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## Traffic-related air pollution and carotid plaque burden in a canadian city with low-level ambient pollution

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ORIGINAL RESEARCH

# Traffic-Related Air Pollution and Carotid Plaque Burden in a Canadian City With Low-Level Ambient Pollution

Markey Johnson , PhD; Jeffrey R. Brook, PhD; Robert D. Brook, MD; Tor H. Oiamo, PhD; Isaac Luginaah, PhD; Paul A. Peters, PhD; J. David Spence, MD

**BACKGROUND:** The association between fine particulate matter and cardiovascular disease has been convincingly demonstrated. The role of traffic-related air pollutants is less clear. To better understand the role of traffic-related air pollutants in cardiovascular disease development, we examined associations between NO<sub>2</sub>, carotid atherosclerotic plaque, and cardiometabolic disorders associated with cardiovascular disease.

**METHODS AND RESULTS:** Cross-sectional analyses were conducted among 2227 patients (62.9±13.8 years; 49.5% women) from the Stroke Prevention and Atherosclerosis Research Centre (SPARC) in London, Ontario, Canada. Total carotid plaque area measured by ultrasound, cardiometabolic disorders, and residential locations were provided by SPARC medical records. Long-term outdoor residential NO<sub>2</sub> concentrations were generated by a land use regression model. Associations between NO<sub>2</sub>, total carotid plaque area, and cardiometabolic disorders were examined using multiple regression models adjusted for age, sex, smoking, and socioeconomic status. Mean NO<sub>2</sub> was 5.4±1.6 ppb in London, Ontario. NO<sub>2</sub> was associated with a significant increase in plaque (3.4 mm<sup>2</sup> total carotid plaque area per 1 ppb NO<sub>2</sub>), exhibiting a linear dose-response. NO<sub>2</sub> was also positively associated with triglycerides, total cholesterol, and the ratio of low- to high-density lipoprotein cholesterol ( $P<0.05$ ). Diabetes mellitus mediated the relationship between NO<sub>2</sub> and total carotid plaque area ( $P<0.05$ ).

**CONCLUSIONS:** Our results demonstrate that even low levels of traffic-related air pollutants are linked to atherosclerotic plaque burden, an association that may be partially attributable to pollution-induced diabetes mellitus. Our findings suggest that reducing ambient concentrations in cities with NO<sub>2</sub> below current standards would result in additional health benefits. Given the billions of people exposed to traffic emissions, our study supports the global public health significance of reducing air pollution.

**Key Words:** air pollution ■ atherosclerosis ■ atherosclerotic plaque ■ cardiovascular disease ■ diabetes mellitus ■ nitrogen dioxide ■ traffic-related air pollution

The role of air pollution in exacerbating and triggering myocardial events, including mortality, has been convincingly demonstrated.<sup>1</sup> There is compelling evidence that atherosclerosis—which forms the basis for much of cardiovascular disease (CVD) pathology—is driven by inflammation, as well as endothelial and metabolic dysfunction,<sup>1–3</sup> and that air pollution contributes to cardiometabolic disease

by promoting systemic inflammation.<sup>4,5</sup> However, the role of air pollution, particularly traffic-related air pollutants (TRAP) such as NO<sub>2</sub> in the development of atherosclerosis, is still poorly understood. This study examines associations between long-term exposure to NO<sub>2</sub>, atherosclerotic plaque burden, and cardiometabolic disorders that may contribute to CVD development.

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## CLINICAL PERSPECTIVE

### What Is New?

- We found that even low levels of traffic-related air pollution were significantly associated with higher carotid plaque burden, triglycerides, and total cholesterol, as well as a higher ratio of low- to high-density lipoprotein cholesterol.
- The relationship between traffic-related air pollution and carotid plaque burden appeared to be mediated by air pollution-induced diabetes mellitus.
- Our results suggest that air pollution-induced atherosclerotic plaque formation may play a role in cardiovascular disease susceptibility, particularly among diabetics; and that these processes occur even in cities with relatively low exposures.

### What Are the Clinical Implications?

- We report that a 10 ppb increase in NO<sub>2</sub>—the range observed in the low-concentration city of London, Ontario, Canada—contributes to a 33.6 mm<sup>2</sup> increase in total plaque area. Plaque burden of this magnitude has previously been associated with a 5% increase in 5-year risk of stroke, myocardial infarction, and vascular mortality.
- The association between NO<sub>2</sub> and plaque in this study is clinically significant because exposure to traffic-related air pollution is ubiquitous; and because previous research suggests that even modest elevation or increase in total carotid plaque area is clinically relevant.
- Although these findings have their most obvious application in public health and regulatory policy, there may be a place for advising patients to follow local air quality indices and take steps to minimize their exposure to traffic-related air pollution, particularly if they are diabetic or have existing cardiovascular disease.

### Nonstandard Abbreviations and Acronyms

<b>CAC</b>	coronary artery calcification
<b>IMT</b>	intima media thickness
<b>LUR</b>	land use regression
<b>SES</b>	socioeconomic status
<b>SPARC</b>	Stroke Prevention and Atherosclerosis Research Centre
<b>TRAP</b>	traffic-related air pollutants
<b>TPA</b>	total carotid plaque area

A growing number of studies have examined associations between air pollution, subclinical atherosclerosis, and vascular disease with varying results.

Fine particulate matter (PM<sub>2.5</sub>) has been positively associated with carotid intima media thickness (IMT) and coronary artery calcification (CAC).<sup>3,6</sup> Other studies reported no association between IMT, CAC, ankle brachial index, and PM<sub>2.5</sub>.<sup>7–10</sup> NO<sub>2</sub> was associated with ankle brachial index but not IMT in 1 study,<sup>11</sup> and left IMT in another.<sup>12</sup> Nitrogen oxides (NOx) were also associated with CAC—but not IMT—progression.<sup>13</sup>

Previous studies examining air pollution and atherosclerosis had several limitations that reduce their interpretability. Most relied on IMT, which is biologically and genetically distinct from atherosclerosis<sup>14–16</sup>; CAC was also used in some studies. CAC is highly correlated with plaque burden,<sup>17</sup> and both CAC and plaque burden are strong predictors of future CVD risk.<sup>15</sup> However, CAC has several notable limitations. It is an indirect marker of atherosclerosis mediated by different biological processes,<sup>18–23</sup> with a much slower response to therapy than plaque burden measured by ultrasound,<sup>24</sup> and CAC scans increase patient exposure to radiation.<sup>24</sup> While MESA-Air was a landmark prospective evaluation that demonstrated important associations between air pollution, CAC, and IMT, the study did not include any direct metric of atherosclerotic plaque. Some studies were also limited by using air pollution data from sparse regulatory monitoring networks rather than spatially refined data.

We examined associations between outdoor residential NO<sub>2</sub>, atherosclerotic plaque burden, and cardiometabolic disorders in London, Ontario, Canada—a city with relatively low TRAP—using accurate, high-resolution methods to assess atherosclerosis and air pollution. Atherosclerotic plaque was quantified using carotid total plaque area (TPA) measured by 2-dimensional carotid ultrasound, a stronger predictor of CVD risk compared with IMT.<sup>25–28</sup> Unlike CAC, TPA is a direct metric of atherosclerosis and is more mechanistically and biologically related to the pathobiological process driving CVD events.<sup>16,19</sup> Only 1 previous study used TPA based on carotid ultrasound to examine air pollution impacts, and they saw no significant associations between TRAP and carotid artery atherosclerosis.<sup>29</sup>

Outdoor residential NO<sub>2</sub> was estimated by land use regression (LUR) modeling, which provides more accurate fine-scale intraurban concentrations compared with proximity models, regulatory monitoring, and satellite-derived estimates<sup>30,31</sup>; and has been shown to have comparable, and in some instances better, performance compared with dispersion models.<sup>30</sup> Cardiometabolic disorders included diabetes mellitus, hypertension, blood pressure (BP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), LDL:HDL ratio, triglycerides, and body mass index (BMI).

## METHODS

### Study Population

The study population comprised patients at the Stroke Prevention and Atherosclerosis Research Centre (SPARC) in London, Ontario, Canada, a small university city (population 385 000). Patients are referred to SPARC following a stroke or transient ischemic attack, or for asymptomatic carotid stenosis, early onset or severe vascular disease, or family history of vascular disease.

Approval to conduct the research described in this article was obtained from the Research Ethics Boards of Health Canada and the University of Western Ontario (protocol number 107051). The requirement to obtain informed consent to analyze the database was waived. Air pollution and neighborhood socioeconomic status (SES) data used in this study are available through The Canadian Urban Environmental Health Research Consortium repository at [canue.ca](http://canue.ca) or by request to the corresponding author. To comply with ethics and privacy requirements, clinical data used in this study will not be made available without additional Research Ethics Boards approval. Requests for clinical data from qualified researchers may be submitted to Dr David Spence at University of Western Ontario ([dspence@robarts.ca](mailto:dspence@robarts.ca)).

