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Ambient air pollution exposure is associated with liver fat and stiffness in Latino youth with a more pronounced effect in those with PNPLA3 genotype and more advanced liver disease

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Abstract

Background: Exposure to ambient air pollutants has emerged as a risk for metabolic-dysfunction associated steatotic liver disease (MASLD).

Objectives: We sought to examine associations between short-term (prior month) and long-term (prior year) ambient air pollution exposure with hepatic fat fraction (HFF) and liver stiffness in

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117234.

CRediT authorship contribution statement

Rachel Beth Schenker: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Fredrick Lurmann:** Methodology. **Hooman Allayee:** Methodology. **Christopher J Machle:** Writing – review & editing, Formal analysis. **Tanya Alderete:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Michael Goran:** Writing – review & editing, Supervision, Conceptualization. **Rohit Kohli:** Writing – review & editing, Supervision, Conceptualization. **William B. Patterson:** Writing – review & editing, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rohit Kohli reports a relationship with Mirum Biopharma that includes: consulting or advisory. Rohit Kohli reports a relationship with Ipsen that includes: consulting or advisory. Michael Goran reports a relationship with YUMI foods that includes: consulting or advisory. Michael Goran reports a relationship with Bobbie Labs that includes: consulting or advisory. Michael Goran receives royalties from Penguin Random House for Sugarproof If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Latino youth with obesity. A secondary aim was to investigate effect modification by patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) genotype and liver disease severity.

Methods: Data was analyzed from 113 Latino youth (age 11–19) with obesity in Southern California. Individual exposure to particulate matter with aerodynamic diameter ≤ 2.5μm ($PM_{2.5}$), ≤ 10μm (PM_{10}), nitrogen dioxide (NO_2), 8-hour maximum ozone (8hrMax-O_3), 24-hr O_3, and redox-weighted oxidative capacity (O_x^{wt}) were estimated using residential address histories and United States Environmental Protection Agency air quality observations. HFF and liver stiffness were measured using magnetic resonance imaging. Linear models were used to determine associations between short-term and long-term exposure to air pollutants with HFF and liver stiffness. Modification by *PNPLA3* and liver disease severity was then examined.

Results: Short-term exposure to 8hrMax-O_3 was positively associated with HFF. Relationships between air pollution exposure and HFF were not impacted by *PNPLA3* genotype or liver disease severity. Long-term exposure to 8hrMax-O_3 and O_x^{wt} were positively associated with liver stiffness. Associations between air pollution exposure and liver stiffness depended on *PNPLA3* genotype, such that individuals with GG genotypes exhibited stronger, more positive relationships between short-term exposure to PM_{10}, 8hrMax-O_3, 24-hr O_3, and O_x^{wt} and liver stiffness than individuals with CC/CG genotypes. In addition, relationships between short-term exposure to NO_2 and liver stiffness were stronger in those with severe liver disease.

Discussion: Air pollution exposure may be a risk factor for liver disease among Latino youth with obesity, particularly in those with other preexisting risks for liver damage.

Keywords

MASLD; Metabolic-dysfunction association steatotic; liver disease; Air pollution; Ozone

1. Introduction

Metabolic-dysfunction associated steatotic liver disease (MASLD) is defined as hepatic steatosis associated with the presence of a cardiometabolic risk factor, including elevated body mass index (BMI), blood pressure, blood glucose, and/or cholesterol (Rinella et al., 2023). MASLD can progress to hepatic inflammation, fibrosis, end-stage liver disease, and need for liver transplant (Vos et al., 2017). Because of the rising rates of pediatric obesity, MASLD is currently the primary cause of child and adolescent liver disease (Vos et al., 2017).

MASLD disproportionately affects Latinos as compared to individuals of other race/ethnicities. This is due, in part, to the high prevalence (50 %) of a C to G polymorphism (rs738409) in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene in Latinos compared to whites and African Americans (Davis et al., 2010; Goran et al., 2010). In whites and African Americans, rs738409 is less than half as common as in Latinos (Davis et al., 2010; Goran et al., 2010). The exact mechanism by which rs738409 predisposes to MASLD remains poorly understood, but animal studies suggest the polymorphism may lead to ineffective hepatic triglyceride breakdown (Wang et al., 2019). Given the prevalence of rs738409 in Latinos, Latino children with obesity are among the most at-risk pediatric

population for MASLD (Vos et al., 2017; Davis et al., 2010; Goran et al., 2010). In fact, studies have shown that the prevalence of MASLD in Latino children is four times that of non-Latino children and that children with obesity have three times the MASLD risk as non-obese children (Schwimmer et al., 2006; Rehm et al., 2014).

Current recommendations for treating pediatric MASLD focus on healthy diet and exercise (Vos et al., 2017). However, real-life implementation of these recommendations can be resource intensive and, as a result, does not always effectively reduce liver fat or fibrosis (Schmidt et al., 2022). Recent emphasis has thus been placed on looking for additional modifiable MASLD risk factors. In this regard, ambient air pollutants, including particulate matter with an aerodynamic diameter ≤ 2.5μm ($PM_{2.5}$) or ≤ 10μm (PM_{10}), nitrogen dioxide (NO_2), ozone (O_3) and redox-weighted oxidative capacity (O_x^{wt}), a value representing the additive effects of both NO_2 and O_3, have emerged as environmental risk factors for cardiometabolic abnormalities in several recent studies (Guo et al., 2022; Kim et al., 2019; Patterson et al., 2023; Xing et al., 2023; Matthiessen et al., 2023; VoPham et al., 2022). For example, a cross-sectional study of 90,000 adults in China found that long-term $PM_{2.5}$, PM_{10}, and NO_2 exposure was associated with increased risk of MASLD (Guo et al., 2022). Two analyses of adolescents from the Meta-AIR cohort of the Children's Healthy Study also found positive associations between short-term exposure to O_3 and O_x^{wt} with liver fat and risk of MASLD, respectively (Kim et al., 2019; Patterson et al., 2023). Finally, an examination of 23,000 Chinese adults found long-term exposure to particulate matter increased risk of advanced liver fibrosis (Xing et al., 2023). To our knowledge, no studies have specifically focused on the relationship between ambient air pollutants and MASLD in Latino children with obesity or examined differential outcomes by *PNPLA3* genotype or liver disease severity.

Our primary aim was to examine associations between short-term (prior-month) and long-term (prior-year) exposure to ambient air pollutants with hepatic fat fraction (HFF) and liver stiffness in Latino youth with obesity. A secondary aim was to investigate whether the associations between ambient air pollution exposure with HFF or liver stiffness differed as a function of *PNPLA3* genotype and liver disease severity. We hypothesized short and long-term ambient air pollutant exposure would be associated with increased HFF and liver stiffness and that these associations would be stronger in those with the GG genotype of *PNPLA3* and more severe liver disease.

