



Ambient Air Pollution, Adiposity, and Hepatic Steatosis: The Framingham Heart Study

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AMBIENT AIR POLLUTION, ADIPOSITY, AND HEPATIC STEATOSIS:
THE FRAMINGHAM HEART STUDY

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The Harvard T.H. Chan School of Public Health
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Abstract

Air pollution-induced systemic inflammation and oxidative stress are among potential underlying mechanisms that mediate the associations between air pollution and metabolic risk factors such as obesity and insulin resistance. Furthermore, both obesity and insulin resistance are two important risk factors for non-alcoholic liver disease, one of the most common liver diseases in the United States. Although in controlled animal studies, exposure to elevated fine particulate matter (PM_{2.5}) has been associated with increased abdominal adiposity and liver fat accumulation, few epidemiologic studies examined these associations among adults.

In this work, we first examined the associations of short-term exposure to air pollution, measured at the central and local air pollution monitors, with biomarkers of oxidative stress, including myeloperoxidase and 8-epi-prostaglandin F_{2α} (8-epi-PGF_{2α}) among participants from the community-based Framingham Heart Study Offspring cohort. We used linear regression models and linear mixed-effects models with random intercepts, and adjusted for demographic variables, individual- and area-level measures of socioeconomic position, clinical and lifestyle factors, weather, and temporal trend. We found positive associations of black carbon, a correlate of local traffic pollution, with myeloperoxidase, and of PM_{2.5} and sulfate with 8-epi-PGF_{2α} across multiple moving averages. Participants with diabetes appeared to be more susceptible.

In the next project, we examined the associations of residential proximity to the nearest major roadway and annual average PM_{2.5} with body mass index (BMI) and abdominal adiposity among participants from the multidetector computed tomography (MDCT) study, a substudy that recruited participants from the Framingham Offspring and Third Generation cohorts. We

estimated residential-based annual average PM_{2.5} concentrations using a spatial-temporal model, and estimated subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) volumes by the MDCT scan. In this study, we found that living closer to a major roadway was associated with higher overall and abdominal adiposity.

Finally, we examined the associations of residential proximity to the nearest major roadway and annual average PM_{2.5} with liver fat attenuation among participants from the MDCT study. In this study, liver CT attenuation in Hounsfield units was measured by abdominal MDCT scan and we defined hepatic steatosis as having a liver-to-phantom ratio ≤ 0.33 . We found more liver fat (lower liver-to-phantom ratio) and higher odds of hepatic steatosis among participants who lived closer to a major roadway than those who lived further away. However, residential-based PM_{2.5} estimations were not associated with liver fat.

Overall, we showed positive associations between short-term exposure to ambient air pollution and biomarkers of oxidative stress. We also showed that participants who lived closer to a major roadway had higher BMI, higher abdominal adiposity, and more liver fat than those who lived further away. Our observations were consistent with previous findings in animal studies and we extended these associations to adults from large community-based cohorts. Future studies are necessary to identify traffic-related factors that are associated with adipose tissue deposition other than particulate matter and to extend these findings by examining changes in abdominal adiposity or liver fat accumulation in relation to traffic-related components.

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Introduction

Air pollution-induced systemic inflammation and oxidative stress may disrupt normal lipid and glucose metabolism, leading to conditions such as obesity and insulin resistance.¹⁻⁷ And both obesity and insulin resistance are important risk factors for non-alcohol fatty liver disease, a common liver disease that is characterized as the presence of hepatic steatosis among individuals in the absence of significant alcohol consumption or other liver diseases that may cause steatosis.^{8,9}

In controlled animal studies, mice exposed to fine particulate matter (PM_{2.5}) had more abdominal fat compared with mice exposed to filtered air,^{3,4} and observational studies conducted among children had reported positive associations between higher traffic density and higher BMI growth.^{10,11} Additionally, mice exposed to higher levels of PM_{2.5} were shown to have increased hepatic inflammation and hepatic lipid accumulation compared with mice exposed to filtered air.^{1,2,6} However, few studies examined the associations of ambient air pollution with overall obesity, abdominal adiposity, and liver fat accumulation among adults.

In Chapter 1, we explored and established the associations between short-term ambient air pollution and biomarkers of oxidative stress among participants from the community-based Framingham Heart Study Offspring cohort. We measured concentrations of 1-7 day moving averages of ambient air pollutants including PM_{2.5}, black carbon, sulfate, nitrogen oxides, and ozone from the central air pollution monitor supersite and local monitors, and biomarkers of oxidative stress including myeloperoxidase and 8-epi-prostaglandin F_{2α} (8-epi-PGF_{2α}) during exam visits. We used linear regression models and linear mixed-effects models with random intercepts for myeloperoxidase and urinary creatinine indexed 8-epi-PGF_{2α}, respectively. Models

were adjusted for demographic variables, individual- and area-level measures of socioeconomic position, clinical and lifestyle factors, weather, and temporal trend.

In Chapter 2, we examined the associations of residential proximity to the nearest major roadway and annual ambient PM_{2.5} concentrations with overall obesity and abdominal adiposity among participants from the multidetector computed tomography (MDCT) study, a substudy that enrolled participants from the Framingham Offspring and Third Generation cohorts. We estimated residential-based annual average PM_{2.5} concentrations using a spatial-temporal model at 1×1 km² resolution. Subcutaneous and visceral adipose tissue volumes were estimated using MDCT scan. We used linear regression models for BMI and volumes of subcutaneous and visceral adipose tissue, and logistic models for the binary indicator of obesity (BMI≥30 kg/m²). Models were adjusted for demographic variables, individual- and area-level measures of socioeconomic position, clinical and lifestyle factors. We additionally adjusted for height in abdominal adiposity analyses.

In Chapter 3, we examined the associations between traffic-related air pollution with hepatic steatosis among participants from the MDCT study. In this study, we measured liver fat attenuation and calculated liver-to-phantom ratio. We additionally defined hepatic steatosis as having a liver-to-phantom ratio ≤ 0.33. We employed linear regression models for continuous liver-to-phantom ratio and logistic models for the binary indicator of hepatic steatosis. Models were adjusted for demographic variables, individual- and area-level measures of socio-economic position, and clinical and lifestyle factors.

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Chapter 1.

Short-Term Exposure to Air Pollution and Biomarkers of Oxidative Stress: The Framingham Heart Study

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Abstract

Background: Short-term exposure to elevated air pollution has been associated with higher risk of acute cardiovascular diseases, with systemic oxidative stress induced by air pollution hypothesized as an important underlying mechanism. However, few community-based studies have assessed this association.

Methods and Results: 2,035 Framingham Offspring Cohort participants living within 50 km of the Harvard Boston Supersite who were not current smokers were included. We assessed circulating biomarkers of oxidative stress including blood myeloperoxidase at the seventh examination (1998-2001), and urinary creatinine-indexed 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF $_{2\alpha}$) at the seventh and eighth (2005-2008) examinations. We measured fine particulate matter (PM $_{2.5}$), black carbon (BC), sulfate, nitrogen oxides, and ozone at the Supersite, and calculated 1-, 2-, 3-, 5-, and 7-day moving averages of each pollutant. Measured myeloperoxidase and 8-epi-PGF $_{2\alpha}$ were \log_e transformed. We used linear regression models and linear mixed effects models with random intercepts for myeloperoxidase and indexed 8-epi-PGF $_{2\alpha}$, respectively. Models were adjusted for demographic variables, individual- and area-level measures of socioeconomic position, clinical and lifestyle factors, weather, and temporal trend. We found positive associations of PM $_{2.5}$ and BC with myeloperoxidase across multiple moving averages. Additionally, 2- to 7-day moving averages of PM $_{2.5}$ and sulfate were positively associated with 8-epi-PGF $_{2\alpha}$. Stronger positive associations of BC and sulfate with myeloperoxidase were observed among participants with diabetes than those without.

Conclusions: Our community-based investigation supports an association of select markers of ambient air pollution with circulating biomarkers of oxidative stress.

Introduction

Increasing evidence indicates that short-term exposure to elevated air pollution is associated with higher risk of incident ischemic stroke, myocardial infarction, and other acute cardiovascular events.¹⁻³ Oxidative stress, an imbalance between the production of the reactive oxygen species and the human body's antioxidant defense mechanism,⁴ has been proposed as an important underlying biological mechanism mediating this association.^{1, 5-7} Increased oxidative stress may induce endothelial dysfunction, which is characterized by increased endothelial permeability, altered vascular tone, platelet adhesion and aggregation, and enhanced thrombogenicity.^{8, 9}

Myeloperoxidase is an enzyme that is abundantly stored in inflammatory cells such as neutrophils, macrophages, and monocytes, and is involved in a wide range of activities that generate reactive oxygen and nitrogen species.¹⁰⁻¹³ Prior studies have yielded mixed results.¹⁴⁻¹⁸ In a recent study, positive associations of short-term exposure to fine particulate matter (diameter ≤ 2.5 μm , $\text{PM}_{2.5}$), black carbon (BC), and nitrogen oxides (NO_x) with myeloperoxidase were found in a group of potentially genetically susceptible participants.¹⁸

8-epi-prostaglandin $\text{F}_{2\alpha}$ (8-epi-PGF $_{2\alpha}$) is formed from peroxidation of arachidonic acid¹⁹ and is detectable in human plasma and urine. The quantification of 8-epi-PGF $_{2\alpha}$ has been widely used as a non-invasive method to assess lipid peroxidation.^{20, 21} Higher short-term air pollution has been associated with higher 8-epi-PGF $_{2\alpha}$ sampled from exhaled breath condensate in children, adolescents, and healthy young adults;²²⁻²⁵ however, few studies have assessed the relationship between exposure to ambient air pollution and urinary 8-epi-PGF $_{2\alpha}$ ^{8, 26} or in older populations more at risk of cardiovascular events.

Epidemiologic studies conducted in the Boston area have reported positive associations of short-term exposure to air pollution with acute stroke onset,²⁷ atrial fibrillation,²⁸ and myocardial infarction onset.²⁹ In the present study, we evaluated whether short-term (1-7 days) ambient air pollution exposure is associated with systemic levels of oxidative stress, measured by plasma myeloperoxidase and urinary creatinine-indexed 8-epi-PGF_{2α}, in the community-based Framingham Heart Study. Our study catchment region and study period largely overlap with the above mentioned studies, and a closer look at the relationship may enable us to elucidate underlying biologic pathways that could in part explain previous findings.

Methods

Study sample

The study participants were from the Framingham Heart Study Offspring cohort living within 50 km of the Harvard Supersite air pollution monitor in Boston, Massachusetts.³⁰ The study design and selection criteria of the Framingham Offspring cohort has been described elsewhere.³¹ We included 2,035 participants from the Offspring cohort seventh examination (1998-2001) and/or eighth examination (2005-2008) who were not current smokers and had at least one valid measurement of plasma myeloperoxidase or urinary creatinine-indexed 8-epi-PGF_{2α} (3,386 observations in total). At each examination, physical examinations were performed following standardized protocols, and data on demographics, medication history, smoking history, and alcohol intake were collected by questionnaires. All participants provided written informed consent for the Framingham Heart Study examinations, and Institutional Review Boards at Beth Israel Deaconess Medical Center and Boston University Medical Center approved the study.

Biomarkers of oxidative stress

Fasting morning plasma samples and urine samples were collected at the examination visits. Plasma myeloperoxidase (ng/ml) was measured in duplicate in examination 7 by commercially available Enzyme Immunoassay Kit (OXIS Health Products, Portland, OR) and 8-epi-PGF_{2α} (pg/ml) was measured in duplicate by Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI) in examinations 7 and 8. Measured 8-epi-PGF_{2α} was adjusted for urinary creatinine and was expressed as ng/mmol creatinine. The levels of myeloperoxidase and indexed 8-epi-PGF_{2α} were log_e transformed.

Air pollution and meteorological variables

Air pollution levels were measured at the Harvard Supersite, located on the rooftop of the Francis A. Countway Library of Medicine (5 stories above ground level) and 50 m from the nearest street. Measurement methods have been described previously.³⁰ PM_{2.5} ($\mu\text{g}/\text{m}^3$) was measured using a tapered element oscillating microbalance (Model 1400A, Rupprecht & Patashnick Co. Inc., Albany, NY); BC ($\mu\text{g}/\text{m}^3$) was measured using an aethalometer (Model AE-16, Magee Scientific Corp., Berkeley, CA). Ozone (O₃, ppm) and NO_x were estimated by averaging available data from local state monitors within the Greater Boston area. Daily sulfate (SO₄²⁻, $\mu\text{g}/\text{m}^3$) was calculated from elemental sulfur measured by X-Ray Fluorescence analysis of the PM_{2.5} filter samples. On days when SO₄²⁻ X-Ray Fluorescence measurements were not available, a SO₄²⁻ analyzer (Model 5020, Thermo Electron Corp., Franklin, MA) was used. Temperature and relative humidity were monitored at the Boston Logan International Airport Weather Station, located 12 km from the Supersite.

