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Complete List of Authors:	Dascalu, Julian ; Murdoch Children's Research Institute Liu, Mengjiao; Murdoch Children's Research Institute; The University of Melbourne, Department of Paediatrics Lycett, kate; Murdoch Children's Research Institute; The University of Melbourne, Department of Paediatrics Grobler, Anneke; Murdoch Children's Research Institute He, Mingguang; The University of Melbourne, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital Burgner, David; Murdoch Children's Research Institute; The University of Melbourne, Department of Paediatrics Wong, , Tien Yin ; The University of Melbourne, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital; Singapore National Eye Centre, Singapore Eye Research Institute Wake, Melissa; Murdoch Children's Research Institute; The University of Melbourne, Department of Paediatrics
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Retinal microvasculature: Population epidemiology, concordance and reliability in 11-12 year old Australians and their parents

Authors: Julian Dascalu,[1] Mengjiao Liu,[1,2] Kate Lycett,[1,2] Anneke Grobler,[1] Mingguang He,[3] David Burgner,[1,2,4] Tien Wong,[3,5,6] Melissa Wake.[1,2,7]

Affiliations: [1]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [2]Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia; [3] Centre for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Australia; [4]Department of Paediatrics, Monash University, Clayton, Victoria, Australia; [5]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; [6]Duke-NUS Medical School, National University of Singapore, Singapore; [7]Department of Paediatrics & The Liggins Institute, The University of Auckland, Auckland, New Zealand.

Corresponding author: Prof. Melissa Wake
Murdoch Children's Research Institute
The Royal Children's Hospital
50 Flemington Road, Parkville 3052, VIC Australia.
Telephone: +61 3 9345 5761
Email: melissa.wake@mcri.edu.au

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Abbreviations: AVR: arteriolar-venular ratio; BIG6: revised Knudston-Parr formula for calculating CRAE and CRVE using IVAN software; BMI: body mass index; CC: Pearson's correlation coefficient; CI: confidence interval; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; GWAS: Genome wide association studies; HV:

home visit assessment; IVAN: Interactive Vessel Analysis software; LSAC: Longitudinal Study of Australian Children; n: number of participants; RC: estimated regression coefficient; SCORM: Singapore Cohort Study of the Risk Factors for Myopia; SD: standard deviation.

ABSTRACT

Objectives: To describe distributions and concordance of the retinal microvasculature measurements in a population-based sample of Australian parent-child dyads at child age 11-12 years.

Design: Cross-sectional study based on the Child Health CheckPoint, between Waves 6 and 7 of the Longitudinal Study of Australian Children (LSAC).

Setting: Assessment centres in seven Australian cities, February 2015-March 2016.

Participants: Of all participating CheckPoint families (n=1874), 1288 children (51% girls, mean age 12.0) and 1264 parents (87% mothers, mean age 43.7) were included.

Outcome measures: Retinal photographs were taken by non-mydriatic fundus camera. Trained graders scored vascular calibre using semi-automated software, yielding estimates of central retinal arteriolar and venular equivalents (CRAE, CRVE) and arteriolar-venular ratio (AVR). Pearson's correlation coefficients and multivariable linear regression models assessed parent-child concordance. Survey weights and methods accounted for LSAC's complex sampling, stratification and clustering within postcodes.

Results: Mean (standard deviations) of CRAE and CRVE were slightly larger in children (159.5 (11.8) and 231.1 (16.5)μm, respectively) than parents (151.5 (14.0) and 220.6 (19.0)μm), yielding similar AVR (children 0.69 (0.05), parents 0.69 (0.06)). Correlation coefficients for parent-child pairs were 0.22 (95% CI 0.16 to 0.27) for CRAE, 0.23 (95% CI 0.17 to 0.28) for CRVE and 0.18 (95% CI 0.13 to 0.24) for AVR. Mother-child and father-child values were similar (0.20 and 0.32 for CRAE, 0.22 and 0.29 for CRVE, respectively). Relationships attenuated slightly on adjustment for parent and child age, sex, blood pressure, diabetes and body mass index. Percentiles and concordance are presented for the whole sample and by sex.

Conclusions: Arteriolar and venular calibre were slightly smaller in midlife than late childhood, contrasting with previous reports of increasing arteriolar calibre across this period. This highlights the need for robust population normative values across the lifecourse. Population parent-child concordance values align with moderate polygenic heritability reported in smaller studies.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the largest Australian population-based study to investigate concordance of microvascular structural measurements
- Our adult sample provides novel data regarding the relatively neglected mid-life phase, with most other adult population samples being elderly
- We used gold-standard methodology and demonstrated high inter- and intra-rater reliability
- Most of the participating parents were mothers, resulting in less precise descriptive and concordance estimates for fathers

INTRODUCTION

The role of the microvasculature (ie small vessels) is increasingly recognised in the pathogenesis of cardiovascular disease.[1-3] Microvascular abnormalities already present in early life are thought to predispose to both cardiovascular risk factors and outcomes (eg stroke, myocardial infarction).[4-6]

The retina offers a fast, non-invasive platform to study microvascular health *in vivo*, with similar pathological changes associated with abnormalities and diseases in the cerebral, coronary and renal microvasculatures.[7-10] The most commonly assessed retinal microvasculature measure is the vessel calibre (diameter),[10] summarised as the central retinal arteriolar and venular equivalents (CRAE, CRVE) and its ratio, the arteriolar-venular ratio (AVR). There is good evidence that retinal vessel calibre is associated with subsequent cardiovascular disease, with cardiovascular risk factors (such as hypertension, diabetes mellitus and obesity) affecting the retinal arterioles and venules likely via both shared and unique underlying pathophysiology.[6, 11, 12] Thus, the retinal vasculature has been suggested to be a robust biomarker of cardio-metabolic diseases and allows studying the natural course of small vessel changes over life and its relationship to cardiovascular outcomes in later years.[10, 11]

However, an important gap in the literature is that the natural course and determinants of the retinal vascular calibre is not fully documented. There is some evidence for distinct but differing curvilinear relationships of arteriolar and venular calibres with age.[10, 11] Thus, from disparate cross-sectional studies, Ikram's 2012 narrative review reported that both arteriolar and venular calibre increased from birth (85.5 and 130.0 μm respectively) to age 6 years (165.6 and 232.0 μm). Arteriolar calibre increased further by midlife (202.3 μm) while venular calibre remained static, with calibres of both arterioles and venules reduced from midlife to old age.[10] However, limitations to this literature include differing protocols for imaging and scoring and a marked under-representation of population studies, with few data from young and mid-adulthood. These limitations preclude robust conclusions regarding reference ranges and predictive cutpoints across the lifespan.

While heritability of retinal vascular calibres appears substantial from small community-based samples using a variety of protocols,[11] large studies of parent-child pairs drawn from the general population are lacking. Parent-child correlations for CRAE and CRVE respectively were 0.24 and 0.27 in 341 American adult pairs (aged 43-86 years) in the

Beaver Dam Eye Study;[13] 0.13 and 0.20 for 174 adult pairs from the Flemish Study on Environment, Genes and Health Outcomes (aged 20+ years)[14] and 0.12 and 0.31 in 304 pairs from the Strabismus, Amblyopia and Refractive Error in Singaporean Children study (mean ages 40 years for parents, 9 years for children).[15] In all three studies spousal concordance was negligible for both metrics. In contrast, monozygotic twins have shown much higher concordance (CRAE 0.60 to 0.80, CRVE 0.63 to 0.88) than dizygotic pairs (CRAE 0.12 to 0.50 and CRVE 0.13 to 0.35),[16-19 20] with higher heritability estimates in twin (CRAE 0.56 to 0.70, CRVE 0.64 to 0.83) than parent-child pairs (CRAE 0.25 to 0.54, CRVE 0.28 to 0.72).[13-19] Genome-wide studies have identified several promising loci associated with retinal vessel calibre.[19, 21] However, it remains unclear whether these values remain constant across the lifecourse or what opportunities these offer for preventive health.

Thus, understanding the population epidemiology of retinal calibre in larger studies of healthy mid-life adults and children would inform age- and sex-specific reference values and facilitate secular trend analyses, international comparisons and, potentially, early risk stratification. The Child Health Check Point, a cross-sectional biophysical assessment nested within the Longitudinal Study of Australian Children (LSAC), provided an opportunity to address this issue/question in a national population-based sample of Australian parent-child dyads at child age 11-12 years. We aimed to (1) assess the distribution of retinal vessel calibre in both age groups, and (2) investigate parent-child concordance of these measures. Additionally, we provide intra- and inter-grader reliability estimates to quantify reliability and measurement error.

METHODS

Study Design and Participants: Details of the initial LSAC study design and recruitment are outlined elsewhere.[22, 23] Briefly, LSAC recruited a nationally representative birth cohort (B cohort) of 5107 infants using a 2-stage clustered design, and followed them up in biennial 'waves' of data collection up to 2015.[23] The initial recruitment rate in 2004 was 57.2%, of whom 73.7% (n=3764) were retained to LSAC wave 6 in 2014. At the wave 6 visit, all contactable and consenting families (n=3513) were invited to consent to their contact details being shared with the Child Health CheckPoint team. In 2015, families that consented to contact were sent an information pack via post and received an information and recruitment phone call. The CheckPoint's detailed cross-sectional biophysical assessment, nested between

LSAC waves 6 and 7 at child age 11-12 years, took place between February 2015 and March 2016. 1874 families participated. A more detailed description of the CheckPoint study design is available elsewhere.[24, 25]

Ethics and Consent: The CheckPoint data collection protocol 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.

Procedure: *Retinal imaging:* Retinal photographs were performed during each participant's 3.5-hour visit to the CheckPoint assessment centres in 7 large cities (mainly state capitals) around Australia. During this 3.4 hour visit, each child and parent rotated sequentially and separately through a number of 15-minute assessment stations.[24] At the "See Here" station, participants sat in front of a fundus camera (EOS 60D SLR) while the procedure was explained; the room was then darkened while the participant rested chin and forehead on the head rest of the fundus camera for several minutes. Two digital photographs (one each centering on the optic disc and macula) were taken for each eye using standard protocols from the Centre for Eye Research Australia. Because CheckPoint was unable to transport its single retinal camera to smaller regional centres or home visits, 518 CheckPoint families did not contribute retinal vessel calibre data (figure 1).

Measures: *Image scoring:* Right eye images were selected as first choice for scoring. Reasonable correlations ($r>0.71$) of retinal vascular diameters between right and left eyes have previously been reported,[26, 27] and this allowed harmonisation with other vascular measures obtained in CheckPoint, such as pulse wave velocity and blood pressure, that also assessed the right-sided circulation. Left eye images were used where right eye images were deemed ungradable. Issues preventing grading of images included poor focus (potentially blurring vessel edges), dark images (increasing the difficulty for graders to visually validate the vessel trace), and confounding pathology (which can obscure the vessels). Images were evaluated at the Zhongshan Ophthalmic Centre in Guangdong, China (77%) and the Centre for Eye Research Australia in Melbourne, Australia (23%), separately. In total, 2624 images were graded from 1307 child images and 1317 parent images (87% and 92% from the right eye for children and parents, respectively). 19 child and 53 parent images did not meet the quality criteria for use in analyses.

