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Dynamics of skin microvascular blood flow in 4–6-year-old children in association with pre- and postnatal black carbon and particulate air pollution exposure

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ABSTRACT

Background: A growing body of evidence indicates that cardiovascular health in adulthood, particularly that of the microcirculation, could find its roots during prenatal development. In this study, we investigated the association between pre- and postnatal air pollution exposure on heat-induced skin hyperemia as a dynamic marker of the microvasculature.

Methods: In 139 children between the ages of 4 and 6 who are followed longitudinally within the ENVIRONAGE birth cohort, we measured skin perfusion by Laser Doppler probes using the Periflux6000. Residential black carbon (BC), particulate (PM_{10} and $PM_{2.5}$) air pollution, and nitrogen dioxide (NO_2) levels were modelled for each participant's home address using a high-resolution spatiotemporal model for multiple time windows. We assessed the association between skin hyperemia and pre- and postnatal air pollution using multiple regression models while adjusting for relevant covariates.

Results: Residential BC exposure during the whole pregnancy averaged (IQR) 1.42 (1.22–1.58) μg/m^3, PM_{10} 18.88 (16.64 – 21.13) μg/m^3, $PM_{2.5}$ 13.67 (11.5 – 15.56) μg/m^3 and NO_2 18.39 (15.52 – 20.31) μg/m^3. An IQR increment in BC exposure during the third trimester of pregnancy was associated with an 11.5 % (95% CI: −20.1 to −1.9; p = 0.020) lower skin hyperemia. Similar effect estimates were retrieved for PM_{10}, $PM_{2.5}$ and NO_2 (respectively 13.9 % [95% CI: −21.9 to −3.0; p = 0.003], 17.0 % [95% CI: −26.7 to −6.1; p = 0.004] and 12.7% [95 % CI: –22.2 to −1.9; p = 0.023] lower skin hyperemia). In multipollutant models, $PM_{2.5}$ showed the strongest inverse association with skin hyperemia. Postnatal exposure to BC, PM_{10}, $PM_{2.5}$ or NO_2, was not associated with skin hyperemia at the age of 4 to 6, and did not alter the previous reported prenatal associations when taken into account.

Conclusion: Our findings support that BC, particulate air pollution, and NO_2 exposure, even at low concentrations, during prenatal life, can have long-lasting consequences for the microvasculature. This proposes a role of prenatal air pollution exposures over and beyond postnatal exposure in the microvascular alterations which were persistent into childhood.

1. Introduction

Particulate matter (PM) pollution embodies emissions from both natural and anthropogenic sources, with the main sources of primary PM originating from the combustion of fossil fuels, biofuels, and biomass (Kelly and Fussell, 2012). Black carbon (BC) particles are emitted as an unwanted by-product during the incomplete combustion of fossil fuels, biofuels, and biomass (Center for Climate and Energy Solutions, 2010; Climate and Clean Air Coalition, 2016; Long et al., 2013). BC particles are considered to be one of the most toxic components of PM (Janssen et al., 2011; Krzyzanowski et al., 2005) due to harmful contaminants adsorbed onto the BC particles. The extent of evidence and the number

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of studies linking air pollution to cardiovascular diseases have grown considerably, and PM plays a substantial role. Both short- and long-term exposures to PM are associated with cardiovascular diseases, including acute (Nawrot et al., 2011) and chronic (Polak et al., 2011) cardiovascular outcomes.

As cardiovascular health's physiology is determined mainly by the microcirculation (Louwies et al., 2013), microcirculatory dysfunction plays an essential role in the onset of cardiovascular disease. A growing amount of evidence suggests that cardiovascular health in adulthood, particularly that of the microcirculation, could find its basis during the prenatal phase (Clough and Norman, 2011; Luyten et al., 2020). During fetal development, the microvasculature undergoes extensive, organ-specific maturation providing several different perinatal "windows of opportunity" during which the maternal and fetal life may influence the development of the offspring's microvasculature (Clough and Norman, 2011). Luyten et al. (Luyten et al., 2020) described a positive association between retinal arterioles' diameter in young children (mean age of 4.6 years) and prenatal exposure to $PM_{2.5}$ and NO_2. Recently we provided evidence for the presence of BC particles in the human placenta (Bové et al., 2019), which suggests direct fetal exposure to those particles during the most susceptible period of life. Various studies have described postnatal effects related to prenatal exposure to combustion-related PM, including BC (World Health Organization, 2013), for example, molecular longevity, reflected by telomere length (Martens and Nawrot, 2016), or clinical factors such as preterm birth (Ritz et al., 2007; Rudra et al., 2011), intrauterine growth restriction (Liu et al., 2007; Winckelmans et al., 2015), and birth weight (Pedersen et al., 2013; Ruttens et al., 2017; Slama et al., 2007). In turn, these effects have been linked to (markers of) disease development later in life, such as increased risks of cardiovascular disease development (Nawrot et al., 2010; Smith et al., 2016). Changes in small vessel structure and function may be perceived very early, long before the onset of cardiovascular disease. Studies of early-life exposure to air pollution on cardiovascular outcomes in offspring are, however, scarce. Here, we investigated the association between pre- and postnatal BC, particulate air pollution, and NO_2 exposure on heat-induced skin hyperemia in four- to six-year-old children as a dynamic marker of the microvasculature. We used a novel method in which skin perfusion was assessed using the Periflux6000 equipped with a laser Doppler system, which exploits the Doppler phenomenon to detect blood flow changes.

