382 (34.7)
Low	124 (11.3)
Child characteristics	
Age at follow-up, years, median (IQR)	8.2 (6.6-9.1)
Sex	
Male	596 (54.1)
Female	506 (45.9)
Liver enzyme concentrations, IU/L, median (IQR)	
ALT	14.4 (11.6-18.1)
AST	28.8 (25.1-34.2)
GGT	12.0 (10.4-14.3)
CK-18	71.2 (60.7-88.9)

N (%), unless otherwise noted.

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK-18, cytokeratin-18; GGT, gamma-glutamyltransferase; IQR, interquartile range.

Table 2.

Distribution of exposures to ambient air pollutants, markers of traffic, and indoor air pollutants in the study population

Ambient air pollutants	
NO_2, μg/m^3	
Pregnancy	18.4 (13.4, 26.2)
Trimester 1	19.0 (13.9, 28.2)
Trimester 2	17.1 (12.5, 26.5)
Trimester 3	17.1 (13.0, 26.5)
Childhood, home	19.7 (11.2, 30.2)
Childhood, school	18.5 (12.0, 29.9)
PM_{10}, μg/m^3	
Pregnancy	22.5 (15.9, 27.7)
Trimester 1	21.9 (16.1, 28.1)
Trimester 2	22.0 (15.6, 28.3)
Trimester 3	20.2 (15.1, 27.5)
Childhood, home	25.2 (18.8, 31.5)
Childhood, school	24.9 (18.4, 31.7)
$PM_{2.5}$, μg/m^3	
Pregnancy	14.9 (13.0, 17.0)
Trimester 1	14.5 (12.2, 17.7)
Trimester 2	14.1 (11.8, 17.2)
Trimester 3	13.7 (11.6, 16.4)
Childhood, home	13.7 (11.5, 14.9)
Childhood, school	13.8 (11.6, 14.9)
Markers of traffic	
Inverse distance, m^{-1}	
Pregnancy	0.051 (0.019, 0.108)
Childhood, home	0.019 (0.007, 0.053)
Childhood, school	0.015 (0.008, 0.03)
Traffic load, vehicles/day-m	
Pregnancy	212,135 (0, 1,501,124)
Childhood, home	229,783 (0, 1,514,536)
Childhood, school	263,992 (0, 1,579,946)
Traffic density, vehicles/day	
Pregnancy	1,210 (500, 4,302)
Childhood, home	2,979 (500, 10,898)
Childhood, school	3,398 (798, 10,000)
Indoor air pollutants	
NO_2, μg/m^3: childhood, home	1.8 (1.4, 2.4)
$PM_{2.5}$, μg/m^3: childhood, home	29.1 (16.9, 94.3)
Benzene, μg/m^3: childhood, home	9.5 (7.7, 13.8)
BTEX, μg/m^3: childhood, home	21.0 (14.4, 30.6)

Median (interquartile range).

BTEX indicates sum of benzene, toluene, ethylbenzene, and xylene; NO_2, nitrogen dioxide; PM_{10}, particulate matter <10 μm; $PM_{2.5}$, particulate matter <2.5 μm.