Each image was graded by one of four experienced graders (two from China and two from Australia) masked to participant characteristics measured the diameters of retinal vessels 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 then selected a segment of each vessel within this area for measurement. Diameters of all the selected segments were measured automatically by the IVAN software. For each participant, summary estimates of the average retinal vascular calibre were calculated according to the Big-Six (revised Knudston-Parr) formula,[26] which combines measurements of the six largest arterioles or venules, represented as the CRAE and CRVE. The AVR was calculated as CRVE divided by the CRAE.

***Other sample characteristics**:* Measures of potential confounding variables were included as detailed elsewhere.[24] Briefly, 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 was self-reported.

Anthropometry was obtained as previously described.[24] Body mass index (BMI) was calculated as kg/m^2. Standing height was obtained using a portable rigid stadiometer (Invicta IP0955, Leicester, UK), measured twice without shoes or socks; a third measurement was taken if the first two measures differed by $\geq$ 0.5cm, and the average of the 2 or 3 values was then calculated. Weight was measured with light clothing and without shoes or socks using 4-limb segmental (InBody230, Biospace, Seoul, South Korea) body composition scales. For children, an age- and sex-adjusted BMI z-score was calculated using the US Centers for Disease Control and Prevention growth reference charts.[28]

Blood pressure was measured using the SphygmoCor XCEL (AtCor Medical Pty Ltd., West Ryde, NSW, Australia). Following seven minutes in supine position at rest, systolic and diastolic blood pressures were measured at the brachial artery up to three times, with mean values reported. Mean arterial blood pressure was calculated from the systolic and diastolic measures.

In Australia, Socio-Economic Indexes for Areas provide standardised scores for socioeconomic position by geographic area (postcode of family domicile) compiled from 2011 Australian Census data. We used the Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage (Disadvantage Index) which numerically summarises

the social and economic conditions of Australian neighbourhoods (national mean of 1000 and standard deviation (SD) of 100, where higher values represent less disadvantage).[29] Parents reported child and parent past medical histories via iPad-administered questionnaires at the Assessment Centre.

Statistical analyses: All analyses were performed using Stata (14.2). Continuous descriptive variables were summarised using weighted means and standard deviations (SD); categorical variables were summarised by number and weighted percentage for children and adults separately and by gender. Survey weights were calculated taking into account the selection probability of each child, and were adjusted for non-response, loss to follow-up and benchmarked to population numbers in major (post stratification) categories of the population of children born in 2004. Standard errors were calculated taking into account the complex design and survey weights.[30] More details on the calculation of survey weights is provided elsewhere.[24, 31]

Concordance between parents and children was assessed by 1) Pearson's correlation coefficients with 95% confidence intervals (CI); 2) linear regression with child variable as dependent variable and parent variable as independent variable adjusted for parent and child age, BMI, mean arterial blood pressure, family disadvantage index score and sex where appropriate; and 3) partial correlation coefficients adjusted for the same covariates. The Pearson's correlation and linear regression analyses were repeated using weighted multi-level survey analyses; as these yielded similar results, unweighted results are displayed.

Reliability of retinal vessel calibre measurements: Inter-grader reliability was assessed by three of the four graders reanalysing a subset of 50 randomly chosen images. To assess intra-grader reliability, the same three graders each re-graded 25 of the 50 randomly chosen images. Hence, an assessment was made as to reproducibility of grading made by different graders and repeatability of gradings made by the same grader. Two-way mixed-effects intraclass correlation coefficients were used for the reliability analysis.

RESULTS

Sample characteristics: A total of 2552 participants (1288 children and 1264 adults) were available for the descriptive Aim 1 analyses (figure 1). This represents 95% of the 1356 pairs who attended CheckPoint assessment centres with retinal photography. As well as the 518 participants already excluded because they attended smaller centres without a retinal camera or had a home visit, a further 160 participants were excluded because they refused at the time, an image could not be taken (eg the camera required repair) or the quality was too poor (figure 1). To address aim 2, 1159 parent-child pairs were assessed; 10 non-biologic adult-child pairs, 29 diabetic parents and 3 diabetic children were excluded from the concordance assessments (figure 1).

Table 1 shows sample characteristics of children and parents stratified by sex. In children, sex was evenly represented (51% girls), while mean age- and sex-specific BMI z-scores were higher than Center for Disease Control historical norms (0.37). The parent sample largely comprised mothers (86.6%), with participants on average being slightly more advantaged (Disadvantage Index mean score 1009, SD 69) than the national Australian population (mean 1000, SD 100). [29]

Descriptive epidemiology of retinal vessel calibre: Summary statistics for parent and child retinal values are displayed in table 1, and broken down for reference into 5th to 95th percentiles in table 2. The corresponding distribution graphs (figure 2) demonstrate approximately normal distribution in all measures for both children and adults. It also highlights that children had larger values for both mean CRAE and CRVE (159.48μm and 231.09μm respectively) than parents (151.41μm and 220.61μm respectively) but narrower distributions (SD 11.8μm vs 14.0μm for CRAE and 16.5μm vs 19.0 for CRVE, respectively, table 1). Boys' CRAE and CRVE mean values were marginally smaller than girls' values (CRAE 156.8μm vs 162.1μm, CRVE 227.8μm vs 234.3μm), and fathers similarly showed smaller mean values than mothers (CRAE 148.4μm vs 152.0μm, CRVE 217.9μm vs 221.0μm). Mean AVR was strikingly similar in parents and children, and between all four groups.

Table 1. Sample characteristics and retinal vessel calibre measures, stratified by sex, of children and parents

Sample characteristics	Mean (SD)*		
	All	Male	Female
Children†	**(n=1288)**	**(n=633)**	**(n=655)**
Age, years	11.96 (0.4)	11.96 (0.4)	11.96 (0.4)
BMI, kg/m^2	19.4 (3.6)	19.2 (3.6)	19.6 (3.6)
BMI z-score	0.37 (1.0)	0.37 (1.0)	0.38 (1.0)
Systolic blood pressure, mmHg	108.7 (8.2)	108.3 (8.4)	109.1 (7.9)
Diastolic blood pressure, mmHg	63.3 (5.7)	63.0 (6.1)	63.6 (5.2)
Neighbourhood disadvantage index	1011.2 (61.0)	1009.6 (63.2)	1012.8 (58.8)
Diabetes (%)	0.4	0.2	0.6
Eye condition or glasses/contact lenses (%)	20.9	17.9	23.8
Retinal vessel measures			
Central retinal arteriolar equivalent, µm	159.5 (11.8)	156.8 (11.8)	162.1 (11.2)
Central retinal venular equivalent, µm	231.1 (16.5)	227.8 (15.6)	234.3 (16.8)
Arteriolar-venular ratio	0.69 (0.05)	0.69 (0.05)	0.69 (0.05)
Parents‡	**(n=1264)**	**(n=169)**	**(n=1095)**
Age, years	43.7 (5.6)	46.2 (6.5)	43.4 (5.3)
BMI, kg/m^2	28.4 (6.5)	28.7 (4.2)	28.3 (6.7)
Systolic blood pressure, mmHg	120.5 (12.9)	238.4 (11.6)	119.3 (12.7)
Diastolic blood pressure, mmHg	73.8 (8.8)	78.3 (8.4)	73.1 (8.6)
Diabetes (%)	2.9	5.3	2.6
Eye condition or glasses/contact lenses (%)	53.2	47.0	54.12
Retinal vessel measures			
Central retinal arteriolar equivalent, µm	151.5 (14)	148.4 (14.3)	152.0 (13.8)
Central retinal venular equivalent, µm	220.6 (19)	217.9 (21.3	221.0 (18.6)
Arteriolar-venular ratio	0.69 (0.06)	0.68 (0.06)	0.69 (0.06)

*Mean (SD), unless otherwise specified; †n for each child variable ranges from 1213-1288; ‡n for each parent variable ranges from 1169-1264. SD, standard deviation; BMI, Body Mass Index

Table 2. Retinal vessel calibre percentiles

Retinal calibre	Child							Parent						
	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
Central retinal arteriolar equivalent, µm														
Male	137.1	141.5	148.0	158.0	165.1	170.7	174.2	123.6	128.7	139.9	148.3	158.2	167.2	171.6
Female	143.8	147.3	153.8	162.5	169.9	175.3	179.3	128.8	134.8	143.3	152.1	162.3	169.1	174.0
All	139.8	144.0	151.3	160.2	167.6	173.1	178.5	128.1	134.1	143.0	151.4	161.7	169.0	173.7
Central retinal venular equivalent, µm														
Male	203.3	208.9	217.7	226.7	237.4	247.4	252.7	186.8	190.9	202.1	215.4	231.5	247.6	261.54
Female	208.8	213.3	222.6	234.0	245.4	256.8	262.8	192.3	197.5	208.4	220.8	233.8	244.8	253.0
All	206.1	210.8	220.4	230.4	241.4	252.1	259.3	190.9	196.8	207.2	220.2	233.7	245.7	253.0
Arteriolar-venular ratio														
Male	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.6	0.61	0.64	0.67	0.73	0.78	0.79
Female	0.61	0.62	0.66	0.69	0.73	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78
All	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78

P: percentile.

Parent-child concordance: Correlations for the 1186 parent-child pairs are displayed in table 3. Pearson's correlations for parent-child dyads were 0.22 for CRAE and 0.23 for CRVE (0.23), and (as expected for a derived ratio value) slightly smaller for AVR (0.18). Mother-child and father-child correlations are presented for reference but not compared statistically, noting slightly higher values for father-child dyads but wider CIs in keeping with the much smaller numbers. In linear regression adjusting for all covariates, estimates attenuated only marginally and all associations remained strong (estimated regression coefficients for parent-child dyads: CRAE 0.19, CRVE 0.21, AVR 0.16) with the exception of father-child AVR. Similarly, partial correlations adjusted for covariates attenuated slightly (correlation coefficient decreased by 0.01 to 0.06, data not shown).

Table 3. Parent-child concordance

	Parent-child			Mother-child			Father-child		
Pearson's Correlation	**N**	**CC**	**95% CI**	**N**	**CC**	**95% CI**	**N**	**CC**	**95% CI**
Central retinal arteriolar calibre,	1186	0.22	0.16 to 0.27	1029	0.20	0.14 to 0.26	157	0.32	0.17 to 0.45
Central retinal venular calibre	1186	0.23	0.17 to 0.28	1029	0.22	0.16 to 0.28	157	0.29	0.14 to 0.42
Arteriolar-venular ratio	1186	0.18	0.13 to 0.24	1029	0.18	0.12 to 0.24	157	0.20	0.04 to 0.35
Adjusted Linear Regression	**N**	**RC**	**P value**	**N**	**RC**	**P value**	**N**	**RC**	**P value**
Central retinal arteriolar calibre, µm	998	0.18	<0.001	867	0.18	<0.001	131	0.21	0.004
Central retinal venular calibre, µm	998	0.20	<0.001	867	0.20	<0.001	131	0.22	0.003
Arteriolar-venular ratio	998	0.16	<0.001	867	0.16	<0.001	131	0.15	0.06

Non-biological caregivers (n=10), diabetic children (n-3) and diabetic adults (n-29) were excluded from these analyses (n=10). Covariates in adjusted linear regression models include parent and child age, BMI and Disadvantage Index, mean arterial blood pressure and parent and child sex in models including both sexes. CC: estimated Pearson's correlation coefficient; CI: confidence interval; N: number of biological child-parent pairs with this measure; RC: estimated regression coefficient.

