Air pollution and retinal vessel diameter and blood pressure in school-aged children in a region impacted by residential biomass burning

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Little is known about the early-life cardiovascular health impacts of fine particulate air pollution (PM_{2.5}) and oxidant gases. A repeated-measures panel study was used to evaluate associations between outdoor PM_{2.5} and the combined oxidant capacity of O_3 and NO_2 (using a redox-weighted average, Ox) and retinal vessel diameter and blood pressure in children living in a region impacted by residential biomass burning. A median of 6 retinal vessel and blood pressure measurements were collected from 64 children (ages 4–12 years), for a total of 344 retinal measurements and 432 blood pressure measurements. Linear mixed-effect models were used to estimate associations between PM_{2.5} or Ox (same-day, 3-day, 7-day, and 21-day means) and retinal vessel diameter and blood pressure. Interactions between PM_{2.5} and Ox were also examined. Ox was inversely associated with retinal arteriolar diameter; the strongest association was observed for 7-day mean exposures, where each 10 ppb increase in Ox was associated with a 2.63 μm (95% CI − 4.63, − 0.63) decrease in arteriolar diameter. Moreover, Ox modified associations between PM_{2.5} and arteriolar diameter, with weak inverse associations observed between PM_{2.5} and arteriolar diameter only at higher concentrations of Ox. Our results suggest that outdoor air pollution impacts the retinal microvasculature of children and interactions between PM_{2.5} and Ox may play an important role in determining the magnitude and direction of these associations.

Abbreviations
AIC  Akaike information criterion
CI  Confidence interval
CRAE  Central retinal arteriolar equivalent
CRVE  Central retinal venular equivalent
CVD  Cardiovascular disease
DBP  Diastolic blood pressure
NO_2  Nitrogen dioxide
O_3  Ozone
Ox  The combined redox-weighted oxidant capacity of NO_2 and O_3
PM_{1.5}  Fine particulate matter air pollution
PM_{10}  Particulate matter < 10 μm
SD  Standard deviation
SBP  Systolic blood pressure

Outdoor air pollution is associated with adverse cardiovascular outcomes\textsuperscript{1,2}. Although cardiovascular disease (CVD) manifests in adulthood, preclinical changes that contribute to and accelerate the development of CVD

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begin in childhood. Therefore, identifying early-life modifiable exposures that adversely affect cardiovascular health may provide important information to help prevent CVD in later life.

Most research on associations between ambient air pollution and cardiovascular outcomes has focused on particulate matter exposure and consistent evidence from epidemiological and animal studies support a causal relationship. Oxidant gases, such as ozone (O₃) and nitrogen dioxide (NO₂), have also been associated with adverse cardiovascular outcomes, although results have been less consistent. Individuals are exposed to both particulate matter and oxidant gases simultaneously, and some evidence suggests these pollutants interact to affect health outcomes. For example, stronger associations between long-term and short-term fine particulate matter air pollution (PM₂.₅) and mortality were found when the combined oxidant capacity of NO₂ and O₃ (using a redox-weighted average, Oₓ) was higher, highlighting the importance of considering Oₓ when evaluating PM₂.₅ health effects.

The microcirculation represents a large component of the circulatory system and microvascular dysfunction is an important predictor of CVD events. Measuring the structure of the retinal microvasculature through fundus photography can serve as a simple, non-invasive method to evaluate microvascular health, as the retinal microcirculation is anatomically and physiologically similar to the cerebrovascular and coronary microcirculation. Of the various parameters that can be estimated with fundus photography, the most common and easily estimated parameters are the diameters of retinal blood vessels. The relationship between air pollution and retinal blood vessel diameter has been examined several times in adults in cross-sectional and repeated-measures studies. In one study of school-aged children living in an urban centre in Belgium, short-term PM₂.₅ (measured on the same day as the retinal image and the day before) was associated with narrower retinal arteriolar diameter and wider venular diameter. In another study of children ages 4–6 years (also living in Belgium), PM₂.₅ measured during the same day as the retinal image, the day before the retinal image, and the week before the retinal image was associated with both narrower and wider retinal arteriolar diameter, depending on the exposure lag, while NO₂ was not associated with retinal vessel diameter. Due to the limited number of studies that have explored these associations in children and inconsistent results, these relationships necessitate further exploration.