Statistical methods

We calculated 1-, 2-, 3-, 5-, and 7-day moving averages for measured pollutants based on the daily means. For each moving average of a pollutant, we fit multivariable linear regression models (for plasma myeloperoxidase), and multivariable linear mixed effects models with subject-specific random intercepts (for indexed urinary 8-epi-PGF_{2 α}). We adjusted for individual- and area-level covariates in the models, including centered age, and (centered age)²; sex; body mass index; smoking status (former or never smoker); pack years; alcohol intake; educational level; and quartile of median household income in the participant's census tract from the 2000 U.S. Census. An examination identifier (examination 7 or 8) was added to the linear

mixed models. We additionally adjusted for season, linear time trend, temperature, and relative humidity.

In secondary analyses, we explored the associations within current U.S. Environmental Protection Agency (EPA) National Ambient Air Quality Standards by excluding observations with any of the seven days prior to the examination date that had a 24-hour $PM_{2.5} > 35 \mu\text{g}/\text{m}^3$. We also explored whether associations differed when we included current smokers. Additionally, we repeated our analyses after restricting the study population to participants who lived within 40 km of the Harvard Supersite air pollution monitor. Furthermore, we examined whether associations varied by age ($>/\leq 65$ years old), sex, obesity (31.8%), diabetes (16.8%), cardiovascular disease (15.0%), antihypertensive medication use (46.8%), statin use (31.5%), and season (warm [April to September] *versus* cold [October to March]) by adding an interaction term to these models.

Analyses were scaled to $5 \mu\text{g}/\text{m}^3$ for $PM_{2.5}$, $0.4 \mu\text{g}/\text{m}^3$ for BC, $2 \mu\text{g}/\text{m}^3$ for SO_4^{2-} , and 0.01 ppm for NO_x and O_3 , which approximated the interquartile range.

Estimated percent changes were reported with 95% confidence intervals (CIs). For primary analyses, we focused on describing the association patterns between pollutants and the biomarkers. For sensitivity analyses in which effect modification was explored, the two-tailed p-value from the Wald test of the interaction term was used to decide whether the observed association differed between subgroups, however, only consistent association patterns were considered important and highlighted. A two-tailed p-value less than 0.05 was considered

statistically significant in these analyses. Primary analyses were performed using PROC GLM and PROC Mixed in SAS 9.4 (SAS Institute, Inc., Cary, NC). Figures were plotted using Stata 13 (StataCorp LP, College Station, TX).

Results

Table 1.1 shows the population characteristics. $PM_{2.5}$ was strongly correlated with BC and SO_4^{2-} . NO_x was moderately correlated with BC, and negatively correlated with O_3 (Table 2). The correlation structure was similar for longer-term moving averages. Figure 1.1 shows the distributions of myeloperoxidase and indexed urinary 8-epi-PGF_{2 α} , and Figure 1.2 shows the distribution of the daily concentrations of each air pollutant.

Table 1.1 Characteristics of the 3,386 observations from the Framingham Offspring cohort examination 7 (1998-2001) and/or 8 (2005-2008) participants.

	N (%) or Mean [SD]
Examination cycle 7	1,878 (55.5%)
Age, years	64.1 [9.7]
Women	1,789 (52.8%)
BMI*, kg/m ²	28.5 [5.4]
Alcohol, drinks/week	4.2 [6.9]
Diabetes	569 (16.8%)
Former Smoker	2,018 (59.6%)
Education	
< High school	161 (4.8%)
High School	1,051 (31.0%)
Some college	1,050 (31.0%)
College graduate	1,094 (32.3%)
Antihypertensive medication use	1,583 (46.8%)
Statins	1,066 (31.5%)
Plasma myeloperoxidase†, ng/ml	40.6 [22.5]
Urinary 8-epi-PGF _{2α} †, pg/ml	897.9 [842.3]
Urine creatinine, mg/100 ml	115.2 [69.1]
Indexed urinary 8-epi-PGF _{2α} †, ng/mmol creatinine	108.7 [69.6]

Abbreviation: SD, standard deviation.

*. weight(kg)/height(m)².

†. geometric mean [standard deviation of the geometric mean].

Table 1.2 Characteristics of the 1-day moving averages of air pollutants previous to the exam date in the study population (1998-2001, 2005-2008).

Pollutant	No. of observations	Mean (SD)	IQR	BC	SO ₄ ²⁻	NO _x	O ₃
PM _{2.5} , µg/m ³	3,380	9.86 (5.34)	6.28	0.76	0.79	0.47	-0.05
BC, µg/m ³	3,376	0.84 (0.46)	0.57		0.53	0.61	-0.25
SO ₄ ²⁻ , µg/m ³	2,758	2.98 (2.25)	2.22			0.33	0.05
NO _x , ppm	3,081	0.04 (0.02)	0.02				-0.52
O ₃ , ppm	3,377	0.02 (0.01)	0.01				

Abbreviation: SD, standard deviation; IQR, interquartile range; PM_{2.5}, fine particulate matter; BC, black carbon; SO₄²⁻, sulfate; NO_x, nitrogen oxides; O₃, ozone.

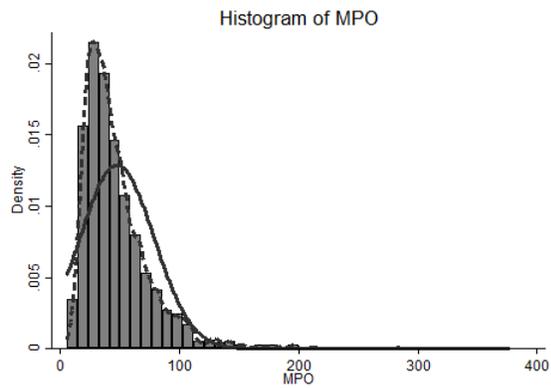
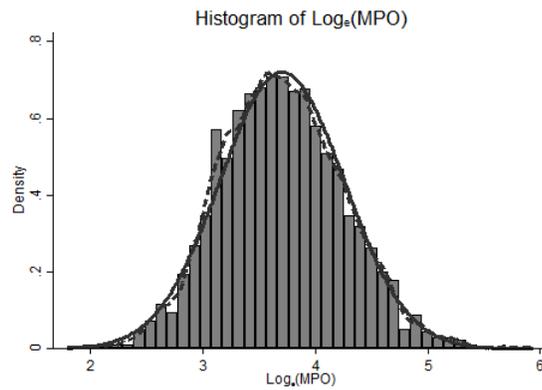
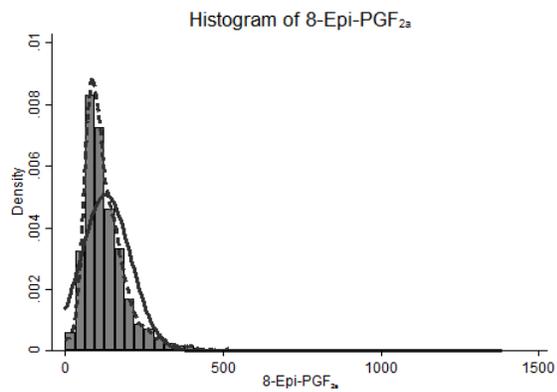
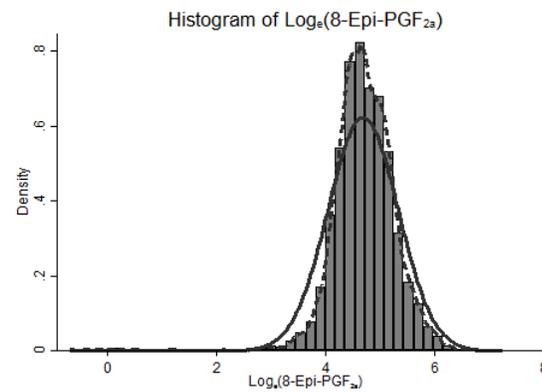
A**B****C****D**

Figure 1.1 Histograms of: (A) myeloperoxidase, (B) Log_e transformed myeloperoxidase, (C) indexed 8-epi-PGF_{2α}, and (D) Log_e transformed indexed 8-epi-PGF_{2α}. Solid line indicates the normal-density plot, dash line indicates the kernel-density plot.

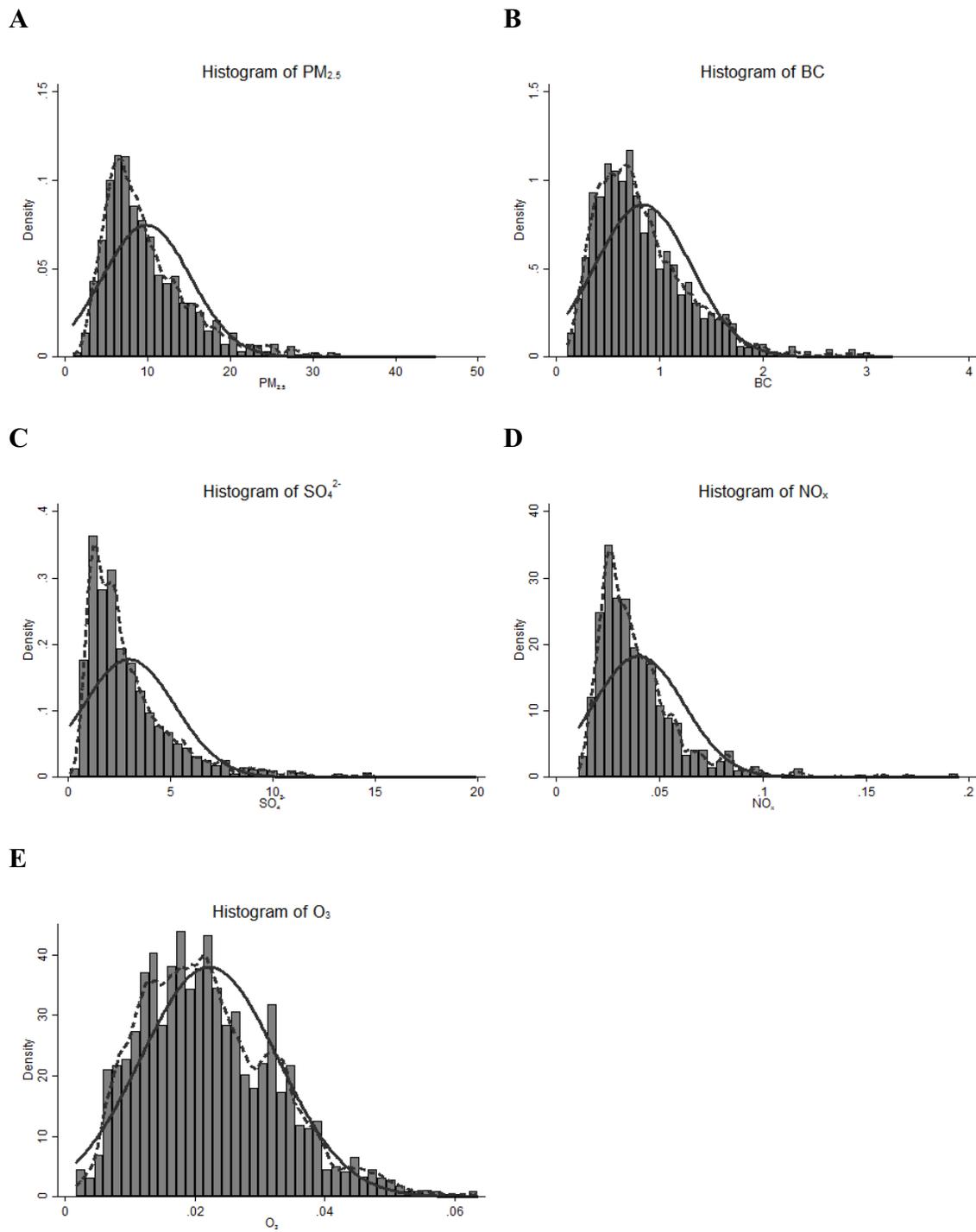


Figure 1.2 Histograms of the daily concentrations of measured air pollutants: (A) PM_{2.5}, (B) BC, (C) SO₄²⁻, (D) NO_x, and (E) O₃. Solid line indicates the normal-density plot, dash line indicates the kernel- density plot.

We found positive associations of PM_{2.5} and BC with plasma myeloperoxidase across multiple moving averages (Figure 1.3A). Additionally, 3- to 7-day moving averages of SO₄²⁻ were weakly associated with plasma myeloperoxidase; however, 95% CIs were rather wide.

We also observed positive associations for PM_{2.5} and SO₄²⁻ with indexed urinary 8-epi-PGF_{2α}, with stronger associations appearing in 3- to 7-day moving averages of PM_{2.5} and 2- to 7-day moving averages of SO₄²⁻ (Figure 1.3B). Similar but weaker positive associations were observed for 2- to 7-day moving averages of BC.