Reliability analysis: Within-grader intraclass correlations were very high for all three graders for CRAE (0.90 to 0.99, 95% CI 0.76 to 0.99) and CRVE (0.92 to 0.98, 95% CI 0.82 to 0.99). This equated to a greater within-grader range for the derived AVR variable (0.69 to 0.97, 95% CI 0.28 to 0.99). Between-grader interclass correlations were also high at 0.79 (95% CI 0.47 to 0.91), 0.92 (95% CI 0.77 to 0.96) and 0.75 (95% CI 0.58 to 0.86) for CRAE, CRVE and AVR respectively.

Post-hoc analysis: As noted above, both CRAE and CRVE were slightly smaller in the mid-life parents than the 11-12 year olds, whereas from Ikram's 2012 narrative review we had expected that CRAE would have increased substantially by midlife while CRVE would remain static (prior to both reducing into old age).[10] Therefore, in post-hoc analyses we plotted mean values for CRAE and CRVE by mean age (figure 3) for 15 community-based studies published since 2004 with participants aged from 6 years to old age.[18, 32-44] We included all adult studies (summarised in supplementary table 1) in Sun et al's systematic review[11] that had a community or population-based sample of >1000 individuals;[37-44] the Norwegian Tromso Eye Study was identified through cited reference searches.[33] Sun's review also identified the child population in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM),[32] and we searched the SCORM study's bibliography and other published reviews for additional children's cohorts.[18, 34-36] We superimposed the results from CheckPoint, separately for adults and children. As visual inspection did not suggest a curvilinear relationship, we then fitted two exploratory linear regression models, with mean age for each study (CheckPoint children and parents considered separately) as the independent variable and with calibre size (arteriolar and venular) as the two dependent variables. It can be seen that venular calibre appears to narrow slightly though not significantly different from 0, at a rate of approximately -1.2 μm per decade from childhood through adulthood (β coefficient -0.12, 95% CI -0.37 to 0.14, $R^2 = 0.03$) while there was no obvious trend for arteriolar calibre (β coefficient -0.02, 95% CI -0.21 to 0.17, $R^2 = 0.002$). Two striking features of the figure are the dearth of published values between childhood and late life, and the marked spread of mean values in the elderly.

DISCUSSION

Principal findings: We provide the first national population-based percentile values for the pattern and distributions of retinal vessel calibre in Australian 11-12 year old children and mid-life

adults, demonstrating an age-related decrease in both mean CRAE and CRVE between mid-childhood and mid-life. Parent-child correlations were similar for all retinal vessel calibre metrics (CRAE 0.20, CRVE 0.22, AVR 0.17) to studies whose offspring probands were already adults, suggesting that the strong element of heritability in microvascular anatomical structure is largely phenotypically overt within the first decade of life.

Strengths and weaknesses: Strengths include the national recruitment basis for this large study, providing a reference point for microvascular measurement for future Australian studies but also filling a significant international gap in population-based studies of these age groups. Results were largely consistent with previous studies and should generalise to the wider Australian child population.

Limitations include our under-representation of the most disadvantaged sector (reflecting social biases in both recruitment and retention, issues common to large longitudinal studies) and the small number of fathers in the parent sample. We adjusted only for a limited range of potential confonders. Parent-child concordance values might have changed slightly had we further adjusted for smoking status, sedentary lifestyle and diet, all previously associated with altered retinal vessel calibre.[45] However, their impact on concordance would likely be small because these factors are all strongly socially patterned. By 2019 this cohort will also be able to consider genome-wide association data, potentially shedding further light on the roles of genetic and shared environmental factors. We did not assess other elements of the microvasculature such as branching angles, tortuosity or fractal dimension. We were not able to control for refractive errors in this study. Retinal vascular calibre measurements may be influenced by refractive errors and refraction is different between children and adults.[46, 47] However, we would expect these effects to be small, particularly as other sources of systematic bias were minimised (measured on the same day with the same equipment by the same person who was blind to dyadic membership).

Meaning and implications for clinicians and policy makers: Findings for all outcomes are consistent with the previous literature. However, direct comparisons of CRAE and CRVE across the lifecourse and between ethnicities remain difficult due to differences in equipment, software and analysis formulae for CRAE and CRVE. Such factors could well account for the differences between our own and three international studies at mean age 11.1-12.7 years: smaller mean CRAE and CRVE in Chinese twins (CRAE 150.1-μm, CRVE 218.4μm) and Singapore Chinese children (CRAE 149.3μm, CRVE 224.6μm), but much larger values in a smaller study of German children

(CRAE 208μm, CRVE 236.2μm).[18, 32, 48] These discrepancies highlight the need for a uniform approach to measurement of retinal parameters in future epidemiological investigations. Our post-hoc analysis of published literature highlights both the dearth of population studies between childhood and old age and a likely fall in venular calibre from childhood through midlife. An individual participant meta-analysis would be the next step to more precisely determine how retinal calibre varies by age.

The moderate parent-child correlations of this Australian population are very similar to those in previous studies with much older adult offspring, suggesting that in terms of microvasculature children do not become more phenotypically similar to their parents between childhood and midlife. Although this is the first study to report intergenerational concordance by parent sex, our marginally stronger father-child than mother-child correlations must be taken with caution due to the small sample of fathers. Like all other parent-child and twin studies except the Beaver Dam Eye Study, our CRVE correlations were marginally larger than those for CRAE.[13-19] Previously, Li et al. have postulated that the retinal arteriolar phenotype may be more sensitive to environmental influences than the venular phenotype, despite having a significant, moderate degree of inheritance.[15] This is further supported by GWAS studies, where only CRVE showed significant associations with the identified gene loci.[19, 21]

Unanswered questions and future research: We provide normative values for retinal vessel calibre for Australian 11-12 year olds and mid-life adults using standardised protocols. Mapped onto other publications (figure 3), our findings make explicit a need for reliable age-specific normative reference values across the lifecourse. This would need to be extended to large long-running cohort studies with access to clinical outcomes if retinal calibre is to realise the potential as a population screening and risk stratification tool for cardiovascular disease, with or without consideration of other retinal vascular features, polygenic risk scores and macrovascular risk.

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CONTRIBUTIONS: MW, TW, DB and KL contributed to the study design. JD, ML and MH contributed to the acquisition of data. AG, KL, ML and JD conducted the data analysis. JD drafted the manuscript with critical input from all authors. MW and KL supervised JD. MW is the

principal investigator of the Child Health CheckPoint and conceived the paper. All authors read and approved the final manuscript.

DATA SHARING STATEMENT: Dataset and technical documents available from Growing Up in Australia: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au

FIGURE CAPTIONS AND FOOTNOTES:

Figure 1. Participant flow through *Child Health Check-Point.*

n: number of families; c: number of children; p: number of attending adults; MAC: Main assessment centre; mAC: Mini assessment centre; HV: Home visit assessment; LSAC: Longitudinal Study of Australian Children.

*Unable to collect image due to equipment failure or time constraints.

~Data excluded for images that did not meet quality criteria for 'Big 6' analysis.

^Data from 10 non-biological child-parent pairs excluded from concordance analyses

Figure 2. Density plots for retinal vessel calibre measures.

AVR: arteriolar-venular ratio; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; BIG6: revised knudston-parr formula for calculating CRAE and CRVE using IVAN software.[26]

Figure 3. Epidemiology of retinal vascular calibre by age.

Mean retinal arteriolar and venular calibre by age from CheckPoint and published community-based studies with n>1000. Each symbol represents the mean value for a single study. Each study is summarised in supplementary table 1, and the paper from which that study's data are drawn is provided in the list of references. Checkpoint_C: Child Health CheckPoint data of children; CheckPoint_P: Child Health CheckPoint data of parents.

SUPPLEMENTARY DOCUMENTS:

Supplementary table 1. Epidemiology of studies reporting retinal vascular calibre

