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Recent *versus* chronic fine particulate air pollution exposure as determinant of the retinal microvasculature in school children

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Abstract

Background - Microvascular changes may represent an underlying mechanism through which exposure to fine particulate matter with a diameter ≤ 2.5 µm ($PM_{2.5}$) contributes to age-related disease development. We investigated the effect of recent and chronic exposure to $PM_{2.5}$ on the microcirculation, exemplified by retinal vessel diameters, using repeated measurements in 8- to 12-year-old children.

Methods - 221 children (49.1% girls; mean age 9.9 years) were examined repeatedly (25 one, 124 two, and 72 three times) adding up to 489 retinal vessel examinations. Same-day exposure to $PM_{2.5}$ was measured at school. In addition, recent (same and previous day) and chronic (yearly mean) exposure was modelled at the child's residence using a high-resolution interpolation model. Residential proximity to major roads was also assessed. Changes in retinal vessel diameters associated with recent and chronic exposures were estimated using mixed models, while adjusting for other known covariates such as sex, age, BMI, blood pressure and birth weight.

Results - Each 10 µg/m³ increment in same-day exposure to $PM_{2.5}$ measured at school was associated with 0.35 µm (95% CI: 0.09 to 0.61 µm) narrower retinal arterioles and 0.35 µm (-0.03 to 0.73 µm) wider venules. Children living 100 meters closer to a major road had 0.30 µm (0.05 to 0.54 µm) narrower arterioles.

Conclusions - Blood vessel diameters of the retinal microcirculation of healthy school-aged children respond to same-day $PM_{2.5}$ exposure. Furthermore, children living closer to major roads had smaller arteriolar diameters. Our results suggest that the microcirculation, with retinal microvasculature as a proxy in this study, is a pathophysiological target for air pollution in children.

Key words: microcirculation, retina, air pollution, children

1. Introduction

The microcirculation constitutes the majority of the circulatory system. Its role in age-related disease development is however less explored than that of the macrocirculation. The microvasculature plays a unifying role in diverse pathological conditions such as hypertension,[1] chronic kidney disease,[2] left ventricular dysfunction,[3] obesity,[4] diabetes mellitus[5], and cognitive impairment.[6] The state of the microvasculature and its physiological response are important factors on the trajectory from healthy to unhealthy ageing.

Numerous epidemiological studies have shown that exposure to particulate air pollution affects the vascular system, reporting associations between particulate matter exposure and increases in both cardio- and cerebrovascular mortality and morbidity.[7-9] However, few studies have addressed the role of the microcirculation in these associations.[10, 11] The retinal microvasculature is considered a proxy for the systemic microcirculation and can be characterized non-invasively by means of fundus photography. Adar and colleagues were the first to associate retinal arteriolar narrowing with air pollution.[12] Among 4,607 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), the retinal arteriolar narrowing associated with chronic ambient exposure to particulate matter with a diameter less than 2.5 μm ($PM_{2.5}$) was equivalent to a 7-year increase in age.[12] A repeated measurements study of the retinal microvessels in healthy volunteers found that increases in recent ambient particulate matter with a diameter less than 10 μm (PM_{10}) exposure were associated with decreases in both arteriolar and venular diameters.[13] Results from these studies show that the retinal microcirculation is influenced by both recent and chronic exposure to particulate air pollution in adults.

The effect of early life exposures on children's health is a growing research field because lasting effects may have major public health implications.[14, 15] Children are potentially more

susceptible than adults when exposed to comparable levels of air pollutants.[16] Not only are they exposed during a critical developmental period, children also differ from adults in physiological characteristics and exposure patterns.[16]

However, to date, no research has evaluated children's retinal blood vessel characteristics in relation to recent *versus* chronic particulate air pollution exposure. Since the microvasculature plays a pivotal role in age-related disease development, we set up a panel study with repeated retinal microvascular measurements in 8- to 12-year-old children.

2. Methods

2.1 Study population

This research was part of the COGNAC (COGNition and Air pollution in Children) study.[17] Children aged 8 to 12 years from two primary schools in Flanders (Belgium) were invited for repeated clinical examinations. The two schools were 3.7 km apart and located in the agglomeration of Hasselt (~70 km east from Brussels). Of the 482 invited children, 221 (45.9%) agreed to participate of which 72 (33%) underwent three clinical examinations, 124 (56%) completed two examinations and 25 (11%) had only one examination, amounting to a total of 489 retinal microvascular examinations. The examinations took place in school years 2012-2013 for one school and 2013-2014 for the other, from November to February on Monday, Tuesday, Thursday, and Friday between 8:30am and 3:30pm. Participating children were examined at their school during school time. The average (SD) time between two consecutive examinations was 49 (19) days. The clinical examinations of each child were scheduled on the same time of day and day of the week to minimize circadian variation.

