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Increased vascular stiffness in children exposed in utero but not children exposed postnatally to emissions from a coal mine fire

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Background: Chronic, low-intensity air pollution exposure has been consistently associated with increased atherosclerosis in adults. However, there was limited research regarding the implications of acute, high-intensity air pollution exposure during childhood. We aimed to determine whether there were any associations between early-life exposure to such an episode and early-life vascular function changes.

Methods: We conducted a prospective cohort study of children (<9 years old) who lived in the vicinity of the Hazelwood coal mine fire (n = 206). Vascular function was measured using noninvasive diagnostic methods including carotid intima-media thickness and pulse wave velocity (PWV). Exposure estimates were calculated from prognostic models and location diaries during the exposure period completed by each participant's parent. Linear mixed-effects models were used to determine whether there were any associations between exposure and changes in vascular outcomes at the 3- and 7-year follow-ups and over time.

Results: At the 7-year follow-up, each 10 μg/m^3 increase in daily $PM_{2.5}$ in utero was associated with increased PWV (β = 0.13 m/s; 95% confidence interval [CI] = 0.02, 0.24; P = 0.02). The association between in utero exposure to daily $PM_{2.5}$ was not altered by adjustment for covariates, body mass index, and maternal fire stress. Each 1 μg/m^3 increase in background $PM_{2.5}$ was associated with increased PWV (β = 0.68 m/s; 95% CI = 0.10, 1.26; P = 0.025), in children from the in utero exposure group. There was a trend toward smaller PWV (β = –0.17 m/s; 95% CI = –0.366, 0.02) from the 3- to 7-year follow-up clinic suggesting that the deficits observed previously in children exposed postnatally did not persist.

Conclusion: There was a moderate improvement in vascular stiffness of children exposed to $PM_{2.5}$ from a local coal mine fire in infancy. There was a mild increase in vascular stiffness in children exposed to $PM_{2.5}$ from a local coal mine fire while their mothers were pregnant.

Keywords: Air pollution; Atherosclerosis; Early childhood; Intima-media thickness; $PM_{2.5}$; Smoke; Vascular stiffness

Introduction

Globally, air pollution accounts for ~4.2 million deaths annually[1] and has been consistently associated with cardiovascular morbidity in adult populations.[2,3] Particulate matter (PM) is a major component of air pollution that contains a range of reactive compounds.[4,5] Fine PM with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) can penetrate to the peripheral regions of the lungs,[6] and ultrafine PM with an aerodynamic diameter of <200 nm can move across the endothelium into the circulation[7] and is strongly associated with cardiovascular morbidity and mortality.[2,3]

Cardiovascular disease is rare in childhood; however, hypertension, the underlying pathology of atherosclerosis, originates in early life.[8] There is clear evidence to suggest that an adverse intrauterine environment contributes to the early development of atherosclerosis, with a long latency period from exposure to cardiovascular diseases.[8–10] Early precursors of vascular changes – subclinical atherosclerosis – measured by noninvasive diagnostic methods such as carotid intima-media thickness (CIMT) and pulse wave velocity (PWV) are considered valid surrogate markers of cardiovascular risk allowing assessment of vascular changes at a very early stage.[11] CIMT has proven to provide reliable and reproducible results in pediatrics.[11] Given that cardiovascular complications occur predominantly in adulthood, investigation into the association between long-term exposure

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

E.J.H. was involved in conceptualization, data curation, formal analysis, methodology, writing – original draft, and writing – review & editing. A.B. and S.A. were involved in data curation, investigation, methodology, and writing – review

What this study adds:
A coal mine fire in the Hazelwood power station (Victoria, Australia) was an extreme air pollution episode that lasted for 6 weeks leading to community concerns regarding the potential health effects. We found evidence for an effect of in utero exposure to $PM_{2.5}$ from the local coal mine fire on vascular stiffness in children, 7 years after the mine fire. Deficits in vascular function that we had previously reported in postnatally exposed children appeared to have resolved.

to air pollution and atherosclerosis has focused on adults.[12–14] However, it is plausible that the underlying pathophysiology of cardiovascular disease and the health consequences observed in adulthood could be driven by PM exposure in childhood.[10] Of the studies that do exist they have focused on chronic, low-intensity air pollution from ambient sources[15] and there is limited research regarding the health consequences of an acute, high-intensity air pollution episode on childhood vascular health. In addition, it has been difficult to separate the ongoing effects of postnatal exposure to PM, from the in utero and early postnatal effects.

In 2014, embers from a landscape fire caused coal in the Hazelwood power station coal mine (Latrobe Valley, Victoria, Australia) to ignite and burn continuously for 45 days.[16] In Australia, the National Environmental Protection Measure (https://www.nepc.gov.au/nepms/ambient-air-quality) air quality guidelines for $PM_{2.5}$ is 25 µg/m^3 as a 24-hour average. Out of the 45 days the fire burned, modeling suggested the standard was exceeded on 23 days and reached a maximum $PM_{2.5}$ of 731 µg/m^3 in the town of Morwell.[16,17] The Early Life Follow-Up (ELF)[18] stream of the Hazelwood Health Study was established to assess the long-term health consequences of exposure to emissions from the fire in early life. We have previously shown that children exposed postnatally to emissions from this fire had mild vascular stiffness, 3 years after the fire.[19] However, it remained unclear whether these effects persisted.

