Cardiovascular health and retinal microvascular geometry in Australian 11-12 year-olds

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Abstract

Traditional retinal microvascular parameters (smaller arteriolar and greater venular calibre) are associated with cardiovascular risk factors, pre-clinical vascular phenotypes and clinical cardiovascular events in adults. Although novel retinal microvascular geometric parameters showed analogous associations in adults, less is known whether these parameters are associated with cardiovascular health from childhood. In a population-based cross-sectional study in children (n=1126, mean age 11.4 years, 50.3% girls), we examined associations of cardiovascular risk factors and pre-clinical arterial phenotypes with retinal geometric parameters. Cardiovascular parameters included body mass index (BMI), an inflammatory marker (GlycA), low-density lipoprotein and high-density lipoprotein (HDL) cholesterol, systolic (SBP) and diastolic blood pressure, large artery functional (pulse wave velocity, PWV and carotid arterial elasticity) and structural (carotid intima-media thickness) phenotypes. Retinal geometric parameters (fractal dimension (D_f) and tortuosity) were quantified from retinal images. Multivariable regression models were performed and adjusted for potential confounders. Higher values for BMI, SBP and PWV showed weak associations with lower (i.e. worse) arteriolar but not venular D_f (standardized mean difference (SMD) ranging from -0.06 to -0.09, 95% CIs -0.13 to -0.01). Higher HDL was associated with greater arteriolar D_f (SMD 0.07, 95%CI 0.01 to 0.13). Only higher SBP was associated with higher (i.e. worse) arteriolar but not venular tortuosity (SMD 0.09, 95% CI 0.02 to 0.16). In generally healthy children, some risk factors and pre-clinical arterial phenotypes show small associations with retinal geometric parameters. In childhood, emerging relationships between microvascular parameters and cardiometabolic risk may be better described by retinal vascular calibre than by geometric parameters.

Keywords: microcirculation, retinal micrvasculature, retinal vascular parameters, children, population-based study, risk factors, pre-clinical vascular phenotypes

INTRODUCTION

The retinal microcirculation can be imaged directly and non-invasively. In adults, retinal microvascular width (or caliber) has been widely studied,[1] with meta-analyses showing that narrower arteriolar and wider venular caliber predict cardiovascular diseases (CVD) including stroke and coronary heart disease.[2-4] However, assessment of retinal microvascular caliber overlooks structural variation and geometry across the overall retinal vasculature, which could be important.

The design of the retinal vascular tree follows the optimization principle, according to the validated theory of minimum work.[5] Thus, it is hypothesized that when the architecture of the vascular tree is compromised, the efficiency of metabolic transport is reduced, reflecting greater CVD risk and microcirculation damage.[5] Image scoring software such as the Singapore I Vessel Assessment (SIVA) software now allows assessment of retinal vascular geometry in large studies, beyond vessel caliber.[6,7] In adults, microvascular geometric parameters have been associated with CVD and risk factors, such as higher blood pressure (BP), and cholesterol levels.[6,8] These results have also been replicated using different scoring software,[9,10] but had mixed findings in the few children's studies,[11,12] when these retinal parameters may be valuable potential early markers of CVD risk.

Using the national, population-based Child Health CheckPoint study nested within the Longitudinal Study of Australian Children (LSAC), we previously reported that retinal microvascular caliber was associated with body mass index (BMI), a chronic inflammation marker (Glycoprotein acetyls, GlycA), and phenotypes of large arterial function and structure in 11-12 year-olds.[13,14] We now investigate whether retinal geometric parameters (considered here to be 'outcomes') show similar associations in the same cohort, and therefore whether they provide additional information about the early development of CVD risk beyond microvascular caliber.

MATERIALS AND METHODS

Study Design and Participants

In 2004, the LSAC study recruited a nationally representative sample of 5107 Australian infants who have since been followed biennially (retention 73.7% to wave 6 in 2014).[15] The Child Health CheckPoint, a cross-sectional data collection wave nested between LSAC's waves 6 and 7, was a one-off comprehensive biophysical assessment of 11- to 12-year old children and one attending parent. The study design and recruitment are described elsewhere[16] and the study flow is shown in the Supplementary Figure. Briefly, CheckPoint collected data on multiple health domains across Australia at assessment centers in major and regional cities. Retinal data were unavailable on 518 children participating in smaller cities or home visits as retinal images were not collected due to limitations in equipment mobility. Retinal geometric parameters are yet to be scored on parents.