### Carotid Plaque Measurements

TPA was defined as the sum of cross-sectional areas of all plaques between the clavicle and the angle of the jaw. TPA measurement has been described in detail previously.<sup>32</sup> Briefly, plaque area was measured using high-resolution duplex ultrasound scanners (Phillips ATL Mark 9, ATL 5000 HDI, and Philips IU-22, Advanced Technology Laboratories). Plaque was defined as a local thickening of the intima >1 mm.

Measurements were performed in magnified longitudinal views of each plaque in the right and left common, internal, and external carotid arteries. The plane of measurement for each plaque was chosen by finding the plane that showed the largest extent of the individual plaque, and then freezing and magnifying the view. Plaque was measured by tracing the perimeter of the magnified plaque image with an onscreen cursor and recording the cross-sectional plaque area calculated by the microprocessor in the scanner. This process was repeated until all visible plaques were measured.

Intraclass correlation for repeated measurements by the same technician was 0.94.<sup>32</sup> For linear regression models, a cube root transformation was used to normalize the distribution of TPA, as previously reported.<sup>17,33</sup> Baseline TPA measurements included in the analyses were collected from 1990 to 2013.

### Other Clinical Measurements and Health Outcomes

All clinical data and health outcomes were obtained from patient medical records. Diabetes mellitus and hypertension were based on a history of physician diagnosis. Clinical measurements were collected by SPARC. Clinical measurements included blood pressure (systolic and diastolic), TC, triglycerides, HDL-C, LDL-C, TC:HDL ratio, LDL:HDL ratio, and BMI. We also examined elevated risk categories defined as follows: high cholesterol (TC >240 mg/dL), high LDL (>160 mg/dL), low HDL (<40 mg/dL), high triglycerides (>200 mg/dL), high TC:HDL ratio (men: >4.5, women: >4.0), high LDL:HDL (men >3.6, women: >3.2), high blood pressure (systolic BP ≥140 and diastolic BP ≥90), overweight or obese (BMI ≥25), and obese (BMI ≥ 30). TPA and clinical measurements were collected during the same examination visit.

### Traffic-Related Air Pollution

The methods used to generate outdoor residential NO<sub>2</sub> concentrations for this study have been described previously.<sup>34</sup> Briefly, Ogawa passive samplers were used to measure 2-week integrated ambient NO<sub>2</sub> concentrations at 50 locations throughout the city of London, Ontario, Canada in spring 2010. A land-use regression model was developed to estimate long-term NO<sub>2</sub> concentrations by regressing spatially varying land use characteristics against NO<sub>2</sub> at the 50 sampling sites. Model predictors included traffic density within 150 meters (m), dwelling density within 1000 m, distance to the nearest highway, industrial land use within 1600 m, and length of railways within 550 m. The LUR model explained 78% of the spatial variability in measured NO<sub>2</sub>.

Exposure surfaces for intraurban variations in NO<sub>2</sub> based on both air monitoring and LUR models have been shown to be stable over time, suggesting that they are representative of long-term gradients of exposure in urban populations.<sup>35–38</sup> Estimates of NO<sub>2</sub> from the LUR model were assigned to each patient based on their residential location, indicated by the 6-character postal code reported during the clinical visit in which TPA was measured. In urban areas, Canadian 6-character postal codes are highly local, typically representing an area smaller than a city block.

### Covariates

Covariates included age, sex, smoking status (ever smoker, current smoker, past smoker, never smoker, and pack-years) and SES. Age, sex, and smoking were obtained from patient medical records. SES was assessed using neighborhood measures from the Canadian census, including average income and percent of population with a university degree or diploma in the census dissemination area where each

patient resided. A Canadian dissemination area is the smallest geographic unit for which all census data are available, with a population of  $\approx 400$  to 700 people.<sup>39</sup> Dissemination area–level SES variables have been used effectively to address residual confounding associated with SES in previous analyses linking air pollution and health outcomes (eg, mortality, CVD, and lung cancer) in large population-based cohorts.<sup>40,41</sup> SES variables were linked to patients based on their residential postal code.

### Inclusion Criteria

Analyses were conducted on patients living within the city of London for whom LUR NO<sub>2</sub> were available. Analyses were limited to patients with at least 2 clinical visits to ensure inclusion of patients with detailed medical history, as well as complete residential location and demographic information including age, sex, smoking history, and SES.

### Statistical Analysis

Multiple linear regression models were used to estimate associations between NO<sub>2</sub>, TPA, and other continuous cardiometabolic outcomes. Logistic regression models were specified for binary outcomes. Covariates were selected based on their relevance and strength of association with the outcome of interest, and optimization of multiple linear regression models based on the Akaike Information Criterion. All models were adjusted for age, sex, smoking, and SES.

Mediation analyses were conducted using methods developed by Dudley et al<sup>42</sup> and Jasti et al.<sup>43</sup> Based on the large sample size, and the similarity in sample size between models used to assess mediation, we used the Sobel test to assess the significance of potential mediation.<sup>44</sup> Effect modification was also considered. Further details are provided in Data S1 and Figure S1.

Data included in these analyses were collected between 1990 and 2013, with 84% of the examinations carried out between 2000 and 2013 (n=1878). We conducted multiple sensitivity analyses to test whether our results were sensitive to the date of the clinical examinations. We considered models that were fully or partially stratified by examination date. LUR models have been consistently shown to provide stable long-term estimates of NO<sub>2</sub> over a 10-year period<sup>35–38</sup>; therefore, we stratified by 10 years from the date of NO<sub>2</sub> data collection in 2010, resulting in 2 cohorts: patients with examination dates from 1990 to 1999 (>10 years from NO<sub>2</sub> data collection, n=349), and patients with examination dates from 2000 to 2013 (within 10 years of NO<sub>2</sub> data collection, n=1878). In fully stratified models, we calculated

separate estimates for all regression parameters in the 1990–1999 versus 2000–2013 patients. In partially stratified models, we reported separate estimates for all parameters that differed between the 1990–1999 and 2000–2013 groups, with combined estimates for parameters that did not vary between the 2 patient groups.

To further assess the potential impacts of examination date, we specified mixed effect linear regression models for TPA with time period of clinical examination date included as a random effect. Mixed models included a 4-level random effect variable (grouped by quartiles) based on the clinical examination date. We also considered the impact of patient age, plaque transformation, and influential observations on reported model results. Sensitivity analyses and effect modifiers are further described in Data S1.

Statistical analyses were performed using SAS 9.3. Dose–response analysis was conducted using a natural cubic spline function in R 3.1.

## RESULTS

### Descriptive Statistics

Descriptive statistics are provided in Table 1. Patients represented an older population, with a mean age of 62.9 (Min: 18.0, Max: 96.0) years. Patients evenly comprised women (49.5%) and men (50.5%). There was a high prevalence of smoking in the patient population; more than half reported either current (17%) or past (42%) smoking. Among ever smokers—comprising both past and current smokers—pack years ranged from 0 to 165, with a mean of 13.5 and median of 5 (Min: 0, Max: 165) pack years. Most patients lived in neighborhoods with a lower proportion of university graduates (Mean: 27%, Min: <1%, Max: 100%) and lower mean neighborhood income compared with national averages.<sup>45</sup> TPA ranged from 0 to 156 mm<sup>2</sup>, with a mean of 109 mm<sup>2</sup>, and median of 70.0 (Min: 0, Max: 873) mm<sup>2</sup>.

The distribution of NO<sub>2</sub> in the city is shown in Figure 1. Outdoor residential NO<sub>2</sub> concentrations at patient homes ranged from 3.0 to 13.0 parts per billion (ppb), with a mean of 5.4 ppb at patient postal codes. NO<sub>2</sub> exposure in this patient population was consistent with the distribution of outdoor residential NO<sub>2</sub> in a population representative cohort; ie, among the 381522 London residents of the Canadian Census Health and Environment Cohort (CanCHEC), outdoor residential NO<sub>2</sub> estimated using the same LUR model ranged from 2.6 to 18.9 ppb, with a mean of 4.7.<sup>41</sup> Ambient pollutant concentrations in Canadian cities are typically much lower than in other cities internationally, falling well below World Health Organization's guideline of 21.2 ppb for mean annual NO<sub>2</sub>.<sup>46</sup> For example, annual average NO<sub>2</sub> ranged from 15 to 19 ppb in Toronto, Edmonton, and

**Table 1. Descriptive Statistics for Sociodemographic Characteristics, Smoking, Air Pollution, Clinical Measurements, and Health Outcomes**