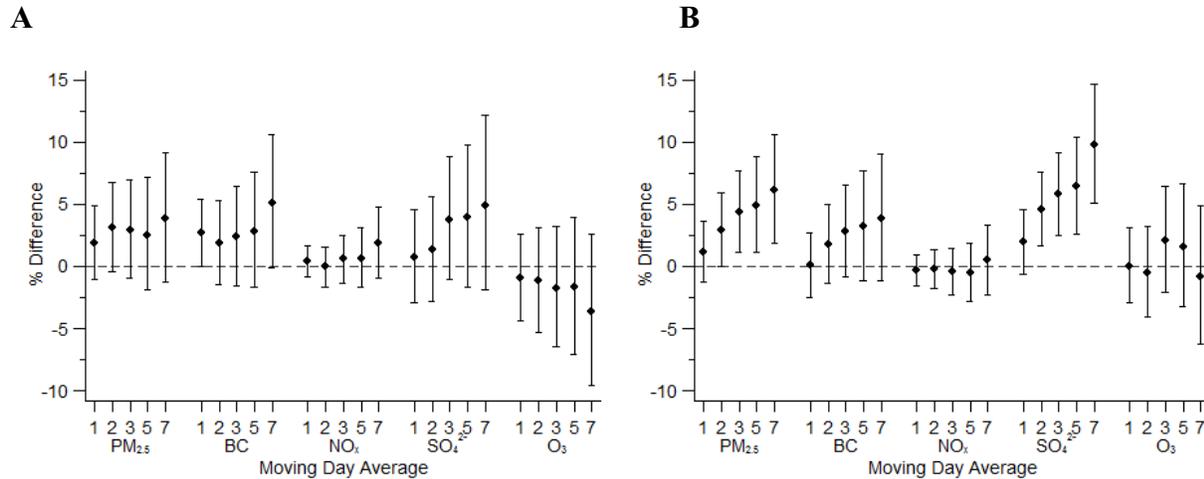


Figure 1.3 Associations of moving averages of air pollutants with (A) myeloperoxidase and (B) indexed 8-epi-PGF_{2α}. Scaled to 5 μg/m³ for fine particulate matter (PM_{2.5}), 0.4 μg/m³ for black carbon (BC), 2 μg/m³ for sulfate (SO₄²⁻), and 0.01 ppm for nitrogen oxides (NO_x) and ozone (O₃). Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

Excluding observations with any 24-hour average $PM_{2.5}$ above the EPA National Ambient Air Quality Standards (19 observations for plasma myeloperoxidase and 38 observations for urinary 8-epi-PGF_{2α}) did not change our findings substantially. As before, 3- to 7-day moving averages of $PM_{2.5}$ and 2- to 7-day moving averages of SO_4^{2-} were positively associated with indexed urinary 8-epi-PGF_{2α} with 95% CIs that did not overlap the null. Results were not materially altered after we included current smokers and adjusted for smoking status and pack years in the primary analyses, or after we restricted study participants to those who lived within 40 km of the Harvard Supersite air pollution monitor. We tested the robustness of our results by including BC and SO_4^{2-} simultaneously; the associations were slightly attenuated but without any substantial change.

There was no consistent evidence of differing associations between pollutants and either biomarker by age, sex, obesity, cardiovascular disease, antihypertensive medication use, statin use, or season. However, stronger associations of BC and SO_4^{2-} with plasma myeloperoxidase were observed among participants with diabetes than those without (Figure 1.4A).

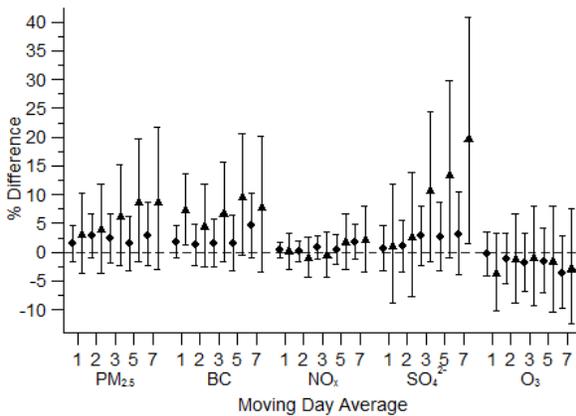
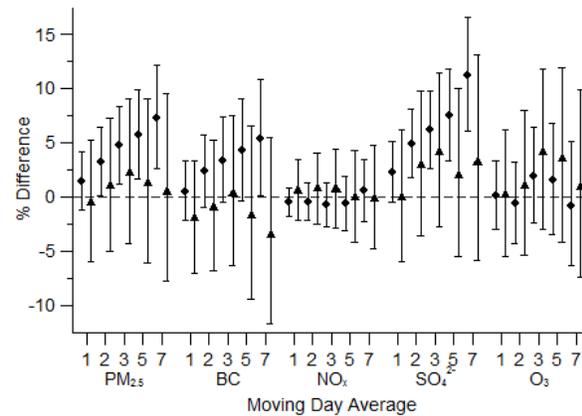
A**B**

Figure 1.4 Associations of moving averages of air pollutants with (A) myeloperoxidase and (B) indexed 8-epi-PGF_{2α} among participants with diabetes and those without. Triangle, participants with diabetes; circle, participants without diabetes. Scaled to 5 μg/m³ for fine particulate matter (PM_{2.5}), 0.4 μg/m³ for black carbon (BC), 2 μg/m³ for sulfate (SO₄²⁻), and 0.01 ppm for nitrogen oxides (NO_x) and ozone (O₃). Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

Discussion

In our community-based study, we found positive associations of PM_{2.5} and BC with plasma myeloperoxidase and of PM_{2.5} and SO₄²⁻ with urinary 8-epi-PGF_{2α} across multiple moving averages. The association of BC and SO₄²⁻ with plasma myeloperoxidase appeared to be stronger among participants with diabetes. To our knowledge, we report the largest community-based study to date on the association of short-term ambient air pollution with oxidative stress biomarkers.

Myeloperoxidase can be involved in diverse oxidation reactions, including lipid peroxidation by acting as an enzyme in generating multiple reactive oxygen and nitrogen species, and may promote endothelial dysfunction.¹³ Accumulation of lipid peroxidation products in vascular walls²² promotes disruption of vulnerable plaques,^{32,33} which likely contributes to the risk of acute cardiovascular events. Some^{14,16,18} but not all^{15,17} prior studies have found an association between short-term air pollution and plasma myeloperoxidase. Ruckerl *et al.* found higher myeloperoxidase was associated with the BC, NO, NO₂, and PM_{2.5} within 5 days in a group of potentially genetically susceptible participants who were free of type 2 diabetes or impaired glucose tolerance.¹⁸ However, Delfino *et al.* reported no association between measured air pollutants and myeloperoxidase among 29 non-smoking elderly participants with history of coronary artery disease.¹⁵

Urinary 8-epi-PGF_{2α} is a reliable and stable biomarker of lipid peroxidation that may promote vasoconstriction and platelet activation.²⁰ Prior studies have found increased 8-epi-PGF_{2α} in exhaled breath condensate following exposure to air pollutants.²²⁻²⁴ However, systemic oxidative

stress may be better reflected by 8-epi-PGF_{2α} measured in plasma or urine. Mixed results have been seen between air pollution and 8-epi-PGF_{2α}^{8, 34, 35} or other oxidative stress markers.³⁶

Prior studies of short-term ambient air pollution exposure with acute cardiovascular outcomes³⁷⁻⁴⁰ and markers of vascular reactivity⁴¹ and inflammation⁴² suggest that individuals with diabetes are more sensitive to air pollution, as a result of baseline chronic inflammation and endothelial dysfunction.⁴³ We observed tendencies for participants with diabetes to have higher levels of myeloperoxidase in relation to BC and SO₄²⁻. There was no evidence suggesting differing associations between pollutants and 8-epi-PGF_{2α}.

In this study region, local traffic sources and regional pollution both contribute to PM_{2.5} mass concentrations.⁴⁴ Locally emitted or transported BC is a product of incomplete combustion and is associated with different sources such as traffic, residential heating and cooking, and biomass burning. SO₄²⁻ is primarily from regional sulfur-related pollution sources such as coal-fired power plants and some is generated from local diesel exhaust.⁴⁵ When we included both BC and SO₄²⁻ in the models, we observed potential positive association between BC and myeloperoxidase, but not SO₄²⁻, suggesting that local sources may play an important role, whereas for 8-epi-PGF_{2α}, the stronger association with SO₄²⁻ suggests that the transported pollutants may play a stronger role, consistent with the finding of Ren *et al.*⁴⁶

There are several limitations that should be noted. We assigned the ambient air pollution level measured by a central monitoring site to all participants, which may decrease precision of our estimates and induce exposure measurement error. Prior studies in our region have demonstrated

moderate correlation between PM_{2.5} measured at the Supersite and personal exposure level.⁴⁷ In daily time series, most of the variability in exposure within the study region is related to temporal, rather than spatial variability,⁴⁴ which supports assigning regional average concentrations to study participants. In the present investigation, the distribution of exposure of the participants was primarily related to the date that participants came for their examination appointment. Thus, we expect the exposure measurement error due to assignment to be non-differential, leading to attenuated point estimates and wider confidence intervals. The participants of the Framingham Offspring Study were predominantly white individuals of European ancestry and middle-aged to older adults, which limits the generalizability of our findings to other ethnicities and to age groups not studied. We acknowledge that we cannot exclude the possibility of residual confounding, and that we cannot prove causal relations.

There are also several strengths. First, our study sample was from a large community-based cohort with standardized protocols for physical examinations and biomarker assessments. Second, we adjusted for demographic characteristics, lifestyle, individual- and area-level of socioeconomic position, weather, and temporal trend. Third, assessments of air pollutants and biomarkers were performed separately. Fourth, we conducted the study in a region that has pollution levels in compliance with current air quality standards, and our findings still suggested adverse associations. Future studies in regions with higher levels of ambient air pollution are needed to determine if these associations are stronger in such regions. Additionally, participants of the Framingham Heart Study scheduled the date of their examination visit months in advance, and this was not likely related to the air pollution level on the days leading up to that prescheduled appointment.

Conclusions

Our findings suggest positive associations of short-term exposure to PM_{2.5} and BC with plasma myeloperoxidase and of short-term exposure to PM_{2.5} and SO₄²⁻ with urinary 8-epi-PGF_{2α}. The associations of BC and SO₄²⁻ with plasma myeloperoxidase appear stronger among participants with diabetes. Our findings provide evidence suggesting potential intermediate biological mechanisms that may in part explain the observed associations between transiently higher air pollution levels and the increase of acute cardiovascular events.

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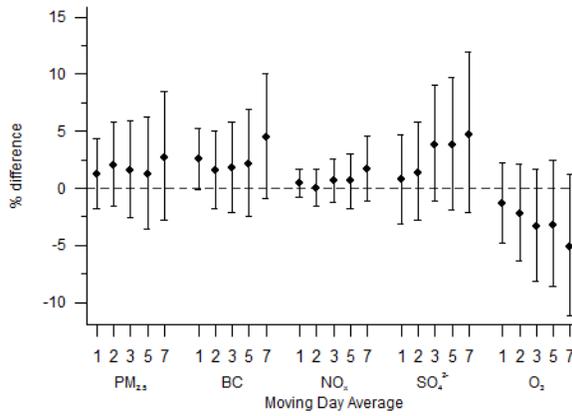
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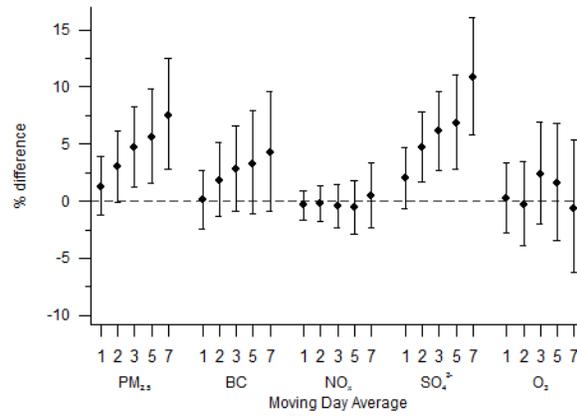
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Supplemental Materials

