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SYMPOSIUM REVIEW

Retinal vascular imaging in early life: insights into processes and risk of cardiovascular disease

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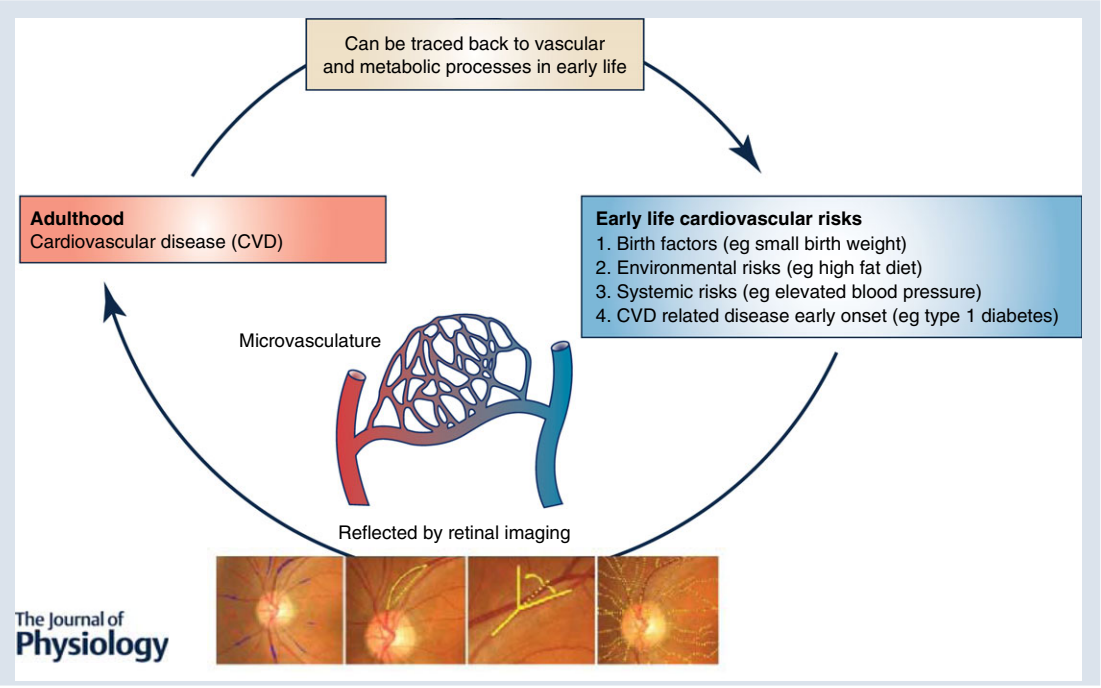

Ling-Jun Li is a clinical research fellow currently working on clinical and epidemiological projects at Singapore Eye Research Institute (SERI) and also collaborating on relevant research at the National University of Singapore, KK Women's and Children's Hospital and Tan Tock Seng Hospital. She has been trained and involved in basic research, clinical practice and epidemiological studies as a whole. The eye is a 'window' to the human circulation and inflammation. Her current research links retinal imaging data, using advanced and state-of-the-art computer-based image analysis, to monitor maternal health (e.g. maternal gestational diabetes and gestational hypertension) and children's health (e.g. fetal growth restriction, Kawasaki disease), and even to predict drug effects (e.g. HIV/AIDS patients undergoing anti-viral therapy). **Mohammad Kamran Ikram** completed dual training in Epidemiology (PhD 2005) and Neurology (Dutch Specialist Board Certified 2010) at the Erasmus Medical Center Rotterdam, the Netherlands. He is currently Associate Professor at the Duke-NUS Graduate Medical School, National University of Singapore and working at the Singapore Eye Research Institute. He is Principal Investigator of the Epidemiology of Dementia in Singapore (EDIS) Study, which is part of the Memory Aging and Cognition Centre (MACC) at the National University Health System in Singapore. His current scientific activities cover a broad spectrum of epidemiological research both in Neurology and Ophthalmology. These include cerebrovascular diseases, dementia, stroke, novel retinal imaging and diabetic retinopathy. He has published more than 100 international peer-reviewed papers. As Medical Director of SNEC, **Tien Y. Wong** helms one of the largest tertiary eye hospital in Asia, with a faculty of >70 ophthalmologists managing >300,000 outpatient visits and >27,000 major surgeries annually. SNEC's research division, the Singapore Eye Research Institute (SERI), is one of the world's leading eye research institutes. Prof Wong balances clinical practice in ophthalmology, focusing on retinal diseases such as diabetic retinopathy and age-related macular degeneration, with a broad-based research program comprising epidemiological, clinical and translational studies of Asian retinal diseases, and on the use of retinal imaging to predict disease risk. He has published >1,000 peer-reviewed papers, including papers in the New England Journal of Medicine and the Lancet. He has given >200 invited plenary, symposium and named lectures globally, and received >US$50 million in grant funding as Principal Investigator.

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Abstract Cardiovascular disease (CVD) is the leading cause of morbidity and mortality globally. In recent years, studies have shown that the origins of CVD may be traced to vascular and metabolic processes in early life. Retinal vascular imaging is a new technology that allows detailed non-invasive *in vivo* assessment and monitoring of the microvasculature. In this systematic review, we described the application of retinal vascular imaging in children and adolescents, and we examined the use of retinal vascular imaging in understanding CVD risk in early life. We reviewed all publications with quantitative retinal vascular assessment in two databases: PubMed and Scopus. Early life CVD risk factors were classified into four groups: birth risk factors, environmental risk factors, systemic risk factors and conditions linked to future CVD development. Retinal vascular changes were associated with lower birth weight, shorter gestational age, low-fibre and high-sugar diet, lesser physical activity, parental hypertension history, childhood hypertension, childhood overweight/obesity, childhood depression/anxiety and childhood type 1 diabetes mellitus. In summary, there is increasing evidence supporting the view that structural changes in the retinal microvasculature are associated with CVD risk factors in early life. Thus, the retina is a useful site for pre-clinical assessment of microvascular processes that may underlie the future development of CVD in adulthood.

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Abstract figure legend The origins of CVD may be traced back to vascular and metabolic processes in early life. Retinal vascular imaging is a new technology that allows detailed non-invasive *in vivo* assessment and monitoring of the microcirculation. Our review supports the view that CVD risk factors are associated with structural and functional changes in the retinal microvasculature in early life. Thus, the microcirculation may be a site for pre-clinical processes underlying the development of CVD in adulthood.

Abbreviations AVR, arteriole-to-venule ratio; CRP, C-reactive protein; CVD, cardiovascular disease; DVA, dynamic vessel analyzer; IUGR, intra-uterine growth retardation; NO, nitric oxide; OCT, optical coherence tomography; sFLT-1, fms-like tyrosine kinase-1; T1DM, type 1 diabetes mellitus.

Introduction

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality globally. There is increasing evidence that the origins of CVD may be traced to vascular, metabolic and other processes that start in early life. This viewpoint is sometimes referred to as the salt hypothesis (Backes *et al.* 2013), the Dorner hypothesis (Kaess *et al.* 1975), or the Barker hypothesis (Barker *et al.* 1989). The effects of early life conditions and diseases that may influence the development of CVD in later life have been studied in several longitudinal studies (Barker *et al.* 1990, 2009; Napoli *et al.* 1999; Harding, 2001; Eriksson *et al.* 2003). In Barker hypothesis, also known as the 'thrifty phenotype hypothesis' (Ellison, 2005), intrauterine growth restriction due to fetal adaptation to metabolic and vascular processes is linked to the development of major CVD in late-life (Barker, 2004*b*).

Population-based studies have also suggested that early life factors may be important determinants of trends and geographical differences in CVD mortality across populations (Forsdahl, 1979; Barker & Osmond, 1986; Ben-Shlomo & Smith, 1991; Elford *et al.* 1992; Dorling *et al.* 2000; Leon & Davey Smith, 2000). Postulated mechanisms include persistent vascular and metabolic damage due to the exposure to CVD risk factors (e.g. high-fat diet, obesity, elevated blood pressure) in early life. This in turn may trigger other epigenetic modifications leading to morphological, pathological and metabolic alterations in major tissues (e.g. fatty tissue), and organs (e.g. liver, pancreas, brain and kidney) (Fig. 1). Consistent with epidemiological studies are autopsy studies from early childhood showing atherosclerosis with fatty streaks in the aorta, and coronary and carotid arteries (Berenson *et al.* 1998; McGill *et al.* 2000*a*,*b*).

While these studies have provided some evidence for vascular damage in early life, most data are cross-sectional in nature, making causal inferences from these studies difficult. Thus, the key question of which pathophysiological mechanisms explain the development and progression of CVD from early to later life remains unanswered. One pathway in the process of vascular damage involves endothelial dysfunction. Endothelial dysfunction generally refers to the reduction of nitric oxide (NO) bioavailability through decreased *endothelial nitric oxide synthase* expression (Griendling & FitzGerald, 2003). Animal studies have shown that endothelial damage leads to inhibition and promotion of the proliferation of smooth muscle cells. This further activates the aggregation of platelet and inflammatory cells disrupting the integrity of the microvasculature (Villar & Belizan, 1982; Nuyt,

2008). However, this area of research requires systematic and continuous long-term monitoring of CVD risk factors and assessment of vascular changes over time.

