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MAJOR REVIEW

Retinal Vascular Caliber: Systemic, Environmental, and Genetic Associations

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Abstract. Quantitative studies of retinal vascular caliber using new computer-assisted retinal imaging systems have allowed physicians and researchers to understand the influence of systemic, environmental, and genetic factors on retinal vascular caliber. Retinal vascular caliber changes reflect cumulative response to aging, cardiovascular risk factors, inflammation, nitric oxide–dependent endothelial dysfunction, and other processes. Recent epidemiological studies have shown that changes in retinal arteriolar and venular caliber size may reflect the differential effects of a range of systemic, environmental, and genetic risk factors. Narrower retinal arteriolar caliber and smaller arteriovenous ratio are associated with older age; higher levels of past, current, and future blood pressure and obesity; and predict the incidence of diabetes and coronary heart disease. Wider retinal venular caliber, in contrast, is associated with younger age; impaired fasting glucose and diabetes; dyslipidemia; obesity; systemic marker of inflammation, endothelial dysfunction, and cigarette smoking; and predicts the risk of stroke and coronary heart disease. New data from family and twin studies indicate a significant genetic contribution to retinal vascular caliber, an area that is under investigation. Elucidating the complete range of systemic, environmental, and genetic factors linked with retinal vascular caliber changes may provide critical insight into the etiology, pathogenesis, and natural history of early vascular disease not only in the eye but elsewhere in the body. (**Surv Ophthalmol 54**:74–95, 2009.

Key words. arteriovenous ratio • generalized arteriolar narrowing • genetics • hypertension • pathophysiology • retinal imaging • retinal arteriolar caliber • retinal vascular caliber • retinal venular caliber • retinal venular dilation

I. Introduction

The retinal blood vessels are accessible for noninvasive visualization, and therefore provide a means to study the early structural changes and pathological features of the human microcirculation.[159] The vascular tree of the retina consists essentially of arterioles and venules, rather than arteries or veins. This is because the widest part of the lumen of the retinal vessels is near the optic disk with diameters of about 100 μm, with neither internal elastic lamina nor a continuous muscular coat.[44,125] Historically, retinal arteriolar narrowing, observed during clinical ophthalmoscopic examination, has been seen as an early sign of hypertensive retinopathy and suggested to be a prognostic indicator in people with hypertension.[41,42,61]

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Advances in retinal photographic techniques and in image analysis have now allowed objective measurement of retinal vascular changes. Various semi-automated, computer-based retinal imaging programs have proven to be highly accurate and reproducible in assessing in vivo architectural changes in the retinal vascular network.[47] In particular, quantitative measurement of retinal vascular caliber has greatly increased knowledge of the clinical significance and influence of systemic, environmental and genetic factors on the retinal vasculature.[104,138,157,178,186] Data from recent population-based studies suggest that retinal arteriolar and venular caliber changes may reflect different vascular pathophysiological processes that link to a range of systemic, environmental, and genetic risk factors.[49,51,163,186]

There are a number of reports associating changes in retinal vascular caliber with clinical cardiovascular outcomes such as stroke and coronary heart disease.[49,164,171,179] There are also increasing data linking changes in retinal vascular caliber with demographic factors (e.g., age, race/ethnicity); various systemic medical conditions (e.g., blood pressure, diabetes); environmental and lifestyle factors (e.g., smoking); and, more recently, genetic factors.[84,163,166,183,186] However, the range of factors influencing variation in retinal vascular caliber have not been fully investigated.

The aims of this review are to 1) summarize findings from recent population-based epidemiologic studies (from 1990 onward) regarding determinants of retinal vascular caliber in terms of systemic, environmental, and genetic factors; 2) explore possible pathophysiological mechanisms underlying retinal vascular caliber changes; and 3) examine potential research questions and clinical implications of these recent findings.

II. Methodological Issues in Measuring Retinal Vascular Caliber

There have been several previous methods to measure retinal vascular caliber from digitized or digital retinal images.[6,31,112,115,137,185] Recent population-based studies have used computer-assisted programs to measure individual arterioles and venules and to combine them according to formulas developed, for example, by Parr and Spear[107,108] and subsequently by Hubbard et al.[47]

Calibration of the computer-assisted program is fundamental when determining the true size of a fundus feature. Pathology series published in the 1990s demonstrated that the true value of one standard vertical disk diameter was equivalent to 1,800–1,900 microns.[113] Later the major digital camera companies (most notably Topcon for the imaging system) standardized to 1,800 microns. This standard has now gained relatively wide acceptance as an internal reference for calibration to compensate the effect of camera magnification on the vessel caliber measurement in the computer-assisted programs. It is also important to emphasize the ethnic difference in terms of variation of normal optic disk size that has been observed in the epidemiological studies,[150] with the range in two-fold from the smallest to largest for disk diameter and four-fold for disk area.[3] Estimation of the ocular effect on the magnification of fundus photograph will be covered later in this review.

