Hazelwood
HEALTH STUDY

The Latrobe Early Life Follow-up (ELF) Cohort Study Volume 3

Investigation of possible associations between coal mine fire emissions and vascular outcomes in the ELF cohort three years after the fire

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Caveat

This report presents a preliminary analysis which has not been submitted to independent peer review. Subsequent scientific manuscripts which undergo independent peer review may vary in their findings or interpretation.

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Abbreviations

95% CI	95 percent confidence interval
aaIMT	Abdominal Aorta Intima-Media Thickness
Ave	Average
BMI	Body Mass Index
cIMT	Carotid Intima-Media Thickness
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Dmax	End-Systolic Diameter
Dmin	End-Diastolic Diameter
ECG	Electrocardiograph
HHS	Hazelwood Health Study
IMT	Intima-Media Thickness
IQR	Interquartile Range
Latrobe ELF Study	Latrobe Early Life Follow-Up Study: The child health and development stream of the Hazelwood Health Study
$PM_{2.5}$	Particulate matter with an aerodynamic diameter less than 2.5 micrometres
PWV	Pulse Wave Velocity

Executive Summary

The Hazelwood open cut coal mine in the Latrobe Valley of Victoria caught fire in February 2014 and burned for nearly six weeks. Several small rural towns near the mine were affected by smoke during this period with air quality impacts ranging from minor to severe. The Latrobe Early Life Follow-up (ELF) Study aims to understand the possible influence of exposure to smoke from the fire on the health and development of young children and children born to women who were pregnant at the time of the fire. The ELF study has two major streams, an identified cohort study of children from the Latrobe Valley who were recruited during 2015-2016, and a series of anonymous data extraction and data linkage studies.

This Report comprises Volume 3 of a set of reports arising from the Latrobe ELF Cohort Study. Volume 1 described the cohort and results of initial investigations of possible associations between mine fire emissions and parent-reported perinatal outcomes. Volume 2 reported the results of respiratory function testing. Here we report results of blood vessel testing in children from the Latrobe ELF cohort.

Little is known about possible long term cardiovascular effects of time-limited exposure to air pollution during early life, such as that associated with smoke from severe fires. However, it is known that exposure to outdoor air pollution can affect blood vessels and heart health in adults,[1-4] and that parental smoking in early life is linked to poorer vascular health in children.[5,6] In response to community concerns, this study was designed to evaluate if smoke from the mine fire had measurable associations with indicators of blood vessel health in young children.

We tested 248 participants from the Latrobe ELF cohort. They were classified into four groups based on their mother's estimated date of conception as follows:

(1) The postnatal exposure group. Children who were exposed to smoke from the fire at some point during their infancy (birth – 2 years of age);

(2) The *in utero* exposure group. Children whose mothers were pregnant at the time of the fire;

(3) The mixed exposure group. This was a smaller group of children who were born during the fire and had some exposure both *in utero* and after birth.

(4) Not exposed. Children conceived after the fire and not exposed to fire smoke at any stage of their development.

The amount of smoke exposure from the fire was calculated for children in the exposed groups (Groups 1 to 3) based on their, or their mother's, reported locations during the fire period. This information was combined with modelled estimates of the concentration of airborne particulate matter less than 2.5 micrometres in diameter ($PM_{2.5}$) at a spatial resolution of 1 x 1 km. The mean and peak daily $PM_{2.5}$ exposures during the fire period (9 February to 31 March 2014) were calculated for each participant.

Three years after the fire, we tested for possible associations between smoke emissions and two main indicators of vascular health:

- The thickness of the inner two layers of the wall of the carotid artery in the neck, and the abdominal aorta. This is known as the arterial intima-media thickness (IMT).
- The stiffness of the vascular system assessed by measuring the pulse wave velocity (PWV).

Multivariable linear regression models were used to test for associations between the daily mean and peak $PM_{2.5}$ exposures, and these indicators of blood vessel health. The analyses took into account other factors that could potentially influence vascular health, such as children's age, birthweight, maternal tobacco smoking during pregnancy, socioeconomic status, parental diabetes and exposure to environmental tobacco smoke.

There were 42 children in the unexposed group (Group 4). These children were not exposed to fire emissions at any stage of their development. With an average age of just 2 years, it was not appropriate to directly compare their results with those of the older children who had been exposed to smoke from the mine fire.

We did not find any associations between early life exposure to $PM_{2.5}$ during the mine fire period and adverse vascular changes when all groups were combined, or in the subgroup of children who were exposed *in utero*. However, an association between mine fire smoke exposure and stiffer blood vessels, indicated by a higher PWV, was found in the postnatal exposure group of 96 children. For that group, each 10 $\mu g/m^3$ increase in exposure to mean $PM_{2.5}$ during the fire period was associated with an increase in PWV by 0.1 meter per second. (0.109; 95%CI 0.008 to 0.211; p = 0.035). There was a weaker trend with peak $PM_{2.5}$ exposure (0.066; 95%CI -0.008 to 0.141; p = 0.080).

We also found that tobacco smoking during pregnancy was independently associated with a higher IMT of the abdominal aorta among children in the postnatal exposure group and higher IMT of the carotid artery in children in the *in utero* exposure group.

When we investigated the subgroup of children whose mothers smoked during pregnancy, regardless of their exposure group (pre or postnatal), we found that exposure to higher amounts of mine fire smoke was associated with higher PWV. However, the number of children in this group was small.

While these results suggested that exposure to mine fire emissions could have been associated with increased stiffness of blood vessels in some children, they should be interpreted with caution. The association was only present in one of several outcomes that were evaluated and it is possible that chance or unmeasured or unknown factors could have contributed to the result. It will be important to confirm these findings with further studies that include data from children who were not exposed to the mine fire emissions, when they are old enough. This is currently planned for 2020.

