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Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Aims To assess the feasibility and reproducibility of non-invasive vascular assessment in a childhood population setting and identify the determinants of vascular phenotype in early life.

Methods and results We studied 7557 children (age 9.8–12.3 years) participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). Six research technicians underwent a 5-month training protocol to enable study of brachial artery endothelial function by flow-mediated dilatation (FMD) and arterial stiffness by carotid to radial pulse wave velocity (PWV) and brachial distensibility [distensibility coefficient (DC)]. Reproducibility studies were performed at the beginning, the middle, and the end of the study. A blinded repeat evaluation of a random selection of 3% of the cohort was also undertaken throughout the study. The effect of anthropometric and environmental factors on each measure was examined. Successful measures were obtained in 88, 95, and 87% of the studied children for FMD, PWV, and DC, respectively. The coefficients of variation between technicians for FMD, PWV, and DC were 10.5, 4.6, and 6.6% at the beginning of the study and reached 7.7, 4.1, and 10% at the end. Baseline vessel diameter and gender were important determinants of all the vascular measures, with a small effect of room and skin temperatures on FMD and PWV. Boys consistently had lower FMD and DC and higher PWV measures ($P < 0.01$ for all).

Conclusion Reproducible, high-quality assessments of vascular structure and function in children can be made on a large scale in field studies by suitably trained non-specialist operators. This study provides an invaluable resource for assessing the impact of early influences, genetic, and environmental factors on arterial phenotype.

Keywords ALSPAC • Vascular • Children • Endothelial function • Reproducibility

Introduction

It is now widely recognized that atherosclerosis starts early in life and that changes in the arterial wall from childhood are associated with altered levels of cardiovascular risk factors.[1,2] This pre-clinical risk profile has been shown to impact on eventual cardiovascular morbidity and mortality.[3] As a result, there is increasing interest in the determinants of the initiation and progression of early arterial disease and the potential for reversibility and prevention from a young age.

Disturbed vascular biology, involving inflammation and endothelial dysfunction, occurs early in atherogenesis.[4] Recently, we have shown that endothelial dysfunction predicts progression of structural changes in the carotid artery in cohort studies of middle-aged asymptomatic individuals and others have demonstrated that it also has prognostic impact.[5,6] Endothelial function and measures of arterial stiffness can be measured non-invasively, reflect the different aspects of the pre-clinical arterial phenotype, and represent endpoints in pre-clinical research.[7,8] For the most part, detailed vascular assessments have been largely confined to

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small groups of subjects, under carefully controlled laboratory conditions. The challenge has been to transfer these technically demanding methods to the study of large unselected populations.

The Avon Longitudinal Study of Parents and Children (ALSPAC), established in 1991, is a two-generational resource which aims to study the genetic and environmental determinants of development and health from the prenatal period into adulthood.[9] It provides an important opportunity to assess causal pathways, by exploring the impact of both genetic and environmental factors on the initiation and progression of cardiovascular disease[9] at an early age, minimizing the confounding effect of cumulative classical risk factors which are present in adulthood. We have assessed vascular function and stiffness in over 7000 children at age 10–11 years participating in ALSPAC. This ambitious large study involved several research technicians over 2 years, using a protocol which required multiple measurements in a restricted time frame. We report the technical, environmental, and biological factors which influence the acquisition of high-quality vascular data relevant to understanding the early phase of atherogenesis.

Methods

ALSPAC population

ALSPAC is a large longitudinal study of the childhood determinants of normal growth and development and of common complex diseases. The cohort and study design are described in detail elsewhere[9] (http://www.alspac.bris.ac.uk). Briefly, 15 541 pregnant women with an expected delivery date between the start of April 1991 and the end of December 1992 were enrolled. This represented 85% of the eligible general population in three health authorities in Bristol, UK. The cohort of 14 062 liveborn children has been followed up, initially with questionnaires through childhood, and at regular annual clinics since the age of 7. We undertook a study of measures of arterial structure and function in all children attending for assessment at age 10–11 years.

Approval for the study was obtained from the ALSPAC Law and Ethics Committee and also from the local NHS Research Ethics Committee; written informed consent/assent was obtained from both the parent/guardian and the child.