	Sample Size (n)	Mean	SD	Median	Min	Max
Age, y	2227	62.9	13.8	64.0	18.0	96.0
Income (average income by DA)*	2227	\$34 215	\$13 160	\$30 062	\$13 842	\$155 960
Education (% University Degree by DA)*	2227	27.0%	17.5%	23.7%	<1%	100%
Pack y	2227	13.5	18.6	5.0	0	165
Nitrogen dioxide (NO <sub>2</sub> )	2227	5.4	1.6	5.1	3.0	13.0
	Sample Size (n)	Frequency (%)				
Female	2227	1102 (49.5)				
Ever smoker	2227	1306 (58.6)				
Current smoker	2227	375 (16.8)				
Past smoker	2227	931 (41.8)				
Never smoker	2227	921 (41.4)				
	Sample Size (n)	Mean	SD	Median	Min	Max
Plaque area, mm <sup>2†</sup>	2227	109	122	70.0	0	873
Systolic BP, mm Hg	2222	144	21.4	142	92.0	240
Diastolic BP, mm Hg	2221	82.3	12.8	82.0	43.0	140
Total cholesterol, mg/dL	1953	195	47.0	192	81.2	583
Triglycerides, mg/dL	1943	160	104	133	6.2	1594
HDL cholesterol, mg/dL	1935	51.9	16.9	49.5	3.9	192
TC:HDL ratio	1935	4.1	2.0	3.8	1.3	52.7
Triglyceride:HDL ratio	1930	3.7	4.0	2.8	0.1	71.1
LDL cholesterol, mg/dL	1898	112	41.3	108	10.0	387
LDL:HDL ratio	1896	2.4	1.2	2.1	0.2	14.9
BMI, kg/m <sup>2</sup>	1875	27.4	5.0	26.7	14.7	51.3
	Sample Size (n)	Frequency (%)				
Diabetes mellitus	2221	328 (14.8)				
High blood pressure‡	2221	485 (21.8)				
Hypertension	2059	1420 (69.0)				
High cholesterol, >240 mg/dL	1953	324 (16.6)				
Medium/high cholesterol, >200 mg/dL	1953	847 (43.4)				
High triglycerides, >200 mg/dL	1943	463 (23.8)				
High TC:HDL ratio§	1935	713 (36.8)				
Low HDL, <40 mg/dL	1935	469 (24.2)				
High LDL, >160 mg/dL	1898	240 (12.6)				
High LDL:HDL ratio	1896	305 (16.1)				
Overweight or obese, BMI ≥25	1875	1240 (66.1)				
Obese, BMI ≥30	1875	482 (25.7)				

BMI indicates body mass index; BP, blood pressure; DA, dissemination area; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Max, maximum; Min, minimum; and TC, total cholesterol.

\*A dissemination area (DA) is a "small, relatively stable geographic unit composed of one or more adjacent dissemination blocks...with a population of 400 to 700 people. It is the smallest standard geographic area for which all census data are disseminated" (Statistics Canada).

†Untransformed plaque area (mm<sup>2</sup>).

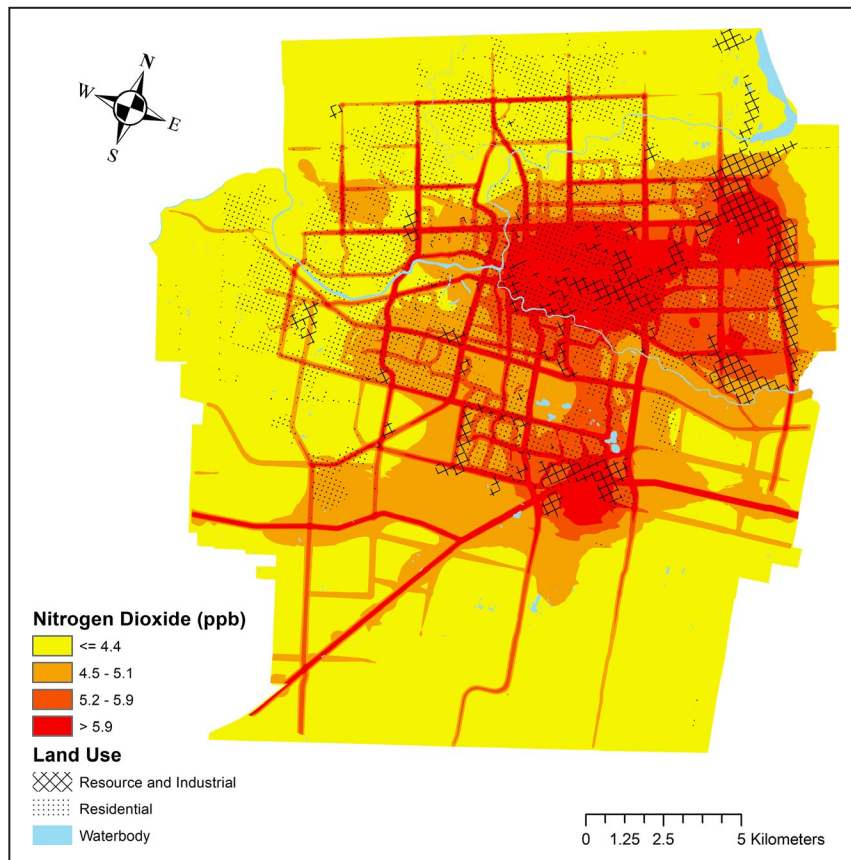
‡High blood pressure was defined as both systolic ≥140 mm Hg and diastolic ≥90 mm Hg.

§High TC:HDL ratio was defined as ≥4.5 for men and ≥4.0 for women.

||High LDL:HDL ratio was defined as ≥3.6 for men and ≥3.2 for women.

Calgary in 2010, while Hong Kong, China had an annual average of almost 40 ppb.<sup>46</sup> London, Ontario displayed the lowest mean NO<sub>2</sub> concentrations among 10 Canadian cities with LUR models.<sup>41</sup>

Descriptive statistics for smoking, obesity, diabetes mellitus, and hypertension stratified by age and sex among study patients compared with the general population are provided in Data S2 and Tables S1 through



**Figure 1. Distribution of NO<sub>2</sub> in the city of London, Ontario.**

Higher NO<sub>2</sub> concentrations are localized mainly in the urban core, with much lower concentrations in the suburbs. NO<sub>2</sub> concentrations were largely driven by traffic indicators, which explained ≈70% of the variability in NO<sub>2</sub> captured by the model. Dwelling density (17%), industrial land use (10%), and railroad lines (4%) explained the remainder of the variation in NO<sub>2</sub> concentrations. LUR indicates land use regression; and NO<sub>2</sub>, nitrogen dioxide. The map of NO<sub>2</sub> in London is reprinted by permission of Taylor & Francis, Ltd, <http://www.tandfonline.com> on behalf of Air & Waste Management Association, from a figure in Oiamo et al.,<sup>34</sup> copyright ©2012 Air & Waste Management Association, [www.awma.org](http://www.awma.org).

S4. The prevalence of current smoking by age and sex among patients in the study was similar to the general population. However, the prevalence of obesity, diabetes mellitus, and hypertension was higher among patients. For obesity and diabetes mellitus, this was driven by elevated prevalence among younger patients, while the prevalence of hypertension was higher among patients in all age and sex groups. See Data S2 for further discussion.

### Associations Among NO<sub>2</sub>, TPA, and Other Clinical Measurements

Linear regression results for NO<sub>2</sub> are reported in Table 2. The NO<sub>2</sub>-TPA dose–response curve is provided in Figure 2. Mean NO<sub>2</sub> by TPA quartile is provided in Data S3 and Figure S2. NO<sub>2</sub> was significantly associated with TPA adjusting for age, sex, smoking,

and SES, with a 1.2 mm<sup>2</sup> increase in cube root transformed TPA per 1 ppb increase in NO<sub>2</sub>. For untransformed TPA, a 1 ppb increase in NO<sub>2</sub> was associated with a 3.4 mm<sup>2</sup> increase in TPA, or 33.6 mm<sup>2</sup> increase in TPA per 10 ppb ( $P<0.05$ ). NO<sub>2</sub> was also positively associated with triglycerides, TC, and LDL:HDL ratio. NO<sub>2</sub> was marginally associated with TC:HDL ratio and triglyceride:HDL ratio. NO<sub>2</sub> was not associated with systolic or diastolic BP, LDL-C, HDL-C, or BMI.

Logistic regression model results for NO<sub>2</sub> are reported in Table 3. NO<sub>2</sub> was significantly associated with increased odds of exceeding clinically relevant thresholds for triglycerides, TC, TC:HDL ratio, and LDL:HDL ratio, as well as with obesity in women. NO<sub>2</sub> was marginally associated with diabetes mellitus and obesity ( $P<0.10$ ), but was not associated with hypertension, high blood pressure, high LDL-C, low HDL-C, or obesity in men.