Essentially, these programs generate three summary variables: the projected caliber size of the central retinal artery (central retinal arteriolar equivalent [CRAE]), the projected caliber size of central retinal vein (central retinal venular equivalent [CRVE]), and the ratio of the two variables (arteriovenous ratio [AVR]). The output of one of the computer programs is shown in Figs. 1 and 2.

More recently, the formulas have been further improved by Knudtson et al.[76] In several large population-based epidemiological studies, these retinal vascular indices have demonstrated substantial reproducibility (intra- and inter-grader correlation coefficient ranged from 0.67–0.99) and thus provide a highly precise and reliable research tool for objective assessment of structural vascular changes (Table 1).

Although measurement of retinal vascular caliber appears to be reproducible for research purposes,[110] its applicability in the clinical setting has yet to be established, due to several methodological issues. First, the current formulas used to combine individual retinal vascular caliber into summarized indices are based on theoretical and empiric models. The Parr and Spear[107,108] and Hubbard[47] formulas for CRAE and CRVE were derived by examining a large number of retinal images with branching points and calculating the relationship between individual trunk vessels and their respective branch vessels using a root mean square deviation model that best fit the observed data.

Although now used widely in epidemiological studies of cardiovascular and ocular diseases,[23,128,162,166] there are some drawbacks in using these formulas. Knudtson et al made an important observation that the Parr-Hubbard formulas were dependent on the number of retinal vessels measured.[76] In addition, the constants included in the formulas were also dependent on the units with which the vessels were measured. Knudtson et al therefore developed a set of revised formulas for summarizing retinal vascular caliber, and demonstrated clear superiority over the previously version of Parr-Hubbard

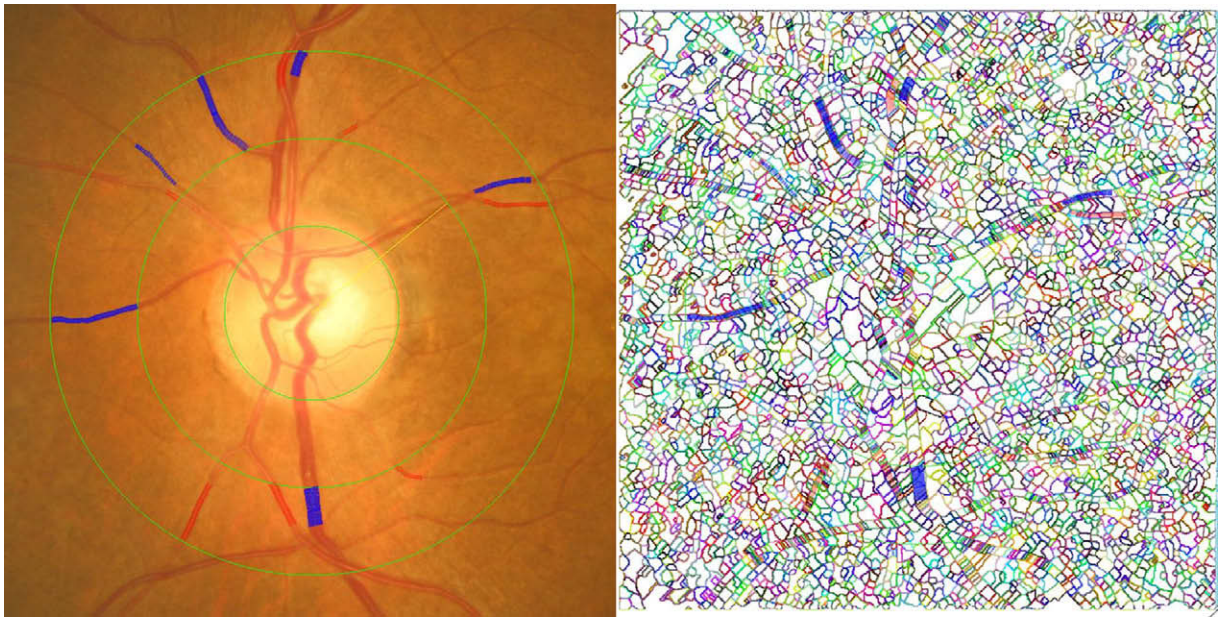

Fig. 1. Retinal vessel measurements by IVAN software: image and splats display showing relatively smaller arterioles (CRAE 148.0 μm) and venules (CRVE 240.42 μm) and an AVR (0.62). Arterioles in red and venules in blue.

formulas.[76] However, it is unclear if there are additional issues regarding the formula that should be addressed, and is likely that further refinement will be needed. Recently, for example, some investigators suggested a revised formula for more accurate estimation of arteriolar branch coefficient.[109] This revised formula used a linear regression model to incorporate the asymmetry index of the vessel branches being measured. Further work is clearly needed to validate the estimates of vessel caliber measurements against entire diameter of these vessels in vivo (e.g., measurements obtained from fluorescent angiographs), and to determine whether the newer formulas are more accurate and have a better predictive value on outcomes compared to older formulas.