Anthropometric and environmental measurements

Infant gender was recorded in the delivery room and abstracted from obstetric records and/or birth notifications. Exact age was determined from the child's date of birth and date of the examination. Weight (to the nearest 0.1 kg), using SECA scales, and height (to the nearest 0.1 cm) using a Leicester height metre were measured. From these, BMI was calculated (weight/height^2, with weight in kilograms and height in metres). Tanner stages of pubertal development were assigned from the mothers' responses to mailed line drawings, on which they indicated stage of development of their offspring. Room and skin temperatures were assessed using a commercial digital thermometer. Immediately before the vascular examination, a short questionnaire was completed to ascertain details of other environmental factors (recent ingestion of fatty food and food/drink containing caffeine, recent vaccination, and recent infection) which may have had an impact on endothelial function.[10,11]

Vascular study protocol

Over a period of 5 months, six research technicians, who had no prior experience of the vascular methods or of ultrasound techniques, were trained to undertake vascular ultrasound and applanation tonometry. The children attended the clinic for multiple assessments that took over 3 h, of which 40 min was allocated to acquisition of the vascular measures. Within this constrained time period, pulse wave velocity (PWV) was assessed first using applanation tonometry, and this was followed by brachial artery distensibility and brachial endothelial function testing, using high-resolution ultrasound techniques.

Pulse wave velocity

Pressure-pulse waveforms were recorded transcutaneously using a high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, TX, USA) from the radial and carotid pulse synchronous with the ECG signal, which provides an R-timing reference. Integral software processed the data to calculate the mean time difference between R-wave and pressure wave on a beat-to-beat basis over 10 s, and the PWV was then calculated using the mean time difference and arterial path length between the two recording points (SphygmoCor version 7.1, Scanmed, UK).

Arterial distensibility

Ultrasound images of the right brachial artery were recorded onto SVHS video using an ALOKA 5500 high-resolution ultrasound system with a 5–10 MHz linear array probe (Keymed, UK). Brachial artery distensibility was assessed on the arterial segment subsequently imaged for flow-mediated dilatation (FMD) measurement (see below). Brachial artery blood pressure was measured in the contralateral arm using an oscillometric blood pressure device (Omron MI-5), at the time of image acquisition. A paediatric cuff was used when the arm circumference was <25 cm and an adult cuff when the arm circumference was >25 cm. Real-time B-mode images, recorded on SVHS video for 20 s, were saved and transported to a core laboratory in London for later offline analysis (Vascular Physiology Unit, Institute of Child Health). The distension of the artery was determined by measuring the luminal diameter excursion from diastole to systole. The distensibility coefficient (DC), which reflects intrinsic vascular wall elasticity, was calculated from the distension and the pulse pressure and was expressed as mean per cent change in cross-sectional area per unit change in blood pressure.

Brachial artery flow-mediated dilatation

Our technique has been reported previously.[12] Briefly, the right brachial artery was imaged, 5–10 cm above the antecubital fossa, using high-resolution ultrasound (ALOKA 5500) with the probe held in a stereotactic clamp that allowed micrometer positional adjustment. Brachial artery FMD was induced by a 5 min inflation of a pneumatic cuff to 200 mmHg, around the forearm immediately below the medial epicondyle, followed by rapid deflation using an automatic air regulator (Logan Research, UK). The diameter of the brachial artery was measured using edge detection software (Brachial Tools, MIA, IA, USA) from ECG-triggered ultrasound images captured at 3 s intervals throughout the 11 min recording protocol. Flow-mediated dilatation was expressed as the maximum percentage change in vessel diameter from baseline. The magnitude of the flow stimulus was recorded continuously by pulse wave Doppler and expressed as per cent reactive hyperaemia (RH%), derived from the maximum change in flow within 15 s of deflation of the pneumatic cuff, relative to the baseline flow.

Reproducibility assessment

Within- and between-technician reproducibility

Within- and between-technician reproducibility for acquisition of the vascular measures was assessed at three different stages of the study over the 2-year duration. Healthy staff volunteers (i.e. adults who were not ALSPAC participants), aged 18–30 years, were studied at the beginning ($n = 23$), middle ($n = 25$), and end ($n = 10$) of the study period (2 years). All of the participants underwent assessment of endothelial function and arterial stiffness on at least two occasions.