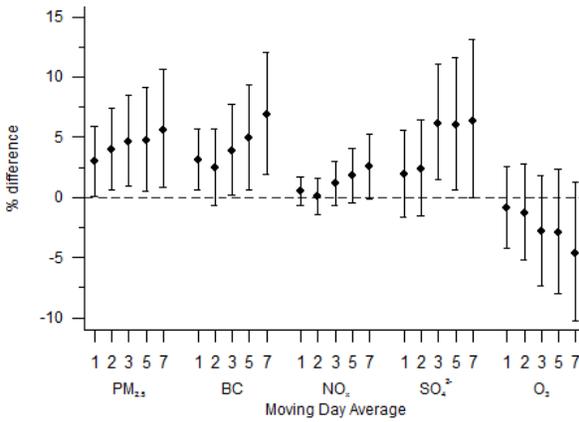
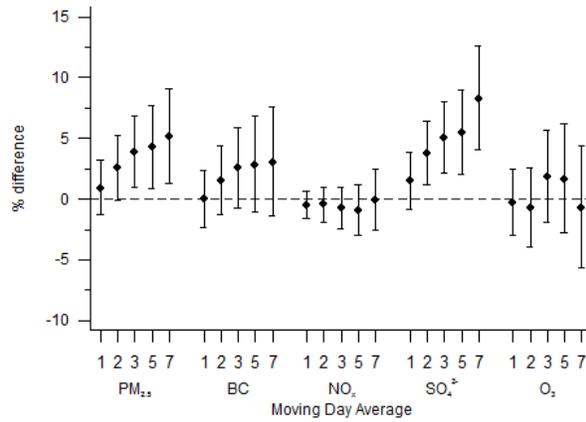
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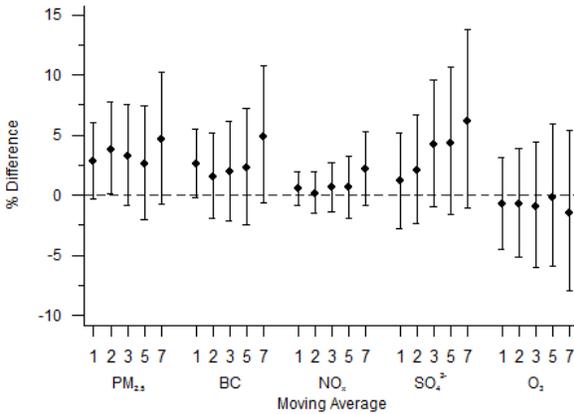
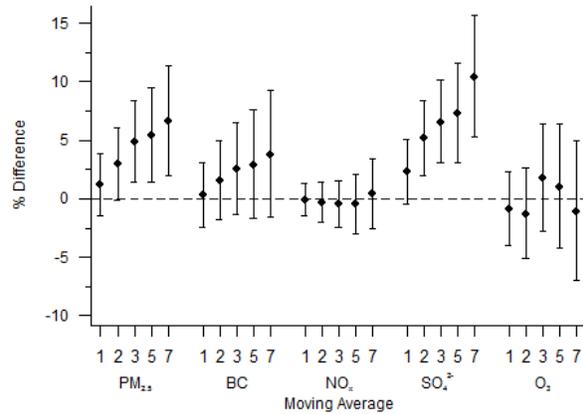
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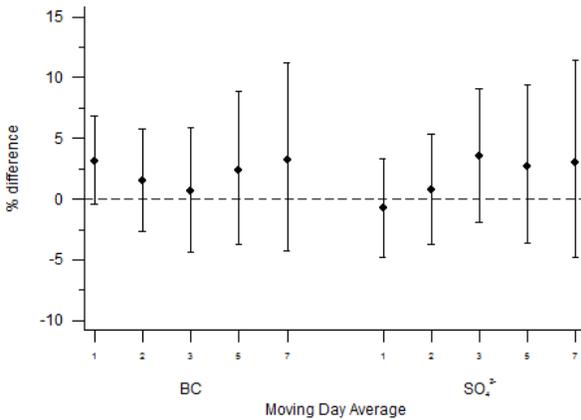
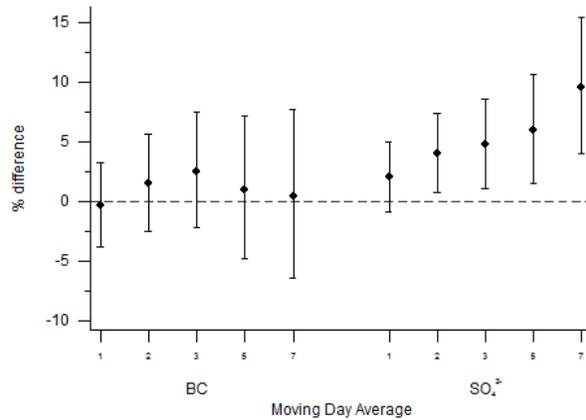
Supplemental Figure 1.1 Associations of moving averages of air pollutants with A) myeloperoxidase and B) indexed 8-epi-PGF_{2α} after excluding observations with any 24-hour average PM_{2.5} above the EPA National Ambient Air Quality Standards. Scaled to 5 μg/m³ PM_{2.5}, 0.4 μg/m³ for BC, 2 μg/m³ for SO₄²⁻, and 0.01 ppm for NO_x and O₃. Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

A**B**

Supplemental Figure 1.2 Associations of moving averages of air pollutants with A) myeloperoxidase and B) indexed 8-epi-PGF_{2α} after including current smokers. Scaled to 5 μg/m³ PM_{2.5}, 0.4 μg/m³ for BC, 2 μg/m³ for SO₄²⁻, and 0.01 ppm for NO_x and O₃. Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

A**B**

Supplemental Figure 1.3 Associations of moving averages of air pollutants with A) myeloperoxidase and B) indexed 8-epi-PGF_{2α} after restricting participants to those who lived within 40km of the Harvard Boston Supersite air pollution monitor. Scaled to 5 μg/m³ PM_{2.5}, 0.4 μg/m³ for BC, 2 μg/m³ for SO₄²⁻, and 0.01 ppm for NO_x and O₃. Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

A**B**

Supplemental Figure 1.4 Associations of moving averages of air pollutants with A) myeloperoxidase and B) indexed 8-epi-PGF_{2α} when adjusted BC and SO₄²⁻ simultaneously. Scaled to 0.4 μg/m³ for BC and 2 μg/m³ for SO₄²⁻. Models are adjusted for centered age, (centered age)², sex, BMI, smoking status, pack years, alcohol intake, education level, and quartile of median household income in the participants' census tracts from the 2000 U.S. Census, sine and cosine of the day of year, exam date, day of the week, temperature, and relative humidity; and an exam identifier is added to models with indexed 8-epi-PGF_{2α} as the dependent variable. Error bars indicate the 95% confidence intervals.

Chapter 2.

Residential Proximity to Major Road, Fine Particulate Matter, and Adiposity: The Framingham Heart Study

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Abstract

Background: Exposure to higher levels of traffic-related air pollution has been associated with higher body mass index (BMI) among children. However, few studies assessed the associations in adults. We therefore examined the associations of residential-based annual fine particulate matter (PM_{2.5}) estimation and proximity to the nearest major roadway with BMI and multidetector computed tomography (MDCT)-based measures of abdominal adiposity in a large cohort of adults.

Methods and Results: The study population consists of 2,850 participants from the Framingham Offspring and Third Generation cohorts who underwent MDCT scans between 2002 and 2005. We calculated residential proximity to the nearest major roadway and used a spatial-temporal model to estimate residential-based PM_{2.5}. BMI was measured at Offspring examination 7 (1998-2001) and Third Generation examination 1 (2002-2005), subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were measured using MDCT. Distance to major roadway was log_e transformed. We used linear regression models for continuous measures of adiposity (BMI, SAT, and VAT) and logistic models for the binary indicator of obesity (BMI \geq 30 kg/m²). Models were adjusted for demographic variables, individual- and area-level measures of socio-economic position, clinical and lifestyle factors. We additionally adjusted for height in SAT and VAT analyses. Compared with participants who lived 416 m (75th percentile) from a major roadway, those who lived 58 m from a major roadway (25th percentile) had 0.37 kg/m² higher BMI (95% CI: 0.10, 0.65 kg/m²), 78.8 cm³ higher SAT (95% CI: 4.9, 152.7 cm³), and 41.9 cm³ higher VAT (95% CI: -4.5, 88.4 cm³). The 2003 annual PM_{2.5} was not associated with adiposity measures.

Conclusions: Living closer to a major roadway was associated with higher overall and abdominal adiposity. Annual PM_{2.5} was not associated with adiposity measures.

Introduction

In 2011-2012, more than one-third of adults (34.9%) in the United States were obese.¹ As obesity is an important and modifiable risk factor for cardiovascular disease, studying the origin and development of obesity is of great interest. Other than overall obesity, both abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) have been associated with adverse cardiometabolic risk profiles, with stronger associations seen for VAT than SAT.²⁻⁶

In controlled animal studies, mice exposed to fine particulate matter (PM_{2.5}; particles with aerodynamic diameter $\leq 2.5\mu\text{m}$) had higher visceral fat or total abdominal fat compared with mice exposed to filtered air.^{7, 8} The hypothesized underlying biological mechanisms were the adverse effects of air pollution on inflammation, oxidative stress, and lipid metabolism.⁹⁻¹² Although exposure to ambient air pollution has been linked to development of diabetes and cardiovascular disease in humans,¹³⁻¹⁶ few studies have examined the association between ambient air pollution and obesity,¹⁷⁻²⁰ and little is known about the associations of ambient air pollution with abdominal adiposity among adults.

Therefore, we conducted the present investigation among participants from the multidetector computed tomography (MDCT) study, a sub-study of the Framingham Offspring and Third Generation cohorts. In the current study, we examined the associations of residential-based estimates of ambient PM_{2.5} exposure and proximity to the nearest major roadway with body mass index (BMI) and MDCT-based measures of abdominal adiposity. Our results may provide insight into the observed associations between air pollution and cardiovascular disease and diabetes.

Methods

Study population

We enrolled participants from the Framingham Offspring cohort examination 7 (1998-2001) or Framingham Third Generation cohort examination 1 (2002-2005) who underwent the MDCT study. Selection criteria and study design of the two cohorts have been previously described.^{21, 22} For inclusion in the MDCT study, men were aged ≥ 35 years old, women were aged ≥ 40 years old and not pregnant, and because of physical constraints of the scanner, all participants weighed < 350 lbs (160 kg).³ Participants were scanned between 2002 and 2005. A total of 3,370 participants had either SAT or VAT measured, 520 were further excluded because of missing annual PM_{2.5} data, leaving 2,850 MDCT participants in the current analyses. At each examination, physical examinations were performed following standardized protocols, and data on demographics, medication history, smoking history, and alcohol intake were collected by questionnaires. All participants provided written informed consent, and Institutional Review Boards at Beth Israel Deaconess Medical Center, Boston University Medical Center, and Massachusetts General Hospital approved the study.

PM_{2.5} assessment

Participants' self-reported residential addresses were collected during the exam visit and geocoded using ArcGIS software. We used a spatial-temporal model to estimate PM_{2.5} concentrations at a 1×1 km² resolution based on residential addresses. The model utilized satellite-based aerosol optical depth data from the advanced Multi-Angle Implementation of Atmospheric Correction (MAIAC) algorithms, daily PM_{2.5} mass concentrations measured from local monitoring stations, land use terms, and meteorological predictors.²³ Briefly, we first fit a

model regressing PM_{2.5} concentrations measured at local monitors against satellite-based aerosol optical depth, adjusting for land use terms and meteorological predictors. Inverse probability weighting was used to address non-random missingness of daily aerosol optical depth data. The predictions from this model had an excellent mean out-of-sample R² of 0.88, with an excellent fit when comparing predictions with observations (slope=0.99).²³ Second, we predicted grid cells with aerosol optical depth measurement but not ground monitors measurement using the above fitted model. Third, for grid cells/days that had missing aerosol optical depth measurements, we imputed data by a generalized additive model with spatial smoothing and a random intercept for each grid cell. Last, we took the residuals (differences between monitor-based PM_{2.5} and predicted PM_{2.5} for each cell) and regressed them against monitor-specific spatial and temporal variables such as traffic density and distance to A1 roadways to generate local predictions for each residential address. The total PM_{2.5} daily estimates were the sum of grid and localized predictions. We used annual average PM_{2.5} of the same index year (2003) for all participants, similar to our previous work.^{24,25}

Distance to the nearest major roadway

We defined major roadways as primary highways with limited-access (A1), primary roads without limited-access (A2), or secondary and connecting roads (A3). And we estimated residential distance to the nearest major roadway based on the geocoded addresses. We restricted our analyses to participants who lived within 1,000 meters from the nearest major roadway (N=2,536) when roadway proximity was the exposure of interest, because distance may not be an informative surrogate of traffic-related air pollution in semirural or rural areas. As in our previous work,^{24,25} we classified participants into five groups based on the distance of their

residential address to the nearest major roadway (0-50, 50-100, 100-200, 200-400, and 400-1,000 m). The cutpoints reflect the decreasing pattern of traffic-related pollutants, such as particulate matter mass and elemental carbon, from mobile sources.²⁶

Overall and abdominal adiposity

Both standing height and weight were measured without shoes according to a standardized protocol. Height was recorded to the nearest 1/4 inch, and weight was recorded to the nearest pound (rounded up if ≥ 0.5 pound). BMI was calculated as weight (kg) / height (m)².