There is normal variation in blood vessel measurements in children. These results do not necessarily mean that children with higher blood vessel stiffness or thickness will develop cardiovascular problems later in life. Blood vessel health in childhood is one of many things, such as genetic make-up, smoking tobacco, stress, diet and physical activity that can influence the risk of cardiovascular disease in adulthood.[7]

In summary, we found that in infants aged up to two years at the time of the fire, exposure to $PM_{2.5}$ during the mine fire period was associated with increases in blood vessel stiffness. We also found that tobacco smoking during pregnancy was associated with thicker blood vessels in children. Further assessments are planned for 2020. At that time, we will be able to further evaluate these findings and test for persistence, remission or emergence of blood vessel changes over time.

1. Introduction

The Latrobe Early Life Follow-up (ELF) Cohort Study forms one stream of the Hazelwood Health Study (HHS). The HHS was established to investigate possible health effects of smoke pollution associated with a fire in the Hazelwood open cut coal mine in the Latrobe Valley of Victoria. The fire caused elevated concentrations of particulate matter ($PM_{2.5}$) and other air pollutants for several weeks, affecting many nearby communities. The ELF Study aims to understand possible associations between exposure to the mine fire smoke and the health and development of children who were aged less than two years, or whose mothers were pregnant at the time of the fire. The ELF Study has two parts. The first part follows an identified cohort of participants who joined the study and completed a baseline survey in 2016. The second part involves the analysis of de-identified data extractions and anonymised data linkage. More information about the Latrobe ELF study is available from the HHS Study website and in the first written report.[8]

This Report comprises Volume 3 of a set of reports arising from the Latrobe ELF Cohort. Volume 1 described the participating cohort and preliminary investigations on the association between mine fire emissions and parent-reported perinatal outcomes. Volume 2 describes the investigation of possible associations between mine fire emissions and clinical measures of lung function. The findings in these reports have not been through external expert peer review. Any subsequent papers in scientific journals, that will undergo peer review, may vary in their findings and interpretation.

This report describes results from the evaluation of vascular function in 248 members of the Latrobe ELF cohort. Measures of vascular function were included in the Latrobe ELF Study because outdoor air pollution, including $PM_{2.5,}$ is known to be associated with impaired vascular function, especially for individuals at higher risk because of illness, such as diabetes, or age, either older or younger.[9-11] In children, parental smoking,[5,6] and exposure to traffic emissions,[12] have been associated with reduced vascular function, and *in utero* exposure to air pollution has been linked with increasing carotid artery stiffness in young adults.[13] However, evidence of long-term cardiovascular outcomes in children exposed to a time limited episode of poor air quality in early life, such as the Hazelwood mine fire pollution episode, is sparse. Cardiovascular disease, like atherosclerosis, is rarely manifest in childhood, yet the origins can begin during the early stages of life.[14-16] Many risk factors can accelerate the development of cardiovascular disease in children, including a genetic predisposition, exposure to tobacco smoke, obesity, and increased sedentary activities.[7] The structural changes of blood vessels are an early indicator of the development of cardiovascular disease. The walls of arteries begin to change in childhood as lipids start to deposit along the inner surface, called the intima. This process can be seen in nearly all children by three years of age.[17] Arterial stiffness gradually increases with age, and can eventually lead to reduced blood supply and damage to organs and tissues such as the heart, brain, kidneys or skin.[18,19]

Our investigation aimed to examine possible associations between exposure to mine fire emissions and vascular health three years after the fire among participants in the Latrobe ELF cohort.

The study was 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.

2. Methods

2.1 Recruitment

All children whose parents completed the ELF baseline survey and consented to clinical testing (N = 438) were invited to participate in clinical testing of vascular structure and function three years after the mine fire. The methods for initial recruitment and data collection for the baseline survey are reported in Volume 1,[8] and are not repeated here. Recruitment for the clinical testing commenced in March 2017. Eligible families were mailed an invitation letter which contained a unique login for an online appointment booking system. Non-responders were followed up by phone. Articles in media outlets including newspaper, TV, radio and social media, and a study newsletter promoting the clinical testing which was sent to all enrolled families, aimed to publicise the clinical testing and increase participation. The commencement of the clinical testing in March 2017 coincided with closure of the Hazelwood power station after 52 years of operation.

2.2 Data collection

2.2.1 Data collected during the baseline survey

In the baseline survey, the parent or carer of each child provided information about demographic, family and health characteristics through telephone interview, mail or an online system. Further details of the baseline survey can be found in our Volume 1 report.[8] The following information was extracted from the baseline survey data.

- Age of child
- Sex of child
- Birthweight
- Gestational age
- Breastfeeding status
- Environmental tobacco smoke
- Maternal age
- Maternal smoking status in pregnancy
- Maternal alcohol consumption in pregnancy
- Maternal stress in pregnancy
- Maternal fire-related stress
- Maternal educational attainment
- Parental heart disease or stroke
- Parental hypertension
- Parental diabetes mellitus
- Parental high cholesterol
- Residential history
- Day and night locations during the fire period

Environmental tobacco smoke (ETS) was defined as the presence of a current smoker in the child's household. For the statistical analyses, a parental history of heart attack, angina or stroke were combined to form a single variable - parental major adverse cardiovascular events.

2.2.2 Data collected at the time of the clinical testing

Demographic characteristics and health-related risk factors

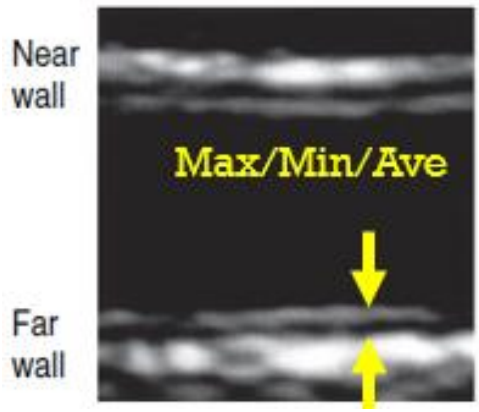
The clinical testing took place in the Latrobe Community Health Centre during 2017, three years after the mine fire. Parents or guardians of participating children were asked not to give their child large meals, medications that affect the cardiovascular system, or caffeine-containing drinks on the day of the test. On arrival at the clinic, parents or carers of participating children completed a survey to complement the clinical information about their child. The date and time of the clinic attendance, child's date of birth, gender, and information about factors which might influence the vascular assessment results were recorded. These included consumption of caffeine-containing drinks in the previous three hours, all medication in the previous 24 hours and any cold or flu symptoms in the previous three weeks. As a child's socio-economic situation can influence child health, we collected updated information about the child's parents' or carers' employment, and any effect of the mine closure on their jobs and overall stress.