Second, existing retinal vascular imaging research has mostly focused on the relative differences in retinal vascular caliber changes within the study population (e.g., people with narrower quintile or quartile of

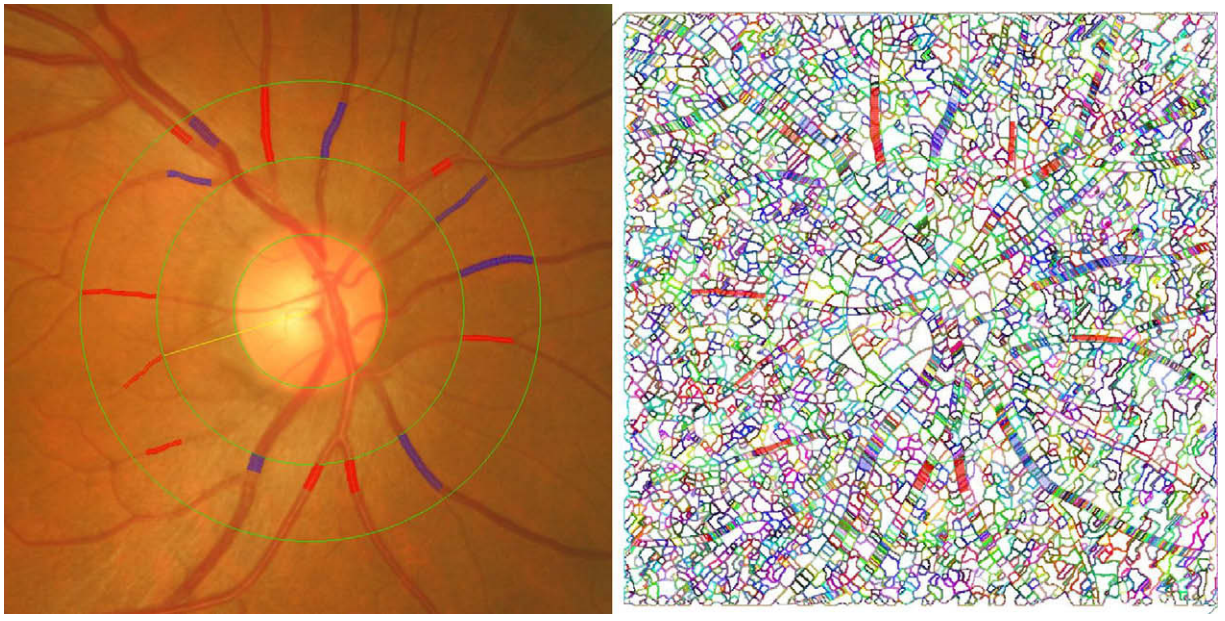

Fig. 2. Retinal vessel measurement by IVAN software: image and splats display showing relatively larger arterioles (CRAE 183.62 μm) and venules (CRVE 251.12 μm) and an AVR (0.73). Arterioles in red and venules in blue.

TABLE 1

Reproducibility of Retinal Vascular Caliber in Selected Studies

Study	n	Age range (years)	Formula	Intra-Grader Intraclass Correlation	Inter-Grader Intraclass Correlation	Reference
ARIC study	12,642	51–72	Parr-Hubbard	0.69 for CRAE 0.89 for CRVE 0.84 for AVR	0.74 for CRAE 0.77 for CRVE 0.79 for AVR	23,47
CHS	2,824	69–97	Parr-Hubbard	0.67	0.91	162
BDES	4,926	43–86	Parr-Hubbard	0.78	0.99	166
BMES	3,654	49 and older	Parr-Hubbard	0.75–0.90[a]	0.80–0.93[a]	128
Rotterdam study	5,674	55 and older	Knudtson	0.69–0.88 for CRAE 0.90–0.95 for CRVE 0.72–0.90 for AVR	0.67–0.80 for CRAE 0.91–0.94 for CRVE 0.75–0.84 for AVR	51
MESA	5,979	45–84	Knudtson	0.78	0.99	163
WESDR	996	15–29 (type 1 diabetes)	Parr-Hubbard	Refer to the ARIC study	Refer to the ARIC study	47,67,68
	1,370	30 and older (type 2 diabetes)				
Hoorn study	256	60–85	Parr-Hubbard	0.08 for CRAE[b] 0.05 for CRVE[b] 0.09 for AVR[b]	Not applicable[c]	149
Funagata study	1,481	35 and older	Parr-Hubbard	Refer to the ARIC study	Refer to the ARIC study	47,60
SCORM study	768	7–9	Knudtson	0.853 for CRAE 0.973 for CRVE	Not applicable[c]	13

