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Fine particulate matter and intima media thickness

Role of endothelial function biomarkers

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Background: Ambient fine particulate matter ($PM_{2.5}$) is a risk factor for atherosclerosis disease. We aimed to assess whether nitric oxide stable metabolites (NOx) and L-arginine mediate the association between $PM_{2.5}$ and carotid intima media thickness (cIMT) increase.

Methods: We selected 251 participants from the control group of GEA (Genetics of Atherosclerosis Disease Mexican) study (2008–2013) in Mexico City. Mediation models were carried out using pathway analyses, a special case of structural equation models.

Results: The median concentration of $PM_{2.5}$ area under the curve (auc) was 25.2 μg/m^3 (interquartile range: 24.2–26.4 μg/m^3). Employing participants with observed values for both biomarkers (n = 117), the total effect of $PM_{2.5auc}$ on mean cIMT at bilateral, right, and left was 19.27 μm (95% confidence interval [CI]: 5.77, 32.78; *P* value = 0.005), 12.69 μm (95% CI: 0.67, 24.71; *P* value = 0.039), and 25.86 μm (95% CI: 3.18, 48.53; *P* value = 0.025) per each 1 μg/m^3 increase of $PM_{2.5auc}$. The direct effect of $PM_{2.5auc}$ (per 1 μg/m^3 increase) was 18.89 μm (95% CI: 5.37, 32.41; *P* value = 0.006) for bilateral, 13.65 μm (95% CI: 0.76, 26.55; *P* value = 0.038) for right, and 24.13 μm (95% CI: 3.22, 45.03; *P* value = 0.024) for left. The indirect effects of NOx and L-arginine were not statistically significant showing that endothelial function biomarkers did not mediate $PM_{2.5}$ and cIMT associations. Although L-arginine was not a mediator in the $PM_{2.5}$ and cIMT pathway, a decrease in L-arginine was significantly associated with $PM_{2.5auc}$.

Conclusions: In this study of adults from Mexico City, we found that $PM_{2.5}$ was associated with an increase in cIMT at bilateral, left, and right, and these associations were not mediated by endothelial function biomarkers (L-arginine and NOx).

Keywords: Intima media thickness; Atherosclerosis; $PM_{2.5}$; l-arginine; Nitric oxide

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Introduction

Ambient air pollution is a complex mixture of thousands of components; however, fine particulate matter ($PM_{2.5}$) drives the most significant health problems and premature mortality.[1,2] In 2021, 97% of urban population around the globe was exposed to $PM_{2.5}$ levels above the World Health Organization Guidelines.[3] $PM_{2.5}$ can penetrate the lung alveoli, is systemically distributed to organs and systems, and exerts a broad spectrum of adverse health effects.[1,2] $PM_{2.5}$ exposure is associated with cardiovascular disease and mortality.[4] Cardiovascular events responsible for cardiovascular mortality, such as stroke, heart failure, and ischemic heart disease,

What this study adds:

This is the first study to assess endothelial function biomarkers as possible mediators between $PM_{2.5}$ exposure and carotid intima media thickness (cIMT) increase. Although previous studies worldwide reported associations between $PM_{2.5}$ and cIMT, there is null evidence of circulating biomarkers that can explain the cIMT increase related to $PM_{2.5}$. Using a particular case of structural equation models, we found no mediation role of endothelial function biomarkers, L-arginine and nitric oxide stable metabolites (NOx), in the $PM_{2.5}$–cIMT pathway. The lack of mediation might be related to the short lifetime of these biomarkers, which do not necessarily reflect the long-term process of cIMT increase. Although L-arginine was not a mediator, we found a significant decrease in L-arginine associated with $PM_{2.5}$ exposure. Our results highlight the need to asses circulating biomarkers that might explain, at least in part, cardiovascular outcomes related to ambient air pollution.

are clinical manifestations of atherosclerosis.[5] A pro-oxidant and proinflammatory state with an imbalance of endothelial function biomarkers, among other factors, contributes to the progression of atherosclerosis.[6–8]

One of the characteristics of endothelial dysfunction is the decrease in endothelial nitric oxide (NO) levels.[7] Endothelial NO has a protective role due to its anti-inflammatory, antithrombotic, and antioxidative properties, and it is considered an antiatherosclerotic molecule.[9] The synthesis of NO is carried out by endothelial NO synthase (eNOS) enzyme using the amino acid L-arginine as substrate, which competes with the amino acid ADMA (asymmetrical dimethylarginine) for the eNOS active site. ADMA is an endogenous inhibitor of NO biosynthesis. Thus, a depletion in NO levels might result from decreased L-arginine or increased ADMA levels.[10] ADMA has been employed as a biomarker of cardiometabolic disease and correlates with intima media thickness (IMT).[11]

The carotid IMT (cIMT) is a surrogate marker of subclinical atherosclerosis and correlates with the progression and regression of the disease. Thus, in clinical practice, cIMT has been used to identify individuals at cardiovascular risk before the appearance of clinical cardiovascular events such as heart attack or stroke.[12]

Environmental exposure to $PM_{2.5}$ is associated with atherosclerosis risk and cIMT increase; however, few studies have been focused on $PM_{2.5}$ and cIMT in the Latin American and the Caribbean (LAC) region.[13–15] Studies suggest different atherosclerosis development and risk factors when comparing cIMT at the left and right carotid sides.[15,16] The differences in atherosclerosis development between cIMT at left and right might be due to flow type and the sensibility to biochemical parameters (glucose, low-density lipoprotein cholesterol [LDL-C], and triglycerides). A previous study reported that the left carotid side is more susceptible to biochemical parameters, while the right carotid side responds mainly to hemodynamic changes.[17] Since NO is involved in vasodilatation and the right carotid side is more susceptible to hemodynamic variables, in this study, we hypothesized that $PM_{2.5}$ exposure would be associated with an increase in right cIMT, finding lower estimates for left cIMT. A decrease in NO stable metabolites (NOx) and L-arginine would mediate, at least in part, the increase in right cIMT associated with $PM_{2.5}$ exposure.

Thus, we aimed to assess the mediation role of endothelial function biomarkers in cIMT increase related to $PM_{2.5}$ exposure in adults belonging to the Genetics of Atherosclerosis Disease study (Spanish acronym, GEA study), one of the largest studies about cardiovascular disease in Mexico and LAC.