**Table 2. Multiple Linear Regression Analysis of NO<sub>2</sub>, Plaque Area, and Other Clinical Measurements**

	Sample Size (n)	Regression Coefficient and 95% CI for NO <sub>2</sub> (per 1 ppb)	
		Unadjusted Models	Adjusted Models
Plaque area, mm <sup>2</sup>	2227	2.55 [1.42, 3.69] <sup>†</sup>	1.22 [0.29, 2.15] <sup>†</sup>
Systolic BP, mm Hg	2222	0.46 [-0.11, 1.02]	0.24 [-0.33, 0.82]
Diastolic BP, mm Hg	2221	-0.09 [-0.43, 0.25]	0.12 [-0.23, 0.48]
Total cholesterol, mg/dL	1953	1.35 [-0.02, 2.71] <sup>‡</sup>	1.73 [0.36, 3.11] <sup>‡</sup>
LDL-C, mg/dL	1898	0.43 [-0.79, 1.65]	0.68 [-0.57, 1.93]
HDL-C, mg/dL	1935	-0.44 [-0.93, 0.05] <sup>‡</sup>	-0.24 [-0.73, 0.24]
TC:HDL ratio	1935	0.06 [0.00, 0.12] <sup>†</sup>	0.05 [-0.01, 0.11] <sup>‡</sup>
LDL:HDL ratio	1896	0.04 [0.01, 0.08] <sup>†</sup>	0.04 [0.01, 0.08] <sup>†</sup>
Triglycerides, mg/dL	1943	5.36 [2.35, 8.37] <sup>†</sup>	4.61 [1.38, 7.84] <sup>†</sup>
Triglyceride:HDL ratio	1930	0.13 [0.01, 0.24] <sup>†</sup>	0.11 [-0.01, 0.23] <sup>‡</sup>
BMI, kg/m <sup>2</sup>	1875	0.17 [0.03, 0.32] <sup>†</sup>	0.09 [-0.06, 0.24]

Models linking NO<sub>2</sub> concentrations (per 1 ppb) with continuous clinical measurements and health outcomes were adjusted for age, sex, smoking, and SES (ie, Plaque Area= $\beta_0+\beta_{NO_2}+\beta_{Age}+\beta_{Sex}+\beta_{Smoking}+\beta_{SES}$ ). Plaque area was modeled as cube root transformed TPA. BMI indicates body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NO<sub>2</sub>, nitrogen dioxide; ppb, parts per billion; SES, socioeconomic status; TC, total cholesterol; and TPA, total plaque area.

\**P*<0.01.

<sup>†</sup>*P*<0.05.

<sup>‡</sup>*P*<0.10.

## Mediation

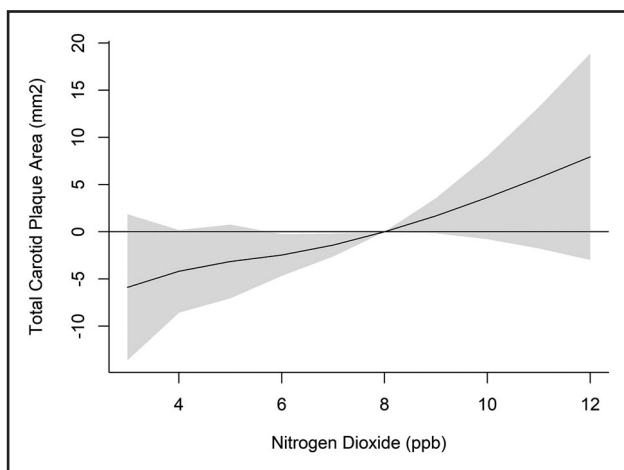
Further analyses were conducted to examine potential mediation of the relationship between NO<sub>2</sub> and TPA by cardiometabolic disorders (Table 4). Diabetes mellitus significantly mediated the relationship between NO<sub>2</sub> and TPA, while elevated TC:HDL was a marginally significant mediator. There were no other statistically significant mediators, and no evidence to suggest that diabetes mellitus and other cardiometabolic disorders significantly modified the association between NO<sub>2</sub> and plaque.

## Sensitivity Analyses

We conducted sensitivity analyses to examine associations between NO<sub>2</sub> and plaque among patients whose ultrasound examination was conducted within 10 years of NO<sub>2</sub> data collection (ie, patients with examination dates from 2000 to 2013) compared with patients with examination dates from 1990 to 1999. Patients with clinical examinations from 1990 to 1999 were younger, and had lower plaque burden compared with patients from 2000 to 2013 (*P*<0.05). In stratified and partially stratified models, the association between NO<sub>2</sub> and TPA was slightly stronger among patients assessed from 1990 to 1999 (2.2 mm<sup>2</sup>/1 ppb) compared with patients from 2000 to 2013 (1.0 mm<sup>2</sup>/1 ppb). The relationship between SES and TPA also varied between patient cohorts, with a strong inverse association among patients from 2000 to 2013, and nonsignificant positive association among patients from 1990 to 1999. Associations between age, sex, smoking, and TPA were similar in both patient cohorts.

Limiting the analyses to older patients did not change the results for the full cohort (see Data S1 for details). However, in analyses stratified by examination date (1990–1999 versus 2000–2013), we saw stronger associations between NO<sub>2</sub> and TPA among older patients (40 years or older) in both patient groups (1990–1999: 2.4 mm<sup>2</sup>/1 ppb, *P*<0.05; 2000–2013: 1.1 mm<sup>2</sup>/1 ppb *P*<0.05).

Mixed model results testing examination date were comparable to linear regression models for TPA (1.1 mm<sup>2</sup>/1 ppb *P*<0.05); therefore, linear regression



**Figure 2. Dose–response curve for NO<sub>2</sub> and TPA.**

Local NO<sub>2</sub> concentrations were significantly associated with TPA after adjustment for age, sex, smoking, and socioeconomic status. There was a 1.2 mm<sup>2</sup> increase in cube root transformed TPA, per 1 ppb increase in NO<sub>2</sub>. ppb, parts per billion; and TPA, total carotid plaque area.

**Table 3. Multiple Logistic Regression Analysis of NO<sub>2</sub>, Clinical Measurements, and Health Outcomes**

	Sample Size (n)	Odds Ratio and 95% Confidence Interval for NO <sub>2</sub> (per 1 ppb)	
		Unadjusted Models	Adjusted Models
Diabetes mellitus	2221	1.13 [1.06, 1.21]*	1.07 [0.99, 1.15]†
High BP	2221	0.96 [0.89, 1.02]	0.97 [0.90, 1.04]
Hypertension	2059	1.06 [0.99, 1.12]†	1.02 [0.95, 1.09]
High TC	1953	1.08 [1.00, 1.16]‡	1.09 [1.01, 1.18]‡
High TC:HDL ratio	1935	1.14 [1.07, 1.22]*	1.08 [1.01, 1.15]‡
High LDL:HDL ratio	1896	1.09 [1.02, 1.15]*	1.11 [1.02, 1.21]‡
High LDL-C	1898	1.04 [0.98, 1.11]	1.07 [0.98, 1.17]
Low HDL-C	1935	1.08 [1.00, 1.18]†	1.04 [0.96, 1.12]
High triglycerides	1943	1.13 [1.05, 1.22]*	1.12 [1.04, 1.20]*
Overweight or obese	1875	1.10 [1.03, 1.17]*	1.02 [0.96, 1.09]
Obese, all	1875	1.05 [0.98, 1.11]	1.06 [1.00, 1.14]†
Obese, women	949	1.14 [1.05, 1.25]*	1.13 [1.03, 1.24]‡
Obese, men	926	1.06 [0.97, 1.16]	1.00 [0.91, 1.10]

Models linking NO<sub>2</sub> concentrations (per 1 ppb) with binary clinical measurements and health outcomes were adjusted for age, sex, smoking, and SES (ie, Diabetes =  $\beta_0 + \beta_{\text{NO}_2} + \beta_{\text{Age}} + \beta_{\text{Sex}} + \beta_{\text{Smoking}} + \beta_{\text{SES}}$ ). BMI indicates body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ppb, parts per billion; SES, socioeconomic status; and TC, total cholesterol.

\* $P < 0.01$ .

† $P < 0.10$ .

‡ $P < 0.05$ .

models were reported in the article. Sensitivity analyses for other clinical health outcomes are described in Data S1.

## DISCUSSION

We found that exposure to low levels of NO<sub>2</sub> was significantly associated with total carotid plaque area—an important measure of systemic atherosclerosis—and that heightened atherogenesis induced by TRAP may be at least partially mediated by air pollution–induced diabetes mellitus. TRAP was also related to other cardiometabolic abnormalities in this study.

### Plaque

Exposure to NO<sub>2</sub> in London, Ontario, a small city with relatively low ambient concentrations, was positively associated with cumulative plaque burden in a high-risk patient population. Previous studies have reported inconsistent results in relating air pollution with atherosclerosis, with interpretation of these findings hampered by methodological limitations.