Another preclinical cardiovascular outcome that may be adversely affected by outdoor air pollution is blood pressure, but associations between short-term air pollution and blood pressure have not been extensively studied in children. In a recent meta-analysis of four studies that looked at associations between short-term air pollution (defined as < 30 days) and blood pressure in children, each 10 μg/m³ increase in particulate matter < 10 μm (PM₁₀) was associated with a very small (< 1 mm Hg) increase in systolic blood pressure, while no clear associations were observed between PM₁₀ or PM₂.₅ and diastolic blood pressure. An understanding of the relationship between air pollution and blood pressure in children is important because childhood blood pressure tracks into adulthood and elevated blood pressure is an important risk factor for the development of cardiovascular disease.

To our knowledge, no studies have explored how the combined oxidant capacity of NO₂ and O₃ (Oₓ) affects retinal blood vessel diameter or blood pressure, or whether associations between PM₂.₅ and these health outcomes are modified by Oₓ. In addition, no studies have focused specifically on the impact of residential biomass burning-related PM₂.₅ to changes in the retinal microvasculature or blood pressure. This is an important consideration because residential biomass burning is a major source of PM₂.₅ in rural Canada due to the prevalence of wood burning to heat homes, and biomass-burning sources of PM₂.₅ may be harmful to cardiovascular health.

To address gaps in our current understanding of air pollution impacts on cardiovascular health of children, we conducted a panel study to examine associations between outdoor PM₂.₅ and Oₓ on changes to retinal vessel diameter and blood pressure in children living in a region of Canada known to be impacted by residential biomass burning. We also considered whether the impact of PM₂.₅ on retinal blood vessel diameter or blood pressure was modified by outdoor concentrations of Oₓ.

**Materials and methods**

**Study design and population.** We conducted a repeated-measures panel study at two elementary schools in the neighbouring communities of Courtenay and Cumberland on the east coast of central Vancouver Island, in the province of British Columbia, Canada. The distance between the two schools is approximately 8 km. This is a rural area of Canada, with a population size of approximately 26,000 in Courtenay and 4,000 in Cumberland in 2016 (the most recent census year). The study took place from September 2018 to June 2019 in Courtenay, and from September 2019–March 2020 in Cumberland (the study was terminated three months early in Cumberland because of school closures due to the COVID-19 pandemic). The study took place over sequential school years (instead of at both schools in the same school year) because study equipment and research staff were limited. This area has elevated outdoor PM₂.₅ concentrations during the cold season (approximately November–April) because many households rely on wood burning as their primary heating source. During the warmer season, outdoor PM₂.₅ concentrations are typically very low (i.e. < 5 μg/m³).

Children at each school were eligible to participate if they were 4–12 years of age at enrollment, lived in a non-smoking home, and resided in the community surrounding either school. Recruitment occurred during September of each school year, and health outcome measurements began in October. Exams were scheduled at intervals of approximately one month and were staggered throughout each month (as opposed to measuring everyone on the same day) in order to increase exposure variation and minimize the impact on regular school activities. Exams took place on Thursday and Friday mornings at the school site in Courtenay, and throughout the week in the morning and early afternoon in Cumberland. Oral assent was obtained from children and written informed consent was obtained from their parent/guardian. At baseline, parents/guardians of each participant completed a questionnaire to collect basic sociodemographic and household information. The study was
approved by McGill University Research Ethics Board and the Health Canada Research Ethics Board and all methods were performed in accordance with the relevant guidelines and regulations.

Air pollutants and meteorological data. In the first year of the study, daily mean outdoor PM$_{2.5}$ concentrations in Courtenay were measured using a BAM (Beta-Attenuation Monitor) 1020 instrument located at the provincial air monitoring station situated on the playground of the school. In case there were any problems or gaps in data collection with the government-run monitor, we also set up a Partisol 2025i sequential air sampler at the same location, which collected daily integrated PM$_{2.5}$ samples that were subsequently sent for gravimetric analysis. However, for this year of the study, we ended up using only PM$_{2.5}$ measurements from the BAM instrument in our analyses because there were fewer missing data. In the second year of the study in Cumberland, the school was not located at a provincial monitoring station so PM$_{2.5}$ was only measured using a Partisol 2025i sequential air sampler that we set up on the roof of the school. Although the PM$_{2.5}$ values used in analysis were from different instruments each year of the study, we observed a strong correlation in duplicate measurements in Courtenay ($r^2 = 0.94$) and both instruments are considered acceptable methods to monitor PM$_{2.5}$ by the United States Environmental Protection Agency$^{23}$.