Measures

Supplementary Table shows the equipment and procedures for cardiovascular risk (BMI, GlycA, systolic blood pressure (SBP), diastolic blood pressure (DBP), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglyceride, carotid intima-media thickness (cIMT), pulse wave velocity (PWV) and carotid arterial elasticity); retinal geometry (retinal fractal dimension (D_f), simple tortuosity; and *a priori* identified potential confounding variables. Collection procedures for each measure have been described previously.[17-20] BMI was converted into age- and gender-specific z-scores using the US CDC growth reference charts.[21]

Figure 1 shows SIVA interface with two novel retinal microvascular geometric parameters. Tortuosity is an index of how much the retinal vessels deviate from a straight line of the same vessel segment; a lower tortuosity index represents straighter (ie better) vessels.[7] D_f summarizes the complexity of the branching of arterioles/venules,[6] where larger values represent a more complex (ie better) branching pattern.

Statistical analysis

Multivariable linear regression models were performed with estimates adjusted for age, sex and socio-economic position (SEP). We considered retinal geometric parameters as the dependent variables, although for the large artery measures the reverse would be equally plausible since they were measured cross-sectionally. To visualize our findings, we internally constructed standardized scores ([observed value - mean]/SD) for all measures except BMI z-score; thus regression coefficients represent the standardized mean difference (SMD). Analyses were performed in Stata 15.0 (StataCorp LP; College Station, TX, USA).

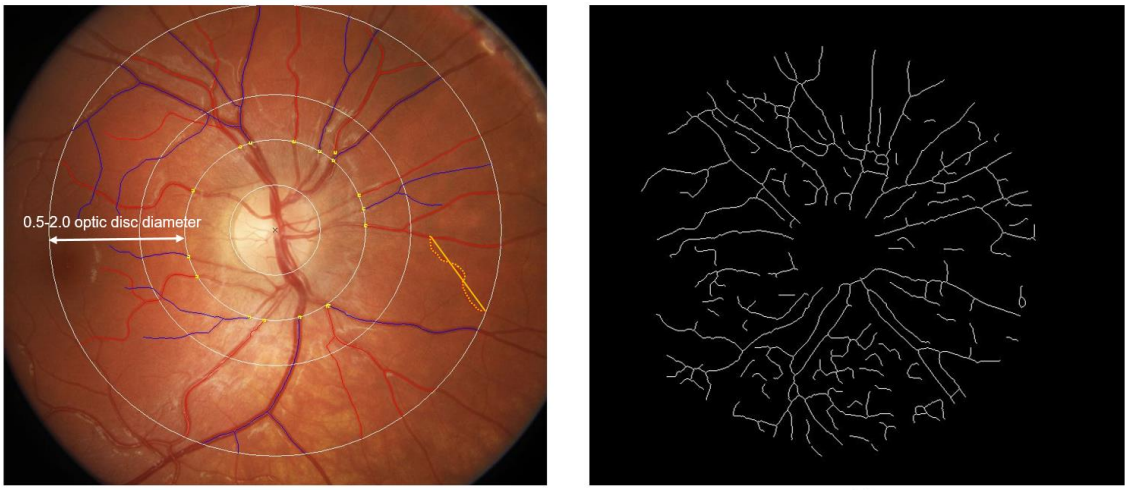

Figure 1. Retinal geometric parameters measured using Singapore I Vessel Assessment (SIVA). (1) Arterioles are highlighted in red and venules in blue. The measured area of retinal microvascular parameters is demarcated by white circles 0.5 to 2.0 disc diameters away from the disc margin. Simple tortuosity is calculated by the actual length of vessel divided by the Euclidean distance between the first and last points of that vessel; (2) Fractal dimension was calculated from the skeletonized line tracing using the box-counting method.

RESULTS

Of the 1874 children participating in CheckPoint, 1126 (mean age 11.4 years, 50.3% girls) had data on all measures (Table 1). The sample mean SEP was 0.26 SD above (i.e. more advantaged) the mean SEP of all families retained at LSAC wave 6 and the mean SEP of all families enrolled at LSAC wave 1 (mean 0, SD 1).