Biometrics

Children's heights in centimetres and weights in kilograms were measured using a calibrated stadiometer and portable scales.

Outcome measures

The main outcome measures were two non-invasive tests for early markers of atherosclerosis: (1) the intima-media thickness (IMT) of the carotid artery in the neck and (2) pulse wave velocity (PWV). Increases in either of these measurements are associated with an increased risk for the subsequent development of cardiovascular disease.[20,21] The IMT of the abdominal aorta was also measured as atherosclerotic changes were thought to develop earlier in the distal aorta.[22,23]



IMT is the thickness of the inner two layers of the artery wall (intima + media). IMT is used to assess the development and progression of atherosclerosis.

Figure 1. An ultrasound image of an artery showing measurement of the IMT.
The white lines at the top and bottom of the image are the artery walls. The yellow arrows point out the inner two layers of the wall, the intima and the media.

Intima-media thickness measurement

The IMT assessments were conducted in a darkened, temperature-controlled room using a commercially available ultrasound system with a 12MHz linear array transducer (Vivid q, GE Healthcare, USA) following published guidelines.[24,25] Participants were connected to a 3-lead electrocardiograph (ECG) and then rested, lying on their backs, for 10 minutes before measurements commenced. The left and right sided common carotid arteries, which run along each side of the neck, were examined separately. As the head needs to be turned 45° away from the side being measured, we played cartoons on an iPad and read story books to keep the children entertained while looking to one side or the other. During measurement, the probe was held as close to perpendicular as possible to the artery while 10 to 30 mm of the distal common carotid artery was scanned. We obtained three longitudinal views on each side with at least three clean cardiac cycles, (i.e. heartbeats), recorded on ECG, and the carotid bifurcation was recorded on the left side of the screen. The abdominal aorta was then imaged in a similar way. If the child was not able to keep still for the entire procedure, we captured and stored the portion of the artery with the best possible quality image.

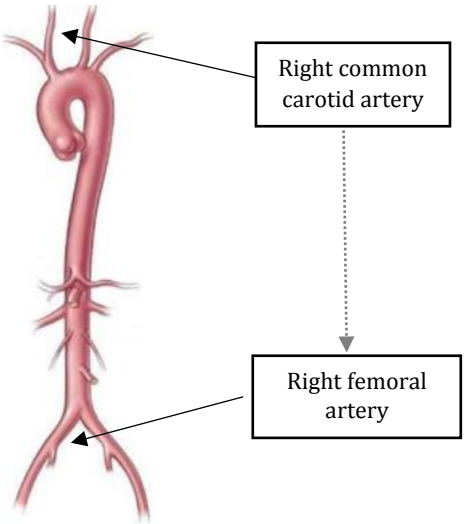
The Vivid q ultrasound system has an in-built IMT measurement procedure which calculated the average, maximum and minimum IMT by automatic contour detection of the region along the artery wall. It also provided the standard deviation and the successful number of pair points among multiple measurements between points on two layers (intima and media). We used this procedure to perform both far wall and near wall measurement of IMT on the region of 10 mm of the common carotid artery below the carotid bifurcation at end-diastole. The abdominal aorta IMT was measured in the same way on the region of interest using the best quality images.

We report the average IMTs from the far wall and near wall of each vessel. As there are two carotid arteries, we also averaged the values from the left and right common carotid arteries. If data from one side were missing, we used the available data for the side that was measured.

Figure 2. Using an ultrasound to measure the IMT of the abdominal aorta.

PWV measurement

To measure the PWV, we first measured the child's blood pressure at the femoral artery on the right side where it runs through the thigh. We used an appropriately-sized femoral cuff attached to a SphygmoCor XCEL device (AtCor Medical, Australia) and recorded systolic and diastolic blood pressure. We measured the PWV distance, which is the sum of the distance between the right carotid artery and the sternal notch, and the sternal notch to the top edge of the femoral cuff. A tonometer was then placed over the carotid artery. The SphygmoCor XCEL then detected the right carotid pulse from the tonometer and the femoral pulse from the blood pressure cuff, compared the timing of these and used the PWV distance to calculate the result.



PWV is the time it takes for a pulse wave to travel from the common carotid artery to the femoral artery. PWV measures vascular stiffness. The greater the stiffness, the faster the speed of the wave.

Figure 3. Diagram of the aorta and its major branches.

Figure 4. PWV measurement.

A blood pressure cuff around the thigh is used together with a tonometer to measure the pressure of the carotid artery in the neck (not shown).

2.3 Exposure allocation

The Hazelwood mine fire smoke exposure period was from 9 February to 31 March 2014. Study participants were classified into four groups based on their mother's estimated date of conception and the fire period:

1. The postnatal exposure group. This group included children who were born between 1 March 2012 and 9 February 2014 and who were therefore aged less than 2 years at the time of the coal mine fire.
2. The *in utero* exposure group. This group was comprised of children who were born between 1 April and 31 December 2014. The mothers of this group of children had been resident in the Latrobe Valley at the time of the fire when they were pregnant with the child participating in the study.
3. The mixed *in utero* and postnatal exposure group. This group comprised a small number of children who were born during the fire period (i.e. between 10 February and 31 March 2014) and were therefore in both the in utero and postnatal exposure group.
4. Not exposed (the control) group. The control group consisted of children who were conceived after the fire and its associated air pollution had resolved.