Methods

Study participants

The GEA study (2008–2013) is a case–control study that includes 1240 patients with premature coronary artery disease (pCAD) and 1500 individuals without a personal or family history of pCAD (GEA control group). GEA study is based on the Instituto Nacional de Cardiología Ignacio Chávez (INCICH). Details on the study aim, inclusion, and exclusion criteria have been previously described. The main aim of the GEA study was to investigate genetic factors associated with pCAD and other coronary risk factors in the Mexican population.[18,19] We defined pCAD as the presence of myocardial infarction history, bypass surgery, angioplasty, or >50% coronary stenosis diagnosed before the age of 55 in men and 65 in women, respectively; age cutoffs were according to the study of Nasir et al.[20] Participants in the GEA study underwent computed tomography of the chest and abdomen to quantify coronary artery calcification using the Agatston scoring method.[21] Participants completed structured questionnaires to provide information on sociodemographic characteristics.

Hypertension and type two diabetes diagnosis

After 10 minutes of sitting, we measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) with a digital sphygmomanometer (Welch Allyn Series 5200, Skaneateles Falls, NY). We calculate the average of the last two of three blood pressure measurements. We defined arterial hypertension as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or the use of high blood pressure medication.[22] Weight in kg and height in m were measured to calculate body mass index (BMI) as kg/m^2. We considered normal weight as a BMI <25 kg/m^2, overweight as a BMI of 25 to 29.9 kg/m^2, and obesity with a BMI ≥ 30 kg/m^2.

Type 2 diabetes was defined as fasting plasma glucose ≥ 126 mg/dl according to the criteria of the American Diabetes Association,[23] or if the patient reported current use of glucose-lowering medication or previous diagnosis of diabetes.

Biochemical parameters

Participants' blood samples were obtained after at least 12 hours of fasting. Lipids measurements were performed at the INCICH endocrinology laboratory, using standardized procedures certified by the Center for Disease Control (Atlanta, GA). Plasma total cholesterol, high-density lipoprotein (HDL-C), triglycerides, and glucose concentrations were quantified using enzymatic-colorimetric methods (Roche/Hitachi, Germany) in a Hitachi 902 autoanalyzer (Hitachi LTD, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were estimated using DeLong's modified Friedewald formula.[24] High-sensitivity C-reactive protein (hs-CRP) concentration was measured by immunonephelometry (SIEMENS Healthcare Diagnostics Products GmbH/BN ProSpec, Germany).

Intima media thickness measurements

The IMT was measured at the time of recruitment (2008–2013) employing a MicroMaxx ultrasound device (SonoSite, Universal Diagnostic Solutions, Inc., Bothell, WA), using a 7.0–14.0 MHz-linear array wideband transducer to obtain B-Mode ultrasound images.[25] Briefly, the trained radiologist

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The study was approved by the Institutional Review Board of the Instituto Nacional de Cardiología Ignacio Chavez (INCICH) (project number 19-1104) and the National Institute of Perinatology (project number 2020-1-41).

Written informed consent was obtained from the patients to publish this paper.

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scanned bilaterally (left and right), over a length of 1 cm, the common carotid artery (CCA), bulb, and internal carotid artery. We used IMT measurements from the CCA (cIMT) at the bilateral, left, and right sides. Mean cIMT (m_cIMT) at left is the average of five measurements of the mean left CCA, m_cIMT at right is the average of five measurements of the mean right CCA, and m_cIMT at bilateral is the average of 10 measurements, five at left and five at right. In addition to the m_cIMT, we reported the average of maximum cIMT (max_cIMT) of CCA at left, right, and bilateral. The max_cIMT is more sensitive to changes than the m_cIMT, but is less reproducible because the value is from a single-point measurement along the 10 mm exploration area.[25] The trained radiologist, who performed cIMT measurements, was blinded to study participants' $PM_{2.5}$ exposure and clinical data. The reproducibility of the measurements was determined in 5% of the cohort, obtaining an intraobserver correlation coefficient of 0.96.[26]

L-arginine and NOx measurements

We employed serum samples collected at recruitment to measure L-arginine concentrations (µg/ml) and NOx in µmol/l. L-arginine was measured using a competitive enzyme-linked immunoassay (ELISA) kit, commercially available (MyBioSource, Vancouver, Canada). The limit of detection (LOD) for L-arginine was 0.1 µg/ml. NO determination was performed as the sum of the stable metabolites: nitrites and nitrates (NOx). First, samples for NOx measurements were deproteinized with $ZnSO_4$, and second, NOx measurements were performed using a commercially available kit based on the Griess reaction (Total Nitric Oxide and Nitrate/Nitrite Assay Kit from R&D Systems, Inc, Minneapolis, MN). The LOD for NOx metabolites ranged between 0.09 and 0.78 µmol/l, and we used the upper limit of 0.78 µmol/l as LOD. L-arginine and NOx measurements were performed by duplicates, and those measurements with more than 15% coefficient of variation (CV) were excluded, treated as missing values, and included in the imputation process. Outliers for endothelial function biomarkers were considered those values above the percentile 99 for NOx (20 µmol/l) and L-arginine (13 µg/ml).

$PM_{2.5}$ exposure assessment

$PM_{2.5}$ exposure assessment was previously described.[15] Briefly, we estimated participants´ exposure to $PM_{2.5}$ at the residential level by reconstructing individual exposure since the year of recruitment and going back 4 years prior. Annual $PM_{2.5}$ exposure was calculated from 24-hour average data from the Mexico City Atmospheric Monitoring System (SIMAT) ground monitoring stations. In total, 26 ground monitoring stations met at least 75% of the completion of hourly data, criteria established by the Mexican Standard NOM-025-SSA1-2014, 2014. We performed exposure assignment through a four-step method, which combines the creation of areas of influence (i.e., buffers around monitoring stations) with the interpolation of $PM_{2.5}$ concentrations as a function of the inverse of the distance weighting between ground monitoring stations. This method assigns exposure to $PM_{2.5}$ considering the location of participants' households within 5 and 10 km radii buffers, outside buffers, or in buffers' intersections, using geocoded addresses and ground monitoring stations as the buffers' centroids.[15,27] Finally, from annual averages of $PM_{2.5}$, we used two long-term measurements of $PM_{2.5}$ exposure: (1) the average of the 5 years (recruitment year and 4 years before), and (2) we calculated the area under the curve (auc), which integrates total $PM_{2.5}$ exposure during 5 years according to the trapezoid approach ($PM_{2.5}$auc).[28] Thus, $PM_{2.5auc}$ combines in a single metric the intensity of $PM_{2.5}$ exposure over a specific time window (5 years).

Analytical population

In this study, we analyzed data only from the GEA control group. In total, 922 of 1500 participants of the GEA control group had at least 5 years of residence in Mexico City, cIMT measurements, and $PM_{2.5}$ exposure assessment. We randomly selected 251 of 922 participants for serological determination of L-arginine and NOx. The analytical population for this study included 251 participants (Figure 1).