The aim of this study was to investigate the associations between acute, early-life exposure to $PM_{2.5}$ emitted from a coal mine fire and vascular changes 3 to 7 years after the fire. The ELF study also provided the opportunity to investigate the impacts of exposure to background air pollution and vascular changes over time.

Methods

Participants

The study participants originated from a clinical testing subsample of the longitudinal ELF study (n = 571). The ELF cohort lived in the vicinity of a local coal mine fire in the Latrobe Valley in Eastern Victoria, Australia. This cohort included children who were <2 years of age during the coal mine fire that occurred in February–March of 2014. The ELF cohort includes three main exposure classifications, children who were infants at the time of the fire and exposed postnatally (date of birth [DOB]: March 1, 2012–March 31, 2014), children of mothers who were pregnant and exposed in utero (DOB: April 1–December 31, 2014), and children who were conceived after all the smoke from the mine fire had cleared and considered unexposed (DOB: January 1–December 31, 2015). A detailed cohort profile was published elsewhere.[18] In summary, in this analysis, children born between March 1, 2012 and March 31, 2014 in the Latrobe Valley (exposed postnatally or in utero) were recruited between February and September 2016 (Figure 1). Participating parents/caregivers completed a baseline survey with multiple choice responses for sociodemographic, health, and family information. The baseline survey also included a location diary for each participant for every 12 hours to track participant movement throughout the fire period. Participants were invited to participate in clinical follow-up studies, 3 and 7 years after the coal mine fire (Figure 1).

All studies were approved by the Tasmanian Health and Medical Human Research Ethics Committee (reference H14875). Additional approval was received from the Human Research Ethics Committees of Monash University, Monash Health, and the University of Melbourne. Parents or caregivers of the participants studied provided signed informed consent for both the baseline survey and clinical testing.

Exposure estimate

Air quality monitoring started 4 days after the fire and was conducted at multiple locations for varying time periods throughout the Latrobe Valley. Air quality was monitored by ambient air quality, and PM monitoring and roving PM monitoring systems were used to assess the impact of smoke across the local region. These emissions were used in a prognostic meteorological, dispersion, and chemical transport model with local wind data assimilation.[16,20,21] The model estimated $PM_{2.5}$ emission rates at hourly time intervals and a 1 × 1 km spatial resolution based on the area of coal burned and other emission factors.[16] Individual exposure estimates were then calculated for each 24-hour period, based on the location diaries completed by the parents for every 12 hours in the baseline survey. Modeled background ambient $PM_{2.5}$ was subtracted from these estimates to provide daily average mean and maximum mine fire-related $PM_{2.5}$ exposure estimates. Background $PM_{2.5}$ exposure estimates were calculated by averaging exposure from conception to the end of follow-up in 2015, based on the smallest Australian Statistical Geography Standard unit at birth. A detailed methodology relating to coal mine fire, exposure models, and residences was published elsewhere.[16]

Vascular function

Vascular function was evaluated using two noninvasive tests for early markers of vessel function: (1) PWV by arterial tonometry and (2) CIMT by ultrasound. Both of these techniques require minimal cooperation from the participant and can be performed on young children resulting in high feasibility in the clinical setting as demonstrated by a study conducted in the ELF cohort.[22]

CIMT assessments were conducted in a darkened, air-conditioned room using a commercially available ultrasound system with a 12 MHz linear array transducer (Vivid q, GE Healthcare, Parramatta, New South Wales) following published guidelines.[11,23] A 3-lead echocardiograph was used to monitor the left and right carotid arteries separately.[19] The participant was lying quietly in the supine position during the measurement. For data acquisition, the probe was held perpendicular to the artery while 10–30 mm of the distal carotid artery was