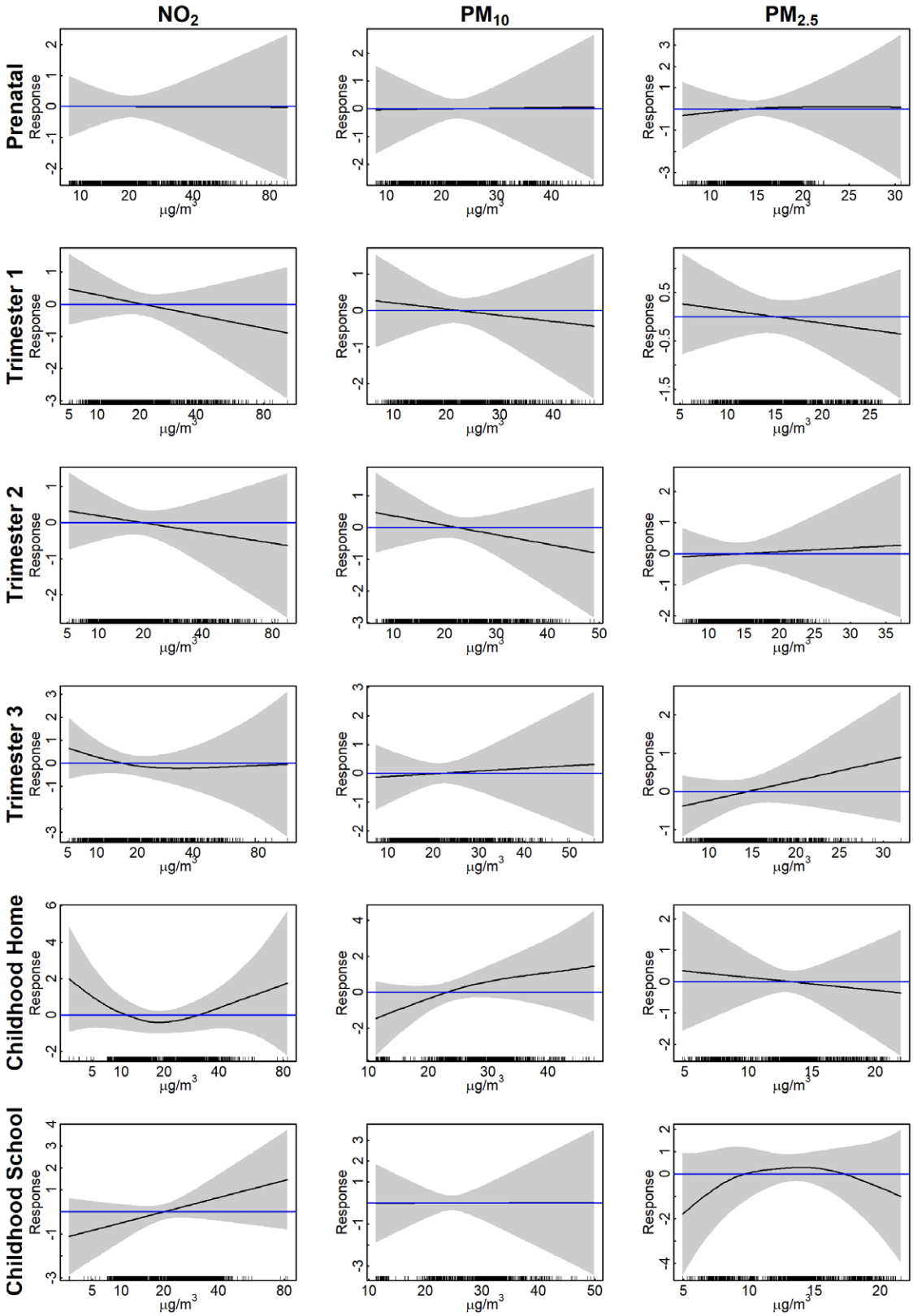

Figure 1. Model results for ambient air pollution and ALT. Associations between ambient air pollution exposure (NO_2, PM_{10}, and $PM_{2.5}$) during different age windows and ALT modeled separately using generalized additive models, adjusting for cohort, maternal age, maternal prepregnancy BMI, maternal education level, paternal education level, and maternal active smoking during pregnancy. Null referent line is shown. No results were statistically significant after adjustment for multiple comparisons. ALT indicates alanine aminotransferase; BMI, body mass index; NO_2, nitrogen dioxide; PM_{10}, particulate matter <10 µm; $PM_{2.5}$, particulate matter <2.5 µm.