Statistical methods

We assessed the quality and consistency of the data and the distribution of the variables of interest. We used the chi-squared test or Mann–Whitney *U* and Kruskal–Wallis test, to evaluate differences between participants for all variables of interest. Continuous variables were described as median and interquartile range (IQR), and for categorical variables, we reported frequencies and percentages. Spearman correlations were used to estimate the strength of association between covariates, cIMT measurements, and observed values of endothelial function biomarkers.

We imputed endothelial function biomarkers for three reasons: (1) high percentage of NOx measurements below the LOD; (2) insufficient sample for L-arginine or NOx measurements, and (3) biomarkers measurements with CV >15%. We imputed NOx negative values below the LOD, missing values because of the lack of sample or measurements with CV (>15%) by applying regression imputation, a method in which we estimated the missing values by linear regression using other variables as parameters. Regression imputation can preserve the relationship between missing values and other variables.[29] Linear regression models for imputations were adjusted for age, sex, education, cIMT, and $PM_{2.5auc}$ based on the biological plausibility and *R*-squared values. cIMT was included as an adjustment variable for the imputation models because the lack of adjustment for the outcome variable results in estimated regression coefficients being biased to the null.[30]

On the other hand, our study's observed values for biomarkers were those above the LOD and NOx positive measurements below the LOD. NOx positive measurements below the LOD were extrapolated using the assay standard curve and treated as observed values.[31] Using observed values below the LOD is an analytic approach previously used in epidemiologic studies.[32]

The mediation analysis was carried out using pathway analyses with the approach proposed by Lange et al.[33] This approach is a particular case of structural equation models (SEM) that test mediators as individual variables in the mediation pathway without testing latent variables constructed from two or more variables. Mediation analyses allow the decomposition of the total effect of a given exposure into a natural direct effect and a natural indirect effect through one or several mediators.[33] The natural direct effect contrasts the effect of $PM_{2.5}$ exposure on cIMT and the indirect effect estimates the percentage of the $PM_{2.5}$–cIMT associations that can be explained through biomarkers.

To assess confounding, we entered covariates into each model one at a time and compared adjusted and unadjusted estimates (exposure–outcome associations and R-squared of the models). Final models included covariates that altered estimates by at least 10% and those that were identified a priori as potential confounders, such as age, BMI, smoking status, education, diabetes, SBP, and LDL-C. Directed acyclic graphs (DAGs) were used to inform our analytical approach to adjusting for confounding using DAGitty v 3.0 software Johannes Textor (Radboudumc, Nijmegen, The Netherlands).[34] The linearity of the models was tested using the Wald test for SEM in Stata. The Akaike information criterion and Bayesian information criterion were used as criteria for the model fit. A lower Akaike information criterion or Bayesian information criterion value

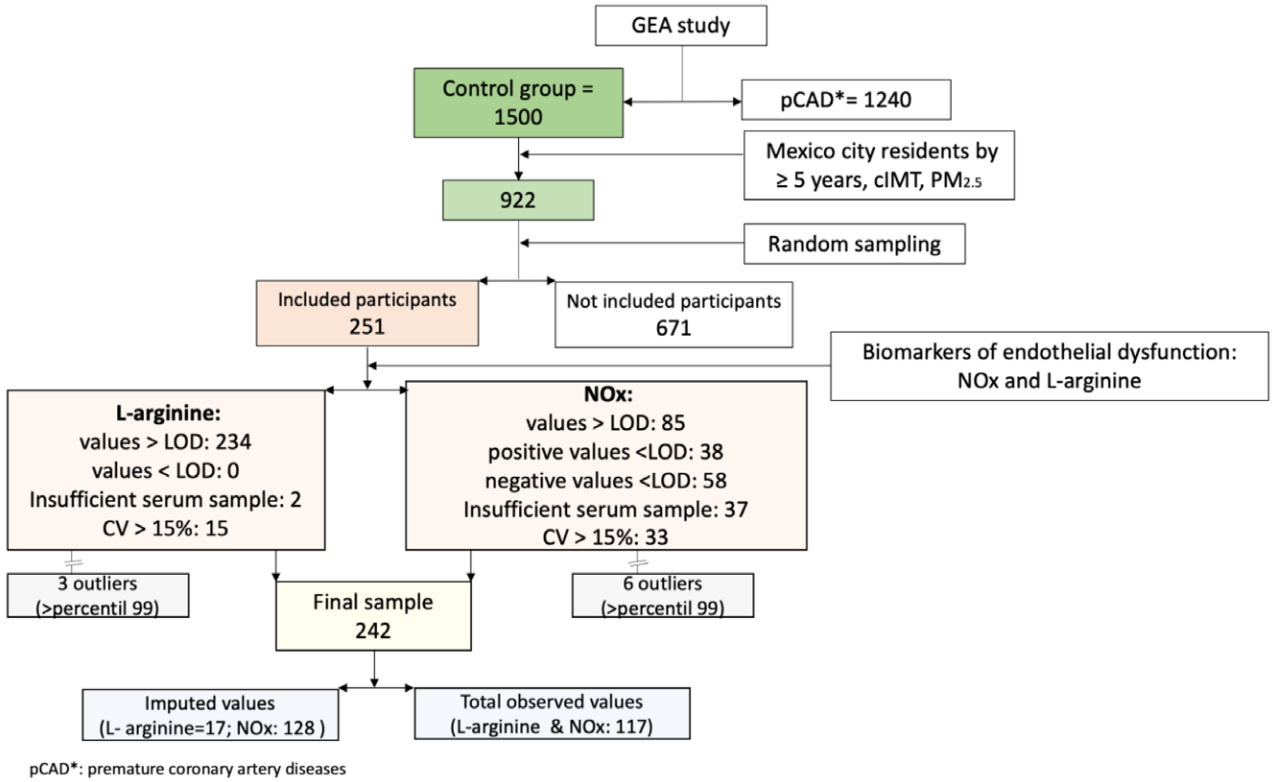

Figure 1. Flowchart of the study population

indicates a better fit.[35] Mediation models were adjusted for age, sex, education, smoking, and LDL-C levels. Figure 2 is the schematic representation of the mediation analyses.

Our main mediation analyses are those conducted with observed values for L-arginine and NOx. However, we also performed mediation analyses using imputed data because, even if noise is introduced in the imputation process, we gained statistical power, and estimates from imputed data should produce more unbiased estimates than nonimputed data.[36,37] To test the hypothesis that endothelial function biomarkers might mediate the increase in right cIMT rather than left cIMT related to $PM_{2.5}$ exposure, we performed mediation analyses for cIMT at bilateral, left, and right for both mean and maximum measurements.