We conducted the study according to the principles outlined in the Helsinki declaration for research on human participants. The ethics committees of Hasselt University and Ziekenhuis Oost-Limburg approved the study. Written informed consent was obtained from the parents as well as oral assent from the children. The parents filled out a questionnaire addressing aspects related to sociodemographics and medical characteristics of the child and its family. Additional information on the indoor and outdoor environment of the residence, including current smoking status of the parents and time spent in traffic, was collected.

2.2 Clinical examination

Clinical examinations were performed by a trained examiner and included imaging of the retinal microvasculature, and measuring of blood pressure and heart rate.

The fundus of the left and right eye of each participant was photographed with a Canon 45° 6.3 megapixel digital non-mydriatic retinal camera (Hospithera, Brussels, Belgium), as described by De Boever and colleagues.[18] The diameters of the retinal blood vessels were measured using IVAN fundus image analysis software according to previously reported protocols.[19] Vessel diameters were summarized per image as the Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE). The equivalents represent a summary of the vessel diameters within an area equal to 0.5-1 times the disc diameter from the optic disc margin. The respective vessel diameters were averaged over both eyes.

Blood pressure and heart rate were measured according to the guidelines of the European Society of Hypertension.[20] The participating children rested for five minutes, after which heart rate, systolic (SBP) and diastolic (DBP) blood pressure were measured five times

consecutively using an automated upper-arm blood-pressure monitor (Stabil-O-Graph®, I.E.M., Stolberg, Germany) with a special sized cuff for children. The last three measurements were averaged and used in the analyses.

2.3 Particulate air pollution exposure assessment

2.3.1 Measured exposure to $PM_{2.5}$ at school

Concentrations of $PM_{2.5}$ on the day of the examination were measured with the portable Aerocet 531 (Met One Instruments Inc. Grants Pass, OR, USA) both inside the classroom and outside at the school playground. The measurements were performed according to the manufacturer's instructions. Recent outdoor exposure was defined as 10-minutes average outside concentrations of $PM_{2.5}$ preceding the clinical examination, during the school breaks when children were playing outside. Recent indoor exposure was defined as the classroom concentrations during the clinical examination averaged over 30 minutes between entering the room until the retinal images were captured.

2.3.2 Modelled residential air pollution

We used a spatial temporal interpolation method to model the daily residential exposure levels ($\mu g/m^3$) of $PM_{2.5}$ at each child's home address. This method takes into account land cover data obtained from satellite images (CORINE land cover data set)[21] and pollution data from fixed monitoring stations in combination with a dispersion model.[22, 23] The model calculates the daily interpolated exposure concentrations in a high resolution receptor grid (25 x 25 m) based on information from the Belgian telemetric air quality networks, point sources, and line sources. Overall model performance was evaluated by leave-one-out cross-validation. Validation statistics of the interpolation tool gave a temporal explained variance

of more than 0.80 and spatial explained variance of 0.60-0.80 for $PM_{2.5}$.[23, 24] We used the model to assess the residential same-day to 48 hours of exposure up to the clinical examination as well as long-term exposure as reflected by the annual mean concentration in the year prior to the examination. When a child had more than one residential address at the moment of the study, we calculated a weighted average using the proportion of time spent at each location. In addition we used a Geographical Information System (ArcGIS version 10.0) to assess the residential proximity to major roads, defined as highways and national roads (Appendix, Figure A.1).

Daily mean ambient temperature and relative humidity were obtained from the Belgian Royal Meteorological Institute in order to calculate apparent temperature on the day of the clinical examination based on a standard formula.[25, 26] The region of Flanders is homogenous regarding temperature since both altitudinal and latitudinal gradients are extremely small. Furthermore, elevations range from 0 to 200 m above sea level and the distance between the most northern and southern part is only 100 km. Meteorological parameters were therefore used from the measuring station in Uccle (Brussels, Belgium), which is central and representative for Flanders.[27]

2.4 Statistical analysis

SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for database management and statistical analysis. The effect of particulate matter exposure on retinal vessel diameters was investigated using the MIXED procedure to account for the clustered data within the same person, i.e. repeated measures of retinal vessel diameter. A random intercept model was used and the coefficients and standard errors were estimated under

143 restricted maximum likelihood estimation (REML) with unstructured autocorrelation. The
144 school where the measurements were performed was included as a random effect.