2. Methods

2.1. Study design, setting, and participants

Our study involved cross-sectional examination of baseline data from during two prior studies conducted at University of Southern California (USC)/Children's Hospital Los Angeles (CHLA). Similar methodology across studies allowed for data pooling. The first study, Probiotic, was a 16-week randomized control trial (RCT) evaluating the impact of the probiotic VSL#3 on the gut microbiome (www.clinicaltrials.gov:NCT03115385) (Jones et al., 2018). The second study, Healthy Eating through Reduction of Excess Sugar (HEROES), was a 12-week RCT that examined the effect of a dietician-led sugar reduction intervention on liver fat and fibrosis (clinical trial registered at www.clinicaltrials.gov:NCT02948647)

(Schmidt et al., 2022). Probiotic participants were recruited from the Los Angeles community between March and November 2015 (Jones et al., 2018). HEROES participants were recruited from USC and CHLA pediatric gastroenterology clinics as well as the community between October 2016 and March 2020 (Schmidt et al., 2022).

At both the Probiotic and HEROES baseline visits, which were conducted prior to any intervention, anthropometries, PNPLA3 genotypes, dietary recalls, liver fat fraction on MRI, and liver stiffness on magnetic resonance elastography (MRE) were collected (Schmidt et al., 2022; Jones et al., 2018). At the Probiotic baseline visit, participants provided their residential address for shipment of VSL#3 (Jones et al., 2018). At the HEROES baseline visit, participants had serum alanine transaminase and lipid panels drawn and were asked to provide prior year residential address history (Schmidt et al., 2022). Informed consent was obtained for all participants at least 18 years old; for participants under 18, informed consent was obtained from parents/guardians, and assent was obtained from participants (Schmidt et al., 2022; Jones et al., 2018). This study was conducted in accordance with Declarations of Helsinki and Istanbul and was approved by the Institutional Review Boards of USC and CHLA (Schmidt et al., 2022; Jones et al., 2018).

Inclusion criteria specific to this analysis were age and sex-specific body mass index (BMI)>95th percentile and Latino self-identification. Exclusion criteria were missing MRI or MRE at baseline visit, missing dietary recall data, or missing address history. A complete list of inclusion and exclusion criteria from Probiotic and HEROES have been previously published (Schmidt et al., 2022; Jones et al., 2018). Thus, 126 individuals involved in HEROES (n=105) and Probiotic (n=21) were evaluated for inclusion. Eight individuals (n=8 from HEROES) were excluded due to lack of MRI and MRE. Four individuals (n=1 from HEROES and n=3 from Probiotic) were excluded for missing or outlier baseline dietary data to ensure results were not biased by unusual eating patterns. Final sample size was 114 (n=18 from Probiotic and n=96 from HEROES).

2.2. Anthropometrics and liver fat and liver stiffness measures

Height, weight, and BMI were measured in standard fashion (Schmidt et al., 2022; Jones et al., 2018). The IDEAL method was used to calculate HFF on MRI. Specifically, eight to nine contiguous abdominal MRI axial cuts starting at the top of the liver and descending to the L5 vertebrate were obtained, 6-echo T2*IDEAL reconstruction with fat spectrum modeling was completed, and the liver was segmented from volume data using Slice-O-Matic (Tomovision) (Schmidt et al., 2022; Hu and Nayak, 2008; Hu et al., 2010; Hamilton et al., 2011; Szczepaniak et al., 2005). We considered HFF ≥5.5 % diagnostic for MASLD (Schmidt et al., 2022). MRE, which used shear wave propagation through the abdomen, was used to assess liver stiffness (Schmidt et al., 2022; Jones et al., 2018; Sawh et al., 2020). We considered liver stiffness >2.74 kilopascals (kPa) diagnostic for clinically significant fibrosis (Sawh et al., 2020). MRI and MRE were both performed on a 3-Tesla GE scanner (Schmidt et al., 2022; Jones et al., 2018). Liver disease severity was then divided into three groupings - healthy liver (HFF<5.5 %), MASLD without fibrosis (HFF ≥5.5 % and liver stiffness of <2.74 kPa, and MASLD with fibrosis (HFF ≥5.5 % and liver stiffness >2.74 kPa).

2.3. PNPLA3 genotyping

Fasting serum samples were obtained at the baseline visit (Schmidt et al., 2022; Jones et al., 2018). Genomic DNA was then isolated from these samples and amplified using polymerase chain reaction (PCR) (Schmidt et al., 2022; Jones et al., 2018). The products were incubated with *Nla*III restriction endonuclease in CutSmart® buffer at 37°C for 4 hours (Schmidt et al., 2022; Jones et al., 2018; Dutta, 2012). The digested pieces were then run with ethidium bromide on agarose gel. G functioned as a *Nla*III restriction site at which 200-base-pair and 133-base-pair fragments were cleaved. Individuals with CC genotypes of *PNLPA3* were identified by the presence of uncut 333-base-pair products, with GG genotypes by both 200-base pair and 133-base pair fragments, and with CG genotypes by 333-base-pair products plus 200-base-pair and 133-base-pair fragments (Schmidt et al., 2022; Jones et al., 2018; Dutta, 2012). As there were only a small number of participants with either CC or CG variants and prior studies have shown liver fat to be twice as high in children with GG as opposed to CC or CG variants, we combined CC and CG variants in our analysis as CC/CG (Rehm et al., 2014).

2.4. Ambient air pollutant exposure estimates

Addresses were input in the Texas A&M geocoder, which assigned specific latitude and longitude coordinates for each unit or home (Goldberg, 2016). Notably, as participants in Probiotic provided their addresses only for shipping purposes, we had to assume that they had resided at these addresses for the entire year prior to the study. Daily averages for ambient air pollutants ($PM_{2.5}$, PM_{10}, NO_2, O_3) at each coordinate were calculated using hourly air quality data from the United States Environmental Protection Agency's Air Quality System (www.epa.gov/ttn/airs/airsaqs) and California Air Resources Board AQMIS System. Inverse distance-squared weighting was utilized to determine air quality data from ≤4 monitoring stations within a 50 km radius of each residence (Goldberg, 2016; Wong et al., 2004). For O_3 only, 8-hour daily max (8hrMax-O_3), and 24-hour daily max (24hr-O_3) were calculated. Based on the daily averages, mean monthly regional air pollutant data for prior 1-month and prior 1-year to the baseline visit at each coordinate were obtained. Seventy-five percent completeness criteria was required for daily averages from hourly data and for monthly averages from daily data. For participants in HEROES who moved addresses within the 1-month or 1-year prior to the baseline visit, pollutant exposures were weighted by time spent at each residential address. Prior 1-month pollutant exposure was considered short-term exposure, and prior 1-year pollutant exposure was considered long-term exposure. O_x^{wt}, a variable assessing the combined oxidative potential of NO_2 and O_3, was calculated according to the following equation: $[(1.07 * NO_2) + (2.075 * O_3]/3.145]$ (Williams et al., 2014; Ravina et al., 2022). Short-term term O_x^{wt} was calculated using the prior 1-month NO_2 value and the prior 1-month 24hr-O_3 value. Long-term term O_x^{wt} was calculated using the prior 1-year NO_2 and the prior 1-year 24hr-O_3 value (Williams et al., 2014; Ravina et al., 2022).