The children in the exposed groups (Groups 1 to 3) were exposed across a gradient of severity to the mine fire air pollution. Their exposure was influenced by many factors, including how close their homes were to the fire, and the amount of time their homes had been in the direct path of the smoke plume from the fire. Their exposure was also determined by the location of their daily activities and if they (or their mother when pregnant), had travelled away from the region during the time of the fire. Information about their daily time activity patterns during the fire period was collected in the baseline questionnaire.[8]

Our collaborators at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) implemented meteorological and chemical transport modelling to estimate hourly $PM_{2.5}$ concentrations at a spatial resolution of 1 x 1 km. Further details about the exposure modelling can be found on the Hazelwood Health Study website.[26] We used this data to calculate 24 hour average exposures for each participant, based on their reported night and day locations during the fire period.

Two exposure metrics were calculated for each participant.

1. The **average** (mean) outdoor concentration of $PM_{2.5}$: the mean of the 24 hourly calculated exposures throughout the fire period.
2. The **peak** (maximum) 24 hour $PM_{2.5}$: the highest 24 hour average $PM_{2.5}$ exposure calculated for each child during the mine fire period.

In this report, we compare the amount of smoke exposure (high to low) with the results of vascular testing in Groups 1 to 3. Children in the control Group 4 were two to three years younger than children in the exposed groups and a direct comparison was not appropriate because vascular function changes with age. All children will be invited to participate in a second round of clinical testing scheduled for 2020, at which time the results from Group 4 will be suitable for an age-matched comparison with the results of those who were tested during 2017.

2.4 Statistical analysis

Sociodemographic and vascular characteristics of the study participants and their parents were expressed as mean ± standard deviation (SD) or percentage using basic descriptive statistics. The associations between exposure to the mine fire emissions and vascular health were examined in the exposure groups combined, with separate sub group analyses of the postnatal and *in utero* exposure groups. We used multivariable linear regression models to look for independent associations between the main vascular outcomes (arterial IMT and stiffness), and daily mean and peak $PM_{2.5}$ exposures during the mine fire period.

We report results per 10 unit increase in mean $PM_{2.5}$ ($\mu g/m^3$) exposure, and per 100 unit increase in peak $PM_{2.5}$, which were close to the interquartile range of each exposure metric. A group of covariates known to be determinants of, or potentially related to, vascular outcomes were selected *a priori* for inclusion in our initial models. These included children's age, sex, maternal smoking status in pregnancy, parental major adverse cardiovascular events, parental hypertension, parental diabetes, parental high cholesterol, birthweight, gestational age, maternal alcohol consumption in pregnancy, breastfeeding status, maternal education attainment, maternal stress in pregnancy, maternal fire stress and maternal age. We retained all variables that influenced the outcome by 10% or more. These were children's age, birthweight, maternal education attainment, maternal smoking status in pregnancy and parental diabetes. Retention of the other covariates did not meaningfully influence the results.

Exposure to ETS was not included in the models *a priori* because of the considerable overlap between this variable, and maternal tobacco smoking during pregnancy. However the influence of adding ETS as an additional variable in the final models was tested in sensitivity analyses. In addition separate subgroup analyses of children whose mothers smoked tobacco during pregnancy was conducted. Diagnostic checks on the residuals were preformed to assess model assumptions. All data analyses were conducted using SPSS Statistics version 23 (IBM, Armonk, NY).

3. Results

3.1 Completeness of vascular assessments

From 438 families who consented, during the 2016 baseline survey, to subsequent vascular testing, 248 children attended. Of those, 213 (86%) were able to complete at least one of the assessments, but 35 were not able to complete any test. After excluding poor quality and incomplete records, the data completeness for each test ranged from 70% to 83% (**Figure 5**).