In the past few decades, novel modalities including retinal vascular imaging have been developed to examine the systemic microvasculature in clinical studies (Liew *et al.* 2008*c*; Strain *et al.* 2012; Struijker-Boudier *et al.* 2012). Due to the non-invasive nature of retinal imaging, it has been applied in a wide range of population-based and clinical studies in persons of different ages (Strain *et al.* 2012). The morphology of retinal microvasculature is represented by a series of vascular parameters such as calibre of retinal arterioles and venules, and their tortuosity, branching angle and fractal dimension (Cheung *et al.* 2012). These parameters have been associated with a range of systemic risk factors (e.g. elevated blood pressure, hyperglycaemia, obesity) (Nguyen *et al.* 2008*b*; Cheung *et al.* 2009*b*, 2012; Jensen *et al.* 2010; Li *et al.* 2012, 2013; Gopinath *et al.* 2013*b*; Xiao *et al.* 2015), and appear to predict the incidence of CVD, including stroke and heart disease, and are related to vascular and metabolic conditions (e.g. hypertension, diabetes, metabolic syndrome) (Wong *et al.* 2002*a*,*b*; Ikram *et al.* 2006*a*,*b*; McGeechan *et al.* 2008; Kawasaki *et al.* 2009; Nguyen *et al.* 2008*a*). Furthermore, these morphological changes in the retinal vessels have been linked to several basic mechanisms involved in the development of CVD, such as inflammation, dyslipidaemia and endothelial dysfunction (Klein *et al.* 2006; Wong *et al.* 2006; Van Hecke *et al.* 2008; Gopinath *et al.* 2009; Yim-Lui Cheung *et al.* 2010; Hanssen *et al.* 2012). In view of these developments, retinal vascular imaging can also be used as a potential tool to study early life factors related to CVD.

The use of retinal vascular imaging as a tool to study early life CVD risk factors was initiated by Hellstrom *et al.* and others, who proposed the concept of studying the retinal microvasculature in children in the 1990s (Hellstrom *et al.* 1997,1998). All these studies found a series of retinal vasculature abnormalities in children with low birth weight and even intra-uterine growth retardation (IUGR), including lower branching points, narrower bifurcation angles, narrower retinal arteriolar calibre and wider retinal venular calibre (Chapman *et al.* 1997; Hellstrom *et al.* 1998, 2004; Minicucci *et al.* 1999; Kandasamy *et al.* 2012*a*,*b*). These initial studies

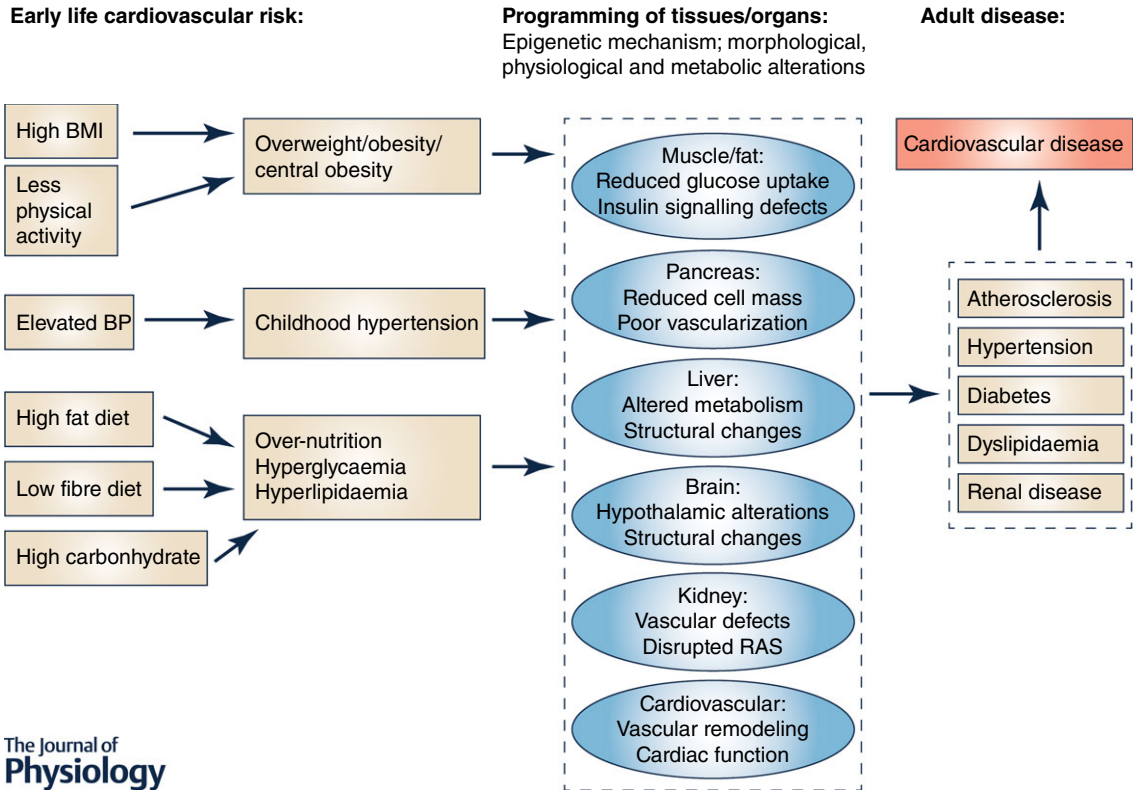

Figure 1. Early life risk factors and adult cardiovascular disease
Early-life risk factors shown in the first panel have been suggested to be associated with tissue or organ programming later in life. Such programming, due to the adaptation to existing environmental conditions, results in different phenotypes shown in the second panel. All together, they are known risk factors for future development of systemic disease and cardiovascular disease.

suggested that such vascular alteration might be associated with increased circulatory energy costs and suboptimal vascular architecture leading to an impairment of fetal development, which subsequently provides a mechanistic link to an increased risk of CVD in late-life. Since then an increasing number of studies have used retinal vascular imaging to elucidate the role of the microvasculature in early life. Therefore, the aim of this systematic review is to summarize the results of retinal vascular imaging applied in studies of children and adolescents, and to determine the relationship of retinal vascular changes to CVD risk factors in early life.

Methods

Data source and study selection. We conducted a systematic review of publications with quantitative retinal vascular assessment in early life performed through two major online searching engines – PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) and Scopus (http://www.scopus.com). The following key words were used in the search criteria: retinal arterioles, retinal venules, retinal vascular calibre, retinal vessel diameter, retinal vessels, retinal microcirculation, retinal microvasculature, retinal vasculature, retinal imaging, childhood, early life, children and adolescents. Relevant papers published until 27 February 2015 were screened by their titles and abstracts. There were nearly 400,000 papers shown on both search engines with keyword searching. After combining the searching schemes, nearly 9000 papers were eligible (Fig. 2). Inclusion criteria of our systemic review were: epidemiological and/or clinical study, studies on children and/or adolescents, written in English, full text available through National University of Singapore library portal, quantitative assessment of retinal vascular parameters, and early life CVD risk factors. Early life CVD risk factors were classified into four groups: birth risk factors (e.g. low birth weight, shorter gestational weeks), environmental risk factors (e.g. parental hypertension, low physical activity, high-fat diet), systemic risk factors (e.g. elevated blood pressure, overweight, obesity), and diseases linked to future CVD development (e.g. diabetes). Finally, 55 papers fitted the criteria and were included for data extraction.

Data extraction and table summary. A standard extraction form was used to summarize all the key findings from 55 papers: information included in the extraction form was first author's name, year of publication, country where data were collected, study design, sample size, response rate (if applicable), age, and changes in exposure and outcomes (either quantitative or qualitative assessment on retinal imaging or CVD risk).

Fundus photography and retinal vessel assessment. Retinal fundus examination allows for non-invasive evaluation of retinal microvasculature. Recent population-based studies have used computer-assisted programmes to measure individual arterioles and venules and to combine them according to formulas developed firstly by Parr & Spears (1974*a*,*b*), subsequently modified by Hubbard *et al.* (1999), and further improved by Knudtson *et al.* (2003). The use of computer-assisted programmes differs in all population-based epidemiological studies. For example, Computer Assisted Image Analysis of the Retina program (CAIAR) and Retinal Image MultiScale Analysis was used in UK adult studies (Mahal *et al.* 2009; Owen *et al.* 2011), retinal Imaging Software Fractal (IRIS-Fractal) was used in an Australian children study (Gopinath *et al.* 2012*a*, 2013*a*), Non-mydriatic Vessel Analyser (SVA-T) was used in a German children study (Hanssen *et al.* 2012), Interactive Vessel Analysis (IVAN) was widely used in US studies (Wong *et al.* 2004; Liew *et al.* 2008*a*) and Asian studies (Li *et al.* 2011*b*), while Singapore I Vessel Assessment (SIVA) was newly developed and applied in recent Singaporean studies (Wong *et al.* 2002*b*; Cheung *et al.* 2011*a*).

Retinal imaging analysis has enabled reproducible assessment of retinal microvascular parameters to quantify structural vascular morphological changes precisely (Wong *et al.* 2004). With the advancement of grading software such as SIVA (Singapore I Vessel Analysis, version 3.0, Singapore) (Fig. 4), a range of retinal static vascular parameters have been explored and widely used, such as retinal vascular calibre, retinal vascular tortuosity, retinal vascular branching angle and retinal vascular fractal dimension. A brief description of these parameters is provided below:

(1) Retinal vascular calibre is represented as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). Pathological changes in such parameters have been identified as retinal arteriolar narrowing and retinal venular widening (Fig. 4) (Ikram *et al.* 2013).
(2) Retinal vascular tortuosity is defined as the integral of the curvature square along the path of the vessel normalized by the total path length; it takes into account the bowing and points of inflection (Fig. 5) (Cheung *et al.* 2011*b*). Increment of retinal vessel curvature tortuosity reflects a curvier vessel and has been identified as part of the pathological changes (Ikram *et al.* 2013).
(3) Retinal vascular fractal dimension, which quantifies the complexity of the branching pattern of the retinal vascular tree, is defined as the gradient of logarithms of the number of boxes and the size of the boxes (Fig. 5) (Liew *et al.* 2008*b*). A lower value for the fractal dimension reflects a sparser vascular network and has been found in diseases such as stroke and Alzheimer's

disease (Cheung *et al.* 2014*a*,*b*, 2015; Hilal *et al.* 2014; Ikram *et al.* 2013; Ong *et al.* 2014, 2015).

(4) Retinal vascular branching angle is defined as the first angle subtended between two daughter vessels at each bifurcation (Fig. 5) (Cheung *et al.* 2011*a*). Larger vessel branching angle might be indicative for pathological changes in retinal vascular geometry (Ikram *et al.* 2013).