145 Separate models were built in order to investigate the association between the
146 different exposure windows (same-day $PM_{2.5}$ exposure measured indoor/outdoor at school;
147 same-day, previous day and chronic $PM_{2.5}$ exposure modelled at the residence; residential
148 proximity to major roads) and the microvascular phenotypes (CRAE or CRVE). All analyses
149 were adjusted for an *a priori* chosen list of covariates including sex, age, body mass index
150 (BMI), blood pressure and heart rate, birth weight, time of day (categorized in tertiles:
151 ≤9:31am, 9:31am to 11:05am, >11:05am) and day of the week of the examination, apparent
152 temperature, maternal occupation (low/high) and passive smoking (yes/no). The analyses
153 were further corrected for fellow vessel diameter, i.e. for CRVE in analyses investigating
154 CRAE and vice versa.[13] Both linear and quadratic terms of age were tested. The quadratic
155 term was not significant and was therefore removed from the model. Q-Q plots of the
156 residuals were used to test the assumptions of the model. Estimates are given as µm change
157 in vessel diameter associated with a 10 µg/m³ increment in recent outdoor measured and
158 residential modelled $PM_{2.5}$ exposure or living 100 meters closer to a major road. $PM_{2.5}$
159 measured in the classroom and chronic $PM_{2.5}$ exposure had low variation in concentrations.
160 Therefore estimated changes in retinal vessel diameters are given for a 1 µg/m³ increment in
161 recent indoor measured or chronic modelled $PM_{2.5}$ exposure.

162 Sensitivity analyses were performed to investigate the robustness of our findings.
163 Models not corrected for blood pressure and heart rate or further corrected for time spent
164 in traffic, prematurity (gestational age <37 weeks), or childhood hypertension as defined in
165 the Fourth report on the diagnosis, evaluation, and treatment of high blood pressure in
166 children and adolescents,[28] were implemented.

3. Results

3.1 Study population and exposure characteristics

Details on the study population of 221 children aged 8 to 12 years are summarized in Table 1. The number of boys and girls was approximately equal. The participating children had an average BMI (SD) of 17.1 (2.6) kg/m². Their average systolic and diastolic blood pressures were 111.2 (10.1) and 63.7 (7.9) mm Hg, respectively. 21 (9.3%) children were exposed to passive smoking. The Central Retinal Arteriolar Equivalent (CRAE) averaged 164.6 (13.1) µm and the Central venular Retinal Equivalent (CRVE) was 224.0 (17.3) µm. The CRAE/CRVE ratio was 0.74 (0.05).

Table 1. Description of the study population.

Anthropometrics	
Age, years	9.9 ± 1.2
Girls	49.1%
Body Mass Index (BMI), kg/m²	17.1 ± 2.6
Systolic blood pressure, mm Hg	111.2 ± 10.1
Diastolic blood pressure, mm Hg	63.7 ± 7.9
Heart rate, beats per minute	85.7 ± 10.5
Birth weight, g	3,403.3 ± 534.1
Gestational age < 37 weeks	13 (5.9)
Exposed to passive smoking	20 (9.1)
Socio-economic status	
Maternal occupation	
Low (no occupation or blue collar workers)	40 (18.1)
High (white collar workers or self-employed)	181 (81.9)
Microvascular endpoints	
CRAE, µm	164.6 ± 13.1
CRVE, µm	224.0 ± 17.3

Values are number (%) or arithmetic mean ± SD. CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal Venular Equivalent.

The exposure characteristics are listed in Table 2. Recent exposure to $PM_{2.5}$ measured on the playground was on average 16.8 (23.2) µg/m³, while modelled daily $PM_{2.5}$ at the residence was 19.2 (14.7) µg/m³. Both indoor and outdoor concentrations as well as measured and modelled $PM_{2.5}$ exposures on the day of the clinical examination were highly correlated (P<0.0001). Children spent on average 9 (6) minutes in traffic. Chronic exposure to $PM_{2.5}$ averaged 15.4 (0.7) µg/m³ and children lived on average 649 (569) meters from a major road. Apparent temperature on the day of the clinical examination ranged from -6.4 to 9.1 °C.

