Associations of retinal microvascular caliber with large arterial function and structure: A population-based study of 11-12 year-olds and mid-life adults

Running title: Co-variation of micro and macrovascular phenotypes

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Abstract

Objective: We examined associations between retinal microvascular and large arterial phenotypes to explore relationships between the micro- and macro-vasculature in childhood and mid-life.

Methods: Participants were 1288 children (11-12 years, 50.9% female) and 1264 adults (mean age 44 years, 87.6% female) in a cross-sectional population-based study. Exposures were retinal arteriolar and venular caliber quantified from retinal images. Outcomes included arterial function (pulse wave velocity, PWV; carotid arterial elasticity) and structure (carotid intima-media thickness, CIMT). Multivariable regression models were performed adjusting for age, sex and family socioeconomic position.

Results: In children, one standard deviation (SD) wider arteriolar caliber was associated with slower PWV (-0.09SD, 95%CI -0.16, -0.02) and higher elasticity (0.13SD, 95%CI 0.06, 0.20); per SD wider venular caliber was associated with faster PWV (0.06SD, 95%CI -0.01, 0.12) and lower elasticity (-0.08SD, 95%CI -0.14, -0.01). The size of adult associations was approximately double. Wider arteriolar caliber was associated with smaller CIMT (-0.09SD, 95%CI -0.16, -0.03) in adults but not children. Venular caliber and CIMT showed little evidence of association.

Conclusions: Narrower retinal arterioles and wider venules are associated with large arterial function as early as mid-childhood. Associations strengthen by mid-life and also extend to arterial structure, although effect sizes remain small.

Keywords: Retinal arteriolar caliber; retinal venular caliber; carotid intima-media thickness; pulse wave velocity; arterial elasticity; population-based study.

1. Introduction

Identifying individuals at increased risk for cardiovascular disease (CVD) early in life could help target interventions to reduce the global disease burden.[1, 2] Decades of epidemiological and clinical evidence show that large artery measures consistently predict atherosclerosis throughout the lifespan, and future CVD outcomes in adults.[3, 4] Commonly-used indicators of poorer large artery cardiovascular phenotypes include faster pulse wave velocity (PWV), reduced arterial elasticity and increased carotid intima-media thickness (CIMT).[5-7]

While generally receiving less public health attention, the microcirculation is also associated with CVD.[8, 9] The retina is a unique site from which the microcirculation can be imaged directly and non-invasively, with repeated measurement possible over time.[10] Structural vascular changes in the retina – in particular, arteriolar narrowing and venular dilation – have been associated with systemic CVD, including coronary heart disease and heart failure.[11-13]

The retinal blood vessels and large arteries are inherently interconnected elements of the vascular tree and are influenced by the same intrinsic and environmental features with systemic circulation.[14, 15] However, the extent to which microvascular and macrovascular phenotypes co-develop, and if so from what age and from what state of health, are unclear. We previously conducted a systematic review of 26 studies, including a meta-analysis of five of these studies, to examine the association between retinal vascular caliber and intermediate phenotypes of large arteries (ie, CIMT, PWV).[16] Most studies involved adults in later life,

with only one study in children.[17] Retinal arteriolar caliber showed small associations with large arterial function (pooled estimates r = -0.17) and arterial structure (r = -0.05), with stronger evidence in cardiometabolic patients than in the general population. Thus, large arteries may share some pathophysiologic processes with the microvasculature, at least by later life and in those already expressing disease endpoints.

However, there is a paucity of data from children and younger adults, and it remains unclear when in the life course interrelationships between the small and large vascular phenotypes emerge and how they change with age. If they already co-vary prior to emergent disease, then this would suggest that the relationship is not solely the result of dysregulation, potentially advancing understanding of CVD pathogenesis. With technology and artificial intelligence putting retinal imaging into clinical and population research, microvascular status could potentially enter precision prediction models for future CVD and other diseases.[18] Before this could take place, it is important to understand the small vessels' unique and shared contributions with more traditional large artery measures. Besides, most previous studies have only assessed one aspect of large arterial health (ie, functional *or* structural intermediate phenotypes), but not both in the same individuals.[16] Assuming that large arterial dysfunction precedes structural changes, retinal microvascular associations with large artery function might emerge earlier than with large artery structure.[19]

The national, population-based Longitudinal Study of Australian Children (LSAC) provided an opportunity to address these evidence gaps in two generations taking part in a large cross-

sectional wave explicitly focusing on phenotypic health measurement. We aimed to examine associations of retinal microvascular caliber with large arterial intermediate phenotypes (measured by PWV, carotid arterial elasticity and CIMT) in Australian children aged 11-12 years and mid-life adults (the children's parents).