2. Materials and methods

2.1. Study population

This study was conducted within the framework of the ENVIR*ON*AGE (ENVIRonmental influence *ON* early AGEing) birth cohort study. The rationale and full methodology of the ENVIR*ON*AGE cohort were previously described (Janssen et al., 2017). In short, mother-newborn pairs are recruited when they arrive for delivery at the East-Limburg Hospital in Genk (Belgium), and they are followed longitudinally. The follow-up examinations in this study population at Hasselt University are performed when children are between four and six years old. This study's only inclusion criteria were to have already participated in the follow-up four years after birth, not to have moved since this follow-up, and not to have planned major renovations close to the house visit planned for this study. Following these inclusion criteria, 255 children were selected to participate. Fifty-one households could not be contacted by phone or e-mail, and forty-nine did not want to participate. In total, 155 children aged between four and six years old were enrolled to join, *i.e.,* a final participation rate of 61%. This study was conducted according to the principles outlined in the Helsinki declaration (World Medical Association, 2013) for research on human participants and was approved by the ethics committee of Hasselt University. We obtained written informed consent from the parents and oral assent from the child during the house visit. House visits were performed in two cycles: April 30th-June 29th, 2017 and April 14th-July 12th, 2018. After the parent (s) and the child's consent to participate, cardiovascular measurements were performed on the child. At the moment of the measurements, all children were healthy and did not suffer from any underlying cardiovascular disease. Of all the children enrolled in this study, the data of sixteen children could not be used: nine children refused to participate, four measurements were not eligible as the children moved or talked too much during the examination, and the skin perfusion data of three children were identified as being outliers and were thus excluded from the analysis. Final statistics were performed on the data of 139 children.

2.2. Exposure assessment

To assess daily air pollutant concentrations of BC, PM_{10}, $PM_{2.5}$, and NO_2, at the maternal residential address during pregnancy and the postnatal period, we used a high-resolution spatial–temporal interpolation method based on land use and dispersion modelling (Janssen et al., 2008). In short, land cover data provided by satellite images from the CORINE land cover dataset were used to interpolate the pollution data provided by the official fixed monitoring stations in the Flemish part of Belgium. Combined with a dispersion model (Lefebvre et al., 2013) that uses emissions from point sources (such as industrial sites) and line sources (such as road traffic ways), this model chain provides interpolated daily air pollution values on a dense, irregular high-resolution receptor point grid. The overall model performance was assessed by leave-one-out cross-validation, including 14 stations for BC, 58 for PM_{10}, 34 for $PM_{2.5}$, and 44 for NO_2. The interpolation tool's validation statistics explained more than 74% of the temporal and spatial variability in Flanders for BC (Lefebvre et al., 2011), 80% for $PM_{2.5}$ and PM_{10} (Maiheu et al., 2013) ,and 78% for NO_2. Furthermore, for prenatal exposure, the accuracy of our exposure model was supported by the correlation between our exposure model and the placental BC load (Bové et al., 2019). For postnatal exposure, our exposure models' accuracy was supported by the correlation between children's urinary environmental carbon load and residential levels of BC (Saenen et al., 2017). The described model provided daily air pollution exposures for each participant from pregnancy to examination.

To explore potentially critical exposures during pregnancy, individual BC, PM_{10}, $PM_{2.5}$, and NO_2 daily values were averaged for specific time windows during pregnancy: first trimester (*i.e.*, date of conception until 13 weeks of pregnancy), second trimester (*i.e.*, 14 weeks until 26 weeks of pregnancy), and third trimester (*i.e.,* 27 weeks of pregnancy until delivery). Ultrasound imaging data combined with the first day of the mother's last menstrual period were used to estimate the date of conception (Janssen et al., 2012). Postnatal BC, PM_{10}, $PM_{2.5}$, and NO_2 exposure were averaged for the month before the house visit as a short-term exposure window, and for the period between birth and the house visit, approximately four years, as a long-term lifetime exposure window. The model was validated and showed that it predicted prenatal exposure by BC load in placenta (Bové et al., 2019) and postnatal exposure by accumulation of particles in urine (Saenen et al., 2017).

2.3. Skin microvascular blood flow measurement

Skin perfusion was assessed using the Periflux6000 (Perimed, Stockholm, Sweden) (Fig. 1A) equipped with a laser Doppler system, which exploits the Doppler phenomenon to detect blood flow changes. The Periflux6000 is a small, stand-alone device operated by a touch screen and offers complete solutions for safe, smooth, and simple workflows to investigate and assess the microcirculation. This method is based on the direction of a low-power laser light beam (780 nm) to tissue, *e.g.*, the skin, at a known frequency and measurement of the Doppler shift that occurs in light that has been scattered by moving red blood cells (Fig. 1C). The Doppler shift's frequency and size are associated with the velocity and amount of red blood cells (Brocq et al., 2008). Skin perfusion measurements were performed in the living room of the children's home,