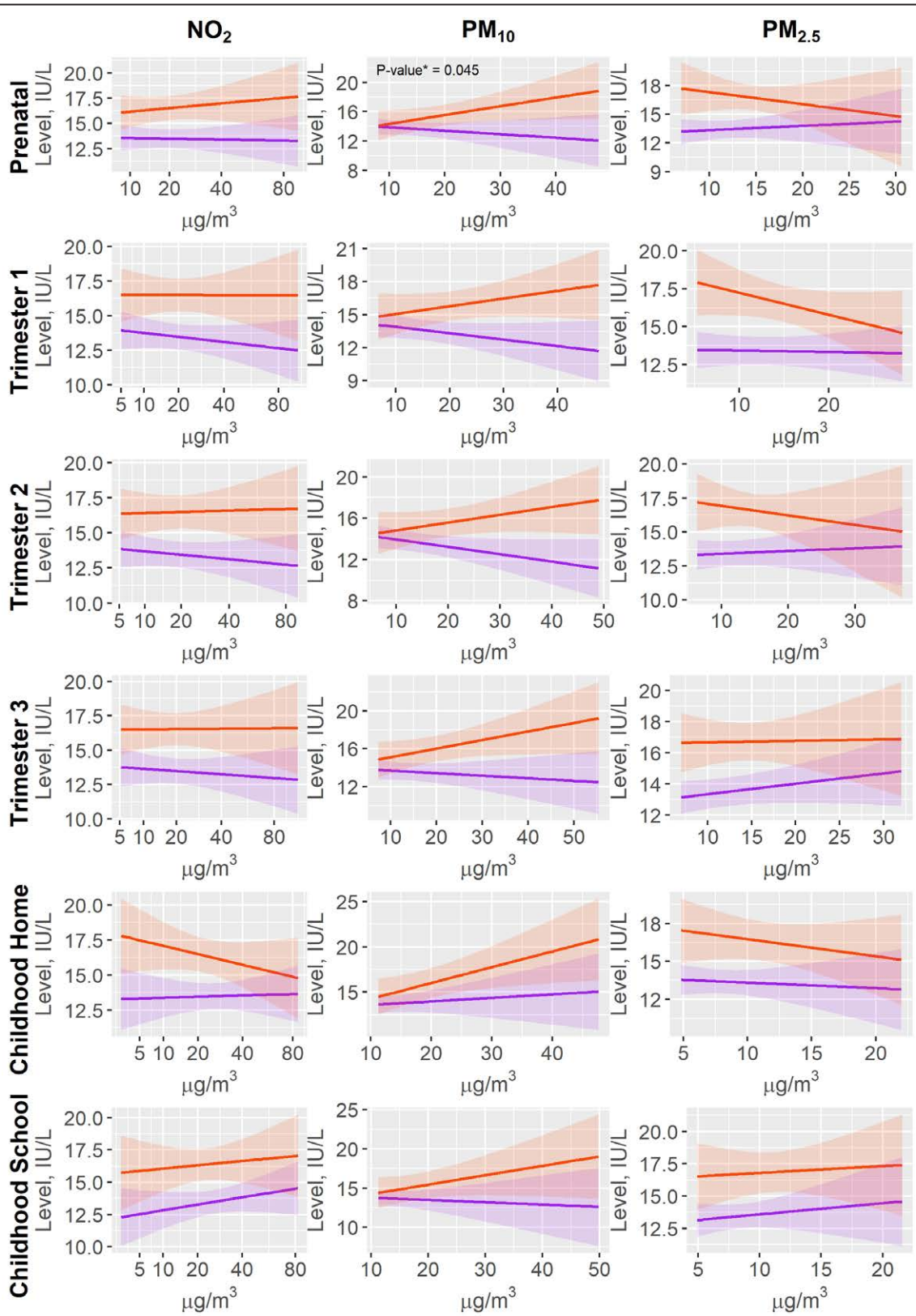

Figure 2. Model results for ambient air pollution with overweight or obese status interaction and ALT. Associations between ambient air pollution exposure (NO_2, PM_{10}, and $PM_{2.5}$) during different age windows and ALT, stratified by overweight or obese status (orange line represents those who are overweight or obese; purple line represents those who are not overweight or obese). Modeled separately using generalized additive models, adjusting for cohort, maternal age, maternal prepregnancy BMI, maternal education level, paternal education level, and maternal active smoking during pregnancy. *Association for prenatal PM_{10} exposure was statistically significant after adjustment for multiple comparisons. No other results were statistically significant. ALT indicates alanine aminotransferase; BMI, body mass index; NO_2, nitrogen dioxide; PM_{10}, particulate matter <10 μm; $PM_{2.5}$, particulate matter <2.5 μm.

who were not overweight or obese (β = –0.047 per 1 μg/m^3; SE = 0.052). Overall ambient PM_{10} exposures associations with ALT, slopes appear larger, although not always statistically significantly different, among participants who were overweight or obese compared with those who were not overweight or obese (Figure 2). No other clear differences by participant overweight/obese status were observed in associations for other air pollution/traffic exposure with any liver enzyme biomarker. For the five exposure-outcomes with significant nonlinearity (eTable 1; http://links.lww.com/EE/A139), plots of exposure smooth with statistically significant interaction with either sex or overweight/obese status are shown in eFigure 26; http://links.lww.com/EE/A139, but these are not adjusted for multiple comparisons and should be interpreted with caution.