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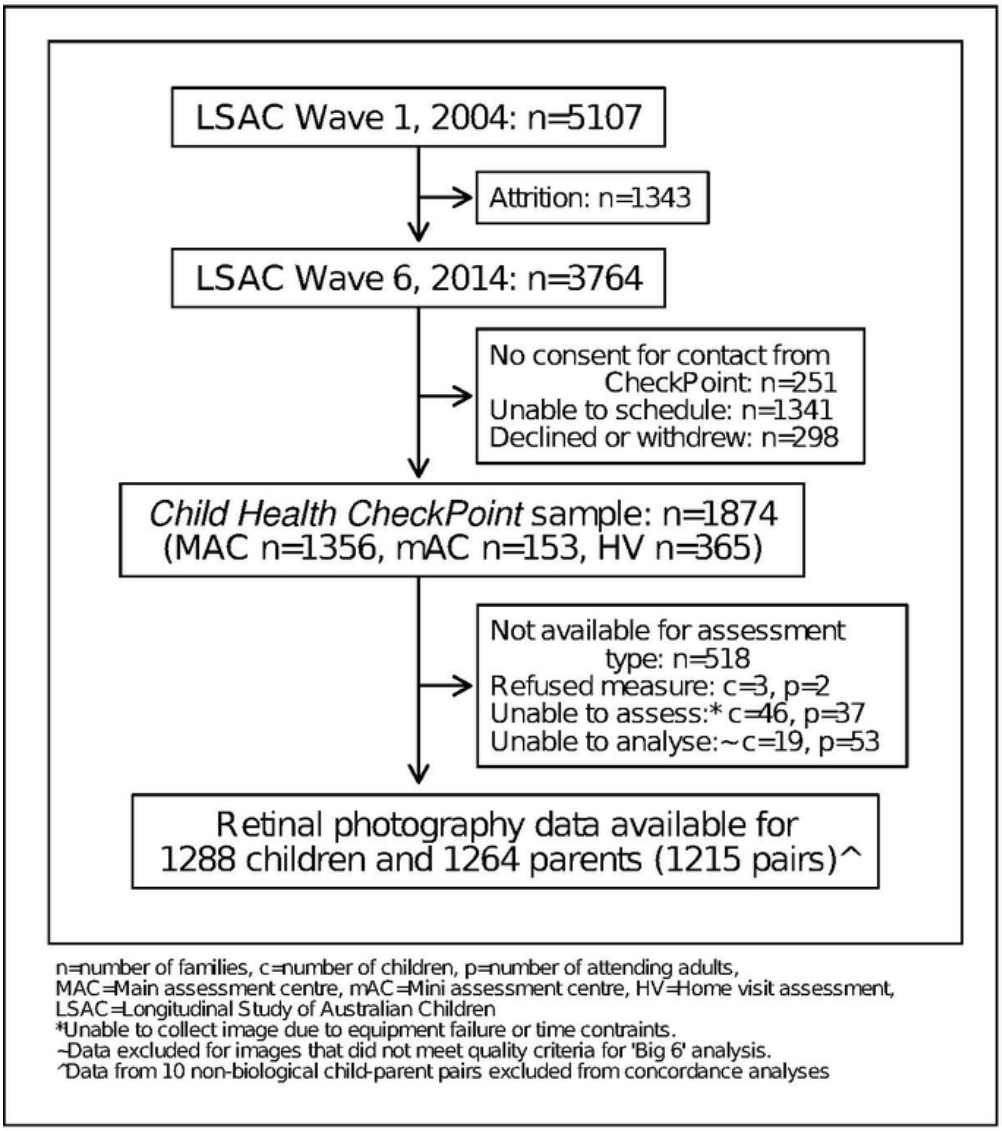
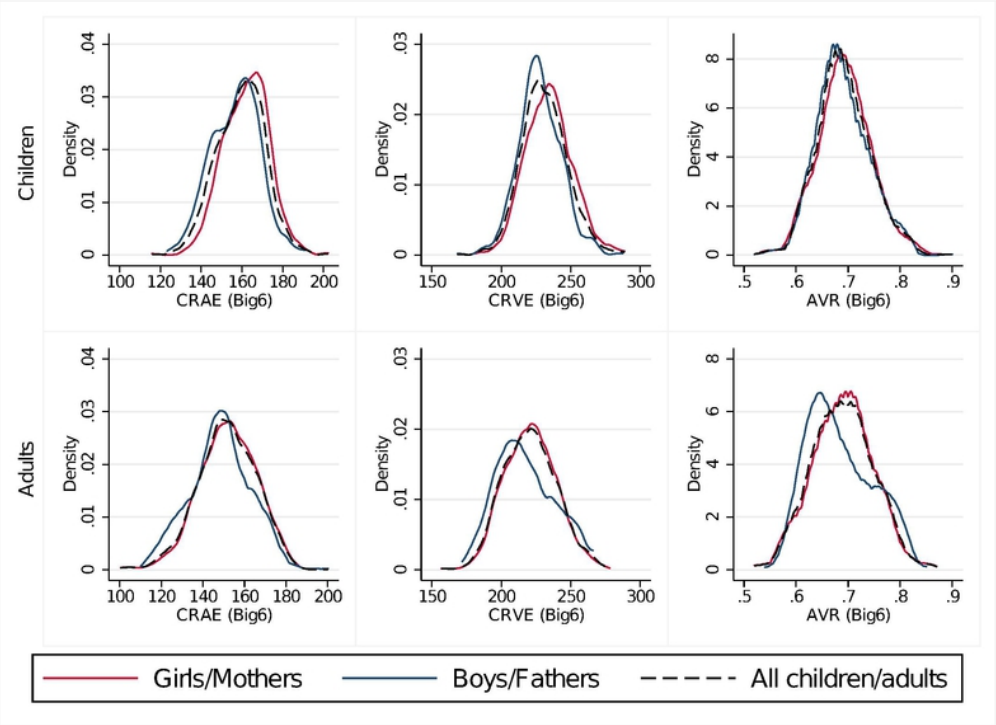


Figure 1. Participant flow through Child Health Check-Point.
n: number of families; c: number of children; p: number of attending adults; MAC: Main assessment centre; mAC: Mini assessment centre; HV: Home visit assessment; LSAC: Longitudinal Study of Australian Children.
*Unable to collect image due to equipment failure or time constraints.
~Data excluded for images that did not meet quality criteria for 'Big 6' analysis.
^Data from 10 non-biological child-parent pairs excluded from concordance analyses

57x65mm (300 x 300 DPI)



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AVR: arteriolar-venular ratio; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; BIG6: revised knudston-parr formula for calculating CRAE and CRVE using IVAN software

66x48mm (300 x 300 DPI)

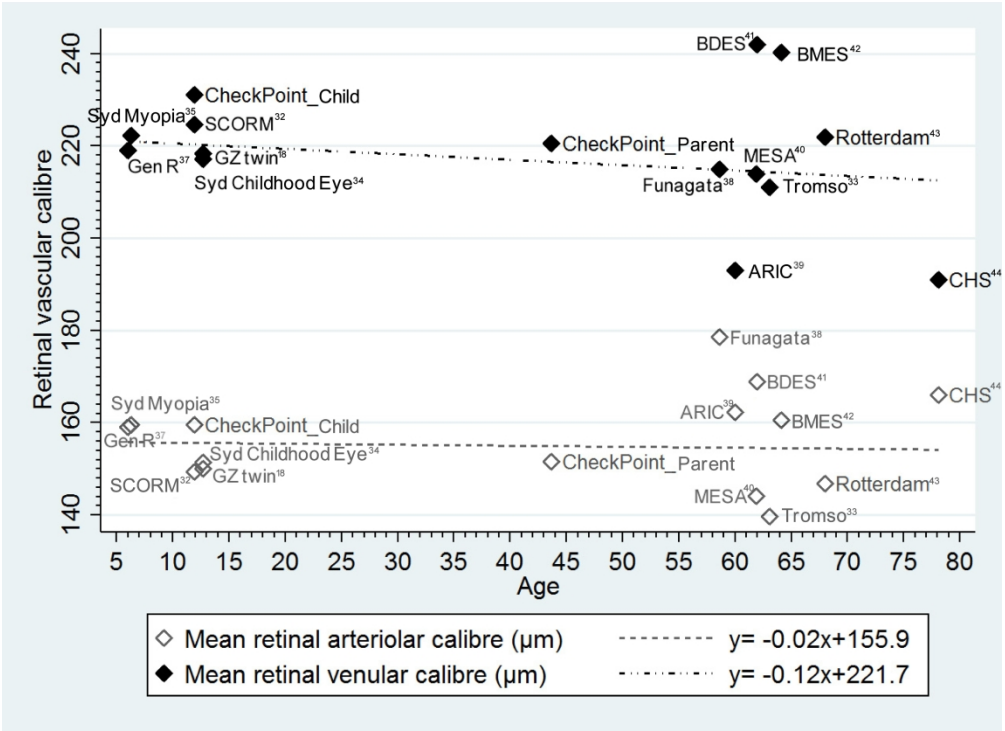

Figure 3. Epidemiology of retinal vascular calibre by age.
Mean retinal arteriolar and venular calibre by age from CheckPoint and published community-based studies with n>1000. Each symbol represents the mean value for a single study. Each study is summarised in supplementary table 1, and the paper from which that study's data are drawn is provided in the list of references. Checkpoint_C: Child Health CheckPoint data of children; CheckPoint_P: Child Health CheckPoint data of parents.

327x238mm (300 x 300 DPI)

Supplementary Table 1. Epidemiology of studies reported retinal vascular calibre

Author; publication year reference number	Study name; country	Examination year	Sample size	Mean age (years)	Arteriolar calibre (mean(SD), µm)	Venular calibre (mean(SD), µm)
Children						
Gishti; 2015[37]	Gen R; Netherlands	2008-2010	4007	6	159.0 (14.9)	219.0 (20.2)
Mitchell; 2008[35]	Syd Myopia; AU	2003-2004	1369	6.3	159.6 (ns)	222.3 (ns)
Cheung; 2012[32]	SCORM; Singapore	2006	1225	11.9	149.3 (12.3)	224.6 (17.8)
	CheckPoint_C; AU	2015-2016	1288	11.9	159.5 (11.8)	231.1 (16.5)
Gopinath; 2010[34]	Syd Childhood Eye; AU	2004-2005	2272	12.7	151.4 (12.1)	151.4 (12.1)
Zheng; 2013[18]	GZ twin; China	2009	2070	12.7	150.1 (13.4)	218.4 (19.1)
Adults						
	CheckPoint_P; AU	2015-2016	1264	43.7	151.5 (14.0)	220.6 (19.0)
Kawasaki-1; 2006[38]	Funagata; Japan	2000-2002	1481	58.6	178.6 (21.0)	214.9 (20.6)
Liew; 2008[39]	ARIC; USA	1993-1995	8794	60	162.3 (ns)	193.1 (ns)
Wong; 2006[40]	MESA; USA	2002-2004	5979	61.9	144.1 (14.4)	214.0 (22.2)
Klein; 2006[41]	BDES; USA	1988-1990	4926	62	169 (ns)	242 (ns)
Von; 2014[33]	Tromso; Norway	2007-2008	6353	63.1	139.7 (14.6)	211.1 (21.1)
Kawasaki-2; 2013[42]	BMES; Australia	2003	2335	64.1	160.6 (14.9)	240.3 (22.4)
Ikram; 2004[43]	Rotterdam; Netherlands	1990-1993	5674	68	146.9 (14.4)	222.0 (20.9)
Kim; 2010[44]	CHS; USA	1997-1998	1744	78.1	166 (19)	191 (18)

Abbreviations: SD, standard deviation; AU, Australia; USA, United State of America; Gen R, Generation R Study; Syd Myopia, The Sydney Myopia Study; SCORM, Singapore Cohort Study of the Risk Factors for Myopia; CheckPoint_C, Child Health CheckPoint data of children; Syd Childhood Eye; The Sydney Childhood Eye Study; GZ twin; The Guangzhou twin eye study; CheckPoint_P, Child Health CheckPoint data of parents; ARIC; Atherosclerosis Risk In Communities; MESA; Multi-Ethnic Study of Atherosclerosis; BDES; Beaver Dam Eye Study; Tromso, The Tromsø Eye Study; BMES; Blue Mountains Eye Study; Rotterdam; The Rotterdam Study; CHS; Cardiovascular Health Study; ns, not stated.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7, 8*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	6, 7, figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7, 8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Figure 1
		(b) Give reasons for non-participation at each stage	7,10, figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 10, table 1
		(b) Indicate number of participants with missing data for each variable of interest	Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	10, table 1, table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13, 14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, 16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16, 17
Generalisability	21	Discuss the generalisability (external validity) of the study results	16, 17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18, 19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Retinal microvasculature: Population epidemiology, concordance and reliability in 11-12 year old Australians and their parents

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Retinal microvasculature: Population epidemiology, concordance and reliability in 11-12 year old Australians and their parents

Authors: Julian Dascalu,[1] Mengjiao Liu,[1,2] Kate Lycett,[1,2] Anneke Grobler,[1] Mingguang He,[3] David Burgner,[1,2,4] Tien Wong,[3,5,6] Melissa Wake.[1,2,7]

Affiliations: [1]Murdoch Children's Research Institute, Parkville, Victoria, Australia; [2]Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia; [3] Centre for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Australia; [4]Department of Paediatrics, Monash University, Clayton, Victoria, Australia; [5]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; [6]Duke-NUS Medical School, National University of Singapore, Singapore; [7]Department of Paediatrics & The Liggins Institute, The University of Auckland, Auckland, New Zealand.