Results

Birth risk factors and retinal microvasculature. A total of six papers (Cheung *et al.* 2007*a*; Tapp *et al.* 2007; Mitchell *et al.* 2008; Sun *et al.* 2009*a*; Gopinath *et al.* 2010*b*; Kandasamy *et al.* 2011; Gishti *et al.* 2015*a*) and one letter-to-the-editor (Cheung *et al.* 2008) were published on the relationship between birth factors and retinal microvasculature (Table 1). Subjects ranging

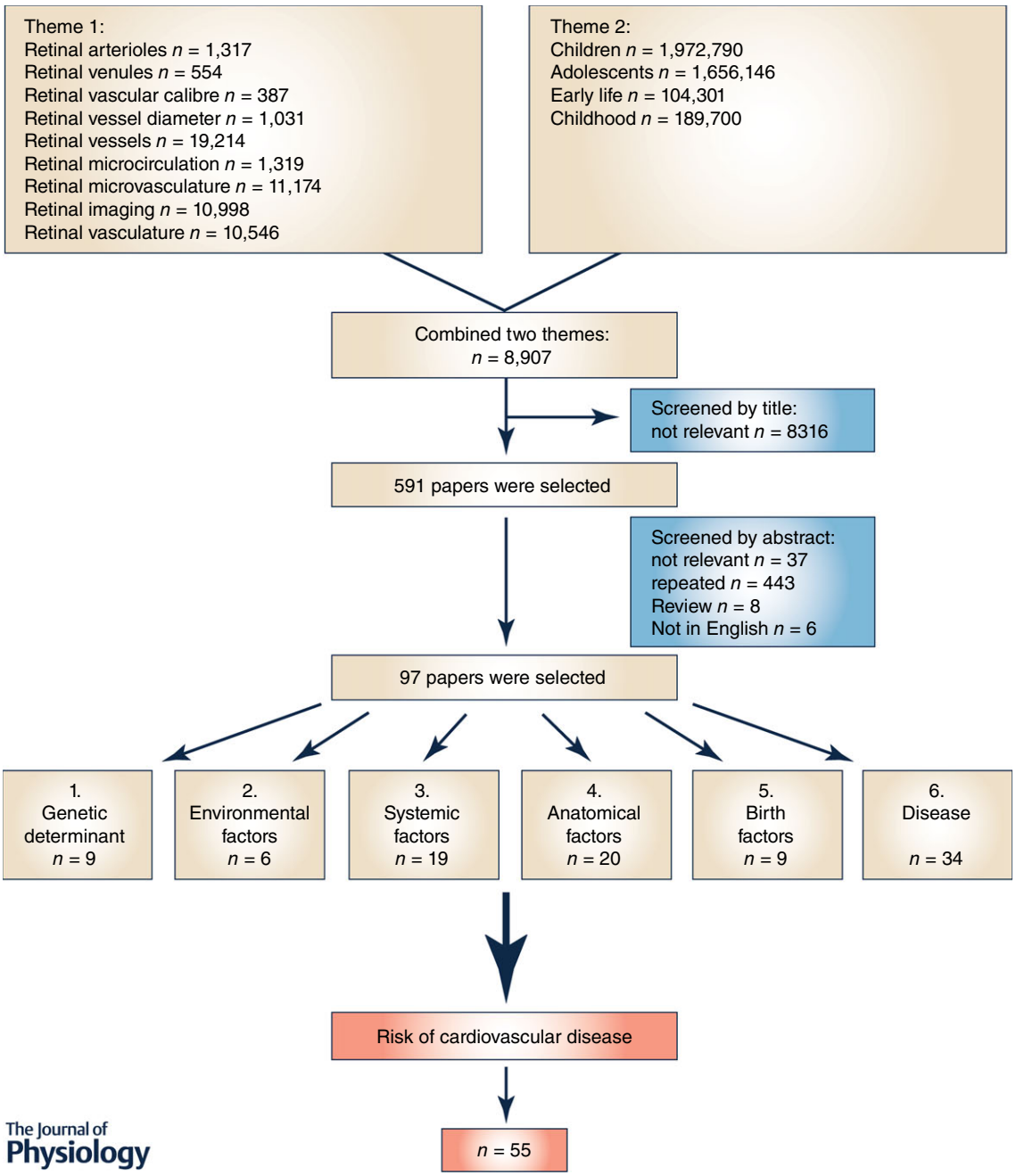

Figure 2. Flow chart illustrating the selection of research papers

from newborn babies to adolescents around 16 years were included; these subjects were mainly of Asian and European origin. All studies were designed in a longitudinal and school-based way. Consistent and significant findings were reported on the association between smaller birth size indexes and retinal arteriolar narrowing and/or retinal venular widening in these children and adolescents (Cheung *et al.* 2007*a*, 2008; Tapp *et al.* 2007; Mitchell *et al.* 2008; Sun *et al.* 2009*a*; Gopinath *et al.* 2010*b*). Furthermore, subjects in the UK with lower birth weight and subjects in Australia with smaller head circumference had higher tortuosity and optimality deviance in retinal arterioles (Tapp *et al.* 2007) and lower retinal vascular fractal dimension (Gopinath *et al.* 2010*b*). However, a recently published clinical study reported that infants born with lower birth weight tend to have both retinal arterioles and venules dilatation (Kandasamy *et al.* 2011). The difference might be due to the small sample size ($n = 24$) of this study.

Environmental risk factors and retinal microvasculature. Seven papers (Gopinath *et al.* 2011*a*,*c*, 2012*b*, 2014; Hanssen *et al.* 2011; Poon *et al.* 2013; Islam *et al.* 2014) and one letter-to-the-editor (Lim *et al.* 2009) published on associations between environmental risks and retinal microvasculature were part of the analysis (Table 2). The environmental risks included diet, parental hypertension and physical activity. All associations between environmental risks and retinal microvasculature were designed in a population/family-based and cross-sectional way. Four papers explored the relationship between unhealthy diet and retinal vasculature; however, the findings were not consistent. The Sydney Children Eye Study (SCES) found that children and adolescents with unhealthy diet including higher intake of sugar and carbohydrate and lower intake of yoghurt and fibre tend to have retinal arteriolar narrowing and suboptimal retinal arteriolar fractal dimension (Gopinath *et al.* 2012*b*, 2014). However, some of findings could not be repeated in Singaporean children (Lim *et al.* 2009). Furthermore, a recent study done on 481 children and adolescents with type 1 diabetes reported no association between vitamin D intake and a series of retinal vascular parameters including calibre, tortuosity, length-to-diameter ratio, branching angle and fractal dimension; the results were the same for association with vitamin D deficiency as well (Poon *et al.* 2013). Two papers found a consistent relationship between parental blood pressure/hypertension history and changes of retinal microvasculature such as higher optimality deviation and larger arteriole-to-venule ratio (AVR) in children (Gopinath *et al.* 2011*a*; Islam *et al.* 2014). Interestingly, physical activity and sedative activity such as TV viewing were also reflected by retinal imaging differently (Gopinath *et al.* 2011*c*; Hanssen *et al.* 2011). Children with less outdoor physical activity and more TV viewing time had narrower retinal arterioles than had their counterparts.

Systemic risk factors and retinal microvasculature.

Elevated blood pressure. A body of evidence has confirmed the relationship between elevated blood pressure, childhood hypertension and retinal microvasculature. Thirteen papers have published consistent findings from pre-schoolers to adolescents (Table 3) (Mitchell *et al.* 2007; Gopinath *et al.* 2010*a*, 2013*b*; Tapp *et al.* 2007, 2013; Li *et al.* 2011*b*; Owen *et al.* 2011;

Figure 3. Retinal microvasculature assessment on the grading platform
A screenshot of a computer-assisted programme for measurement of new geometrical retinal vascular parameters from retinal fundus photograph. Zone C is marked in SIVA software by 0.5 to 2.0 optic disc diameters away from the margin of the optic disc. All retinal arterioles and venules larger than 25 μm are marked and assessed within zone C.

Hanssen *et al.* 2012; Kurniawan *et al.* 2012; Murgan *et al.* 2013; Sasongko *et al.* 2010; Zheng *et al.* 2013; Gishti *et al.* 2015*b*). A wide range of retinal vascular parameters were studied among all these original articles, such as retinal vessel width, fractal dimension, tortuosity and length-to-diameter ratio. Elevated peripheral and central blood pressure and subsequent childhood hypertension were associated with narrower retinal arteriolar calibre, wider retinal venules, more tortuous retinal arterioles and lower retinal arteriolar fractal dimension and length-to-diameter ratio (Mitchell *et al.* 2007; Gopinath *et al.* 2010*a*, 2013*b*; Sasongko *et al.* 2010; Li *et al.* 2011*b*; Owen *et al.* 2011; Hanssen *et al.* 2012; Kurniawan *et al.* 2012; Murgan *et al.* 2013; Tapp *et al.* 2013; Zheng *et al.* 2013). In a group of 166 UK children aged 9 years, increased heart rate was also found to be associated with lower simple tortuosity (Tapp *et al.* 2007).

Anthropometric indexes. As a phenotypic indication for overweight and obesity, anthropometric indexes and retinal microvasculature was widely investigated across all races and ages (Table 4) (Cheung *et al.* 2007*b*; Tapp *et al.* 2007, 2013; Taylor *et al.* 2007; Sasongko *et al.* 2010; Gopinath *et al.* 2011*b*, 2013*b*; Li *et al.* 2011*a*; Owen *et al.* 2011; Hanssen *et al.* 2012; Zheng *et al.* 2013; Siegrist *et al.* 2014; Xiao *et al.* 2015; Gishti *et al.* 2015*d*).

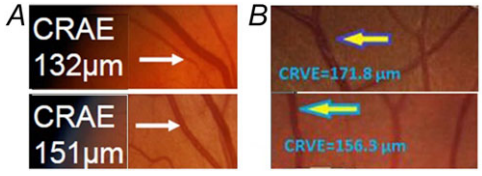

Figure 4. Retinal arteriolar narrowing and retinal venular widening
Retinal arteriolar narrowing is shown in (a). Retinal arteriolar caliber listed in the image on top has narrower (132 μm) than the image in the bottom (151 μm). Retinal venular widening is shown in (b). Retinal venular caliber listed in the image on top has wider (171.8 μm) than the image in the bottom (156.3 μm).