ARIC = Atherosclerosis Risk In Communities; CHS = Cardiovascular Health Study; BDES = Beaver Dam Eye Study; BMES = Blue Mountains Eye Study; MESA = Multi-Ethnic Study of Atherosclerosis; WESDR = Wisconsin Epidemiological Study of Diabetic Retinopathy; SCORM = Singapore Cohort Study of the Risk Factors for Myopia.
[a]BMES reported kappa statistics.
[b]Hoorn study used coefficients of variation [Standard deviation of the mean difference/($\sqrt{2}$ × pooled mean)].
[c]One grader did all retinal measurements in this study.

retinal arteriolar caliber are more likely to develop cardiovascular disease than people with wider quintile or quartile of arteriolar caliber). Currently, no age-, sex-, and blood pressure level–specific reference levels of retinal vascular caliber have been established. Normative reference levels are critical for these measurements to be implemented in clinical settings. However, due to numerous systemic and environmental effects on the variation of the measurements for retinal arteriolar and venular caliber, it may be difficult to determine a uniformly standard normal value across different individuals or different populations. In adult populations, it is also difficult to completely account for the confounding effect of systemic (e.g., hypertension, diabetes) and ocular (e.g., diabetic retinopathy, glaucoma) disease processes on retinal vessel measurement. One recent approach by researchers has been to study retinal vascular caliber in healthy children, who are presumably free of these systemic and environmental influences, and may therefore provide better normative reference values.[97] However, a value considered normal for children may not be normal for adults.

Third, there remain unresolved issues related to magnification effects from camera and ocular refractive media in studies focusing on ocular outcomes, especially when the ocular condition is related to refraction status and when the study population has large variation with regard to refraction status. Determining the actual retinal vascular caliber size is of particular importance when comparing between individuals in the clinical settings. Although there are already a few methods to adjust for magnification using ocular biometric data (e.g., axial length),[111] most were designed for telecentric cameras. For non-telecentric cameras, Rudnicka et al have described a method to adjust for magnification using plain films,[123] but its applicability for digitized retinal photographs remains unknown. There has also been development of techniques for correcting ocular magnification by the Bengtsson and Krakau formula[4] and the Bennett formula,[5] based on ocular biometric parameters. The effect of refraction on the absolute magnitude of retinal vascular caliber measured from fundus photographs has been demonstrated in the Beaver Dam Eye Study,[177] the Blue Mountains Eye

Study,[184] and the Singapore Cohort Study of the Risk Factors for Myopia.[16,17] These studies found no relationship between refraction/axial length and retinal vascular caliber after ocular magnification has been accounted for, suggesting no biological link between ocular dimension and structural changes in the retinal vasculature.[16,17,184] Other studies have also sought alternative geometric attributes of retinal vasculature that are dimensionless in nature to account for magnification effects and to allow for comparison of measurements of retinal vascular changes between individuals.[111] These include the retinal AVR, junctional exponents, vascular bifurcation angles, vascular tortuosity, and length-to-diameter ratios.[110] Among these, the AVR is the most commonly used measure, as it is simple to understand and is attractive to clinicians. Advances in the computer-assisted system have also allowed automatic and precise measurement of the AVR.[88] However, it is important to emphasize that the AVR has significant limitations, including the inability to separately capture the information of the individual arteriolar and venular caliber components.[92] For example, both narrower arterioles and wider venules may produce a smaller AVR. Thus, a small AVR cannot differentiate specific changes in arteriolar and venular caliber. There is already increasing evidence that narrowing retinal arterioles and widening venules carry different information as to their associations with different systemic diseases. Whereas smaller retinal arteriolar caliber is associated with hypertension, and may even precede clinical hypertension development, larger retinal venular caliber has been associated with inflammation, smoking, hyperglycemia, obesity, and dyslipidemia.[163] These observations suggest that changes in retinal arteriolar and venular caliber may reflect different pathophysiological processes underlying the associated systemic diseases. Combining these two components into one estimate, the AVR, without consideration of separate arteriolar or venular caliber measurements, would therefore mask such associations.