Table 2. Exposure characteristics.

EXPOSURE	MIN	Q1	Q3	MAX	IQR
Recent exposure to $PM_{2.5}$ (µg/m³)					
Measured at playground	0.5	3	19	142	16
Measured in classroom	1	2	4	17	2
Modelled at residence (lag 0)	1.6	7	27	73	20
Modelled at residence (lag 1)	3	8	25	83	17
Chronic exposure to $PM_{2.5}$ (µg/m³)					
Modelled at residence	13	15	16	17	1
Residential proximity to major roads (m)	20	176	1,059	2,230	883
Apparent temperature (°C)	-6.4	-0.5	3.4	9.1	3.9

Values represent minimum (min), 25th percentile (Q1), 75th percentile (Q3), maximum (max), and interquartile range (IQR) concentrations of $PM_{2.5,}$ residential proximity to major roads or apparent temperature. Recent exposure on same-day (lag 0) or previous day (lag 1) of the clinical examination. Chronic exposure is annual mean concentration 1 year prior to the clinical examination.

3.2 Determinants of retinal vessel diameter

CRAE and CRVE were not significantly correlated with age in our study population (*P*=0.73 and 0.49, respectively). Girls had 3.7 µm (95% CI: 0.8 to 6.6 µm) wider retinal arteriolar diameters than boys. Venular diameter, however, did not significantly differ between boys and girls (*P*=0.27). Having a 1 mm Hg higher diastolic blood pressure was associated with 0.13 µm (95% CI: 0.00073 to 0.25 µm) narrower arterioles. Systolic blood pressure (*P*=0.51) and heart rate (*P*=0.46) were not significantly associated with CRAE. Children for which a lower birth weight was reported had narrower arterioles (estimate: -0.28 µm per 100 g lower birth weight; 95% CI: -0.0073 to -0.55 µm). BMI (*P*=0.65), passive smoking (*P*=0.80), occupation of the mother (*P*=0.58), time of the day (*P*=0.99) and day of the week (*P*=0.28) of

the examination and apparent temperature (*P*=0.81) were not significantly associated with CRAE. Finally, a 1 µm widening of venular diameter was associated with 0.34 µm (95% CI: 0.28 to 0.40 µm) wider arterioles. BMI was the only statistically significant correlate of venular diameter, expressed as CRVE (estimate: +1.08 µm per 1 kg/m² increase in BMI; 95% CI: 0.19 to 1.96 µm).

3.3 Recent exposure to $PM_{2.5}$ and retinal vessel diameter

Same-day exposure to particulate air pollution was significantly associated with retinal vessel diameters (Table 3). Each 10 µg/m³ increment in same-day exposure to $PM_{2.5}$ measured on the playground was associated with a 0.35 µm (95% CI: 0.09 to 0.61 µm) narrowing of the retinal arteriolar diameter (CRAE), while venular diameter (CRVE) widened 0.35 µm (95% CI: -0.03 to 0.73 µm). Likewise, CRAE narrowed 0.39 µm (95% CI: 0.13 to 0.65 µm) and CRVE widened 0.41 µm (95% CI: 0.04 to 0.79 µm) in association with a 1 µg/m³ increase in $PM_{2.5}$ concentrations measured in the classroom. Similar results were found with recent exposures to $PM_{2.5}$ modelled at the residence, showing a 0.62 µm (95% CI: 0.12 to 1.12 µm) narrowing of CRAE and a 0.59 µm (95% CI: -0.12 to 1.30 µm) widening of CRVE associated with a 10 µg/m³ increase in $PM_{2.5}$ on the day of the clinical examination (lag 0). We found no significant associations with $PM_{2.5}$ concentrations modelled at the residence on the day before the clinical examination (lag 1). Not correcting for blood pressure and heart rate or further correcting for prematurity or childhood hypertension did not substantially alter the reported associations (Appendix, Tables A.1- A.3).

229 **Table 3.** Estimated change in retinal vessel diameter (µm) associated with recent or chronic exposure to particulate air pollution $PM_{2.5}$ or

230 residential proximity to major roads.