For both years of the study, ozone and nitrogen dioxide were measured at the provincial air monitoring site in Courtenay with an API T400 UV Absorption O$_3$ analyzer and an API T200 chemiluminescence NO/NO$_2$/NO analyzer, respectively; due to equipment limitations, we were unable to set up our own monitors for O$_3$ and NO$_2$ in Cumberland so relied on measurements from Courtenay as approximations. The combined weighted oxidant capacity (O$_x$) of NO$_2$ and O$_3$ was calculated as a weighted average of NO$_2$ and O$_3$, with weights equivalent to the respective redox potentials using the formula $O_x = \frac{[(1.07 \times NO_2) + (2.075 \times O_3)]}{3.145}$, as previously described$^{24,25}$. Indoor air pollution was not measured in this study. Meteorological data, including mean daily temperature, wind speed, precipitation, and humidity were available from a provincial monitoring station located approximately 8 km from the school in Courtenay and 15 km from the school in Cumberland.

In the second year of the study (in Cumberland), there were some days with missing PM$_{2.5}$ data due to a delay in setting up the PM$_{2.5}$ monitor at the start of the study and occasional technical issues throughout the study. A model to predict missing PM$_{2.5}$ was developed, and predicted values were used to impute missing PM$_{2.5}$. The prediction model regressed log-transformed PM$_{2.5}$ on several predictors including same-day PM$_{2.5}$, NO$_2$, temperature, wind speed, and precipitation measured at a nearby provincial monitoring station. Global search regression using the ggrep command in Stata was used to select the final prediction model, considering all possible combinations of interactions and square terms of predictor variables. The best fitting model had a R$^2$ of 0.72. There was a total of 58 days in which PM$_{2.5}$ was imputed (approximately 12% of PM$_{2.5}$ values in the time series).

Clinical exams. Clinical exams were conducted by two trained research assistants (one research assistant at each site) and involved imaging the retinal microvasculature and measuring blood pressure, height, and weight. All exams took place in a designated, quiet room in each school.

The fundus of the left and right eye of participants was photographed with a Canon CR2-AF 45° 20.2-megapixel digital nondiagnostic retinal camera in a darkened room. Images were analyzed by one grader (J.K.) using the semi-automatic MONA-REVA software (version 3.0.0, VITO Health, Mol, Belgium). For each participant, images from either the left or right eye were analyzed; the choice of whether to analyze the left or right eye of each participant depended on which eye had the most high quality images (where image quality was judged by how sharp the image was, whether the optic disc was centered, and whether the arterioles and venules were distinguishable from one another). Epidemiological studies have demonstrated a high correlation in retinal vessel diameters between the left and right eye$^{26,27}$. When analyzing the images, the diameter of the optic disc was first determined, then the width of the retinal arterioles and venules were measured within an area equal to 0.5–1 times the disc diameter from the optic disc margin (Figure S1 in the Supplemental Material). Diameters of the 6 largest arterioles and venules were used in the revised Parr Hubbard formula$^{28}$ to estimate Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE), summary measures reflecting average arteriolar and venular diameter. For each participant, the same 6 arterioles and venules were used to calculate CRAE and CRVE in repeated measurements.

Following fundus photography, blood pressure was measured with the SunTech CT40 vital signs device. While sitting upright with their non-dominant arm resting on a table, an appropriately sized arm cuff was selected based on the circumference of the child’s upper arm, and blood pressure was measured twice with one minute between each reading. If systolic or diastolic blood pressure from the two successive readings were >10 mm Hg apart, a third reading was done. The average of the two closest readings was calculated and used for analysis.

With shoes and bulky clothing removed, height was measured to the nearest 0.1 cm with the Seca 213 Stadiometer, and weight was measured to the nearest 0.1 kg using the Seca 874 Digital Scale. Measurements were taken in duplicate, and an average was calculated. Body mass index-for-age z-scores were then calculated based on the World Health Organization child growth standards$^{29}$.