2. Materials and methods

2.1 Study design and participants

The Child Health CheckPoint (CheckPoint) is a cross-sectional population-derived wave nested within the national LSAC study.[20] Details of the initial study design and recruitment are outlined elsewhere.[21, 22] Briefly, LSAC recruited a nationally representative birth cohort (B cohort) of 5107 infants using a 2-stage clustered design and has since followed them in biennial 'waves' of data collection up to 2016. Most of families retained to wave 6 in 2014 (73.7%, n=3764).

At the wave 6 visit, all participating families (n=3513) were invited to consent to their contact details being shared with the CheckPoint team. CheckPoint took place between February 2015 and March 2016, nested between LSAC waves 6 and 7 at child age 11-12 years. In total, 1874 children and their parents participated in this detailed cross-sectional biophysical assessment.[23] Most attended the CheckPoint Main Assessment Centre in seven large cities around Australia, where all measures were performed. A small minority of families who attended more limited assessments in regional cities or home visits are not included in the analyses as the retinal photograph was not collected at these visits, and home visits did not include CIMT and arterial elasticity because the large and delicate equipment could not be readily transported.

The CheckPoint study was approved by the Royal Children's Hospital Melbourne Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics

Committee (14-26). The attending parents/caregivers provided written informed consent for themselves and their children to participate in the study.

2.2 Retinal vascular caliber assessment

Two optic disc-centered digital photographs from each eye were taken by a fundus camera (EOS 60D SLR) in a darkened room. Details of scoring procedures are described elsewhere.[24] Briefly, right eye images were selected as the first choice for scoring. Each image was graded by one of four experienced graders (two from the Zhongshan Ophthalmic Centre, China and two from the Centre for Eye Research Australia, Australia) masked to participant age and sex using the software program Integrative Vessel Analysis (IVAN, University of Wisconsin, Madison, USA). Retinal vessels were identified as arterioles or venules from a specific area (one-half to one-disc diameter from the margin of the optic disc). Each grader selected a segment of each vessel within this area for measurement. Diameters of all the selected segments were measured automatically by the IVAN software. Summary estimates of the average retinal vascular caliber were calculated combining measurements of the six largest arterioles or venules according to the revised Knudston-Parr formula.[25] Reproducibility of measurement by IVAN was high, with inter- and intra-grader intraclass correlation coefficients ranging of 0.76 to 0.99.[24]

2.3 Large arterial intermediate phenotypes

Details of each measure are described elsewhere.[26, 27] Carotid-femoral PWV measures arterial function and was assessed using the Sphygmocor XCEL (AtCor Medical Pty Ltd., West

Ryde, Australia) with participants in the supine position. Briefly, the arterial waveforms of the right carotid and femoral artery were measured by blood pressure cuffs on the arm and leg. Data were collected one to three times for each participant and the mean was calculated. PWV was calculated as the ratio of the distance traveled by the pulse wave and the time delay between the waveforms, as expressed in meters per second (m/s). The agreement of PWV waveforms between two analysts was high (0.99).[26] A quicker pulse wave indicates higher levels of arterial stiffness.

CIMT measures large arterial structure, an indicator of subclinical atherosclerosis.[28] It was assessed using standardized protocols via B-mode ultrasound (GE Healthcare Vivid-I BT06 with 10MHz linear array probe, Chicago, IL, USA).[29, 30] To image the right carotid artery, the participants lay in the supine position with the neck slightly extended and the head turned 45° towards the left. Measurements were made at the distal 10mm of the right common carotid artery, and images were taken across five sites within a minimum of three longitudinal loops of five to ten cardiac cycles. CIMT is defined as the distance from the lumen-intima interface to the media-adventitia interface. Images were analyzed using semi-automated edge-detection software (Carotid Analyser, Medical Imaging Applications, Coralville, IA, USA) to calculate CIMT far wall mean, where higher scores represent a thicker carotid artery wall. The inter- and intra-grader intraclass correlation coefficients of CIMT analyses were moderate, ranging of 0.59 to 0.71.[26]

Carotid arterial elasticity (%/mmHg) measures the ability of the arteries to expand as a

response to pulse pressure caused by cardiac contraction and relaxation.[31] We calculated elasticity from carotid artery images using intima-intima lumen diameter (LD) and the different value of systolic blood pressure and diastolic blood pressure (ΔP), according to previously published work from the Cardiovascular Risk in Young Finns Study:[32]

$$\frac{\left(\frac{LD_{max} - LD_{min}}{LD_{min}}\right)}{\Delta P} \times 100\%$$

Lower arterial elasticity is considered to represent lower arterial compliance.