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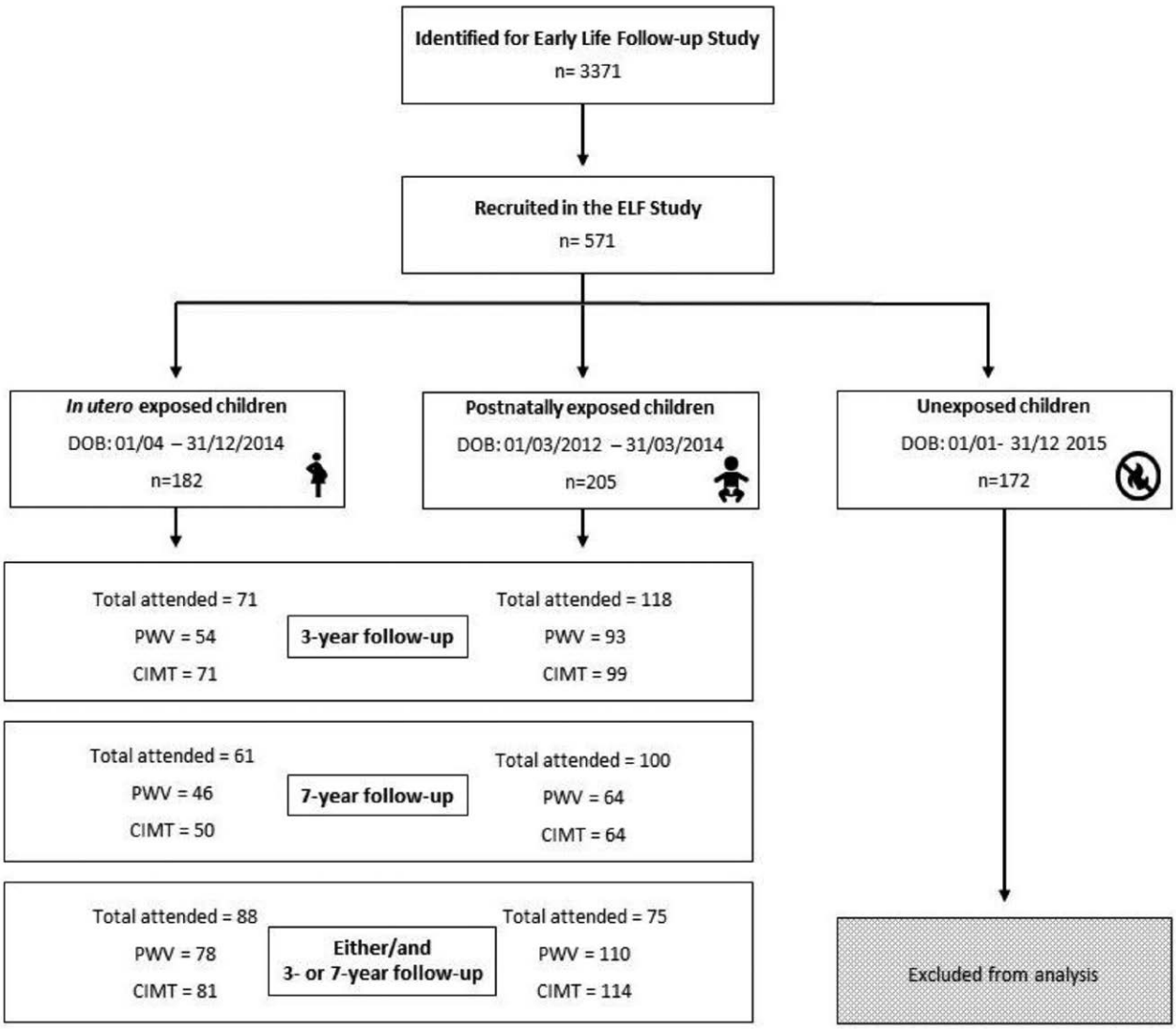

Figure 1. Flowchart of participants in the study. Outcomes: CIMT (mm) and PWV (m/s).

scanned.[19] Three longitudinal views were obtained for each side with at least three clean R-R intervals on the electrocardiogram, and the carotid bifurcation as a landmark on the left side of the screen.[19] A 10-mm region of the common carotid artery below the carotid bifurcation at end-diastole was used to calculate the far wall and near wall measurements.[19] We reported the CIMT from averaging the far wall and near wall of the two common carotid arteries.[19] If data from one side was missing then average CIMT was not reported.

To measure PWV, a pediatric-sized femoral cuff was inflated at the right femoral artery and attached to a commercially available device (SphygmoCor XCEL, AtCor Medical, NSW, Australia).[19] The PWV distance is the sum of the distance between the right carotid artery and the sternal notch, and the sternal notch to the edge of the femoral cuff.[19] A tonometer was placed over the right carotid artery.[19] The device then detected the pulse from the tonometer and the femoral pulse from the cuff and used the PWV distance to calculate the velocity.[19]

Statistical analysis

As a cross-sectional relationship between postnatal exposure to $PM_{2.5}$ from the coal mine fire and vascular stiffness at the 3-year follow-up has been previously reported,[19] longitudinal models were employed in this study. We fitted linear mixed-effects regression models with random intercepts to evaluate changes in vascular function (PWV, CIMT) over time attributable to mine fire exposure by including interactions between mine fire exposure and follow-up time point. To facilitate interpretation, we also reported the estimated exposure effects to mine fire $PM_{2.5}$ in both follow-up rounds from this model. The exposure effect at the 3-year follow-up was estimated directly from the linear mixed-effect regression model as the coefficient for the exposure variable. The exposure effect at the 7-year follow-up was estimated using the linear combination of the coefficient of the exposure variable and the coefficient of the interaction between the exposure variable and the follow-up time point variable. The exposure effect of change over time was estimated using the coefficient of the interaction between the exposure variable and the time point variable (Figure 2).

To ensure the robustness of the findings, we estimated the exposure effects in two sets of models: (1) base models, which only included the exposure variable and the time point variable and the interaction between them, and (2) covariance-adjusted models, which also included a body mass index and maternal fire stress. These covariates were selected based on a data and theory-driven approach from a range of a priori covariates based on previous work including birthweight, gestational age at birth, breastfeeding duration, maternal education, smoking during pregnancy, alcohol consumption during pregnancy,