Discussion

Using data from a well-characterized multi-cohort study across several European countries, we examined for the first time in a population-based prospective study the associations between prenatal and childhood air pollution and traffic exposure with biomarkers of child liver enzymes. We found no clear association of air pollution and traffic both in the prenatal and postnatal periods with liver enzyme levels in the overall study population. Notably, stratified analysis by child obesity status revealed a stronger association between prenatal PM_{10} exposure and ALT among children who were overweight or obese compared with children who were not overweight or obese; the same pattern was observed in trimester-specific associations and for childhood PM_{10} exposures.

There is emerging evidence that environmental factors may play a role in the onset and progression of NAFLD.[7,37] Air pollution exposure is linked with oxidative stress, systemic low-grade inflammation, and alterations in insulin/insulin-like growth factor and insulin resistance, which are all etiological factors related to NAFLD.[37] Among the few studies in adult populations, associations have been reported for $PM_{2.5}$ with ALT,[38,39] AST,[38] and GGT[40]; PM_{10} with ALT[41,42] and AST[42]; NO_2 with ALT and AST[38,41,42]; and blood benzene with CK-18.[43] Only two studies have examined the effect of air pollution or markers of traffic exposure on liver injury and NAFLD risk in children. In contrast to our findings, a small cross-sectional study on 150 newborns from Sabzevar, Iran, reported higher maternal exposure to PM <1 μm, $PM_{2.5}$, and PM_{10} to be associated with increased ALT, AST, and GGT in newborn cord blood.[11] Positive associations were also observed with higher street length in a 100 m buffer around the home for ALT, AST, and GGT, and an inverse association for distance to major roads with AST. Pollution levels were a lot higher in this newborn study in comparison with the present analysis; for example, the median (IQR) $PM_{2.5}$ was 46.8 (40.1–73.3) μg/m^3 compared with 14.9 (13.0–17.0) μg/m^3, respectively. It is possible air pollution effects may differ by level of pollutant, with larger effects at higher pollutant concentrations and smaller, less detectable effects at lower concentrations, such as those in the present study. A prospective study of 74 children with mean age of 14 years who were overweight or obese from the Yale Pediatric Obesity Clinic followed for two years reported association for CK-18 at follow-up with NO_2 and traffic volume at baseline residence.[10] An IQR increase in NO_2 (1.91 ppb) was associated with 11 U/L higher CK-18 (SE = 5.4), and an IQR increase in residential traffic volume within a 1-km buffer was associated with 15 U/L higher CK-18 (SE = 5.2) per 110,000 vehicle-km. No statistically significant associations of AST or ALT with NO_2 or traffic volume were found. This study, however, comprised only overweight and obese children recruited from an obesity clinic, whereas our study sample is population based. Given their health status, the children in the Yale study may have been more susceptible to the effects of pollution. Although we did not observe the same positive association between CK-18 and NO_2 and traffic volume, we did find similar stronger associations between PM_{10} and ALT among children who were overweight or obese. These findings of higher pollution effects in children who are overweight/obese should be more carefully examined in future research.

Our analysis revealed an interaction between prenatal PM_{10} exposure and overweight or obese status in childhood for liver injury biomarkers and especially for ALT. This suggests that prenatal air pollution might make the liver vulnerable to effects of increased weight status. This observation is consistent with the multiple-hits hypothesis for NAFLD pathogenesis,[44] whereby prenatal PM_{10} exposure serves as an initial hit, leaving the liver compromised and sensitive to further insults, such as obesity or being overweight (possibly a proxy for high-fat/proinflammatory diet), acting as an additional hit promoting disease progression. Synergistic interaction between air pollutant exposure and high-fat diet, a precursor to increased weight status, have been reported based on animal experiments. Mice exposed to an average of 15 μg/m^3 $PM_{2.5}$ and fed high-fat chow showed significantly increased lobular inflammation, hepatocyte ballooning, and Mallory bodies compared with either $PM_{2.5}$ exposure or diet alone.[8] It has also been reported that $PM_{2.5}$ acts synergistically with high-fat diet to promote other metabolic outcomes, such as adiposity, insulin resistance and type 2 diabetes.[45,46] It has been posited that environmental exposure—the first hit—may compromise the liver's protective responses against overnutrition—a subsequent hit—, promoting fatty liver disease from high-fat diets.[47] The synergistic interaction between prenatal PM_{10} exposure and overweight or obese status merits further investigation. If these findings hold, then regulatory effects to improve air quality may potentially reduce the hits to the liver and lower the risk of NAFLD development.