Among children and adolescents, BMI, ponderal index, waist circumference, skinfold thickness indexes, fat mass index, body water percentage and trunk fat percentage parameters were all used to evaluate the body composition. It was observed that if a child or adolescent had higher index for body composition or fat deposition, he/she had retinal venular widening consistently with or without retinal arteriolar narrowing. Interestingly, retinal venular calibre seemed to be the most sensitive index to reflect body composition among all retinal vascular parameters such as retinal arteriolar calibre, fractal dimension, tortuosity, length-to-diameter ratio and optimality deviation. Aside from structural retinal vasculature, functional changes were also investigated in 77 children and adolescents with either type 1 diabetes mellitus (T1DM) or overweight or obesity. Retinal venular dilatory response under flicker light examination was found to be associated with increased BMI (Schiel *et al.* 2009).

Inflammation, hyperglycaemia, dyslipidaemia and angiogenesis. Systemic conditions such as inflammation, hyperglycaemia, dyslipidaemia and angiogenesis were well recognized to be on the path of developing atherosclerosis and future CVD. In the last 5 years, researchers have started to look into the early indication of such a process in children and adolescents. Five papers published cross-sectional data and one paper published longitudinal data on relevant topics (Table 5) (Owen *et al.* 2011; Sasongko *et al.* 2010; Hanssen *et al.* 2012; Siegrist *et al.* 2014; Gishti *et al.* 2015*c*,*d*). Inflammation biomarkers such as C-reactive protein (CRP), hyperglycaemia (indicated by high glucose, HbA1C and insulin level), dyslipidaemia (indicated by cholesterol and low density lipoprotein, high density lipoprotein, leptin and triglyceride) were all associated with retinal venular widening and more tortuous retinal arterioles (Sasongko *et al.* 2010; Owen *et al.* 2011; Hanssen *et al.* 2012; Siegrist *et al.* 2014). Interestingly, a group of Dutch researchers also found significant associations between indexes for maternal angiogenesis (e.g. placental growth factor,

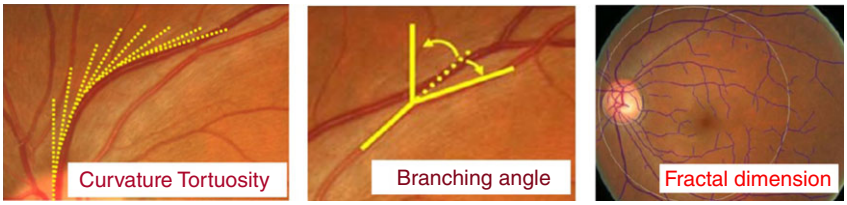

Figure 5. Retinal vascular geometry parameters
Retinal vascular tortuosity, retinal vascular branching angle and retinal vascular fractal dimension are shown. Tortuosity is derived from the integral of the curvature square along the path of the vessel, normalized by the total path length, which takes into account the bowing and points of inflection. Branching angle is defined as the first angle subtended between two daughter vessels at each bifurcation. Fractal dimension quantifies the complexity of the whole branching pattern of the retinal vascular tree and is defined as the gradient of logarithms of the number of boxes and the size the boxes.

Table 1. Birth risk factors and retinal vasculature in early life

	Study	Study population and study design	Sample size and response rate	Age, male %	Cardiovascular risk factors	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
1 (2015)	Olta G Generation R	Population-based prospective cohort study	4122 61%	6.2 years 50%	Birth weight: Low *vs.* Normal	0.26 SDS ↓ (P trend ≤0.001)	n.s.
2 (2011)	Kandasamy Y	Clinical observational study	24 newborn infants	Term newborn	Birth weight: Low *vs.* Normal	Calibre: 113.1 *vs.* 86.4 μm ($P = 0.0009$)	Calibre: 151.7 *vs.* 128.4 μm ($P = 0.0040$)
3 (2010)	Gopinath B SCES	School-based, longitudinal study	2353 out of 3144 75.3%	12.7 years 50.4%	Birth weight: 1st *vs.* 4th quartile	Calibre: 149.4 *vs.* 151.9 μm (P trend = 0.001)	Calibre: n.s.
					Birth length: 1st *vs.* 4th quartile	Calibre: 148.9 *vs.* 151.2 μm (P trend = 0.005)	Calibre: n.s.
					Head circum: 1st *vs.* 4th quartile	Calibre: 150.0 *vs.* 152.3 μm (P trend = 0.03)	Calibre: n.s.
					Birth weight: 1st *vs.* 4th quartile		Fractal dimension: n.s.
					Birth length: 1st *vs.* 4th quartile		n.s.
					Head circum: 1st *vs.* 4th quartile		1.466 *vs.* 1.469 (P trend = 0.03)
4 (2009*a*)	Sun C TEST	Population-based, longitudinal	266 twins (49 monozygotic and 84 dizygotic pairs)	5–16 years MZ: 43% DZ: 53%	Birth length: each 5 cm ↓	Calibre: −7.27 μm (−11.54, −3.01) ($P < 0.001$)	Calibre: n.s.
					Head circum: each 2 cm ↓	Calibre: −2.55 μm (−4.92, −0.18) ($P = 0.04$)	Calibre: n.s.
					Birth weight: each 1 kg ↓	Calibre: n.s.	Calibre: n.s.

(continued)

Table 1. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Cardiovascular risk factors	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
5 (2008)	Mitchell P SCES	Population-based, longitudinal study	1369 out of 2238 78.7%	12.7 years 50.4%	Birth weight: each SD ↓ SD = 569 g	Calibre: −1.28 μm (−2.23, −0.32) ($P = 0.01$)	Calibre: n.s.
					Birth weight: Low *vs.* Normal	Calibre: −3.73 μm (−7.09, −0.38) ($P = 0.03$)	Calibre: n.s.
					Birth length: each SD ↓ SD = 3.1 cm	Calibre: −1.00 μm (−1.89, −0.12) ($P = 0.03$)	Calibre: n.s.
					Head circum: each SD ↓ SD = 1.8 cm	Calibre: −1.24 μm (−2.09, −0.38) ($P = 0.006$)	Calibre: n.s.
					Gestational age: Premature *vs.* Full-term	Calibre: −3.43 μm (−5.95, −0.91) ($P = 0.009$)	Calibre: n.s.
6 (2008)	Cheung N SCORM	School-based, longitudinal study 73.0%	561 out of 768 children	7–9 years 52.5%	Birth weight: Each SD ↓ (SD = 453 g)	Calibre: n.s.	Calibre: 1.49 μm (0.18, 2.80) ($P = 0.03$)
					Birth Weight: Very low, < 2500 g	n.s	171.8 μm 167.6 μm
					Low, 2500–2999 g Normal, ≥ 3000 g		165.0 μm (P trend = 0.006)
7 (2007)	Tapp RJ ALSPAC	Population-based, longitudinal study	166 children	9 years 40%	Birth weight: each 1 kg ↓	Optimality deviance: 0.190 (P = 0.021)	Optimality deviance: n.s.
						Simple tortuosity: 0.179 (P = 0.026)	Simple tortuosity: n.s.
						Bifurcation angle: n.s.	Bifurcation angle: n.s.
						Bifurcation angle: n.s.	Length–diameter ratio: n.s.

Abbreviation: 95% CI, 95% confidence interval; SD, standard deviation; circum, circumference; TEST, the Twins Eye Study in Tasmania; SCES: Sydney Children Eye Study; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia; ALSPAC, the Avon Longitudinal Study of Parents and Children.

Table 2. Environmental risk factors and retinal microvasculature in early life

	Study	Study design	Sample size and response rate	Age, male %	Environmental factors	Arteriolar parameters β or mean, 95% CI	Venular parameters β or mean, 95% CI
1 (2014)	Gopinath B SCES	School-based, cross-sectional study	888	17 years 49.4%	Yoghurt, serves/day: 1st *vs*. 3rd tertile	160.9 *vs*. 162.2 μm (P trend = 0.05)	237.9 *vs*. 235.9 μm (P trend = 0.04)
2 (2013)	Poon M	Clinical study, cross-sectional	481	mean age: 14.9 years 52%	Vitamin D deficiency:	Calibre/tortuosity/length–diameter ratio/ branching angle/fractal dimension: n.s.	
3 (2012)	Gopinath B SCES	School-based, cross-sectional study	2353	12 years 49.4%	Carbohydrate intake: 1st *vs*. 3rd tertile	Calibre: Girls: 153.1 *vs*. 151.7 μm (P trend = 0.03) Fractal dimension: Girls: 1.461 *vs*. 1.465 (P trend = 0.003)	Calibre: Boys: 217.6 *vs*. 219.9 μm (P trend = 0.02) Fractal dimension: Boys and girls: n.s.
					Total sugar intake: 1st *vs*. 3rd tertile	Calibre: Boys: 150.5 *vs*. 148.7 μm (P trend = 0.04) Fractal dimension: Girls: 1.462 *vs*. 1.465 (P trend = 0.002)	Calibre: Boys and girls: n.s. Fractal dimension: Boys and girls: n.s.
					Total fibre intake: 1st *vs*. 3rd tertile	Calibre: Boys and girls: n.s.	Calibre: Boys and girls: n.s.
					Mean dietary GI: 1st *vs*. 3rd tertile	Calibre: Girls: 153.5 *vs*. 151.7 μm (P trend = 0.03)	Calibre: Boys and girls: n.s.
					Mean dietary GL: 1st *vs*. 3rd tertile	Calibre: Girls: 153.5 *vs*. 151.9 μm (P trend = 0.05) Fractal dimension: Girls: 1.461 *vs*. 1.464 (P trend = 0.01)	Calibre: Boys and girls: n.s. Fractal dimension: Boys and girls: n.s.