Fourth, researchers have raised the issue of a potential confounding effect between retinal arteriolar caliber and venular caliber. Liew et al observed that retinal arteriolar and venular caliber size are highly correlated and individuals with narrower arterioles are, not surprisingly, more likely to have narrower venules.[93] One approach to account for this confounding effect has been to adjust for the fellow vessels in statistical analysis (e.g., adjustment for retinal arteriolar caliber in analysis of retinal venular caliber, and vice versa).[93] The confounding effect of arteriolar caliber on the association of venular caliber with blood pressure was elegantly documented in the Blue Mountains and Rotterdam studies.[54,93] In the Rotterdam study, an association between retinal venular narrowing and incident hypertension was initially reported.[55] However, using the new analytical approaches of simultaneously adjusting for retinal arteriolar and venular calibers, retinal venular narrowing was no longer associated with incident hypertension.[54]

Fifth, there are dynamic and physiological changes to retinal vascular caliber, and measurement of retinal caliber from a single, static retinal image may not capture all the information. For example, retinal caliber varies with the cardiac cycle.[11] The Atherosclerosis Risk In Community (ARIC) study showed that vessel pulsation was detectable, mainly in the largest venules of the retina.[75] Additionally, Dumskyj et al reported that small changes in retinal vessel diameter could be accurately detected by multiple frame electrocardiograph synchronized fundus photography.[29] However, because the variation caused by cardiac pulse appears to be small and random, it may only induce non-differential misclassification.

Finally, new research has shown that the retinal background color may be a potential source of measurement error on the retinal vessel caliber. This was demonstrated in the Sydney Myopia Study.[119] The study observed that retinal vascular caliber was significantly wider in children of East Asian ethnicity than those of white children, which was in line with the findings from other two population-based studies suggesting racial/ethnic differences of retinal vascular caliber.[13,163] However, retinal arteriolar caliber was no longer significantly different between white and East Asian children when the analysis was confined to children with dark brown iris, a surrogate of retinal pigment color. The investigators therefore hypothesized that the reported racial/ethnic differences may be due to differences in retinal pigmentation, represented by iris color in their study. This finding has raised an important question: whether the variation of retinal vascular caliber across different ethnicities is induced by measurement error due to different levels of retinal pigmentation associated with different ethnic groups. Alternatively, the noted association of retinal vascular caliber with iris color may partly reflect genetic differences among different races as iris color itself is a complex human trait with very high heritability,[80] and has been suggested to link to potential genes.[190] This is an area that clearly requires further research.

III. Pathophysiology of Retinal Vacular Caliber Changes

A complete understanding of the underlying mechanisms of retinal vascular caliber changes remains lacking.[10,147,167] It has been postulated that

retinal vascular caliber changes may reflect the cumulative structural vascular damage from multiple processes, including aging,[84,166] long-term hypertension,[87] arteriosclerosis,[146] inflammation,[74] endothelial dysfunction,[27,43,74,145] and other vascular processes.[167] Variations in arteriolar and venular caliber may also be influenced by physiological blood flow parameters such as oxygenation and shear stress.[101,103]

There are distinct pathophysiological factors influencing retinal arteriolar and venular caliber. The endothelial cells of arterioles and venules are molecularly distinct from the earliest stages of angiogenesis and may reflect genetic factors.[152] This view is shared by a recent genome-wide linkage analysis demonstrating that retinal arteriolar and venular caliber are linked to different genetic loci.[186]

A. MECHANISMS OF RETINAL ARTERIOLAR CALIBER CHANGES

The pathophysiological changes in retinal arterioles in response to blood pressure elevation are well documented and often referred to as hypertensive retinopathy.[44,100,146,180] Raised blood pressure initiates vasospasm and an increase in vasomotor tone owing to local autoregulation, leading to consequent elevation in capillary pressures and flows. This stage is seen clinically as generalized narrowing of the retinal arterioles. With persistent blood pressure elevation, chronic arteriosclerotic changes, such as intimal thickening, media-wall hyperplasia, and hyaline degeneration, develop. These changes manifest as diffuse and focal arteriolar narrowing, opacification of arteriolar walls (clinically described as silver or copper wiring), and compression at the venules by arterioles at their junction (termed arteriovenous nicking or nipping). An exudative stage follows with breakdown of the blood–retinal barrier as a result of autoregulation failure caused by severe elevation in blood pressure. Focal or generalized dilatation of arterioles follows,[45] along with increased permeability, necrosis of smooth muscles and endothelial cells, exudation of blood (hemorrhage) and lipids (hard exudates), and retinal ischemia. Narrowing of the arteriolar caliber is thus part of the initial stages of hypertensive retinopathy. Impairment of autoregulation in the retinal circulation has also been implicated in the pathogenesis of various retinal diseases, including diabetic retinopathy,[116] diabetic maculopathy,[35] and glaucoma.[40]