Within-participant clinic variability

The variability of the measures made in the ALSPAC clinic was assessed by collection of repeat measures in 3% of the cohort ($n = 231$). These children were selected randomly and invited to return for a second vascular assessment within 6 weeks of their initial visit. At the repeat scan, no attempt was made to replicate the scanning conditions of the first visit. This test of variability therefore includes the influence of the technician, study environment (time of day, temperature, position of ultrasound probe, and tonometer), and the physiological variations inherent in the haemodynamic and arterial measures (e.g. flow, vessel size, and reactive hyperaemia).

Image measurement reproducibility for flow-mediated dilatation

Reproducibility of the baseline diameter and the %FMD measurement from the stored images was assessed for inter- and intra-observer reader variability (70 and 50 studies, respectively, were repeated by different technicians).

Statistical analysis

Variability of the vascular measures was assessed by ANOVA. The impact of subject, technician, set, and study on the estimated variability of the method was assessed using multiple classification analysis and confidence intervals were obtained by the bootstrapping method,

Table 1 Characteristics of cohort participants

Characteristic (unit)	Number (%) with data	Distribution mean (SD) (range)
Female, *n* (%)	7557 (100)	3823 (50.6)
Age (years)	7557 (100)	10.7 (0.26) (9.8–12.3)
Height (cm)	7490 (99)	144 (6.73) (113.7–173.8)
Weight (kg)	7517 (99)	38.1 (8.63) (20.2–106.6)
BMI	7472 (99)	18.3 (3.16) (12.2–43.2)
Supine systolic blood pressure (mmHg)	7474 (99)	104.3 (9.23) (65–177)
Supine diastolic blood pressure (mmHg)	7474 (99)	60.2 (8.02) (30–105)
Pulse rate (b.p.m.)	7473 (99)	72 (11.2) (6–127)
Brachial artery diameter (mm)	6614 (88)	2.7 (0.31) (1.6–3.9)
Brachial artery blood flow (mL/min)	6197 (82)	63.5 (26.2) (13.4–278)
Reactive hyperaemia ratio (%)	6340 (84)	555 (144) (186–1341)
Flow-mediated dilation (%)	6614 (88)	8.1 (3.34) (−1.8 to 29.9)
Flow-mediated dilation absolute (mm)	6614 (88)	0.2 (0.08) (−0.1 to 0.7)
Pulse wave velocity (m/s)	7209 (95)	7.6 (1.22) (3.7–16.8)
Distensibility (% per mmHg)	6814 (90)	12.6 (6.11) (1.3–78.9)

Table 2 Reproducibility for vascular measures

	Study	Mean	% variation due to technician	% variation due to subject	SD differences for technician	SD/mean%
Baseline diameter	R1	3.4	0.3	71.2	0.06	1.9
	R2	3.1	0.6	84.9	0.07	2.3
	R3	3.3	0.7	89.1	0.08	2.4
FMD (%)	R1	7	3.1	66.8	0.7	10.5
	R2	9.5	1.6	68.6	0.8	7.7
	R3	9.8	2.8	86.5	0.8	7.7
PWV (m/s)	R1	8	3.3	41.7	0.4	4.6
	R2	8.5	1.4	43.9	0.3	3.4
	R3	8.5	3.4	35.8	0.3	4.1
DC (% per mmHg)	R2	0.07	1.4	68.7	0.005	6.6
	R3	0.09	6.4	48.2	0.009	10

R = reproducibility assessment (1 at beginning, 2 at middle, and 3 at end of the study). FMD, flow-mediated dilatation; PWV, pulse wave velocity; DC, distensibility coefficient; SD, standard deviation.

using 100 samples of size equal to data set, with replacement. Within cohort and within measurement reproducibility are expressed as coefficient of variation. Univariable and multivariable linear regression models have been used to describe anthropometric and environmental determinants of the vascular measurements. In the multivariate models, adjustment was made for age, sex, baseline diameter, BMI, systolic and diastolic blood pressure, and environmental factors (i.e. temperature, food, etc). Pearson's correlation coefficients were used to assess the interrelationships between vascular measures. All statistical analyses were performed using Stata version 8 statistical package and a *P*-value of <0.05 was considered statistically significant with a two-sided test.