(continued)

Table 2. continued

	Study	Study design	Sample size and response rate	Age, male %	Environmental factors	Arteriolar parameters β or mean, 95% CI	Venular parameters β or mean, 95% CI
4	Cheung N	School-based, cross-sectional study	823	12.8 years	Fibre/sugar/total fat/protein/energy intake:	Calibre:	Calibre:
(2009)	SCORM			52.5%		n.s.	n.s.
5	Islam M	Family-based, cross-sectional study	751	9–14 years	Paternal SBP:	Optimality deviation:	
(2014)				49.6%	1st *vs.* 5th quintile	115.1 *vs.* 145.7 μm (P trend = 0.032)	
					each 10 mmHg ↑	0.0053 (0.0001, 0.0106) (P = 0.047)	
					Paternal DBP:	115.5 *vs.* 145.4 (P trend = 0.010)	
					1st *vs.* 5th quintile	0.0109 (0.0025, 0.0193) (P = 0.011)	
					each 10 mmHg ↑	AVR:	
					Maternal SBP:	0.83 *vs.* 0.80 (P trend = 0.013)	
					1st *vs.* 5th quintile	0.82 *vs.* 0.80 (P trend = 0.008)	
					Maternal DBP:	−0.0102 (−0.0198, −0.00007) (P = 0.035)	
					1st *vs.* 5th quintile		
					each 10 mmHg ↑		
6	Gopinath B	School-based, cross-sectional study	1739 out of 2238	6 years	Parental HTN:	Calibre:	Calibre:
(2011)	SCES		77.7%	50.4%	Yes *vs.* No	Girls:	Boys and girls:
						161.6 *vs.* 165.9 μm	n.s.
						(P = 0.0004)	
					Parental HTN:	Calibre:	Calibre:
					Yes *vs.* No	Girls:	Boys and girls:
						161.6 *vs.* 165.9 μm	n.s.
						(P = 0.0004)	
					Maternal HTN:	Calibre:	Calibre:
					Yes *vs.* No	Girls:	Boys and girls:
						161.2 *vs.* 165.7 μm	n.s.
						(P = 0.01)	
7	Hanssen H	School-based, cross-sectional study	578 out of 792	11.1 years	Physical inactivity:	AVR:	
(2011)	JuvenTUM 3 (2008)		73.0%	41.5%	each 1 h/week ↑	<0.001 (< 0.001, < 0.001) (P = 0.032)	
8	Gopinath B	School-based, cross-sectional study	1765 out of 2238	6 years	Outdoor sporting activities:	Calibre:	Calibre:
(2011)	SCES			50.4%	Low *vs.* High	162.5 *vs.* 164.7 μm	n.s.
					Television viewing:	(P trend = 0.004)	
					1st *vs.* 4th quartile	Calibre:	Calibre:
						164.2 *vs.* 161.9 μm	n.s.
						(P trend = 0.003)	

Abbreviation: 95% CI, 95% confidence interval; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; AVR, arteriovenous ratio; SCES: Sydney Children Eye Study; SCORM: the Singapore Cohort Study of the Risk Factors of Myopia.

Table 3. Elevated blood pressure and retinal vasculature in early life

	Study	Study population and study design	Sample size and response rate	Age, male %	Blood pressure	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
1	Olta G	Population-based, cross-sectional study	4007, 61.4%	6.0		per SDS ↓	per SDS ↑
(2015)	Generation R			50.2%	SBP:	0.19 SDS ↑ ($P < 0.05$)	0.05 SDS ↑ ($P < 0.05$)
					DBP:	0.13 SDS ↑ ($P < 0.05$)	n.s.
					Pulse pressure:	0.10 SDS ↑ ($P < 0.05$)	0.05 SDS ↑ ($P < 0.05$)
					Carotid femoral pulse wave velocity:	n.s.	0.04 SDS ↑ ($P < 0.05$)
2	Gopinath B	Population-based, cross-sectional study	1077	3–6 years	SBP:	Calibre:	Calibre:
(2013)	SCES			50.8%	each 10 mm Hg ↑	−1.70 μm ($P = 0.02$)	n.s.
3	Zheng YF	Population-based, cross-sectional study	1035 twin pairs	7–19 years	MAP:	Calibre:	Calibre:
(2013)	The Guangzhou Twin Eye Study		(657 MZ, 378 DZ)	51.8%	each 10 mm Hg ↑	−1.48 μm ($P < 0.005$)	n.s.
4	Tapp RJ	Population-based, cross-sectional study	1067	12 years	SBP at 9 years:	Calibre:	Calibre:
(2013)	ALSPAC			49%	each SD ↑	−0.21 μm (−0.33, −0.09)	n.s.
					DBP at 9 years:	($P < 0.001$)	n.s.
					each SD ↑	−0.16 μm (−0.27, −0.05)	Calibre:
					SBP at 11 years:	($P = 0.004$)	n.s.
					each SD ↑	Calibre:	n.s.
					DBP at 11 years:	−0.20 μm (−0.13, −0.08)	
					each SD ↑	($P = 0.001$)	
						−0.13 μm (−0.24, −0.02)	
						($P = 0.019$)	
5	Murgan I	Clinical research, cross-sectional study	121	16 years	Central SBP:	Calibre:	
(2013)				56.2%	each 1 mm Hg ↑	−0.096 ($P = 0.023$)	
6	Hanssen H	School-based, cross-sectional study	578 out of 792	11.1	SBP:	Calibre:	Calibre:
(2012)	JuvenTUM 3		73.0%	41.5%	each 10 mm Hg ↑	−1.34 μm (−2.61, −0.06)	n.s.
					DBP:	($P = 0.040$)	n.s.
					each 10 mm Hg ↑	−2.53 μm (−4.15, −0.92)	
						($P = 0.002$)	
7	Li LJ	Population-based, cross-sectional study	385	4–5 years	SBP:	Calibre:	Calibre:
(2011)	STARS			50.7%	each 10 mm Hg ↑	−2.00 μm (−3.61, −0.39)	2.51 (0.35, 4.68)
					DBP:	($P = 0.02$)	($P = 0.02$)
					each 10 mm Hg ↑	n.s.	n.s.
					MABP:	−1.39 μm (−2.95, 0.17)	2.15 (0.06, 4.24)
					each 10 mm Hg ↑	($P = 0.08$)	($P = 0.04$)
					Hypertensive stage:	160.15 *vs.* 156.06 μm	n.s.
					No *vs.* Yes	($P = 0.02$)	
8	Owen CG	School-based, cross-sectional study	986	10–11	DBP:	Tortuosity:	Tortuosity:
(2011)	CHASE			Years	each SD ↑	2.3% (0.1%, 4.6%)	n.s.
				46.9%	SD = 9.1 mm Hg	($P = 0.01$)	

(continued)

Table 3. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Blood pressure	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
9 (2011)	Kurniawan ED SCORM	School-based, cross-sectional study	1174	10–14 years 49.1%	SBP: each 10 mm Hg ↑ DBP: each 10 mm Hg ↑ MABP: each 10 mm Hg ↑	Fractal dimension: −0.74 (−1.37, −0.11) ($P = 0.021$) n.s. −0.71 (−1.31, −0.10) ($P = 0.022$)	Fractal dimension: n.s
10 (2010)	Gopinath B SCES	Australian adolescents Population-based, cross-sectional study	2353 out of 3144 75.3%	12.7 years 50.4%	SBP: 1st *vs.* 4th quartile DBP: 1st *vs.* 4th quartile MABP: 1st *vs.* 4th quartile Hypertensive stage: No *vs.* Yes	Calibre: 153.9 *vs.* 149.7 μm (P trend < 0.0001) 153.0 *vs.* 149.4 μm (P trend < 0.0001) 153.4 *vs.* 149.6 μm (P trend < 0.0001) 151.9 *vs.* 149.9 μm ($P = 0.002$)	Calibre: n.s. n.s. n.s. n.s.
11 (2010)	Sasongko MB SPDS	Clinic-based, cross-sectional study	944 out of 1159 81.4% with type 1 diabetes	12–20 years 43.7%	SBP: each SD ↑ (SD = 12 mm Hg)	Length–diameter ratio: −10.0 (−16.3, −3.69) ($P = 0.003$)	Length–diameter ratio: n.s.
12 (2007)	Tapp RJ ALSPAC	Population-based, cross-sectional study	166 children	9 years 40%	Heart rate: each 1 BPM ↑	Simple tortuosity: −0.190 ($P = 0.019$)	Optimality deviance/ simple tortuosity/ bifurcation angle/ length–diameter ratio: n.s.
13 (2007)	Mitchell P SCES, SCORM	School-based, cross-sectional study	1572 Australian children 380 Singapore children	SCES: 6–8 years 50.8% SCORM: 7–9 years 56.8%	SBP: each 10 mm Hg ↑ DBP: each 10 mm Hg ↑ MABP: each 10 mm Hg ↑	Calibre: SCES: −2.08 μm (−2.79, −1.38) ($P < 0.0001$) SCORM: −1.43 μm (−2.59, −0.27) ($P = 0.016$) SCES: −1.46 (−2.16, −0.78) ($P < 0.001$) SCORM: −2.30 (−4.00, −0.59) ($P = 0.008$) SCES: −2.00 (−2.76, −1.24) ($P < 0.001$) SCORM: −2.45 (−4.11, −0.79) ($P = 0.004$)	Calibre: SCES: −1.12 μm (−2.00, −0.24) ($P = 0.013$) SCROM: n.s. SCES: n.s. SCORM: n.s. SCES: −0.99 (−1.95, −0.04) ($P = 0.041$) SCORM: n.s.

Abbreviation: 95% CI: 95% confidence interval; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP: mean arterial blood pressure; BPM, beats per minute; SCES, Sydney Children Eye Study; ALSPAC, the Avon Longitudinal Study of Parents and Children; STARS, the Strabismus, Amblyopia and Refractive Error Study in Singapore Chinese Preschoolers; SCORM, the Singapore Cohort Study of the Risk Factors for Myopia; CHASE, the Child Heart and Health Study in England; SPDS, Sydney Pediatric Diabetes Study.