The retinal blood vessels have no adrenergic vasomotor nerve supply to initiate changes in vascular tone.[81,95,188] Furthermore, retinal blood flow has been postulated to be dependent on myogenic changes[30,78] in arteriolar tone and may also partly involve other mechanisms such as endothelial function and metabolic autoregulation.[28] Previous studies demonstrated that cultured brain endothelial cells directly interact with smooth muscle cells and pericytes via gap junctions, and actively regulate arteriolar tone and caliber size by elaborating vasodilators (nitric oxide [NO], adenosine, prostanoids) and vasoconstrictors (endothelin 1, angiotensin II) in response to local metabolic needs.[79,129] Among these factors, NO plays a central role in the maintenance of vascular homeostasis by regulating vascular tone and inhibiting platelet and leukocyte adhesion to endothelial cells. Recent studies have demonstrated that NO synthase may have a vasoregulatory role in the retina.[101,122]

NO-dependent endothelial dysfunction has been postulated as a key feature of essential hypertension, and decreased levels of NO may contribute to impaired endothelium-dependent vasodilatation in essential hypertension. In support of this hypothesis, recent clinical studies have shown that patients with hypertension have lower levels of NO production than normotensive subjects,[2,8,27,105] along with increased oxidative stress.[2] A recent animal study found that a novel polymorphism in the 3′UTR of the L-arginine transporter gene contributed to hypertension and endothelial dysfunction.[187]

Therefore, failure of NO-dependent endothelial function is likely to be the underlying mechanism involved in narrowing of retinal vascular caliber.[27,59] Data from the Beaver Dam Eye Study support this hypothesis by demonstrating several genes in the linkage region of retinal arteriolar and venular caliber involved in endothelial NO-related pathways.[186] Linkage regions of retinal vessel caliber in this study were also overlapped with the regions of hypertension, which provided a strong genetic basis for the consistent observations from four prospective studies showing retinal arteriolar narrowing precedes hypertension and may contribute to the pathogenesis of hypertension.[55,132,170,183]

In diabetes, endothelial dysfunction and inflammation are likely to have a major effect on the retinal microvasculature as well.[34,134,148] Both in vitro and in vivo studies have shown that the synthesis and release of vasoconstrictors by the vascular endothelium are increased in patients with diabetes.[9,141] Consistent with this concept, in the ARIC[173] and Beaver Dam Eye studies[182] narrower retinal arteriolar caliber predicted the incidence of diabetes, independent of other established factors.

B. MECHANISMS OF RETINAL VENULAR CALIBER CHANGES

There is less understanding of the pathophysiological mechanisms of retinal venular caliber changes.

Epidemiological studies have consistently shown associations of retinal venular caliber with systemic inflammatory markers. The Beaver Dam Eye Study showed that participants with the highest levels of both inflammatory and endothelial dysfunction markers had the largest retinal venular caliber.[66] Animal studies demonstrated that administration of lipid hydroperoxide into the vitreous humour of rats increased the number of leukocytes in the retinal microvasculature and also the size of retinal venule caliber, but not arterioles.[139] Another study reported that intravitreous administration of low doses of an *Escherichia coli* endotoxin to humans led to an increase in white blood cell count and retinal venular dilation.[77]

Inflammation and endothelial dysfunction have also been hypothesized to be the mechanisms underlying the link between obesity and larger retinal venular. Evidence has shown that obese subjects have increased markers of endothelial activation and chronic vascular inflammation.[7,22] Furthermore, obesity was also linked with increased blood volume[106] and leptin levels,[136] which might modulate vascular caliber through local mechanisms involving NO release.[151] Although an exact mechanism needs to be fully elucidated, it has been proposed that several plausible theories including inflammation, oxidative stress, hyperleptinemia, and NO dysregulation may explain the association between larger retinal venules and obesity development.[51,74,156] These factors might interact to produce the larger venular diameter in obese individuals.[105]

It has been postulated that the association of smoking with venular dilation may involve higher carbon monoxide levels[133] and endothelium-dependent relaxation,[114] which may lead to a decrease in oxygen supply to retinal tissue thus resulting in retinal venular dilatation. In people with diabetes and hyperglycemia, arteriolar and venular dilation may also reflect hyperperfusion resulting from hyperglymia and lactic acidosis from retinal hypoxia.[39]

IV. Systemic Factors and Retinal Vascular Caliber

There is growing evidence that variations in retinal vascular caliber are associated with a range of systemic conditions and diseases (Tables 2–4).