2.4 Assessment of covariates

We considered several covariates when examining associations between retinal microvascular caliber and large artery phenotypes. The technical details of these measures are summarized in Table 1. For example, age, sex and family socioeconomic position (SEP) were considered as covariates, given that both large arterial and microvascular phenotypes have been shown to vary by age (worse with age), have distinct sex differences, and to be worse in those from poorer socioeconomic groups.[12, 26] Body mass index (BMI, z-score for children),[33] low-density lipoprotein (LDL) cholesterol, smoking exposure (passive smoking for children and ever smoked for adults) and early life risk factors for children (birth weight and gestational age) were also included as covariates. These are commonly reported risk factors for cardiovascular disease that have been reported to influence both large arterial and microvascular variations.[12, 34-36] Additionally, we considered mean arterial blood pressure as a covariate as it might lie on the causal pathway linking microvascular variations to large

arterial changes.[15]

2.5 Statistical analyses

Linear regression assumptions were tested when fitting models to assess associations of retinal vascular caliber with large arterial intermediate phenotypes in children and adults. We considered retinal arteriolar or venular caliber as the independent variable and large arterial intermediate phenotypes as the dependent variable,[37, 38] although the reverse would be equally plausible since they were measured cross-sectionally. It has been proposed that modeling arteriolar and venular caliber simultaneously may give less biased results.[39] We thus performed all analyses with both arteriolar and venular caliber in the same model.

We fitted two main models and ran one sensitivity analysis. Model 1 estimates were adjusted for age, sex, SEP and jointly for arteriolar or venular caliber. Model 2 estimates for children additionally adjusted for BMI z-score, LDL cholesterol, passive smoking, birth weight and gestational age; for adults, we further adjusted estimates for BMI, LDL cholesterol and ever smoking. This fully adjusted model could inform whether the association is independent of these common potential risk factors. We also conducted a sensitivity analysis further adjusting estimates for mean arterial blood pressure to examine whether it could lie on the causal pathway. For all analyses, we internally standardized exposures and outcomes (ie, mean of zero and a standard deviation [SD] of 1) to present standardized regression coefficients. All analyses were conducted in Stata 14.2 (StataCorp LP, TX, USA).

3. Results

3.1 Sample characteristics

Supplementary Figure presents the study flow from wave 1 of LSAC onwards. Of the 1874 parent-child dyads that took part in CheckPoint, 1288 children and 1264 adults had retinal caliber data available. The CheckPoint sample mean family SEP (0.26) was above the mean SEP of all families enrolled in LSAC at wave 1 (mean 0, SD 1), indicating more advantage in participating families. Participants with retinal data available were similar to those without these images, except for family SEP, which was around a quarter of a SD lower (ie, less advantaged) in those missing retinal data (Table 2). In addition, children without retinal images were slightly older (11.7 vs. 11.4 years of age) and more likely to be male (51% vs. 45%).

3.2 Main results

Table 3 shows the associations of retinal vascular caliber with large arterial phenotypes in children and adults. In Model 1 (estimates adjusted for age, sex, SEP and jointly considering arteriolar and venular caliber), both worse arteriolar and venular caliber were found to consistently associate with adverse large arterial function. For example, in children, a one SD higher arteriolar caliber was associated with slower PWV (-0.15SD, 95% CI -0.21 to -0.09) and higher arterial elasticity (0.14SD, 95% CI 0.06 to 0.20), while a one SD higher venular caliber was associated with faster PWV (0.09SD, 95% CI 0.03 to 0.15) and lower arterial elasticity (-0.07SD, 95% CI -0.13 to -0.01). Similar patterns were mirrored in adults, but associations were about twice the size seen in children (Figure).

In Model 2, when estimates were additionally adjusted for common risk factors (ie BMI, LDL and smoking exposure), the associations of arteriolar and venular caliber with PWV were attenuated in both children and adults by 30-40%. For example, in children per SD higher

arteriolar caliber was associated with slower PWV (-0.09SD, 95% CI -0.16 to -0.02), while higher venular caliber was associated with faster PWV (0.06SD, 95% CI -0.01 to 0.12). The associations with arteriolar elasticity changed little after the further adjustment in Model 2. The model R^2 for the association of retinal vascular caliber and functional phenotypes was about 6% to 13% in children and about 30% in adults.

Results were mixed for associations of retinal microvascular caliber and large arterial structure. There was little evidence of an association between arteriolar caliber and CIMT in children, while in adults, per SD higher arteriolar caliber was associated with smaller CIMT scores (-0.09SD, 95% CI -0.16 to -0.03, Model 1), and this association attenuated by 44% after common risk factor adjustment (-0.05SD, 95% CI -0.12 to 0.01, Model 2). Unexpectedly, higher retinal venular caliber showed small associations with smaller CIMT in children (-0.08SD, 95% CI -0.15 to -0.01), but there was little evidence of an association in adults. The proportion of variance explained in model 2 was about 4% for children and 20% for adults.

3.3 Sensitivity analysis

Further adjustment for mean arterial blood pressure attenuated, but did not eliminate, associations of arteriolar and venular caliber with arterial elasticity in children and adults. However, it fully attenuated associations with PWV (Table 3). Adding mean arterial blood pressure to the models did not change the lack of adjusted associations with CIMT.