Statistical analyses. Associations between outdoor air pollution and retinal blood vessel diameter. Linear mixed-effect models with a random subject intercept (with a first order autoregressive correlation structure) were used to evaluate associations between PM$_{2.5}$ (as a continuous variable, in units of μg/m$^3$) or O$_3$ (a continuous variable, in units of ppb) and within-person changes in CRAE or CRVE (continuous variables, in units of μm). We assessed associations between CRAE or CRVE with four different exposure lags: PM$_{2.5}$ or O$_3$ on the day of the retinal image, 3-day mean (mean of PM$_{2.5}$ or O$_3$ on the day of the retinal image and two preceding days), 7-day mean, and 21-day mean. These time periods were selected to examine both acute and sub-chronic expo-
sures. For each exposure-outcome relationship, we ran crude models, and models adjusted for a priori list of potential confounders or predictors of retinal blood vessel diameter, including 7-day mean temperature (degrees Celsius) and humidity (%) (which may be correlated with seasonal differences in air pollution concentrations), body mass index-for-age z-score at the time of the retinal image, sex, age (years), highest level of maternal education (high school or less/ community or technical college/ university), and time of day of outcome assessment (≤11:00 AM or > 11:00 AM). We also explored whether associations between PM$_{2.5}$ and retinal vessel diameter were modified by concentrations of O$_3$ by running models with an interaction term between PM$_{2.5}$ and O$_3$ (as continuous variables using the same exposure lag for both air pollutants) while adjusting for the same set of covariates identified above. A p-value less than 0.05 for the interaction term was interpreted as evidence of effect modification (on the additive scale). We explored whether including a fixed effect for school was necessary to account for potential clustering within schools, but it did not improve model fit based on the minimum Akaike Information Criterion (AIC) so was not included in the final models. We also explored potential non-linear relationships between continuous covariates and CRAE or CRVE using spline terms, but as splines did not improve model fit (based on the minimum AIC), final models included linear terms for all continuous covariates. Residual plots were generated to verify model assumptions. All estimates are expressed as a change in retinal arteriolar or venular diameter per 5 μg/m$^3$ increase PM$_{2.5}$ or 10 ppb increase in O$_3$, which reflect the approximate interquartile ranges of PM$_{2.5}$ and O$_3$.

Associations between outdoor air pollution and blood pressure. Linear mixed-effect models with a random subject intercept (and a first order autoregressive correlation structure) were used to evaluate associations between short-term and sub-chronic PM$_{2.5}$ or O$_3$ (the same exposure lags described above) and systolic and diastolic blood pressure. Similar to analyses for retinal vessel diameter, crude models, adjusted models (including the same set of covariates identified above), and models with an interaction term between PM$_{2.5}$ and O$_3$ were examined.

Sensitivity analyses. Several sensitivity analyses were conducted. First, analyses were repeated excluding retinal images or blood pressure measurements in which the relevant PM$_{2.5}$ exposure lags included imputed PM$_{2.5}$ values. Second, instead of evaluating associations between O$_3$ and retinal blood vessel diameter and blood pressure, we looked at associations with each gas (O$_3$ or NO$_2$) individually. Third, we additionally adjusted our models for season (fall/winter/spring/summer).

All data cleaning and manipulation were conducted using Stata v.15 (StataCorp, College Station, TX), and all modelling was conducted using R (R-project.org).

### Results

#### Study population.
A description of the study population is presented in Table 1. A total of 71 children (median age of 8 years) enrolled in the study and high-quality retinal images were available for 64 of these children. Most participants (N = 54, 76%) enrolled during the second year (2019–2020) of the study. The sample was predominantly Caucasian (N = 64, 90%), there were a similar number of boys and girls, and most mothers of participants had some post-secondary education. The majority of participants lived in households that used electricity (N = 46, 65%) or natural gas (N = 21, 30%) as their primary heating source, while few households used wood burning as their primary heating source (N = 3, 4%). The use of woodstoves or wood fireplaces as a secondary source of heating was uncommon in this sample (N = 6, 8%), and 17 participants (24%) lived in households that used an air filter. The average (± standard deviation) body mass index-for age z-score was 0.7 ± 1.3, indicating body mass index of children was slightly higher than the age and sex-specific reference population. Mean (± standard deviation) systolic and diastolic blood pressure at baseline were 106 ± 7 and 63 ± 5 mm Hg, respectively, while mean (± standard deviation) CRAE and CRVE at baseline were 181.51 ± 11.88 and 260.34 ± 15.70 μm.

There was a total of 344 high quality retinal images and 432 blood pressure measurements. The median number of retinal images and blood pressure measurements per child was 6 but some children had as few as three measurements. The maximum number of retinal images was 6 per child, and for blood pressure the maximum number of measurements was 10 per child. Median time between retinal images and blood pressure measurements was 28 days (range 20–63 days).