231

	CRAE		CRVE	
	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE
Recent exposure to $PM_{2.5}$				
Measured on the playground (+10 µg/m³)	-0.35 (-0.61 to -0.09)	0.009	0.35 (-0.03 to 0.73)	0.07
Measured in the classroom (+1 µg/m³)	-0.39 (-0.65 to -0.13)	0.004	0.41 (0.04 to 0.79)	0.03
Modelled at residence, lag 0 (+10 µg/m³)	-0.62 (-1.12 to -0.12)	0.02	0.59 (-0.12 to 1.30)	0.10
Modelled at residence, lag 1 (+10 µg/m³)	-0.33 (-0.80 to 0.13)	0.16	0.46 (-0.19 to 1.10)	0.16
Chronic exposure to $PM_{2.5}$				
Modelled at residence (+1 µg/m³)	-1.15 (-3.28 to 0.97)	0.28	2.52 (-0.35 to 5.40)	0.08
Residential proximity to major roads (+100 m)	-0.30 (-0.54 to -0.05)	0.02	0.13 (-0.21 to 0.47)	0.46

232 Analyses adjusted for sex, age, body mass index, birth weight, maternal occupation, passive smoking, hour of the day and day of the week, apparent

233 temperature, systolic and diastolic blood pressure, heart rate, and fellow vessel diameter. CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal

234 Venular Equivalent.

3.4 Chronic exposure to PM and retinal vessel diameter

Allowing for the aforementioned covariates, each 1 μg/m³ increment in chronic exposure to $PM_{2.5}$ modelled at the child's residence was associated with a trend towards 2.52 μm (95% CI: -0.35 to 5.40 μm) wider venules, while no association was found with retinal arteriolar diameter (estimate: -1.15 μm; 95% CI: -3.28 to 0.97 μm). Living close to a major road, as a proxy for chronic exposure to traffic-related air pollution, was significantly associated with retinal arteriolar diameter (Table 3). Children living 100 meters closer to a major road had 0.30 μm (95% CI: 0.05 to 0.54 μm) narrower arterioles, while no association was found between residential proximity to major roads and venular diameter (estimate: 0.13 μm; 95% CI: -0.21 to 0.47 μm). Modelled chronic exposure estimates and residential proximity to major roads were highly correlated (P<0.001), but variation in the latter data was larger. Further correction for time spent in traffic did not alter the reported associations (Appendix, Table A.4).

4. Discussion

In a panel of 221 healthy school children aged 8 to 12 years, with a total of 489 retinal microvascular examinations, we found that increases in same-day exposure to $PM_{2.5}$ were associated with narrower retinal arteriolar diameters and wider venular diameters. In addition, those children living closer to major roads, a proxy for long-term PM exposure, had narrower arterioles. There was a trend for wider venules associated with modelled chronic residential exposure based on annual $PM_{2.5}$ concentrations. The associations were independent of other risk factors or correlates of microvascular changes such as sex, age,

BMI, birth weight, blood pressure and fellow vessel diameter. Hypertension, diabetes mellitus, chronic kidney disease, coronary heart disease and dementia follow trajectories that can have their roots in early phases of life, but track over life and become clinically overt in adulthood. Studies suggest that subclinical differences in risk factors for cardiovascular disease in childhood are related to the development of cardiovascular disease in later life.[15, 29] Microcirculatory alterations can be early phenotypic markers for disease development.[30-32] Therefore, the current findings may have significance in the context of the developmental origin of diseases.

To our knowledge, this is the first study to investigate microvascular effects of particulate air pollution in children using retinal imaging. Our results are in line with previously reported retinal arteriolar narrowing associated with particulate matter exposure in adult populations. Adar and colleagues documented retinal arteriolar narrowing associated with higher exposure to $PM_{2.5}$ in a cohort of 4,607 participants (mean age 64 years) of the Multi-Ethnic Study of Atherosclerosis.[12] These authors reported that retinal arteriolar diameter narrowed with 0.8 µm in association with an interquartile range (3 µg/m³) increase in long-term exposure to $PM_{2.5}$ estimated at the participant's residence. Furthermore, previous day exposure to particulate air pollution was independently associated with narrower arteriolar diameter, estimated cross-sectionally at -0.4 µm for a 9 µg/m³ increase in $PM_{2.5}$ levels. In another repeated measures study with adult volunteers, a 0.93 µm narrowing of retinal arteriolar diameter in association with a 10 µg/m³ higher recent PM_{10} exposure was reported.[13] These effect estimates are comparable in size to our reported results, but it has to be noted that our study population consists of primary school children, who in comparison with adult volunteers have a low burden of vascular or metabolic risk factors.