Corresponding author: Prof. Melissa Wake
Murdoch Children's Research Institute
The Royal Children's Hospital
50 Flemington Road, Parkville 3052, VIC Australia.
Telephone: +61 3 9345 5761
Email: melissa.wake@mcri.edu.au

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Abbreviations: AVR: arteriolar-venular ratio; BIG6: revised Knudston-Parr formula for calculating CRAE and CRVE using IVAN software; BMI: body mass index; CC: Pearson's correlation coefficient; CI: confidence interval; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; GWAS: Genome wide association studies; HV: home visit assessment; IVAN: Interactive Vessel Analysis software; LSAC: Longitudinal

Study of Australian Children; n: number of participants; RC: estimated regression coefficient; SCORM: Singapore Cohort Study of the Risk Factors for Myopia; SD: standard deviation.

ABSTRACT

Objectives: To describe distributions and concordance of the retinal microvasculature measurements in a population-based sample of Australian parent-child dyads at child age 11-12 years.

Design: Cross-sectional Child Health CheckPoint study, between Waves 6 and 7 of the national population-based Longitudinal Study of Australian Children (LSAC).

Setting: Assessment centres in seven Australian cities, February 2015-March 2016.

Participants: Of all 1874 participating families, 1288 children (51% girls) and 1264 parents (87% mothers, mean age 43.7) were analysed. Diabetic participants and non-biological pairs were excluded from concordance analyses.

Outcome measures: Retinal photographs were taken by non-mydriatic fundus camera. Trained graders scored vascular calibre using semi-automated software, yielding estimates of central retinal arteriolar and venular equivalents (CRAE, CRVE) and arteriolar-venular ratio (AVR). Pearson's correlation coefficients and multivariable linear regression models assessed parent-child concordance. Survey weights and methods accounted for LSAC's complex sampling, stratification and clustering within postcodes.

Results: Mean (standard deviations) of CRAE and CRVE were larger in children (159.5 (11.8) and 231.1 (16.5)μm, respectively) than parents (151.5 (14.0) and 220.6 (19.0)μm), yielding similar AVR (children 0.69 (0.05), parents 0.69 (0.06)). Correlation coefficients for parent-child pairs were 0.22 (95% CI 0.16 to 0.27) for CRAE, 0.23 (95% CI 0.17 to 0.28) for CRVE and 0.18 (95% CI 0.13 to 0.24) for AVR. Mother-child and father-child values were similar (0.20 and 0.32 for CRAE, 0.22 and 0.29 for CRVE, respectively). Relationships attenuated slightly on adjustment for age, sex, blood pressure, diabetes and body mass index. Percentiles and concordance are presented for the whole sample and by sex.

Conclusions: Arteriolar and venular calibre were similar to previously documented measures in midlife adult and late childhood populations. Population parent-child concordance values align with moderate polygenic heritability reported in smaller studies.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the largest Australian population-based study to investigate concordance of microvascular structural measurements
- Our adult sample provides novel data regarding the relatively neglected mid-life phase, with most other adult population samples being elderly
- We used gold-standard methodology and demonstrated high inter- and intra-rater reliability
- Most of the participating parents were mothers, resulting in less precise descriptive and concordance estimates for fathers

INTRODUCTION

The role of the microvasculature (ie small vessels) is increasingly recognised in the pathogenesis of cardiovascular disease.[1-3] Microvascular abnormalities already present in early life are thought to predispose to both cardiovascular risk factors and outcomes (eg stroke, myocardial infarction).[4-6]

The retina offers a fast, non-invasive platform to study microvascular health *in vivo*, with similar pathological changes associated with abnormalities and diseases in the cerebral, coronary and renal microvasculatures.[7-10] The most commonly assessed retinal microvasculature measure is the vessel calibre (diameter),[10] summarised as the central retinal arteriolar and venular equivalents (CRAE, CRVE) and its ratio, the arteriolar-venular ratio (AVR). Meta-analyses have demonstrated statistically significant correlations of smaller retinal arteriolar and wider retinal venular vessel calibre, with subsequent cardiovascular disease, including stroke, obesity and coronary heart disease.[11-14] Cardiovascular risk factors (such as hypertension, diabetes mellitus and obesity) have been similarly associated with smaller retinal arterioles and larger retinal venules via both shared and unique underlying pathophysiology.[6, 15, 16] Thus, the retinal vasculature has been suggested to be a robust biomarker of cardio-metabolic diseases and allows studying the natural course of small vessel changes over life and its relationship to cardiovascular outcomes in later years.[10, 15]

However, an important gap in the literature is that the natural course and determinants of the retinal vascular calibre is not fully documented. There is some evidence for distinct but differing curvilinear relationships of arteriolar and venular calibres with age.[10, 15] Thus, from disparate cross-sectional studies, Ikram's 2012 narrative review reported that both arteriolar and venular calibre increased from birth (85.5 and 130.0 μm respectively) to age 6 years (165.6 and 232.0 μm). Arteriolar calibre increased further by midlife (202.3 μm) while venular calibre remained static, with calibres of both arterioles and venules reduced from midlife to old age.[10] However, limitations to this literature include differing protocols for imaging and scoring and a marked under-representation of population studies, with few data from young and mid-adulthood. These limitations preclude robust conclusions regarding reference ranges and predictive cutpoints across the lifespan.

While heritability of retinal vascular calibres appears substantial from small community-based samples using a variety of protocols,[15] large studies of parent-child pairs drawn from the general population are lacking. Parent-child correlations for CRAE and CRVE respectively were 0.24 and 0.27 in 341 American adult pairs (aged 43-86 years) in the Beaver Dam Eye

Study;[17] 0.13 and 0.20 for 174 adult pairs from the Flemish Study on Environment, Genes and Health Outcomes (aged 20+ years)[18] and 0.12 and 0.31 in 304 pairs from the Strabismus, Amblyopia and Refractive Error in Singaporean Children study (mean ages 40 years for parents, 9 years for children).[19] In all three studies spousal concordance was negligible for both metrics. In contrast, monozygotic twins have shown much higher concordance (CRAE 0.60 to 0.80, CRVE 0.63 to 0.88) than dizygotic pairs (CRAE 0.12 to 0.50 and CRVE 0.13 to 0.35),[20-23 24] with higher heritability estimates in twin (CRAE 0.56 to 0.70, CRVE 0.64 to 0.83) than parent-child pairs (CRAE 0.25 to 0.54, CRVE 0.28 to 0.72).[17-23] Genome-wide studies have identified several promising loci associated with retinal vessel calibre.[23, 25] However, it remains unclear whether these values remain constant across the lifecourse or what opportunities these offer for preventive health.

Thus, understanding the population epidemiology of retinal calibre in larger studies of healthy mid-life adults and children would inform age- and sex-specific reference values and facilitate secular trend analyses, international comparisons and, potentially, early risk stratification. The Child Health Check Point, a cross-sectional biophysical assessment nested within the Longitudinal Study of Australian Children (LSAC), provided an opportunity to address this issue/question in a national population-based sample of Australian parent-child dyads at child age 11-12 years. We aimed to (1) assess the distribution of retinal vessel calibre in both age groups, and (2) investigate parent-child concordance of these measures. Additionally, we provide intra- and inter-grader reliability estimates to quantify reliability and measurement error.

METHODS

Study Design and Participants: Details of the initial LSAC study design and recruitment are outlined elsewhere.[26, 27] Briefly, LSAC recruited a nationally representative birth cohort (B cohort) of 5107 infants using a 2-stage clustered design. First, 10% of Australian postcodes (stratified by state and urban/rural locations) were randomly selected, then in-age children (born between March 2003 and February 2004) within those enrolled in the Medicare Australia database (Australia's universal healthcare system, into which 98% of children are enrolled by their first birthday) were selected. Study participants were then followed up in biennial 'waves' of data collection up to 2015.[27] The initial recruitment rate in 2004 was 57.2%, of whom 73.7% (n=3764) were retained to LSAC wave 6 in 2014. At the wave 6 visit, all contactable and consenting families (n=3513) were invited to consent to their contact

details being shared with the Child Health CheckPoint team. In 2015, families that consented to contact were sent an information pack via post and received an information and recruitment phone call. The CheckPoint's detailed cross-sectional biophysical assessment, nested between LSAC waves 6 and 7 at child age 11-12 years, took place between February 2015 and March 2016. 1874 families participated. A more detailed description of the CheckPoint study design is available elsewhere.[28, 29]

Ethics and Consent: The CheckPoint data collection protocol 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.

Procedure: *Retinal imaging:* Retinal photographs were performed during each participant's 3.5-hour visit to the CheckPoint assessment centres in 7 large cities (mainly state capitals) around Australia. During this 3.5 hour visit, each child and parent rotated sequentially and separately through a number of 15-minute assessment stations.[28] At the "See Here" station, participants sat in front of a fundus camera (EOS 60D SLR) while the procedure was explained; the room was then darkened while the participant rested chin and forehead on the head rest of the fundus camera for several minutes. Two digital photographs (one each centering on the optic disc and macula) were taken for each eye using standard protocols from the Centre for Eye Research Australia. Because CheckPoint was unable to transport its single retinal camera to smaller regional centres or home visits, 518 CheckPoint families did not contribute retinal vessel calibre data (figure 1).

Measures: *Image scoring:* Right eye images were selected as first choice for scoring. Reasonable correlations ($r>0.71$) of retinal vascular diameters between right and left eyes have previously been reported,[30, 31] and this allowed harmonisation with other vascular measures obtained in CheckPoint, such as pulse wave velocity and blood pressure, that also assessed the right-sided circulation. Left eye images were used where right eye images were deemed ungradable. Issues preventing grading of images included poor focus (potentially blurring vessel edges), dark images (increasing the difficulty for graders to visually validate the vessel trace), and confounding pathology (which can obscure the vessels). Images were evaluated at the Zhongshan Ophthalmic Centre in Guangdong, China (77%) and the Centre for Eye Research Australia in Melbourne, Australia (23%), separately. In total, 2624 images were graded, including from 1307 children and 1317 parents (87% and 92% from the right

eye for children and parents, respectively). 19 child and 53 parent images did not meet the quality criteria for use in analyses.

Each image was graded by one of four experienced graders (two from China and two from Australia) masked to participant characteristics measured the diameters of retinal vessels 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 then selected a segment of each vessel within this area for measurement. Diameters of all the selected segments were measured automatically by the IVAN software. For each participant, summary estimates of the average retinal vascular calibre were calculated according to the Big-Six (revised Knudston-Parr) formula,[30] which combines measurements of the six largest arterioles or venules, represented as the CRAE and CRVE. The AVR was calculated as CRVE divided by the CRAE.

***Other sample characteristics**:* Measures of potential confounding variables were included as detailed elsewhere.[28] Briefly, 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 was self-reported.