4. Discussion

Principal findings

In this study, we found that the microvasculature is associated with functional artery phenotypes (ie, PWV, arterial elasticity) from early in life, and this association strengthens with age. By midlife, there is evidence of an association between the microvasculature and large artery structural phenotypes (ie, greater CIMT). It is likely that these associations will increase with age and with the development of manifest cardiometabolic disease.[16]

Strengths and limitations

Our study has several strengths. It adds important new knowledge to the only other study (to our knowledge) to consider the association of the microvascular and large arterial intermediate phenotypes in generally healthy children.[17] Our study drew on a large, randomly-selected national sample of Australian children and their parents, spanning diverse geographical areas across a very large country. All measures were objectively measured and processed under standard operating procedures, with moderate to high inter- and intra-rater reliability, and were conducted at the same time using the same equipment and processes for the two generations. This cross-generational design enabled us to efficiently infer temporal strengthening of associations with age, which would otherwise take decades to observe. Our well-phenotyped sample allowed us to consider the effects of important covariates. Unlike other studies, we were able to examine relationships of retinal microvascular caliber with both arterial function and structure simultaneously in the same individuals, across the two generations.

There are also limitations. Firstly, as our analytic sample was on average somewhat more advantaged than the national average, our findings may not generalize well to the most disadvantaged families (some of whom were nonetheless included). Secondly, limited data

were available for adult men as most adults in the CheckPoint sample were mothers, but similar patterns of associations were nonetheless seen in adult women and men (Supplementary Table). Thirdly, the cross-sectional nature of the study precludes inference regarding the temporal sequence of the associations.

Interpretation in light of other studies

To date, only one study has examined similar associations in healthy children.[17] The Generation R Study found that in children (median age six years) each SD increase in retinal arteriolar and venular caliber was associated with 0.02SD (95% CI -0.01 to 0.05) and 0.04SD (95% CI 0.01 to 0.07) higher carotid-femoral PWV (SD 0.9 cm/s).[17] In our study with children aged 11-12 years, we found a much stronger association with PWV and replicated this finding with another arterial function measure, arterial elasticity. In turn, we now show that the magnitude of associations between retinal vascular caliber and arterial functional phenotypes roughly doubles between mid-childhood and mid-adulthood. The explanatory power (ie, R^2 values) was small at age 11-12 years and much stronger in adults. These results are consistent with our meta-analysis of slightly older adults (mean age 54 years), which showed narrower retinal arteriolar caliber was associated with faster PWV (r = -0.17, 95%CI -0.25 to -0.10).[16]

We found little evidence that retinal arteriolar caliber was associated with CIMT in children, but a small association was observed in adults. In children of similar age (mean age 13.6 years) with type 1 diabetes, retinal vascular caliber has been associated with CIMT.[40] In our meta-analysis with older adults (mean age 63 years), narrower retinal arteriolar caliber was weakly associated with higher CIMT scores (r = -0.05, 95%CI -0.09 to -0.02),[16] and effect sizes were greater in patients with cardiometabolic disease. Taken together, both age and cardiometabolic disease conditions may contribute to the degree of co-variation between

small and large artery structure.

Higher CIMT is associated with adverse changes in arterial structure, which has been shown to be linked with cardiovascular risk factors such as obesity and hypertension in children.[41] The association we observed between wider (worse) venular caliber and lower (better) CIMT in children may be a chance finding, given its very small magnitude, unexpected direction and full attenuation on adjustment and in the sensitivity analysis.

Implications

The potential association between the macro- or microcirculation and CVD risk are usually investigated separately. While it is plausible that their genetic determinants could be quite separate, biologically the two vascular systems are functionally related to the pulsatile transmission of pressure and flow.[15] We have provided population-based evidence that the phenotypes of macro- and microcirculation co-vary from mid-childhood, and other studies suggest that this relationship is emerging from as young as six years onwards.[17] The strength of the association increases with both age and with manifest cardiometabolic disease.[16]

Some of the observed associations between the macro- and microcirculation may arise from shared cardiometabolic risk factors.[42] For example, we found that cardiovascular risk factors such as BMI, LDL cholesterol, and smoking exposures attenuated the association of retinal vascular caliber with functional and structural large artery phenotypes to a varying extent. However, these factors only contributed to a small amount of co-variations between small and large vascular phenotypes. It may be that small and large vessels are differentially susceptible to risk factors,[8] or that they are mainly determined by distinct factors. In addition, the fully attenuated relationships between retinal caliber and PWV after adjusting for mean arterial blood pressure suggests the association may be mediated via blood pressure. This further emphasizes the likely importance of public health interventions to reduce blood

pressure throughout life in order to protect all elements of the vascular tree.