283 Microvascular changes may contribute to increased susceptibility for sustained
284 hypertension and other cardiovascular diseases associated with particulate matter exposure.
285 Gishti and colleagues showed an increased risk of hypertension associated with narrower
286 retinal vessel diameters in a cohort of 4,007 school-age children in the Generation-R study.[33]
287 Likewise, Gonipath et al. showed that children in the highest quartile of blood pressure had
288 significantly narrower retinal arterioles than those in the lowest quartiles.[34] In a sensitivity
289 analysis, we showed that either no correction for continuous blood pressure (Appendix,
290 Table A.2) or additional correction for childhood hypertension (Appendix, Table A.3) did not
291 alter our reported associations, indicating that the observed changes in retinal vessel
292 diameters due to air pollution exposure are independent of blood pressure. Nonetheless,
293 our estimated effect size of a 10 μg/m³ increment in same-day $PM_{2.5}$ exposure on retinal
294 arteriolar diameter was equal to the effect estimate of a 4.6 mm Hg higher diastolic blood
295 pressure. We found no significant association between systolic blood pressure and the
296 retinal microcirculation. It is established that diastolic blood pressure correlates more closely
297 with indicators of vascular stiffness below the age of 50.[35, 36] Beyond cardiovascular health
298 effects, air pollution exposure has also been associated with cerebrovascular disease[37] and
299 impaired cognitive functioning[38], all of which may be potentially reflected in retinal
300 microvascular changes.[39] The microvascular changes observed in the retina may therefore be
301 part of a risk phenotype that puts children on a trajectory for age-related disease
302 development. In this respect, retinal arteriolar narrowing and venular widening are
303 independent predictors of an increased risk for dementia,[40] coronary heart disease,[41] and
304 stroke[42] among adults and these retinal changes are associated with measures of
305 atherosclerosis and inflammation.[43] Inflammatory responses and oxidative stress have been
306 suggested as underlying pathways by which particulate air pollution can exert its effects.

307 Animal studies have shown that exposure to particulate matter attenuates nitric oxide
308 bioavailability due to the activation of inflammatory mechanisms and oxidative stress.[44, 45] In
309 addition, controlled exposure studies in humans have reported impaired nitric oxide-
310 mediated endothelial vasomotor function in response to acute diesel exhaust exposure.[46, 47]
311 Furthermore, retinal venular diameter has been associated with systemic inflammation in a
312 cohort of 5,979 participants of the Multi-Ethnic Study of Atherosclerosis.[43]

313 Our findings are based on exposure measurements at school and high resolution
314 modelling at home. We studied the effect of recent and chronic exposure and used repeated
315 examinations of the retinal microcirculation within the same child. Within-subject repeated
316 measures allow each subject to act as its own control, reducing potential confounding by
317 variables that do not change over the time period of the study (e.g. socio-economic status).
318 Planning the repeated examinations of individual children on the same day of the week and
319 time of day further controls for circadian variation and daily activity patterns. In addition, the
320 children in our study are free of vascular diseases and other major traditional risk factors and
321 therefore introduce less confounding.[48] Although we cannot exclude some level of residual
322 confounding, it is unlikely that unmeasured confounders would cancel out the observed
323 associations due to the study design, particularly with regard to the short-term effects.
324 Furthermore, results based on both measured and modelled recent $PM_{2.5}$ were in
325 agreement, further validating the use of modelled exposure data for the investigation of PM-
326 related health effects. Nevertheless, our current study must also be interpreted within the
327 context of its limitations. Our participation rate was just under 50%, which might result in a
328 not completely representative sample of the target population (e.g. low maternal
329 occupation was 18% in our study population while we expected 26%[49]). However, we do not
330 expect this to have an impact on the mechanistic associations reported in this study. We

only assessed capillary properties in the retina. To what extent these findings can be extrapolated to other microvascular beds in children, including physiological consequences, remains to be elucidated. However, studies indicate that changes in the retinal microvessels of adults parallel those in the coronary and cerebral microcirculation.[50, 51] The observations warrant further research into the potential value of retinal microvascular changes as an early phenotype for age-related disease development.