Body mass index (BMI) was calculated as kg/m^2, using measured height and weight.[28] For children, an age- and sex-adjusted BMI z-score was calculated using the US Centers for Disease Control and Prevention growth reference chats.[32]

Brachial blood pressure was measured using the SphygmoCor XCEL (AtCor Medical Pty Ltd., West Ryde, NSW, Australia), following seven minutes rest. Mean arterial blood pressure was calculated from the systolic and diastolic measures.

In Australia, Socio-Economic Indexes for Areas provide standardised scores for socioeconomic position by geographic area (postcode of family domicile) compiled from 2011 Australian Census data. We used the Socio-Economic Indexes for Areas Index of Relative Socioeconomic Disadvantage (Disadvantage Index) which numerically summarises the social and economic conditions of Australian neighbourhoods (national mean of 1000 and standard deviation (SD) of 100, where higher values represent less disadvantage).[33] Parents reported child and parent past medical histories via iPad-administered questionnaires at the Assessment Centre.

Child and parent participants who attended CheckPoint assessment centres also completed a visual acuity assessment (not conducted in home visits). As part of this assessment, they were

asked if they "usually wear glasses or contact lenses". Staff members recorded their response as yes or no; the strength of prescription was not captured.

Statistical analyses: All analyses were performed using Stata (14.2). Aim 1: Continuous descriptive variables were summarised using weighted means and standard deviations (SD); categorical variables were summarised by number and weighted percentage for children and adults separately and by gender. Survey weights were calculated taking into account the selection probability of each child, and were adjusted for non-response, loss to follow-up and benchmarked to population numbers in major (post stratification) categories of the population of children born in 2004. Standard errors were calculated taking into account the complex design and survey weights.[34] More details on the calculation of survey weights is provided elsewhere.[28, 35]

Aim 2: Concordance between parents and children was assessed by 1) Pearson's correlation coefficients with 95% confidence intervals (CI); 2) linear regression with child variable as dependent variable and parent variable as independent variable adjusted for parent and child age, BMI, mean arterial blood pressure, family disadvantage index score and sex where appropriate; and 3) partial correlation coefficients adjusted for the same covariates. The Pearson's correlation and linear regression analyses were repeated using weighted multi-level survey analyses; as these yielded similar results, unweighted results are displayed. Twenty nine diabetic participants, and 10 non-biological child-parent pairs were excluded from concordance analyses.

Reliability of retinal vessel calibre measurements: Inter-grader reliability was assessed by three of the four graders reanalysing a subset of 50 randomly chosen images. To assess intra-grader reliability, the same three graders each re-graded 25 of the 50 randomly chosen images. Hence, an assessment was made as to reproducibility of grading made by different graders and repeatability of gradings made by the same grader. Two-way mixed-effects intraclass correlation coefficients were used for the reliability analysis.

Patient and Public Involvement: Because LSAC is a population-based longitudinal study, no patient groups were involved in its design or conduct. To our knowledge, the public was not involved in the study design, recruitment or conduct of LSAC study or its CheckPoint module. Parents received a summary health report for their child and themselves at or soon after the assessment visit. They consented to take part knowing that they would not otherwise receive individual results about themselves or their child.

RESULTS

Sample characteristics: A total of 2552 participants (1288 children and 1264 adults) were included in the descriptive Aim 1 analyses (figure 1). This represents 95% of the 1356 pairs who attended CheckPoint assessment centres (where retinal photography was offered). Reasons for participants not having retinal photography images and data areattendence at smaller assessment centres without a retinal camera or home visit (n=518 participants), participant refusal (n=160 participants), and an image not able to be taken (eg the camera required repair) or the quality was too poor (n=5 participants, figure 1). A total of 1186 parent-child pairs were included in Aim 2 analyses; 10 non-biologic adult-child pairs and 29 diabetic participants were excluded from the concordance assessments (figure 1).

Table 1 shows sample characteristics of children and parents stratified by sex. In children, sex was evenly represented (51% girls), while mean age- and sex-specific BMI z-scores were higher than Center for Disease Control historical norms (0.37). The parent sample largely comprised mothers (86.6%), with participants on average being slightly more advantaged (Disadvantage Index mean score 1009, SD 69) than the national Australian population (mean 1000, SD 100). [33]

Descriptive epidemiology of retinal vessel calibre: Summary statistics for parent and child retinal values are displayed in table 1, and broken down for reference into 5th to 95th percentiles in table 2. The corresponding distribution graphs (figure 2) demonstrate approximately normal distribution in all measures for both children and adults. Mean (standard deviations) of CRAE and CRVE were around 0.6 standard deviation larger in children (159.5 (11.8) and 231.1 (16.5)μm, respectively) than parents (151.5 (14.0) and 220.6 (19.0)μm), yielding similar AVR (children 0.69 (0.05), parents 0.69 (0.06)), table 1). Boys' CRAE and CRVE mean values were marginally smaller than girls' values (CRAE 156.8μm vs 162.1μm, CRVE 227.8μm vs 234.3μm), and fathers similarly showed smaller mean values than mothers (CRAE 148.4μm vs 152.0μm, CRVE 217.9μm vs 221.0μm). Mean AVR was strikingly similar in parents and children, and between all four groups.

Table 1. Sample characteristics and retinal vessel calibre measures, stratified by sex, of children and parents

Sample characteristics	Mean (SD)*		
	All	Male	Female
Children†	**(n=1288)**	**(n=633)**	**(n=655)**
Age, years	11.96 (0.4)	11.96 (0.4)	11.96 (0.4)
BMI, kg/m^2	19.4 (3.6)	19.2 (3.6)	19.6 (3.6)
BMI z-score	0.37 (1.0)	0.37 (1.0)	0.38 (1.0)
Systolic blood pressure, mmHg	108.7 (8.2)	108.3 (8.4)	109.1 (7.9)
Diastolic blood pressure, mmHg	63.3 (5.7)	63.0 (6.1)	63.6 (5.2)
Neighbourhood disadvantage index	1011.2 (61.0)	1009.6 (63.2)	1012.8 (58.8)
Diabetes (%)	0.4	0.2	0.6
Eye condition or glasses/contact lenses (%)	20.9	17.9	23.8
Retinal vessel measures			
Central retinal arteriolar equivalent, µm	159.5 (11.8)	156.8 (11.8)	162.1 (11.2)
Central retinal venular equivalent, µm	231.1 (16.5)	227.8 (15.6)	234.3 (16.8)
Arteriolar-venular ratio	0.69 (0.05)	0.69 (0.05)	0.69 (0.05)
Parents‡	**(n=1264)**	**(n=169)**	**(n=1095)**
Age, years	43.7 (5.6)	46.2 (6.5)	43.4 (5.3)
BMI, kg/m^2	28.4 (6.5)	28.7 (4.2)	28.3 (6.7)
Systolic blood pressure, mmHg	120.5 (12.9)	128.4 (11.6)	119.3 (12.7)
Diastolic blood pressure, mmHg	73.8 (8.8)	78.3 (8.4)	73.1 (8.6)
Diabetes (%)	2.9	5.3	2.6
Eye condition or glasses/contact lenses (%)	53.2	47.0	54.12
Retinal vessel measures			
Central retinal arteriolar equivalent, µm	151.5 (14)	148.4 (14.3)	152.0 (13.8)
Central retinal venular equivalent, µm	220.6 (19)	217.9 (21.3)	221.0 (18.6)
Arteriolar-venular ratio	0.69 (0.06)	0.68 (0.06)	0.69 (0.06)

*Mean (SD), unless otherwise specified; †n for each child variable ranges from 1213-1288; ‡n for each parent variable ranges from 1169-1264. SD, standard deviation; BMI, Body Mass Index

Table 2. Retinal vessel calibre percentiles

Retinal calibre	Child							Parent						
	P5	P10	P25	P50	P75	P90	P95	P5	P10	P25	P50	P75	P90	P95
Central retinal arteriolar equivalent, µm														
Male	137.1	141.5	148.0	158.0	165.1	170.7	174.2	123.6	128.7	139.9	148.3	158.2	167.2	171.6
Female	143.8	147.3	153.8	162.5	169.9	175.3	179.3	128.8	134.8	143.3	152.1	162.3	169.1	174.0
All	139.8	144.0	151.3	160.2	167.6	173.1	178.5	128.1	134.1	143.0	151.4	161.7	169.0	173.7
Central retinal venular equivalent, µm														
Male	203.3	208.9	217.7	226.7	237.4	247.4	252.7	186.8	190.9	202.1	215.4	231.5	247.6	261.54
Female	208.8	213.3	222.6	234.0	245.4	256.8	262.8	192.3	197.5	208.4	220.8	233.8	244.8	253.0
All	206.1	210.8	220.4	230.4	241.4	252.1	259.3	190.9	196.8	207.2	220.2	233.7	245.7	253.0
Arteriolar-venular ratio														
Male	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.6	0.61	0.64	0.67	0.73	0.78	0.79
Female	0.61	0.62	0.66	0.69	0.73	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78
All	0.61	0.63	0.66	0.69	0.72	0.76	0.78	0.59	0.61	0.65	0.69	0.73	0.77	0.78

P: percentile.

Parent-child concordance: Correlations for the 1186 parent-child pairs are displayed in table 3. Pearson's correlations for parent-child dyads were 0.22 for CRAE and 0.23 for CRVE (0.23), and (as expected for a derived ratio value) slightly smaller for AVR (0.18). Mother-child and father-child correlations are presented for reference but not compared statistically, noting slightly higher values for father-child dyads but wider CIs in keeping with the much smaller numbers. In linear regression adjusting for all covariates, estimates attenuated only marginally and all associations remained strong (estimated regression coefficients for parent-child dyads: CRAE 0.19, CRVE 0.21, AVR 0.16) with the exception of father-child AVR. Similarly, partial correlations adjusted for covariates attenuated slightly (correlation coefficient decreased by 0.01 to 0.06, data not shown).

Table 3. Parent-child concordance

	Parent-child			Mother-child			Father-child		
Pearson's Correlation	**N**	**CC**	**95% CI**	**N**	**CC**	**95% CI**	**N**	**CC**	**95% CI**
Central retinal arteriolar calibre,	1186	0.22	0.16 to 0.27	1029	0.20	0.14 to 0.26	157	0.32	0.17 to 0.45
Central retinal venular calibre	1186	0.23	0.17 to 0.28	1029	0.22	0.16 to 0.28	157	0.29	0.14 to 0.42
Arteriolar-venular ratio	1186	0.18	0.13 to 0.24	1029	0.18	0.12 to 0.24	157	0.20	0.04 to 0.35
Adjusted Linear Regression	**N**	**RC**	**P value**	**N**	**RC**	**P value**	**N**	**RC**	**P value**
Central retinal arteriolar calibre, µm	998	0.18	<0.001	867	0.18	<0.001	131	0.21	0.004
Central retinal venular calibre, µm	998	0.20	<0.001	867	0.20	<0.001	131	0.22	0.003
Arteriolar-venular ratio	998	0.16	<0.001	867	0.16	<0.001	131	0.15	0.06

Non-biological caregivers (n=10), diabetic children (n=3) and diabetic adults (n=29) were excluded from these analyses. Covariates in adjusted linear regression models include parent and child age, BMI and Disadvantage Index, mean arterial blood pressure and parent and child sex in models including both sexes. CC: estimated Pearson's correlation coefficient; CI: confidence interval; N: number of biological child-parent pairs with this measure; RC: estimated regression coefficient.

