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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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1 **Abstract**

2 **Background** - Microvascular changes may represent an underlying mechanism through
3 which exposure to fine particulate matter with a diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) contributes to
4 age-related disease development. We investigated the effect of recent and chronic exposure
5 to $\text{PM}_{2.5}$ on the microcirculation, exemplified by retinal vessel diameters, using repeated
6 measurements in 8- to 12-year-old children.

7 **Methods** - 221 children (49.1% girls; mean age 9.9 years) were examined repeatedly (25 one,
8 124 two, and 72 three times) adding up to 489 retinal vessel examinations. Same-day
9 exposure to $\text{PM}_{2.5}$ was measured at school. In addition, recent (same and previous day) and
10 chronic (yearly mean) exposure was modelled at the child's residence using a high-resolution
11 interpolation model. Residential proximity to major roads was also assessed. Changes in
12 retinal vessel diameters associated with recent and chronic exposures were estimated using
13 mixed models, while adjusting for other known covariates such as sex, age, BMI, blood
14 pressure and birth weight.

15 **Results** - Each $10 \mu\text{g}/\text{m}^3$ increment in same-day exposure to $\text{PM}_{2.5}$ measured at school was
16 associated with $0.35 \mu\text{m}$ (95% CI: 0.09 to $0.61 \mu\text{m}$) narrower retinal arterioles and $0.35 \mu\text{m}$ (-
17 0.03 to $0.73 \mu\text{m}$) wider venules. Children living 100 meters closer to a major road had 0.30
18 μm (0.05 to $0.54 \mu\text{m}$) narrower arterioles.

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

24 **Key words:** microcirculation, retina, air pollution, children

25 **1. Introduction**

26 The microcirculation constitutes the majority of the circulatory system. Its role in age-related
27 disease development is however less explored than that of the macrocirculation. The
28 microvasculature plays a unifying role in diverse pathological conditions such as
29 hypertension,¹ chronic kidney disease,² left ventricular dysfunction,³ obesity,⁴ diabetes
30 mellitus⁵, and cognitive impairment.⁶ The state of the microvasculature and its physiological
31 response are important factors on the trajectory from healthy to unhealthy ageing.

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

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

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

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

56

57

58 **2. Methods**

59 **2.1 Study population**

60 This research was part of the COGNAC (COGNition and Air pollution in Children) study.¹⁷
61 Children aged 8 to 12 years from two primary schools in Flanders (Belgium) were invited for
62 repeated clinical examinations. The two schools were 3.7 km apart and located in the
63 agglomeration of Hasselt (~70 km east from Brussels). Of the 482 invited children, 221
64 (45.9%) agreed to participate of which 72 (33%) underwent three clinical examinations, 124
65 (56%) completed two examinations and 25 (11%) had only one examination, amounting to a
66 total of 489 retinal microvascular examinations. The examinations took place in school years
67 2012-2013 for one school and 2013-2014 for the other, from November to February on
68 Monday, Tuesday, Thursday, and Friday between 8:30am and 3:30pm. Participating children
69 were examined at their school during school time. The average (SD) time between two
70 consecutive examinations was 49 (19) days. The clinical examinations of each child were
71 scheduled on the same time of day and day of the week to minimize circadian variation.

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

80

81 **2.2 Clinical examination**

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

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

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

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

99

100 **2.3 Particulate air pollution exposure assessment**

101 ***2.3.1 Measured exposure to PM_{2.5} at school***

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

110

111 ***2.3.2 Modelled residential air pollution***

112 We used a spatial temporal interpolation method to model the daily residential exposure
113 levels ($\mu\text{g}/\text{m}^3$) of PM_{2.5} at each child's home address. This method takes into account land
114 cover data obtained from satellite images (CORINE land cover data set)²¹ and pollution data
115 from fixed monitoring stations in combination with a dispersion model.^{22, 23} The model
116 calculates the daily interpolated exposure concentrations in a high resolution receptor grid
117 (25 x 25 m) based on information from the Belgian telemetric air quality networks, point
118 sources, and line sources. Overall model performance was evaluated by leave-one-out cross-
119 validation. Validation statistics of the interpolation tool gave a temporal explained variance

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

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

136

137 **2.4 Statistical analysis**

138 SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for database
139 management and statistical analysis. The effect of particulate matter exposure on retinal
140 vessel diameters was investigated using the MIXED procedure to account for the clustered
141 data within the same person, i.e. repeated measures of retinal vessel diameter. A random
142 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.

167

168

169 **3. Results**

170 **3.1 Study population and exposure characteristics**

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

178

179 **Table 1.** Description of the study population.