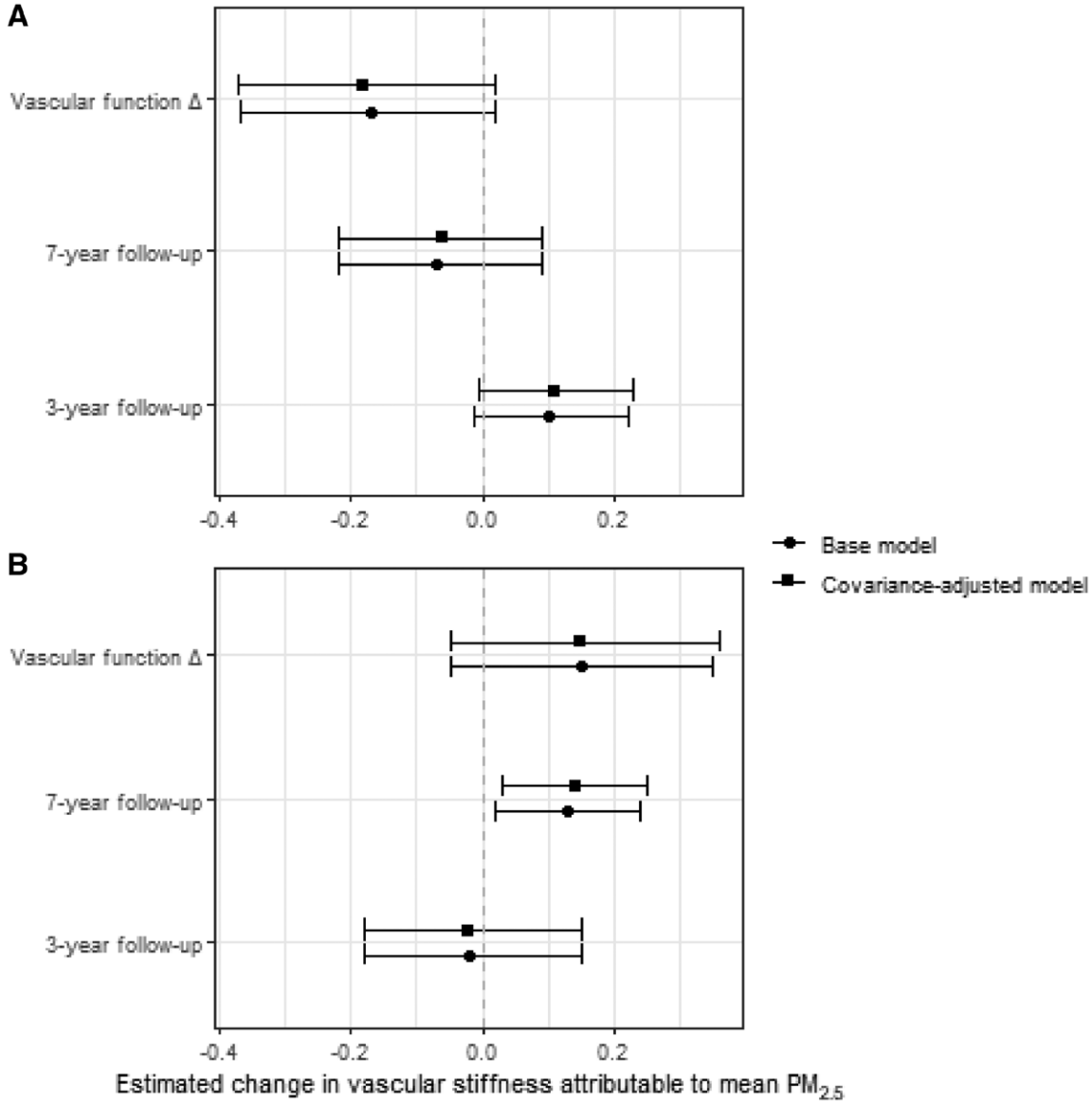

Figure 2. Coefficient plot with mixed-effect linear regression analysis attributable to mean daily $PM_{2.5}$. Exploring the relationship between vascular stiffness of children exposed to mine fire smoke over time. Estimates from (A) refer to children exposed postnatally to mine fire-related $PM_{2.5}$ and (B) refer to children with in utero exposure to mine fire-related $PM_{2.5}$.

overall stress, fire stress, maternal asthma, exposure to second-hand smoke, cold or flu or medication usage in the last 24 hours, and index of relative socioeconomic disadvantage. The exposure estimate was investigated as mean daily $PM_{2.5}$ in increments of 10 μg/m^3, maximum daily $PM_{2.5}$ in increments of 100 μg/m^3, and background $PM_{2.5}$ in 1 μg/m^3.

However, due to the limited sample size, the inclusion of all of these covariates resulted in overfitting. To address this challenge, stepwise regression models (using R package olsrr version 0.5.3) were used to select covariates that were 80% retained to be included in the covariance-adjusted models to develop the final multivariate regression model. Multiple imputation by chained equations with predicted mean matching was used to address missing data in outcome variables and covariates. A total of 20 imputed datasets were used in stepwise variable selection as well as final analysis. Covariates were selected if they were retained in 80% of stepwise models across imputed datasets and pooled with Rubin's rule to calculate estimated regression coefficients in the linear regression models.[24]

R Studio Version 4.1.3 (Boston, Massachusetts) was used for the statistical analysis (Table S2 and supplementary information; http://links.lww.com/EE/A273). Summary data are reported as means (SD) or median with ranges. β coefficients, 95% confidence intervals (95% CIs), and P values are reported for all regression analyses.

Results

In utero exposure to $PM_{2.5}$

Participant characteristics

Of the 206 children who attended clinical testing, 88 children were exposed in utero to fire-related $PM_{2.5}$. Of those children exposed in utero, 71 attended the 3-year follow-up and 61 the 7-year follow-up (Table 1 and Figure 1). At the 3-year follow-up, PWV had a mean of 4.1 m/s ± 0.45 and CIMT 0.49 nm ± 0.03. At the 7-year follow-up, PWV had a mean of 3.0 m/s ± 0.56 and CIMT 0.42 nm ± 0.03.