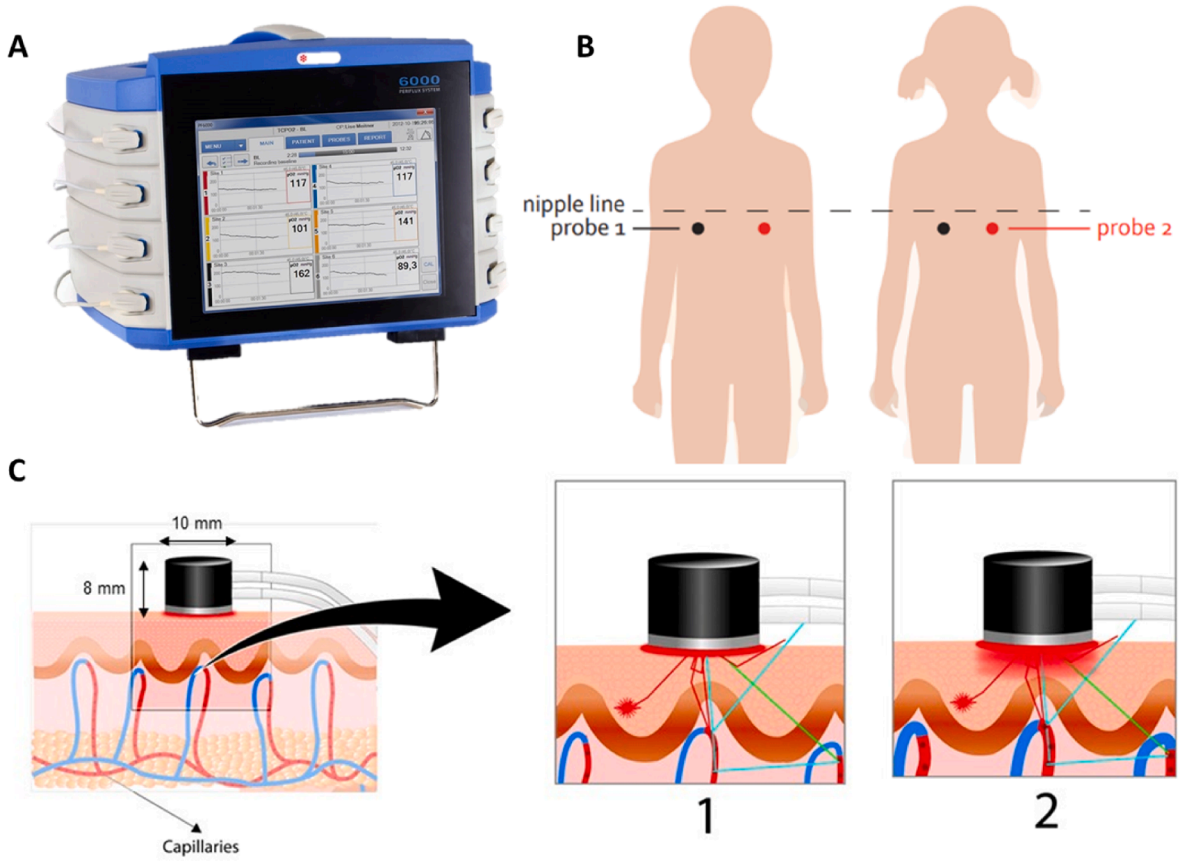

Fig. 1. Set-up of the cardiovascular assessment. (A) The main unit of the Periflux6000 (Perimed, Stockholm, Sweden) is operated by a touch screen and can be equipped with up to eight functions units such as the thermostatic small-angled probe used for blood perfusion measurements. (B) Placement of the two thermostatic laser Doppler probes to the child's chest, directly under the left and the right nipple, using double-sided tape. The probes were placed avoiding bony prominences, areas of edema, large superficial vessels, callused skin, and infected or inflamed areas. (C) The 457 Thermostatic Small-Angled Probe is a combined laser Doppler and thermostatic probe for simultaneous blood perfusion measurement and local heat provocation. Double-sided tapes are used for fixation. (1) A fiber-optic probe emits a laser light (780 nm), which is scattered and partly absorbed by the tissue being studied. Light hitting the moving red blood cells changes frequency (Doppler shift) while light hitting static objects is unchanged. The information is picked up by a returning fiber, converted into an electronic signal, and analyzed. (2) Rapid localized heating to 42 °C induces thermal hyperemia. The increase in blood perfusion as a response to local heating indicates tissue reserve capacity and endothelial function.

with the children sitting in an upright position. They were asked to move and talk as little as possible during the measurement and were allowed to watch television. Skin perfusion was measured non-invasively using two thermostatic laser-Doppler probes (PROBE 457 Thermostatic Small-Angled Probe; Perimed) (Fig. 1C), which were attached to the chest of the child using double-sided tape (PF 105–3 Double-Sided Tape Strips, Perimed, Stockholm, Sweden). Alcohol tissues were used to wipe the chest before applying the probes (Soft-Zellin, Hartmann, 70% alcohol).

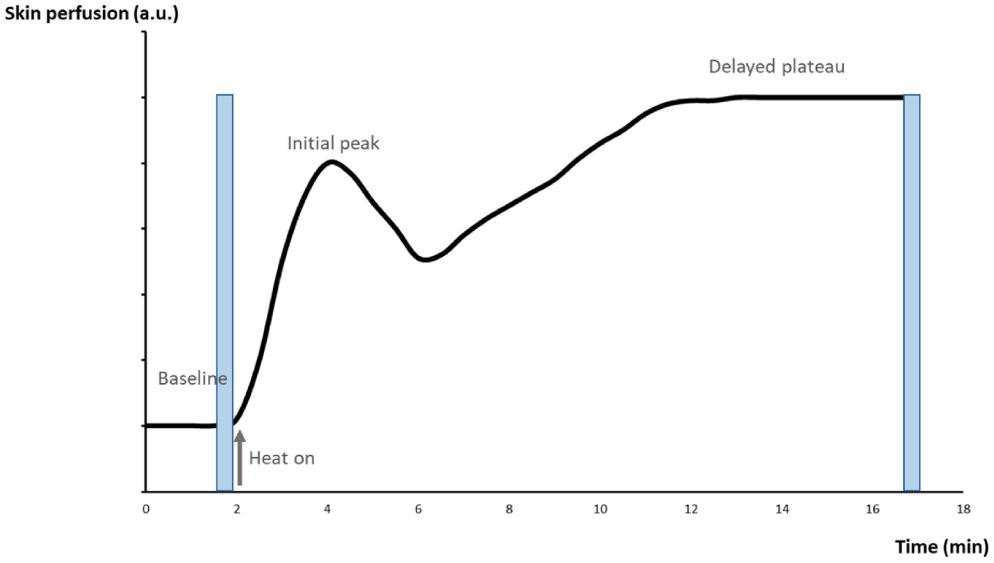

Fig. 2. Graphic representation of the skin perfusion assessment. Skin perfusion was first recorded unheated for 2 min to serve as a baseline. After the 2 min of baseline, the probes' temperature was rapidly and locally increased to 42 °C and was then kept constant until the end of the registration, *i.e.*, after 17 min. The initial rapid phase is predominantly mediated by local sensory nerves, while NO predominantly mediates the delayed plateau. The heat-induced skin hyperemic response was expressed as the average perfusion units increase during the approximately 15-minute heating phase over the average 2-minutes baseline perfusion units. Average perfusion units for the baseline and the heating phase were calculated during the last 30 s of that phase (indicated by the blue bars).