Anthropometrics	
Age, years	9.9 ± 1.2
Girls	49.1%
Body Mass Index (BMI), kg/m ²	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

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

182

183 The exposure characteristics are listed in Table 2. Recent exposure to PM_{2.5} measured on the
 184 playground was on average 16.8 (23.2) μg/m³, while modelled daily PM_{2.5} at the residence
 185 was 19.2 (14.7) μg/m³. Both indoor and outdoor concentrations as well as measured and
 186 modelled PM_{2.5} exposures on the day of the clinical examination were highly correlated
 187 (*P*<0.0001). Children spent on average 9 (6) minutes in traffic. Chronic exposure to PM_{2.5}
 188 averaged 15.4 (0.7) μg/m³ and children lived on average 649 (569) meters from a major
 189 road. Apparent temperature on the day of the clinical examination ranged from -6.4 to 9.1
 190 °C.

191

192 **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

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

197

198 **3.2 Determinants of retinal vessel diameter**

199 CRAE and CRVE were not significantly correlated with age in our study population ($P=0.73$
 200 and 0.49, respectively). Girls had 3.7 µm (95% CI: 0.8 to 6.6 µm) wider retinal arteriolar
 201 diameters than boys. Venular diameter, however, did not significantly differ between boys
 202 and girls ($P=0.27$). Having a 1 mm Hg higher diastolic blood pressure was associated with
 203 0.13 µm (95% CI: 0.00073 to 0.25 µm) narrower arterioles. Systolic blood pressure ($P=0.51$)
 204 and heart rate ($P=0.46$) were not significantly associated with CRAE. Children for which a
 205 lower birth weight was reported had narrower arterioles (estimate: -0.28 µm per 100 g
 206 lower birth weight; 95% CI: -0.0073 to -0.55 µm). BMI ($P=0.65$), passive smoking ($P=0.80$),
 207 occupation of the mother ($P=0.58$), time of the day ($P=0.99$) and day of the week ($P=0.28$) of

208 the examination and apparent temperature ($P=0.81$) were not significantly associated with
209 CRAE. Finally, a 1 μm widening of venular diameter was associated with 0.34 μm (95% CI:
210 0.28 to 0.40 μm) wider arterioles. BMI was the only statistically significant correlate of
211 venular diameter, expressed as CRVE (estimate: +1.08 μm per 1 kg/m^2 increase in BMI; 95%
212 CI: 0.19 to 1.96 μm).

213

214 **3.3 Recent exposure to $\text{PM}_{2.5}$ and retinal vessel diameter**

215 Same-day exposure to particulate air pollution was significantly associated with retinal
216 vessel diameters (Table 3). Each 10 $\mu\text{g}/\text{m}^3$ increment in same-day exposure to $\text{PM}_{2.5}$
217 measured on the playground was associated with a 0.35 μm (95% CI: 0.09 to 0.61 μm)
218 narrowing of the retinal arteriolar diameter (CRAE), while venular diameter (CRVE) widened
219 0.35 μm (95% CI: -0.03 to 0.73 μm). Likewise, CRAE narrowed 0.39 μm (95% CI: 0.13 to 0.65
220 μm) and CRVE widened 0.41 μm (95% CI: 0.04 to 0.79 μm) in association with a 1 $\mu\text{g}/\text{m}^3$
221 increase in $\text{PM}_{2.5}$ concentrations measured in the classroom. Similar results were found with
222 recent exposures to $\text{PM}_{2.5}$ modelled at the residence, showing a 0.62 μm (95% CI: 0.12 to
223 1.12 μm) narrowing of CRAE and a 0.59 μm (95% CI: -0.12 to 1.30 μm) widening of CRVE
224 associated with a 10 $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ on the day of the clinical examination (lag 0).
225 We found no significant associations with $\text{PM}_{2.5}$ concentrations modelled at the residence on
226 the day before the clinical examination (lag 1). Not correcting for blood pressure and heart
227 rate or further correcting for prematurity or childhood hypertension did not substantially
228 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 $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$				
Measured on the playground (+10 $\mu\text{g}/\text{m}^3$)	-0.35 (-0.61 to -0.09)	0.009	0.35 (-0.03 to 0.73)	0.07
Measured in the classroom (+1 $\mu\text{g}/\text{m}^3$)	-0.39 (-0.65 to -0.13)	0.004	0.41 (0.04 to 0.79)	0.03
Modelled at residence, lag 0 (+10 $\mu\text{g}/\text{m}^3$)	-0.62 (-1.12 to -0.12)	0.02	0.59 (-0.12 to 1.30)	0.10
Modelled at residence, lag 1 (+10 $\mu\text{g}/\text{m}^3$)	-0.33 (-0.80 to 0.13)	0.16	0.46 (-0.19 to 1.10)	0.16
Chronic exposure to $\text{PM}_{2.5}$				
Modelled at residence (+1 $\mu\text{g}/\text{m}^3$)	-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.