Most studies to date relied on measurement of IMT, which is biologically and genetically distinct from atherosclerosis.<sup>16,25</sup> MESA-Air investigators cited several major limitations in using IMT to examine air pollution and atherosclerosis in observational studies, including the weakness of IMT as a marker for atherosclerosis,<sup>13</sup> and the sensitivity of the method to ultrasound device quality.<sup>47</sup> Some meta-analyses

reported statistically or marginally significant associations between PM<sub>2.5</sub> and IMT.<sup>10,48</sup> However, both MESA-Air<sup>13</sup> and a meta-analysis of 4 cohorts in the ESCAPE study using high-resolution (LUR) estimates of PM<sub>2.5</sub> and NO<sub>2</sub><sup>9</sup> found no statistically significant associations between air pollution and IMT. The current study, which used a direct measure of atherosclerotic plaque burden and spatially resolved pollutant concentrations, provides additional evidence for a link between TRAP and atherosclerosis.

Although total carotid plaque area is a stronger indicator of atherosclerosis<sup>16</sup> and CVD risk<sup>26–28,49,50</sup> compared with IMT, only 1 previous study, the Multicultural Community Health Assessment Trial (M-CHAT) in Vancouver, BC, examined associations between air pollution and TPA.<sup>29</sup> Like the current study, M-CHAT used high-resolution LUR models for TRAP coupled with ultrasound measures of TPA. However, they found no associations between TRAP and plaque burden. Ambient NO<sub>2</sub> levels among M-CHAT participants were slightly higher than in the London study population, with similar spatial variation. Compared with the current study, the M-CHAT population was much younger—with a median age of 46 years in M-CHAT versus 64 in London patients, and the sample size was much smaller (N=509). These limitations likely explain their null findings.

CVD is a multifactorial disease impacted by environmental, behavioral, and genetic risk factors. The association between NO<sub>2</sub> and plaque in this study is clinically significant because exposure to traffic pollution is ubiquitous; and because previous research

**Table 4. Mediating Effects of Cardiometabolic Disorders on the Association Between NO<sub>2</sub> and Plaque**

	N	Percent of the Total Effect That Is Mediated	Ratio of the Indirect to the Direct Effect	Sobel P Value
Continuous				
Systolic BP, mm Hg	2222	7.12%	0.08	0.32
Diastolic BP, mm Hg	2221	0.36%	<0.01	0.75
Total cholesterol, mg/dL	1953	≈(0.83%)	−0.01	0.77
Triglycerides, mg/dL	1943	6.07%	0.06	0.12
HDL-C, mg/dL	1935	3.05%	0.03	0.37
TC:HDL ratio	1935	2.27%	0.02	0.33
Trig:HDL ratio	1930	1.64%	0.02	0.45
LDL:HDL ratio	1896	3.89%	0.04	0.28
LDL-C, mg/dL	1898	0.31%	<0.01	0.83
BMI, kg/m <sup>2</sup>	1875	≈(0.83%)	−0.01	0.56
Binary				
Diabetes mellitus	2221	9.25%	0.41	0.03
High blood pressure	2221	≈(2.75%)	−0.09	0.29
Hypertension	2059	6.07%	0.19	0.49
High cholesterol	1953	1.42%	0.06	0.57
High Trig	1943	2.22%	0.06	0.55
Low HDL-C	1935	3.63%	0.11	0.27
TC:HDL level	1935	6.30%	0.16	0.10
High LDL-C	1898	4.46%	0.22	0.21
LDL:HDL level	1896	6.65%	0.27	0.16
Obese	1875	≈(1.38%)	−0.04	0.52
Overweight or obese	1875	≈(0.10%)	<0.01	0.83

Models were adjusted for age, sex, smoking, and SES. BMI indicates body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SES, socioeconomic status; TC, total cholesterol; and Trig, triglycerides.

suggests that even modest elevation or increase in TPA is clinically relevant.<sup>32,51</sup> For example, Spence et al.<sup>32</sup> reported that elevation of plaque burden was a strong predictor of the 5-year risk of stroke, myocardial infarction, and vascular mortality; after adjustment for a broad panel of risk factors, the 5-year risk of those events by TPA quartile was 5.6%, 10.7%, 13.9%, and 19.5%. Our results suggest that exposure to TRAP was associated with an elevation of ≈3.4 mm<sup>2</sup> per ppb of NO<sub>2</sub>—a difference of ≈33.6 mm<sup>2</sup> between patients with the highest and lowest levels of exposure—which suggests a possible increase in 5-year risk of 5% for those patients. Thus, our results may be regarded as clinically important as well as statistically significant.

## Diabetes Mellitus

There was a marginally significant positive association between NO<sub>2</sub> and diabetes mellitus in this study, which is consistent with emerging evidence that PM<sub>2.5</sub>, NO<sub>2</sub>, and other ambient pollutants may be associated with diabetes mellitus risk. Long-term exposure to NO<sub>2</sub>, NOx, PM<sub>2.5</sub>, and PM<sub>10</sub> has been positively associated with incident<sup>52–55</sup> and prevalent<sup>53</sup> diabetes mellitus,

as well as diabetes mellitus–related hospitalizations and mortality<sup>52–56</sup> and insulin resistance.<sup>53,57</sup> Recent reviews<sup>53,58</sup> highlight the importance of TRAP such as NO<sub>2</sub> in the development of diabetes mellitus.

Our results provide evidence that diabetes mellitus significantly mediated the relationship between NO<sub>2</sub> and plaque, suggesting that some of the observed association between NO<sub>2</sub> and plaque may be attributable to pathophysiology of diabetes mellitus. This finding is consistent with the increased risk of air pollution–mediated CVD outcomes among diabetics, and the hypothesized role of diabetes mellitus in atherosclerosis. Diabetes mellitus has been associated with increased risk of air pollution–mediated CVD morbidity and mortality,<sup>54,57</sup> as well as atherosclerosis and plaque development.<sup>59–61</sup> Metabolic syndrome severity was also associated with carotid plaque area in a small adult cohort,<sup>62</sup> providing additional evidence for a linkage between metabolic disease and plaque burden.

Several potential mechanisms have been proposed to explain susceptibility to air pollution–mediated–CVD outcomes among diabetics including increased inflammation, vascular reactivity, and endothelial dysfunction.<sup>4,57</sup> Similar mechanisms have

been proposed to explain the increased severity of CVD and atherosclerosis observed among diabetics.<sup>63–66</sup> Renin–angiotensin–aldosterone-system activation,<sup>60</sup> overexpression of microRNA,<sup>67</sup> and altered mineral metabolism<sup>66</sup> may also contribute to atherosclerosis among diabetics.

### Other Cardiometabolic Disorders

NO<sub>2</sub> was significantly associated with total cholesterol, elevated cholesterol, elevated TC:HDL ratio, total triglycerides, and elevated triglycerides, suggesting that air pollution–mediated atherosclerosis may be related to metabolic changes including dyslipidemia and hypertriglyceridemia. These results are consistent with the limited evidence available from previous studies, which reported associations between air pollutants (eg, PM<sub>2.5</sub>, NO<sub>2</sub>, and ozone), triglycerides, fasting glucose, apolipoprotein B, hemoglobin A1c, and elevated TC and LDL-C, as well as reduced HDL-C.<sup>68–71</sup> Our results provide further evidence that air pollution contributes to cardiometabolic disorders.

NO<sub>2</sub> was not significantly associated with obesity, BMI, BP, or hypertension in this patient population. Previous literature reviews found clear evidence for a causal association between ambient PM<sub>2.5</sub> and increased arterial BP,<sup>72</sup> as well as growing evidence for an association between PM<sub>2.5</sub> and hypertension.<sup>6,73,74</sup> However, evidence for NO<sub>2</sub> is limited.<sup>73</sup> A meta-analysis of 15 population-based cohorts in Europe (N=113 926) also reported no association between air pollutants (including PM<sub>2.5</sub> and NO<sub>2</sub>) and hypertension or BP.<sup>75</sup> Mixed results in the literature may be attributable to differences in study design, BP collection methods (eg, standardized day, time, and activity before measurement), pollutant of interest, and statistical analysis methods.

Mechanistic studies in animals suggest that the inflammatory effects of air pollution could promote obesity,<sup>53,76</sup> and several longitudinal studies linked TRAP with the development of obesity in children.<sup>53,76</sup> However, there is less convincing evidence linking air pollution and obesity in adults.<sup>76</sup> Associations between air pollution, BMI, and obesity in adults, particularly from longitudinal studies, are limited. Our results suggest that further work is needed to characterize associations between air pollution and obesity in adults.

Finally, there was a high prevalence of hypertension and obesity in the SPARC patients. Associations among NO<sub>2</sub>, hypertension, high blood pressure, BMI, and obesity may differ in healthy populations.