There was a reasonable gender balance of children with 48% female, while 30% of the children were overweight or obese (Table 1). The subsample of children who attended clinical testing had some characteristics that differed from the remainder

Table 1.

Participant characteristics and covariates

	Children exposed in utero to fire-related $PM_{2.5}$			Children exposed postnatally to fire-related $PM_{2.5}$		
	3-year follow-up (n = 71)	7-year follow-up (n = 61)		3-year follow-up (n = 100)	7-year follow-up (n = 75)	
Participant characteristics	**Mean ± SD (range)**	**Mean ± SD (range)**	***P* value**	**Mean ± SD (range)**	**Mean ± SD (range)**	***P* value**
Age (years)	2.9 ± 0.3 (2.4–3.7)	6.8 ± 0.3 (6.3–7.3)	<0.001	4.3 ± 0.6 (2.5–5.6)	8.1 ± 0.6 (6.9–9.3)	<0.001
Height (cm)	93.9 ± 4.2 (85–109)	122.8 ± 5.0 (110–134)	<0.001	105.9 ± 6.6 (88–129)	130.3 ± 6.4 (114–146)	<0.001
Weight (kg)	14.9 ± 1.5 (11.2–17.8)	25.6 ± 5.8 (19.2–44.7)	<0.001	19.3 ± 4.7 (12.9–48.5)	31.0 ± 9.4 (18.9–74.0)	<0.001
IRSD decile	3.6 ± 2.8 (1.0–10.0)	3.5 ± 2.7 (1.0–9.0)	0.87	3.5 ± 3.0 (1.0–10.0)	3.4 ± 3.0 (1.0–10.0)	0.84
Birth weight (kg)	3.5 ± 0.5 (2.0–4.5)	3.4 ± 0.5 (2.0–4.5)	0.53	3.4 ± 0.6 (1.4–5.3)	3.5 ± 0.6 (2.1–5.7)	0.66
Gestational age (weeks)	39.4 ± 1.7 (35.0–42.0)	39.4 ± 1.8 (35.0–42.0)	0.88	39.5 ± 1.8 (33.0–43.0)	39.8 ± 1.4 (36.0–43.0)	0.42
BMI for age	16.8 ± 1.1	16.9 ± 2.9	0.89	17.0 ± 2.4	18.2 ± 5.5	0.058
Underweight (<5th)	0 (0%)	0 (0%)		1 (1%)	1 (1%)	
Normal (5–85th)	50 (70%)	44 (72%)		59 (59%)	45 (60%)	
Overweight (≥85th)	21 (30%)	17 (28%)		40 (40%)	29 (39%)	
Participant covariates	n (%)	n (%)		n (%)	n (%)	
Gender: female	34 (48%)	27 (44%)	0.73	52 (52%)	36 (48%)	0.76
Breastfeeding: <3 months	17 (24%)	12 (20%)	0.52	65 (65%)	53 (71%)	0.32
Maternal education: >year 12	49 (69%)	47 (77%)	0.52	58 (58%)	45 (60%)	0.62
Maternal smoking during pregnancy	8 (11%)	4 (7%)	0.38	17 (17%)	8 (11%)	0.28
Maternal alcohol consumption during pregnancy	1 (1%)	1 (1%)	0.99	1 (1%)	2 (2%)	0.89
Maternal overall stress: mostly stressed	46 (65%)	38 (62%)	0.71	64 (64%)	48 (64%)	0.99
Maternal fire stress: increased a lot	59 (83%)	49 (80%)	0.81	74 (74%)	57 (76%)	0.72
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Background $PM_{2.5}$ ($\mu g/m^3$)	5.08 (4.9–5.2)	5.09 (4.9–5.2)	0.40	5.3 (5.0–5.3)	5.2 (5.0–5.3)	0.17
Mean daily $PM_{2.5}$ ($\mu g/m^3$)	3.8 (2.3–9.2)	3.9 (2.5–13.6)	0.27	2.75 (1.9–11.5)	3.2 (2.0–11.5)	0.82
Maximum daily $PM_{2.5}$ ($\mu g/m^3$)	75.7 (40.6–156.1)	81.7 (35.7–188.6)	0.32	61.8 (22.4–131.5)	72.7 (29.6–149.8)	0.50

IRSD indicates index of relative social disadvantage.

Table 2.

Mixed-effect model for linear regression analysis, reported as base models and covariance-adjusted models

	3-year follow-up		7-year follow-up		Vascular Δ over time[a]	
Vascular	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**
Mean daily $PM_{2.5}$ (per 10 μg/m³)						
Base model						
PWV	−0.02 (−0.18, 0.15)	0.82	0.13 (0.02, 0.24)	0.02	0.15 (−0.05, 0.35)	0.15
CIMT	0.002 (−0.01, 0.01)	0.64	−0.003 (−0.01, 0.009)	0.59	−0.006(−0.02, 0.01)	0.45
Covariance-adjusted model						
PWV	−0.02 (−0.18, 0.15)	0.84	0.14 (0.03, 0.25)	0.016	0.15 (−0.05, 0.36)	0.14
CIMT	0.003 (−0.01, 0.01)	0.60	−0.005 (−0.02, 0.007)	0.61	−0.006 (−0.02, 0.01)	0.43
Maximum daily $PM_{2.5}$ (per 100 μg/m³)						
Base model						
PWV	0.02 (−0.10, 0.14)	0.75	0.02 (−0.06, 0.09)	0.67	−0.003 (−0.14, 0.14)	0.96
CIMT	0.002 (−0.003, 0.01)	0.44	−0.003(−0.01, 0.003)	0.28	−0.006 (−0.01, 0.002)	0.15
Covariance-adjusted model						
PWV	0.021 (−0.10, 0.14)	0.73	0.02 (−0.06, 0.10)	0.59	0.001 (−0.14, 0.14)	0.99
CIMT	0.002 (−0.003, 0.01)	0.42	−0.003 (−0.01, 0.003)	0.30	−0.006 (−0.01, 0.002)	0.15