TABLE 2

Relationship of Systemic and Environmental Factors with the Retinal Arterio-Venous Ratio (AVR)

	ARIC	BDES	BMES	Rotterdam	CHS	MESA	WESDR	Hoorn	Funagata	Reference
Older age		n.s.	–				–		–	60,71,84,166
Sex (Female)		–	+		+					69,84,127,172
Race (blacks vs whites)	–				–	–				74,163,172
Higher current blood pressure	–	–	–	–	–		–		–	51,60,71,84, 127,162,166
Higher past blood pressure	–		–		–					87,127,162
Incident hypertension	–	–	–	–						55,132,170,183
Incident diabetes mellitus	–	–		–						52,173,182
Higher total holesterol	n.s.		n.s.	n.s.	n.s.					51,74,86,172
Higher triglyceride	–		n.s.							74,86
Higher HDL	+		n.s.	+						51,74,86
Higher LDL			n.s.							86
measures of obesity				–			n.s.			51,71
Measures of atherosclerosis	–			–	n.s.			n.s.		74,149
Measures of inflammation	–			–						51,74
Incident clinical stroke	–		n.s.							98,165
Subclinical cerebral disease	–									21,169
Cardiovascular mortality		n.s.	n.s.				–			64,98,154,168
Proteinuria and renal dysfunction	n.s.				n.s.		n.s.			32,71,160
Cigarette smoking	–			–			n.s.			51,71,74
Alcohol consumption	–			–						51,74
Medication use			–							85

Significant associations: + denotes larger caliber; – denotes smaller caliber and inverse association; n.s. denotes not significant associations.

TABLE 3

Effect of Systemic and Environmental Factors on Retinal Arteriolar Caliber

	ARIC	BDES	BMES	Rotterdam	CHS	MESA	WESDR	Hoorn	Funagata	SCORM	References
Older age		–	–			–	–		–		57,60,71,72,84, 163,164,166
Sex (Female)			+			+				n.s.	13,84,163
Race (blacks vs whites)						+				+	13,163
Higher current blood pressure	–	–	–	–	–	–	–		–		51,60,71,84,163, 164,166
Higher past blood pressure			–								87
Incident hypertension			–	–							55,93
Higher glucose or diabetes	+		+	n.s.		+	+				51,52,62,71,161, 163,164
Higher total cholesterol			n.s.	+							51,86,164
Higher triglyceride	+		n.s.			n.s.					86,161,163
Higher HDL	n.s.		–	–		n.s.					86,161,163,164
Higher LDL			n.s.			n.s.					86,161
Measures of Obesity	–		–	–	n.s.	–					51,156,161, 163,164
Measures of inflammation		n.s.		+							51,66
Endothelial dysfunction		n.s.						n.s.			66,149
Incident clinical stroke					n.s.						164
Incident coronary heart disease					–						164
Cardiovascular mortality			–				–				70,154
Proteinuria and renal dysfunction	–						n.s.				71,160,181
Cigarette smoking				+		+	+				51,66,71,163,164
Alcohol consumption				n.s.		–					51,163
Medication use		–[a] –[b]	n.s.[a] +[c]								58,85,90,174

Significant associations: + denotes larger caliber; – denotes smaller caliber and inverse association; n.s. denotes not significant associations.
[a]Hormone-replacement therapy.
[b]Antiglaucoma medication.
[c]Aspirin and antihypertensive agents.

A. ASSOCIATION WITH AGE, SEX, AND RACE/ETHNICITY

Older people have narrower retinal vascular caliber, and this inverse association between age and retinal vascular caliber has been uniformly demonstrated across different study populations.[60,72,84,163,166] In the Beaver Dam and the Blue Mountains Eye studies, retinal arteriolar and venular caliber decreased from 1.8–4.8 μm for each decade increase in age, independent of sex, hypertension, and other risk factors.[84,166] Comparable results have also been found in other racial/ethnic groups, such as the Multi-Ethnic Study of Atherosclerosis (MESA)[163] and a study involving a non-diabetic Japanese population.[60] This relationship has also been consistently shown in people with type 1[71] and type 2[72] diabetes in the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR). After taking the correlation between retinal arteriolar and venular caliber into account, the inverse association between retinal vascular (both arteriolar and venular) caliber and age was unchanged.[57]

Whereas many studies have investigated the relationship between age and retinal vascular caliber, few population-based studies have evaluated the influence of sex or race/ethnicity on retinal