The probes were placed directly under the left and the right nipple (Fig. 1B) while avoiding bony prominences, areas of edema, large superficial vessels, callused skin, and infected or inflamed areas.

The laser-Doppler output was recorded for 17 min with a sample rate of 16 Hz, providing an assessment of skin blood flow expressed in arbitrary perfusion units (*i.e.,* the product of the velocity and concentration of moving red blood cells) (Braverman et al., 1992). As the microcirculation exhibits large variations, provocations are often used. It allows us to look at the response to a specific provocation rather than just a basal value of microcirculatory flow. First, skin perfusion was recorded for 2 min (unheated) to serve as a baseline. After 2 min of baseline, the probes' temperature was rapidly and locally increased to 42 °C, to reach maximal vasodilatation (Christen et al., 2004; Humeau-Heurtier et al., 2013; Minson, 2010). The temperature was then kept constant until the end of the recording (Fig. 2). The heat-induced skin hyperemic response was expressed as the average perfusion units increase during the 15 min heating phase over the average 2 min baseline perfusion units. Average perfusion units for the baseline and the heating phase were calculated during the last 30 sec of that phase (Fig. 2). Reports containing graphs and data on temperature and perfusion were controlled visually before inclusion in the study. Graphs of both the left and the right probe required to show a stable baseline, an initial peak in cutaneous blood flow after heating the probe, and a plateau phase following the initial peak (Fig. 2).

The children's blood pressure was measured with an automated oscillometric upper-arm blood- pressure monitor (Omron 705IT, Omron Corporation, Japan) using cuffs depending on the child's right upper arm circumference. Measurements were performed by a standardized method (Parati et al., 2008). After assessing the skin perfusion, five consecutive readings of the diastolic (DBP) and systolic (SBP) blood pressure of the right arm were obtained with 1 min intervals. Average DBP and SBP were based on the last three readings. Mean arterial pressure (MAP) was calculated through the equation: MAP = (2/3 × DBP) + (1/3 × SBP). MAP data based on the last three readings were available for 138 children.

2.4. Statistical analysis

For each individual, the mean heat-induced skin hyperemic response measured by the left and right probe was calculated and used as the dependent variable in the statistical model. Associations between pre- and postnatal BC, particulate air pollution, and NO_2 exposure and natural log-transformed skin perfusion were investigated using multiple regression models. In the main analyses, the effect of prenatal and postnatal exposure was assessed separately. To investigate the association of skin perfusion with prenatal or postnatal BC, PM_{10}, $PM_{2.5}$, and NO_2 exposure, we accounted for *a priori* selected variables including determinants of skin perfusion and variables with a potential link with skin perfusion and BC, PM_{10}, $PM_{2.5}$, and NO_2 exposure: year of skin perfusion assessment, date of delivery, age of the child at the moment of the house visit (months), sex of the child, child's birth weight (grams), parity, age of the mother at birth (years), maternal pre-pregnancy BMI, gestational age (weeks), gestational weight gain (kg), maternal smoking behavior, child ethnicity, and maternal education. Maternal smoking behavior was classified as never smoked, stopped smoking before pregnancy, and smoked during pregnancy. Maternal education level was coded as low (no high school diploma), middle (high school diploma), and high (college degree or higher). To assess whether the prenatal associations were driven by postnatal exposures, in a secondary analysis, we combined exposure during pregnancy and postnatal exposures during either the month before the house visit (short-term exposure) or the period between birth and the house visit (long-term exposure) in the same model. In a sensitivity analysis, we additionally corrected the main model and the model of the secondary analysis for MAP. In a second sensitivity analysis, we examined individual effects of pollutant combinations, pairing exposure to two different pollutants during the third trimester in the same main model. We created multipollutant models in which we paired the main prenatal model of $PM_{2.5}$ exposure with BC or NO_2 exposure during the third trimester. We also paired the main prenatal model of BC and NO_2 exposure with $PM_{2.5}$ exposure during the third trimester. The multipollutant models used the same basic structure as the single-pollutant models but with the inclusion of one more pollutant variable term. For all the analyses, pregnancy trimester-averaged BC, PM_{10}, $PM_{2.5}$, and NO_2, exposure levels were entered into the same model to estimate independent trimester-specific effects. Final estimates are presented as a percentage change (with 95% CI) in skin perfusion for each IQR increment in BC, PM_{10}, $PM_{2.5}$, and NO_2. Outliers were identified as differing three standard deviations from the mean. Statistical significance was set at $p < 0.05$. All analyses were performed with the statistical software R, version 3.5.1 (R Core Team (2018)).

3. Results

3.1. Study population characteristics

Table 1 displays the general characteristics of the study population (n = 139). At the time of birth, mothers had a mean (SD) age of 30.56 (3.83) years. They had a mean (SD) pre-pregnancy BMI of 23.55 (3.63) kg/m^2 and gained a mean (SD) of 13.86 (4.71) kg during their pregnancy. During pregnancy, 7.2% of the mothers smoked, 23% stopped smoking before pregnancy, and 69.8% never smoked. Most mothers (70.5%) were highly educated, while 4.3% of the mothers had a low education level. For 48.2% of the participating mothers, this was their first child, for 42.4% their second, and for 7.2% and 2.2% their third and fourth child, respectively. Of the children participating, 51.8% were boys. The children had a mean (SD) gestational age of 39.1 (1.5) weeks and a mean (SD) weight of 3406 (491) grams at birth. Most of them (94.2%) were Europeans of white ethnicity. At the time of the house visit, they had a mean (SD) age of 57.04 (6.41) months.

Table 1
Characteristics of the study population (n = 139).