Table 4. Anthropometric indexes and retinal vasculature in early life

	Study	Study population and study design	Sample size and response rate	Age, male %	Anthropometric measurements	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
1	Gishti O	Population-based, cross-sectional study	4145	6.0 years		Per SDS ↓:	n.s..
(2015)	Generation R		61%	50%	BMI:	0.06 SDS ↑ ($P < 0.05$)	
					Total body fat mass:	0.05 SDS ↑ ($P < 0.05$)	
2	Xiao Wei	Population-based, cross-sectional study	444 twins	12–19 years	Fat mass index	Calibre:	Calibre:
(2015)	The Guangzhou Twin Eye Study			46.4%	(kg m^{-2})	147.2 *vs.* 148.4 μm	211.7 *vs.* 218.2 μm
					1st *vs.* 4th quartile	(P trend = 0.005)	(P trend = 0.005)
					Body water %,	Calibre:	Calibre:
					1st *vs.* 4th quartile	148.3 *vs.* 147.9 μm	218.9 *vs.* 213.2 μm
					Trunk fat %,	(P trend = 0.009)	(P trend = 0.001)
					1st *vs.* 4th quartile	Calibre:	Calibre:
					Triceps skinfold, mm	147.0 *vs.* 148.7 μm	211.7 *vs.* 218.8 μm
					1st *vs.* 4th quartile	(P trend = 0.007)	(P trend = 0.001)
					BMI (kg m^{-2})	Calibre:	Calibre:
					1st *vs.* 4th quartile	147.7 *vs.* 148.2 μm	211.5 *vs.* 218.0 μm
						(P trend = 0.007)	(P trend = 0.002)
						Calibre:	Calibre:
						149.4 *vs.* 147.1 μm	213.1 *vs.* 216.3 μm
						(P trend = 0.026)	(P trend = 0.020)
3	Siegrist M	Randomized controlled school-and family-based lifestyle interventional trial	792	10–11 years	BMI:	AVR:	
(2014)				58.5%	each 1 kg m^{-2}	−0.003 (−0.004, −0.001) ($P = 0.003$)	
4	Zheng YF	Population-based,	1035 twin pairs	7–19 years	BMI:	Calibre:	Calibre:
(2013)	The Guangzhou Twin Eye Study	cross-sectional study	(657 MZ, 378 DZ)	51.8%	each 1 kg m^{-2} ↑	−0.30 μm ($P = 0.01$)	0.43 μm ($P < 0.05$)
5	Tapp RJ	Population-based, cross-sectional study	1067	12 years	Fat mass at 9 years:	Calibre:	Calibre:
(2013)	ALSPAC			49%	each SD ↑	n.s.	0.19 (0.03, 0.35)
					Fat mass at 11	Calibre:	($P = 0.022$)
					years:	n.s.	Calibre:
					each SD ↑		0.25 (0.10, 0.40)
							($P = 0.001$)

(continued)

Table 4. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Anthropometric measurements	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
6 (2013)	Gopinath B SCES	Australian pre-schoolers, population-based, cross-sectional study	1077	3–6 years 50.5%	Healthy *vs.* Overweight *vs.* Obese	Calibre: 159.5 μm *vs.* 156.3 μm *vs.* 153.4 μm (P trend = 0.01)	Calibre: 214.1 μm *vs.* 218.7 μm *vs.*220.5 μm (P trend = 0.01)
7 (2012)	Hanssen H JuvenTUM 3	German school children at 5th grade School-based cross-sectional study	578 out of 792 73.0%	11.1 years 41.5%	BMI : each 1 kg m^{-2} ↑	Calibre: −0.374 μm (−0.724, −0.025) (P = 0.036)	Calibre: 0.369 μm (0.007, 0.731) (P = 0.046)
					Obese *vs.* Overweight *vs.* Normal weight	AVR: 0.85 *vs.* 0.87 *vs.* 0.89 ($P \leq 0.05$)	
					PBF: each 1% ↑	AVR: −0.001 (−0.002, <0.001) (P = 0.002)	
					Waist circum: each 1 mm ↑	AVR: −0.001 (−0.002, <0.001) ($P < 0.001$)	
8 (2012)	Li LJ STARS	Population-based, cross-sectional study	136	6–16 years 45.6%	BMI: each SD ↑ SD = 3.53 kg m^{-2} Above *vs.* Below threshold	Calibre: n.s. n.s. n.s. n.s.	Calibre: 3.40 μm (P = 0.005) 227.38 *vs.* 218.05 (P = 0.021)
					TSF: each SD ↑ SD = 4.49 mm Above *vs.* Below threshold		2.94 μm (P = 0.012) 227.96 *vs.* 217.75 (P = 0.001)
9 (2011)	Gopinath B SCES	Australian adolescents Population-based, cross-sectional study	2353 out of 3144 75.3%	12.7 years 50.4%	BMI: 4th *vs.* 1st quartile	Calibre: 150.0 *vs.* 152.8 μm ($P < 0.0001$) Fractal dimension: n.s.	Calibre: 221.1 *vs.* 216.9 μm (P = 0.0009)
					Obese *vs.* Overweight *vs.* Normal weight	Calibre: 149.2 *vs.* 150.6 *vs.* 152.0 μm (P = 0.01)	Calibre: 222.6 *vs.* 220.4 *vs.* 218.1 μm (P = 0.01)

(continued)

Table 4. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Anthropometric measurements	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
10 (2009)	Schiel R	Clinical study, cross-sectional	77	6–16 Years	BMI (kg m^{-2})	Dilatation: n.s.	Dilatation: $r = 0.336$ ($P = 0.026$)
11 (2007)	Taylor SCES	Population-based, cross-sectional study	1608 out of 1740 92.4%	6 years 50.8%	BMI: each SD ↑ SD = 2.14 kg m^{-2}	Calibre: −0.76 μm↓ (−1.43, −0.08)	Calibre: 1.13 μm (0.11, 2.15)
					Above *vs.* Below BMI threshold	Calibre: 162.0 *vs.* 163.7 μm ($P = 0.0029$)	Calibre: 231.7 *vs.* 229.0 ($P = 0.0007$)
					Waist circum: each SD ↑ SD = 5.14 cm	Calibre: n.s.	Calibre: 0.99 μm (0.15, 1.84)
					BSA: each SD ↑ SD = 0.099 m^2	Calibre: n.s.	Calibre: 1.97 μm (0.86, 3.09)
12 (2006)	Cheung N SCORM	Singapore Chinese School-based, cross-sectional study	768	7–9 Years 52.5%	BMI: each SD ↑ SD = 3.1 kg m^{-2}	Calibre: n.s.	Calibre: 2.19 (0.23, 4.15) ($P = 0.03$)
13 (2011)	Owen CG CHASE	School-based, cross-sectional study	986	10–11 years 46.9%	Ponderal index: each 1 kg m^{-3} ↑	Tortuosity: n.s.	Tortuosity: n.s.
					Waist circum: each 1 cm ↑	n.s.	n.s.
					Sum of skinfolds: each 1 mm ↑	n.s.	n.s.
					Fat mass index: each 1 kg m^{-5} ↑		
14 (2010)	Sasongko MB SPDS	Clinic-based, cross-sectional study	944 out of 1159 81.4% with type 1 diabetes	12–20 years 43.7%	BMI: each SD ↑ SD = 3.5 kg m^{-2}	Tortuosity/branchingnangle/optimality deviation/length–diameter ratio: n.s.	
15 (2007)	Tapp RJ ALSPAC	Population-based, cross-sectional study	166 children	9 years 40%	BMI: each 1 kg m^{-2} ↑	All vascular parameters: n.s.	All vascular parameters: n.s.

Abbreviation: 95% CI: 95% confidence interval; SD, standard deviation; PBF, percentage body fat; BMI, body mass index; circum, circumference; BSA, body surface area; SCES, the Sydney Children Eye Study; ALSPAC, the Avon Longitudinal Study of Parents and Children; STARS, the Strabismus, Amblyopia and Refractive Error Study in Singapore Chinese Preschoolers; SCORM, the Singapore Cohort Study of the Risk Factors for Myopia; CHASE, the Child Heart and Health Study in England; SPDS, Sydney Pediatric Diabetes Study.

Table 5. Inflammation, dyslipdemia and angiogenesis and retinal microvasculature in early life

	Study	Study population and study design	Sample size and response rate	Age, male %	Serum biomarkers	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
1	Gishti O	Population-based, longitudinal study	3505	6 years	PIGF at 2nd trimester:	Calibre:	Calibre:
(2015)	Generation R		61%	50%	$<$ 145.80 pg ml^{-1} *vs.* $>$ 145.80 pg ml^{-1} (reference)	−0.11 (−0.19, −0.03)	n.s.
					sFLT-1 at 2nd trimester:	($P = 0.008$)	Calibre:
					$>$ 7.35 ng ml^{-1} *vs.* $<$ 7.35 ng ml^{-1} (reference)	Calibre:	0.09 (0.01, 0.17)
					Per SDS↑ in CRP:	0.10 (0.02, 0.17)	($P = 0.03$)
						($P = 0.02$)	0.09 SDS ↑
						n.s.	($P < 0.05$)
2	Siegrist M	Randomized controlled school-and family-based lifestyle interventional trial		10–11 years	Adiponectin/IL-6:	Calibre:	Calibre:
(2014)			792	58.5%	each unit ↑	n.s.	n.s.
					Insulin:	Calibre:	Calibre:
					each 1 μU ml^{-1} ↑	0.152 (0.001, 0.305)	n.s.
					Leptin:	($P = 0.046$)	Calibre:
					1st *vs.* 4th quartile	Calibre:	232.8 *vs.* 239.9 μm
						n.s.	(P trend $= 0.009$)
3	Hanssen H	School-based, cross-sectional study	578 out of 792	11.1 years	CRP:	Calibre:	Calibre:
(2012)	JuvenTUM 3		73.0%	41.5%	each 1 mg l^{-1} ↑	n.s.	11.01 μm (4.164, 17.859) ($P = 0.002$)
					HDL-C:	Calibre:	Calibre:
					each 1 unit ↑	n.s.	n.s.
					Triglyceride:	Calibre:	Calibre:
					each 1 unit ↑	n.s.	n.s.
					Glucose:	Calibre:	Calibre:
					each 1 unit ↑	n.s.	n.s.