Participants underwent MDCT scan of the abdomen in the supine position by an eight-slice scanner (LightSpeed Ultra, General Electric, Milwaukee, WI) and 25 contiguous 5 mm thick slices (120 kVp, 400 mA, gantry rotation time 500 ms, table feed 3:1) were obtained above the S1 level. A semiautomatic segmentation technique that required manual definition of the abdominal muscular wall (Aquarius 3D Workstation, TeraRecon Inc., San Mateo, CA) was used to measure SAT and VAT. An image display window with width of -195 to -45 Hounsfield units was used to identify pixels containing fat. The MDCT-based volumetric measurements of abdominal adipose tissue have been shown to be reproducible with high intra-class correlation (ICC) for both intra-reader (ICC=0.99) and inter-reader (ICC=0.99) comparisons.²⁷

Statistical methods

We fit multivariable linear regression models for continuous BMI, SAT, and VAT, and multivariable logistic regression models for a binary indicator of obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$). We adjusted for age at examination, and (age at examination)²; sex; smoking status (current, former, or never); pack years of smoking; alcohol intake (drinks/week; calculated based on self-reported

questionnaires and standardized to 0.5 oz alcohol/drink);²⁸ educational level; physical activity index (estimated based on self-reported questionnaires);²⁹ anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; diabetes; and a cohort identifier. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height. Distance to the nearest major roadway was \log_e transformed to account for hypothesized air pollution dispersion pattern and the log-linear relationship between residential distance to major roadway and cardiovascular outcomes as in our previous work.^{30, 31}

For sensitivity analyses, we excluded participants who were current smokers (N=368), or had previous cardiovascular disease or diabetes (N=373). We additionally explored whether associations differed by median age ($>/\leq 51$ years old), sex, or a binary educational level indicator (high school or less vs. some college or higher) by adding interaction terms to the model. To estimate the influence of choosing the year 2003 as the index year, we assessed the associations between three-year average PM_{2.5} (2003-2005) and adiposity measures. We also evaluated the influence of adjusting for time by additionally including year of CT scan (categorical variable), date of CT scan (continuous variable), and days between CT scan and cohort examination visit for MDCT analyses. Additionally, we used quantile regression models to explore whether the associations differed at each quartile (25th, 50th, and 75th) of the distributions of these adiposity measures.

Results from the PM_{2.5} analyses were scaled to 1.5 $\mu\text{g}/\text{m}^3$, which is approximately the interquartile range of the 2003 annual average of PM_{2.5} concentration. Results from the residential proximity analyses contrasted participants lived 58 m from the major roadway (25th percentile) to those who lived 416 m from the major roadway (75th percentile). We examined the

linear trend for road category analyses by assigning participants in each road category the median value of \log_e -transformed distance within that category and then replaced the road category variables with this continuous variable in the models.

Scaled regression coefficients and odds ratios (ORs) were reported with 95% confidence intervals (CIs). A two-tailed p-value < 0.05 was considered statistically significant. Analyses were performed using PROC GLM, PROC GENMOD, and PROC QUANTREG in SAS 9.4 (SAS Institute, Inc., Cary, NC). Figures were plotted using Stata 13 (StataCorp LP, College Station, TX).

Results

Table 2.1 shows the characteristics of the study population. The mean age at the time of MDCT scan was 52.9 (standard deviation (SD): 11.9) years old and 49% were women. Of all the participants, 12.9% were current smokers, 6.4% had cardiovascular disease, and 6.7% had diabetes. The 2003 annual average PM_{2.5} concentration was 10.6 µg/m³, which was lower than the current U.S. Environmental Protection Agency's National Ambient Air Quality Standard (annual average PM_{2.5}: 12.0 µg/m³).

Table 2.1 Characteristics of the 2,850 participants from the multidetector computed tomography (MDCT) study (2002-2005).

	N (%) or Mean [SD]
Offspring cohort	1,200 (42.1%)
Age *, years	52.9 [11.9]
Women	1,395 (49.0%)
Alcohol, drinks/week	4.9 [7.1]
Current smoker	368 (12.9%)
Former Smoker	1,139 (40.0%)
Education	
< High school	52 (1.8%)
High School	623 (21.9%)
Some college	925 (32.5%)
College graduate	1,245 (43.7%)
Antihypertensive medication use	576 (20.2%)
Statins use	379 (13.3%)
Cardiovascular disease	183 (6.4%)
Diabetes	190 (6.7%)
PM _{2.5} †, µg/m ³	10.6 [1.4]
Distance ‡, m	271 [253]
Distance categories ‡	
0-50 m	589 (20.7%)
50-100 m	245 (8.6%)
100-200 m	444 (15.6%)
200-400 m	586 (20.6%)
400-1000 m	672 (23.6%)

Abbreviation: SD, standard deviation; PM_{2.5}, fine particulate matter.

* Age at the time of CT scan.

† 2003 annual average.

‡ 314 participants lived more than 1,000 meters from the nearest major roadway.

Table 2.2 shows the characteristics of the adiposity measures and Spearman partial correlation coefficients adjusting for sex. Of the 2,843 participants who had BMI data available, 27.1% (N=773) were obese ($\text{BMI} \geq 30 \text{ kg/m}^2$). BMI was highly correlated with both SAT and VAT, and SAT was moderately correlated with VAT.

Table 2.2 Characteristics of adiposity measures and the Spearman partial correlation between the measures.

	N	Mean [SD]	Interquartile range	Correlation coefficients*	
				SAT	VAT
BMI, kg/m ²	2,843	27.8 [5.2]	6.3	0.83	0.72
SAT, cm ³	2,850	2,882 [1,375]	1,672		0.65
VAT, cm ³	2,850	1,820 [1,027]	1,485		

Abbreviation: SD, standard deviation; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

* Spearman partial correlation coefficients, adjusted for sex. Correlation coefficients were calculated from 2,843 participants who had data available on BMI, SAT, and VAT.

Living closer to a major roadway was associated with higher BMI and higher SAT (Table 2.3 and Figure 2.1). Compared with participants who lived 416 m from the major roadway (75th percentile), those who lived 58 m from the major roadway (25th percentile) had higher BMI ($\beta=0.37 \text{ kg/m}^2$, 95% CI: 0.10, 0.65 kg/m^2) and higher SAT ($\beta=78.8 \text{ cm}^3$, 95% CI: 4.9, 152.7 cm^3). The positive association between living closer to a major roadway and higher VAT was weaker and the 95% CI overlapped the null ($\beta= 41.9 \text{ cm}^3$, 95% CI: -4.5, 88.4 cm^3). The 2003 annual average $\text{PM}_{2.5}$ was not associated with adiposity measures.

Table 2.3 Associations of distance to the nearest major roadway and PM_{2.5} with adiposity measures.

	Closer to a major roadway		2003 annual average PM _{2.5}	
	β*	95% CI	β*	95% CI
BMI, kg/m ²	0.37	0.10, 0.65	0.07	-0.15, 0.29
SAT, cm ³	78.8	4.9, 152.7	31.7	-26.7, 90.1
VAT, cm ³	41.9	-4.5, 88.4	-11.6	-48.1, 24.8
	Odds Ratio*	95% CI	Odds Ratio*	95% CI
Obesity †	1.10	0.97, 1.25	1.01	0.91, 1.12

Abbreviation: BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

* Models were adjusted for age at examination, and (age at examination)²; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height. Results from distance analyses were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway. Results from PM_{2.5} analyses were scaled to be as equivalent to per 1.5 μg/m³ increase in 2003 annual PM_{2.5} concentrations.

† Defined as BMI ≥ 30 kg/m².

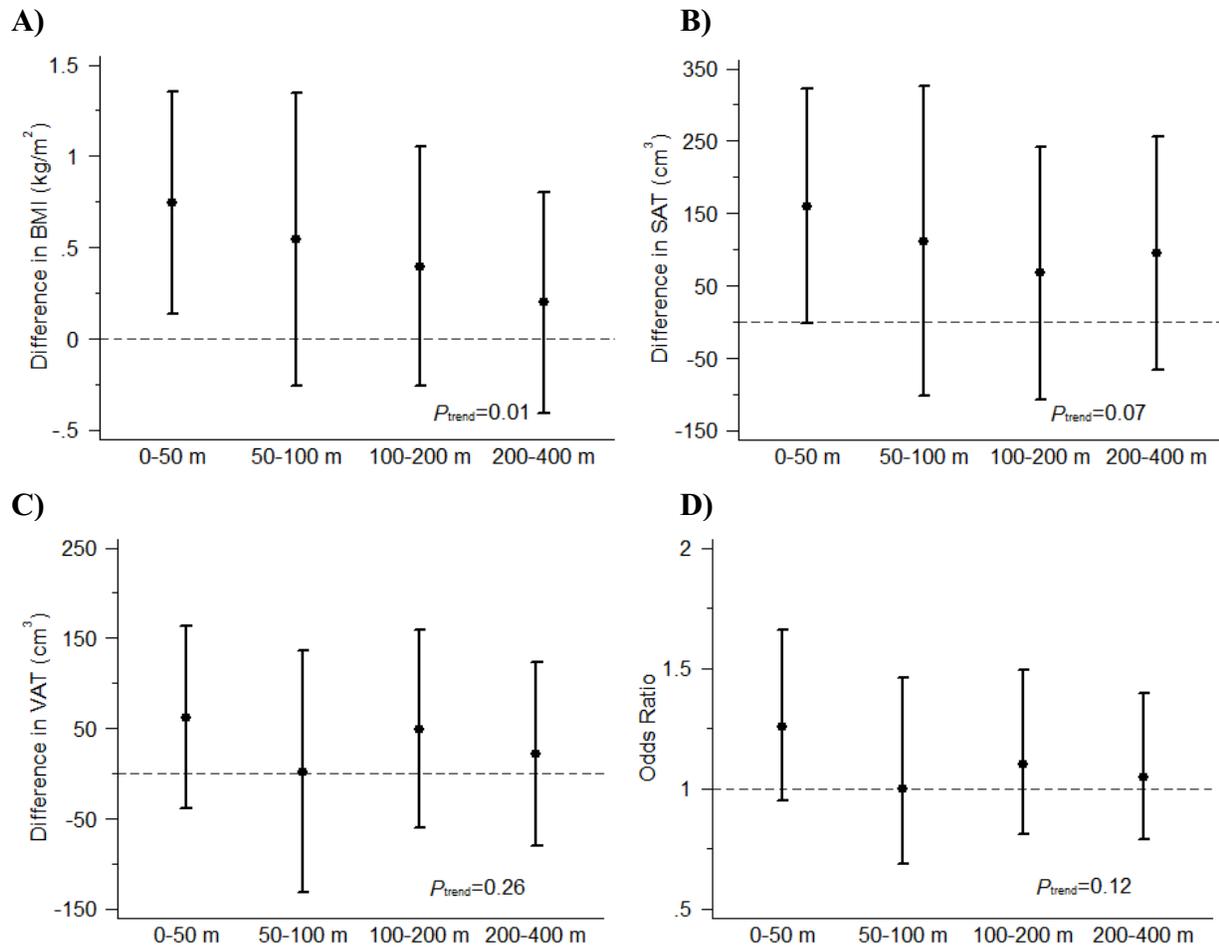


Figure 2.1 The associations between distance categories and (A) body mass index (BMI), (B) subcutaneous adipose tissue (SAT), (C) visceral adipose tissue (VAT), and (D) obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$). Models were adjusted for age at examination, and $(\text{age at examination})^2$; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height. P-values from trend tests are shown in the plots. Error bars indicate the 95% confidence intervals.

Excluding participants who were current smokers or participants who had cardiovascular disease or diabetes did not alter our results substantially. The associations were not altered when using the 2003-2005 average PM_{2.5} or additionally adjusting for time covariates.

We found some evidence of stronger associations between living closer to a major roadway and BMI among younger participants, female participants, or participants with an educational level less than or equal to high school (Supplemental Table 2.2).

Quantile regression analyses between proximity to the nearest major roadway and adiposity measures confirmed our findings in the primary analyses: participants who lived closer to a major roadway had higher BMI, SAT, and VAT than those who lived further away, and the associations were consistent across quartiles of the distribution of each adiposity measure. Additionally, the associations of distance to a major roadway with higher percentiles of VAT appeared stronger than the associations with lower percentiles of VAT (Figure 2.2).