2.5. Statistical analysis

We used analysis of variance (ANOVA) to evaluate mean differences in continuous variables and Chi-square tests to evaluate differences in categorical variables when separating by

group (i.e., *PNPLA3* genotype and liver disease severity). Pearson correlation coefficients were used to examine the correlation among pollutants and exposure windows.

The current study included ten co-habitant sibling pairs. To ensure results were not impacted by these sibling pairs, we ran linear mixed effect models that included random effects for siblings. The variance estimates for sibling pairs were small in all models. Additionally, the estimates and p values were essentially unchanged between linear mixed effect models and multivariable linear models when examining associations between ambient air pollution exposures with liver outcomes. Thus, we opted to use multivariable linear models for all analyses with HFF (in %) and liver stiffness (kPa). All models were adjusted for sex (male/female), BMI (kg/m^2), study (HEROES/Probiotic), and season of visit (warm/cool). Season was defined as warm (April - September) and cool (January, February, March, October, November, December). Sex and BMI were included as covariates as both have been associated with increased liver fat and fibrosis (Halaoui et al., 2020; Gopalakrishna et al., 2023).

To assess the impact of *PNPLA3* genotype and liver disease severity on the relationship between air pollutants and HFF and liver stiffness, *PNPLA3* genotype (GG vs. CC/CG) and liver disease severity (healthy liver, MASLD without fibrosis, and MASLD with fibrosis) were added as effect modifiers to separate models using interactions terms (e.g., exposure * *PNPLA3* genotype). Because we hypothesized that the association between air pollution exposure and liver stiffness would be strongest in the MASLD with fibrosis group, we used a contrast that specifically tested the difference between MASLD with fibrosis and the average of MASLD without fibrosis and healthy liver. To ensure orthogonal contrasts, the second contrast we used examined the difference between healthy liver and MASLD without fibrosis. Both the *PNPLA3* genotype and liver disease severity models incorporated sex, BMI, study, and season of visit as covariates. Finally, to contextualize our findings when interaction terms were statistically significant, we utilized our multivariable linear models to calculate the predicted liver stiffness for those at the 75th versus 25th percentile for a given air pollutant exposure. To accomplish this, we set continuous covariates at their mean values and categorical variables were set to their most common level (mode). We then describe the difference in predicted liver stiffness between the 75th and 25th percentiles for a given exposure.

We performed sensitivity analyses to determine the influence of removing high leverage observations by examining residual vs leverage plots and Cook’s distance. Based on this, one observation was found to be highly influential and was removed from all analyses. This observation had a HFF of 14.2 % and liver stiffness of 4.4 kPa compared to the mean of 14.6 % and 2.5 kPa, respectively. Model diagnostics were then performed to ensure assumptions of linear modeling were satisfied. We accounted for violations of heteroscedasticity with robust standard errors. Residual normality of all models was assessed and determined to be satisfactory using Q-Q plots. Multicollinearity in each model was calculated and found to be low. Statistical analyses were conducted using R version 4.2.2 (R Development Core Team, 2011). Statistical significance was set at $p<0.05$ for all analyses.

3. Results

Baseline characteristics of the 113 participants are shown in Table 1. Mean participant age was 14.8 years (range: 11.3–19.0), ALT was 47 IU/L (range: 7–433, upper limit of normal for females is 26 IU/L and for males is 22 IU/L), HFF was 12.2 % (range: 2.3–37.0), and liver stiffness was 2.4kPa (range: 1.8–3.7) (Vos et al., 2017). HFF and liver stiffness values were significantly different across *PNPLA3* genotypes and liver disease severity categories (with individuals with the GG genotype of *PNPLA3* or MASLD and fibrosis having significantly greater HFF and liver stiffness values). As expected, we observed moderate to strong correlations among and between short-term and long-term ambient air pollution exposure (Fig. 1).

3.1. Air pollution exposure was associated with liver fat, but was not impacted by PNPLA3 genotype or liver disease severity

Short-term exposure to 8hrMax-O_3 (β=0.22, p=0.04) was positively associated with HFF (Supplementary Table 1, Fig. 2). Short-term exposure to PM_{10} (β=0.31, p=0.06), NO_2 (β=0.37, p=0.06), O_x (β=0.57, p=0.07) and long-term exposure to O_x (β=1.51, p=0.09) were also positively associated with HFF, however, these results did not reach statistical significance. There were no statistically significant associations between HFF and short-term exposure to $PM_{2.5}$ (β=0.25, p=0.39) or 24hr-O_3 (β=0.12, p=0.48) or long-term exposure to $PM_{2.5}$ (β=−0.75, p=0.54), PM_{10} (β=0.25, p=0.41), NO_2 (β=0.36, p=0.28), 8hrMax-O_3 (β=0.45, p=0.19), or 24hr-O_3 (β=0.54, p=0.37) (Supplementary Table 1). These findings were not modified by *PNPLA3* genotype or liver disease severity (Supplementary Table 2 and 3).

3.2. Air pollution exposure was associated with liver stiffness

Long-term exposure to 8hrMax-O_3 (β=0.02, p=0.03) and O_x^{wt} (β=0.08, p<0.01) were positively associated with liver stiffness (Supplementary Table 4, Fig. 3). Long-term exposure to 24hr-O_3 was also positively associated with liver stiffness (β=0.03, p=0.06), but did not reach statistical significance. No other exposures were associated with liver stiffness (Supplementary Table 4).

3.3. Associations between air pollution exposure and liver stiffness depends on PNPLA3 genotype

Individuals with the GG genotype exhibited a stronger and more positive relationship between liver stiffness and long-term exposure to PM_{10} (β-interaction=0.04, p-interaction=0.04), 8hrMax-O_3 (β-interaction=0.05, p-interaction=0.02), and 24hrMax-O_3 (β-interaction=0.08, p-interaction=0.01), and O_x^{wt} (β-interaction=0.11, p-interaction=0.03) than individuals with CC and CG genotypes (Fig. 4, Supplementary Table 5). For all models demonstrating a significant interaction, simple slope analysis for the GG group confidence intervals (CIs) that did not contain zero (95 % CIs for long-term exposure to PM_{10}, 8hrMax-O_3, 24hrMax-O_3 and O_x^{wt} are [0.001, 0.05], [0.01, 0.05], [0.01, 0.08], and [0.06, 0.16], respectively). These differences suggest varying degrees of impact from different types of pollutant exposure on the GG group, indicating that individuals within the GG group have a significant positive association between long-term exposure to PM_{10}, 8hrMax-O_3,

24hrMax-O_3 and O_x^{wt} with liver stiffness. To contextualize these findings, adolescents with the GG genotype of *PNPLA3* were predicted to have a 0.17 kPa higher liver stiffness at the 75th percentile of long-term PM_{10} exposure compared to the 25th percentile (27.63 μg/m^3 vs 20.72 μg/m^3). Similarly, liver stiffness was predicted to be 0.10 kPa higher at the 75th percentile of long-term O_3-8hrMax exposure compared to the 25th percentile (43.62 ppb vs 40.08 ppb), and 0.15 kPa higher at the 75th percentile of long-term O_x^{wt} exposure compared to the 25th percentile (23.47 vs 22.02). *PNPLA3* genotype did not significantly modify the relationships between any other short-term or long-term ambient air pollution exposure with liver stiffness (Supplementary Table 5).