(continued)

Table 5. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Serum biomarkers	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
4 (2011)	Owen C CHASE	Cross-sectional study	968 UK children	10–11 years 46.9%	Triglyceride: each 1 mmol l^{-1} ↑	Tortuosity:' 3.8% (1.0%, 6.6%) ($P = 0.01$)	Tortuosity: n.s.
					Total cholesterol: each 1 mmol l^{-1} ↑	Tortuosity: 3.2% ↑ (0.8%, 5.7%) ($P = 0.01$)	Tortuosity: n.s.
					LDL-C: each 1 mmol l^{-1} ↑	Tortuosity: 2.9% ↑ (0.4%, 5.5%) ($P = 0.02$)	Tortuosity: n.s.
					Glucose/HbA1C/ insulin/CRP/HDL-C: each unit ↑	Tortuosity: n.s.	Tortuosity: n.s.
5 (2010)	Sasongko MB SPDS	Clinic-based, cross-sectional study	944 out of 1159 81.4%	12–20 years 43.7%	HbA1C: ≤ 8.5% *vs.* > 8.5%	Tortuosity: 1.75 (0.45, 3.05) ($P = 0.014$)	Tortuosity: n.s.
					Cholesterol: each SD ↑ SD = 0.90 mmol l^{-1}	Tortuosity: n.s. Branching angle: n.s. optimality deviation ($\times 10^2$): n.s. LDR: 6.92 (0.19, 13.6) ($P = 0.041$)	Tortuosity: n.s. Branching angle: n.s. optimality deviation ($\times 10^2$): −1.90 (−3.75, −0.06) ($P = 0.015$) LDR: n.s.

Abbreviation: 95% CI: 95% confidence interval; SD, standard deviation; CRP, C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HbA1C, glycosylated haemoglobin; LDR, length-to-diameter ratio; CHASE, the Child Heart and Health Study in England; SPDS, Sydney Pediatric Diabetes Study.

soluble fms-like tyrosine kinase-1 (sFLT-1)) and their ongoing impact on children turning 6 years (Gishti *et al.* 2015*c*).

Disease linked to future CVD and retinal microvasculature. There are a series of diseases in adults that have been identified as being associated with vascular damage with the possibility of leading to future cardiovascular disease, such as hypertension, diabetes, depression and metabolic syndrome. A total of 14 papers were published on a wide range of early life diseases including childhood hypertension, T1DM, metabolic syndrome, carotid plaque, and microvascular complications (e.g. retinopathy and nephropathy) in T1DM paediatric patients (Table 6) (Alibrahim *et al.* 2006; Kifley *et al.* 2007; Cheung *et al.* 2009*a*; Gopinath *et al.* 2010*a*; Sasongko *et al.* 2010, 2011, 2012; Benitez-Aguirre *et al.* 2011, 2012; Li *et al.* 2011*b*, 2014; Bronson-Castain *et al.* 2012; Hosking *et al.* 2013; Meier *et al.* 2014; Yau *et al.* 2014; Gishti *et al.* 2015*b*). Except for four studies that were longitudinal (on T1DM young patients) (Kifley *et al.* 2007; Benitez-Aguirre *et al.* 2011, 2012; Cheung *et al.* 2009*a*), the rest of the studies were published on cross-sectional data (four on T1DM, two on hypertension, one on depression and anxiety, one on metabolic syndrome, and one on carotid plaque) (Bronson-Castain *et al.* 2012; Gopinath *et al.* 2010*a*; Hosking *et al.* 2013; Li *et al.* 2011*b*, 2014; Meier *et al.* 2014; Sasongko *et al.* 2010, 2011, 2012; Yau *et al.* 2014). The majority of the papers (9 out of 14) had investigated microvascular changes and complications in T1DM (Kifley *et al.* 2007; Cheung *et al.* 2009*a*; Sasongko *et al.* 2010, 2011, 2012; Benitez-Aguirre *et al.* 2011, 2012; Bronson-Castain *et al.* 2012; Hosking *et al.* 2013). Consistent cross-sectional findings in five papers suggested that retinal venular calibre widening was commonly seen in T1DM. Moreover, longer duration of T1DM was also associated with more tortuosity of retinal arterioles and higher retinal arteriolar and venular optimality deviation (Bronson-Castain *et al.* 2012; Hosking *et al.* 2013; Sasongko *et al.* 2010, 2011, 2012). Abnormal retinal vascular morphology such as wider retinal venular calibre, higher retinal arteriolar tortuosity and larger length-to-diameter ratio was related to concurrent and incident microvascular complications in both nephropathy and retinopathy among T1DM young patients (Kifley *et al.* 2007; Cheung *et al.* 2009*a*; Benitez-Aguirre *et al.* 2011, 2012). As for childhood-specific hypertension, two studies on Singaporean pre-schoolers and Sydney adolescent children reported similar findings on significant narrowing of retinal arterioles (Gopinath *et al.* 2010*a*; Li *et al.* 2011*b*). There were three papers published on mental health, carotid plaque and metabolic syndrome in children and adolescents. Narrowing in retinal arteriolar calibre was suggested to be associated with higher risks in carotid plaque (Li *et al.* 2014), smaller white matter volume (Yau *et al.* 2014) and presence of metabolic syndrome (Yau *et al.* 2014), yet with lower risks in depression and anxiety (Meier *et al.* 2014).

Discussion

CVD is the leading cause of mortality, morbidity and hospitalization worldwide (Visentin *et al.* 2014). Although the clinical manifestation is acute, CVD is a chronic disease that evolves gradually and may interfere with quality of life, physical disability, and lifelong dependence on health services and medications (Visentin *et al.* 2014). Establishing the mechanisms that link these factors with vascular and metabolic changes could provide essential insights for the development of preventative and therapeutic strategies.

Early life CVD risk factors might exert their influence through a series of complicated mechanisms, including adverse *in utero* programming (Barker, 2004*a*, 2005), IUGR (Barker, 2004*a*, 2005), lack of physical activity (Malina, 1996), unbalanced nutrition (Barclay *et al.* 2008), childhood hypertension and obesity (Berenson *et al.* 1998; Brion *et al.* 2007), depression (Glassman & Shapiro, 1998; Nemeroff & Goldschmidt-Clermont, 2012) and type 1 diabetes (de Ferranti *et al.* 2014*a*,*b*; Nathan *et al.* 2005). All CVD risk factors will impose an adverse impact on endothelium and subsequently lead to vascular remodelling. In this systematic review, we summarized 55 papers published on the topic of retinal imaging and early life CVD risk factors. Consistent and strong trends were found in children and adolescents between the presence of early life CVD risk factors and suboptimal structural changes in the retinal microvasculature.

Possible mechanisms for retinal vascular changes. In the general adult population, changes in the retinal microvasculature may reflect different changes in the systemic microvasculature. A range of morphological changes has been studied and they may reflect different underlying physiological and pathological states.

For example, generalized retinal arteriolar narrowing has been suggested to be related to hypertension. The pathophysiological changes in retinal arteriolar narrowing are related to initial vasospasm, followed by chronic arteriosclerotic changes in relation to elevated blood pressure (Wong & Mitchell, 2007; Sun *et al.* 2009*b*). As systemic blood pressure remains chronically elevated, generalized retinal arteriolar narrowing will develop as a consequence of an auto-regulatory process and result in intimal thickening, media-wall hyperplasia and hyaline degeneration (Sun *et al.* 2009*b*).

As for retinal venular dilatation, inflammatory-induced NO-dependent endothelial dysfunction has been mostly postulated (Sun *et al.* 2009*b*). One animal study found that

Table 6. Disease linked to future CVD and retinal microvasculature in early life

	Study	Study population and study design	Sample size and response rate	Age, male %	Diseases that will linked to CVD	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
1	Olta G	population-based, cross-sectional study	4007, 61.4%	6.0		per SDS ↓	per SDS ↓
(2015)	Generation R			50.2%	Childhood HTN:	OR: 1,35 (1.21, 1.45)	OR: 1.19 (1.00, 1.32)
2	Meier MH	Population-based, longitudinal study	865	mean age: 16.5 years	Depression/axiety:	Calibre:	Calibre:
(2014)	Brisbane Twin Study			42.8%	each 1 score↑	0.08 μm (SE, 0.036)	n.s.
					Overall mental health:	($P = 0.025$)	Calibre:
					each 1 score ↑	Calibre: 0.08 μm (SE, 0.036) ($P = 0.028$)	n.s.
3	Li LX	Clinical study, cross-sectional	2970	15–90 years	Carotid intima-media thickness (CIMT):	Retinal microvascular abnormalities (retinal arteriolar narrowing, retinopathy)	
(2014)				55.8%	0.078 mm (0.080, 0.262) ($P < 0.001$)	Presence	
					Carotid plaque: OR = 1.72 (1.32, 2.24) ($P < 0.001$)	Presence	
4	Yau PL	Clinical research, cross-sectional study	90 obese adolescents	14–21 years	Mets:	Calibre:	
(2014)			39 with Mets	42.2%			
			51 without Mets		Presence *vs.* Non-presence	182.35 *vs.* 198.62 μm	
					Mets criteria present:	($P < 0.01$)	
					each 1 score ↑	Calibre:	
					White matter:	−8.61, $P < 0.001$	
					smaller than 3150 voxel	Calibre:	
						Reduction (β not mentioned) ($P < 0.001$)	
5	Hosking SPM	Clinical study	26 T1DM	8–18 years	Microvascular complications:	AVR:	
(2013)		cross-sectional design		50%	Absent *vs.* Present	n.s.	