5. Conclusions

We show that vessel diameters of the retinal microcirculation of children aged 8 to 12 years respond to recent particulate air pollution exposure. Additionally, children living closer to major roads had smaller arteriolar diameters. Our results suggest that the microcirculation is a pathophysiological target for air pollution from a young age onwards.

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Disclosures

None.

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Appendix

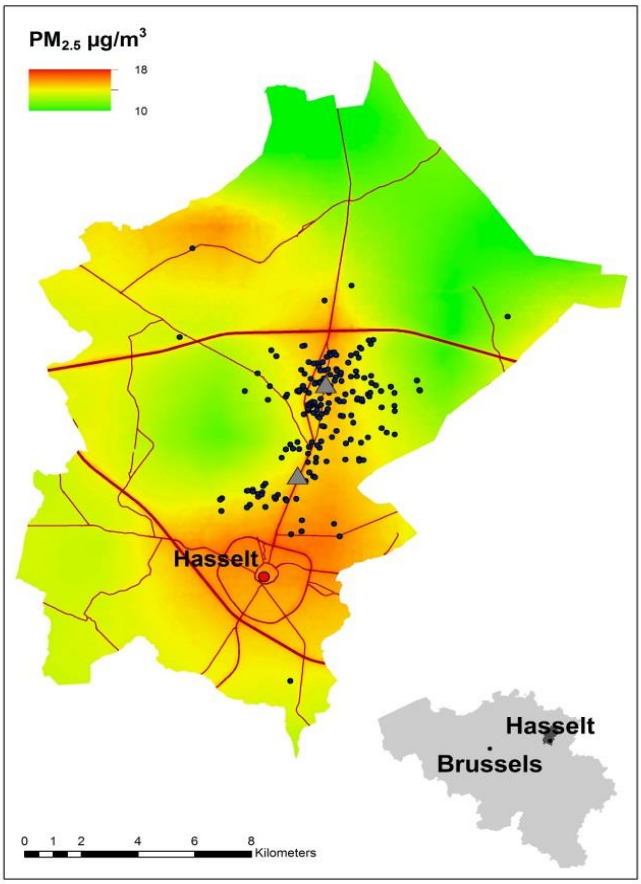

Figure A.1. Heat map of the study area with yearly average $PM_{2.5}$ concentrations (2013) and major road network. Dots represent home addresses of the participating children while triangles represent the locations of the schools.

Table A.1. Estimated change in retinal vessel diameter (µm) associated with recent or chronic exposure to particulate air pollution $PM_{2.5}$ or residential proximity to major roads – Sensitivity analysis with additional correction for prematurity (gestational age <37 weeks).

	CRAE		CRVE	
	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE
Recent exposure to $PM_{2.5}$				
Measured on the playground (+10 µg/m³)	-0.35 (-0.62 to -0.09)	0.009	0.34 (-0.03 to 0.72)	0.07
Measured in the classroom (+1 µg/m³)	-0.38 (-0.65 to -0.12)	0.005	0.38 (0.007 to 0.76)	0.05
Modelled at residence, lag 0 (+10 µg/m³)	-0.62 (-1.13 to -0.11)	0.02	0.60 (-0.11 to 1.31)	0.10
Modelled at residence, lag 1 (+10 µg/m³)	-0.31 (-0.78 to 0.16)	0.19	0.44 (-0.20 to 1.09)	0.17
Chronic exposure to $PM_{2.5}$				
Modelled at residence (+1 µg/m³)	-1.06 (-3.17 to 1.05)	0.32	2.40 (-0.45 to 5.26)	0.10
Residential proximity to major roads (+100 m)	-0.29 (-0.54 to -0.028)	0.02	0.13 (-0.21 to 0.47)	0.46

Analyses adjusted for sex, age, body mass index, birth weight, maternal occupation, passive smoking, hour of the day and day of the week, apparent temperature, systolic and diastolic blood pressure, heart rate, fellow vessel diameter, and prematurity (gestational age <37 weeks). CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal Venular Equivalent.

Table A.2. Estimated change in retinal vessel diameter (µm) associated with recent or chronic exposure to particulate air pollution $PM_{2.5}$ or residential proximity to major roads – Sensitivity analysis without correction for blood pressure and heart rate.