### Limitations

The results of the current study may be limited by several factors. Lack of residential history is a common limitation in air pollution epidemiology. In this study,

NO<sub>2</sub> concentrations were assigned to patients based on their residential address at the time of TPA measurement. While residential mobility is typically lower among noninstitutionalized older adults,<sup>77</sup> who comprise the majority of the study population, the potential impact of past exposures, particularly among patients who moved, is unknown.

LUR models have been widely used to estimate long-term exposure in epidemiological studies because they accurately characterize the long-term intraurban gradients required to support epidemiological analyses.<sup>30,31</sup> LUR modeling does not account for individual exposures that may vary as a result of exposure to air pollution at nonresidential (eg, work and school) locations, during daily commutes, or to indoor-generated pollutants such as those produced by heating, cooking, and other combustion sources. However, ambient NO<sub>2</sub> has been shown to be highly correlated with total personal exposure in previous meta-analyses,<sup>78</sup> likely attributable to the infiltration of outdoor pollutants into the home, and the large percentage of time North Americans spend indoors at home. For example, Canadians spend 70% of their time, on average (≈17 hours/d), indoors at home.<sup>79</sup> Furthermore, ambient NO<sub>2</sub> was more strongly associated with air-pollution–related health outcomes in the few studies that attempted to differentiate between ambient and nonambient exposures.<sup>78</sup> Therefore, outdoor residential concentrations generated by LUR models provide an appropriate measure of exposure in air pollution health studies.

NO<sub>2</sub> has been widely used as a marker for TRAP<sup>80</sup> because it better reflects the local scale heterogeneity in traffic emissions compared with PM<sub>2.5</sub> and O<sub>3</sub>,<sup>78</sup> and because NO<sub>2</sub> can be more reliably measured and modeled compared with other traffic pollutants.<sup>31</sup> However, there is growing evidence to suggest that NO<sub>2</sub> may be directly linked to some health outcomes<sup>81,82</sup> rather than simply acting as a marker for other TRAP species.

Previous studies reported strong associations between NO<sub>2</sub> and CVD and diabetes mellitus development.<sup>82</sup> In addition, previous experimental studies provide evidence that NO<sub>2</sub> exposure may lead to an increase in circulating and tissue (heart) specific inflammatory mediators generated from reactions with inhaled NO<sub>2</sub> in the epithelial lining, suggesting a potential mechanism for NO<sub>2</sub>-induced systemic inflammation.<sup>82</sup> However, while the US Environmental Protection Agency's Integrated Science Assessment concluded that long-term and short-term exposure to NO<sub>2</sub> were causally associated with respiratory outcomes, they determined that the evidence for a causal association between NO<sub>2</sub> and the development of CVD and diabetes mellitus was “suggestive, but not sufficient,” because past studies do not sufficiently address potential

confounding by other TRAP pollutants in studies linking NO<sub>2</sub> with CVD and diabetes mellitus development.<sup>82</sup>

While we reported associations between NO<sub>2</sub> and atherosclerosis, NO<sub>2</sub> can be highly correlated with other TRAP species, and this study was not designed to differentiate between effects of NO<sub>2</sub> and other TRAP. NO<sub>2</sub> concentrations were estimated using a LUR model, which may not easily distinguish between highly correlated pollutants with similar spatial distribution and sources. There are also nontraffic sources of NO<sub>2</sub>, which are reflected in the LUR model predictors. Therefore, further evidence is needed to isolate the impact of disparate pollutants and sources on cardiometabolic disease.

We also reported only cross-sectional associations. These results are supported by recent findings in MESA-Air that NO<sub>2</sub> and PM<sub>2.5</sub> were significantly associated with CAC progression.<sup>13</sup> However, it is important to consider this in more detail. The most significant limitation of cross-sectional analyses is the greater potential for exposure misclassification during the period of time that played a biological role in TPA development (ie, if patients relocated from differing exposure settings or if relative exposures in London changed over time). However, previous studies suggest that LUR pollution estimates and long-term concentration gradients are stable over the time-frame of this study,<sup>35–38</sup> and that seasonal LUR models adequately capture long-term concentration gradients, making these models appropriate for estimating long-term exposure in health studies.<sup>82</sup> Cesaroni et al.<sup>36</sup> reported that NO<sub>2</sub> models from samples collected 12 years apart showed good agreement, and similar associations with mortality. Eeftens et al.<sup>35</sup> found that LUR models for NO<sub>2</sub> measured in 2007 predicted a high proportion (77%) of the variation in NO<sub>2</sub> measurements from 1999 to 2000. Similarly, Wang et al.<sup>37</sup> reported that LUR models for NO<sub>2</sub> predicted a high proportion of the variability in measurements collected 7 years prior. Finally, de Hoogh et al.<sup>38</sup> found that NO<sub>2</sub> models developed using measurements collected 10 years apart showed good agreement across multiple countries in Western Europe. Furthermore, the population included in the analysis has been shown to have low residential mobility.<sup>77</sup>

Otherwise, longitudinal analyses are not unequivocally superior. Longitudinal studies of atherosclerosis have been limited by reliance on IMT and CAC progression. IMT represents a phenotype distinct from atherosclerosis,<sup>14–16</sup> and IMT progression does not accurately predict CVD risk compared with single-time measures.<sup>83</sup> Furthermore, the extremely small and variable changes in the progression of IMT decrease the power of longitudinal studies relying on IMT. CAC progression has been inconsistently associated with atherosclerosis and adverse cardiovascular events, with evidence that CAC progression can be associated

with plaque rupture, as well as with plaque stability and reduced CVD risk.<sup>21–23</sup> Thus, we believe that our cross-sectional analysis—given the robust methods of exposure and atherosclerosis assessment—is not a major limitation.

Mediation tests assume that models are correctly specified and assume a causal relationship between potential mediators (ie, diabetes mellitus) and plaque. Causality cannot be verified in the current study because the timing of diabetes mellitus diagnosis relative to plaque measurement is unknown. However, the assumption that diabetes mellitus contributes to plaque burden rather than vice versa is consistent with the existing literature.<sup>60,61,66</sup>

A further limitation is that we did not have data on medication use by individual patients. All patients received similar treatment (ie, usual care or aggressive therapy). Thus, it is unlikely that medication use confounded reported associations between NO<sub>2</sub> and cardiometabolic disorders. However, we cannot account for potential effect modification by medication subtypes.

Another limitation is the long period during which study data were collected. The analyses include data from patients under treatment at SPARC from 1990 to 2013, with 84% of the data collected from 2000 onward. Sensitivity analyses indicate a positive association between NO<sub>2</sub> and TPA in patients across the time span of the study. The association between NO<sub>2</sub> and TPA was stronger in patients with plaque measured from 1990 to 1999. Interestingly, patients from 1990 to 1999 were younger and had lower TPA. Furthermore, SES indicators were not strongly associated with TPA in the 1990–1999 patients (in contrast with the strong association between SES and TPA in 2000–2013 patients), suggesting that some of the increase in estimates of association between NO<sub>2</sub> and TPA among 1990–1999 patients may be the result of uncontrolled confounding by SES in that patient group. In addition to age, TPA, and SES, differences in unmeasured factors such as referral criteria and medication trends (ie, increases in statin use from 2000 onward) may have contributed to stronger associations between NO<sub>2</sub> and TPA in the 1990–1999 patient group. Despite the differences in magnitude, our results suggest a consistently positive association across time periods. Furthermore, hierarchical model results provide additional confirmation that the positive association between NO<sub>2</sub> and plaque was significant, accounting for differences between patients from 1990 to 1999 versus 2000 to 2013.

Finally, the patients in this study are not representative of the general population. Rather, they represent a subset of the general population who may be at higher risk for air pollution-mediated health impacts. Our results suggest that even low levels of TRAP may

contribute to atherosclerosis and CVD development in high-risk populations. Further research is needed to assess impacts of TRAP on atherosclerosis and CVD among healthy adults.

## CONCLUSIONS

The potential role of air pollution as a contributor to CVD development has enormous public health implications. This study provides unique new evidence for a link between long-term exposure to TRAP and atherosclerotic plaque burden, as well as cardiometabolic disorders including dyslipidemia, hypertriglyceridemia, and impaired glucose metabolism. Furthermore, our results suggest that air pollution-induced atherosclerotic plaque formation may play a role in CVD susceptibility, particularly among diabetics; and that these processes occur even in cities with relatively low exposures.