The results of all analyses are presented in Table 2. Data are presented as the association per SD higher of BMI z-score, standardized GlycA, BP, LDL, HDL, triglyceride, cIMT, PWV and arterial elasticity with standardized regression coefficient change in retinal parameters. Higher BMI, SBP and PWV showed weak evidence of an association with lower (ie worse) arteriolar D_f (SMD ranging from -0.07 to -0.09, 95% CIs -0.13 to -0.01), whereas higher HDL was associated with greater arteriolar D_f 0.07 (95%CI 0.01 to 0.13). Only higher SBP was associated with higher (ie worse) arteriolar tortuosity (SMD 0.09, 95% CI 0.02 to 0.16). There was no evidence of associations between any exposure measures with venular D_f or tortuosity.

Table 1. Sample characteristics of the analytic sample; all values are mean (SD) except sex (%)

Variable	Value (n=1126)
Age (years)	11.4 (0.5)
% females	50.3
Family socioeconomic position	0.26 (1.0)
BMI z-score	0.28 (0.97)
Glycoprotein acetyls (mmol/L)	0.99 (0.12)
Low-density lipoprotein cholesterol (mmol/L)	1.36 (0.31)
High-density lipoprotein cholesterol (mmol/L)	1.48 (0.27)
Triglyceride (mmol/L)	1.03 (0.44)
Systolic blood pressure (mmHg)	108.0 (7.8)
Diastolic blood pressure (mmHg)	62.8 (5.4)
cIMT(μm)	575.4 (41.0)
Arterial elasticity (%/10mmHg)	4.8 (0.8)
Pulse wave velocity (m/s)	4.5 (0.5)
Fractal dimension	
Arteriole fractal dimension	1.24 (0.05)
Venule fractal dimension	1.21 (0.05)
Tortuosity	
Simple tortuosity arteriole	1.12 (0.03)
Simple tortuosity venule	1.10 (0.02)

Table 2. Differences in retinal outcomes per SD higher of cardiovascular risk factors and pre-clinical arterial phenotypes; regression estimates adjusted for age, sex and SEP.

Exposures	Fractal dimension				Simple tortuosity			
	Arteriole		Venule		Arteriole		Venule	
	SMD (95%CI)	*p*	SMD (95%CI)	*p*	SMD (95%CI)	*p*	SMD (95%CI)	*p*
BMI z-score	**-0.08 (-0.13, -0.02)**	**0.01**	-0.01 (-0.06, 0.05)	0.83	0.00 (-0.06, 0.07)	0.92	-0.02 (-0.09, 0.05)	0.57
GlycA	-0.06 (-0.13, 0.00)	0.05	0.02 (-0.04, 0.09)	0.52	0.04 (-0.03, 0.12)	0.22	0.05 (-0.02, 0.12)	0.17
LDL cholesterol	0.00 (-0.06, 0.06)	1.00	-0.02 (-0.09, 0.04)	0.43	-0.00 (-0.06, 0.06)	0.99	0.01 (-0.06, 0.07)	0.83
HDL cholesterol	**0.07 (0.01, 0.13)**	**0.02**	0.01 (-0.05, 0.07)	0.68	-0.05 (-0.11, 0.02)	0.14	-0.01 (-0.08, 0.05)	0.74
Triglyceride	-0.05 (-0.11, 0.02)	0.15	-0.03 (-0.09, 0.04)	0.43	0.06 (-0.00, 0.13)	0.06	0.06 (-0.00, 0.13)	0.07
Systolic BP	**-0.07 (-0.13, -0.01)**	**0.03**	-0.00 (-0.06, 0.06)	0.92	**0.09 (0.02, 0.16)**	**0.01**	-0.01 (-0.07, 0.06)	0.86
Diastolic BP	-0.02 (-0.08, 0.04)	0.52	-0.00 (-0.07, 0.06)	0.93	0.07 (-0.00, 0.14)	0.06	0.01 (-0.06, 0.09)	0.68
cIMT	-0.02 (-0.08, 0.04)	0.44	-0.05 (-0.11, 0.01)	0.12	-0.01 (-0.07, 0.06)	0.80	-0.03 (-0.09, 0.04)	0.46
Arterial elasticity	0.05 (-0.01, 0.11)	0.11	0.01 (-0.05, 0.08)	0.64	-0.03 (-0.10, 0.04)	0.35	0.01 (-0.06, 0.08)	0.71
PWV	**-0.09 (-0.15, -0.03)**	**0.00**	-0.01 (-0.07, 0.04)	0.63	0.05 (-0.01, 0.12)	0.10	0.02 (-0.05, 0.09)	0.58

Abbreviations: SMD, standardized mean difference; SEP, socioeconomic position; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure; cIMT, carotid intima-media thickness; PWV, pulse wave velocity. Results with p-value less than 0.05 were in bold.