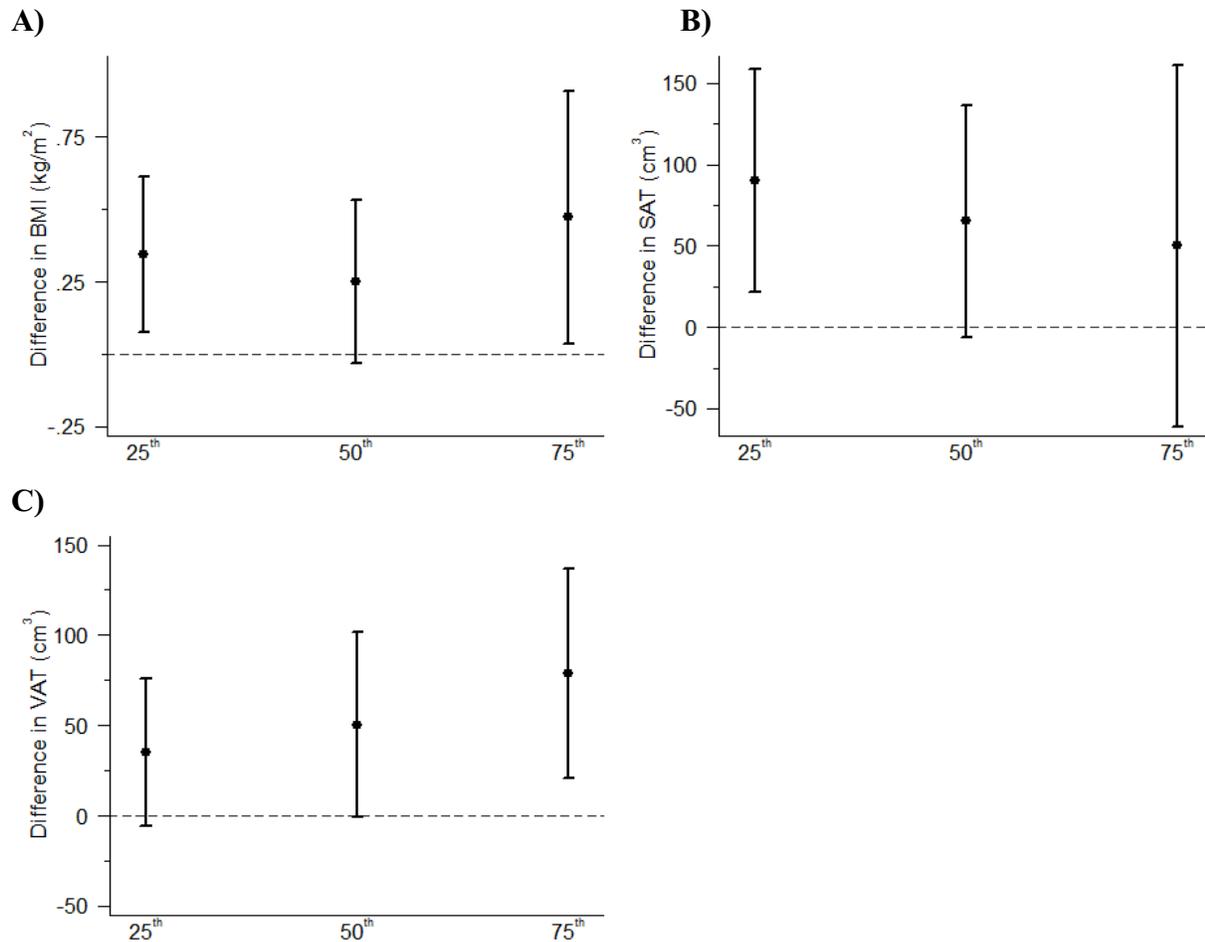


Figure 2.2 Associations between distance to the nearest major roadway and quartiles of the distributions of (A) body mass index (BMI), (B) subcutaneous adipose tissue (SAT), and (C) visceral adipose tissue (VAT). Models were adjusted for age at examination, and (age at examination)²; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant’s census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height. Results were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway. Error bars indicate the 95% confidence intervals.

Discussion

In the present study, living closer to a major roadway was associated with higher BMI. We also observed similar associations between distance to the nearest major roadway and SAT but weaker for VAT. We found no association between annual average PM_{2.5} and BMI or measures of abdominal adiposity. We further found some evidence suggesting stronger associations among participants who had higher VAT.

Few studies have examined and reported the associations of residential proximity to the nearest roadway and ambient PM_{2.5} with BMI and abdominal adipose tissue measures among adults. Our results are consistent with findings from the Southern California Children's Health Study to some extent. In that study, higher levels of traffic-related pollution were associated with higher BMI growth and BMI after four¹⁷ and eight years^{18, 19} of follow-up. In a study conducted among adults, Ponticello *et al.* found higher mean BMI among adult outdoor workers compared with indoor workers.²⁰ However, the authors did not adjust for any characteristics that may have differed between the policemen and indoor workers.

Controlled animal studies have reported that mice exposed to PM_{2.5} had more visceral adipose tissue than mice exposed to filtered air. Among male C57BL/6 mice (6-week old) fed with high-fat chow, after a 24-week PM_{2.5} exposure (72.7 µg/m³; 6 hours/day, 5 days/week), Sun *et al.* found significantly higher visceral fat mass but not subcutaneous fat mass, compared to mice exposed to filtered air.⁷ Among a group of 3-week old male C57BL/6 mice fed with normal diet, exposure to PM_{2.5} (111.0 µg/m³; 6 hours/day, 5 days/week) was associated with an increase in both visceral and subcutaneous fat content compared to mice exposed to filtered air.⁸ In both experiments, exposure to PM_{2.5} induced adipose tissue inflammation and metabolic dysfunction.^{7, 8} However, the exposure concentrations in those animal studies were higher than

the annual average PM_{2.5} concentration in our study, and the relevance of animal data to assessing the association between ambient air pollution and abdominal adiposity in humans is unknown. In the current study, we observed no association between residential-based estimates of annual average PM_{2.5} with adiposity measures among adults living in a region with lower levels of ambient PM_{2.5} concentrations.

This discrepancy between results for the residential proximity to the nearest major roadway and the satellite model-based annual average PM_{2.5} might be explained by differences between these two exposures. The proximity to a major roadway could be viewed as an integrated measure of near-road exposures. It captures information about traffic-related components besides PM_{2.5}, such as traffic-related gaseous pollutants, road dust, traffic noise, traffic light, vibration, and possible psychological stress.^{17, 32} However, the satellite model-based PM_{2.5} estimation captures information about regional background pollution levels, local PM_{2.5} components that may be from emission sources far from major roadways, transported PM_{2.5} components from other regions, and local meteorology conditions.²³ Residual confounding may also cause this discrepancy if there were some unmeasured variables that were consistently associated with living closer to a major roadway and higher adiposity measures but were not associated with satellite model-based PM_{2.5} predictions. However, this was unlikely to be the explanation in our study because we have adjusted for a large set of potential confounders in our models and the influence of residual confounding was expected to be small.

There are several limitations of our study that should be noted. First, participants enrolled in the MDCT study were a relatively healthy cohort and they were predominantly white individuals of European ancestry and middle-aged. Thus, our findings may not be generalizable to other ethnicities and age groups. Second, although we have adjusted for demographic factors,

lifestyle factors, and socio-economic position, we cannot exclude the possibility of residual confounding. Third, the adipose tissue volumes were measured only once for each participant, we were not able to assess progression of adiposity. Last, we did not have statistical power to examine whether the observed associations differ by disease status, such as cardiovascular disease or diabetes. However, excluding participants with cardiovascular disease or diabetes did not materially change our results.

Our study also has several strengths. Our study population was from large well-characterized cohorts with standardized protocols for physical examinations and adipose deposition assessments. The large sample size in our study allowed us to adjust for demographic characteristics, lifestyle factors, and individual- and area-level of socioeconomic position. Although we were not able to directly measure walkability, we have adjusted for individual-level physical activity in the models. We used a novel spatial-temporal model to estimate PM_{2.5} at a high resolution, and we precisely quantified abdominal adipose tissue volumes using MDCT. The technique has high inter- and intra-reader reliability. Last, assessment of air pollution and adiposity were performed independently of each other, which decreased potential differential measurement errors in exposure and outcome assessments.

Conclusion

Among this relatively large cohort of adults living predominantly in the Northeastern U.S., we found evidence that living closer to a major roadway was associated with higher overall and abdominal adiposity. However, we observed no association of annual PM_{2.5} with adiposity measures. Future longitudinal studies with repeated adiposity measures are necessary to confirm or refute our findings and to extend our findings by exploring changes in adiposity measures associated with traffic-related air pollution.

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Supplemental Materials

Supplemental Table 2.1 The associations of 2003 annual PM_{2.5}^a with adiposity measures stratified by median age at the time of CT scan, sex, and education levels.

	BMI		Obesity ^b		SAT		VAT	
	β	95% CI	OR	95% CI	β	95% CI	β	95% CI
Median age ^c								
≤ 51 yr	-0.07	-0.36, 0.23	0.97	0.85, 1.12	-9.0	-87.0, 68.9	-49.2	-97.7, -0.7
> 51 yr	0.22	-0.11, 0.56	1.06	0.91, 1.23	73.5	-15.4, 162.3	28.9	-26.4, 84.2
Sex ^d								
Women	0.19	-0.10, 0.48	1.10	0.96, 1.27	55.3	-22.8, 133.5	17.5	-31.2, 66.3
Men	-0.08	-0.4, 0.25	0.92	0.79, 1.07	2.2	-85.2, 89.5	-48.1	-102.6, 6.4
Educational level ^e								
≤ High school	0.14	-0.41, 0.69	1.01	0.79, 1.29	26.7	-119.9, 173.3	-12.4	-104.0, 79.1
≥ College	0.04	-0.20, 0.28	1.01	0.90, 1.12	26.8	-37.0, 90.6	-13.7	-53.5, 26.2

a. Results from PM_{2.5} analyses were scaled to be as equivalent to per 1.5 μg/m³ increase in 2003 annual PM_{2.5} concentrations.

b. Defined as BMI ≥ 30 kg/m².

c. Models were additionally adjusted for sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we additionally adjusted for height.

d. Models were additionally adjusted for age at examination, and (age at examination)²; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height.

e. Models were additionally adjusted for age at examination, and (age at examination)²; sex; smoking status (current, former, or never); pack years; alcohol intake; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height.

Supplemental Table 2.2 The associations of distance to the nearest major roadway^a with adiposity measures stratified by median age at the time of CT scan, sex, and education levels.

	BMI		Obesity ^b		SAT		VAT	
	β	95% CI	OR	95% CI	β	95% CI	β	95% CI
Median age ^c								
≤ 51 yr	0.55	0.14, 0.97	1.06	0.88, 1.29	154.1	43.5, 264.7	41.3	-28.1, 110.6
> 51 yr	0.20	-0.18, 0.57	1.12	0.95, 1.33	6.8	-93.3, 106.8	36.7	-26.0, 99.4
Sex ^d								
Women	0.52	0.14, 0.90	1.19	1.00, 1.42	108.3	7.1, 209.5	55.6	-7.9, 119.2
Men	0.21	-0.19, 0.61	1.02	0.85, 1.22	45.5	-61.9, 153.0	26.5	-41.0, 93.9
Educational level ^e								
\leq High school	0.50	-0.04, 1.04	1.21	0.95, 1.53	115.2	-28.5, 258.9	86.4	-3.8, 176.6
\geq College	0.34	0.02, 0.67	1.07	0.92, 1.24	70.2	-16.1, 156.4	29.5	-24.6, 83.7

a. Results from distance analyses were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway.

b. Defined as BMI ≥ 30 kg/m².

c. Models were additionally adjusted for sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we additionally adjusted for height.

d. Models were additionally adjusted for age at examination, and (age at examination)²; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height.

e. Models were additionally adjusted for age at examination, and (age at examination)²; sex; smoking status (current, former, or never); pack years; alcohol intake; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. For MDCT analyses, we replaced age at examination with age at CT scan and additionally adjusted for height.

Chapter 3.

Residential Proximity to Major Roadway, Fine Particulate Matter, and Hepatic Steatosis:

The Framingham Heart Study

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Abstract

Background: Exposure to elevated fine particulate matter (PM_{2.5}) has been associated with hepatic steatosis in controlled animal studies. However, few studies assessed the association in humans. We therefore examined the associations of residential-based estimates of PM_{2.5} and proximity to the nearest major roadway with hepatic steatosis, measured by multidetector computed tomography (MDCT), in a large cohort of adults.

Methods and Results: We enrolled 2,590 participants from the Framingham Offspring and Third Generation cohorts who underwent an MDCT scan between 2002 and 2005, after excluding men who reported > 21 drinks/week and women who reported > 14 drinks/week. Based on participants' residential addresses, we calculated each participant's distance to the nearest major roadway and used a spatial-temporal model to estimate the annual mean PM_{2.5}. Liver attenuation was measured by the MDCT scan and liver-to-phantom ratio (LPR) was calculated. A lower value of the LPR represents more liver fat. Distance to major roadway was log_e transformed. We used linear regression models for continuous LPR and logistic models for a binary indicator of hepatic steatosis, defined as LPR≤0.33. Models were adjusted for demographic variables, individual- and area-level measures of socio-economic position, and clinical and lifestyle factors. Participants who lived 58 m (25th percentile) from the nearest major roadway had more liver fat (β =-0.004, 95% CI: -0.007, -0.001) and higher odds of hepatic steatosis (odds ratio=1.26, 95% CI: 1.09, 1.47) than those who lived 416 m (75th percentile) from the nearest major roadway. Residential-based annual average PM_{2.5} was not associated with liver fat attenuation as assessed by MDCT scan.

Conclusions: Living closer to a major roadway was associated with more liver fat and higher odds of hepatic steatosis in this large cohort of adults.