Characteristic	Mean (SD)/Frequency (%)
Mother	
Age at birth child, years	30.6 (3.8)
Pre-pregnancy BMI, kg/m^2	23.6 (3.6)
Gestational age, weeks	39.1 (1.5)
Gestational weight gain, kg	13.9 (4.7)
Smoking behavior during pregnancy	
Never smoked	97 (69.8)
Stopped smoking before pregnancy	32 (23.0)
Smoked during pregnancy	10 (7.2)
Education level	
Low (no high school diploma)	6 (4.3)
Middle (high school diploma)	35 (25.2)
High (college degree or higher)	98 (70.5)
Parity	
1	67 (48.2)
2	59 (42.4)
3	10 (7.2)
4	3 (2.2)
Child	
Weight at birth, grams	3406 (491)
Age at time of house visit, months	57.04 (6.4)
Sex	
Male	72 (51.8)
Female	
Ethnicity[a]	
European	131 (94.2)
Non-European	8 (5.8)
MAP[b]	76.57 (11.44)

MAP mean arterial pressure

[a] Based on the native country of the newborn's grandparents. European when two or more grandparents were European, or non-European when at least three grandparents were of non-European origin.

[b] MAP data were available for 138 children.

3.2. Exposure characteristics

Table 2 summarizes the BC, NO_2, PM_{10}, and $PM_{2.5}$ exposure characteristics of the study population during different time windows of pregnancy and at different moments during childhood. For BC, PM_{10}, $PM_{2.5}$, and NO_2 mean exposures were comparable between the three trimesters.

3.3. Microvascular characteristics

The heat-induced skin hyperemic response measured with the left and right probes showed a moderate positive correlation (Spearman coefficient = 0.44, 95% CI 0.29 to 0.57, $p < 0.0001$). A Bland-Altman analysis for the heat-induced skin hyperemic response revealed a bias of 13.20 PU and a SD of 47.53 PU with upper and lower limits of agreement of −79.95 and 106.36 PU (Figure S1 Supplementary Material). Further analyses were performed using the mean perfusion measured by the left and right probe. An assessment of the effect of both probes separately can be found in Figure S2 in the Supplementary Material. In Table S1, a summary of the population's microvascular characteristic is given.

Table 2
Exposure details on BC, NO_2, PM_{10}, and $PM_{2.5}$ ($\mu g/m^3$) during different time windows of pregnancy and at different moments during childhood.

Type of air pollution exposure and time window		Mean (SD) ($\mu g/m^3$)	25th percentile ($\mu g/m^3$)	75th percentile ($\mu g/m^3$)	IQR ($\mu g/m^3$)
BC					
	Pregnancy				
	Trimester 1	1.43 (0.39)	1.14	1.71	0.57
	Trimester 2	1.43 (0.46)	1.12	1.66	0.55
	Trimester 3	1.38 (0.40)	1.05	1.62	0.58
	Childhood				
	Month[a]	0.73 (0.15)	0.62	0.85	0.23
	Lifetime[b]	1.12 (0.18)	1.02	1.21	0.20
NO_2					
	Pregnancy				
	Trimester 1	18.65 (5.70)	14.15	22.94	8.79
	Trimester 2	18.31 (5.48)	14.16	21.35	7.19
	Trimester 3	18.17 (5.58)	13.79	22.28	8.49
	Childhood				
	Month	13.02 (3.05)	11.03	15.32	4.30
	Lifetime	17.12 (3.31)	14.65	19.47	4.82
PM_{10}					
	Pregnancy				
	Trimester 1	19.04 (5.89)	14.52	22.58	8.06
	Trimester 2	18.89 (5.16)	14.91	21.93	7.01
	Trimester 3	18.72 (5.78)	14.44	22.78	8.34
	Childhood				
	Month	19.79 (5.51)	14.58	24.31	9.73
	Lifetime	17.58 (1.91)	16.46	18.77	2.30
$PM_{2.5}$					
	Pregnancy				
	Trimester 1	13.92 (5.45)	9.25	17.52	8.28
	Trimester 2	13.65 (4.93)	9.60	16.71	7.11
	Trimester 3	13.44 (5.37)	9.19	17.26	8.07
	Childhood				
	Month	11.12 (4.07)	7.07	14.51	7.43
	Lifetime	12.34 (0.96)	11.65	13.01	1.36

BC black carbon, IQR interquartile range, NO_2 nitric dioxide, PM_{10} particulate matter with a diameter smaller than 10 μm, $PM_{2.5}$ particulate matter with a diameter smaller than 2.5 μm, SD standard deviation

[a] Exposure calculated for the month before the house visit.

[b] Exposure calculated for the period between birth and the house visit.

3.4. Main analysis

Postnatal exposure, both short-term and long-term, to BC, PM_{10}, $PM_{2.5}$, or NO_2, was not associated with skin hyperemia at the age of four to six years (Table S2, Figure S3 in Supplementary Material).

In contrast, for prenatal exposure, we found an inverse association between skin hyperemia and prenatal residential air pollution exposure during the third trimester but not for the other two trimesters (Fig. 3). Therefore, for the further analysis, only the estimates for the third trimester are shown. For the mean perfusion measured by the left and the right probe (Fig. 3), an IQR increment in BC exposure during the third trimester of pregnancy was associated with an 11.5% (95% CI: −20.1 to −1.9; $p = 0.020$) lower skin hyperemia. A 12.7% (95 % CI: –22.2 to −1.9; $p = 0.023$) lower skin hyperemia was observed for an IQR increment in exposure to NO_2 during the third trimester. Similar effect estimates were retrieved for PM_{10} and $PM_{2.5}$: respectively a 13.9 % (95% CI: −21.9 to −3.0; $p = 0.003$) and 17.0 % (95% CI: −26.7 to −6.4; $p = 0.004$) lower skin hyperemia.

3.5. Secondary analysis

Adding postnatal exposure to our prenatal model did not alter the previously reported prenatal associations (Fig. 4, Table S3 Supplementary Material). When correcting for short-term exposure, an 11.9% (95% CI: −20.8 to −1.9; $p = 0.021$), 13.8% (95% CI: –22.0 to −4.7; $p = 0.004$), 17.4% (95% CI: −27.1 to −6.3; $p = 0.003$) and a 14.1 % (95% CI: –23.9 to −3.1; $p = 0.014$), lower skin hyperemia was found for an IQR increment in BC, PM_{10}, $PM_{2.5}$, and NO_2 exposure respectively during the third trimester. Correcting for long-term exposure did not mitigate the results (Fig. 4).