	CRAE		CRVE	
	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE
Recent exposure to $PM_{2.5}$				
Measured on the playground (+10 µg/m³)	-0.41 (-0.67 to -0.15)	0.002	0.33 (-0.04 to 0.71)	0.08
Measured in the classroom (+1 µg/m³)	-0.43 (-0.68 to -0.17)	0.001	0.41 (0.035 to 0.79)	0.03
Modelled at residence, lag 0 (+10 µg/m³)	-0.61 (-1.10 to -0.011)	0.02	0.46 (-0.25 to 1.16)	0.20
Modelled at residence, lag 1 (+10 µg/m³)	-0.27 (-0.72 to 0.17)	0.23	0.29 (-0.33 to 0.92)	0.36
Chronic exposure to $PM_{2.5}$				
Modelled at residence (+1 µg/m³)	-0.96 (-3.12 to 1.19)	0.38	2.55 (-0.29 to 5.38)	0.08
Residential proximity to major roads (+100 m)	-0.28 (-0.53 to -0.028)	0.03	0.15 (-0.17 to 0.49)	0.38

Analyses adjusted for sex, age, body mass index, birth weight, maternal occupation, passive smoking, hour of the day and day of the week, apparent temperature, and fellow vessel diameter. CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal Venular Equivalent.

Table A.3. Estimated change in retinal vessel diameter (µm) associated with recent or chronic exposure to particulate air pollution $PM_{2.5}$ or residential proximity to major roads – Sensitivity analysis with additional correction for childhood hypertension.

	CRAE		CRVE	
	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE
Recent exposure to $PM_{2.5}$				
Measured on the playground (+10 µg/m³)	-0.35 (-0.62 to -0.08)	0.01	0.32 (-0.06 to 0.70)	0.10
Measured in the classroom (+1 µg/m³)	-0.39 (-0.65 to -0.13)	0.004	0.41 (0.027 to 0.78)	0.04
Modelled at residence, lag 0 (+10 µg/m³)	-0.61 (-1.12 to -0.01)	0.02	0.55 (-0.17 to 1.26)	0.13
Modelled at residence, lag 1 (+10 µg/m³)	-0.32 (-0.79 to 0.14)	0.17	0.42 (-0.23 to 1.07)	0.21
Chronic exposure to $PM_{2.5}$				
Modelled at residence (+1 µg/m³)	-1.15 (-3.27 to 0.97)	0.28	2.31 (-0.68 to 5.30)	0.13
Residential proximity to major roads (+100 m)	-0.30 (-0.54 to -0.05)	0.02	0.14 (-0.50 to 0.22)	0.43

Analyses adjusted for sex, age, body mass index, birth weight, maternal occupation, passive smoking, hour of the day and day of the week, apparent temperature, systolic and diastolic blood pressure, heart rate, fellow vessel diameter, and childhood hypertension. CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal Venular Equivalent.

Table A.4. Estimated change in retinal vessel diameter (µm) associated with recent or chronic exposure to particulate air pollution $PM_{2.5}$ or residential proximity to major roads – Sensitivity analysis with additional correction for time spent in traffic.

	CRAE		CRVE	
	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE	ESTIMATED CHANGE IN µM (95% CI)	P-VALUE
Recent exposure to $PM_{2.5}$				
Measured on the playground (+10 µg/m³)	-0.32 (-0.59 to -0.06)	0.02	0.28 (-0.10 to 0.67)	0.14
Measured in the classroom (+1 µg/m³)	-0.41 (-0.68 to -0.14)	0.003	0.42 (0.03 to 0.81)	0.03
Modelled at residence, lag 0 (+10 µg/m³)	-0.56 (-1.07 to -0.04)	0.03	0.47 (-0.25 to 1.19)	0.20
Modelled at residence, lag 1 (+10 µg/m³)	-0.26 (-0.73 to 0.21)	0.27	0.35 (-0.30 to 1.0)	0.29
Chronic exposure to $PM_{2.5}$				
Modelled at residence (+1 µg/m³)	-1.23 (-3.44 to 0.97)	0.27	2.58 (-0.38 to 5.55)	0.08
Residential proximity to major roads (+100 m)	-0.31 (-0.56 to -0.05)	0.02	0.16 (-0.19 to 0.51)	0.38

Analyses adjusted for sex, age, body mass index, birth weight, maternal occupation, passive smoking, hour of the day and day of the week, apparent temperature, fellow vessel diameter, and time spent in traffic. CRAE: Central Retinal Arteriolar Equivalent, CRVE: Central Retinal Venular Equivalent.