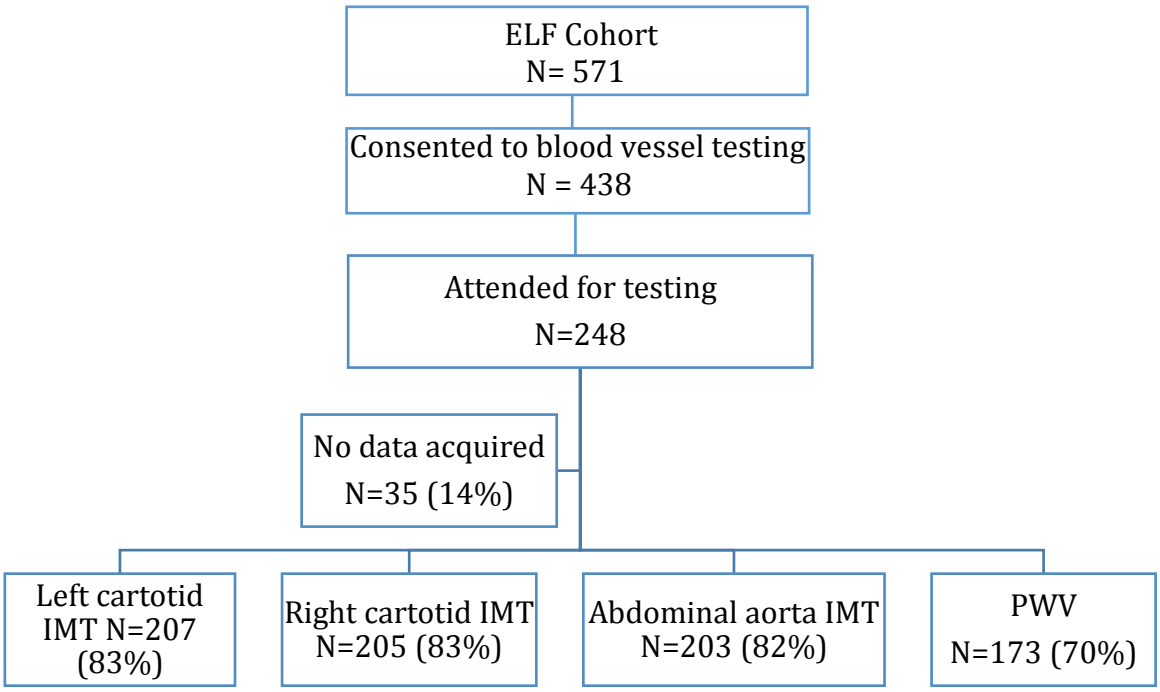


Figure 5. Flow diagram for completeness of vascular assessment

3.2 Description of participants

A total of 171 children were exposed to coal mine fire smoke either *in utero* (Group 2, N=60), in the two years following their birth (Group 1, N=96), or both (Group 3, N=15). We report detailed results for all three groups combined, and for Groups 1 and 2 alone. For Group 3 we report only descriptive data as there were insufficient numbers to implement detailed statistical modelling and analyses. There were 42 children in the non-exposed Group 4 who were tested, however in this Report they were not compared with Groups 1 - 3 as discussed in section 2.3.

The sociodemographic characteristics of the exposed children are summarised in **Table 1**. Nearly 99% of the children were born in Australia. The gender distributions were similar in postnatal and *in utero* groups. The age differences between groups were due to the classification of their exposure by age. The proportions of children exposed to maternal tobacco smoking during pregnancy and environmental tobacco smoke were slightly higher in the postnatal than the *in utero* exposure group. Maternal age and maternal alcohol consumption in pregnancy were similar among exposure groups.

Table 1. Sociodemographic characteristics of participants categorised by exposure group.

	Exposure classification			
	Postnatal (Group 1) N = 96	***In utero* (Group 2) N = 60**	**Mixed (Group 3) N = 15**	**All N = 171**
	mean ± SD	**mean ± SD**	**mean ± SD**	**mean ± SD**
Child's age (months)	50.9 ± 6.5	33.6 ± 2.6	38.2 ± 1.3	43.7 ± 9.7
Height (cm)	106.3 ± 6.4	93.3 ± 3.8	97.2 ± 4.1	101.0 ± 8.2
Weight (kg)	19.4 ± 4.7	14.7 ± 1.5	15.7 ± 1.2	17.4 ± 4.3
BMI (kg/m^2)	17.0 ± 2.5	16.9 ± 1.1	16.6 ± 1.0	16.9 ± 2.0
Birth weight (g)	3435 ± 597	3477 ± 490	3556 ± 429	3460 ± 547
Gestational age (week)	39.6 ± 1.8	39.4 ± 1.6	39.0 ± 2.1	39.5 ± 1.8
Maternal age (years)	28.7 ± 5.8	30.4 ± 4.3	31.7 ± 4.9	29.6 ± 5.3
	n(%)	**n(%)**	**n(%)**	**n(%)**
Child's gender				
Male	49 (51.0)	28 (46.7)	9 (60.0)	86 (50.3)
Female	47 (49.0)	32 (53.3)	6 (40.0)	85 (49.7)
Child's country of birth				
Australia	95 (99.0)	60 (100)	14 (93.3)	169 (98.8)
Other	1 (1.0)	0 (0)	1 (6.7)	2 (1.2)
Breastfeeding status				
Breastfed until 3 months of age or less	31 (32.3)	20 (33.3)	3 (20.0)	54 (31.6)
Breastfed longer than 3 months of age	63 (65.6)	39 (65.0)	12 (80.0)	114 (66.7)
Not stated	2 (2.1)	1 (1.7)	0 (0)	3 (1.8)
Maternal cigarettes smoking status in pregnancy				
Smoker	17 (17.7)	7 (11.7)	1 (6.7)	25 (14.6)
Non-smoker	79 (82.3)	53 (88.3)	14 (93.3)	146 (85.4)
Quantity of cigarettes smoked in first 20 weeks of pregnancy				
9 or less per day	93 (96.9)	58 (96.7)	15 (100)	166 (97.1)
10 or more per day	3 (3.1)	2 (3.3)	0 (0)	5 (2.9)
Quantity of cigarettes smoked in last 20 weeks of pregnancy				
9 or less per day	93 (96.9)	59 (98.3)	15 (100)	167 (97.7)
10 or more per day	2 (2.1)	1 (1.7)	0 (0)	3 (1.8)
Not stated	1 (1.0)	0 (0)	0 (0)	1 (0.6)
Maternal alcohol consumption in pregnancy				
Yes	9 (9.4)	5 (8.3)	2 (13.3)	16 (9.4)
No	87 (90.6)	55 (91.7)	13 (86.7)	155 (90.6)
Alcohol consumption in the first 20 weeks of pregnancy				
No alcohol in early pregnancy	87 (90.6)	55 (91.7)	13 (86.7)	155 (90.6)
Alcohol in early pregnancy	9 (9.4)	4 (6.7)	2 (13.3)	15 (8.8)
Unsure	0 (0)	1 (1.6)	0 (0)	1 (0.6)
Alcohol consumption in the last 20 weeks of pregnancy				
No alcohol in late pregnancy	93 (96.9)	59 (98.3)	14 (93.3)	166 (97.1)
Alcohol in late pregnancy	2 (2.1)	1 (1.7)	1 (6.7)	4 (2.4)
Not stated	1 (1.0)	0 (0)	0 (0)	1 (0.6)

Table 1 (Continued) Sociodemographic characteristics of participants categorised by exposure group.