Introduction

Non-alcohol fatty liver disease (NAFLD) refers to the presence of hepatic steatosis, or fatty liver, among individuals in the absence of excessive alcohol use or other causes of secondary fat accumulation.^{1,2} NAFLD is now the most common liver diseases in the United States and NAFLD patients may show a spectrum of alterations ranging from simple hepatic steatosis to steatohepatitis, and cirrhosis.^{1,2}

In animal studies, higher exposure to fine particulate matter (PM_{2.5}; aerodynamic diameter $\leq 2.5 \mu\text{m}$) has been associated with abdominal obesity^{3,4} and hepatic lipid accumulation⁵⁻⁷. Air pollution-induced oxidative stress and inflammation have been proposed as possible underlying mechanisms.⁸⁻¹⁰ Inhaled air pollutants could induce hepatotoxicity by promoting release of pro-inflammatory cytokines into circulation or possibly by translocating into circulation through the alveolar wall.¹¹ Among C57BL/6 mice fed with a high fat chow diet, exposure to PM_{2.5} for 6 weeks was associated with inflammation in Kupffer cells (macrophages in the liver) and greater hepatic steatosis progression compared to mice exposed to filtered air.⁶ Zheng *et al.* found development of hepatic steatosis, inflammation, and fibrosis in the liver of C57BL/6 mice fed a regular chow diet after a 10-week exposure to PM_{2.5}.⁷ It has been hypothesized that higher air pollution may be associated with more liver fat in humans.¹² A few human studies have examined associations between air pollution exposure and aminotransferase levels but have shown mixed results.¹³⁻¹⁶ However, aminotransferase levels are not good measures of hepatic steatosis because the large majority of participants with hepatic steatosis may have normal aminotransferase levels.¹⁷ Computer tomography (CT) is a practical and reliable non-invasive method that assesses hepatic steatosis by measuring attenuation, a marker

of liver fat accumulation.¹⁸ Currently there is a lack of data about the association between air pollution and CT-based measures of hepatic steatosis.

We therefore examined the associations of residential ambient PM_{2.5} pollution and proximity to the nearest major roadway with hepatic steatosis, assessed by multidetector computed tomography (MDCT), among participants from the Framingham Offspring and Third Generation cohorts. In the Framingham Heart Study, MDCT-based liver fat measurement has been shown to have high reproducibility.¹⁹ Our results may provide insight into the hypothesized association between ambient air pollution and hepatic steatosis.

Methods

Study population

We enrolled participants from the Framingham Heart Study Offspring cohort examination 7 (1998-2001) or Third Generation cohort examination 1 (2002-2005) who participated in the MDCT study. Selection criteria and study design of the two cohorts have been previously described.^{20, 21} For inclusion in the MDCT study, men were aged ≥ 35 years old, women were aged ≥ 40 years old and not pregnant, and all participants weighed < 350 lbs (160 kg) because of the physical constraints of the scanner.²² Among 3,206 participants who underwent abdominal CT scan and had valid liver fat measurement, 492 participants did not have valid measurement of annual $PM_{2.5}$, and thus were excluded. Participants in the current analysis were scanned between 2002 and 2005, and data on demographics, medication history, smoking history, and alcohol intake were collected by questionnaires at each cohort's examination visit. Physical examinations were performed following standardized protocols. Alcohol intake was self-reported and standardized as 0.5 oz pure alcohol per drink.²³ We further excluded men who reported > 21 and women who reported > 14 alcoholic drinks/week ($N=122$),²⁴ as in a previous publication,²⁵ or those with missing information on alcohol consumption ($N=2$), leaving a total of 2,590 participants in the current analysis. All participants provided written informed consent, and Institutional Review Boards at Beth Israel Deaconess Medical Center, Boston University Medical Center, and Massachusetts General Hospital approved the study.

$PM_{2.5}$ assessment

We geocoded participants' self-reported home addresses using the ArcGIS software and then estimated ambient $PM_{2.5}$ concentrations at each residential address using a spatio-temporal

model. The model utilized the satellite-based aerosol optical depth data from advanced Multi-Angle Implementation of Atmospheric Correction (MAIAC) algorithms, local monitor-based daily PM_{2.5} mass concentrations, land use terms, and meteorological covariates.²⁶ Briefly, we first fit a model regressing monitor-based PM_{2.5} concentrations against satellite-based aerosol optical depth product, adjusting for land use terms and meteorological variables. We used inverse probability weighting to address non-random missingness of daily aerosol optical depth data. Predictions from this model had an excellent mean out-of-sample R² of 0.88 and an excellent fit when comparing predictions with observations (slope=0.99).²⁶ Second, we predicted grid cells that only had aerosol optical depth data available using the above fitted model. Third, for grid cells/days that had missing aerosol optical depth measurements, we imputed data using a generalized additive model with smoothing and a random intercept for each grid cell. Last, for each residential address, we regressed residuals (differences between monitor-based measurement and predicted values for each cell) against monitor-specific spatial and temporal variables to generate daily local predictions. The total PM_{2.5} daily estimates were then calculated as the sum of grid and localized predictions. We used the annual PM_{2.5} concentration of the same index year (2003) for all participants, similar to our previous work.^{27, 28}

Distance to the nearest major roadway

A major roadway was defined as a primary highway with limited-access (A1), a primary road without limited-access (A2), or a secondary or connecting road (A3). For each participant we calculated residential distance to the nearest major roadway. We restricted our analyses to participants who lived within 1,000 meters from the nearest major roadway (N=2,299) for proximity analyses because distance may not be an informative surrogate of traffic-related air

pollution in semirural or rural areas. As in our previous work,^{27,28} we classified participants into five groups based on the distance of their residential address to the nearest major roadway (0-50, 50-100, 100-200, 200-400, and 400-1,000 m) in order to reflect the typical decreasing pattern of traffic-related air pollutants with distance to road.²⁹

MDCT protocol and assessment of liver fat attenuation

The attenuation of the liver on a CT scan relative to the CT penetrance of a calibration control is a non-invasive method to assess liver fat.^{19,30} Details of liver fat assessment in the MDCT study have been previously described.¹⁹ Briefly, each participant underwent abdominal MDCT scan in the supine position. An eight-slice scanner (LightSpeed Ultra, General Electric, Milwaukee, WI) was used to obtain 25 contiguous 5 mm thick slices (120 kVp, 400 mA, gantry rotation time 500 ms, table feed 3:1) covering 125 mm above the S1 level. The CT Hounsfield units (HU) were measured in three areas of the liver and one area from a white external phantom control (calibration control). We then calculated the liver-to-phantom ratio (LPR) by dividing the average HU of the liver by the HU of the phantom control.¹⁹ A lower value of the LPR represents more liver fat. In a validation study with 100 MDCT participants, the MDCT-based LPR was shown to be reproducible with high intra-class correlation (ICC) for both intra-reader (ICC=0.99) and inter-reader (ICC=0.99) comparisons.¹⁹

To maximize our statistical power, we analyzed the LPR as a continuous variable and we additionally defined the presence of hepatic steatosis as a $LPR \leq 0.33$. This cutpoint has been shown to have a sensitivity of 70% and specificity of 98% for detecting hepatic steatosis compared to using liver-to-spleen ratio of 1.1 as the gold standard.³¹

Statistical methods

We used multivariable linear regression models for continuous LPR, and multivariable logistic regression models for binary indicator of hepatic steatosis. We adjusted for age at CT scan, and (age at CT scan)²; sex; smoking status (current, former, or never); pack years of smoking; alcohol intake (drinks/week); educational level; physical activity index (self-reported questionnaire);³² anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; diabetes; and a cohort identifier. Based on our previous work^{33, 34} and our hypothesis about the dispersion pattern of air pollutants, residential distance to the nearest major roadway was log_e transformed.

We conducted several sensitivity analyses. We assessed the extent of possible residual confounding by adding BMI to the models or excluding participants with cardiovascular disease or diabetes. To assess the influence of our exclusion criteria on alcohol, we excluded men who reported >14 and women who reported >7 alcohol containing drinks/week, which is stricter than our inclusion criteria. We also explored whether associations differed by median age, sex, or a binary educational level indicator (high school or less vs. some college or higher) by adding interaction terms to the model. To explore whether participants who had certain levels of liver fat were more affected by exposure, we applied quantile regression analyses and examined differences in associations at the 25th, 50th, and 75th percentiles of LPR. Additionally, we evaluated the influence of choosing the year 2003 as the index by examining the associations between three-year average PM_{2.5} (2003-2005) and liver fat attenuation, and we evaluated the influence of adjusting for time by including date of CT scan (continuous variable), days between CT scan and examination visit, and the year of CT scan (categorical variable) in the models.

Results for the residential-based estimated 2003 annual average PM_{2.5} analyses were scaled to 1.5 µg/m³, which approximated the interquartile range. Results from distance to the nearest major roadway analyses were scaled as contrasting participants who lived 58 m (25th percentile) from the major roadway to those who lived 416 m (75th percentile) from the major roadway. Linear trend tests for road category analyses were conducted by assigning the median value of log_e-transformed distance of each category to participants in that category and then replacing the categorical distance indicator variables in the models with the new continuous variable.

Scaled regression coefficients and odds ratios (ORs) were reported with 95% confidence intervals (CIs). A two-tailed p-value of less than 0.05 was considered statistically significant. Analyses were performed using Proc GLM, Proc GENMOD, and PROC QUANTREG in SAS 9.4 (SAS Institute, Inc., Cary, NC). Figures were plotted using Stata 13 (StataCorp LP, College Station, TX).

Results

Table 3.1 shows the characteristics of the study population. The mean age of the study population at the time of MDCT study was 52.9 (standard deviation [SD]: 12.0) years; 52.7% were women. The residential-based estimated 2003 annual average PM_{2.5} concentration was 10.6 µg/m³, which was lower than the current U.S. Environmental Protection Agency's National Ambient Air Quality Standard (12.0 µg/m³). Of the whole study sample (N=2,590), 17.3% (N=448) had hepatic steatosis (LPR ≤0.33).

Table 3.1 Characteristics of the 2,590 participants from the multidetector computed tomography (MDCT) study (2002-2005)

	N (%) or Mean [SD]
Offspring cohort	1,090 (42.1%)
Age*, years	52.9 [12.0]
Women	1,364 (52.7%)
Alcohol, drinks/week	3.8 [4.6]
Current smoker	313 (12.1%)
Former Smoker	1,021 (39.4%)
Education	
< High school	45 (1.7%)
High School	562 (21.7%)
Some college	831 (32.1%)
College graduate	1,147 (44.3%)
Antihypertensive medication use	491 (19.0%)
Statins use	329 (12.7%)
Cardiovascular disease	151 (5.8%)
Diabetes	171 (6.6%)
Liver-to-phantom ratio	0.36 [0.05]
Hepatic steatosis‡	448 (17.3%)
2003 annual PM _{2.5} , µg/m ³	10.6 [1.4]
Distance†, m	270 [252]
Distance categories†	
0-50 m	533 (20.6%)
50-100 m	220 (8.5%)
100-200 m	407 (15.7%)
200-400 m	534 (20.6%)
400-1000 m	605 (23.4%)

Abbreviation: SD, standard deviation; PM_{2.5}, fine particulate matter.

* Age at the time of CT scan.

† 291 participants lived more than 1,000 meters from the nearest major roadway and were excluded.

‡ Liver-to-phantom ratio ≤ 0.33.

Comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m), living closer to a major roadway was associated with more liver fat (lower LPR) ($\beta=-0.004$, 95% CI: -0.007, -0.001) (Table 3.2). Consistent with this observation, participants who lived within 100 m from the nearest major roadway had more liver fat (lower LPR) than those who lived 400-1000 m from the nearest major roadway (Figure 3.1A). We also observed higher odds of hepatic steatosis (LPR \leq 0.33) for participants who lived closer to a major roadway (OR=1.26, 95% CI: 1.09, 1.47) (Figure 1B). However, the 2003 annual PM_{2.5} was not associated with LPR or hepatic steatosis (LPR \leq 0.33) (Table 3.2).

Table 3.2 Associations of distance to a major roadway and 2003 annual average PM_{2.5} with liver-to-phantom ratio and presence of hepatic steatosis.

	Closer to a major roadway		2003 annual average PM _{2.5}	
	β^*	95% CI	β^*	95% CI
Liver-to-phantom ratio	-0.004	-0.007, -0.001	-0.0001	-0.002, 0.002
	Odds Ratio*	95% CI	Odds Ratio*	95% CI
Hepatic steatosis †	1.26	1.09, 1.47	0.95	0.84, 1.08

* Models were adjusted for age at CT scan, and (age at CT scan)²; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes. Results from distance analyses were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway. Results from PM_{2.5} analyses were scaled to be as equivalent to per 1.5 $\mu\text{g}/\text{m}^3$ increase in 2003 annual PM_{2.5} concentrations.

† Defined as liver-to-phantom ratio ≤ 0.33 .