#### Exposure characteristics.
Distributions of daily mean outdoor PM$_{2.5}$ and O$_3$ concentrations throughout the study are shown in Fig. 1 and additional exposure characteristics are provided in Table S1 of the Supplemental Material. Overall, mean daily PM$_{2.5}$ ranged from <1 μg/m$^3$ to 32 μg/m$^3$ over the entire study period, and was slightly higher and more variable in the first year of the study (mean ± standard deviation: 9 ± 7 μg/m$^3$) than in the second year of the study (mean ± standard deviation: 6 ± 4 μg/m$^3$). Average PM$_{2.5}$ on the day of the retinal image was the same as the 3-day mean, 7-day mean, and 21-day mean concentrations (7 μg/m$^3$), although the standard deviation was slightly larger on the day of the retinal image (standard deviation: 6 μg/m$^3$) compared to the 3-day and 7-day means (standard deviation of 4 μg/m$^3$ for both lags), and the 21-day mean (standard deviation: 3 μg/m$^3$). O$_3$ ranged from 3 to 27 ppb over the entire study period, and was slightly higher and more variable during the first year of the study (mean ± standard deviation: 14 ± 6 ppb) compared to the second year of the study (mean ± standard deviation: 13 ± 5 ppb). Mean O$_3$ for all exposure lags was 13 ppb, and the standard deviation was slightly larger on the day of the retinal image (6 ppb) compared to the 3-day, 7-day, and 21-day means (5 ppb). There was a moderate inverse correlation between PM$_{2.5}$ and O$_3$ based on Pearson's correlation coefficient ($r^2 = -0.43$).
Associations between outdoor PM$_{2.5}$ or Ox and retinal blood vessel diameter. Associations between PM$_{2.5}$ or Ox from single-pollutant models and retinal arteriolar and venular diameter are presented in Fig. 2 and Tables S2 and S3 of the Supplemental Material. In adjusted models, PM$_{2.5}$ was associated with a small increase in CRAE but 95% confidence intervals included the null. The strength of this association was largest for the 21-day exposure lag: a 5 μg/m$^3$ increase in 21-day mean PM$_{2.5}$ was associated with a 1.42 μm increase in CRAE (95% CI $-$ 0.47, 3.32). On the other hand, Ox was consistently associated with a reduction in CRAE and the strongest association was for the 7-day exposure lag: a 10 ppb increase in Ox was associated with a 2.63 μm decrease in CRAE (95% CI $-$ 4.63, $-$ 0.63).

In general, positive association were observed between PM$_{2.5}$ and venular diameter and inverse associations were observed between Ox and venular diameter but the strength of these associations was small and 95% confidence intervals included the null in all adjusted models. There were no notable differences in associations between PM$_{2.5}$ and CRAE or CRVE when analyses excluded retinal images with imputed PM$_{2.5}$ (Table S4 of the Supplemental Material). In sensitivity analyses, estimated associations between Ox and retinal blood vessel diameter were similar to that of Ox (Table S5 of the Supplemental Material), while NO$_2$ was positively associated with retinal arteriolar and venular diameter, but estimates were imprecise and all confidence intervals included the null (Table S6 of the Supplemental Material). When models were additionally adjusted for season, conclusions remain the same (Table S7 and S8 of the Supplemental Material).

Models including an interaction term between PM$_{2.5}$ and Ox suggested that Ox modified associations between outdoor PM$_{2.5}$ and retinal arteriolar diameter (p-values from interaction terms for same-day, 3-day mean, 7-day mean and 21-day mean exposures: 0.10, 0.04, 0.02, and 0.03, respectively). To visualize the associations between PM$_{2.5}$ and CRAE modified by Ox, we plotted predicted values of CRAE across a range of PM$_{2.5}$ concentrations (2–16 μg/m$^3$) stratified by Ox concentrations 1 standard deviation above or below the mean (Fig. 3). This figure suggests that when Ox is low there is a weak positive association between PM$_{2.5}$ and CRAE, while when Ox concentrations are higher there is a weak inverse association between PM$_{2.5}$ and CRAE. These trends were more pronounced in the 3-day, 7-day, and 21-day lags compared to same-day exposure. Similar figures were generated to visualize how concentrations of PM$_{2.5}$ modified the associations between Ox and CRAE and suggest that a negative association between Ox and CRAE is only present when concentrations of PM$_{2.5}$ were high (i.e., 1 standard deviation above the mean) (Figure S2 of the Supplemental Material). There was no evidence of