(continued)

Table 6. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Diseases that will linked to CVD	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
6	Benitez-Aguirre P	Hospital based,	511 baseline with T1DM	12–20 years	Renal dysfunction:	Length–diameter ratio:	Length–diameter ratio:
(2012)	Clinical research	prospective cohort study	174 developed renal dysfunction	48.6%	OR = 1.69	n.s.	4th *vs.* 1–3rd quartile
		Median 3.7 years' follow-up			(1.17, 2.44) ($P = 0.02$)		
					OR = 1.55 (1.08, 2.22) ($P = 0.02$)	Simple tortuosity: n.s.	Simple tortuosity 1st *vs.* 2–4th quartile
7	Bronson-Castain KW	Clinical observational, cross-sectional study	26 control	Ctrl: 17.6	Type 2 DM:	Calibre:	Calibre:
(2012)	Clinical study		32 T1 DM 15 T2 DM	38.5% T1 15.6 56.2% T2: 16.0 40%	Yes *vs.* No	n.s.	283.4 *vs.* 270.9 μm ($P < 0.05$)
8	Sasongko MB	Clinic-based, cross-sectional study	944 out of 1159	12–20 years	Early retinopathy:	Tortuosity:	Tortuosity:
(2011–2012)	SPDS		81.4%	43.7%	OR = 1.42 (1.11, 1.83) ($P = 0.005$)	each SD ↑ SD = 10.2 ($\times 10^3$)	n.s. n.s. n.s.
					Early kidney dysfunction: OR = 1.56 (1.06, 2.28) ($P = 0.023$)	each SD ↑ SD = 10.2 ($\times 10^3$)	
					Both: OR = 1.46 (1.13, 1.89) ($P = 0.004$)		
9	Benitez-Aguirre P	Hospital based,	736 baseline with T1DM	12–20 years	DR:	Simple tortuosity:	Simple tortuosity:
(2011)	Clinical research	prospective cohort study	287 developed retinopathy	48.6%	OR = 1.5	4th *vs.* 1st quartile	n.s.
		Median 3.8 years' follow-up			(1.0, 2.2) ($P = 0.03$)	Length–diameter ratio: 4th *vs.* 1st quartile	Length–diameter ratio: 4th *vs.* 1st quartile
					n.s.		
	Study	Study population and study design	Sample size and response rate	Age, male %	Diseases that will linked to CVD	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI

(continued)

Table 6. continued

	Study	Study population and study design	Sample size and response rate	Age, male %	Diseases that will linked to CVD	Arteriolar parameters β or mean, 95 % CI	Venular parameters β or mean, 95 % CI
10	Li LJ	Population-based, cross-sectional study	385	4–5 years	Hypertensive stage:	Calibre:	Calibre:
(2011)	STARS			50.7%	No *vs*. Yes	160.15 *vs*. 156.06 μm ($P = 0.02$)	n.s.
11	Sasongko MB	Clinic-based, cross-sectional study	944 out of 1159	12–20 years	T1DM duration:	Tortuosity:	Tortuosity:
(2010)	SPDS		81.4%	43.7%	each 3.3 years ↑	n.s. Branching angle: 1.15 (0.52, 1.78) ($P = 0.045$) Optimality deviation ($\times 10^2$): 1.29 (0.22, 2.35) ($P = 0.007$) Length–diameter ratio: n.s.	n.s. Branching angle: n.s. Optimality deviation ($\times 10^2$): 1.29 (0.22, 2.35) ($P = 0.007$) Length–diameter ratio: n.s.
12	Gopinath B	Australian adolescents	2353 out of 3144	12.7 years	Hypertensive stage:	Calibre:	Calibre:
(2010)	SCES	Population-based, cross-sectional study	75.3%	50.4%	No *vs*. Yes	151.9 *vs*. 149.9 μm ($P = 0.002$)	n.s.
13	Cheung N	Hospital based,	645 baseline with T1DM	12–20 years	DR:	Calibre:	Calibre:
(2009)		prospective cohort study	274 developed retinopathy	44.2%	HR = 1.46	each SD ↑	n.s.
		Median 2.5 years' follow-up			(1.22, 1.74) ($P < 0.001$) HR = 0.82 (0.69, 0.98) ($P = 0.028$)	SD = 18.90 μm	each SD ↑ SD = 22.63 μm
14	Alibrahim E	Hospital based,	668 baseline with T1DM	12–20	DR:	Calibre:	Calibre:
(2006)		prospective cohort study	172 developed retinopathy	Years	OR = 1.44	each SD ↑	n.s.
		Median 3.1 years' follow-up	180 age, sex-matched control	48.2%	(1.11. 1.86) ($P = 0.006$)		

Abbreviation: 95% CI: 95% confidence interval; SD, standard deviation; DR, diabetic retinopathy; HR, hazard ratio; STARS, the Strabismus, Amblyopia and Refractive Error Study in Singapore Chinese Preschoolers; SPDS, Sydney Pediatric Diabetes Study.

administration of lipid hydroperoxide into the vitreous humour of rats increased the number of leucocytes in the retinal microvasculature, which led to retinal venular dilatation (Tamai *et al.* 2002). In human subjects, low dosage of an injected *Escherichia coli* endotoxin will cause an increase in peripheral white blood cell count and dilatation in retinal venules (Kolodjaschna *et al.* 2004).

As described earlier, besides retinal vascular calibre, retinal vascular geometry represents different parameters of the retinal blood vessel network. These parameters include tortuosity, branching angle, fractal dimension and a series of others. Although the exact pathophysiological substrates of all these vascular geometric parameters are not fully understood, the main idea is that they reflect increased circulatory energy costs and a decreased efficacy in the distribution of blood to the tissue (e.g. retina).

There are also other ways to assess structural and functional changes of retinal microcirculation through different newly developed and advanced retinal imaging tools, such as ultra-wide field retinal imaging, retinal oximetry and scanning laser Doppler flowmetry. All these new techniques can measure and analyse peripheral retinal vasculature, foveal capillary network, retinal oxygen saturation, retinal blood flow and choroidal vasculature (Cheung *et al.* 2012).

Other methods to measure retinal microcirculation. Optical coherence tomography. In the past few years, optical coherence tomography (OCT) has made accessible in-depth high-resolution information on the retina with its vessels, including quantitative analysis of the vessel diameter. The retinal vasculature in OCT scans can be derived from direct recognition of the smooth musculature of the vessel wall and the vessel lumen. With advances in software algorithms, there are possibilities to perform OCT angiography, which provides a better approach to invasively visualize blood flow in the retina and the choroid capillary network and to detect the growth of neovascularization (Spaide *et al.* 2015). Therefore, future application of the OCT technique may be quite promising to image the capillary network around the optic nerve head either by vascular static parameters (e.g. OCT scan (Muraoka *et al.* 2013; Schuster *et al.* 2015)) or by vascular dynamic parameters (e.g. fourier-domain OCT (Wang *et al.* 2008), Doppler OCT (Konduru *et al.* 2012; Tan *et al.* 2012) and *en face* OCT angiography (Dansingani *et al.* 2015)).

Dynamic vessel analyzer. Dynamic vessel analyzer (DVA) is a new technology to determine dynamic retinal vessel responses through a series of stimulation techniques including flickering light (Nagel & Vilser, 2004), carbogen and oxygen inhalation (Wimpissinger *et al.* 2004; Heitmar *et al.* 2010), and intravenous vasoactive substance infusions (Jeppesen *et al.* 2007). After stimulation with flickering light, DVA analysis software calculates maximum retinal vessel response to 20 s of flickering light over three stimulation cycles. The average response, within a 17–23 s window after the start of the stimulation, is taken to be the maximum diameter response. This analysis generates a maximum artery dilatory response index, as well as similar outputs for minimum response, peak response and maximum venous dilatory response to flicker (Heitmar *et al.* 2010).

Adaptive optics imaging. Adaptive optics imaging is an opto-electronic technology that improves the resolution of fundus images (Koch *et al.* 2014). Current adaptive optics-based fundus cameras enable visualization of microstructures such as photoreceptors (Liang *et al.* 1997), capillaries (Martin & Roorda, 2005) and vascular wall (Chui *et al.* 2012) noninvasively in humans.

However, these imaging techniques may be difficult to implement in children, since most of them require full understanding by the subject of the instructions given by the examiner. In view of these limitations, thus far retinal fundus imaging is the most feasible and widely used technique in children and young adults. Future child-friendly technologies with quantitative measurements targeting structural (e.g. En Face OCT (Dansingani *et al.* 2015; Savastano *et al.* 2015) and speckle variance OCT (Chan *et al.* 2015)) and functional (e.g. DVA (Lim *et al.* 2013) and oximetry) aspects of the microvasculature may yield promising results.

The current gap in research and future perspectives. There is increasing interest in epidemiological studies of early origins of CVD. In the past three decades, Barker's hypothesis has been widely debated and modified. Early life CVD risks such as IUGR, malnutrition, T1DM, elevated blood pressure and obesity may lead to damage in several target organs, subsequently increasing the risk of a variety of diseases later in life. Microvascular changes have been implicated as one of the pathways through which these early life factors may be related to the risk of CVD in late-life. However, thus far the exploration of various pathways related to the microvasculature has been limited due to the inability to employ any invasive examinations in these young subjects. To some extent the implementation of retinal imaging has made it now possible to interrogate the role of the microvasculature non-invasively during early life. In this systematic review, we summarized 55 papers published on the topic of retinal imaging and early life CVD risks. We found a consistent and strong trend that children and adolescents exposed to early life CVD risk factors had suboptimal structural changes in the retinal microvasculature. Furthermore, all these studies have shown the feasibility, safety and reliability of retinal

imaging in young children. However, there are also a few major gaps in the current research employing retinal imaging: first, most studies thus far are cross-sectional, hence limiting our ability to draw causal inferences and examine the predictive value of retinal imaging; second, functional retinal imaging has so far been difficult to implement due to limited cooperation of study subjects; and third, retinal imaging has not been implemented in the very young (< 3 years old).

Despite these limitations, the current literature shows strong 'proof of concept' that retinal imaging can provide additional information on the status of the microvasculature in children. Besides, the need for longitudinal studies to assess the additional value of retinal imaging, there is a need to implement some of the advanced retinal imaging techniques described earlier, which may allow us to examine functional aspects of the microvasculature.

Conclusion

In summary, there is now substantial evidence that CVD risk factors are associated with structural changes in the retinal microvasculature in early life. The retinal microvasculature may therefore be an indicator of future CVD risk. These findings emphasize early life predisposition to microvascular damage due to the presence of CVD risk factors during that period. Further longitudinal studies are required to investigate specific pathophysiological mechanisms, and to examine the additional value of retinal imaging in early life in the prediction of CVD during adulthood.

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Additional information

Competing interests

None declared.

Author contributions

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