There is strong evidence that microvascular dysfunction plays an important role in the pathogenesis of CVD.[9] The intermediate phenotype of large arteries represents a snapshot of preclinical cardiovascular health. In the current study, we found that phenotypic measures of microcirculation were associated with large arterial phenotypes, which in keeping with experiment models showing that abnormality of small and large vessels often coexist in the pathogenesis of CVD.[43] Given the association between microvascular and large arterial phenotypes was relatively small, each phenotype may contribute to CVD through largely separate causal pathways. Further studies with clinical CVD outcomes would determine whether combining phenotypes of the microvasculature and large arteries improves risk prediction and inform the optimal use of these measures.

Conclusion

Retinal microvascular caliber is associated with large arterial function by mid-childhood, with stronger (though still small) associations observed in mid-life. Cumulative exposure to shared risk factors may underlie the age-dependent co-variation between small and large arterial phenotypes. Longitudinal studies with clinical outcomes would inform the optimal use of these parameters in early cardiovascular risk management.

Perspectives

Variations in the micro- and macro-vasculature underlie CVD pathogenesis, but how they may influence each other at different life stages is not well understood. We showed a small amount of co-variation between the two in childhood, which doubled in strength by mid-life. This suggests it is important to understand the unique contribution of both the micro- and

macro-vasculature to CVD across the life course, and whether the assessment of both the small and large vessels could improve risk prediction for CVD**.**

Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

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Author contributions

MW conceived and led the CheckPoint study with the CheckPoint team, and was LSAC's Health Design Leader. KL was the primary student supervisor, along with MW, DB, TW, MH, and oversaw all aspects of the study and the manuscript preparation. ML performed data analysis and wrote the main paper. All authors provided expert advice and critical review of this manuscript, and had final approval of the submitted and published version of this paper.

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Legends

TABLES

Table 1. Measures of covariates

Measure	Equipment	Brief protocol
Model 1		
Age	Parental questionnaire	Age was calculated to nearest week using date of birth, either imported from Medicare Australia's database at the time of LSAC enrolment (child) or self-reported (parent), and date of assessment.
Sex	Parental questionnaire	Sex was self-reported in the CheckPoint questionnaire by children and parents respectively.
Family socioeconomic position (SEP)	Parental questionnaire	SEP was measured in LSAC wave 6 (child age 10-11), approximately 12 months prior to measurement of biophysical measures in CheckPoint. This SEP variable summarized parent-reported combined household income, current or most recent occupation of each parent and highest achieved educational qualification of each parent. Each component of the score was scaled and an unweighted average was calculated over three values in a single parent household, or over five values in a dual parent household. The unweighted average variable at LSAC was then standardized within the wave to have a mean 0, and standard deviation (SD) of 1.
Model 2		
Body mass index (BMI)	Height: Invicta Stadiometer	Height (cm): Participants were measured in bare feet. Mean of two measurements used. If two measures differ by >0.5cm, a third was taken and mean of all measurements was used.
	Weight: 4-limb	Weight (kg): Bare feet and in light clothing, participants' weight were measured to the nearest 50g.

Measure	Equipment	Brief protocol
	segmental body composition scales	BMI was calculated as kg/m^2. For children, an age- and sex-adjusted BMI z-score was calculated using the US Centres for Disease Control and Prevention growth reference charts.[33]
Low-density lipoprotein cholesterol	Proton nuclear magnetic resonance (NMR)	Venous blood samples of 30 ml were collected from each participant at the main and mini assessment centers. Participants were at a minimum semi-fasted (ie, no food/drink for at least 2 hours). A high-throughput NMR metabolomics platform was used to quantify the lipid and metabolite measures from stored EDTA plasma samples. The platform applies a single experimental setup, which allows for the simultaneous quantification of routine lipids
Smoking exposure	Parental questionnaire	At each LSAC wave, parents reported the number of smokers at home. We created a binary variable of "ever exposed to passive smoke" from this data; it was positive for the child if the answer to the smokers at home question in any LSAC wave (age 0 to 11) was greater than zero.
Birth weight	Parental questionnaire	At LSAC Wave 1, the parent was asked "How much did the child weigh at birth?"
Gestational age	Parent Health Record Parental questionnaire	At LSAC Wave 1, the interviewer copied gestational age from the Parent Health Record recorded prospectively by the birthing hospital (the great majority). If the record was not available, interviewers asked parents the question "After how many weeks of pregnancy was [child's name] born?"
Sensitivity analysis		
Mean arterial	SphygmoCor	Following seven minutes in the supine position at rest, systolic and

Measure	Equipment	Brief protocol
blood pressure	XCEL	diastolic blood pressures were measured at the brachial artery up to three times, with mean values reported. Mean arterial blood pressure was calculated as 1/3 systolic and 2/3 diastolic blood pressure.