Results

Population characteristics

Between February 2002 and October 2003, 7557 children agreed to attend for study (*Table 1*). The mean age of the participants was 10.7 (range 9.8–12.3) years. Only, 27 (0.4%) of the 7557 children attending the clinic refused to take part in the vascular session, which was successfully completed by 6484 (85.8%). A partial session was undertaken by the remaining 1046 (13.8%) children. Thirteen of the studied children had undergone vaccination within 2 weeks of the vascular study and 1149 (15%) were

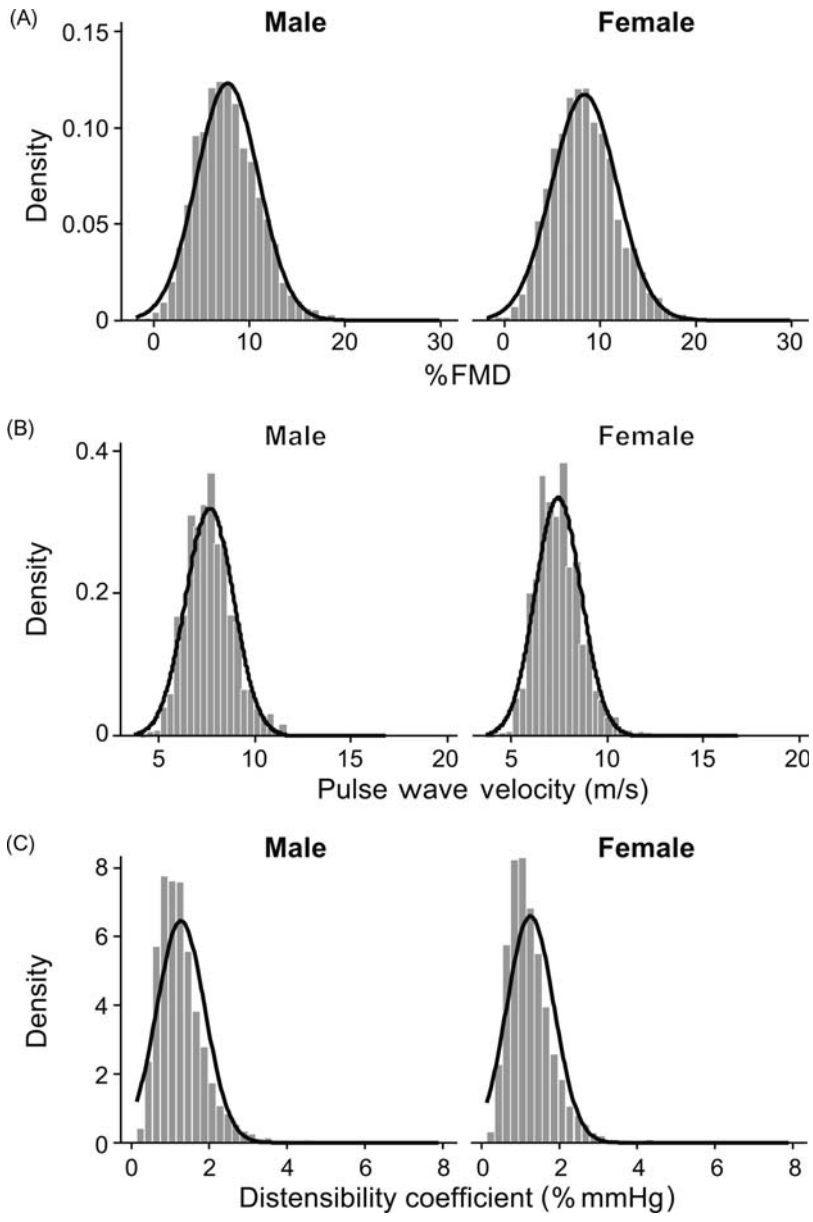

Figure 1 Distributions of vascular measures by sex. Distribution of flow-mediated dilatation, pulse wave velocity, and distensibility coefficient in males and females in the ALSPAC cohort.

convalescing from a mild upper respiratory infection but were well at the time of study.

No dietary restrictions and fasting protocols were applied before the vascular studies. Six hundred and sixty-four children documented consumption of either fried food ($n = 378$) or caffeine ($n = 584$) in the 2 h prior to the vascular assessment. Tanner questionnaires for pubertal status had been completed for 3931 (52%) of the children who attended for vascular studies.