Reliability analysis: Within-grader intraclass correlations were very high for all three graders for CRAE (0.90 to 0.99, 95% CI 0.76 to 0.99) and CRVE (0.92 to 0.98, 95% CI 0.82 to 0.99). This equated to a greater within-grader range for the derived AVR variable (0.69 to 0.97, 95% CI 0.28 to 0.99). Between-grader interclass correlations were also high at 0.79 (95% CI 0.47 to 0.91), 0.92 (95% CI 0.77 to 0.96) and 0.75 (95% CI 0.58 to 0.86) for CRAE, CRVE and AVR respectively.

Unplanned post-hoc analysis: As noted above, both CRAE and CRVE were around 0.6 standard deviations smaller in the mid-life parents than the 11-12 year olds. This contrasts with Ikram's 2012 review, from which we had expected that CRAE would be substantially larger by midlife, but that CRVE would remain static (prior to both reducing into old age).[10] Because these results were surprising, we therefore conducted some unplanned post-hoc analyses to determine how our findings fit within the exisitng literature. These should be considered as exploratory and hypothesis-generating only.

We plotted mean values for CRAE and CRVE by mean age (figure 3) for 15 community-based studies published since 2004 with participants aged from 6 years to old age.[11, 22, 36-47] We included all adult studies (summarised in supplementary table 1) in Sun et al's systematic review[15] that had a community or population-based sample of >1000 individuals;[40-47] the Norwegian Tromso Eye Study was identified through cited reference searches.[37] Sun's review also identified the child population in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM),[36] and we searched the SCORM study's bibliography and other published reviews for additional children's cohorts.[11, 22, 38, 39] We superimposed the results from CheckPoint, separately for adults and children. As visual inspection did not suggest a curvilinear relationship, we then fitted two exploratory linear regression models, with mean age for each study (CheckPoint children and parents considered separately) as the independent variable and with calibre size (arteriolar and venular) as the two dependent variables. It can be seen that venular calibre appears to narrow slightly (though not significantly differently from 0) at a rate of approximately -1.2 μm per decade from childhood through adulthood (β coefficient -0.12, 95% CI -0.37 to 0.14, R^2 = 0.03). However, there was no obvious trend for arteriolar calibre (β coefficient -0.02, 95% CI -0.21 to 0.17, R^2 = 0.002). Two striking features of the figure are the dearth of published values between childhood and late life, and the marked spread of mean values in the elderly.

DISCUSSION

Principal findings: We provide the first national population-based percentile values for the pattern and distributions of retinal vessel calibre in Australian 11-12 year old children and mid-life adults. Surprisingly, in light of previous literature, mean scores for both CRAE and CRVE were around 0.6 standard deviations smaller in parents than in children. Parent-child correlations were similar for all retinal vessel calibre metrics (CRAE 0.20, CRVE 0.22, AVR 0.17) to studies whose offspring probands were already adults, suggesting that the strong element of heritability in microvascular anatomical structure is largely phenotypically overt within the first decade of life.

Strengths and weaknesses: Strengths include the national recruitment basis for this large study, providing a reference point for microvascular measurement for future Australian studies but also filling a significant international gap in population-based studies of these age groups. Results were largely consistent with previous studies and should generalise to the wider Australian child population.

Limitations include our under-representation of the most disadvantaged sector (reflecting social biases in both recruitment and retention, issues common to large longitudinal studies) and the small number of fathers in the parent sample. We adjusted only for a limited range of potential confounders. While the distributions of parent retinal vessel caliber might have changed slightly had we further adjusted for smoking status, sedentary lifestyle and diet (all previously associated with altered retinal vessel calibre[48]), their impact on concordance would likely be small because these factors are all strongly socially patterned. By 2019 this cohort will also be able to consider genome-wide association data, potentially shedding further light on the roles of genetic and shared environmental factors. We were not able to measure refractive errors in this study. Retinal vascular calibre measurements may be influenced by refractive errors and refraction is different between children and adults.[49, 50] However, we would expect these effects to be small, particularly as other sources of systematic bias were minimised (measured on the same day with the same equipment by the same person who was blind to dyadic membership).

Meaning and implications for clinicians and policy makers: Findings for all outcomes are consistent with the previous literature. We showed a larger standard deviation in the parent than the child group, indicating a greater spread of variation in retinal calibre with age. This would be in keeping with greater physiologic dysregulation for some inidividuals with age in response to genetic and risk exposure (eg higher blood pressure, obesity etc) over multiple decades. Direct

comparisons of CRAE and CRVE across the lifecourse and between ethnicities remain difficult due to differences in equipment, software and analysis formulae for CRAE and CRVE. Such factors could well account for the differences between our own and three international studies at mean age 11.1-12.7 years: smaller mean CRAE and CRVE in Chinese twins (CRAE 150.1-µm, CRVE 218.4µm) and Singapore Chinese children (CRAE 149.3µm, CRVE 224.6µm), but much larger values in a smaller study of German children (CRAE 208µm, CRVE 236.2µm).[22, 36, 51] These discrepancies highlight the need for a uniform approach to measurement of retinal parameters in future epidemiological investigations. Our post-hoc analysis of published literature suggest both a dearth of population studies between childhood and old age and a likely fall in venular calibre from childhood through midlife. An individual participant meta-analysis would be the next step to more precisely determine how retinal calibre varies by age, incorporating any additional large-scale studies that now exist.

The moderate parent-child correlations of this Australian population are very similar to those in previous studies with much older adult offspring, suggesting that in terms of microvasculature children do not become more phenotypically similar to their parents between childhood and midlife. Although this is the first study to report intergenerational concordance by parent sex, our marginally stronger father-child than mother-child correlations must be taken with caution due to the small sample of fathers. Like all other parent-child and twin studies except the Beaver Dam Eye Study, our CRVE correlations were marginally larger than those for CRAE.[17-23] Previously, Li et al. have postulated that the retinal arteriolar phenotype may be more sensitive to environmental influences than the venular phenotype, despite having a significant, moderate degree of inheritance.[19] This is further supported by GWAS studies, where only CRVE showed significant associations with the identified gene loci.[23, 25]

Unanswered questions and future research: We provide normative values for retinal vessel calibre for Australian 11-12 year olds and mid-life adults using standardised protocols. Our findings make explicit a need for reliable age-specific normative reference values across the lifecourse. Ideally, this would be extended to large long-running cohort studies with access to clinical outcomes; to exploration of other retinal vascular features such as branching angles, tortuosity and fractal dimension; and to consider other factors such as polygenic risk scores and macrovascular risk. Such studies could help retinal calibre realise its potential as a clinical, population screening and/or risk stratification tool for cardiovascular disease.

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CONTRIBUTIONS: MW, TW, DB and KL contributed to the study design. JD, ML and MH contributed to the acquisition of data. AG, KL, ML and JD conducted the data analysis. JD drafted the manuscript with critical input from all authors. MW and KL supervised JD. MW is the principal investigator of the Child Health CheckPoint and conceived the paper. All authors read and approved the final manuscript.

DATA SHARING STATEMENT: Dataset and technical documents available from Growing Up in Australia: The Longitudinal Study of Australian Children via low-cost license for bone fide researchers. More information is available at www.growingupinaustralia.gov.au

FIGURE CAPTIONS AND FOOTNOTES:

Figure 1. Participant flow through *Child Health CheckPoint.*

n: number of families; c: number of children; p: number of attending adults; MAC: Main assessment centre; mAC: Mini assessment centre; HV: Home visit assessment; LSAC: Longitudinal Study of Australian Children.

*Unable to collect image due to equipment failure or time constraints.

~Data excluded for images that did not meet quality criteria for 'Big 6' analysis.

^Data from 10 non-biological child-parent pairs and 29 diabetic participants excluded from concordance analyses

Figure 2. Density plots for retinal vessel calibre measures.

AVR: arteriolar-venular ratio; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; BIG6: revised knudston-parr formula for calculating CRAE and CRVE using IVAN software.[30]

Figure 3. Epidemiology of retinal vascular calibre by age.

Mean retinal arteriolar and venular calibre by age from CheckPoint and published community-based studies with n>1000. Each symbol represents the mean value for a single study. Each study is summarised in supplementary table 1, and the paper from which that study's data are drawn is provided in the list of references. Checkpoint_C: Child Health CheckPoint data of children; CheckPoint_P: Child Health CheckPoint data of parents.

SUPPLEMENTARY DOCUMENTS:

Supplementary table 1. Epidemiology of studies reporting retinal vascular calibre

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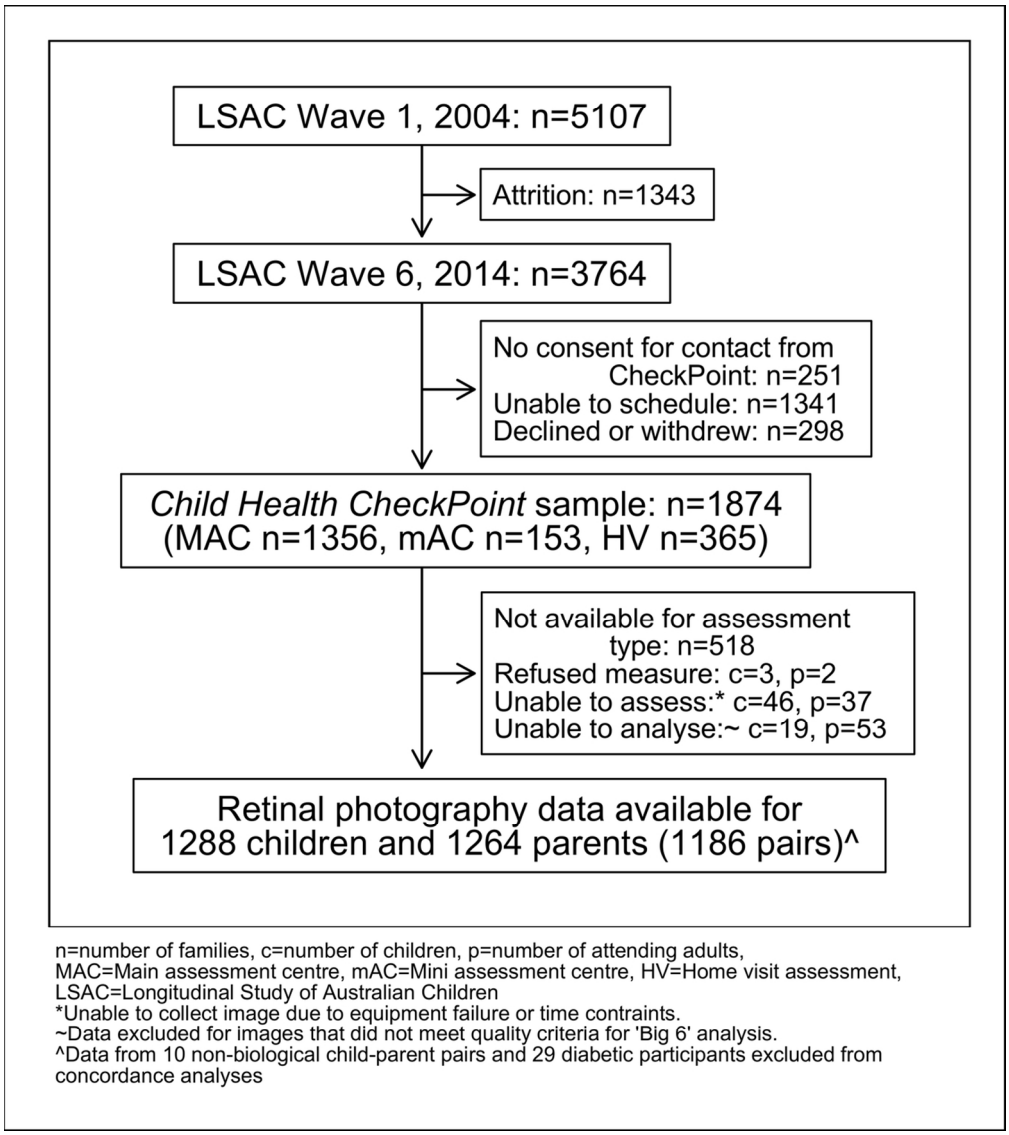