Sensitivity analyses

We performed a series of sensitivity analyses to assess whether the associations between $PM_{2.5}$ and cIMT measurements and the mediation role of endothelial function biomarkers were robust, including:

(1) Since cIMT measurements were nonnormally distributed, we also performed pathway mediation analyses using Satorra–Bentler errors for variables with non-normal distributions to assess the robustness of our results.[38]
(2) SBP increase can mediate $PM_{2.5}$ and cIMT associations.[39,40] Thus, we added SBP as a third mediator in the model.
(3) We conducted sensitivity analyses using a fully adjusted model with variables previously described to be associated with $PM_{2.5}$ exposure or cIMT, such as BMI and glucose levels.[41,42] In this fully adjusted model, we used age, sex, smoking, and education level as confounders and L-arginine, NOx, glucose, BMI, and LDL-C as mediators (Supplemental Figure 2; http://links.lww.com/EE/A314).

All analyses were performed employing Stata/MP version 14.2. Mediation path analyses were performed using medsem Stata package. A significance level of 0.05 with a two-tailed distribution was considered statistically significant.

Results

Among 251 participants randomly selected for biomarker measurements, 234 (93.2 %) samples were above the LOD for L-arginine and 85 (33.9%) for NOx. Samples with measurements below the LOD for L-arginine and NOx were 0 and 96, respectively. A total of 15 and 33 samples had CV > 15% for L-arginine and NOx, respectively. We excluded 3 outliers for L-arginine and 6 outliers for NOx. We considered as observed values those

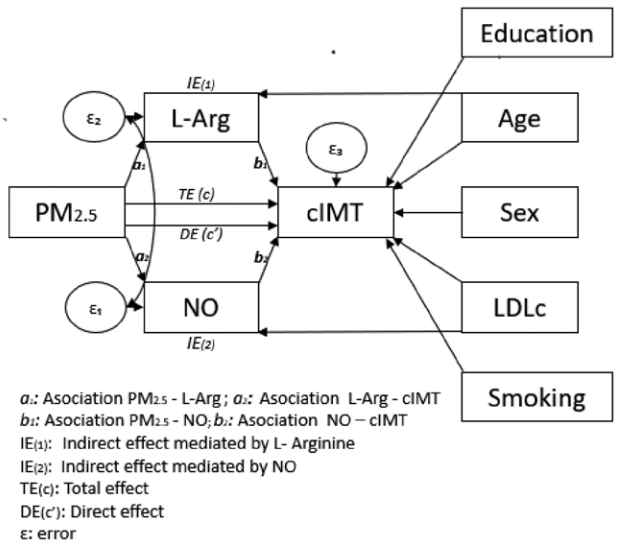

Figure 2. Schematic representation of mediation analysis.

Table 1.

Characteristics of the GEA control group participants comparing not-included participants with imputed and nonimputed data for endothelial function biomarkers

	Not-included participants (n = 671)	Participants with imputed data for biomarkers (n = 242)		Participants with nonimputed data for biomarkers (n = 117)	
	% or median (IQR)	% or median (IQR)	*P* value[a]	% or median (IQR)	*P* value[b]
Demographic characteristics					
Sex			0.62		0.56
Women	50.1	51.7		49.6	
Men	50.2	48.4		50.4	
Age (years)	54 (47–61)	54 (49–60)	0.64	54 (49–60)	0.98
Marital status			0.96		0.73
Union	74.9	74.8		74.4	
No union	25.0	25.1		25.6	
Level of education			0.77		0.95
<Elementary	31.0	28.5		28.2	
Junior high school	33.8	34.7		35.9	
>Senior high school	35.2	36.8		35.9	
Atherosclerosis risk factors					
Smoking			0.03		0.66
Never	37.8	45.9		47.9	
No current active smoker	40.5	31.0		30.8	
Current active smoker	21.8	23.1		21.4	
Alcohol consumption			0.27		0.23
Occasional	50.5	48.7		47.8	
1–3 per month	27.6	28.9		31.1	
1–5 per week	21.3	20.3		21.1	
Daily	0.6	2.1		0.0	
Physical activity	7.9 (7.0–8.6)	8 (7.1–8.9)	0.09	7.9 (7.1–8.9)	0.19
BMI (kg/m^2)	28.3 (25.7–31.3	28.1 (25.2–31.2)	0.35	28.1 (25.1–31.2)	0.41
SBP (mmHg)	112 (102–123)	109(100–123)	0.44	109 (101–123)	0.78
DBP (mmHg)	74 (67–83)	75(68–82)	0.99	72 (66–84)	0.17
Hypertension			0.83		0.80
Yes	25.3	26.0		26.5	
No	74.7	74.0		73.5	
Diabetes			0.71		0.14
Yes	14.7	15.3		18.8	
No	85.0	84.7		81.2	
BMI categories			0.436		0.22
Normal weight	19.3	23.1		24.8	
Overweight	46.6	44.2		37.6	
Obese	34.2	32.6		37.6	
Biochemical parameters					
Glucose (mg/dl)	91 (85–101)	91 (85–99)	0.97	91.0 (85.0–99.0)	0.54
Total cholesterol (mg/dl)	189.8 (163.2–212.7)	190.7 (174.2–218.3)	0.07	189.8 (169.3–208.8)	0.11
LDL-C (mg/dl)	114.1 (93.6–136.4)	119.6 (98.9–136.9)	0.07	118.4 (97.6–132.8)	0.45
HDL-C (mg/dl)	43.9 (36.2–53.7)	44.0 (36.1–54.6)	0.9	43 (36–52.8)	0.24
Triglycerides (mg/dl)	147.5 (113–203.3)	153.7 (116.2–219.8)	0.26	149.7 (113.8–214.2)	0.69
hs-CRP (mg/dl)	1.6 (0.8–3.3)	1.3 (0.7–2.9)	0.1	1.5 (0.8–3.1)	0.05
	Median (IQR)	Median (IQR)	*P* value	Median (IQR)	*P* value
$PM_{2.5}$ 5 years average (μg/m^3),	24.5 (23.5–25.5)	24.4 (23.5–25.1)	0.03	24.4 (23.2–24.9)	0.04
PM2.5auc, (μg/m^3)	25.9 (24.8–27.2)	25.7 (24.5–26.8)	0.02	25.2 (24.2–26.4)	0.01
m_cIMT (μm)					
At bilateral	630 (555– 735)	630 (560– 730)	0.97	635 (550–730)	0.29
At right	620 (530– 720)	615 (540–720)	0.91	615 (530–730)	0.47
At left	640 (550–740)	629 (550–760)	0.74	635 (550–760)	0.33
max_cIMT					
At bilateral	700 (615– 840)	702 (615– 820)	0.79	728 (615–835)	0.59
At right	690 (595– 830)	690 (600– 810)	0.97	690 (590–860)	0.74
At left	710 (610– 860)	720 (620– 860)	0.50	720 (630–880)	0.52
NOx (μmol/l)					0.03
Observed values				2.22 (0.69–4.13)	
Observed + imputed values		2.88(2.13– 3.42)			
L-arginine (μg/ml)					0.34
Observed values				0.75 (0.38–1.59)	
Observed + imputed values		0.76 (0.37– 1.59)			

Differences between groups tested with Mann–Whitney *U* test.