Reproducibility

We found high levels of reproducibility within and between technicians at the beginning, in the middle, and the end of the data collection (assessed using healthy adult volunteers) (*Table 2*). The coefficient of variability remained unchanged over the 2-year study for the less technically demanding technique of PWV but improved for FMD. Less than 5% of the variation in the individual vascular measures could be explained by technician performance.

In the 3% random sample of the cohort who had a repeat set of measurements (with likely differences in time of day, technician, and position of probe in relation to cuff), the coefficients of variation for baseline diameter, FMD, PWV, and DC were 4.9, 10.9, 8.7, and 18. The inter- and intra-observer variability for analysis of the stored images was 1.6 and 1.2% for baseline diameter and 5.6 and 5.3% for FMD, respectively.

Vascular measurements

Flow-mediated dilatation was measured in 6614 (88%), arterial distensibility in 6814 (90%), and PWV in 7209 (95%) of the 7557 children who attended the 10-year follow-up clinic. Poor image quality or significant image movement prevented FMD analysis in 193 (3%) of the children. All vascular measures were normally distributed, showing a wide range of values that were similar in both sex (*Figure 1*). No systematic error in repeated measurements was detected for any vascular measures (*Figure 2*). Room and skin temperatures were positively associated with baseline diameter ($r = 0.2$ and $r = 0.4$, respectively, $P < 0.001$ for both). Baseline flow was strongly positively correlated with baseline diameter ($r = 0.7$, $P < 0.001$). Vessel size was inversely associated with %FMD (*Figure 3*). There was, however, no association between absolute FMD and baseline diameter ($P = 0.53$).

On univariate analysis, arterial DC showed a weak inverse relation with PWV ($r = -0.2$, $P < 0.001$). This association did not substantially change after adjustment for age, sex, brachial diameter, systolic and diastolic blood pressure, and temperature in multivariable analysis.

Vascular measures and anthropometric and environmental factors

Boys had larger vessels than girls (2.8 ± 0.3 vs. 2.6 ± 0.3 mm, respectively, $P < 0.001$) and they had consistently lower FMD and DC and higher PWV values ($P < 0.01$ for all), even after adjusting for vessel size (*Tables 3–5*). Increasing age, even within our narrow age range, was positively associated with increased PWV.

Weak associations were noted between room and skin temperatures and other environmental influences, i.e. ingestion of a fatty meal or caffeine within a 2 h period prior to the vascular assessment. Following multivariable analysis, these parameters only explained a very small proportion of the variation in vascular measures (R^2: 0.02 for FMD, 0.006 for DC, and 0.03 for PWV).

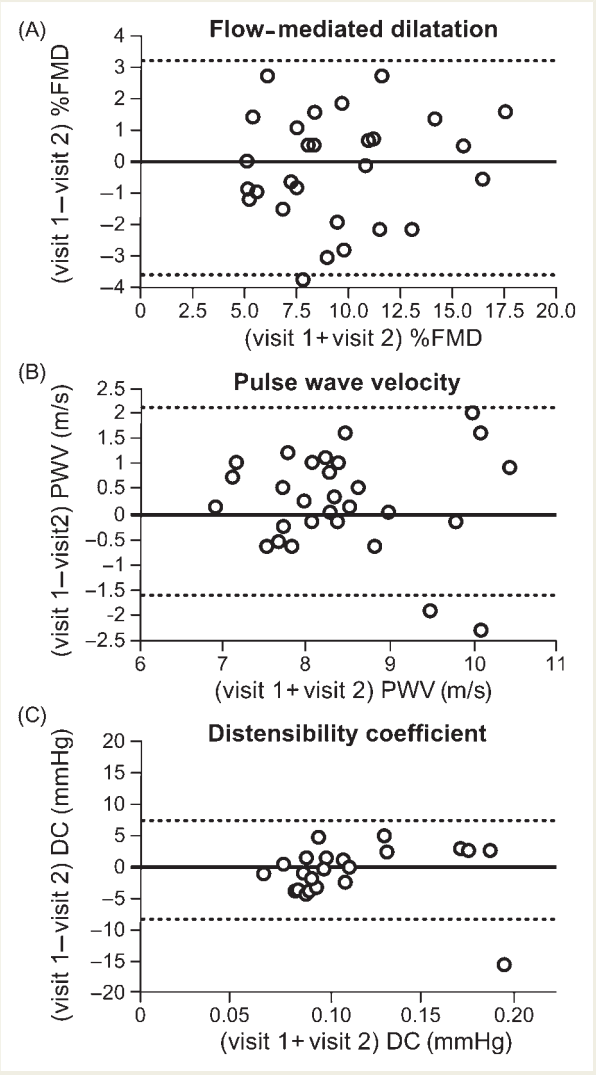

Figure 2 Reproducibility of vascular measures (Bland–Altman plots). The Bland–Altman plot for (*A*) flow-mediated dilatation, (*B*) pulse wave velocity, and (*C*) distensibility coefficient. Dotted lines represent the 95% confidence intervals.