Figure 1. Participant flow through Child Health CheckPoint.
n: number of families; c: number of children; p: number of attending adults; MAC: Main assessment centre; mAC: Mini assessment centre; HV: Home visit assessment; LSAC: Longitudinal Study of Australian Children.
*Unable to collect image due to equipment failure or time constraints.
~Data excluded for images that did not meet quality criteria for 'Big 6' analysis.
^Data from 10 non-biological child-parent pairs and 29 diabetic participants excluded from concordance analyses

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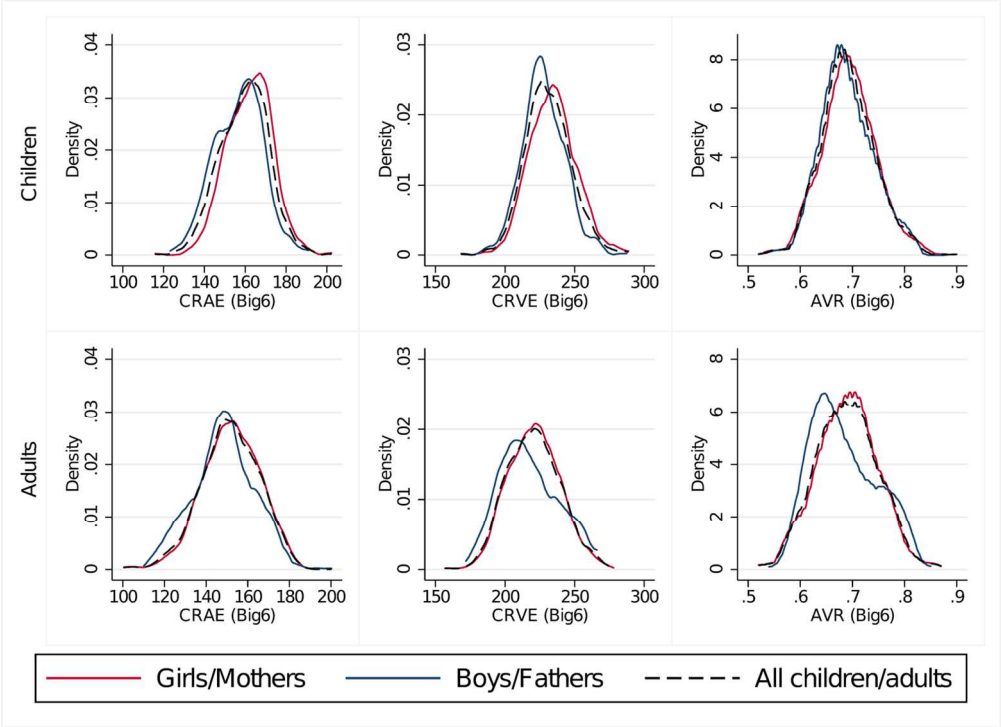

Figure 2. Density plots for retinal vessel calibre measures.
AVR: arteriolar-venular ratio; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; BIG6: revised knudston-parr formula for calculating CRAE and CRVE using IVAN software.30

66x48mm (600 x 600 DPI)

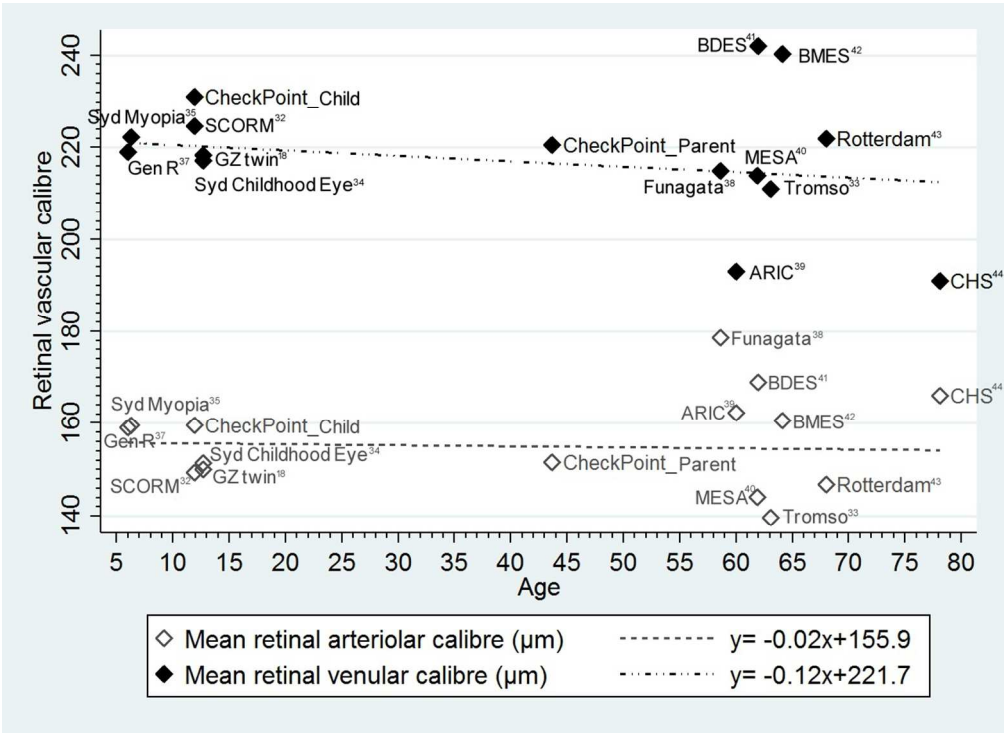

Figure 3. Epidemiology of retinal vascular calibre by age. ├ ┬ Mean retinal arteriolar and venular calibre by age from CheckPoint and published community-based studies with n>1000. Each symbol represents the mean value for a single study. Each study is summarised in supplementary table 1, and the paper from which that study's data are drawn is provided in the list of references. Checkpoint_C: Child Health CheckPoint data of children; CheckPoint_P: Child Health CheckPoint data of parents.├ ┬

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Supplementary Table 1. Epidemiology of studies reported retinal vascular calibre

Author; publication year reference number	Study name; country	Examination year	Sample size	Mean age (years)	Arteriolar calibre (mean(SD), µm)	Venular calibre (mean(SD), µm)
Children						
Gishti; 2015[37]	Gen R; Netherlands	2008-2010	4007	6	159.0 (14.9)	219.0 (20.2)
Mitchell; 2008[35]	Syd Myopia; AU	2003-2004	1369	6.3	159.6 (ns)	222.3 (ns)
Cheung; 2012[32]	SCORM; Singapore	2006	1225	11.9	149.3 (12.3)	224.6 (17.8)
	CheckPoint_C; AU	2015-2016	1288	11.9	159.5 (11.8)	231.1 (16.5)
Gopinath; 2010[34]	Syd Childhood Eye; AU	2004-2005	2272	12.7	151.4 (12.1)	151.4 (12.1)
Zheng; 2013[18]	GZ twin; China	2009	2070	12.7	150.1 (13.4)	218.4 (19.1)
Adults						
	CheckPoint_P; AU	2015-2016	1264	43.7	151.5 (14.0)	220.6 (19.0)
Kawasaki-1; 2006[38]	Funagata; Japan	2000-2002	1481	58.6	178.6 (21.0)	214.9 (20.6)
Liew; 2008[39]	ARIC; USA	1993-1995	8794	60	162.3 (ns)	193.1 (ns)
Wong; 2006[40]	MESA; USA	2002-2004	5979	61.9	144.1 (14.4)	214.0 (22.2)
Klein; 2006[41]	BDES; USA	1988-1990	4926	62	169 (ns)	242 (ns)
Von; 2014[33]	Tromso; Norway	2007-2008	6353	63.1	139.7 (14.6)	211.1 (21.1)
Kawasaki-2; 2013[42]	BMES; Australia	2003	2335	64.1	160.6 (14.9)	240.3 (22.4)
Ikram; 2004[43]	Rotterdam; Netherlands	1990-1993	5674	68	146.9 (14.4)	222.0 (20.9)
Kim; 2010[44]	CHS; USA	1997-1998	1744	78.1	166 (19)	191 (18)

Abbreviations: SD, standard deviation; AU, Australia; USA, United State of America; Gen R, Generation R Study; Syd Myopia, The Sydney Myopia Study; SCORM, Singapore Cohort Study of the Risk Factors for Myopia; CheckPoint_C, Child Health CheckPoint data of children; Syd Childhood Eye; The Sydney Childhood Eye Study; GZ twin; The Guangzhou twin eye study; CheckPoint_P, Child Health CheckPoint data of parents; ARIC; Atherosclerosis Risk In Communities; MESA; Multi-Ethnic Study of Atherosclerosis; BDES; Beaver Dam Eye Study; Tromso, The Tromsø Eye Study; BMES; Blue Mountains Eye Study; Rotterdam; The Rotterdam Study; CHS; Cardiovascular Health Study; ns, not stated.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7, 8*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	6, 7, figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7, 8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Figure 1
		(b) Give reasons for non-participation at each stage	7,10, figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 10, table 1
		(b) Indicate number of participants with missing data for each variable of interest	Figure 1
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	10, table 1, table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13, 14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15, 16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16, 17
Generalisability	21	Discuss the generalisability (external validity) of the study results	16, 17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18, 19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.