DISCUSSION

Principal findings

In this population-based cross-sectional study of 11-12 year-olds, we found weak evidence of associations between some cardiovascular health measures and retinal arteriolar, but not venular geometry. However, there was no clear generalized pattern across all factors considered. For example, some risk factors (ie higher BMI, HDL and SBP) were weakly associated with arteriolar D_f, while only higher SBP was associated with arteriolar tortuosity. Of the clinical arterial phenotypes examined, only PWV showed weak evidence of an association with retinal arteriolar D_f.

Strengths and limitations

To the best of our knowledge, this is the first study in children to examine the relationship between pre-clinical cardiovascular risk factors and retinal geometric parameters. Our study drew on a large, national sample of Australian children spanning diverse geographical areas. Intermediate phenotypes of small and large vessels were assessed contemporaneously at the right-sided circulation.

Longitudinal studies would perhaps reveal stronger associations and their temporal patterns. Further, our sample is slightly more socioeconomically advantaged compared to the original LSAC samples and is predominantly Anglo-European, which may limit the generalizability of findings to other populations.

Interpretation in light of other studies

D_f and tortuosity are indicators of vasculature complexity. In adults with cardiometabolic conditions, such as hypertension and diabetes, microvascular remodeling results in less structural complexity but more tortuous vessel patterns.[22,23] There are few studies in children and results are not consistent. One study of 166 Malaysian children (mean age 9.6 years) found that venular D_f and tortuosity were slightly higher in children with obesity (mean difference: D_f 0.015 (95% CI 0.001, 0.030), tortuosity 0.006 (95% CI 0.001, 0.012)).[11] In contrast, our study revealed evidence of an association with only with arteriolar D_f, with per z-score higher BMI associated with 0.08SD (0.10 unit) lower arteriolar D_f.

A smaller study of 88 children with type 1 diabetes (mean age 13.6 years) reported that greater cIMT was associated with greater arteriolar but not venular tortuosity.[12] This was not evident in our sample. If large arterial dysfunction precedes structural changes,[24] it is plausible that the association of arterial phenotypes with retinal geometry emerge from mid-childhood and may become more evident later in life and in those with cardiometabolic disease conditions.[25]

Implications

Several studies have demonstrated that retinal microvascular parameters correlate with changes in the microcirculation in the kidney, brain, and heart, highlighting the clinical value of evaluating the retinal microcirculation.[26] Advanced deep-learning algorithms from retinal images can predict a range of cardiovascular risk factors, including age, sex, SBP and BMI.[27] Although some large population-based studies report associations between retinal geometric parameters and CVD in late adulthood,[6] these parameters have only recently been described and there are fewer and less consistent data compared to retinal microvascular caliber, particularly in children. Likewise, we found a smaller size of associations with these geometric parameters than we have previously reported for retinal microvascular caliber in the same cohort.[13,14] This might reflect lower reliability in geometric parameters than calibers.[28] Longitudinal studies with repeated measurements of both cardiovascular risk factors and retinal parameters across different life

stages could clarify the relationships and inform the usefulness of the geometric measures of retinal vasculature.

CONCLUSIONS

In a largely healthy sample of Australian children, CVD risk factors were only weakly associated with retinal arteriolar geometric parameters. In childhood, emerging relationships between microvascular parameters and cardiometabolic risk may be better described by retinal vessel caliber than by retinal vessel geometry.

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Conflicts of interest

The authors declare no potential conflicts of interest, including no specific financial interests relevant to the subject of this manuscript.