Discussion

In this study, we demonstrate that pre-clinical vascular characterization of a very large childhood population is feasible, with high success rates for a range of vascular measures and excellent reproducibility, comparable to those reported in specialized vascular laboratories. There was a relatively small contribution of environmental factors to the variability of the vascular measures. These therefore should not be considered as limiting factors for implementation of vascular assessment both in field and clinical studies. Interestingly, within this 'healthy' UK pre-pubertal childhood population, we identified children with values of each of

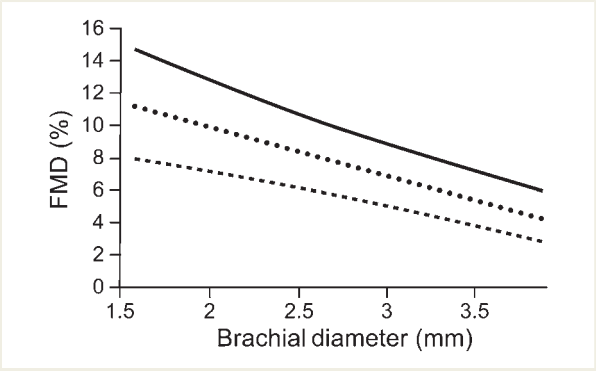

Figure 3 Nomogram for flow-mediated dilatation according to the vessel baseline diameter. A negative linear association exists between baseline diameter and flow-mediated dilatation. For a specific baseline diameter, flow-mediated dilatation can be estimated for a 10- to 11-year-old child. The black line shows the 75th percentile, the circle dashed line the 50th percentile, and the rectangular dashed line the 25th percentile of the population.

the three vascular measures which are comparable to those seen in adults with multiple cardiovascular risk factors or clinical cardiovascular disease.[13,14] This provides an excellent opportunity to explore causal pathways for the development of arterial disease from early life.

Atherosclerosis has a long pre-clinical phase from childhood, during which functional and structural arterial changes develop and evolve into advanced atherosclerotic lesions in adult life.[15] We chose to study endothelial function in conduit arteries because the endothelium plays a key role in regulating many of the vascular changes involved in the initiation and progression of early arterial disease. We measured FMD, a technique developed in our department. We and others have shown this to be both accurate and reproducible in the laboratory setting.[16] It reflects nitric oxide bioavailability and we have recently demonstrated that FMD predicts progression of structural arterial disease in middle-age asymptomatic individuals.[5] We also undertook, as part of our vascular assessment, measures that reflect changes in arterial 'stiffness' (PWV and distensibility).[17] These have been shown to progress in pre-clinical cohorts and to relate to future cardiovascular events.[18] Pulse wave velocity and distensibility