Exploring the relationship between vascular health of children exposed in utero to mine fire smoke over time. Regression models included imputed data and covariates identified using stepwise regression. Outcomes: CIMT (mm) and PWV (m/s). Delta (Δ) denotes change. Body mass index (BMI) and maternal fire stress (< or > due to fire) added to covariance-adjusted models investigating the relationships between vascular health and $PM_{2.5}$. Significant relationships are bold.

[a]The interaction between exposure and follow-up rounds.

of the cohort. This subsample had a relatively high mother's educational level (72% of mothers reporting education beyond year 12 among those who attended clinic compared with 48% among those that did not; Table S1; http://links.lww.com/EE/A273). Mean and maximum daily mine fire-related $PM_{2.5}$ exposure for the 88 children in this analysis had a median (interquartile range [IQR]) of 3.8 (2.3–10.4) μg/m³ and 78.7 (41.1–177.4) μg/m³, respectively (Table S1; http://links.lww.com/EE/A273). Background $PM_{2.5}$ had a median (IQR) of 5.1 (4.9–5.2) μg/m³ (Table S1; http://links.lww.com/EE/A273).

$PM_{2.5}$ and vascular outcomes

There were no associations between in utero exposure to $PM_{2.5}$ and vascular outcomes (Table 2), at the 3-year follow-up. Interestingly, at the 7-year follow-up, each 10 μg/m³ increase of in utero exposure to mean daily $PM_{2.5}$ was associated with a 0.13 m/s increase in PWV (95% CI = 0.02, 0.24; P = 0.02; Table 2), due to a positive interaction effect between exposure and follow-up time (vascular Δ over time: β = 0.15 m/s; 95% CI = −0.05, 0.35; P = 0.15; Table 2). This indicates a trend toward increased vascular stiffness over time, suggesting that there may be a longer latency period before subclinical changes appear.

Background $PM_{2.5}$ and vascular changes

There were no associations between background $PM_{2.5}$ and vascular outcomes, at the 3-year or 7-year follow-up clinics. However, from the 3-year to the 7-year follow-up clinic, there was some evidence to suggest that stiffer vessels, indicated by PWV, were due to background $PM_{2.5}$ (β = 0.68 m/s; 95% CI = 0.10, 1.26; P = 0.025; Table 4). These associations were not altered by adjustment for covariates (Table 4) and there was minimal effect on the precision of the estimates.

Postnatal exposure to $PM_{2.5}$

Participant characteristics

Of the 206 children who attended clinical testing, 118 children were exposed postnatally to fire-related $PM_{2.5}$, 100 attended the 3-year follow-up clinic, and 75 attended the 7-year follow-up clinic (Table 1 and Figure 1). At the 3-year follow-up, PWV had a mean of 4.1 m/s ± 0.47 and CIMT 0.5 nm ± 0.04. At the 7-year follow-up, PWV had a mean of 3.0 m/s ± 0.63 and CIMT 0.43 nm ± 0.07.

There was a reasonable gender balance of children with 49% female, while 40% of the children at the 3-year follow-up were overweight or obese (Table 1). The subsample of children who attended clinical testing had some characteristics that differed from the remainder of the cohort including slightly higher gestational age (39.6 weeks vs. 39 weeks, Table S1; http://links.lww.com/EE/A273), fewer children being breastfed for less than 3 months (15% vs. 23%, Table S1; http://links.lww.com/EE/A273), and lower rates of maternal smoking (15% vs. 23%, Table S1; http://links.lww.com/EE/A273). Mean and maximum daily mine fire-related $PM_{2.5}$ exposure for the 88 children in this analysis had a median (IQR) of 2.8 (1.9–11.9) μg/m³ and 62.9 (22.8–137.5) μg/m³, respectively (Table S1; http://links.lww.com/EE/A273). Background $PM_{2.5}$ had a median (IQR) of 5.2 (5.0–5.3) μg/m³ (Table S1; http://links.lww.com/EE/A273).

$PM_{2.5}$ and vascular outcomes

There was a trend toward stiffer vessels, indicated by PWV, at the 3-year follow-up in the covariance-adjusted model (Table 3); however, the relationship was not statistically significant (β = 0.11 m/s; 95% CI = −0.005,0.23; P = 0.062) and was attenuated at the 7-year follow-up (β = −0.06 m/s; 95% CI = −0.22, 0.09; P = 0.43) due to a negative interaction effect (β = −0.18 m/s; 95% CI = −0.37, 0.02; P = 0.079). These results align with previously published work in this cohort at this timepoint.[19] There were no statistically significant relationships between mean or maximum daily $PM_{2.5}$ and CIMT, at the two follow-up time points. These associations were not altered by adjustment for covariates and there was minimal effect on the precision of the estimates.