3.4. Associations between air pollution exposure and liver stiffness depends on liver disease severity

The positive relationships between short-term exposure to NO_2 was stronger in those with MASLD and fibrosis compared to those with healthy livers and MASLD without fibrosis (Fig. 5, Supplementary Table 6). Specifically, compared to the average of the healthy liver group and MASLD without fibrosis group, we observed that higher NO_2 (β-interaction=0.03, p-interaction=.04) exposure was associated with greater liver stiffness among those that already had MASLD with fibrosis. For all models with significant interactions, simple slope analysis for the MASLD with fibrosis group had CIs that did not contain zero (95 % CIs for short-term exposure to NO_2 are [0.01, 0.06]). These findings indicate significant positive associations between short-term exposure to NO_2 and liver stiffness for those with MASLD with fibrosis. However, liver disease severity did not significantly moderate the relationships between short-term exposure to $PM_{2.5}$, PM_{10}, 8hrMax-O_3, 24hr-O_3, O_x^{wt} or long-term exposure to $PM_{2.5}$, PM_{10}, NO_2, 8hrMax-O_3, 24hr-O_3, or O_x^{wt} and liver stiffness (Supplementary Table 6). There were also no significant differences in the associations between short-term or long-term exposure to air pollutants with liver stiffness in individuals with healthy livers as compared to those with MASLD without fibrosis.

4. Discussion

In the present study, we examined associations between short-term and long-term exposure to ambient air pollutants with HFF and liver stiffness in Latino youth with obesity and evaluated whether these associations were moderated by *PNPLA3* genotype or liver disease severity. Significant associations were found between air pollution exposure and HFF. Additionally, significant associations were seen between air pollution exposure and liver stiffness, with notable differences in the strength of these relationships based on *PNPLA3* genotype and liver disease severity. Our findings suggest that air pollution exposure is a risk factor for HFF and for liver stiffness, particularly among those with preexisting risks for liver damage. To our knowledge, this is the first study to show that air pollution exposure is associated with liver stiffness in high-risk Latino youth with *PNPLA3* risk alleles and greater liver disease severity. Thus, our collective results further add to the body of evidence that ambient air pollution is an environmental risk factor for HFF and fibrosis. Given that liver fat can progress to fibrosis, which significantly impairs liver function and can lead to

cirrhosis, these findings underscore the importance of addressing environmental factors in the prevention and management of liver disease in vulnerable populations.

We found that ambient air pollution exposure was significantly positively associated with HFF, specifically short-term 8 hrMax-O_3, but was not impacted by *PNPLA3* genotype or liver disease severity. This finding supports a prior study that examined young adults (17–22 years) from Los Angeles County and noted significant positive associations between short-term 8hr-O_3 exposure and HFF (Kim et al., 2019; Patterson et al., 2023). Notably, our participants had a similar mean short-term 8hr-O_3 exposure those in the prior study. Our lack of statistically significant associations between $PM_{2.5}$, PM_{10}, and NO_2 and HFF, however, differs from a prior study that found significant positive relationships between prior three-year $PM_{2.5}$, PM_{10}, and NO_2 exposure and hepatic steatosis in adults living in China (Guo et al., 2022). It is possible these results differ as our participants had lower ambient air pollutant exposures than those in China - our participants had long-term mean $PM_{2.5}$, PM_{10}, and NO_2 exposure values of 12.1 μg/m^3, 24.9 μg/m^3, and 17.1 ppb compared to the Chinese participants whose respective exposures were 38.1 μg/m^3, 65.6 μg/m^3, 27.8ppb (Guo et al., 2022).

We also found that exposure to ambient air pollutants, specifically long-term exposure to 8hrMax-O_3 and O_x^{wt}, was associated with greater liver stiffness. Additionally, long-term exposure to 24hr-O_3 was associated with greater liver stiffness but did not reach statistical significance. To date, only one prior human study has examined the associations between ambient air pollution exposure with liver stiffness. This prior study was conducted among adults in China and found that three years of exposure to $PM_{2.5}$ was positively associated with advanced liver fibrosis (Xing et al., 2023). It is difficult to compare our findings to this Chinese study; however, given higher levels of air pollution in China compared to Los Angeles County. In addition to this prior human study, however, there was also an *in vitro* study that exposed Huh-7 human hepatocellular carcinoma cells (HSCs) to O_3 (200 ppb) and found that heightened O_3 exposure led to increased oxidative cellular stress, increased HSC activity, and disruption of normal hepatocyte metabolism, suggesting a physiologic basis for the association between O_3 exposure and fibrosis (Peng et al., 2023).

The current study also investigated the impact of ambient air pollution exposure on liver health in Latino youth with obesity, with a particular focus on the role of *PNPLA3* genotype. We found that ambient air pollution exposure was associated with liver stiffness among children with two copies of *PNPLA3* alleles that increase risk of MASLD. Specifically, those with the GG genotype of *PNPLA3* exhibited stronger, more positive relationships between long-term exposure to PM_{10}, 8hrMax-O_3, 24hrMax-O_3, and O_x^{wt} with liver stiffness than individuals with CC/CG genotypes. It is also important to note that the effect sizes we observed were clinically relevant. For example, at average levels of long-term PM_{10} exposure, liver stiffness among youth with the GG genotype was 0.99 kPa higher than youth in the CC/CG group. Given that 2.74 kPa on MRE is diagnostic of clinically relevant liver fibrosis, a 0.99 kPa increase in the GG compared to the CC/CG group is substantial. These findings suggest limiting long-term exposure to PM_{10}, 8hrMax-O_3, 24hrMax-O_3, and O_x^{wt} in Latino youth with obesity may be an effective strategy to reduce liver fibrosis.