Table 2. Sample characteristics

Characteristics [a]	Children				Adults			
	With data on retinal image		Without data on retinal image		With data on retinal image		Without data on retinal image	
	N	Mean (SD [b]) or %	N	Mean (SD [b]) or %	N	Mean (SD [b]) or %	N	Mean (SD [b]) or %
Age (years)	12 88	11.4 (0.5)	58 6	11.7 (0.5)	12 64	43.8 (5.1)	610	43.6 (5.6)
Gender (% female)	12 88	50.9	58 6	45.1	12 64	87.6	610	90.0
Birth weight (kg)	12 85	3.44 (0.55)	58 5	3.43 (0.62)				
Gestational age (weeks)	12 79	39.2 (2.0)	58 6	38.9 (4.0)				
Socioeconomic position	12 83	0.26 (0.97)	58 4	-0.02 (1.01)	12 60	0.26 (0.97)	607	-0.01 (1.01)
Smoking exposure	12 87	20.7	58 6	27.8	12 63	8.4	610	11.2
Body mass index z-score [c]	12 88	0.30 (0.96)	58 4	0.31 (1.04)				
Body mass index (kg/m^2)					12 59	27.86 (6.15)	602	27.74 (6.11)
Mean arterial blood pressure (mmHg)	12 13	78.07 (5.66)	56 4	76.65 (6.03)	11 69	89.05 (9.70)	580	87.20 (8.98)

Retinal arteriolar calibre (μm)	12 88	159.1 (11.9)			12 64	151.0 (14.0)		
Retinal venular calibre (μm)	12 88	230.7 (16.6)			12 64	218.9 (18.5)		
Pulse wave velocity (m/s)	12 42	4.44 (0.54)	56 4	4.49 (0.63)	11 34	6.96 (1.14)	541	6.66 (1.08)
Arterial elasticity (%/10mmHg)	11 51	4.83 (0.87)	19 3	4.66 (1.00)	10 53	2.44 (0.06)	199	2.50 (0.66)
Carotid intima-media thickness (μm)	12 73	580.03 (46.40)	21 2	589.01 (47.59)	12 36	663.55 (97.01)	232	667.04 (99.37)

[a.] Most characteristics measured at the Child Health CheckPoint; birth weight, gestation were collected at LSAC wave 1 in 2004, SEP was measured at LSAC Wave 6 in 2015, and smoking exposure was generated combing the questionnaire from LSAC Wave 1 to 6; [b.] Standard deviation; [c.] Body mass index was transformed to z-score with widely used Centres for Disease Control and Prevention (US)-growth charts.

Table 3. Associations of retinal microvascular caliber with large arterial intermediate phenotypes

Table 3. Associations of retinal microvascular caliber with large arterial intermediate phenotypes

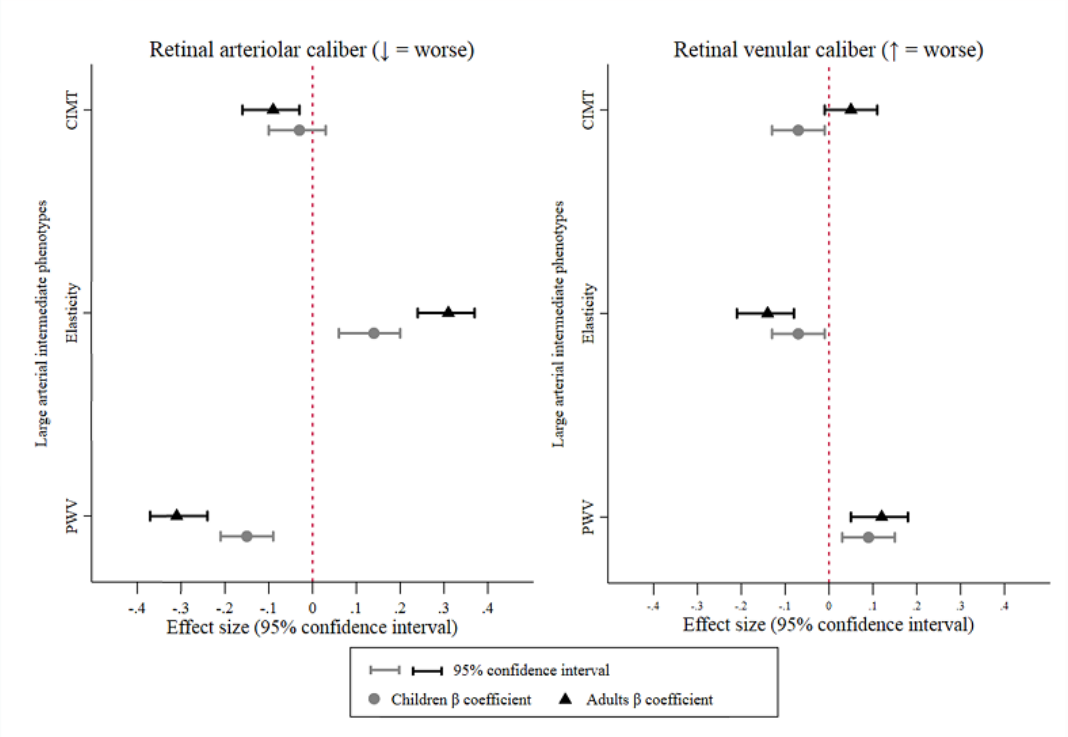
Retinal caliber (per SD increase)	Pulse wave velocity			Carotid arterial elasticity			Carotid intima-media thickness		
	Standardized β (95% CI)	*p-value*	R^2	Standardized β (95% CI)	*p-value*	R^2	Standardized β (95% CI)	*p-value*	R^2
Children									
Model 1: adjusted for age, sex, socioeconomic position and jointly considering arteriolar and venular caliber									
Arteriolar	-0.15 (-0.21, -0.09)	<0.001	7.3	0.13 (0.06, 0.20)	<0.001	3.3	-0.03 (-0.10, 0.03)	0.30	2.2
Venular	0.09 (0.03, 0.15)	<0.01		-0.07 (-0.13, -0.01)	0.03		-0.07 (-0.13, -0.01)	0.03	
Model 2: Model 1 plus BMI z-score, low-density lipoprotein cholesterol, passive smoking, birth weight and gestational age									
Arteriolar	-0.09 (-0.16, -0.02)	<0.01	12.8	0.13 (0.06, 0.20)	<0.001	6.1	-0.02 (-0.09, 0.05)	0.51	3.6
Venular	0.06 (-0.01, 0.12)	0.10		-0.08 (-0.14, -0.01)	0.03		-0.08 (-0.15, -0.01)	0.02	
Sensitivity analysis: Model 2 plus mean arterial blood pressure									
Arteriolar	-0.02 (-0.09, 0.04)	0.51	20.1	0.08 (0.00, 0.15)	0.04	10.0	-0.03 (-0.10, 0.04)	0.42	3.3

