Long-Term Exposure to Ambient Ozone and Progression of Subclinical Arterial Disease: The Multi-Ethnic Study of Atherosclerosis and Air Pollution

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BACKGROUND: Long-term ozone (O3) exposure is associated with cardiovascular mortality, but little is known about the associations between O3 and subclinical arterial disease.

OBJECTIVES: We studied the longitudinal association of exposure to O3 and progression of key subclinical arterial markers in adults: intima-media thickness of common carotid artery (IMTCCA), carotid plaque (CP) burden, and coronary artery calcification (CAC).

METHODS: CAC was measured one to four times at baseline and at follow-up exams (1999–2012) by computed tomography (CT) in 6,619 healthy adults, recruited at age 45–84 y without cardiovascular disease (CVD), over a mean of 6.5 y (standard deviation: 3.5 y). IMTCCA and CP burden were quantified in 3,392 participants using carotid artery ultrasound imaging acquired over a mean of 9 y (1.7 y). Over 91% and 89% participants had at least one follow-up IMTCCA and CAC measurement, respectively. Residence-specific O3 concentrations were estimated by a validated spatiotemporal model spanning from 1999 to 2012. This model relied on comprehensive monitoring data and geographical variables to predict individualized long-term average concentrations since baseline. Linear mixed models and logistic regression model were used to evaluate relationships of long-term average exposure to O3 with longitudinal change in IMTCCA, CAC, and CP formation, respectively.

RESULTS: Mean progression rates of IMTCCA and CAC were 0.12 ± 0.5 μm and 25 ± 1.4 Agatston units per year. CP formation was identified in 55% of the subjects. A 3-ppb increase in long-term average O3 exposure was associated with a 5.6-μm [95% confidence interval (CI): 1.4, 9.7] greater increase in IMTCCA over 10 y. A 3-ppb increase in O3 was also associated with new CP formation [odds ratio (OR): 1.2 (95% CI: 1.1, 1.4)] but not CAC progression [8 Agatston units (95% CI: –18, 2)]. Associations were robust in the analysis with extended covariate adjustment, including copollutants, i.e., nitrogen oxides (NOx) and particulate matter with diameter <2.5 μm (PM2.5).

CONCLUSION: Over almost a decade of follow-up, outdoor O3 concentrations were associated with increased rate of carotid wall thickness progression and risk of new plaque formation, suggesting arterial injury in this cohort. https://doi.org/10.1289/EHP3325

Introduction

Ground-level ozone (O3) is a powerful oxidizing agent and is one of the most harmful air pollutants currently addressed by air quality standards in the European Union and United States. Over recent decades, O3 has not shown a discernible trend of decline in Europe and the United States, and it will likely remain an important environmental health issue, especially given projected increases in temperature related to climate change (EEA 2015), since ground-level ozone is formed when a complex set of chemical reactions is triggered by heat and sunlight (U.S. EPA 2013). Strong and consistent evidence for health effects of O3 exposure has been demonstrated for the short-term exposure effects on the respiratory system (EEA 2011; U.S. EPA 2013). The literature on cardiovascular effects of long-term exposure to O3 have been considered less consistent (U.S. EPA 2013; Jerrett et al. 2009) and focused primarily on mortality effects reported previously.

The underlying association between long-term exposure to O3 and subclinical vascular disease (Breton et al. 2012). Intima-media thickness of common carotid artery (IMTCCA) and coronary artery calcification (CAC) are noninvasive markers of subclinical arterial disease that predict risk of coronary heart disease and stroke in people without cardiovascular diseases (CVDs) (Stein et al. 2008). Compared to IMTCCA, carotid plaque (CP) represents a later stage of arterial injury where diffuse thickening of the intima media complex accelerates focally, leading to atherosclerotic plaque formation (Stein et al. 2008). CP presence and burden are associated with increased risk of CVD events, and this measure improves the predictive accuracy of IMTCCA (Gepner et al. 2015).