Table 3 Association of biological and environmental factors with %FMD

	Univariable mean FMD (95% CI)	*P*-value	Multivariable (95% CI)	*P*-value
Age (months)	−0.01 (−0.04, 0.01)	0.34	0.001 (−0.02, 0.03)	0.90
Gender				
Male	Reference		Reference	
Female	0.67 (0.51, 0.83)	<0.001	0.22 (0.06, 0.39)	0.01
BMI	0.01 (−0.01, 0.04)	0.32	0.11 (0.08, 0.14)	<0.001
Brachial diameter (mm)	−3 (−3.26, −2.75)	<0.001	−3.99 (−4.28, −3.7)	0.00
Systolic blood pressure (mmHg)	0.003 (−0.01, 0.01)	0.52	−0.001 (−0.01, 0.01)	0.79
Diastolic blood pressure (mmHg)	0.02 (0.01, 0.03)	<0.001	0.02 (0.01, 0.03)	<0.001
Room temperature (°C)	0.14 (0.1, 0.18)	<0.001	0.12 (0.08, 0.17)	<0.001
Skin temperature (°C)	0.15 (0.13, 0.18)	<0.001	0.24 (0.21, 0.26)	<0.001
Time of day (h)	0.07 (0.03, 0.11)	<0.001	−0.01 (−0.05, 0.03)	0.61
Recent infection				
No	Reference		Reference	
Yes	−0.16 (−0.39, 0.06)	0.15	−0.26 (−0.47, −0.05)	0.02
Recent vaccination				
No	Reference		Reference	
Yes	−1.83 (−3.81, 0.15)	0.07	−1.42 (−3.26, 0.42)	0.13
Consumed fried food in last 2 h				
No	Reference		Reference	
Yes	−0.04 (−0.41, 0.33)	0.83	−0.15 (−0.51, 0.21)	0.42
Consumed caffeine in last 2 h				
No	Reference		Reference	
Yes	0.3 (−0.01, 0.6)	0.05	0.29 (−0.01, 0.59)	0.06

FMD, flow-mediated dilatation (%); BMI, body mass index (kg/m^2). In the multivariate models, adjustment was made for age, sex, baseline diameter, BMI, systolic and diastolic blood pressure, and environmental factors (i.e. room and skin temperatures, time of day, recent infection, recent vaccination, and consumption of food or caffeine in the last 2 h).

Table 4 Association of biological and environmental factors with PWV

	Univariable mean PWV (95% CI)	*P*-value	Multivariable (95% CI)	*P*-value
Age (months)	0.009 (0, 0.02)	0.03	0.01 (0, 0.02)	0.014
Gender				
Male	Reference		Reference	
Female	−0.16 (−0.22, −0.1)	<0.001	−0.24 (−0.3, −0.18)	<0.001
BMI (kg/m^2)	−0.07 (−0.08, −0.06)	<0.001	−0.07 (−0.08, −0.06)	<0.001
Brachial diameter (mm)	−0.51 (−0.61, −0.41)	<0.001	−0.25 (−0.36, −0.14)	<0.001
Systolic blood pressure (mmHg)	0.01 (0.01, 0.02)	<0.001	0 (−0.01, 0)	0.48
Diastolic blood pressure (mmHg)	0.03 (0.03, 0.04)	<0.001	0.03 (0.03, 0.04)	<0.001
Room temperature (°C)	−0.06 (−0.08, −0.05)	<0.001	−0.03 (−0.04, −0.01)	0.002
Skin temperature (°C)	−0.03 (−0.05, −0.02)	<0.001	−0.004 (−0.02, 0.01)	0.43
Time of day (h)	−0.04 (−0.05, −0.03)	<0.001	−0.01 (−0.02, 0.01)	0.2
Recent infection				
No	Reference		Reference	
Yes	0.11 (0.03, 0.19)	0.01	0.08 (−0.002, 0.15)	0.06
Recent vaccination				
No	Reference		Reference	
Yes	0.16 (−0.64, 0.95)	0.7	0.15 (−0.61, 0.91)	0.69
Consumed fried food in last 2 h				
No	Reference		Reference	
Yes	−0.24 (−0.37, −0.1)	<0.001	−0.19 (−0.32, −0.06)	0.006
Consumed caffeine in last 2 h				
No	Reference		Reference	
Yes	0.06 (−0.05, 0.17)	0.31	0.04 (−0.07, 0.15)	0.48

PWV, pulse wave velocity (m/s); BMI, body mass index. In the multivariate models, adjustment was made for age, sex, baseline diameter, BMI, systolic and diastolic blood pressure, and environmental factors (i.e. room and skin temperatures, time of day, recent infection, recent vaccination, and consumption of food or caffeine in the last 2 h).

provide a composite assessment which includes structural arterial wall changes and are therefore less susceptible to acute influences than FMD. These measures are relatively easy to perform. This was reflected in lower variability from the onset of the study, and a less pronounced learning curve over the study period than that seen for FMD. The three techniques evaluate different aspects of vascular function and structure and, only a weak correlation was detected between the measures at this early stage of disease evolution.