Retinal caliber (per SD increase)	Pulse wave velocity			Carotid arterial elasticity			Carotid intima-media thickness		
	Standardized β (95% CI)	*p-value*	R^2	Standardized β (95% CI)	*p-value*	R^2	Standardized β (95% CI)	*p-value*	R^2
Venular	0.02 (-0.04, 0.08)	0.47		-0.05 (-0.12, 0.02)	0.15		-0.07 (-0.14, 0.00)	0.06	
Adults									
Model 1: adjusted for age, sex, socioeconomic position and jointly considering arteriolar and venular caliber									
Arteriolar	-0.31 (-0.37, -0.24)	<0.001	16.1	0.31 (0.24, 0.37)	<0.001	18.0	-0.09 (-0.16, -0.03)	<0.01	16.8
Venular	0.12 (0.05, 0.18)	<0.001		-0.14 (-0.21, -0.08)	<0.001		0.05 (-0.01, 0.11)	0.12	
Model 2: Model 1 plus BMI, low-density lipoprotein cholesterol and ever smoking									
Arteriolar	-0.20 (-0.27, -0.14)	<0.001	26.7	0.24 (0.17, 0.30)	<0.001	29.5	-0.05 (-0.12, 0.01)	0.12	20.9
Venular	0.06 (-0.00, 0.13)	0.06		-0.10 (-0.16, -0.04)	<0.001		0.05 (-0.02, 0.11)	0.17	
Sensitivity analysis: Model 2 plus mean arterial blood pressure									
Arteriolar	0.01 (-0.06, 0.07)	0.82	43.7	0.12 (0.06, 0.19)	<0.001	36.0	-0.01 (-0.08, 0.06)	0.72	21.9
Venular	-0.02 (-0.08, 0.04)	0.57		-0.06 (-0.12, -0.00)	0.05		0.01 (-0.05, 0.08)	0.72	