To date, the association of long-term exposure to O3 and IMTCCA has only been reported in a cross-sectional study of schoolchildren (Breton et al. 2012). Findings on the effects of O3 exposure on progression of IMTCCA, CAC, and CP have not been reported previously.

The Multi-Ethnic Study of Atherosclerosis (MESA, https://www.mesa-nhlbi.org/) is a population-based prospective cohort study of adults free of CVD at baseline with repeated measurements of IMTCCA, CAC, and CP for up to 10 y. This provides a unique opportunity to assess the longitudinal relationship between long-term exposure to O3 and progression of IMTCCA, CAC, and CP in a well-characterized cohort of adults.

Methods

Study Population

Study objectives and design have been previously published (Kaufman et al. 2012). MESA enrolled 6,814 participants aged 45–84 y without a clinical history of CVD in six U.S. city regions (Baltimore, Maryland; Chicago, Illinois; Los Angeles County,
Carotid Artery Measurements

Ultrasound images were acquired from the baseline examination (Exam 1, 2000–2002) and follow-up examinations between 2005–2007 (Exam 4) and/or between 2010–2012 (Exam 5).

Details of the measurements of the common carotid artery IMTCCA and CP have been described previously (Gepner et al. 2015; Tattersall et al. 2014). In brief, high-resolution B-mode ultrasound images of the right and left common, bifurcation, and internal carotid artery segments were recorded at all exams with the same ultrasound model (Logiq 700, GE Medical Systems) using the M12L transducer (GE Medical Systems) with a standardized imaging protocol. Trained and certified sonographers from all six MESA sites acquired images for all three examinations. All ultrasound images for this analysis were centrally read and interpreted by the University of Wisconsin Atherosclerosis Imaging Research Program’s MESA Carotid Ultrasound Reading Center.

IMTCCA was defined as the IMT measured as the mean of the left and right mean far wall distal CCA wall thickness. CP burden was defined as the number of CPs (0–200) – 20–30, one to four) for CAC.

Exposure Assessment

A detailed description of the methodology for estimating long-term outdoor, residence-specific O₃ concentrations has been published elsewhere (Wang et al. 2015). In brief, region-specific spatiotemporal exposure models developed for O₃ were based upon continuous daily measurements (1999–2013) from the Air Quality System of the U.S. Environmental Protection Agency (U.S. EPA), and the spatially dense supplementary monitoring data specific to the MESA Air Study to capture time-varying trends and spatial gradients of O₃ included monitoring at a sample of participant homes. The models incorporated a large number of geographical variables covering a wide diversity of geographic features, such as traffic, industrial emissions, population density, and land use. The performance of city-specific models ranged from good to excellent as assessed by the overall cross-validation $R^2$ (range: 0.61–0.90) at participants’ homes (Wang et al. 2015).

Exposures were estimated longitudinally and cross-sectionally. Longitudinal exposures, i.e., long-term average, were time-varying mean predictions between the baseline exam and each follow-up exam. To avoid possible seasonal differences of exposure due to different starting and ending dates among the participants, we averaged the time- and location-specific 2-wk predictions, rounding the time period to the nearest whole year between recruitment and each examination. Cross-sectional exposures were assigned the concentrations for the year preceding the baseline exam.

In the sensitivity analysis, we explore the different exposure time windows, including the 1-y average exposure prior to each exam and O₃ exposures averaged only in the warm season (April to September) between baseline and follow-up exams.

Statistical Analysis

Our primary interest is to assess the longitudinal relationship between long-term O₃ exposure and progression of mean IMTCCA and CAC over time. This association was estimated using linear mixed-effect models with random slopes and intercepts for each participant and for each Census tract. The model analyzed longitudinal relationships of the outcome (IMTCCA or CAC) with long-term average air pollution exposure and other risk factors (Kaufman et al. 2016). We controlled for cross-sectional effects in this analysis because the cross-sectional relationships of outcomes with air pollution and other risk factors can produce biased results in a progression analysis (Yanez et al. 2002). This model has three components: a) the cross-sectional relationship between the baseline outcome and values of covariates at baseline, b) the longitudinal relationship for rate of change, and c) measurement error associated with outcome, as follows:

$$ Y_{iv} = (x_{0} + X_{0}x_{1} + e_{0}) + (t_{0} + t_{1} + W_{iv}x_{2} + e_{iv}$$

where $Y_{iv}$ is the outcome measurement (IMTCCA or CAC) for subject $i$ at $v$th follow-up exam, and $X_{0}$, $t_{0}$, and $W_{iv}$ are the time-invariant covariates at baseline for subject $i$, including cross-sectional confounders, risk factors, and mean $O_3$ exposure during the baseline year. $W_{iv}$ are the time-varying longitudinal covariates at exam $v$ for subject $i$, including possible confounders, other risk factors, and mean $O_3$ exposure during the time period between baseline ($v = 0$) and $v$th follow-up exam, rounded to the nearest whole year or annual average $O_3$ prior.
to each exam, \( t_i \), is the time in years from baseline (\( v = 0 \)) to the \( i \)th follow-up exam for subject \( i \), \( \beta_0 \) is the average outcome progression (annual rate of change) when \( W_0 = 0 \), \( \beta_1 \) are the coefficients for the interaction between longitudinal covariates and time; this includes the \( O_3 \) exposure by time interaction, which is interpreted as a rate (association between air pollution and annual progression). \( \gamma_0 \) is the average outcome at baseline when \( X_0 = 0 \). \( \gamma_1 \) are the coefficients for cross-sectional associations between baseline outcome and covariates (including baseline \( O_3 \) exposure). \( \epsilon_i \) is the subject-specific random slope and intercept. \( \epsilon_v \) is the error associated with \( Y_{iv} \).

We developed models in stages. The base model (Model 1) included age, sex, race/ethnicity, and study region. For CAC, the base model also adjusted for CT scanner types. The moderate adjustment model (Model 2) added various risk factors [body mass index, smoking (status and pack-years), secondhand smoke exposure].
exposure, alcohol consumption, physical activity (metabolic equivalents: minutes/week by quartiles from low to vigorous activity at baseline), employment status, high-density lipoprotein cholesterol, total cholesterol, and statin use. Pack-years smoking provided information on smoking duration in addition to smoking status. The primary model (Model 3) for reporting results further adjusted for neighborhood (2000 U.S. Census tract level) socioeconomic status (SES) index [continuous variable constructed by factor analysis of six indicators of neighborhood-level SES, i.e., wealth, income, education, employment, and occupation, with higher value indicating more socioeconomic disadvantage (Diez Roux et al. 2001)], individual baseline social economic factors, e.g., income (continuous) and education categories (less than high school, high school, some college/technical college, or graduate school), blood pressure, hypertension (defined as systolic blood pressure $\geq 140$ mm Hg, diastolic blood pressure $\geq 90$ mm Hg, or reported use of antihypertensive medication), antihypertensive medications, and diabetic categories (normal, impaired fasting glucose, diabetic). Medication use was defined as any positive report of a statin and/or antihypertensive medication use on the medication inventory for the participants at each of the five clinical exams. These covariates were selected \textit{a priori} according to the findings from former studies that may potentially influence the exposure–outcome association (Gassett et al. 2015). In secondary analysis (Model 4), we extended our regression models by adjusting for CVD risk biomarkers and family history of premature CVD. Family history of premature CVD was defined as myocardial infarction/heart attack, stroke/brain attack, or cardiovascular procedure (coronary bypass or balloon angioplasty) in a female primary relative (parent, sibling, or child) aged $<65$ y or a male primary relative aged $<55$ y.

For CP, logistic regression models were used to estimate an odds ratio (OR) for association between long-term O3 exposure (between the last exam and baseline) and CP formation (any increase in CP burden from baseline to the last measured visit) in the same cohort for all individuals with repeat measurements. Furthermore, CP incidence among the population with no CP at baseline (i.e., baseline CP burden is zero) was also examined.