[a]*P* value to test statistical differences between not-included participants and participants with imputed data.

[b]*P* value for differences between participants with imputed data and nonimputed data.

Significant results (*P* value < 0.05) are highlighted in bold

measurements above the LOD for both biomarkers and NOx measurements below the LOD that were positive. Thus, we had 117 observed values for L-arginine and NOx (Figure 1).

Compared to nonincluded participants (n = 671), participants with imputed data for biomarkers (N = 242) were more likely to be never smokers and had slightly higher total cholesterol and LDL-C (Table 1). The $PM_{2.5}$ levels for 5 years were slightly lower in participants with imputed data (vs. nonincluded) (Table 1).

Except for $PM_{2.5}$ levels for 5 years and hs-CRP, we found no statistically significant differences in sociodemographic characteristics and biochemical parameters in participants with imputed data compared to nonimputed data (Table 1).

For those participants with nonimputed data for biomarkers, the median age was 54 years (IQR: 49–60 years), 49.6% were women, 28.2% reported having less than basic education, and 21.4% reported being active smokers. Regarding alcohol consumption, 47.8% reported occasional alcohol consumption, and 21.1% drank alcohol 1–5 times per week. The average physical activity index was 7.9. The prevalence of hypertension and diabetes was 26.5% and 18.8%, respectively. The percentage of normal weight was 24.8%. All biochemical parameters were within the reference values reported for the Mexican population.[43]

Median value for m_cIMT in participants with nonimputed data was 635 μm (IQR: 550–730 μm) for bilateral, 615 μm (IQR: 530–730 μm) for right, and 635 μm (IQR: 550–760 μm) for left. The median value for max_cIMT was 728 μm (IQR: 615–835) at bilateral, 690 μm (IQR: 590–860 μm) at right, and 720 μm (IQR: 630–880 μm) at left. We found nonstatistical differences in cIMT measurements (mean or maximum) between participants with imputed or nonimputed data (Table 1).

$PM_{2.5auc}$ over the study period ranged from 21.9 to 30.0 μg/m^3 for participants with nonimputed data. Figure 3 shows the geographic distribution of the study participants and their average 5-year $PM_{2.5}$ levels. The median value for the 5 years of $PM_{2.5}$ exposure was 24.4 μg/m^3 (IQR: 23.2–24.9 μg/m^3), and the median value for $PM_{2.5auc}$ was 25.2 μg/m^3 (IQR: 24.2–26.4 μg/m^3) for participants with nonimputed data (Table 1).

The median NOx concentrations for imputed and observed values were 2.88 μmol/l (IQR: 2.13–3.42 μmol/l) and 2.22 μmol/l (IQR: 0.69–4.13 μmol/l), respectively.

The median L-arginine concentrations for imputed values was 0.76 μg/ml (IQR: 0.37–1.59 μg/ml) and for observed values was 0.75 μg/ml (IQR: 0.38–1.59 μg/ml) (Table 1).

We found no statistical significant correlation between the two biomarkers (L-arginine and NOx) or between any of the biomarkers and blood pressure, biochemical parameters, age, BMI, or cIMT measurements. The biomarker concentrations were statistically similar across all categorical variables; we observed marginal differences across educational degrees and marital status (data not shown). Regarding $PM_{2.5}$ exposure, we observed a significant correlation only for $PM_{2.5auc}$ and L-arginine ($r = -0.29$; $P < 0.01$) (data not shown).

Mediation analyses using observed and imputed values for endothelial function biomarkers

Overall, our results showed significant associations between $PM_{2.5auc}$ and m_cIMT for the direct and total effects. Both, the direct and total effects were positively and significantly associated with m_cIMT at bilateral, right, and left (Table 2).

In participants with observed values for both biomarkers (n = 117), the total effect of $PM_{2.5auc}$ on m_cIMT increase at bilateral, right, and left was 19.27 μm (95% CI: 5.77, 32.78; *P* value = 0.005), 12.69 μm (95% CI: 0.67, 24.71; *P* value = 0.039), and 25.86 μm (95% CI: 3.18, 48.53; *P* value = 0.025) per each increase in 1 μg/m^3 of $PM_{2.5auc}$. The total effect is the contribution of the direct and indirect effects mediated by the endothelial function biomarkers. The direct effect of $PM_{2.5auc}$ (per 1 μg/m^3 increase) was 18.89 μm (95% CI: 5.37, 32.41; *P* value = 0.006) for bilateral, 13.65 μm (95% CI: 0.76, 26.55; *P* value = 0.038) for right, and 24.13 μm (95% CI: 3.22, 45.03; *P* value = 0.024) for left. The indirect effect of NOx and L-arginine were not statistically significant for all the cIMT measurements (Table 2).

Considering max_cIMT, the total effect at bilateral was 19.21 μm (95% CI: 2.37, 36.05; *P* value = 0.025) and the direct effect was 19.29 μm (95% CI: 2.19, 36.38; *P* value = 0.027) (Table 2). The total and direct effect for max_cIMT at left and right did not reach statistical significance (Table 2). Similar to that observed for mean cIMT measurements, the indirect effects mediated by the biomarkers were not statistically significant (Table 2).

Figure 4 shows the percentage of mediation for each biomarker in the $PM_{2.5auc}$ and cIMT associations. Although not statistically significant, the percentage mediated by L-arginine was higher for max_cIMT at right than left, 5.4% and 1.8%, respectively. The percentage mediated by NOx was 1.6% for right cIMT and 2% for left cIMT, which was not statistically significant (Figure 4).

Except for the direct effect of $PM_{2.5auc}$ on m_cIMT at left, we found no significant associations for $PM_{2.5auc}$ and mean or maximum cIMT for all the effects (direct, indirect, and total) or carotid sides (bilateral, left, and right) when employing imputed data for endothelial function biomarkers (Table 2).

Sensitivity analyses

The direction and significance of the estimates were similar to those of our main model when applying mediation analyses with Satorra–Bentler errors (data not shown).

Including SBP as a third mediator gave similar results to those of the main model for m_cIMT and max_cIMT (Supplemental Table 1 and Supplemental Figure 1; http://links.lww.com/EE/A314).