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Reference

1. Struijker-Boudier, H. A., et al., 2012. Phenotyping the microcirculation. Hypertension.60(2):523-527.
2. Ikram, M. K., et al., 2013. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. Ophthalmologica.229(3):125-136.
3. McGeechan, K., et al., 2009. Meta-analysis: retinal vessel caliber and risk for coronary heart disease. Ann Intern Med.151(6):404-413.
4. McGeechan, K., et al., 2009. Prediction of incident stroke events based on retinal vessel caliber: a systematic review and individual-participant meta-analysis. Am J Epidemiol.170(11):1323-1332.
5. Zamir, M., 1976. Optimality principles in arterial branching. J Theor Biol.62(1):227-251.
6. Liew, G., et al., 2011. Fractal analysis of retinal microvasculature and coronary heart disease mortality. Eur Heart J.32(4):422-429.
7. Cheung, C. Y., et al., 2011. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. Ophthalmology.118(5):812-818.
8. Campbell, M. D., et al., 2018. Impact of ideal cardiovascular health in childhood on the retinal microvasculature in midadulthood: Cardiovascular Risk in Young Finns Study. J Am Heart Assoc.7(20):e009487.
9. Witt, N., et al., 2006. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. Hypertension.47(5):975-981.
10. McGrory, S., et al., 2018. Towards standardization of quantitative retinal vascular parameters: Comparison of SIVA and VAMPIRE measurements in the Lothian Birth Cohort 1936. Transl Vis Sci Technol.7(2):12.
11. Tai, E. L. M., et al., 2018. Comparison of retinal vascular geometry in obese and non-obese children. PLoS One.13(2):e0191434.
12. Pena, A. S., et al., 2018. Early atherosclerosis is associated with retinal microvascular changes in adolescents with type 1 diabetes. Pediatr Diabetes.19(8):1467-1470.
13. Liu, M, et al., 2019 Does inflammation mediate the relationship between obesity and retinal vascular calibre in children and mid-life adults? Sci Rep Under review (manuscript no. SREP-19-29746).
14. Liu, M; , et al., 2019. Associations of retinal microvascular caliber with large arterial function and structure: A population-based study of 11-12 year-olds and mid-life adults. Microcirculation.Under review (manuscript no. UMIC-2019-0067).
15. Ann S., Johnstone R., 2004. Growing Up in Australia takes its first steps. Family Matters. (67):46-53.
16. Clifford, S. A., et al., 2019. Child Health CheckPoint: cohort summary and methodology of a physical health and biospecimen module for the Longitudinal Study of Australian Children. BMJ Open.9(Suppl 3):3-22.
17. Liu, R. S., et al., 2019. Carotid artery intima-media thickness, distensibility and elasticity: population epidemiology and concordance in Australian children aged 11-12 years old and their parents. BMJ Open.9(Suppl 3):23-33.
18. Dascalu, J., et al., 2019. Retinal microvasculature: population epidemiology and concordance in Australian children aged 11-12 years and their parents. BMJ Open.9(Suppl 3):44-52.
19. Clifford, S. A., et al., 2019. Body composition: population epidemiology and concordance in Australian children aged 11-12 years and their parents. BMJ Open.9(Suppl 3):95-105.
20. Ellul, S., et al., 2019. Metabolomics: population epidemiology and concordance in Australian children aged 11-12 years and their parents. BMJ Open.9(Suppl 3):106-117.
21. Kuczmarski, R. J., et al., 2000. CDC growth charts: United States. Adv Data. (314):1-27.
22. Zhu, P., et al., 2014. The relationship of retinal vessel diameters and fractal dimensions with blood pressure and cardiovascular risk factors. PLoS One.9(9):e106551.
23. Klein, R., et al., 2018. The Relationship of retinal vessel geometric characteristics to the incidence and progression of diabetic retinopathy. Ophthalmology.125(11):1784-1792.
24. Balletshofer, B. M., et al., 2003. Insulin resistant young subjects at risk of accelerated atherosclerosis exhibit a marked reduction in peripheral endothelial function early in life but not differences in intima-media thickness. Atherosclerosis.171(2):303-309.
25. Liu, M.;, et al., 2019. Associations of retinal microvascular caliber with intermediate phenotypes of large arterial function and structure: A systematic review and meta-analysis. Microcirculation.26(7):e12557.
26. Grassi, G., Mancia, G., 2018. 'Keep an eye' on the heart: retinal microcirculation disarray in congestive heart failure. Eur Heart J.39(1):57-59.
27. Poplin, R., et al., 2018. Prediction of cardiovascular risk factors from retinal fundus photographs via deep learning. Nat Biomed Eng.2(3):158-164.
28. Huang, Q. F., et al., 2018. Reproducibility of retinal microvascular traits decoded by the Singapore I Vessel Assessment software across the human age range. Am J Hypertens.31(4):438-449.