3.6. Sensitivity analysis

In a first sensitivity analysis, we additionally corrected the main model and the secondary model for MAP. Adding MAP did not change the outcome of the main model or the secondary model (Figure S4 in Supplementary Material).

In a second sensitivity analysis, we created multipollutant models in which $PM_{2.5}$ showed the strongest association (Figure S5 in Supplementary Material). It has to be taken into account that the risk estimates from the multipollutant models can be biased by the inclusion of the second pollutant variable that can be correlated with the first pollutant. For all the multipollutant models tested, the VIF varied between 1.3 and 4.1. A table of Pearson correlations between the various pollutants is presented in Table S4 in the Supplementary Material.

4. Discussion

To the best of our knowledge, this is the first study investigating the association between pre- and postnatal BC, particulate matter, and NO_2 air pollution exposure and heat-induced skin hyperemia as a dynamic marker of the microvasculature. We have evaluated this association by performing a cardiovascular assessment in four- to six-year-old children. The study's key finding is that skin hyperemia in these children is inversely associated with exposure to BC, PM_{10}, $PM_{2.5}$, and NO_2 during the third trimester of pregnancy. Postnatal exposures did not alter these associations either by long-term or short-term exposure windows.

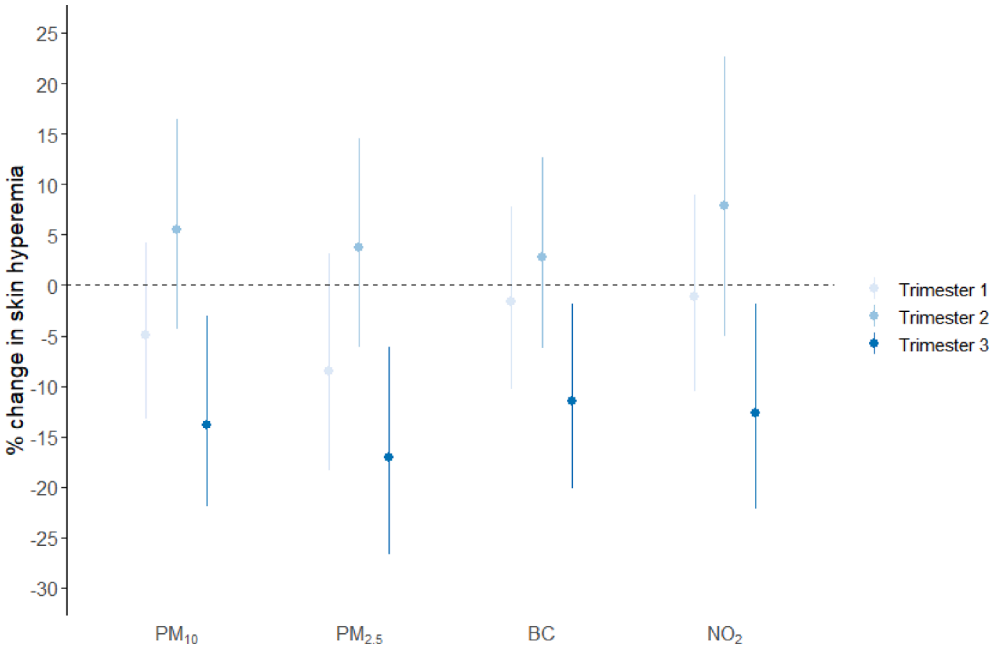

Fig. 3. Prenatal associations between skin hyperemia and the investigated pollutants. An inverse association was found between skin hyperemia and prenatal residential air pollution exposure during the third trimester but not for the other two trimesters. Estimates of each trimester are provided as a percentage change (95% CI) in skin hyperemia per IQR increment in BC, PM_{10}, $PM_{2.5}$, and NO_2, during the first, second or third trimester of pregnancy, estimated by multiple regression models. Prenatal models were mutually adjusted for the first, second and thirst trimester, and adjusted for the year of skin perfusion assessment, date of delivery, age of the child at the house visit, sex, child's birth weight, parity, age of the mother at birth, pre-pregnancy BMI, gestational age, gestational weight gain, maternal smoking, child ethnicity, and maternal education. Abbreviations: BC, black carbon; CI, confidence interval; NO_2, nitric dioxide; PM_{10}, particulate matter with an aerodynamic diameter of 10 μm or less; $PM_{2.5}$, particulate matter with an aerodynamic diameter of 2.5 μm or less.