Supplemental Table 2 and Supplemental Figure 2; http://links.lww.com/EE/A314 show sensitivity analyses using fully adjusted models, and the magnitude and significance of the direct effect of $PM_{2.5auc}$ on m_cIMT measurements at bilateral, right, and left were similar to those found in the main model (adjusted for age, sex, LDL-C, smoking, and education). Overall, we observed changes in estimates less than 10% for the total and direct effects. Similar to that observed for our main model, in the fully adjusted model, the indirect effect of endothelial function biomarkers (NOx and L-arginine) was not statistically significant (Supplemental Table 2; http://links.lww.com/EE/A314).

Discussion

The main findings of our study were the significant associations between $PM_{2.5auc}$ and m_cIMT at bilateral, left, and right; however, these associations were not mediated by endothelial function biomarkers (L-arginine and NOx). Although L-arginine did not mediate $PM_{2.5auc}$ and m_cIMT associations, L-arginine was negatively associated with $PM_{2.5auc}$ suggesting L-arginine as a possible molecular target of $PM_{2.5}$. Overall, we found no significant associations between $PM_{2.5auc}$ and NOx metabolites.

Endothelial function biomarkers concentrations

The median NOx concentrations in our study were 2.22 μmol/l (95% CI: 0.69, 4.13 μmol/l) for observed values; these concentrations were below than the 95% reference values for men (11.7–76.4 μmol/l) and women (10.1–65.6 μmol/l) previously reported in serum by Ghasemi et al.[44] Similarly, NOx concentrations in our study were below than those reported by Binh et al[45]

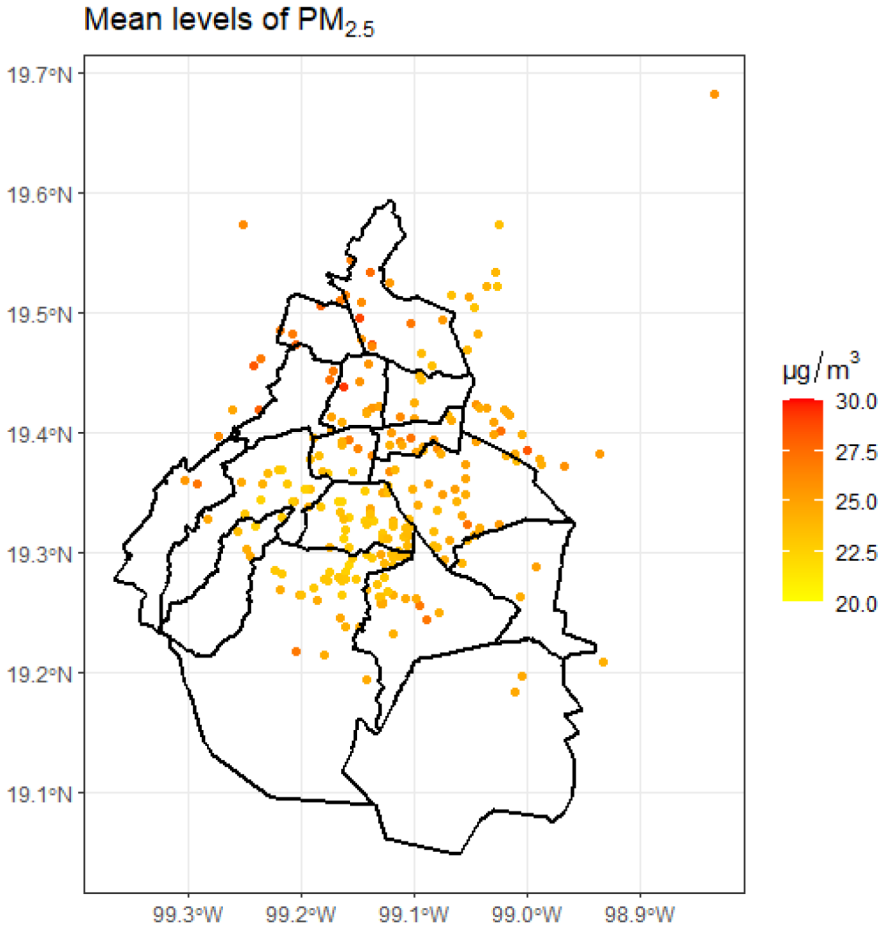

Figure 3. $PM_{2.5}$ concentrations for the 5 years of exposure in study participants (N = 251 belonging to GEA control group) according to geographic location in Mexico City.

(26.4 ± 17.6 µmol/l) in adults with normal glucose tolerance. NOx concentrations are influenced by endogenous NO synthesis, dietary intake, and liver and kidney functions; these factors might explain differences among populations.[46] Additionally, low NOx concentrations might be related to a high prevalence of overweight and obesity (76.8%, participants with imputed data) in our study setting. Previous experimental and epidemiologic studies have shown that BMI increase is associated with a decrease in eNOS expression, and the ROS production linked to obesity might lead to peroxynitrite formation with a subsequent decrease in NOx concentrations.[47,48]

L-arginine levels in our study setting, 0.75 (95% CI: 0.38, 1.59) µg/ml for observed values, were lower than L-arginine levels for healthy adults from Framingham Heart Study (7.14–19.86 µg/ml) and the control group (16.85 [SD, standard deviation: 3.99] µg/ml) of the study conducted by Corso et al.[49,50] Differences might be related to dietary patterns, L-arginine supplementation, and the sensitivity of the techniques.[51] For example, ELISA has a sensibility of 0.1 µg/ml, 100 times higher than the method used by Luneburg et al (12.89 µg/ml), who reported only values above this cutoff point.[49,52]

$PM_{2.5}$ exposure and carotid intima media increase

Regarding cIMT measurements, the mean and maximum cIMT measurements at bilateral (left and right), left, and right in our study were within the range (250–1500 µm) previously reported in the literature for healthy adults from the Region of the Americas (PAHO).[53] To note, cIMT measurements (left and right) were within the ranges previously reported for the Hispanic population according to sex and age.[25]

Our results showed increased cIMT bilaterally, left and right associated with $PM_{2.5auc}$. Although no previous studies specifically assessed associations between $PM_{2.5auc}$ for 5 years and cIMT, our findings align with previous studies showing significant associations between long-term $PM_{2.5}$ exposure and cIMT increase.[14,54]

In this study focused on mediation analyses (n = 117), we found that the increase in cIMT related to $PM_{2.5auc}$ (direct effects) was similar for m_cIMT and max_cIMT ([18.89 µm; 95% CI: 5.37, 32.41] and [19.29 µm; 95% CI: 2.19, 36.38], respectively). Additionally, we found no differential susceptibility to $PM_{2.5}$ between the left and right carotid sides, as we previously reported in this population with a larger sample size (n = 914). Comparing our findings with our previous paper is challenging since we have different sample sizes, research questions, statistical analyses, and $PM_{2.5}$ exposure variables (annual lags and auc).[15] However, our study fills a gap in the literature regarding the lack of studies suggesting possible biomarkers related to $PM_{2.5}$ and cIMT increase.