Finally, our study provides compelling evidence that ambient air pollution exposure is significantly associated with increased liver stiffness among children with greater liver disease severity. Notably, among those with the MASLD and fibrosis, there were stronger and more positive relationships between short-term exposure to NO_2 with liver stiffness than in those with healthy livers and those with MASLD without fibrosis. The effect sizes we observed were also clinically relevant. For example, at average levels of short-term NO_2 exposure, liver stiffness amongst youth with MALSD and fibrosis was 0.51 kPa higher than youth in the average of the healthy liver and MASLD without fibrosis group. These findings suggest that fibrotic livers are more sensitive to damage from oxidative stress secondary to air pollution compared to non-fibrotic livers. Mechanistically, this fits with the theory that inflammation in liver cells activates HSCs, which then deposit collagen and disrupt the integrity of the liver (Zhang et al., 2016). Once activated, HSCs cannot be turned off. Thus, individuals with baseline fibrosis may have more activated HSCs, particularly in the presence of air pollution exposure.

Our study has several limitations. First, as this was a post-hoc secondary analysis, we could not account for participants' air pollutant exposure beyond one year prior to the study due to incomplete historical address data. While life-long air pollutant exposure may have impacted our findings, numerous studies have shown significant results when considering only prior-month and prior-year exposure. Second, we used magnetic resonance imaging (MRI) rather than the gold standard of liver biopsy to determine hepatic fat fraction (HFF) and liver stiffness. Although liver biopsy is associated with risk, its absence may have led to the underdiagnosis of MASLD and fibrosis, potentially biasing our results towards the null. Third, non-differential exposure misclassification is possible, as we estimated ambient air pollution based solely on residential address history rather than direct measurement. Finally, due to our relatively small sample size, one individual with higher liver fibrosis was found to be highly influential. Although this individual's values were within the physiological range, we chose to remove this observation from our analysis. This decision limits our ability to evaluate how ambient air pollution affects those with the most significant levels of liver fibrosis. Future studies should address this by including larger sample sizes and focusing specifically on individuals with advanced liver fibrosis to better understand the impact of air pollution on this group.

Despite potential limitations, our study has multiple strengths. It is the only study to examine the associations between ambient air pollutants with HFF and liver stiffness in a population made up entirely of Latino youth with obesity. Given that Latino youth - particularly those with obesity - have significantly higher rates of MASLD than their peers, it is essential to learn about modifiable risk factors that can help reduce the burden of MASLD and liver fibrosis in this population. While many studies have examined the relationship between ambient air pollutants and liver fat, our study is one of the very few to investigate the link between air pollutants and liver fibrosis in a human population. This is significant because it is liver fibrosis, not liver fat, that leads to the severe long-term consequences of MASLD, making it a critical area for future research. Additionally, our study is the first to provide evidence that ambient air pollutants are more strongly associated with liver stiffness in individuals with the GG genotype of *PNPLA3* and those with more severe liver disease.

5. Conclusions

Our findings suggest that strategies aimed at reducing exposure to ambient air pollutants may help limit the burden of MASLD, particularly in high-risk Latino youth with obesity, those with the GG *PNPLA3* genotype, and those with more advanced liver disease. Further investigation into Latino youth in regions beyond Los Angeles, as well as Latino adults, and the long-term cumulative exposure among Latino individuals, will be crucial for enhancing our understanding of the relationship between ambient air pollutants and MASLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Author responsibilities — RBS, MIG, TLA: designed research and wrote paper; RBS: conducted research; RBS, CM: analysis; RBS, CM, TLA, RK, WP: interpretation; FL: determined air pollution exposure estimates; HA: *PNPLA3* genotyping; All authors: read and approved final manuscript.

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Data availability

Data will be made available on request.

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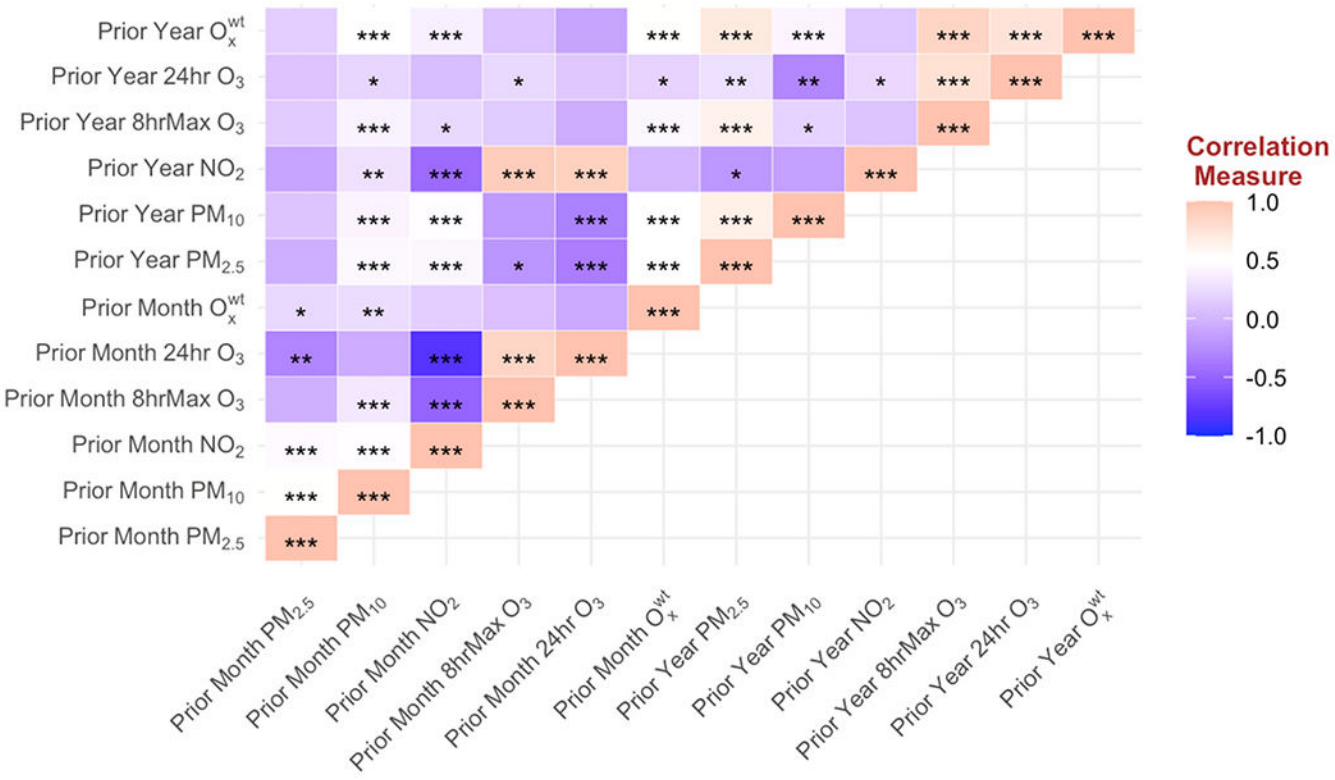

Fig. 1.