	Exposure classification			
	Postnatal (Group 1) N = 96	***In utero* (Group 2) N = 60**	**Mixed (Group 3) N = 15**	**All N = 171**
	n(%)	**n(%)**	**n(%)**	**n(%)**
Maternal highest level of education				
Year 12 or below	38 (39.6)	14 (23.3)	3 (20.0)	55 (32.2)
Post-secondary	58 (60.4)	46 (76.7)	11 (73.3)	115 (67.3)
Not stated	0 (0)	0 (0)	1 (6.7)	1 (0.6)
Maternal stress in pregnancy				
Hardly ever/sometimes stressed	84 (87.5)	50 (83.3)	13 (86.7)	147 (86.0)
Stressed often/nearly all of the time	12 (12.5)	9 (15.0)	1 (6.7)	22 (12.9)
Not stated	0 (0)	1 (1.7)	1 (6.7)	2 (1.2)
Mine fire on maternal stress				
Stress not affected / increased a little	59 (61.5)	38 (63.3)	9 (60.0)	106 (62.0)
Stress increased a lot	36 (37.5)	21 (35.0)	4 (26.7)	61 (35.7)
Not stated	1 (1.0)	1 (1.7)	2 (13.3)	4 (2.3)
Exposure to environmental tobacco smoke				
Yes	23 (24.0)	10 (16.7)	3 (20.0)	36 (21.1)
No	73 (76.0)	50 (83.3)	12 (80.0)	135 (78.9)
Parental major adverse cardiovascular events				
Yes	2 (2.1)	1 (1.7)	0 (0)	3 (1.8)
No	94 (97.9)	59 (98.3)	15 (100)	168 (98.2)
Parental hypertension				
Yes	19 (19.8)	7 (11.7)	2 (13.3)	28 (16.4)
No	77 (80.2)	53 (88.3)	13 (86.7)	143 (83.6)
Parental diabetes mellitus				
Yes	6 (6.3)	7 (11.7)	0 (0)	13 (7.6)
No	89 (92.7)	53 (88.3)	15 (100)	157 (91.8)
Not stated	1 (1.1)	0 (0)	0 (0)	1 (0.6)
Parental high cholesterol				
Yes	13 (13.5)	6 (10)	1 (6.7)	20 (11.7)
No	83 (86.5)	54 (90)	14 (93.3)	151 (88.3)

3.3 Exposure to $PM_{2.5}$ during the mine fire period

The children's exposure to $PM_{2.5}$ during the mine fire period is summarised in **Table 2**. The median of the average $PM_{2.5}$ exposures was 8.8 µg/m^3 while the median of the peak $PM_{2.5}$ exposures was 101.3 µg/m^3. **Figure 6** illustrates the distributions of the mean and peak 24-hour $PM_{2.5}$ exposure during the mine fire period by exposure group.

Table 2. Distribution of estimated $PM_{2.5}$ exposure by study group.

	Exposure classification			
	Postnatal N = 96	***In utero* N = 60**	**Mixed N = 15**	**All N = 171**
	Median [IQR]	**Median [IQR]**	**Median [IQR]**	**Median [IQR]**
Mean $PM_{2.5}$ (µg/m^3)	8.4 [10.8]	8.9 [10.2]	8.3 [7.6]	8.8 [9.8]
Peak $PM_{2.5}$ (µg/m^3)	113.1 [121.1]	104.9 [121.2]	88.1 [93.9]	101.3 [116.8]

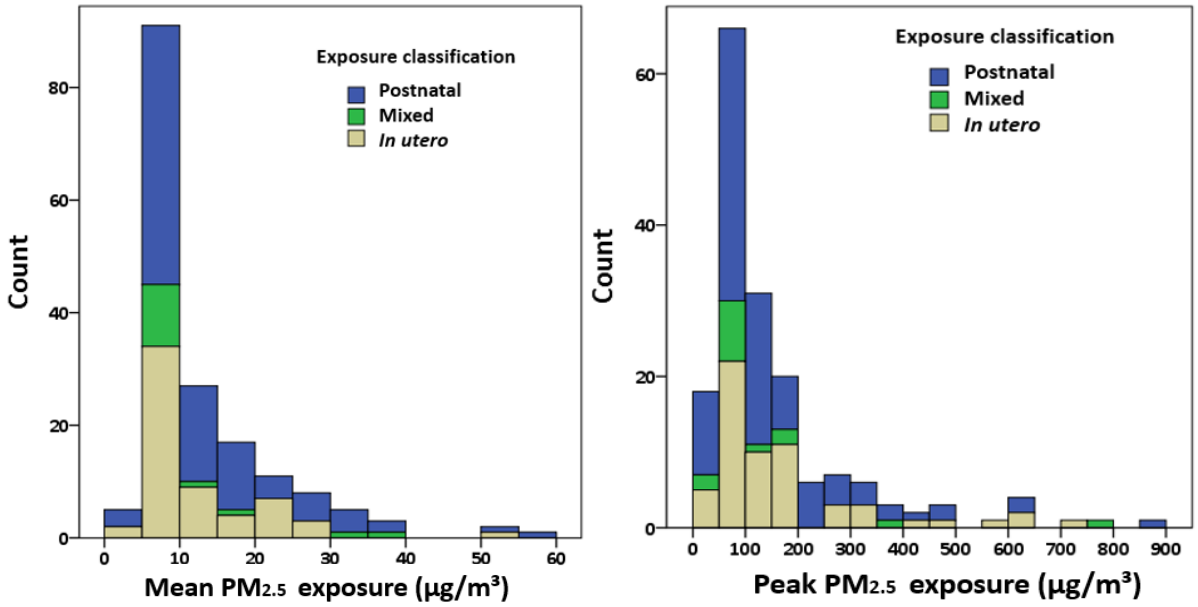


Figure 6. Distribution of estimated mean (left) and peak (right) 24-hour $PM_{2.5}$ exposure during the mine fire period by exposure group.

3.4 Vascular characteristics of study participants

Arterial IMT and stiffness characteristics for study participants categorised by exposure group are presented in **Table 3**. When all groups were combined, the mean carotid IMT was 0.497mm, the mean abdominal aorta IMT was 0.406mm and the mean PWV was 4.15m/s.

Table 3. Descriptive statistics for vascular assessment categorised by study group.