We evaluated potential effect modification by age category ($<60$ y or $\geq 60$ y), gender, race (white vs. black, Hispanic, and Chinese), smoking status (never vs. former and current), diabetes, hypertension, statin therapy, and presence of CP at baseline using three-way interaction terms between study time ($t_{\text{obs}}$), the effect modifier, and exposure concentration.

To assess the concentration–response relationship of O3 with the subclinical outcomes, we refitted the model using a natural spline with 4 degrees of freedom for long-term average O3 concentrations. In sensitivity analyses, we explored health effects using the 1-y average exposure prior to each exam and O3 exposures in the warm season (April to September) for each participant as a proxy for long-term exposure. Additional sensitivity analyses included addition of copollutants, i.e., nitrogen oxides (NO$_x$) and particulate matter with diameter $<2.5$ lm (PM$_{2.5}$), in the models. Interquartile range (IQR) increases in O3 (3 ppb) for each study region were used to express the model parameter estimates. All analyses were performed using SAS (version 9.4; SAS Institute).

**Results**

**Study Participants**

Of the 3,640 participants with IMT$_{CCA}$ and any CP measurements, 3,392 had estimated outdoor residential O3 concentrations for the year of their baseline exam and also during the follow-up period (Figure 1), and 91% of the participants had at least one follow-up ultrasound measurement. Table 1 shows the population characteristics for all the participants by study regions. Mean follow-up duration ranged from 8.4 (Los Angeles) to 9.2 y (St. Paul and Chicago), and participants were, on average, 60 y old at baseline. Approximately equal numbers of men and women were included. Almost half were lifelong nonsmokers (49%), one-third were non-Hispanic white (39%), and two-thirds had at least a college education (70%). Moreover, 41% of participants had hypertension, 10% had diabetes, and nearly 47% had CP at baseline.

For CAC, 6,619 participants had estimated O3 concentrations, and 89% had at least one follow-up CAC measurement, with mean follow-up duration of 6.5 y (Figure S1; Table S2). The
small subgroup with only baseline data had similar characteristics compared with the majority with longitudinal data.

Long-term average O₃ concentrations at baseline varied substantially within and across the study regions (Figure 2A; Table S3), with the highest mean value and variation in Winston-Salem and the lowest in New York (Table 1). Mean O₃ concentrations at the participants' homes remained constant over time in each study region (Figure 2B). Correlations of predictions of O₃ with NOₓ and PM₂.₅ were negative and relatively low within each study region (Table S4). Unadjusted mean IMTCCA and CAC at baseline were 760 μm and 194 Agatston units, respectively. Mean (± standard deviation) progression of IMTCCA and CAC were 12 ± 0.5 μm/year and 25 ± 1.4 Agatston units/year (Table 1; Table S2). Among all participants, 1,767 (53%) had CP progression, of which 1,036 (31%) without CP at baseline developed new CP during follow-up.

O₃ and IMTCCA. In the longitudinal analysis, an IQR increment in long-term average O₃ exposure (≈3 ppb) was associated with a 5.6-μm (95% CI: 1.4, 9.7) faster increase in IMTCCA over 10 y (Table 2). The association was not sensitive to additional adjustment for family history of CVD and relevant biomarkers. In sensitivity analyses, the measure of association became slightly larger with long-term average O₃ exposures that were based only on the warm season (6.5 μm; 95% CI: 2.6, 10.3) but was much smaller using the 1-y average exposure prior to each exam (3.5 μm; 95% CI: 0.1, 7.1). Addition of PM₂.₅ or NOₓ as covariates did not weaken the associations with exposure to O₃. There is little evidence of a nonlinear relationship between O₃ concentration and change in IMTCCA (Figure S2). We found suggestive evidence of effect modification by CP, with larger effect estimates in those without CP at baseline (Table 3).

O₃ and carotid plaque. The 3-ppb increment in long-term O₃ exposure was also associated with CP formation (OR: 1.2; 95% CI: 1.1, 1.4) (Table 2), with little evidence of nonlinearity observed (Figure S2). These ORs were stable with little change for the different covariate adjustments. Effect estimates were smaller when different exposure metrics were used, but were robust to adjustment for NOₓ or PM₂.₅. O₃ exposure was also associated with CP incidence (OR: 1.2; 95% CI: 1.1, 1.4; full model) among those without CP at baseline (Table S5).