235

236 **3.4 Chronic exposure to PM and retinal vessel diameter**

237 Allowing for the aforementioned covariates, each 1 $\mu\text{g}/\text{m}^3$ increment in chronic exposure to
238 $\text{PM}_{2.5}$ modelled at the child's residence was associated with a trend towards 2.52 μm (95%
239 CI: -0.35 to 5.40 μm) wider venules, while no association was found with retinal arteriolar
240 diameter (estimate: -1.15 μm ; 95% CI: -3.28 to 0.97 μm). Living close to a major road, as a
241 proxy for chronic exposure to traffic-related air pollution, was significantly associated with
242 retinal arteriolar diameter (Table 3). Children living 100 meters closer to a major road had
243 0.30 μm (95% CI: 0.05 to 0.54 μm) narrower arterioles, while no association was found
244 between residential proximity to major roads and venular diameter (estimate: 0.13 μm ; 95%
245 CI: -0.21 to 0.47 μm). Modelled chronic exposure estimates and residential proximity to
246 major roads were highly correlated ($P < 0.001$), but variation in the latter data was larger.
247 Further correction for time spent in traffic did not alter the reported associations (Appendix,
248 Table A.4).

249

250

251 **4. Discussion**

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

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

267 To our knowledge, this is the first study to investigate microvascular effects of
268 particulate air pollution in children using retinal imaging. Our results are in line with
269 previously reported retinal arteriolar narrowing associated with particulate matter exposure
270 in adult populations. Adar and colleagues documented retinal arteriolar narrowing
271 associated with higher exposure to PM_{2.5} in a cohort of 4,607 participants (mean age 64
272 years) of the Multi-Ethnic Study of Atherosclerosis.¹² These authors reported that retinal
273 arteriolar diameter narrowed with 0.8 μm in association with an interquartile range (3
274 $\mu\text{g}/\text{m}^3$) increase in long-term exposure to PM_{2.5} estimated at the participant's residence.
275 Furthermore, previous day exposure to particulate air pollution was independently
276 associated with narrower arteriolar diameter, estimated cross-sectionally at -0.4 μm for a 9
277 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} levels. In another repeated measures study with adult volunteers, a
278 0.93 μm narrowing of retinal arteriolar diameter in association with a 10 $\mu\text{g}/\text{m}^3$ higher
279 recent PM₁₀ exposure was reported.¹³ These effect estimates are comparable in size to our
280 reported results, but it has to be noted that our study population consists of primary school
281 children, who in comparison with adult volunteers have a low burden of vascular or
282 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 $\mu\text{g}/\text{m}^3$ increment in same-day $\text{PM}_{2.5}$ 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 disease³⁷ and
299 impaired cognitive functioning³⁸, 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 stroke⁴² 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

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

337

338

339 **5. Conclusions**

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

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347

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352

353 **Disclosures**

354 None.

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Appendix

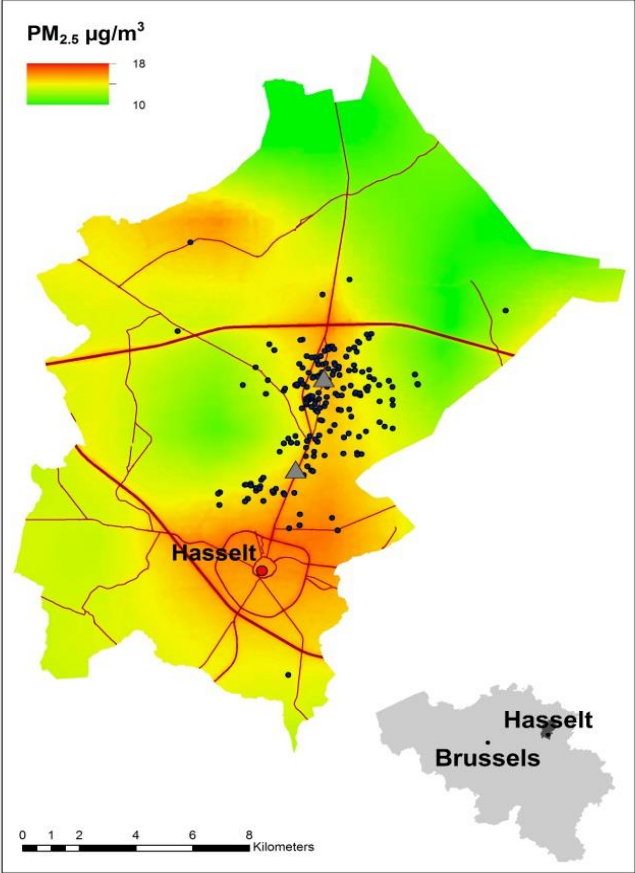


Figure A.1. Heat map of the study area with yearly average PM_{2.5} concentrations (2013) and major road network. Dots represent home addresses of the participating children while triangles represent the locations of the schools.