Correlation Among Short-Term and Long-Term Ambient Air Pollutants. Heatmap shows the correlation among the short-term and long-term ambient air pollutants. Pearson correlation coefficients are colored according to the strength of the correlation coefficient, and statistical significance is reported as *p<0.05, **p<0.01, and *** p<0.001.

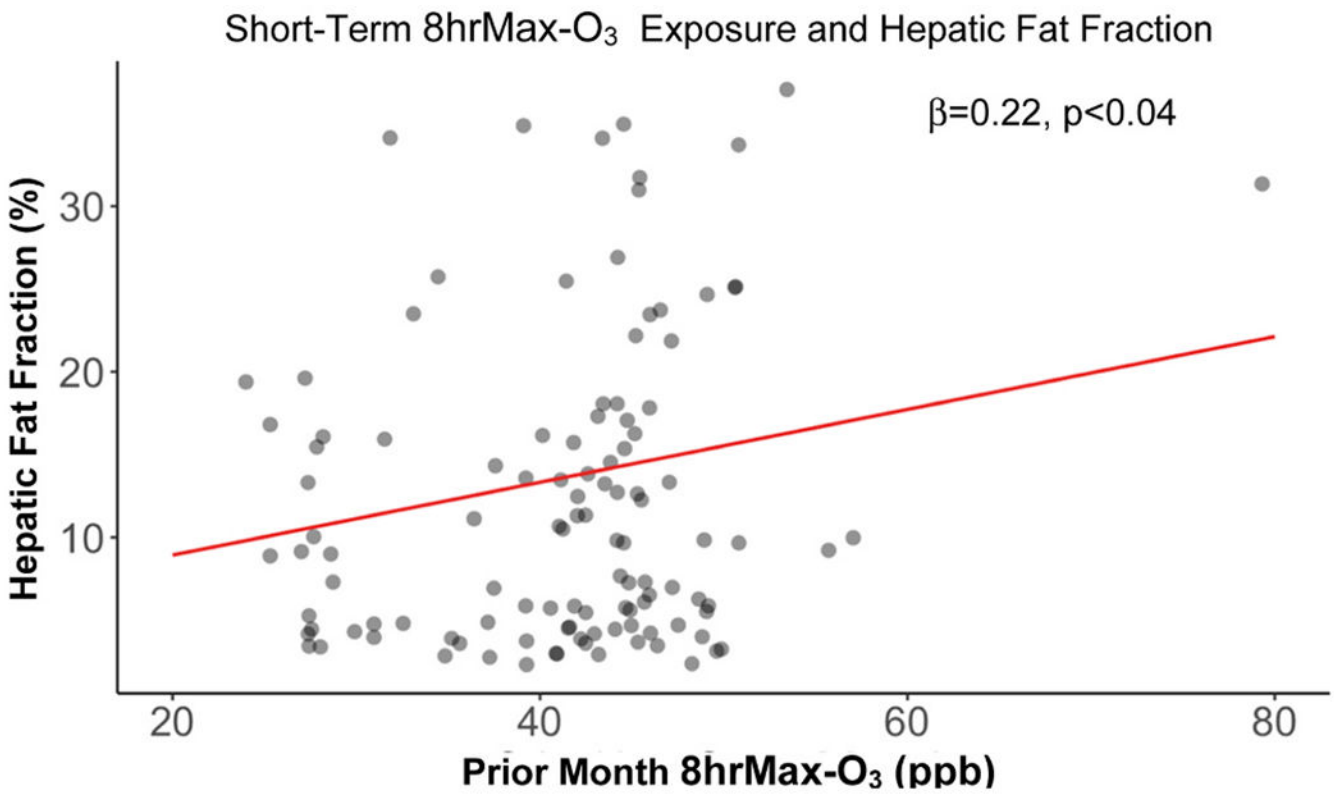

Fig. 2. Short Exposure to Ambient Air Pollutants were Associated with Hepatic Fat Fraction (HFF).

Multivariable linear models with robust standard errors were used to assess the associations between ambient air pollution exposure with hepatic fat fraction (HFF) after adjusting for sex (male/female), body mass index (BMI), study (HEROES/Probiotic), and season of visit (warm/cool). Plots show the raw (unadjusted) data with the best fit line derived from the multivariable linear model.

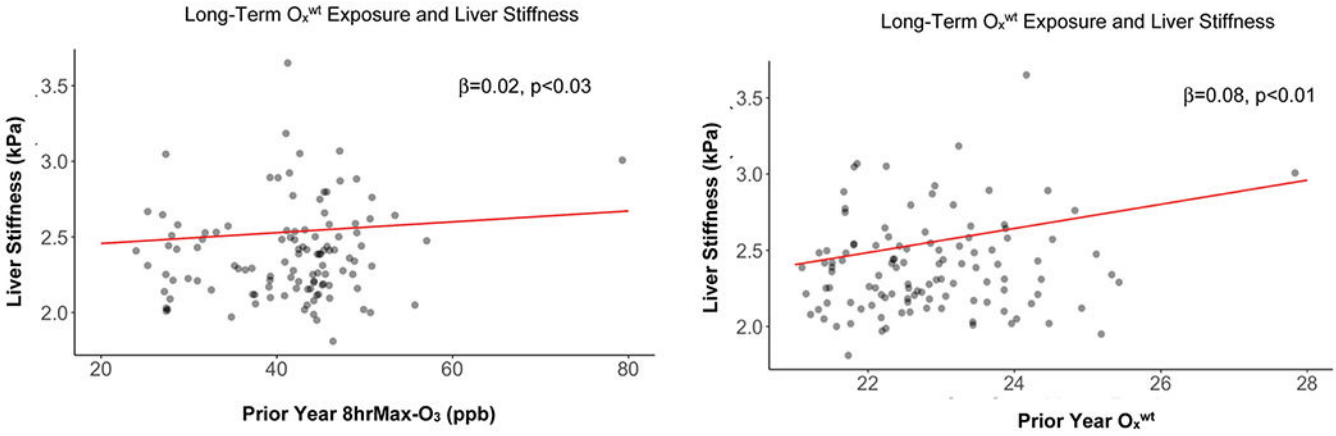

Fig. 3.

Long-Term Exposure to Ambient Air Pollutants were Associated with Greater Liver Stiffness. Multivariable linear models with robust standard errors were used to assess the associations between ambient air pollution exposure with liver stiffness after adjusting for sex (male/female), body mass index (BMI), study (HEROES/Probiotic), and season of visit (warm/cool). Plots show the raw (unadjusted) data with the best fit line derived from the multivariable linear model.