O₃ and coronary artery calcification. We did not observe association between O₃ exposure and CAC change in any

| Change in IMTCCA over 10 y | CP formation over 10 y |
|---------------------------|------------------------|
| (μm/10 y, 95% CI)         | Odds ratio (95% CI)    |

Note: CI, confidence interval; CP, carotid plaque; IMTCCA, intima-media thickness of common carotid artery; NOₓ, nitrogen oxides; PM₂.₅, particulate matter diameter <2.5 μm.

- Main analyses*: 
  - Base: 5.6 (1.6, 9.6) 1.3 (1.2, 1.4)
  - Moderate: 5.5 (1.4, 9.6) 1.2 (1.1, 1.4)
  - Full: 5.6 (1.4, 9.7) 1.2 (1.1, 1.4)

- Sensitivity analyses: 
  - O₃, warm seasons: 6.5 (2.6, 10.3) 1.0 (0.9, 1.1)
  - O₃, 1-y mean: 3.6 (0.1, 7.1) 1.0 (0.9, 1.2)
  - Adjustment for NOₓ: 15.2 (7.8, 23.2) 1.3 (1.1, 1.4)
  - Adjustment for PM₂.₅: 7.8 (3.1, 12.6) 1.3 (1.1, 1.5)

- Full (no site adjustment): 5.3 (1.1, 9.4) 1.0 (1.0, 1.1)

*Main analyses [within-city interquartile range (IQR) of O₃ exposure = 3 ppb].

**Base model includes age, sex, race/ethnicity, and study region.

***Moderate model = base model + body mass index, smoking status, pack-years, second-hand smoke exposure, alcohol consumption, physical activity, employment status, high-density lipoprotein cholesterol, total cholesterol, statin use.

****Full model (primary analysis) = moderate model + neighborhood socioeconomic status index, income, education, systolic and diastolic blood pressure, hypertension, antihypertensive medication, diabetes; full model is the primary model for report in the main text and discussion.

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Figure 2. Ambient ozone (O₃) exposure estimated at the participants’ residential locations. (A) Distribution of O₃ concentrations at baseline by study regions, and (B) mean O₃ estimated concentrations at all participants’ homes from 1999 to 2013. Boxes cover the 25–75th percentile (interquartile range; IQR) with a center line for the median concentration. Whiskers extend to the highest observation within 3 IQRs of the box, with more extreme observations shown as points. Note: W-S, Winston-Salem; NYC, New York; LA, Los Angeles.
analyses. The estimate for the effect of a 3-ppb higher long-term exposure to O₃ in CAC was ~8 Agatston units (95% CI: −18, 2) over 10 y (Table S6).

Discussion

In this well-characterized cohort using fine spatial-scale exposure predictions for O₃, we found that chronic O₃ exposure was associated with evidence of progressive arterial injury as assessed by both IMTCCA and CP, independent from exposure to PM2.5 and NO₂, suggesting a different mechanism from the effect of traffic-related air pollution. The findings were robust to control for all major known CVD risk factors. Strengths of the study include its relatively large sample size, long period of follow-up (>10 y), use of advanced methods for estimating individual-level long-term outdoor O₃ concentrations, and high-quality individual information on the outcome measures and potential confounding factors.

Findings on the associations of long-term exposure to O₃ with mortality in large-scale studies in the United States and Europe have not been consistent. Although respiratory effects of long-term exposure to O₃ have been reported (Jerrett et al. 2009), evidence for long-term O₃ effects on the cardiovascular system is limited (U.S. EPA 2013). For example, in Jerrett et al. (2009), associations between ozone and cardiovascular mortality were not robust to adjustment for fine particles. O₃ concentrations were associated with total mortality (Di et al. 2017) and cardiovascular mortality (Turner et al. 2016) and its subtypes, including ischemic heart disease (Jerrett et al. 2013), cerebrovascular disease (Turner et al. 2016), and mortality risk among participants with congestive heart failure (Zanobetti and Schwartz 2011) in the United States but not with cardiovascular mortality in the United Kingdom (Carey et al. 2013). The causal nature of the association between O₃ exposure and cardiovascular risk therefore remains uncertain.