Apart from our research group, only two studies have evaluated associations between ambient air pollution and cIMT in the LAC region. One study conducted in Ecuador used as a proxy of ambient air pollution exposure, the distance to high-traffic avenues in school children, while one study in adults from the same country employed differential ambient air pollution exposure in occupational groups. Despite differences in study design, exposure assessment, and target population, our results are according to these two studies in Ecuador, reporting an increase in cIMT associated with ambient air pollution.[13,15,55] More studies are needed in the LAC region focused on the link between ambient air pollution and IMT because of differences in $PM_{2.5}$ sources and composition, the high prevalence of diabetes and hypertension in the LAC that can increase the cardiovascular

Table 2.

Mediation analyses: $PM_{2.5auc}$–cIMT through endothelial function biomarkers (observed values and imputed data)

	At bilateral		At right		At left	
	μm (95% CI)	*P* value	μm (95% CI)	*P* value	μm (95% CI)	*P* value
Observed values (n = 117)						
m_cIMT						
Direct effect	18.89 (5.37, 32.41)	0.006	13.65 (0.76, 26.55)	0.038	24.13 (3.22, 45.03)	0.024
Indirect effect	0.39 (–3.72, 4.49)	0.854	–0.96 (–5.32, 3.40)	0.666	1.73 (–3.18, 6.65)	0.49
Total effect	19.27 (5.77, 32.78)	0.005	12.69 (0.67, 24.71)	0.039	25.86 (3.18, 48.53)	0.025
max_cIMT						
Direct effect	19.29 (2.19, 36.38)	0.027	**15.56 (–1.28, 32.40)**	**0.07**	**23.02 (–2.03, 48.06)**	**0.072**
Indirect effect	–0.08 (–4.69, 4.53)	0.973	–1.04 (–6.55, 4.48)	0.713	0.88 (–4.25, 6.00)	0.737
Total effect	19.21 (2.37, 36.05)	0.025	**14.52 (–0.87, 29.92)**	**0.064**	**23.89 (–3.05, 50.84)**	**0.082**
Imputed data (n = 242)						
m_cIMT						
Direct effect	**8.42 (–0.36, 17.21)**	**0.06**	5.77(–2.81, 14.35)	0.188	11.08 (0.38, 21.78)	0.042
Indirect effect	–0.14 (–1.78, 1.51)	0.871	–0.41(–2.18, 1.35)	0.646	0.14 (–1.92, 2.21)	0.893
Total effect	**8.29 (–0.45, 17.02)**	**0.063**	5.36(–2.99, 13.70)	0.209	**11.22 (0.07, 22.37)**	**0.049**
max_cIMT						
Direct effect	**9.74(–0.33, 19.81)**	**0.058**	**8.89 (–1.19, 18.98)**	**0.084**	**10.58 (–1.63, 22.79)**	**0.089**
Indirect effect	–0.23 (–2.34, 1.89)	0.833	–0.94 (–3.62, 1.74)	0.492	0.48 (–1.87, 2.84)	0.687
Total effect	**9.51 (–0.41, 19.43)**	**0.06**	7.96 (–1.63, 17.54)	0.104	**11.06 (–1.68, 23.81)**	**0.089**

Adjusted for age, sex, LDL-C smoking, and education level. Significant results (*P* value < 0.05) are highlighted in bold, and marginally significant results (*P* value < 0.10) are given in italics.

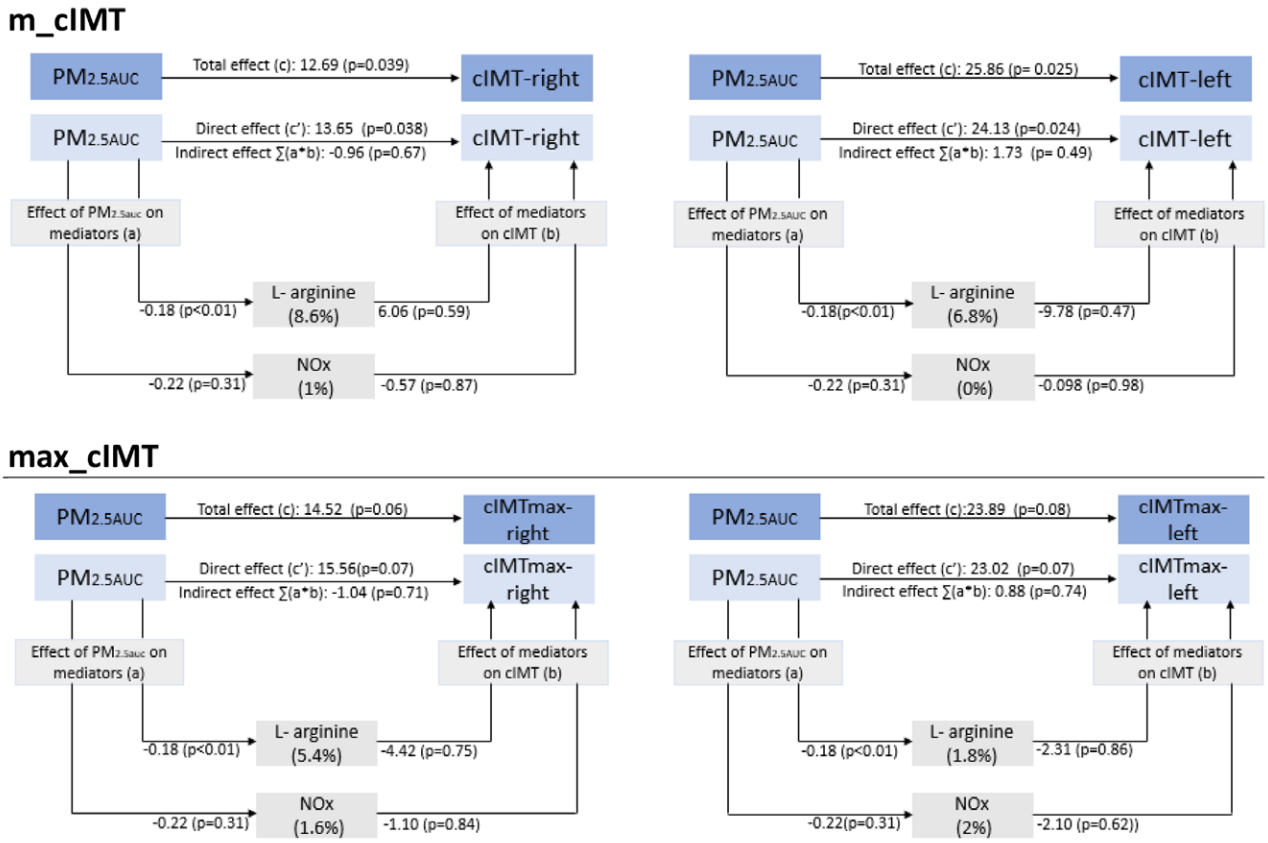

Figure 4. Mediation analyses for $PM_{2.5auc}$ and cIMT (mean and maximum) associations using as mediators endothelial function biomarkers (n = 117). *Adjusted for sex, age, LDL-C, smoking, and education level.