Several toxicological studies have examined the role of air pollution in NAFLD and possible mechanisms, with most focusing on PM. Inhaled PM particles can reach the liver where they activate Kupffer cells and induce an inflammatory response through the activation of several molecular pathways, such as c-Jun *N*-terminal kinases-activator protein 1, nuclear factor-κB, and Toll-like receptor 4.[7] In a mouse study, $PM_{2.5}$ exposure significantly increased Kupffer secretion of cell interleukin-6,[8] a proinflammatory cytokine associated with human NAFLD and those with higher steatohepatitis compared with simple fatty liver.[48] PM particles may also affect peroxisome proliferator-activated receptors activity, altering lipid and glucose metabolism in Kupffer cells, hepatocytes, and hepatic stellate cells.[7,9] In mice with 6-month $PM_{2.5}$ exposure, accelerated upregulation of tumor necrosis factor alpha (TNF-α) caused hepatic inflammation and oxidative stress, disrupting the balance of lipid metabolism in the liver.[9] However, given the species-specific toxico-kinetics of PM, extrapolation from animals to humans is difficult.[49] Although the toxicological literature supports an effect of air pollution on NAFLD development, we did not observe such associations in our longitudinal epidemiologic study. It may be that liver injury biomarkers being examined here in a population of apparently healthy children are not sufficiently sensitive to detect small perturbations in hepatic inflammation and lipid metabolism or that the levels of exposure in our analysis are below some effect threshold.

The main strength of our study is that it is the first large-scale epidemiologic study of the impact of air pollution on child liver injury, using data from six European birth cohorts with prospectively collected data. In addition to using the same protocol for the outcome assessment, these six cohorts have detailed information on air pollution exposure assessed using standardized protocols in two critical developmental age periods, in utero (including estimates of trimester-specific outdoor air pollution exposure) and early childhood. Our study has also a number of limitations. First, we used serum liver enzymes as our measure of liver injury rather than the current diagnostic gold standard

of liver biopsy for NAFLD. Large-scale liver biopsies, however, are not feasible in large population studies due to ethical considerations and high costs; any outcome misclassification is not expected to have been differential by exposure level. Second, although we had detailed exposure assessment regarding participants' residential addresses, we did not have information about total exposure, including maternal occupational exposure during pregnancy or exposure at other locations such as school for indoor pollutants, which might have affected the observed results. Third, we were not able to examine other potentially informative markers of traffic, such as distance to nearest major road or total street length in select buffers since such estimates were not available for the HELIX study. It is important to note that traffic exposure captures not only near-roadway air pollution but also traffic noise and possibly aspects of the built environment, such as green space and opportunity for outdoor physical activity. Finally, because of the large number of exposures being tested and our appropriate adjustment for multiple comparisons, the statistical power was limited. A narrower list of focused exposures could have partly addressed this issue, however, we wanted to take full advantage of the rich air pollution and traffic exposure data available in HELIX. Regardless of statistical significance, researchers will be able to examine the exposure-response curves reported for the four liver enzymes with the pollution and traffic exposures to inform their own work. Furthermore, *P* values not adjusted for multiple comparisons are presented in the Supplement for the benefit of the reader.

This multicohort study of over 1,100 European children did not find prenatal or childhood air pollution or traffic exposure to be associated with biomarkers of liver injury in children. Findings from interaction analyses suggest PM_{10} effect estimates may be higher in children who are overweight or obese, consistent with the multiple-hits hypothesis for NAFLD pathogenesis. Although additional research is needed to confirm these findings, this synergistic interaction suggests that reduction of particulate air pollution levels may be a possible intervention to lower the risk of liver injury and NAFLD development in children.

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