	Exposure classification							
	Postnatal (Group 1)		***In utero* (Group 2)**		**Mixed (Group 3)**		**All**	
	n	**mean ± SD**	**n**	**mean ± SD**	**n**	**mean ± SD**	**n**	**mean ± SD**
Carotid IMT (mm)	95	0.499 ± 0.034	60	0.495 ± 0.033	15	0.487 ± 0.035	170	0.497 ± 0.034
Abdominal aorta IMT (mm)	93	0.405 ± 0.057	57	0.409 ± 0.055	14	0.399 ± 0.047	164	0.406 ± 0.055
PWV (m/s)	91	4.12 ± 0.45	46	4.19 ± 0.48	10	4.21 ± 0.50	147	4.15 ± 0.46

3.4 Associations between fire emissions and vascular outcomes

The results of multivariable linear regression models to examine the association between daily mean and peak $PM_{2.5}$ exposures during the fire period, and vascular outcomes for different exposure groups, are presented in **Table 4**. All models were adjusted for children's age, birthweight, maternal educational attainment, maternal smoking status in pregnancy and parental diabetes. Retention of other covariates did not meaningfully influence the results.

The β value represents the change in IMT or PVW associated with every 10 $\mu g/m^3$ increase in mean $PM_{2.5}$, or every 100 $\mu g/m^3$ increase in peak $PM_{2.5}$. There was no association between either the mean or the peak $PM_{2.5}$ exposures and vascular measurements in the combined exposure groups, those being all children exposed either *in utero* or during their infancy. However, when the pre and postnatal exposure groups were evaluated separately a slightly different picture emerged for each group. (**Table 4**)

3.4.1 Factors associated with vascular outcomes in the postnatal exposure group

In the postnatal exposure group (Group 1, N = 96), each 10 $\mu g/m^3$ increase in mean $PM_{2.5}$ exposure was associated with an increase in PWV of 0.109 m/s (95%CI 0.008 to 0.211; p = 0.035). The result for the association between increases in peak $PM_{2.5}$ exposure and PWV was in a similar direction but not statistically significant (β = 0.066; 95%CI -0.008 to 0.141; p = 0.08). (**Table 4**)

Exposure to ETS was not included in the core models *a priori* because there was overlap between this and maternal tobacco smoking. However, in a sensitivity analysis including ETS in addition to all variables in the core model, the results were unchanged (mean $PM_{2.5}$: β = 0.116; 95%CI 0.013 to 0.218; p = 0.028; peak $PM_{2.5}$: β = 0.067; 95%CI -0.008 to 0.143; p = 0.08).

Maternal tobacco smoking during pregnancy was independently associated with greater abdominal aortic IMT. There was a 0.036mm increase in mean abdominal aortic IMT (95%CI 0.002 to 0.069; p = 0.04) among children exposed to maternal smoking during pregnancy compared to children without that exposure.

3.4.2 Factors associated with vascular outcomes in the *in utero* exposure group

We did not find any associations between exposure to fire smoke and any markers of vascular health in the *in utero* exposure group (Group 2, N = 60).

In this group, maternal tobacco smoking during pregnancy was associated with a 0.034mm increase in mean carotid IMT (95%CI 0.003 to 0.065; p = 0.032 in the mean $PM_{2.5}$ model; 95%CI 0.004 to 0.065; p = 0.029 in the peak $PM_{2.5}$ model).

Table 4. $PM_{2.5}$ exposure during the fire period and vascular outcomes by exposure group.

	Exposure groups combined		**Postnatal**		***In utero***	
	Mean $PM_{2.5}$	**Peak $PM_{2.5}$**	**Mean $PM_{2.5}$**	**Peak $PM_{2.5}$**	**Mean $PM_{2.5}$**	**Peak $PM_{2.5}$**
Carotid IMT β (95% CI)	-0.001 (-0.007, 0.005)	-0.0004 (-0.004, 0.003)	-0.002 (-0.008, 0.005)	-0.001 (-0.006, 0.004)	0.003 (-0.008, 0.014)	0.002 (-0.004, 0.007)
Abdominal aorta IMT β (95% CI)	-0.009 (-0.018, 0.0004)	-0.006 (-0.012, 0.0001)	-0.011 (-0.023, 0.0003)	-0.007 (-0.016, 0.001)	-0.003 (-0.023, 0.016)	-0.005 (-0.016, 0.005)
PWV β (95% CI)	0.058 (-0.027, 0.144)	0.028 (-0.029, 0.084)	**0.109 (0.008, 0.211)**	0.066 (-0.008, 0.141)	-0.062 (-0.262, 0.138)	-0.001 (-0.120, 0.119)

The β value for each variable represents the change per 10 μg/m^3 increase in mean $PM_{2.5}$ and per 100 μg/m^3 increase in peak $PM_{2.5}$ adjusted for maternal smoking status during pregnancy, parental diabetes, children's age, birthweight and maternal education level. **Bold** p<0.05.

3.4.3 Subgroup analysis by status of maternal smoking in pregnancy in the combined exposure group including both *in utero* and postnatal exposure.

We observed an association between coal mine fire smoke exposure and higher blood vessel stiffness in the subgroup of children whose mothers smoked during pregnancy. Each 10-unit increase in mean $PM_{2.5}$ was associated with a 0.151 m/s increase in PWV 95%CI 0.039 to 0.263; p = 0.011). A similar result was also observed in the peak $PM_{2.5}$ model (0.098; 95%CI 0.016 to 0.180; p = 0.023).

No associations were found with the thickness of either the abdominal aorta or the carotid arteries in this subgroup.

There was no evidence of associations between mean or peak $PM_{2.5}$ exposures and higher PWV or measures of IMT in children whose mothers did not smoke during pregnancy.

4. Discussion

This report summarised the findings of the first clinical assessment of vascular health in the Latrobe ELF Cohort Study. A total of 171 children were included in our analysis of the association between air pollution from the Hazelwood mine fire and vascular health.