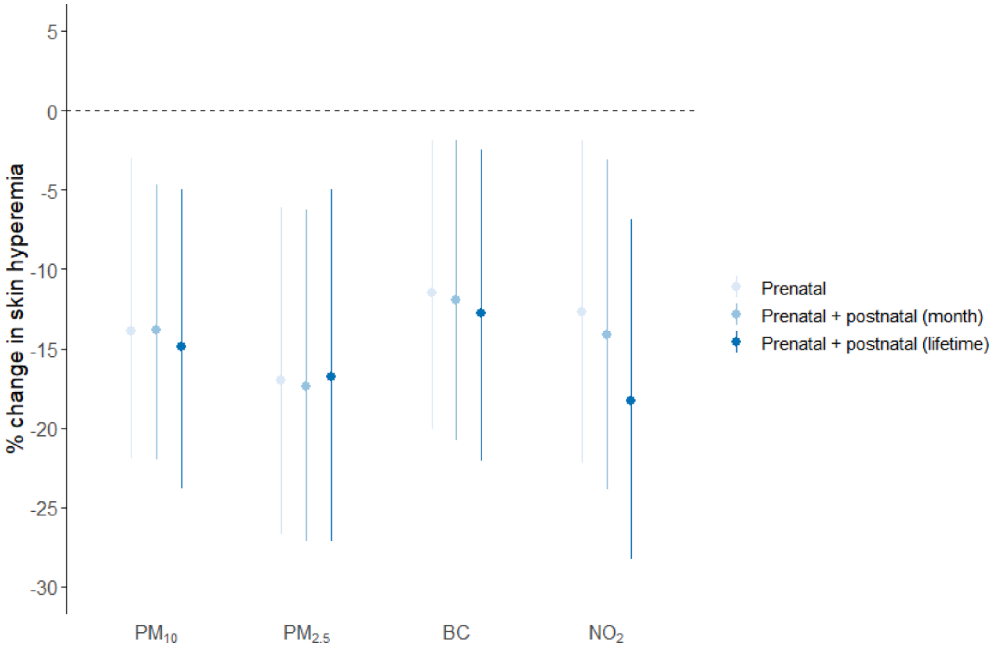

Fig. 4. Associations between skin hyperemia and the investigated pollutants. An inverse association between mean skin hyperemia, measured by two probes placed under the right and the left nipple, and PM_{10}, $PM_{2.5}$, BC, or NO_2 exposure during the third trimester of pregnancy was found. Estimates of prenatal exposure in the third trimester are given as percentage change (95% CI) for every IQR increase in PM_{10}, $PM_{2.5}$, BC, or NO_2. Prenatal models were mutually adjusted for the first, second and thirst trimester, and adjusted for the year of skin perfusion assessment, date of delivery, age of the child at the house visit, sex, child's birth weight, parity, age of the mother at birth, pre-pregnancy BMI, gestational age, gestational weight gain, maternal smoking, child ethnicity, and maternal education. Prenatal models were additionally adjusted for postnatal exposures during either the month before the house visit or for the period between birth and the house visit, *i.e.,* lifetime exposure. For the described models, n = 139. Abbreviations: BC, black carbon; CI, confidence interval; NO_2, nitric dioxide; PM_{10}, particulate matter with an aerodynamic diameter of 10 μm or less; $PM_{2.5}$, particulate matter with an aerodynamic diameter of 2.5 μm or less.

The knowledge of mechanisms underlying air pollution–mediated cardiovascular risks is still evolving, but endothelial dysfunction/disruption is one of the possible effector pathways (Rajagopalan et al., 2018). In the cardiovascular system, reactive oxygen species (ROS) play a pivotal role in controlling endothelial function and vascular tone. PM can cause toxicity by inducing the generation of ROS on their surface, leading to oxidative stress, which plays a crucial role in altering endothelial function (Silva et al., 2012). The endothelium, a mono-cellular layer lining the inner wall of vessels, plays an essential role in regulating vascular tone by releasing both vasorelaxing, *i.e.,* nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factors (EDHFs), and vasoconstrictor mediators, *i.e.,* endothelin and prostanoid. Endothelial dysfunction typically corresponds to a decrease of NO bioavailability and plays an essential role in the pathogenesis of a wide range of cardiovascular diseases (Anderson, 1999; Kellogg et al., 1999; Minson et al., 2001). Feto-neonatal endothelial damage may extend long after an insult in the perinatal period and may lead to limitations in microvascular vasodilatory reserve. To non-invasively determine endothelial function in the human skin microcirculation, the laser Doppler technique is an attractive tool to evaluate the cutaneous blood flow over time and its alterations following a given challenge, such as thermal provocation used in this study (Christen et al., 2004; Cracowski

et al., 2006; Humeau-Heurtier et al., 2013; Minson, 2010). The response to thermal provocation is characterized by an initial peak in skin blood perfusion, involving the sensory afferents, and a secondary rise and plateau mediated by NO release (Charkoudian, 2003; Kellogg, 2006; Minson, 2010) (Fig. 2). ROS production, induced by PM, can decrease NO bioavailability, possibly leading to a lower plateau phase resulting in lower skin hyperemia.

In our study, we found negative associations between skin hyperemia and prenatal exposure for all the pollutants we investigated, *i.e.,* BC, PM_{10}, $PM_{2.5}$, and NO_2. Our data demonstrate the importance of the susceptibility of the prenatal window. We did not observe any association with postnatal exposure, suggesting that the prenatal period is critical for adequately modelling small vessel structures. The Developmental Origins of Health and Disease or Barker hypothesis (Barker, 1999) states that fetal development is indeed a critical window of exposure-related susceptibility. Germ and fetal cells are particularly sensitive to external exposure events due to their faster rates of replication, faster differentiation, and higher sensitivity to surrounding signals compared with mature cells (Proietti et al., 2013). Any disruption in the efficiency of transplacental function *in utero* can negatively impact fetal growth and development, particularly during critical periods of organogenesis (Backes et al., 2013). The cardiovascular system is one of the first body systems to appear within the embryo. It is critical to the survival of the developing human that the circulatory system forms early, *i.e.,* in the first months of pregnancy, to supply the growing tissue with nutrients and gases, and to remove waste products (Clough and Norman, 2011; Johnson and Holbrook, 1989; Kiserud and Acharya, 2004). During fetal life, the microvasculature expands and remodels to increase capillary density and decrease diffusion distances. The retinal vascularization starts *in utero* at about 16 weeks and is completed at approximately 40 weeks of gestation (Sun and Smith, 2018). After birth, in early postnatal life, a further rapid enlargement and maturation of many organs and increase in tissue metabolic demands are observed, all of which are accompanied by an extensive growth and adaptation of the microvascular networks (Clough and Norman, 2011; Johnson and Holbrook, 1989; Kiserud and Acharya, 2004). There are thus a number of different "windows of opportunity'' during which the maternal, fetal, and infant environment may influence development of the offspring's microvasculature. That prenatal exposure to air pollutants affects the cardiovascular system is supported by the studies of Madhloum et al. (Madhloum et al., 2019) and Van Rossem et al. (van Rossem et al., 2015), which show that prenatal exposure to air pollutants during the third trimester affects the microvasculature as exemplified by elevated newborn blood pressure. We previously found that changes in the diameter of retinal venules are associated with prenatal exposure during the entire pregnancy (Luyten et al., 2020). This could possibly be attributed by the long developmental period of retinal vascularization, *i.e.*, 16–40 weeks. Our findings also support the hypothesis that subclinical microvascular changes are associated with air pollution exposures, even within the range of concentrations common in many developed countries. More than 80% of people living in urban areas are exposed to air quality levels that exceed WHO guideline limits (WHO | Ambient (outdoor) air pollution [WWW Document], 2018). The WHO and the European Union (EU) have stipulated limits on short- and long-term exposures to different air pollutants (European Environment Agency, 2019; WHO, 2006). The associations described in this research have been determined for a mean exposure of 13.44 $\mu g/m^3$ to $PM_{2.5}$ during the third trimester of pregnancy (Table 2), which is well below the yearly EU limit value for $PM_{2.5}$, which is set at 25 $\mu g/m^3$ (Table S5 in Supplementary Material), but above the WHO guideline value of 10 $\mu g/m^3$. For PM_{10} and NO_2, the mean exposures during the third trimester of pregnancy, 18.72 and 18.17 $\mu g/m^3$ respectively, are below the WHO annual guideline value, 20 and 40 $\mu g/m^3$. This corresponds to the statement that there exists no evidence for a safe level of exposure or a threshold below which no adverse health effects occur during susceptible periods of life.