| Table 1. Description of the study population. *High-quality images were unavailable for some participants due to blinking, inability to sit still, and general discomfort with getting their eyes photographed. **Excludes participants in which a woodstove/wood fireplace is the main source of heating. ***Body mass index-for-age-and-sex z-score calculated based on World Health Organization growth charts. |  |
|---|---|
| **Socio-demographic characteristics** |  |
| Total enrolled participants, N | 71 |
| Participants with retinal images available*, N | 64 |
| **Date on study, n (%)** |  |
| September 2018–June 2019 | 17 (24) |
| September 2019–March 2020 | 54 (76) |
| Age (years) at baseline, median (range) | 8 (4–12) |
| Girls, n (%) | 33 (46) |
| Caucasian, n (%) | 64 (90) |
| **Highest level of maternal education complete, n (%)** |  |
| Graduated high school or less | 11 (15) |
| Some or graduated community/technical college | 15 (21) |
| Some or graduated university | 45 (63) |
| **Household characteristics** |  |
| **Main heating source in home, n (%)** |  |
| Wood | 3 (4) |
| Natural gas | 21 (30) |
| Electricity | 46 (65) |
| Oil | 1 (1) |
| Use of a woodstove or wood fireplace in home as a secondary heating source**, n (%) | 6 (8) |
| Use of air filter in home, n (%) | 17 (24) |
| **Cardiovascular measures** |  |
| Central retinal arteriolar equivalent (μm), mean ± SD | 181.51 ± 11.88 |
| Central retinal venular equivalent (μm), mean ± SD | 260.34 ± 15.70 |
| Systolic blood pressure (mm Hg), mean ± SD | 106 ± 7 |
| Diastolic blood pressure (mm Hg), mean ± SD | 63 ± 5 |
| Body mass index-for-age z-score***, mean ± SD | 0.7 ± 1.3 |
interaction between PM$_{2.5}$ and O$_x$ for CRVE (p-values from interaction terms for same-day, 3-day mean, 7-day mean, and 21-day mean exposures: 0.52, 0.63, 0.14, and 0.83, respectively).

**Associations between outdoor PM$_{2.5}$ or O$_x$ and blood pressure.** Associations between outdoor PM$_{2.5}$ or O$_x$ concentrations and blood pressure are presented in Fig. 2 and Tables S2 and S3 of the Supplemental Material. In adjusted models, each 5 μg/m$^3$ increase in 3-day mean PM$_{2.5}$ was associated with a 0.95 mm Hg reduction in systolic blood pressure (95% CI −1.86, −0.05), 7-day mean PM$_{2.5}$ was associated with a 1.11 mm Hg reduction in systolic blood pressure (95% CI −2.12, −0.09), and 21-day mean PM$_{2.5}$ was associated with a 1.70 mm Hg reduction in systolic blood pressure (95% CI −2.98, −0.41), but these associations were slightly attenuated and 95% confidence intervals included the null in sensitivity analyses excluding exams where PM$_{2.5}$ was imputed (Table S4 of the Supplemental Material). Conversely, positive associations were observed between O$_x$ and systolic blood pressure, with the largest association detected for the 21-day exposure lag (estimated change per 10 ppb increase in 21-day mean O$_x$ from an adjusted model: 1.59 (95% CI −0.06, 3.25)), but confidence intervals included the null for all exposure lags. There were no clear associations between PM$_{2.5}$ or O$_x$ and diastolic blood pressure. In sensitivity analyses, associations between O$_x$ and blood pressure were similar to those found for O$_x$ and no clear relationship was observed between NO$_2$ and blood pressure (Tables S5 and S6 of the Supplemental Material). When models were additionally adjusted for season, conclusions are similar except the confidence intervals for associations between 3-day and 7-day mean PM$_{2.5}$ now include the null (Table S7 and S8 of the Supplemental Material).

There was evidence that 7-day mean O$_x$ modified the associations between 7-day mean PM$_{2.5}$ and systolic blood pressure (p-value from interaction term: 0.04), but there was no evidence of a significant interaction for the same-day, 3-day mean, or 21-day mean exposures (p-values from interaction terms for same-day, 3-day mean, and 21-day mean exposures: 0.63, 0.26, 0.55). Figure S3 in the Supplemental Material suggests that an inverse relationship between 7-day mean PM$_{2.5}$ and systolic blood pressure is present when 7-day mean O$_x$ concentrations are above average (i.e., 1 standard deviation above the mean), while there is no association when O$_x$ concentrations are lower (i.e., 1 standard deviation below mean). O$_x$ did not modify associations between PM$_{2.5}$ or diastolic blood pressure for any exposure lags (p-value for interaction term for same-day, 3-day mean, 7-day mean, and 21-day mean exposures: 0.57, 0.46, 0.51 and 0.61).
Discussion

Our findings suggest that outdoor air pollution in a region impacted by residential biomass burning has a measurable impact of the microvasculature of school-age children. Specifically, Ox was consistently associated with retinal arteriolar narrowing in single-pollutant models. Our findings also suggest that an important interaction may exist between outdoor concentrations of oxidant gases and PM$_{2.5}$, as PM$_{2.5}$ was only associated with arteriolar narrowing when Ox concentrations were elevated. We also found inverse associations between PM$_{2.5}$ and systolic blood pressure and evidence of effect modification by Ox for the 7-day exposure lag, while in single-pollutant models there were trends towards positive associations between Ox and systolic blood pressure. No clear associations between PM$_{2.5}$ or Ox and retinal venular diameter or diastolic blood pressure were observed.