When the exposed children were grouped together, irrespective of whether their exposure was in utero, postnatal or both, increases in mine fire $PM_{2.5}$ were not found to be associated with adverse vascular outcomes measured three years later. However, when the children in each exposure group were evaluated separately, a different pattern emerged in each group. We did not find any association with blood vessel changes in children whose mothers were pregnant with them at the time of the fire. However, increased PWV was observed in the postnatal exposure group who were aged up to two years at the time of the fire. In this group, higher mean $PM_{2.5}$ exposure during the mine fire period was associated with increased PWV, indicating greater vascular stiffness, but not with any measures of arterial thickness. Early life exposure to $PM_{2.5}$ during the mine fire period was also associated with higher PWV in the 14.6% of children with a history of maternal tobacco smoking in pregnancy. This finding was in a relatively small subgroup of children. However, smoking during pregnancy is a well-established risk factor for the reduced vascular health of children and it is plausible that it could increase the sensitivity to adverse impacts of other sources of air pollution.[27,28] We also observed that maternal tobacco smoking during pregnancy was independently associated with higher IMT in the carotid artery in the *in utero* exposure group, and higher IMT in the abdominal aorta in the postnatal exposure group of children in this study.

Studies investigating possible influences of early life exposure to smoke from landscape fires on blood vessel stiffness in young children are scarce. In studies of adults, there is wide variation in the types of air pollution studied, sample sizes, study designs and study populations. One double blind randomized study involving 12 healthy men found that acute exposure to diesel exhaust was associated with increasing arterial stiffness.[29] Another randomised, crossover study with older adults demonstrated that PWV was decreased after walking in less polluted areas but that the benefit was reduced when walking on streets with high traffic related air pollution.[30] Further, a cross-sectional study reported that average ambient 12-month PM_{10} was independently associated with higher PWV in a group of hemodialysis patients.[31]

Tobacco smoking during pregnancy has been associated with adverse foetal outcomes, such as preterm birth, foetal growth restriction and low birth weight.[32,33] It is also associated with long-term impairment of children's cardiovascular health,[33] higher aortic and carotid artery IMT,[27,28] and higher blood pressure and reduced cardiac function in six-year-old children.[34] Other cardiovascular effects documented to date have included a greater risk of atherosclerotic lesions, higher cholesterol levels and higher systolic blood pressure in childhood.[35,36] Similarly, studies have shown that smoking by either parent in early life increases the risk of adverse vascular health outcomes including greater carotid IMT and atherosclerotic plaque formation in adulthood.[5,6] These results are consistent with our findings about the associations between maternal tobacco smoking during pregnancy and higher IMT in children.

The development of cardiovascular disease in children exposed to tobacco smoke in early life may follow a different physiological trajectory. The adverse foetal environment with maternal smoking has been associated with foetal blood flow adaptations,[37] increased arterial stiffness[34] and enduring changes to blood pressure regulation.[38] Further, reprogramming of cardiovascular

control systems have been demonstrated in animal studies of prenatal nicotine exposure.[39] It is possible that such changes could lead to different reactions and a decreased ability to adapt to later environmental insults such as that produced by the coal mine fire smoke.

The strengths of our study include the detailed and personalised individual $PM_{2.5}$ exposure estimates, which were based on the daily locations of the children, or their pregnant mothers, during the fire. Further, we were able to evaluate a wide range of potential risk factors, confounders and effect modifiers for vascular outcomes via comprehensive information collected in the baseline survey. We used state of the art vascular measurements. These included automatic contour detection of IMT which provides a more accurate and objective result than manual measurements.[24] We used applanation tonometry in our PWV measurements, which is the gold standard for assessing arterial stiffness.[40]

However, our study also has several limitations. The information from the baseline survey relied on parent or carers' self-report of medical conditions and lifestyle factors rather than medically verified diagnoses. Further, clinical testing in young children can be challenging and a number of test results in the youngest children had to be excluded due to inadequate quality. Although we obtained good quality data for 42 children who were not exposed to the fire smoke episode at any stage, these results were not appropriate to compare directly with the results from the older children who had been exposed to the fire emissions because of the age differences between those exposed and not exposed. The control group will become old enough to be an appropriate age-matched comparison group in the second round of clinical testing which is currently planned for 2020. Reanalysis at that time will be important to confirm these initial findings.

Another limitation is that the quality of IMT images from the abdominal aorta were generally not as good as those obtained from the carotid artery. However, atherosclerotic changes develop earlier in the distal aorta than carotid artery.[22,23] Performing an ultrasound on the abdominal area in younger children was initially expected to be more convenient than imaging the neck area.[23] However, we found younger children tolerated the carotid measurements much better than the abdominal measurements. This was because they were much more sensitive to pressure from the transducer on their abdomen than on their necks. Less pressure is required for carotid artery measurement because these blood vessels are closer to the skin.

There is normal variation in blood vessel measures in children, so these results do not mean that children with higher blood vessel stiffness or thickness will necessarily develop cardiovascular problems. Blood vessel health in childhood is one of many things, such as genetic make-up, smoking tobacco, stress, diet and physical activity, that can influence the risk of cardiovascular disease in later life.[7] Further, many factors including socioeconomic disadvantage and lower health literacy are associated with smoking and other environmental and lifestyle risk factors which could impact upon cardiovascular risk in children.[41,42] However, the existing literature and the results from the ELF study demonstrate the importance of reducing exposure to tobacco smoke in unborn babies and children because of the large influence this has on shaping children's health.

In summary, we found no associations between mine fire exposure and blood vessel health in the exposure groups combined nor the *in utero* exposure group. However, in the postnatal exposure group and the subgroup of children whose mothers smoked during pregnancy there were associations between $PM_{2.5}$ exposure and vascular stiffness. This observation requires further evaluation. Tobacco smoking in pregnancy was also identified as an independent risk factor for increased vascular thickness. This highlighted the importance of introducing practical support for quitting cigarette smoking, especially among women of reproductive age.

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