risk related to $PM_{2.5}$, and the need for more strict surveillance of $PM_{2.5}$ emissions in urban LAC areas.[56]

Mediation analysis for $PM_{2.5}$ and carotid intima media associations

We hypothesized that a decrease in L-arginine or NOx would mediate $PM_{2.5auc}$–cIMT associations. Overall, we observed no mediation through L-arginine or NOx in the $PM_{2.5auc}$–cIMT associations for all cIMT measurements (mean or maximum) and carotid sides.

The lack of mediation through these biomarkers might be because the thickening of the intima media layers of the arteries is a long-term process that takes place over the years. Therefore, endothelial function biomarkers, biomarkers with a short life in circulation (days and months), do not necessarily reflect the

cIMT chronic process. For example, the half-life of NOx ranges from 90–900 minutes in physiological conditions and oscillates between 3.8 and 6.2 seconds in pro-oxidant states.[57,58]

Although NOx and L-arginine were not mediators, a decrease in L-arginine was significantly associated with $PM_{2.5auc}$, indicating that $PM_{2.5}$ might alter L-arginine's protective role in the endothelium.[59] No significant association was found for $PM_{2.5auc}$ and NOx.

The lack of significant association between $PM_{2.5auc}$ and NOx metabolites might be related to NOx coming from different enzymes eNOS, iNOS (inducible NO synthase), and nNOS (neuronal NO synthase) that have different functions, including endothelium protective effects (eNOS and iNOS), but also are involved in inflammation pathways and proatherogenic process (iNOS).[60] Contrary to our findings, experimental evidence suggested that $PM_{2.5}$ might decrease NO levels through eNOS downregulation, ROS production, and inflammatory pathways.[61]

The inverse association between $PM_{2.5}$ and L-arginine aligns with Liang and colleagues'[62] findings; they found alterations in arginine metabolome in commuters exposed to traffic-related air pollution with and without asthma. On the other hand, we found no significant associations between L-arginine and cIMT measurements, and this finding is consistent with the study conducted by Jazwinska-Kozuba et al, which shows no significant correlations between L-arginine analog (homoarginine) and cIMT at bilateral in 40 healthy children and adolescents. Authors suggest that the role of other enzymes like arginase that competes with eNOS for L-arginine should be considered when testing arginine and carotid vascular structure.[63]

Additionally, the small sample size might not allow us to capture the mediation effect of endothelial function biomarkers in the $PM_{2.5}$–cIMT associations. Newman highlighted that in multifactorial diseases, with the interplay of many risk factors, it is relevant to consider a large sample size that allows us to detect slight effects related to ambient air pollution.[64]

Finally, future studies should focus on the mediation role of other molecules in the $PM_{2.5}$ and cIMT pathway and involved in the pathophysiology of atherosclerosis, such as cytokines, selectins, endothelin-1, endothelial adhesion molecules, and NO endogenous inhibitors such as ADMA.[65–67]

Strengths and limitations

Our study has several strengths: (1) the study is nested in a large sample size study with detailed individual-level cardiovascular examinations, extensive covariate data, and long-term evaluation of ambient air pollution; (2) our study has detailed cIMT evaluation that includes mean and maximum parameters for bilateral, left, and right carotid sides; (3) endothelial function biomarkers measurements in a population without diagnosed pCAD.

The limitations of our study include: (1) $PM_{2.5}$ ground monitoring stations are inequally distributed and a limited number in some geographical areas[68] and satellite-derived $PM_{2.5}$ compared to ground monitoring stations better estimates spatial variability. Even with some limitations, $PM_{2.5}$ from monitoring stations better captures $PM_{2.5}$ temporal variations and peaks of exposure compared with satellite data in the Mexico City Metropolitan area.[27] (2) We do not have information on $PM_{2.5}$ exposure at work or during commuting, which might impact individual $PM_{2.5}$ exposure levels.[69] (3) We do not have data on $PM_{2.5}$ composition, which is a key factor for CVD effects related to $PM_{2.5}$ exposure.[70,71] (4) The high percentage of NOx imputed data might underestimate the mediation effect of the biomarkers in the $PM_{2.5}$–cIMT associations due to the loss of variability of imputed data. (5) We excluded 33 samples with CV > 15% for NOx and imputed them as missing values; the high percentage of samples with CV > 15% might be because measurements are closer to the technique's LOD, and CV increases with decreasing target concentrations.[72] (6) We did not include eNOS polymorphism determination. A previous study showed that eNOS polymorphisms affect the oxidative stress level regarding PM_{10} exposure.[73] Thus the eNOS genotype might also participate in the NOx levels related to $PM_{2.5}$ exposure.

Despite the limitations, our study is the first to examine the mediation role of endothelial function biomarkers in the $PM_{2.5}$–cIMT associations in Mexico City. Capturing early stages of subclinical atherosclerosis disease through anatomic measurements like cIMT or biomarkers might lead to the prevention of atherosclerosis diseases and related clinical outcomes. In the future, our final aim is to generate scientific evidence that might lead stakeholders to plan actions to reduce or more strictly surveil ambient air pollution levels in Mexico City, one of the most polluted cities worldwide.

Conclusions

$PM_{2.5}$ exposure was associated with m_cIMT at bilateral, left, and right, and endothelial function biomarkers, L-arginine and NOx, did not mediate $PM_{2.5}$–cIMT associations. Although L-arginine did not mediate $PM_{2.5}$ and cIMT associations, $PM_{2.5}$ was associated with a decrease in L-arginine concentrations. Further studies should confirm our results and explore the pathways underlying $PM_{2.5}$ and L-arginine associations. Other biomarkers associated cIMT increase and L-arginine should be evaluated, such as ADMA.

Conflicts of interest statement

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

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