Although this study did not conduct any source apportionment of PM$_{2.5}$, it is known that residential biomass burning affects air quality in this region of Canada. For example, Hong et al. developed an algorithm that was applied to 23 communities in British Columbia, Canada, to identify smoky vs. non-smoky days, and classified 30% of days in Courtenay between 2014–2016 as smoky, making it the second smokiest community of the 23 studied. Moreover, Weichenthal et al. identified biomass burning as a major contributor to ambient PM$_{2.5}$ in Courtenay by measuring daily levoglucosan (a tracer of biomass burning) levels from January 2014–March 2015. Furthermore, traffic-related air pollution is very minimal in this region because it is a rural location on an island with a small population size, and there are no major industries in the area that would affect air quality.

The biological mechanisms underlying air pollution impacts on the microcirculation and blood pressure are thought to be related to oxidative stress, inflammation, and disturbances to the autonomic nervous system. Inhaled particles can stimulate the generation of reactive oxygen species causing both pulmonary and systemic oxidative stress and inflammation which contributes to endothelial dysfunction and vasoconstriction. Arteriolar narrowing may contribute to elevated blood pressure because arterioles are the main regulators of peripheral blood flow and are essential in the maintenance of blood pressure. In addition, air pollution exposure may lead

Figure 2. Estimated change (95% confidence interval) in (A) central retinal arteriolar diameter (CRAE, μm); (B) central retinal venular diameter (CRVE, μm); (C) systolic blood pressure (SBP, mm Hg) and; (D) diastolic blood pressure (DBP, mm Hg) per 5 μg/m$^3$ increase in PM$_{2.5}$ or 10 ppb increase in Ox. Models adjusted for 7-day mean temperature and humidity, body mass index-for-age z-score on the day of the retinal image, sex, age (years), maternal education (high school or less vs. community/technical college vs. university), and time of day of outcome assessment (≤ 11:00 AM vs. > 11:00 AM). $^a$PM$_{2.5}$ or Ox on the same day as the outcome assessment. $^b$Mean PM$_{2.5}$ or Ox on the day of the outcome assessment and two preceding days. $^c$Mean PM$_{2.5}$ or Ox on the day of the outcome assessment and 6 preceding days. $^d$Mean PM$_{2.5}$ or Ox on the day of the outcome assessment and 20 preceding days.
to an imbalance of the autonomic nervous system which favours sympathetic pathways, and can contribute to endothelial dysfunction, vasoconstriction, and elevated blood pressure. In general, existing evidence from observational studies related to the associations between outdoor air pollution and blood pressure in children is inconsistent. For example, Yang et al. found that short-term exposure to PM$2.5$ was associated with very small increases in both systolic and diastolic blood pressure (< 1 mm Hg increase in systolic and diastolic blood pressure per 10 μg/m$^3$ PM$2.5$) in a large study of approximately 190,000 children in China, but a smaller study in the Netherlands found no clear associations between short-term PM$10$, NO$_2$, or O$_3$ and systolic or diastolic blood pressure. In another study in Belgium, consistent positive associations were detected between ultrafine particles and systolic blood pressure in children, but trends of an inverse association was observed for PM$2.5$. Inverse associations between systolic blood pressure and short-term particulate matter and ozone have also been observed in adult populations. We are not sure why we observed inverse associations between air pollution and systolic blood pressure because our existing knowledge of physiological responses to air pollution generally would support positive associations; however, these inconsistent findings highlight uncertainty in our current understanding of air pollution impacts on cardiovascular health. In this study, although we found limited evidence of effect modification by O$_3$ for the associations between PM$2.5$ and blood pressure, it is still possible that complex interactions between air pollutants exist and contribute to the heterogeneity of results observed between studies.