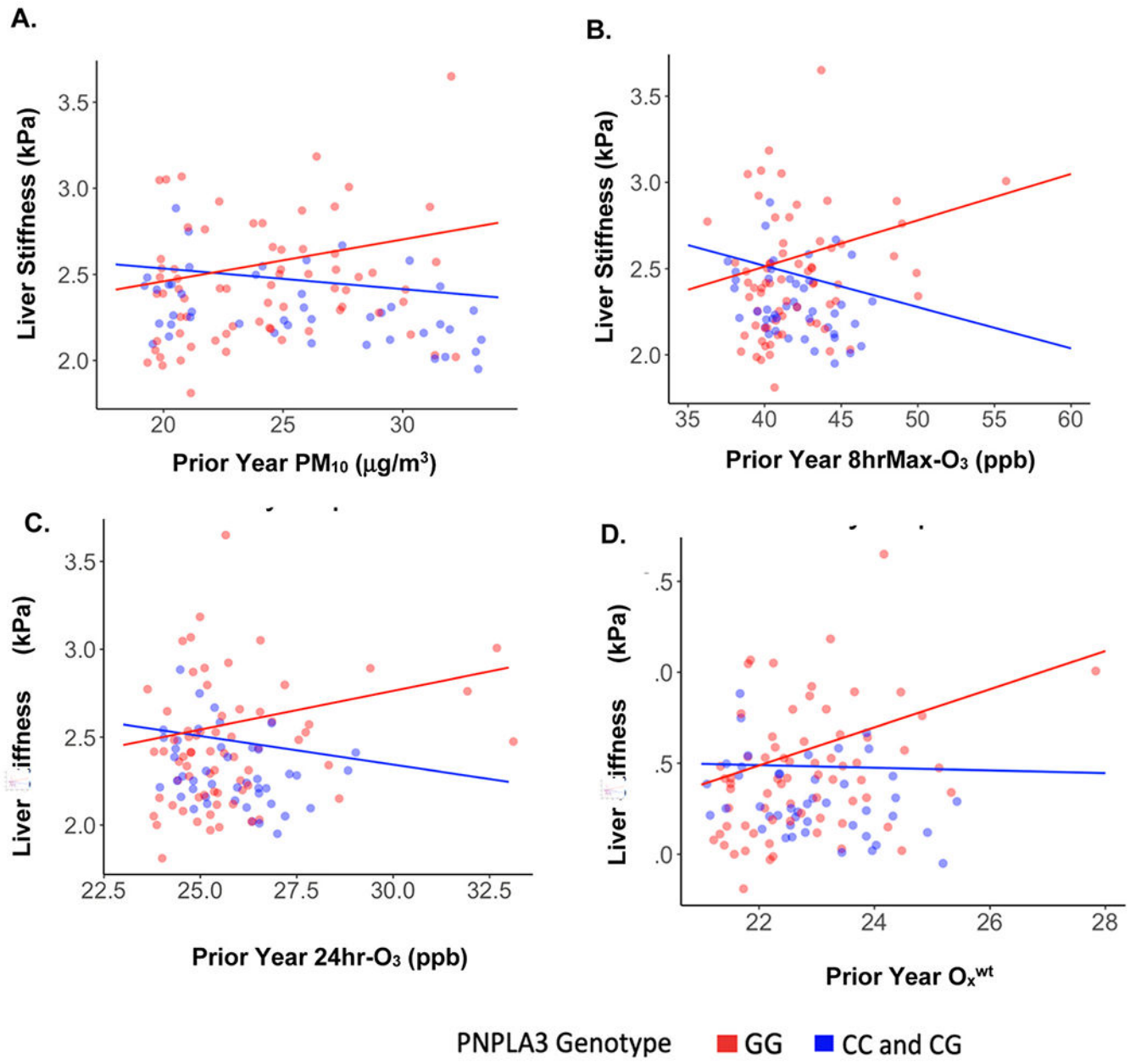

Fig. 4.

Long-Term Ambient Air Pollution Exposure is Associated with Greater Liver Stiffness Among those with Two PNPLA3 Risk Alleles. Multivariable linear models with robust standard errors were used to assess the associations between ambient air pollution exposure with liver stiffness after adjusting for sex, body mass index (BMI), season, and study. Interaction terms between exposure and *PNPLA3* genotype were additionally included in each model. Plots show the raw (unadjusted) data with the best fit line derived from the multivariable linear model. Figures show that the associations between **A)** prior year PM_{10} (β interaction=0.04, p-interaction=0.04), **B)** prior year 8hrMax-O_3 (β interaction=0.05, p-interaction=0.02), **C)** prior year 24hr-O_3 ((β interaction=0.08, p-interaction=0.01), and **D)** prior year O_x^{wt} exposure (β interaction=0.11, p-interaction=0.03) with liver stiffness

was stronger among those with GG genotypes of *PNPLA3* (red) than those with CC/CG genotypes of *PNPLA3* (blue).

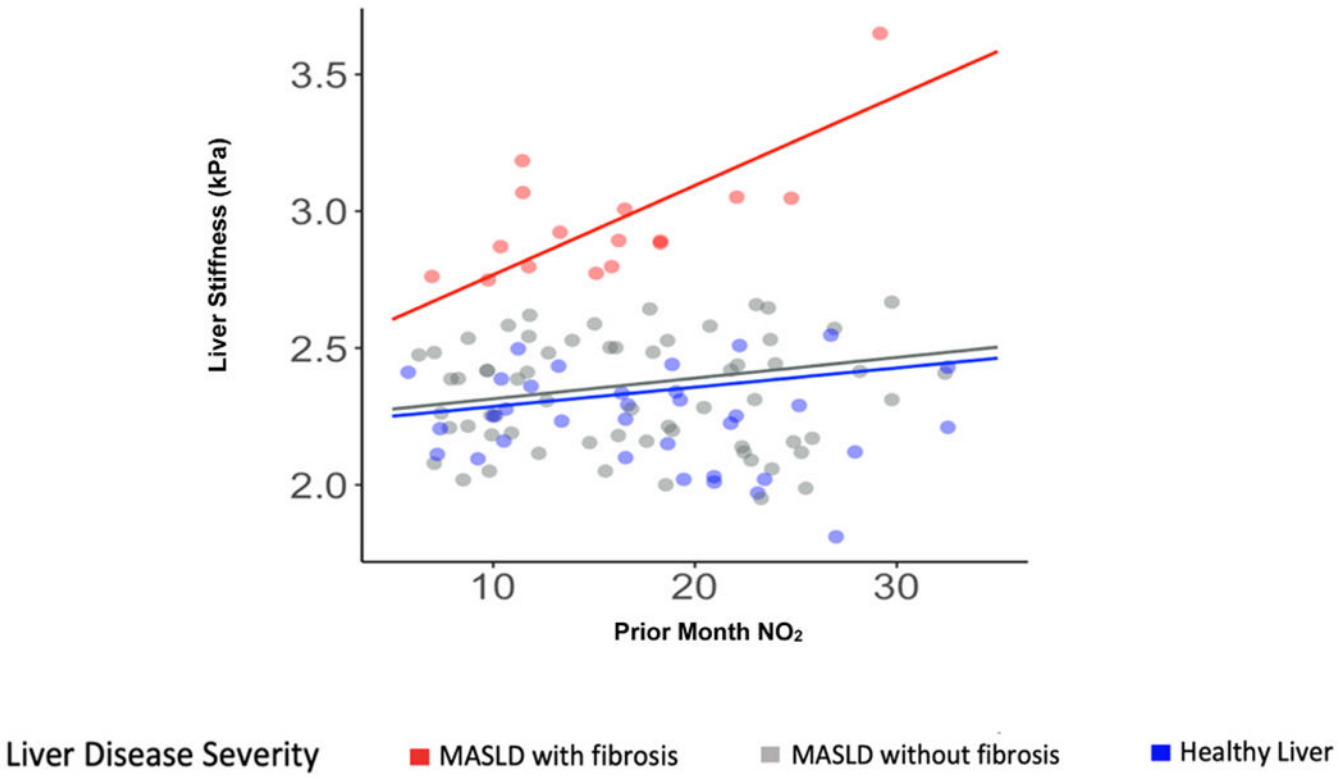

Fig. 5.