Our paper does not address clinical outcomes or mortality, and focuses on subclinical arterial injury. The pathophysiological mechanisms underlying the associations between O₃ exposure and progression of arterial injury are not well elucidated. One proposed pathway is generation of oxidative reaction products from the reaction of O₃ with lipids or cellular membranes in the lung, which are subsequently released into the circulatory system and initiate or propagate a systemic inflammatory response. Persistent or repeated activation of this pathway is associated with development of arterial injury (Cosselman et al. 2015). Both human and animal studies support involvement of this pathway. In an animal study using a mouse model, inhaled O₃ promoted increased arterial dysfunction, oxidative stress, mitochondrial DNA damage, and atherogenesis (Chuang et al. 2009). In a human study of young, healthy volunteers, exposure to outdoor O₃ caused platelet activation and an increase in blood pressure and vascular markers of inflammation relative to clean air after controlling the effects of PM2.5 and NO₂ (Day et al. 2017).

Progression of IMTCCA and CAC have not been examined previously with regard to O₃ exposure in epidemiological studies, but have been investigated with PM2.5 and traffic-related air pollution, where findings have been inconsistent (Adar et al. 2013; Kaufman et al. 2016). Varying associations may be influenced by study design, geography, quality of exposure assessment, characteristics of the study populations, and approaches to analysis and reporting. Compared to the elevated association between O₃ and IMTCCA observed in our study, a parallel analysis in the MESA study that used the same set of IMTCCA readings and the same disease models did not find progression of IMTCCA associated with PM2.5 or NO₂, but did for CAC (Kaufman et al. 2016), which was not associated with O₃ in our study. In that paper, the difference could be attributed...
to the fact that IMT$_{\text{CCA}}$ and CAC are measured in different arterial beds and/or differences in effects due to the different underlying pathophysiological processes reflected by IMT$_{\text{CCA}}$ and CAC measures. IMT$_{\text{CCA}}$ reflects early arterial injury from many processes, including inflammation, intimal thickening, and medial hypertrophy (Kiechl and Willett 1999; Stein et al. 2008), whereas CAC indicates a more advanced stage of atherosclerosis with vessel wall calcification (O’Rourke et al. 2000). IMT$_{\text{CCA}}$, CP, and CAC all predict adverse CVD events (Gepner et al. 2015; Lorenz et al. 2012; Stein et al. 2008). CAC is a more robust predictor of coronary artery disease events, whereas IMT$_{\text{CCA}}$ and CP are similar or better predictors of stroke in the general population (Gepner et al. 2015). Also, CAC and IMT$_{\text{CCA}}$ may be associated with different risk factors and do not appear to share common genes (Rampersaud et al. 2008). For example, hypertension causes a significant increase in IMT and is considered a major CVD risk factor, whereas hypertension does not appear to contribute strongly to the occurrence of plaque (Baroncini et al. 2015).

Although O$_3$ and PM$_{2.5}$ may share similar etiologic pathways, their mechanisms of action on the vasculature may differ. Toxicological studies have suggested that cardiac functional changes in response to O$_3$ are different from exposure to PM (Tankersley et al. 2013). In human studies, the effect estimate of personal exposure to O$_3$ on cardio-ankle vascular index, a measure of arterial stiffness, was twice as large as that of PM$_{1-2.5}$ exposure in a small-panel study (Wu et al. 2010). In a cross-sectional study, greater exposure to O$_3$ rather than PM was associated with increased risk of IMT in young adulthood (Breton et al. 2012). Indeed, in the MESA cohort, we found the association between O$_3$ exposure and progression of IMT$_{\text{CCA}}$ was insensitive to copollutant adjustment, and Kaufman et al. 2016 showed there was no independent effect of PM$_{2.5}$ over the same time period. Further study is needed to better understand the relative potency of ozone and PM, and pathways of effects on arterial injury.