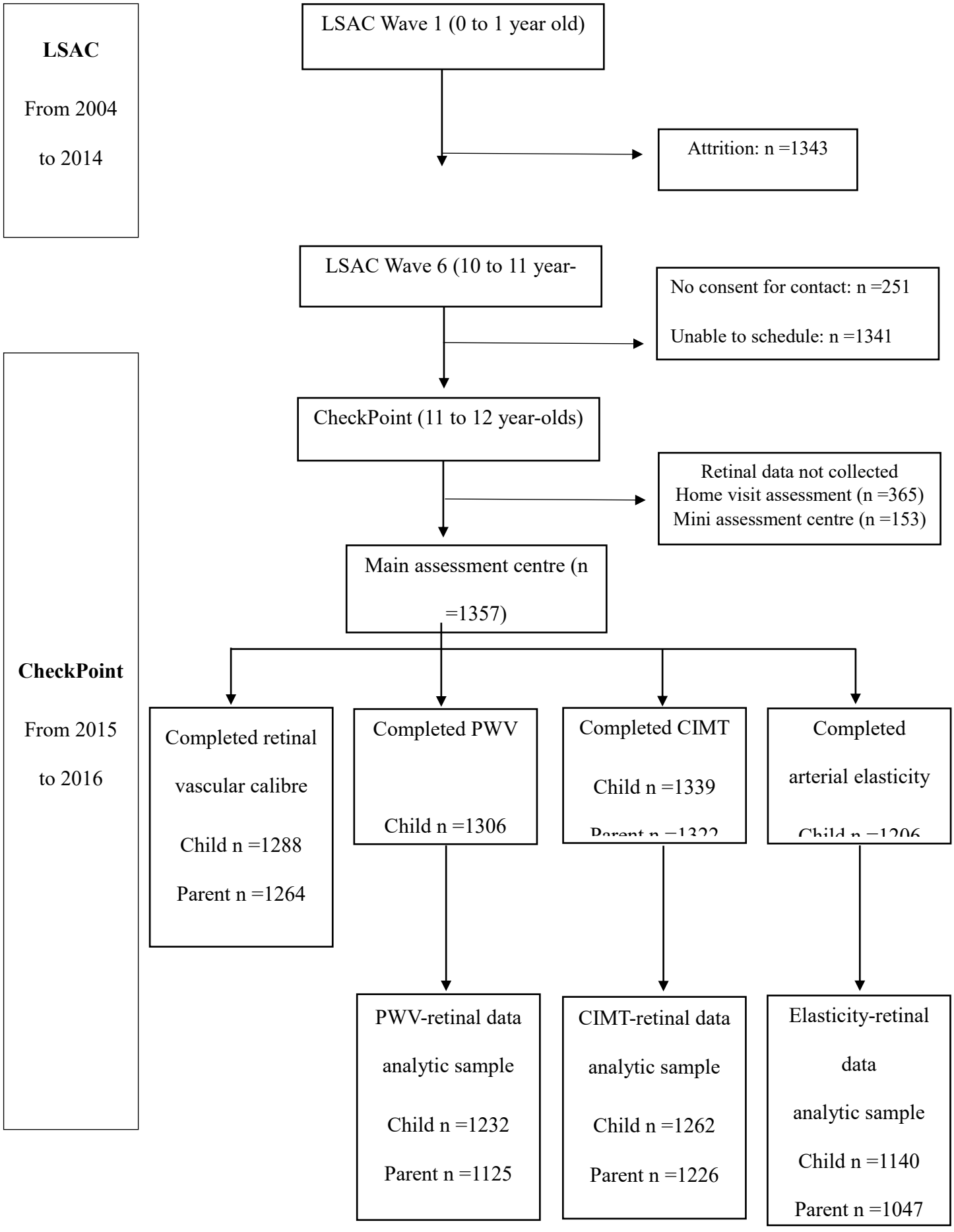
All models included arteriolar and venular caliber, plus other covariates. SDs of arteriolar and venular caliber for children were 11.9 and 16.6 μm, and for adults were 14.0 and 18.5 μm, respectively. Abbreviations: SD, standard deviation; CI, confidence interval; BMI, body mass index; R^2, model R square.

FIGURE: Associations of retinal microvascular caliber and large arterial intermediate phenotypes. Models included arteriolar and venular caliber simultaneously, adjusting for age, sex and socioeconomic position (Model 1)

Footnotes: Effect size = standardized regression coefficients β, representing per standard deviation increase in arteriolar/venular caliber for a standard deviation change in large arterial phenotypes. Abbreviations: PWV, pulse wave velocity; CIMT, carotid intima-media thickness



Supplementary figure. Participant flowchart



Supplementary table. Associations between retinal microvascular caliber and large arterial intermediate phenotypes in adults stratified by gender; standardized regression coefficients for each standard deviation higher in retinal vascular caliber scores.

Retinal vascular calibre (Per SD increase)	Pulse wave velocity (SD: 1.1 m/s)		Carotid arterial elasticity (SD: 0.6%/10mmHg)		Carotid intima-media thickness (SD: 97.4 μm)	
	Standardized β (95% CI)	*p*	Standardized β (95% CI)	*p*	Standardized β (95% CI)	*p*
Male						
Arteriolar	-0.32 (-0.51, -0.14)	<0.01	0.20 (0.05, 0.35)	<0.05	-0.10 (-0.31, 0.12)	0.38
Venular	0.27 (0.08, 0.46)	<0.01	-0.09 (-0.26, 0.07)	0.27	0.05 (-0.17, 0.27)	0.65
Female						
Arteriolar	-0.31 (-0.38, -0.24)	<0.001	0.32 (0.25, 0.40)	<0.001	-0.09 (-0.16, 0.03)	<0.01
Venular	0.09 (0.02, 0.16)	0.01	-0.15 (-0.22, -0.08)	<0.001	0.05 (-0.02, 0.11)	0.14

SDs of retinal arteriolar and venular calibre for adults were 14.0 and 18.5 μm, respectively.

Abbreviations: SD, standard deviation; CI, confidence interval.