Cardiovascular changes in early life may persist into later life and eventually lead to cardiovascular diseases. Our study provides insight into early life exposures in the childhood origin of adult health diseases using the DOHaD paradigm (Heindel and Vandenberg, 2015). Microvascular changes may represent an underlying mechanism through which exposure to $PM_{2.5}$ contributes to age-related disease development. In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort study (Adar et al., 2010), Adar et al. (2010) examined the association between exposure to air pollution and retinal arteriolar narrowing and venular widening as independent predictors for cardiovascular diseases. In their population of healthy adults (mean age of 64 years, $n = 4607$), narrower retinal arterioles and wider venules were observed with increased 2-year exposure to $PM_{2.5}$. The retinal arteriolar narrowing associated with chronic ambient exposure to $PM_{2.5}$ was equivalent to a 7-year increase in age. The same effects were observed in young children (Provost et al., 2017). In a panel of 221 healthy school children aged 8–12 years, Provost et al. revealed that short-term exposure to $PM_{2.5}$ was associated with narrower retinal arteriolar diameters and wider venular diameters. Luyten et al. (Luyten et al., 2020) measured the retinal microvascular diameters in young children (mean age of 4.6 years, $n = 245$) and suggests an opposite effect. They described a positive association between the diameter of retinal arterioles and prenatal exposure to $PM_{2.5}$ and NO_2. Both narrowing and widening of the retinal arterioles have been associated with detrimental health outcomes later in life (Jeganathan et al., 2009; Rhee et al., 2016; Tano et al., 2016; Triantafyllou et al., 2014), providing a clear indication that the diameter of retinal venules is affected by ambient air pollution exposure. Endothelial dysfunction and elevated oxidative stress are potential mechanisms explaining these associations.

Our study has several strengths. We used a novel method by which skin perfusion was assessed non-invasively using a stand-alone device equipped with a laser Doppler system. The advantage of this method is that it measures microvascular functioning instead of structure. In addition, the skin is an ideal site for the evaluation of microvascular dysfunction because it is easily accessible, stimuli can be applied with non– or minimally invasive approaches, and there is growing evidence that changes in skin vascular reactivity may precede overt clinical signs of disease (Minson, 2010). Our study should also be interpreted within the context of its potential limitations. Our study population is relatively small, which can limit the statistical power. Nevertheless, we observed statistically significant associations. Another limitation is the potential misclassification of exposure. Our results are based on daily residential PM exposure levels during prenatal and postnatal life but do not account for exposures other than residential. However, the accuracy of our exposure models and relevance for personal and internal exposures have been proven in our cohort since air pollution levels at the residential address showed that prenatal concentrations were associated with BC load in placenta (Bové et al., 2019) and cord blood and placental telomere length (Martens et al., 2017), and postnatal exposures with children's urinary BC load (Saenen et al., 2017). A third limitation is that the laser Doppler technique is very sensitive to movements (movements of the subjects, of the skin), which is a severe drawback when recordings are performed on people unable to remain still (children, patients with tremors, among others). However, children who moved or talked too much, see description of the study population in the Material and Methods section, were excluded from the analysis.

As functional changes in the microcirculation may be an early sign of microvascular dysfunction, measuring the cutaneous microcirculation has often been proposed as a model of generalized microvascular function. Our results show that the effects of ambient air pollution exposure on the microcirculation cannot be underestimated and should be further studied in terms of the effects of prenatal exposures on development later in life.

5. Conclusions

Endothelial dysfunction plays an essential role in the development and progression of cardiovascular diseases. This study opens new areas of investigation regarding thermal hyperemia as an integrative tool to assess microvascular function. Our findings support the hypothesis that BC, particulate matter, and NO_2 air pollution exposure during prenatal life can have long-term consequences on the microvasculature. This suggests a role for prenatal air pollution exposures in the microvascular origin of cardiovascular disease development later in life. Therefore, we believe that studies exploring the correlation between air pollution and the microcirculation of children can add knowledge to the complicated relationship between early-life exposure to ambient air pollution and cardiovascular disease development later in life.

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CRediT authorship contribution statement

Katrien Witters: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Project administration. **Yinthe Dockx:** Resources, Writing - review & editing. **Jos Op't Roodt:** Methodology, Writing - review & editing. **Wouter Lefebvre:** Software, Resources, Writing - review & editing. **Charlotte Vanpoucke:** Software, Resources, Writing - review & editing. **Michelle Plusquin:** Conceptualization, Methodology, Writing - review & editing. **Jaco Vangronsveld:** Conceptualization, Writing - review & editing. **Bram G. Janssen:** Conceptualization, Methodology, Software, Formal analysis, Writing - review & editing. **Tim S. Nawrot:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106799.

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