We observed an association between long-term O$_3$ exposure and CP formation over a decade in the same population as the IMT$_{\text{CCA}}$ cohort. CP formation represents a later stage of arterial injury than increased IMT$_{\text{CCA}}$, and it is more predictive of CVD events than IMT$_{\text{CCA}}$ (Lorenz et al. 2012). A clinical consensus statement recommended that ultrasound assessment of the carotid arteries for CVD risk prediction should include CP assessment in addition to IMT$_{\text{CCA}}$ (Stein et al. 2008). Our study suggests that chronic O$_3$ exposure may affect both diffuse carotid arterial injury (IMT$_{\text{CCA}}$) and focal lesions of carotid atherosclerosis (CP). Exposure to traffic-related air pollution, on the other hand, has not been shown to be associated with plaque progression (Gan et al. 2014).

We found stronger associations between chronic O$_3$ exposure and progression of IMT$_{\text{CCA}}$ among the subset of healthier subjects without CP. One potential explanation is that there is less exposure misclassification among healthy subjects who would be expected to spend more time outdoors (confirmed by data on time-activity from the baseline questionnaire) and thus have more personal O$_3$ exposure for a given amount of estimated outdoor residential exposure. Moreover, IMT$_{\text{CCA}}$ may become an imprecise indicator of arterial injury in later stages (as better reflected by CP) due to the effect of risk-reducing medications on the end points (Gepner et al. 2015; Lorenz et al. 2012). Therefore, the estimated association between O$_3$ exposure and progression of IMT$_{\text{CCA}}$ may be stronger in the early stages of arterial injury.

Our estimates of associations with progression of IMT$_{\text{CCA}}$ and CP formation were stronger for long-term concentration averages than with a single-year average exposure. This suggests that specification of exposure over a long time period more accurately captures the critical aspects of exposure on subclinical cardiovascular effects than specifying exposure over shorter time windows.

Our study has some limitations that could affect the findings. First, although we employed advanced statistical modeling methods to produce accurate O$_3$ predictions at the time and location of each residence during follow-up, exposure misclassification remains a concern. O$_3$ is scavenged rapidly by nitric oxide near roadways and therefore varies substantially at local scale. Our measurement data included a large number of sites at the residential locations, some of which were near roadways, but did not use a monitoring design intended to capture fine-scale near-road gradients. Furthermore, indoor O$_3$ concentrations differ from outdoor concentrations because of the rapid deposition and reaction with indoor surfaces and gases. Therefore, outdoor concentrations of O$_3$ do not fully reflect personal O$_3$ exposures (Spalt et al. 2015). Second, because of the time-integrated sampling frame in our monitoring campaigns, our concentrations included the entire 24-h period, which is not directly comparable with the 8-h maximum form of the O$_3$ standard in the United States. However, correlations between daily average and daily 8-h maximum O$_3$ observations have been reported to be high across the U.S. regulatory sites (median $r = 0.89$) (U.S. EPA 2013). Third, there may be potential selection bias operating in our analysis because some participants have no follow-up data. However, the percentage with only baseline data is small (9%). Finally, we cannot rule out biases due to unmeasured confounders or changes in neighborhood characteristics that occurred during the study period. Our analysis minimized this concern by incorporating extensive data on potential confounding variables, and we found the results were robust across multiple confounder models.

**Conclusion**

Chronic exposure to O$_3$ was associated with accelerated progression of IMT$_{\text{CCA}}$ and higher risk of CP formation over a decade of follow-up in a cohort of elderly adults. This may indicate that the association between long-term exposure to O$_3$ and cardiovascular mortality that has been observed in some studies is due to arterial injury and acceleration of atherosclerosis. This is the first epidemiological study to provide evidence that O$_3$ might accelerate subclinical arterial disease, and provides insight into a relationship between O$_3$ and CVD risk.

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