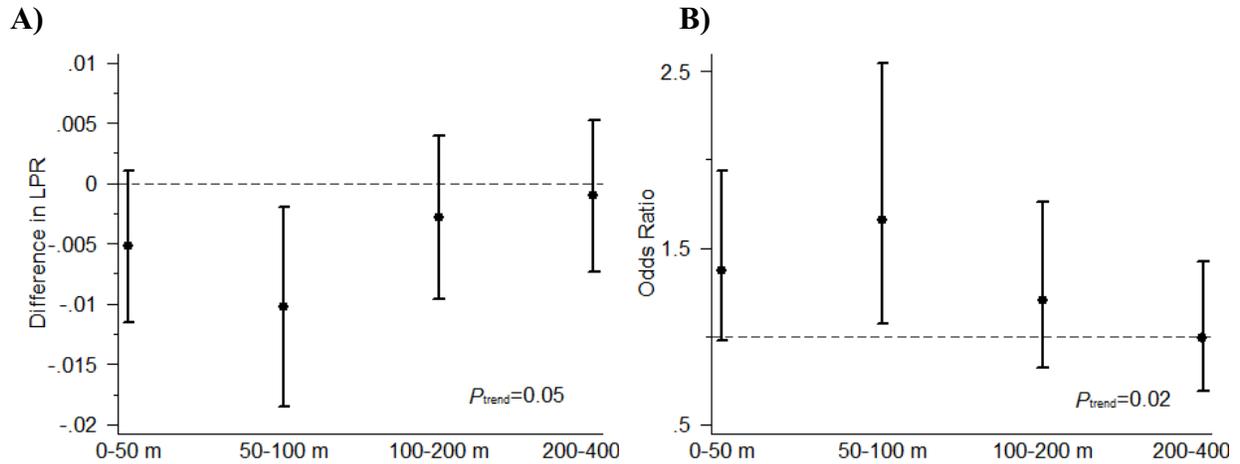


Figure 3.1 The associations between distance to the nearest major roadway and (A) liver-to-phantom ratio (LPR) and (B) hepatic steatosis ($LPR \leq 0.33$). Models were adjusted for age at CT scan, and (age at CT scan)²; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant’s census tract in 2000; cardiovascular disease; diabetes; and an exam identifier. P-values from trend tests are shown in the plots. The Error bars indicate the 95% confidence intervals.

Results from the stratified analyses showed stronger associations between distance to the major roadway and LPR among women or participants who had lower educational levels across multiple distance categories (Supplemental Table 3.2). Quantile regression analyses suggested stronger associations between proximity and liver fat among participants who had LPR in the lower quartiles (i.e. had more liver fat) than those who had less liver fat (Figure 3.2).

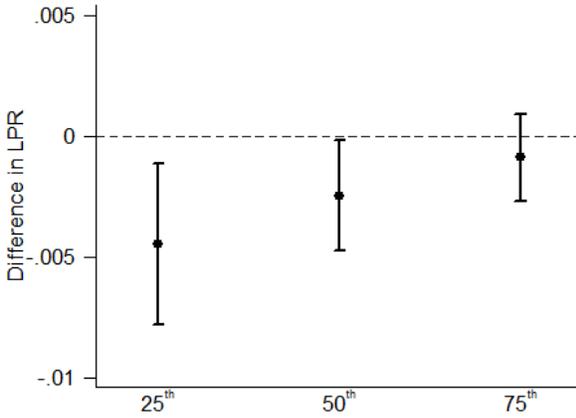


Figure 3.2 Associations between distance to the nearest major roadway and the 25th, 50th, and 75th percentiles of the distribution of liver-to-phantom ratio (LPR). Models were adjusted for age at CT scan, and (age at CT scan)²; sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant’s census tract in 2000; cardiovascular disease; diabetes; and cohort identifier. Results were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway. Error bars indicate the 95% confidence intervals.

Adding BMI to the models, excluding men who reported >14 and women who reported >7 drinks/week, or excluding participants with cardiovascular disease or diabetes did not change our results substantially. The associations were not altered when using the 2003-2005 average PM_{2.5} or adjusting for time covariates.

Discussion

In the current study, we observed more liver fat and higher odds of hepatic steatosis ($LPR \leq 0.33$) among participants who lived closer to a major roadway than those who lived further away. We found no association between residential-based estimates of annual average $PM_{2.5}$ and liver fat attenuation. We further showed that the observed associations might be stronger among participants who had more liver fat. To our knowledge, few studies have examined and reported these associations in adults.

Our current study extended findings in animal studies to a large cohort of adults. Reports from controlled animal studies suggested positive associations between $PM_{2.5}$ and overall and abdominal obesity^{3,4} as well as liver fat accumulation.⁵⁻⁷ For example, after exposing mice fed high fat chow to $PM_{2.5}$ for 6 weeks ($85 \mu\text{g}/\text{m}^3$, 6 hours/day), Tan *et al.* found a higher degree of hepatic inflammation and fibrosis than mice that were exposed to filtered air.⁶ In another study, mice fed with regular chow that were exposed to $PM_{2.5}$ for 10 weeks ($74.6 \mu\text{g}/\text{m}^3$, 6 hours/day) showed a non-alcoholic steatohepatitis-like phenotype, with disrupted hepatic glycogen storage, glucose tolerance, and insulin resistance.⁷ However, the $PM_{2.5}$ concentrations in the controlled animal studies were higher than the ambient $PM_{2.5}$ levels in our study and the relevance of animal studies to assessing the associations of ambient air pollution with hepatic steatosis in humans is unclear.

Individuals with diabetes may be more susceptible to the effects of air pollution on the liver fat. In an animal study, Tomaru *et al.* found fatty changes in livers of diabetic obese mice after intra-tracheally administering $100 \mu\text{g}$ diesel exhaust particles every two weeks for 12 to 18 weeks, but not among nondiabetic mice.⁵ We did not have sufficient statistical power to examine

whether the observed associations differ by diabetes status. However, excluding participants with cardiovascular disease or diabetes did not materially change our results.

In the current study we found positive associations between living closer to a major roadway and more liver fat, whereas the positive associations were not observed for the annual average PM_{2.5}. This discrepancy might be explained by differences in these two exposure metrics. We conceptualize the distance to a major roadway as a measure that is more related to near-road exposure than the satellite model-based PM_{2.5} predictions; it represents multiple traffic-related factors such as vehicle emissions (particulate and gaseous pollutants), road dust, traffic noise, traffic light, and possible psychological stress induced by these near-road exposures.^{35, 36} Whereas residential-based PM_{2.5} concentrations estimated by the spatial-temporal model may take account of some factors that are not closely related to local traffic, such as regional background pollution levels, pollutants transported from other regions, pollutants from point emissions far from major roadways, and local weather conditions.²⁶ Additionally, as in any observational study, this discrepancy could also be due to residual confounding that was highly correlated with living closer to a roadway and more liver fat but not with PM_{2.5} predictions. However, considering that we have adjusted for a large set of potential confounders in our models, the impact of residual confounding is expected to be small.

There are several limitations of our study that should be considered. Participants who joined the MDCT study were relatively healthy and were predominantly white individuals of European ancestry and middle-aged. As a result, our observations may not be generalizable to populations of different ethnicities or age groups. As in any observational study, we cannot exclude the possibility of residual confounding or unmeasured confounding. However, we

adjusted for potential confounders including demographic characteristics, lifestyle, and individual- and area-level of socioeconomic position in our models.

Our study also has several strengths. The study population was composed of participants enrolled in relatively large and well-characterized cohorts. Data were collected using standardized protocols for physical examinations and MDCT scan. We constructed models that adjusted for a robust set of potential confounders including demographic characteristics, lifestyle, and individual- and area-level measures of socioeconomic position. We also adjusted for individual-level physical activity in the analyses. We employed a novel spatial-temporal model to estimate annual average PM_{2.5} concentrations at participants' home addresses. We used MDCT, a technique with high inter- and intra-reader reliability, to quantify liver fat attenuation. Finally, the assessment of air pollution and liver fat were performed independently of each other.

Conclusions

Living closer to a major roadway was associated with more liver fat (lower LPR) and higher odds of hepatic steatosis ($LPR \leq 0.33$). However, we observed no association of residential-based annual average $PM_{2.5}$ with liver fat measurement. Future longitudinal studies with repeated liver fat measures are warranted to confirm or refute our findings, and to extend these results by examining progression of liver fat accumulation in relation to ambient air pollution.

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Supplemental Materials

Supplemental Table 3.1 The associations of 2003 annual PM_{2.5}^a with liver-to-phantom ratio (LPR) and presence of hepatic steatosis stratified by median age at the time of CT scan, sex, and education levels.

	Liver-to-phantom ratio		Hepatic steatosis ^b	
	β	95% CI	OR	95% CI
Median age ^c				
≤ 51 yr	0.0017	-0.0013, 0.0046	0.84	0.71, 1.00
> 51 yr	-0.0026	-0.0061, 0.0010	1.10	0.91, 1.33
Sex ^d				
Women	-0.0016	-0.0046, 0.0014	1.11	0.94, 1.31
Men	0.0020	-0.0015, 0.0054	0.81	0.68, 0.97
Educational level ^e				
≤ High school	-0.0002	-0.0057, 0.0053	1.01	0.75, 1.35
≥ College	-0.0001	-0.0026, 0.0024	0.94	0.82, 1.08

a. Results from PM_{2.5} analyses were scaled to be as equivalent to per 1.5 μg/m³ increase in 2003 annual PM_{2.5} concentrations.

b. Defined as liver-to-phantom ratio ≤ 0.33.

c. Models were additionally adjusted for sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

d. Models were additionally adjusted for age at scan, and (age at scan)²; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

e. Models were additionally adjusted for age at scan, and (age at scan)²; sex; smoking status (current, former, or never); pack years; alcohol intake; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

Supplemental Table 3.2 The associations of distance to the nearest major roadway^a with liver-to-phantom ratio (LPR) and presence of hepatic steatosis stratified by median age at the time of CT scan, sex, and education levels.

	Liver-to-phantom ratio		Hepatic steatosis ^b	
	β	95% CI	OR	95% CI
Median age ^c				
\leq 51 yr	-0.0043	-0.0086, 0.0001	1.27	1.01, 1.61
$>$ 51 yr	-0.0036	-0.0075, 0.0003	1.25	1.03, 1.52
Sex ^d				
Women	-0.0050	-0.0088, -0.0012	1.49	1.22, 1.82
Men	-0.0027	-0.0071, 0.0017	1.03	0.82, 1.30
Educational level ^e				
\leq High school	-0.0072	-0.0127, -0.0016	1.52	1.16, 1.98
\geq College	-0.0029	-0.0063, 0.0005	1.18	0.98, 1.41

a. Results from distance analyses were scaled to approximate comparing participants who lived at the 25th percentile (58 m) to those who lived at the 75th percentile (416 m) from the nearest major roadway.

b. Defined as liver-to-phantom ratio \leq 0.33.

c. Models were additionally adjusted for sex; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

d. Models were additionally adjusted for age at scan, and (age at scan)²; smoking status (current, former, or never); pack years; alcohol intake; educational level; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

e. Models were additionally adjusted for age at scan, and (age at scan)²; sex; smoking status (current, former, or never); pack years; alcohol intake; physical activity; anti-hypertensive medication use; statin use; quartile of median household income in the participant's census tract in 2000; cardiovascular disease; and diabetes.

Final Remark

Obesity is a major public health problem in the United States, and it is an important risk factor for non-alcoholic liver disease, one of the common liver diseases in the United States. Previous controlled animal studies and limited human observational studies suggested associations of air pollution with obesity and liver fat accumulation. Our work presented supportive evidence by demonstrating that participants who lived closer to the major roadways had higher body mass index (BMI), higher abdominal adiposity, and more liver fat than those who lived further away.

In Chapter 1, we examined the associations between short-term air pollution with biomarkers of oxidative stress. We found positive associations of black carbon with myeloperoxidase, and of fine particulate matter (PM_{2.5}) and sulfate with 8-epi-prostaglandin F_{2α}. Our findings added evidence to the current literature and provided rationale to explore the associations between ambient air pollution with abdominal adiposity and liver fat accumulation, because oxidative stress and inflammation were hypothesized as the potential underlying mechanisms that mediate the associations between air pollution and adiposity. The differential associations by diabetes status indicated potential susceptibility among participants with diabetes.

In Chapter 2, we analyzed existing data from the multidetector computed tomography (MDCT) study, a substudy that enrolled participants from the Framingham Offspring and Third Generation cohorts. This study provided us with valuable opportunities to examine the associations between ambient air pollution and abdominal adiposity. In this study, we found higher BMI and higher abdominal adiposity among participants who lived closer to a major roadway than those who lived further away. These findings confirmed and extended the

associations observed in controlled animal studies to adults. Our findings also indicated that some traffic-related components besides fine particulate matter may be associated with overall and abdominal adiposity.

In Chapter 3, by using the same exposure assessment, we analyzed liver attenuation data from the MDCT study and found that participants who lived closer to a major roadway had more liver fat than those who lived further away.

Our analyses were conducted among adults from a large community-based cohort. While we have a relatively large sample size, our participants were middle-aged and were white individuals with European ancestry, which limited the generalizability of our findings to other age groups or ethnicity groups. Our study region had relatively low air pollution levels which limited the extrapolation capability of our results. Furthermore, the discrepancy in associations of residential proximity to the major roadways and the residential-based annual average PM_{2.5} with adiposity measures suggested there might be other traffic-related components that mediate the hypothesized associations between air pollution and adiposity. Future studies, especially in heavier polluted areas or with study designs that could evaluate traffic-related components, are necessary.