## ARTICLE INFORMATION

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### Supplementary Materials

**Data S1-S3**

**Tables S1-S4**

**Figures S1 and S2**

**References 33, 42-44**

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# SUPPLEMENTAL MATERIAL

## DATA S1. Mediation Analyses, Sensitivity Analyses and Effect Modification

### Methods

**Mediation Analyses:** Mediation analyses were conducted using methods developed by Dudley et al. (42) and Jasti et al. (43). The goal of our analyses was to test whether the reported association between nitrogen dioxide (NO<sub>2</sub>) and total plaque area (TPA) was potentially mediated by cardiometabolic (CM) disorders that are known to be associated with plaque and that may be affected by NO<sub>2</sub>. Specifically, we hypothesized that in addition to a direct association between NO<sub>2</sub> and plaque, there might be an indirect association between NO<sub>2</sub> and plaque, influenced by the cardiometabolic conditions associated with NO<sub>2</sub> (TRAP) that were assessed within our study population. These hypothesized relationships are illustrated in Figure S1. Direct effects (NO<sub>2</sub> → Plaque) are represented by 1. Indirect effects acting through a mediator (i.e., NO<sub>2</sub> → CM → Plaque) are represented by 2 and 3.

We used the Sobel test (44) to evaluate whether the observed reduction in our estimated association between NO<sub>2</sub> and TPA, after accounting for potential mediation, was statistically significant. The Sobel test was appropriate for our data based on our large sample size and similarity in sample size between the models used to assess mediation (i.e., models 1, 2, and 3). Results from alternate mediation tests, i.e., Goodman I and II, were consistent with the results of the Sobel test.

The statistical approach we used is described in further detail as follows.

$$\begin{aligned}\text{Model 1:} & \quad \text{TPA} = \beta_{\text{NO}_2} + \beta_{\text{Age}} + \beta_{\text{Sex}} + \beta_{\text{Smoking}} + \beta_{\text{SES}} \\ \text{Model 2:} & \quad \text{Mediator} = \beta_{\text{NO}_2} + \beta_{\text{Age}} + \beta_{\text{Sex}} + \beta_{\text{Smoking}} + \beta_{\text{SES}} \\ \text{Model 3:} & \quad \text{TPA} = \beta_{\text{Mediator}} + \beta_{\text{NO}_2} + \beta_{\text{Age}} + \beta_{\text{Sex}} + \beta_{\text{Smoking}} + \beta_{\text{SES}}\end{aligned}$$

Model 1 estimates the association between NO<sub>2</sub> and TPA without the potential mediator. Model 2 examines the association between NO<sub>2</sub> and the mediator. Model 3 estimates the association between NO<sub>2</sub> and TPA controlling for the potential mediator. The parameter estimates and standard errors from these models are used to estimate the Sobel test statistic, the percent of the total effect that is mediated, and the ratio of indirect to direct effects, as shown below.

$$\begin{aligned}\text{Sobel test statistic} & \quad = (a*b) / [\sqrt{(b^2*sa^2)+(a^2*sb^2)}] \\ \text{Percent of the total effect that is mediated} & \quad = (a*b) / [(a*b)+(c-(a*b))] \\ \text{Ratio of the indirect to the direct effect} & \quad = (a*b) / [(c-(a*b))]\end{aligned}$$

#### Where:

a = parameter estimate for NO<sub>2</sub> from Model 2  
sa = standard error for NO<sub>2</sub> from Model 2  
b = parameter estimate for the mediator from Model 3  
sb = standard error for the mediator from Model 3  
c = parameter estimate for NO<sub>2</sub> from Model 1  
sc = NO<sub>2</sub> standard error for NO<sub>2</sub> from Model 1

**Sensitivity Analyses and Effect Modification:** We further tested potential differences in exposure classification by examining associations between NO<sub>2</sub>, plaque, and other clinical outcomes among patients with clinical exams conducted within 10 years of NO<sub>2</sub> data collection (i.e., patients with clinical

exam from 2000-2013) compared with patients with exams from 1990-1999. We also considered the impact of age, plaque transformation, and influential observations on reported model results. We tested for potential effect modification using multiplicative interaction terms.

## Results

**Sensitivity Analyses:** Associations between NO<sub>2</sub> and plaque were robust with respect to plaque transformation; e.g., NO<sub>2</sub> was significantly associated with plaque in models specifying untransformed measures of plaque area. However, model diagnostics suggested a better model fit for cube root transformed plaque. Therefore, cube root transformed TPA was reported throughout the paper.

Sensitivity analyses for TPA in patients with clinical exams from 1990-1999 versus patients from 2000-2013 are reported in the main text. Among patients with clinical exams from 2000-2013, associations with NO<sub>2</sub> were slightly stronger for LDL:HDL ratio and obesity. The association between NO<sub>2</sub> and diabetes was slightly stronger among patients from 1990-1999. The associations with NO<sub>2</sub> for TC:HDL ratio and TC:HDL level were similar between 1990-1999 and 2000-2013 patients, but did not achieve statistical significance in either cohort alone.

We observed no difference in the association between NO<sub>2</sub> and plaque when younger participants were excluded from the full cohort; i.e., the difference in estimate of association was < 1% when patients aged < 30 years were excluded from the analyses and less than < 15% when patients aged < 40 or < 50 were excluded from the analyses. There was also no significant effect modification of the association between NO<sub>2</sub> and plaque by age group. However, there was some evidence of sensitivity to age in the 1990-1999 and 2000-2013 cohorts; i.e., associations between NO<sub>2</sub> and TPA were stronger among older patients. Furthermore, Spence (33) previously reported a dramatic increase in plaque burden among patients 40 years of age and older. Therefore, we conducted additional sensitivity analyses (reported in the main paper) excluding patients < 40 years from the stratified (1990-1999 vs 2000-2013) models.

Associations between NO<sub>2</sub> and other clinical outcomes did not vary significantly when younger patients were excluded from the analyses, with a difference of < 5% for most estimates of associations, and < 8% for all estimates of association, except for LDL which had a difference of 19%.

For all multiple linear regression models in which air pollution was (marginally or significantly) associated with clinical health outcomes, the inclusion of influential observations (absolute studentized residual  $\geq 3$ ) resulted in estimates that were closer to the null hypothesis pollution – i.e., when influential points were removed, the associations we observed were stronger than the associations reported in the paper. Although some influential observations were identified as statistical outliers, we had no reason to believe the measurements were erroneous; therefore, the more conservative results (including influential observations/outliers) were reported in the paper.

**Effect Modification:** There was some evidence to suggest a possible interaction between NO<sub>2</sub> and pack years in models for elevated (but not continuous) triglycerides ( $p < 0.10$ ). However, this interaction did not achieve statistical significance in this patient population. There was no significant effect modification by smoking status in any of the reported models.

There was some evidence to suggest that the association between NO<sub>2</sub> and obesity was stronger among women compared with men (interaction  $p$  value = 0.069). Similarly, the association between NO<sub>2</sub> and BMI was stronger among women, but neither the interaction nor the association between NO<sub>2</sub> and BMI

in women achieved statistical significance. There was no other evidence of effect modification by sex in these analyses. There was also some evidence to suggest that the association between NO<sub>2</sub> and high LDL was stronger among non-diabetics (p=0.056) compared with diabetics (p=0.41); however, this interaction was not statistically significant (p=0.13), likely due to the small number of diabetics in the stratified model (n=271).

TPA varied by season of measurement, but season was not a significant predictor of TPA in multiple regression models, and did not significantly modify the association between NO<sub>2</sub> and TPA in multiple regression models. There was no variation in other clinical measurements based on the season during which the measurement took place. Therefore, we did not adjust or stratify the models based on season.

## **DATA S2. Smoking, Obesity, Diabetes and Hypertension Prevalence in London Patients versus the General Population.**

Prevalence of current smoking, obesity, diabetes and hypertension among London patients versus the general population of Canada are provided in Tables S1 to S4. Results are stratified by age and sex. The prevalence of current smoking was generally similar between patients in the study compared with the general population, although the prevalence of current smoking was slightly higher among young female patients (20-34 years). Conversely, the prevalence of current smoking in young male patients (20-34 years) was slightly lower than that of the general population. These findings should be interpreted cautiously due to the small number of younger patients, and because the smoking status was based on current smoking rather than smoking history. We used current smoker to compare smoking prevalence because survey data assessing past or lifetime smoking status are less widely available for the general population. However, a comparison of lifetime smoking history between study patients and the general population would likely be more illuminating, given that many patients were referred to SPARC due to an acute, life threatening event that may have provided them with a strong motivation to quit smoking.

The prevalence of obesity was modestly higher among patients in the study compared with the general population, with the exception of older adults (65+) of both sexes, and among males aged 45-54. The greatest difference was among men aged 20-34 (2.5 times higher) and among women aged 35-44 (2 times higher). Diabetes showed a similar pattern, with the greatest differential between patients and general population in the younger age groups for both sexes, with a maximum differential of approximately 14.5 times higher among men aged 20-34 and approximately 4.6 times higher among women aged 35-44. The prevalence of diabetes between patients and general population was slightly higher among adults aged 45-64 of both sexes, and similar among older adults (65+ years) of both sexes.