Regarding the retinal microvasculature, previous evidence in adults and children have observed arteriolar narrowing in response to PM$2.5$ exposure. For example, Provost et al. found that same-day residential outdoor PM$2.5$ was associated with a 0.62 μm decrease in retinal arteriolar diameter (95% CI = 1.12, −0.12) per 10 μg/m$^3$ increase in PM$2.5$ in school-aged children in Belgium. However, a second study by Luyten et al. found that the direction of associations between PM$2.5$ and retinal arteriolar diameter in children was sensitive to the exposure lag that was selected. Results for retinal venular diameter have been less conclusive but tend to suggest positive associations with air pollution. To our knowledge, no studies to date have examined associations between O$_3$ and retinal blood vessel diameter but Luyten et al. investigated the impact of NO$_2$ and did not find any clear associations.

Figure 3. Predicted values and 95% confidence intervals for central retinal arteriolar equivalent (CRAE) at different concentrations of PM$2.5$, stratified by O$_3$ (1 standard deviation below and above mean O$_3$). Plots correspond to (A): Same-day exposure lag; (B): 3-day mean exposure lag; (C): 7-day mean exposure lag; (D): 21-day mean exposure lag.
The most interesting finding in our study is the interaction observed between PM$_{2.5}$ and O$_x$ in models for retinal arteriolar diameter. Specifically, the direction of the association between PM$_{2.5}$ and arteriolar diameter was modified by concentrations of O$_x$, with weak positive associations observed at lower concentrations of O$_x$ and inverse associations observed at higher concentrations of O$_x$. Similarly, the inverse association between O$_x$ and retinal arteriolar diameter was only observed when concentrations of PM$_{2.5}$ were high. This modifying role of O$_x$ in PM$_{2.5}$ health effects has been observed previously for other outcomes. For example, Weichenthal et al.\(^6\) found stronger associations between PM$_{2.5}$ and all-cause, cardiovascular, and respiratory mortality when concentrations of O$_x$ were higher, while Lavigne et al.\(^7\) observed similar results with short-term PM$_{2.5}$ and all-cause and cardiovascular mortality. Together, this evidence highlights the importance of considering O$_x$ when evaluating the health impacts of PM$_{2.5}$ and also suggests possible co-benefits of regulatory interventions aimed at reducing outdoor air pollution (i.e. reducing O$_x$ may also reduce the health impacts of PM$_{2.5}$ even if PM$_{2.5}$ mass concentrations remain unchanged).

Existing evidence suggests several possible mechanisms underlying the observed interaction between PM$_{2.5}$ and O$_x$. First, elevated ozone depletes antioxidants in the epithelial lining fluid of the respiratory tract\(^4\), and this may lower our defenses against reactive oxygen species produced in response to PM$_{2.5}$ exposure, contributing to greater oxidative stress. In addition, ozone has been shown to increase the permeability of the lung epithelial barrier\(^40-44\), which may contribute to greater absorption of particles into the systemic circulation and greater health impacts of PM$_{2.5}$. Lastly, oxidant gases can increase the toxicity of PM$_{2.5}$ through photochemical aging processes; for example, exposure to ozone has been shown to increase the oxidative potential of particles from both engine exhaust\(^42,44\) and biomass burning\(^45\).

There are several strengths of this study, including the repeated measures design that eliminates potential confounding by variables that do not change within individuals over a short time period, exposure information for multiple air pollutants, and the study setting that allows us to evaluate air pollution primarily from residential biomass burning. However, this study also had limitations. Foremost, this study is subject to non-differential, Berkson-type exposure measurement error because true personal PM$_{2.5}$ or O$_x$ exposures may differ from outdoor concentrations. The result of Berkson measurement error is a reduction in precision without any systematic bias\(^46\). Another limitation is we are evaluating short-term changes in retinal blood vessel diameter but how this may impact future health is not clear. We (and others\(^47\)) hypothesize that repeated short-term damage to microvascular structure can lead to chronic microvascular changes in later life, but there are no longitudinal studies demonstrating this. In addition, there is likely some classical measurement error in estimating arteriolar and venular diameter, but this is almost certainly non-differential with respect to outdoor air pollution concentrations.

**Conclusion**

In summary, these results suggest that short-term and sub-chronic exposures to air pollution impact the retinal microvasculature and blood pressure of children, and highlight the importance of considering potential interactions between air pollutants when evaluating cardiovascular health impacts. Given the small number of studies that have investigated the impact of outdoor air pollution on the retinal microvasculature or blood pressure in children, additional work is needed to confirm these findings.

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Author contributions
J.K. coordinated the study, analyzed the retinal images, performed statistical analyses and wrote the manuscript. K.L.P. and N.G.P. organized the fieldwork and collected all clinical data. R.K. coordinated the collection and analysis of exposure data. S.W. designed the study, obtained funding, oversaw all aspects of data collection and statistical analyses and provided critical revisions of the manuscript. All authors have read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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