Our vascular study in the ALSPAC cohort is the largest reported evaluation of pre-pubertal children and multiple technicians were required to study up to 32 children per day. With appropriate training, individuals with no prior ultrasound experience were capable of competently performing the technically challenging vascular assessments and were able to achieve high reproducibility figures similar to those reported in specialized vascular laboratories.[12] The variability assessment in the randomly selected cohort of 3% of the ALSPAC children incorporates all the technical, physiological, and logistical factors that influence these measures in children. It is not surprising therefore that there was greater variability in this measure for all tests than in the structured reproducibility evaluations. It is important to standardize methodology so that comparisons can be made between different studies.[12] A number of environmental influences have been shown to affect both PWV and ultrasound tests, especially FMD.[11,19,20] We were able to examine the effect of room and skin temperatures (over a modest range), time of day, and consumption of caffeine or fried foods and we confirmed the previously reported relationships with all three vascular measures. Their contribution, however, to the variability was very small and this is reassuring for future field studies as it is unlikely that environmental factors, unless extreme, will confound study of the underlying arterial phenotype, its determinants, and response to treatment.

Vessel size contributes to FMD responses both by an impact on blood flow-related shear stress on the vessel wall and by its component in the calculation of %FMD as usually reported. However, the absolute change in FMD (millimetres) was unrelated to resting vessel size. We provide a nomogram, relating resting vessel size to %FMD in childhood, based on our large cohort which can be used for adjustment for differences in baseline diameters.

Flow-mediated dilatation values were higher in girls and this could only partially be explained by the larger resting vessel diameter in boys. There was an unexpected positive relation between

Table 5 Association of biological and environmental factors with distensibility coefficient

	Univariable mean FMD difference (95% CI)	*P*-value	Multivariable (95% CI)	*P*-value
Age (months)	0.05 (0, 0.1)	0.03	0.07 (0.03, 0.12)	0.002
Gender				
Male	Reference		Reference	
Female	−0.12 (−0.41, 0.18)	0.44	−0.75 (−1.05, −0.44)	<0.001
BMI	0.04 (−0.01, 0.09)	0.12	0.17 (0.12, 0.22)	<0.001
Brachial diameter (mm)	−2.29 (−2.77, −1.8)	<0.001	−2.93 (−3.48, −2.38)	<0.001
Systolic blood pressure (mmHg)	−0.07 (−0.09, −0.06)	<0.001	−0.16 (−0.18, −0.14)	<0.001
Diastolic blood pressure (mmHg)	0.06 (0.04, 0.08)	<0.001	0.15 (0.13, 0.18)	<0.001
Room temperature (°C)	−0.06 (−0.13, 0.01)	0.08	0.01 (−0.07, 0.1)	0.72
Skin temperature (°C)	0.01 (−0.04, 0.06)	0.68	0.07 (0.02, 0.13)	0.01
Time of day (h)	−0.06 (−0.13, 0.01)	0.08	−0.05 (−0.13, 0.03)	0.20
Recent infection				
No	Reference		Reference	
Yes	−0.07 (−0.47, 0.33)	0.73	−0.24 (−0.63, 0.15)	0.23
Recent vaccination				
No	Reference		Reference	
Yes	3.59 (−0.41,7.6)	0.08	3.99 (0.11,7.88)	0.04
Consumed fried food in last 2 h				
No	Reference		Reference	
Yes	−0.33 (−1, 0.33)	0.32	0.06 (−0.62, 0.73)	0.87
Consumed caffeine in last 2 h				
No	Reference		Reference	
Yes	−0.55 (−1.1, 0)	0.05	−0.58 (−1.14, −0.02)	0.04

BMI, body mass index. In the multivariate models, adjustment was made for age, sex, baseline diameter, BMI, systolic and diastolic blood pressure, and environmental factors (i.e. room and skin temperatures, time of day, recent infection, recent vaccination, and consumption of food or caffeine in the last 2 h).

BMI and FMD as well as a negative relation to PWV. This differs from findings in smaller post-pubertal cohorts reported by our group and others. These differences between sex and the impact of changes in adiposity after puberty require further study.

Reduction in the population burden of atherosclerosis will require effective prevention strategies, with increasing emphasis in the young. Many adverse lifestyle risk factors are established in childhood and track in adult life. We have demonstrated that important aspects of vascular structure and function relevant to atherogenesis can be characterized non-invasively in a large young population. This should enable better understanding of the initiation and progression of early arterial disease and provide opportunities for prevention.

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