The prevalence of hypertension was the most dramatically elevated among patients in the study compared to the general population. As with obesity and diabetes, the greatest differential prevalence between patients and general population occurred among younger adults of both sexes, and the difference in prevalence decreased among older age groups. However, unlike obesity and diabetes, the prevalence of hypertension remained higher among our patients compared to the general population for all age and sex groups, including the oldest adults. The prevalence in younger patients was 25 and 33 times higher than in the general population for men and women, respectively. Again, relative prevalence in the younger age groups should be interpreted cautiously due to the small number of younger patients in the study.

### **DATA S3: Mean Total Plaque Area by Nitrogen Dioxide (NO<sub>2</sub>) Quartile**

Figure S2 shows the mean cube root transformed total plaque area (TPA) by nitrogen dioxide (NO<sub>2</sub>) quartile for the London study population.

**Table S1. Prevalence of Current Smoker by Age and Sex in Study Patients and the General Population.**

Age	Female			Male		
	Study Population (n)	Study Population (%)	Canadian Population (%)	Study Population (n)	Study Population (%)	Canadian Population** (%)
20-34	31*	32.26	23.2	23	21.74	30.3
35-44	75	22.67	19.8	90	26.67	27.1
45-54	191	21.47	22.2	222	32.88	26.3
55-64	227	18.94	16.5	292	17.47	21.1
65+	578	9.00	9.3	498	11.85	10.1

**Table S2. Prevalence of Obesity by Age and Sex in Study Patients and the General Population**

Age	Female			Male		
	Study Population (n)	Study Population (%)	Canadian Population (%)	Study Population (n)	Study Population (%)	Canadian Population <sup>&amp;</sup> (%)
20-34	23*	21.74	13.8	16	43.75	17.4
35-44	64	39.06	19.3	68	32.35	23.3
45-54	163	28.22	20.4	171	26.90	26.4
55-64	191	36.65	23.7	232	34.05	25.3
65+	508	18.50	19.7	439	20.05	20.3



**Table S3. Prevalence of Diabetes by Age and Sex in Study Patients and the General Population.**

Age	Female			Male		
	Study Population (n)	Study Population (%)	Canadian Population (%)	Study Population (n)	Study Population (%)	Canadian Population# (%)
20-34	31*	3.23	0.9	23	13.04	0.9
35-44	75	10.67	2.3	90	11.11	3
45-64	418	12.44	8	513	13.65	9.1
65+	575	15.65	16.4	496	18.95	20.6

**Table S4. Prevalence of Hypertension by Age and Sex in Study Patients and the General Population.**

Age	Female			Male		
	Study Population (n)	Study Population (%)	Canadian Population (%)	Study Population (n)	Study Population (%)	Canadian Population <sup>##</sup> (%)
20-34	29*	37.93	1.14	22	45.45	2.3
35-44	71	46.48	5.76	84	54.76	8.87
45-54	176	52.27	15.16	202	62.38	20.45
55-64	209	65.55	29.77	272	66.91	32.06
65-74	259	76.06	44.2	270	71.85	45.5
75+	275	84.36	53.27	190	84.21	50.11

\*Female 20-34 age group in the study population includes one 19-year old patient

\*\*Canadian population data from: [www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health74b-eng.htm](http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health74b-eng.htm); [www.statcan.gc.ca/pub/82-624-x/2012001/article/desc/11676-04-desc-eng.htm](http://www.statcan.gc.ca/pub/82-624-x/2012001/article/desc/11676-04-desc-eng.htm); and [www.statcan.gc.ca/pub/82-624-x/2012001/article/desc/11676-03-desc-eng.htm](http://www.statcan.gc.ca/pub/82-624-x/2012001/article/desc/11676-03-desc-eng.htm)

&Canadian population data from: [www.statcan.gc.ca/pub/82-625-x/2015001/article/14185/c-g/desc/desc04-eng.htm](http://www.statcan.gc.ca/pub/82-625-x/2015001/article/14185/c-g/desc/desc04-eng.htm)

#Canadian population data from: [www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health53b-eng.htm](http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health53b-eng.htm)

##Canadian population data from: [www.statcan.gc.ca/pub/82-625-x/2014001/article/14020/c-g/desc/14020-02-desc-eng.htm](http://www.statcan.gc.ca/pub/82-625-x/2014001/article/14020/c-g/desc/14020-02-desc-eng.htm)

Figure S1. Potential mediation of NO<sub>2</sub>-plaque associations by cardiometabolic disorders.

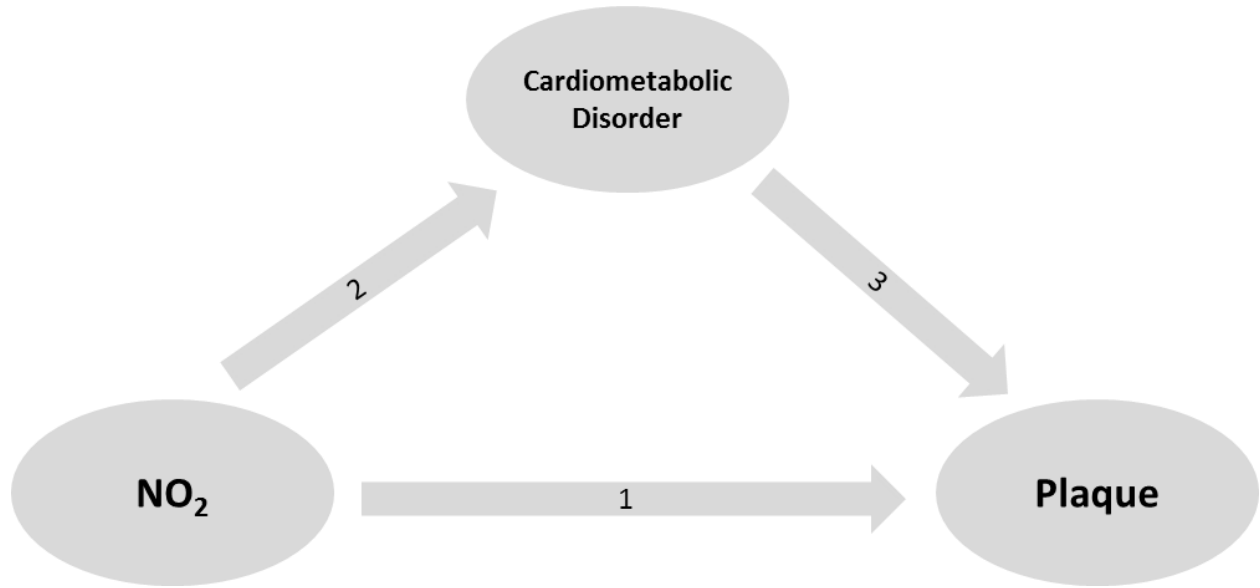
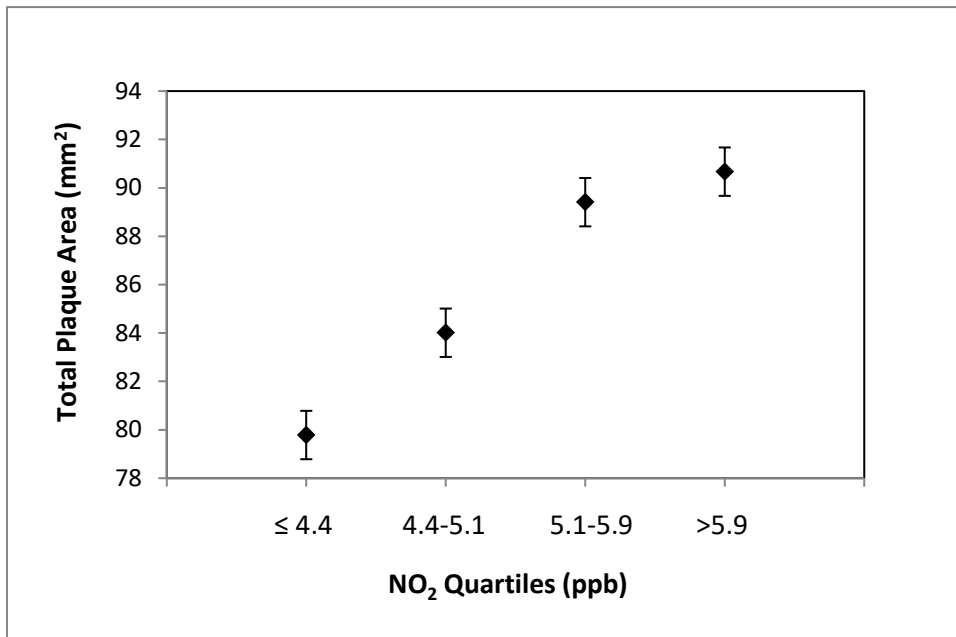


Figure S2. TPA by NO<sub>2</sub> quartile.



NO<sub>2</sub> = nitrogen dioxide  
TPA = total plaque area  
mm<sup>2</sup> = square millimeter  
ppb = parts per billion