Short-Term Ambient Air Pollution Exposure is Associated with Greater Liver Stiffness Among those with Liver Disease. Multivariable linear models with robust standard errors were used to assess the associations between ambient air pollution exposure with hepatic fat fraction (HFF) after adjusting for sex, body mass index (BMI), season, and study. Interaction terms between exposure and liver disease severity were additionally included in each model. Plots show the raw (unadjusted) data with the best fit line derived from the multivariable linear model. Figure shows that the MASLD with fibrosis group (red) demonstrated stronger positive associations between prior month NO_2 exposure (β interaction=0.03, p-interaction=0.04) and liver stiffness when compared to the average of the MASLD without fibrosis group (grey) and healthy liver group (blue).

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Table 1

Baseline Participant Characteristics of Study Participants (n=113), Grouped by PNPLA3 Genotype and Liver disease Severity[a].

	PNPLA3 Genotype				Liver Health			
	All Genotypes N=113	**GG N=69**	**CC and GC N=44**	**P-value**	**Healthy Liver N=34**	**MASLD without Fibrosis N=63**	**MASLD with Fibrosis N=16**	**P Value**
General Characteristics								
Age (years)	14.8±2.1	14.6±2.2	15.0±2.1	0.36	14.3±1.8	14.5±2.	14.7±2.2	0.31
Female Sex (n, % Female)	62 (55 %)	34 (49 %)	28 (64 %)	0.19	27 (79 %)	32 (51 %)	3 (19 %)	<0.01
Calories (kcal/day)	1541±523	1511±494	1587±586	0.45	1583±625	1537±463	1463±538	0.75
BMI (kg/m^2)	34.4±6.3	34.1±6.5	34.8±6.0	0.57	32.8±5.6	34.9±6.3	35.4±7.6	0.21
Markers of Liver Health								
ALT (IU/L)[b]	47±65	55.1±75.9	29.5±28.7	0.07	18.3±14.7	44.9±60.2	95.7±97.6	<0.01
AST (IU/L)[b]	30±27	33.0±30.6	23.5±15.2	0.10	17.9±5.8	29.7±26.4	48.5±36.9	<0.01
HFF (%)	12.2±9.1	14.6±9.6	8.3±6.8	<0.01	3.8±0.76	16.0±8.8	14.8±8.5	<0.01
GG *PNPLA3* (n, % GG)	69 (61 %)	69 (100 %)	0 (0 %)	<0.01	13 (38 %)	42 (67 %)	14 (88 %)	<0.01
Liver Stiffness (kPa)	2.4±0.3	2.5±0.4	2.3±0.2	0.02	2.2±0.2	2.3±0.2	3.0±0.2	<0.01
Short-Term Ambient Air Pollution Exposures								
Prior-Month $PM_{2.5}$ ($\mu g/m^3$)	12.2±2.9	12.8±3.1	11.3±2.4	0.01	11.6±2.3	12.3±3.2	14.7±2.2	0.31
Prior-Month PM_{10} ($\mu g/m^3$)	24.2±5.7	24.6±6.5	24.1±5.9	0.92	24.6±5.5	23.5±5.4	25.9±7.1	0.27
Prior-Month NO_2 (ppb)	17.0±6.8	17.1±6.7	16.9±7.1	0.89	17.9±7.1	16.8±6.9	15.7±5.9	0.55
Prior-Month 8hrMax-O_3 (ppb)	41.2±8.3	41.8±8.6	40.3±7.8	0.34	39.0±7.2	41.4±7.9	45.3±10.5	0.04
Prior-Month 24hr-O_3 (ppb)	25.3±6.9	25.4±6.9	25.0±6.9	0.73	23.6±6.1	25.6±7.2	27.6±6.4	0.13
Prior-Month O_x^{wt}	22.5±3.0	22.6±3.0	22.2±2.9	0.52	21.7±2.6	22.6±3.0	23.5±3.2	0.09
Long-Term Ambient Air Pollution Exposures								
Prior-Year $PM_{2.5}$, $\mu g/m^3$	12.1±0.9	12.0±1.0	12.3±0.8	0.11	12.3±0.8	12.0±0.8	12.1±1.5	0.30
Prior Year PM_{10}, $\mu g/m^3$	24.7±4.2	24.2±3.7	25.6±4.8	0.09	25.9±4.6	23.5±3.3	24.1±3.9	0.15
Prior-Year NO_2, ppb	17.1±2.3	17.0±2.2	17.4±2.5	0.42	17.5±2.3	17.0±2.2	17.0±2.7	0.57
Prior-Year 8hrMax-O_3, ppb	42.1±3.0	42.0±3.3	42.0±2.5	0.99	42.2±2.7	41.7±2.6	42.6±4.8	0.48
Prior-Year 24hr-O_3, ppb	25.8±1.7	25.8±1.9	25.8±1.3	0.94	25.9±1.4	25.6±1.4	26.4±2.7	0.25
Prior-Year O_x^{wt}	22.8±1.1	22.8±1.2	22.9±1.1	0.53	23.3±1.1	22.7±1.0	23.2±1.6	0.17

[a] Values are means ± SDs and n (%) for categorical variables. Analysis of variance (ANOVA) and chi square were respectively used to assess for differences in continuous and categorical variables based on grouping. ALT, alanine transaminase; IU/L, international units per liter; AST, aspartate aminotransferase; HFF, hepatic fat fraction; *PNPLA3*, patatin-like phospholipase 3; kPa, kilopascals; $PM_{2.5}$, particulate matter with aerodynamics ≤2.5μm; μg/m3, micrograms per meter cubed; PM_{10}, particulate matter with aerodynamics ≤10μm NO_2, nitrogen dioxide; 8hr-O_3, 8-hour daily max ozone; 24hr-O_3, 24-hour daily max ozone; ppb, parts per billion; O_x^{wt}, redox-weighted oxidative capacity.

[b] ALT and AST were only collected in 95 individuals from HEROES