Background $PM_{2.5}$ and vascular changes

There were no associations between exposure to background $PM_{2.5}$ and vascular outcomes (P > 0.10; Table 4).

Discussion

This study evaluated the long-term effects of both postnatal and in utero exposures to fire-related $PM_{2.5}$ on vascular outcomes. Children exposed in utero to fire-related $PM_{2.5}$ had increased

Table 3.

Mixed-effect model for linear regression analysis, reported as base models and covariance-adjusted models

	3-year follow-up		7-year follow-up		Vascular Δ over time[a]	
Vascular	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**
Mean daily $PM_{2.5}$ (per 10 μg/m^3)						
Base model						
PWV	0.10 (−0.013, 0.22)	0.083	−0.07 (−0.22, 0.09)	0.38	−0.17 (−0.366, 0.02)	0.084
CIMT	−0.002 (−0.01, 0.01)	0.68	−0.008 (−0.02,0.01)	0.32	−0.006 (−0.03, 0.01)	0.53
Covariance-adjusted model						
PWV	0.11 (−0.005, 0.23)	0.062	−0.06 (−0.22, 0.09)	0.43	−0.18 (−0.370, 0.02)	0.079
CIMT	−0.001 (−0.01, 0.01)	0.84	−0.008 (−0.02, 0.008)	0.34	−0.007 (−0.03, 0.01)	0.48
Maximum daily $PM_{2.5}$ (per 100 μg/m^3)						
Base model						
PWV	0.03 (−0.07, 0.12)	0.60	0.01 (−0.11, 0.13)	0.82	−0.01 (−0.16, 0.14)	0.88
CIMT	−0.001 (−0.01, 0.01)	0.78	0.007 (−0.005, 0.02)	0.39	0.008 (−0.01, 0.02)	0.28
Covariance-adjusted model						
PWV	0.03 (−0.07, 0.12)	0.57	0.02 (−0.10, 0.14)	0.79	−0.01 (−0.16, 0.14)	0.88
CIMT	0.0002 (−0.01, 0.01)	0.96	0.008 (−0.004, 0.02)	0.24	0.008 (−0.01, 0.02)	0.30

Exploring the relationship between vascular health of children exposed postnatally to mine fire smoke over time. Regression models included imputed data and covariates identified using stepwise regression. Outcomes: CIMT (mm) and PWV (m/s). Delta (Δ) denotes change. Body mass index (BMI) and maternal fire stress (< or > due to fire) added to covariance-adjusted models investigating the relationships between vascular health and $PM_{2.5}$.

[a]The interaction between exposure and follow-up rounds.

vascular stiffness at the 7-year but not at the 3-year follow-up. There was also a trend toward increased vascular stiffness over time in this group suggesting that there may be a longer latency period for children exposed in utero before subclinical changes appear. Similarly, children from the in utero exposure group also had increased vascular stiffness over time attributable to background $PM_{2.5}$ exposure. However, children exposed postnatally to fire-related $PM_{2.5}$ had increased vascular stiffness at the 3-year follow-up but this effect disappeared by the 7-year follow-up. There was a trend toward less vascular stiffness over time in this group suggesting that the mild effects demonstrated early on did not persist over time. Similarly, children from the postnatally exposed group did not have any associations between vascular outcomes and background $PM_{2.5}$ exposure.

There are a number of possible explanations for the longer latency period for in utero exposed children and vascular changes. First, $PM_{2.5}$ has been shown to provoke an inflammatory response and increase cytokine release from the pulmonary vascular bed, leading to altered vasomotor tone and lipid peroxidation.[25,26] This in turn increases the release of prooxidant and proinflammatory mediators that play key roles in the pathogenesis of atherosclerosis.[25,26] Similarly, a study conducted by McGuinn et al[27] found associations between in utero exposure to $PM_{2.5}$ and increased blood lipid levels in children 4–6 years of age. Therefore, this process may be occurring for a period of time before we are able to quantify vascular changes in the clinical setting.

Second, changes in microvascular blood flow associated with $PM_{2.5}$ have been observed in pediatric clinical studies.[28,29] A prospective cohort study conducted in Belgium found an inverse relationship between $PM_{2.5}$ exposure in the third trimester and skin microvascular blood flow, skin hyperemia, in 4- to 6-year-old children.[28] Similarly, the study found similar relationships between $PM_{2.5}$ and other markers of microvasculature function including retinal arteriolar diameter in 4- to 6-year-old children.[29] Although we did not observe any changes by the 3-year follow-up, we were able to detect a clear association between in utero exposure to PM and vascular stiffness by the 7-year follow-up. By the 7-year follow-up, the children were 6–7 years of age, which is a similar age range to the studies discussed. The

Table 4.