Table A.1. Estimated change in retinal vessel diameter (μm) associated with recent or chronic exposure to particulate air pollution $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$				
Measured on the playground (+10 $\mu\text{g}/\text{m}^3$)	-0.35 (-0.62 to -0.09)	0.009	0.34 (-0.03 to 0.72)	0.07
Measured in the classroom (+1 $\mu\text{g}/\text{m}^3$)	-0.38 (-0.65 to -0.12)	0.005	0.38 (0.007 to 0.76)	0.05
Modelled at residence, lag 0 (+10 $\mu\text{g}/\text{m}^3$)	-0.62 (-1.13 to -0.11)	0.02	0.60 (-0.11 to 1.31)	0.10
Modelled at residence, lag 1 (+10 $\mu\text{g}/\text{m}^3$)	-0.31 (-0.78 to 0.16)	0.19	0.44 (-0.20 to 1.09)	0.17
Chronic exposure to $\text{PM}_{2.5}$				
Modelled at residence (+1 $\mu\text{g}/\text{m}^3$)	-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 $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$				
Measured on the playground (+10 $\mu\text{g}/\text{m}^3$)	-0.41 (-0.67 to -0.15)	0.002	0.33 (-0.04 to 0.71)	0.08
Measured in the classroom (+1 $\mu\text{g}/\text{m}^3$)	-0.43 (-0.68 to -0.17)	0.001	0.41 (0.035 to 0.79)	0.03
Modelled at residence, lag 0 (+10 $\mu\text{g}/\text{m}^3$)	-0.61 (-1.10 to -0.011)	0.02	0.46 (-0.25 to 1.16)	0.20
Modelled at residence, lag 1 (+10 $\mu\text{g}/\text{m}^3$)	-0.27 (-0.72 to 0.17)	0.23	0.29 (-0.33 to 0.92)	0.36
Chronic exposure to $\text{PM}_{2.5}$				
Modelled at residence (+1 $\mu\text{g}/\text{m}^3$)	-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 $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$				
Measured on the playground (+10 $\mu\text{g}/\text{m}^3$)	-0.35 (-0.62 to -0.08)	0.01	0.32 (-0.06 to 0.70)	0.10
Measured in the classroom (+1 $\mu\text{g}/\text{m}^3$)	-0.39 (-0.65 to -0.13)	0.004	0.41 (0.027 to 0.78)	0.04
Modelled at residence, lag 0 (+10 $\mu\text{g}/\text{m}^3$)	-0.61 (-1.12 to -0.01)	0.02	0.55 (-0.17 to 1.26)	0.13
Modelled at residence, lag 1 (+10 $\mu\text{g}/\text{m}^3$)	-0.32 (-0.79 to 0.14)	0.17	0.42 (-0.23 to 1.07)	0.21
Chronic exposure to $\text{PM}_{2.5}$				
Modelled at residence (+1 $\mu\text{g}/\text{m}^3$)	-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 $\text{PM}_{2.5}$ 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 $\text{PM}_{2.5}$				
Measured on the playground (+10 $\mu\text{g}/\text{m}^3$)	-0.32 (-0.59 to -0.06)	0.02	0.28 (-0.10 to 0.67)	0.14
Measured in the classroom (+1 $\mu\text{g}/\text{m}^3$)	-0.41 (-0.68 to -0.14)	0.003	0.42 (0.03 to 0.81)	0.03
Modelled at residence, lag 0 (+10 $\mu\text{g}/\text{m}^3$)	-0.56 (-1.07 to -0.04)	0.03	0.47 (-0.25 to 1.19)	0.20
Modelled at residence, lag 1 (+10 $\mu\text{g}/\text{m}^3$)	-0.26 (-0.73 to 0.21)	0.27	0.35 (-0.30 to 1.0)	0.29
Chronic exposure to $\text{PM}_{2.5}$				
Modelled at residence (+1 $\mu\text{g}/\text{m}^3$)	-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.