Mixed-effect model for linear regression analysis, reported as base models and covariance-adjusted models

	3-year follow-up		7-year follow-up		Vascular Δ over time[a]	
Vascular	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**
Children exposed in utero to PM2.5						
Base model						
PWV	−0.35 (−0.80, 0.10)	0.126	0.33 (−0.04, 0.70)	0.085	0.68 (0.10, 1.26)	0.025
CIMT	0.004 (−0.02, 0.03)	0.76	−0.10 (−0.04, 0.02)	0.45	−0.01 (−0.05, 0.02)	0.44
Covariance-adjusted model						
PWV	−0.37 (−0.83, 0.09)	0.123	0.32 (−0.05, 0.71)	0.094	0.69 (0.09, 1.29)	0.027
CIMT	0.003 (−0.03, 0.03)	0.82	−0.011(−0.04, 0.02)	0.42	−0.01 (−0.05, 0.02)	0.44
Children exposed postnatally to $PM_{2.5}$						
Base model						
PWV	−0.02 (−0.46, 0.42)	0.93	−0.50 (−1.11, 0.09)	0.10	−0.48 (−1.22, 0.26)	0.20
CIMT	0.008 (−0.03, 0.05)	0.67	−0.001 (−0.03, 0.03)	0.96	−0.008 (−0.06, 0.04)	0.76
Covariance-adjusted model						
PWV	−0.02 (−0.47, 0.43)	0.93	−0.49 (−1.10, 0.12)	0.12	−0.47 (−1.21, 0.28)	0.22
CIMT	0.01 (−0.03, 0.05)	0.59	−0.001 (−0.03, 0.03)	0.93	−0.01 (−0.06, 0.04)	0.69

Exploring the relationship between vascular health of children exposed to background $PM_{2.5}$ over time. Regression models included imputed data and covariates identified using stepwise regression. Outcomes: CIMT (mm) and PWV (m/s). Delta (Δ) denotes change. Body mass index (BMI) and maternal fire stress (< or > due to fire) added to covariance-adjusted models investigating the relationships between vascular health and $PM_{2.5}$. Significant relationships are bold. Significant relationships are bold.

[a]The interaction between exposure and follow-up rounds.

changes in vascular function may have been too subtle to detect before this time point.

Our study also found that the deficits previously observed in postnatally exposed children appear to have resolved over time. There are a number of possible explanations, the strongest being disturbances in the early environment, including postnatal stressors, result in catch-up growth.[30] Vascular development is controlled by growth and shear stress produced by blood flow on the vessel wall leading to changes in the microvasculature or endothelial dysfunction.[30] Postnatal stressors such as environmental air pollution increase changes in endothelial function and oxidative stress.[31] However, there is limited research evaluating the health consequences of acute air pollution exposure during different windows of childhood. A retrospective cohort study in Australia found a relationship between ambient $PM_{2.5}$ and increased CIMT in 11- to 12-year-old children.[15] Interestingly, a study by Poursafa et al[32] found associations between $PM_{2.5}$ exposure and increased blood lipid levels in children 8–12 years of age. Life-time exposure to $PM_{2.5}$ may drive the alterations to vascular health observed in epidemiological studies.[15] Clearly, further work is required to understand the health consequences of air pollution exposure at different stages of childhood development.

This study includes a number of strengths such as the accurate exposure classification given the clear time interval of the exposure event. Exposure estimates were calculated for each individual child based on exposure models and the child's location for every 12 hours during the fire. Noninvasive subclinical measurements of carotid artery thickness and stiffness were used to assess vascular function and are recognized as reliable subclinical measures of arterial damage and atherosclerosis.[11] We were able to adjust the base models for gender and age, both of which have considerable influence in childhood, but we were not able to calculate standardized values as no reference pediatric datasets were available. However, we were still able to directly comment on stiffness in m/s and thickness in mm. In order to detect a clinically meaningful change in vascular stiffness (1 SD), we required 135 children per group based on a two-group comparison.[33] Therefore, the study may have been underpowered.

There were a number of challenges relating to the study population; however, the ELF cohort provided the unique opportunity to investigate acute air pollution exposure against a background of relatively low ambient levels. There was also a biased subgroup who attended clinical testing compared to children who did not. Children from the most severely impacted areas were more likely to attend clinical testing. Mothers with higher education attainment were also more likely to understand the value in attending clinics. The differences in the characteristics of children who attended clinical testing may have impacted the representativeness of the sample and affected the translatability of the findings. Unexposed children were not included in this analysis due to the limited sample size and power. Finally, although the total number of children in each follow-up clinic was limited, an advantage of using a mixed-effect model enabled the inclusion of all children across both years. Boosting the capacity of the model to detect differences if they indeed existed.

The prenatal period may be more sensitive to the vascular health consequences of $PM_{2.5}$ compared with the early postnatal environment. Overall, there were no associations between postnatal exposure to fire-related $PM_{2.5}$ and vascular function, 7 years later. The deficits previously observed in children exposed postnatally to fire-related $PM_{2.5}$ appear to have resolved over time. However, there was increased vascular stiffness of children exposed in utero to fire-related $PM_{2.5}$, 7 years later. Clearly, there are differences in the vascular effects observed between children based on when they were exposed to mine fire-related $PM_{2.5}$. Children exposed postnatally had short-term changes in vascular health that resolved over time. Whereas children exposed in utero may have a longer latency period from exposure to the vascular outcomes observed. These findings have important implications for the public health response to short-term severe air pollution events. Further studies are needed to determine if these vascular effects persist over time.

Conflicts of interest statement

E.J.H., A.B., S.A., A.J.W., M.D., G.J.W., C.X.G., K.N., F.H.J., and G.R.Z. declare that they have no conflicts of interest with regard to the content of this report. M.J.A. reports a relationship with Pfizer Global Research and Development that includes funding grants. M.J.A. reports a relationship with Boehringer Ingelheim GmbH that includes funding grants. M.J.A. reports a relationship with Sanofi Australia that includes consulting or advisory, and funding grants. M.J.A. reports a relationship with GlaxoSmithKline that includes funding grants and speaking and lecture fees.

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