TABLE 4

Effect of Systemic and Environmental Factors on Retinal Venular Caliber

	ARIC	BDES	BMES	Rotterdam	CHS	MESA	WESDR	Hoorn	Funagata	SCORM	References
Older age		–	+		–	–	–		–		57,60,71,72,84, 163,164,166
Sex (Female)			n.s.		n.s.	n.s.				n.s.	13,84,163,164
Race (blacks vs whites)					+	+				+	13,163,164
Higher current blood pressure	n.s.	n.s.	+	–	–	n.s.	–		–		51,57,60,71,84, 161,163,164,166
Incident hypertension			+	+							54,93
Higher glucose or diabetes	+			+		+	+				52,71,161,163
Higher total cholesterol			n.s.	n.s.	+						51,86,163,164
Higher triglyceride	+		n.s.			+					86,161,163
Lower HDL	n.s.		–	–	n.s.	–					51,86,161,163,164
Higher LDL			n.s.			+					86,163
Measures of Obesity	+			n.s.	+	+	+			+	14,51,66,71,161, 163,164
Measures of atherosclerosis				+				n.s.			51,149
Measures of inflammation		+		+	+	+		n.s.			26,51,66,149, 163,164
Endothelial dysfunction		n.s.				+		n.s.			66,149,163
Incident clinical stroke				+	+						49,164
Sub-clinical cerebral disease				+							49,50
Incident coronary heart disease					+						164
Cardiovascular mortality			+				+				70,154
Proteinuria and renal dysfunction	–						+				70,71,160,181
Cigarette smoking				+	+	+	+				51,66,71,163,164
Alcohol consumption				n.s.		n.s.					51,163
Medication use		–[a] –[b]	n.s.[a] n.s.[c]								58,85,90,174

Significant associations: + denotes larger caliber; – denotes smaller caliber and inverse association; n.s. denotes not significant associations.
[a]Hormone-replacement therapy.
[b]Antiglaucoma medication.
[c]Aspirin and antihypertensive agents.

vascular caliber, with less consistent results. In the Blue Mountains Eye Study, both mean retinal arteriolar caliber and AVR were consistently higher in women than men across all age groups,[84] in good agreement with findings from the Cardiovascular Health Study[172] and MESA.[163] Sex difference, possibly due to a protective effect of estrogen on the coronary artery, has been reported in previous studies.[158] However, this hypothesis could not be confirmed in retinal arterioles in reports from the Blue Mountains[58,85] or the Beaver Dam Eye studies.[174] There is no adequate explanation for these apparently inconsistent findings for men and women.

Data from both the ARIC and Cardiovascular Health studies demonstrated a lower AVR in black patients than white patients, even after adjusting for age and sex.[74,172] However, neither of the two studies investigated the racial/ethnic contribution to the variation in arteriolar or venular caliber separately. Data from the MESA revealed that AVR was consistently larger in white participants than in black, Hispanic, and Chinese participants. Furthermore, it demonstrated that both retinal arteriolar and

venular caliber, the two separate components of AVR, were significantly larger in black and Hispanic participants than in white and Chinese persons, reflecting smaller AVR in the former compared to the latter groups.[163] In 7- to 9-year-old children, Chinese individuals showed significantly narrower retinal arteriolar and venular caliber than Malay and Indian children.[13]

Some of the racial/ethnic differences in retinal vascular caliber may be explained by the significant differences in the distribution of cardiovascular risk factors between the ethnic groups. In MESA, for example, black and Hispanic participants were more likely to have diabetes, obesity, hyperlipidemia, and systemic inflammation than whites.[163] It is also possible that the racial/ethnic differences in retinal vascular caliber may partly reflect variations in susceptibility of the retinal vasculature to cardiovascular risk factors or other processes not examined in these studies, including genetic factors[83,186] and potential measurement error caused by ocular pigmentation.[119]

B. ASSOCIATION WITH CURRENT BLOOD PRESSURE

It has long been recognized that elevated blood pressure exerts profound effects on the retinal microcirculation. The impact of blood pressure on retinal arteriolar caliber, in particular, is strong and consistent, and seen in both adults[51,60,84,87,127,155,162,163,166,172] and children.[97]

The inverse relationship between higher blood pressure and retinal arteriolar caliber, as measured using new imaging methods, was initially reported in the ARIC study,[127] and has been subsequently confirmed across different study populations, including Beaver Dam,[166] Blue Mountains,[84] Cardiovascular Health Study,[162] MESA,[163] Rotterdam studies,[51] and in the diabetic population from the WESDR.[71] Importantly, this association has also been consistently shown in two populations of children aged 6–8 years. A higher blood pressure was associated with narrower retinal arteriolar caliber in this study, with each 10 mm Hg increase in mean arterial blood pressure associated with narrowing of the retinal arterioles by 2.0 to 2.4 μm.[97]

Whereas most earlier studies have used smaller AVR as the only measure of generalized retinal arteriolar narrowing, later studies evaluating retinal arteriolar and venular caliber separately in diabetic adults,[71] the non-diabetic general population,[51,161,163] and children[97] have confirmed the strong inverse association between elevated blood pressure and retinal arteriolar narrowing.

Recent data from the Blue Mountains Eye Study using the new approach to model retinal arteriolar and venular caliber simultaneously have clearly demonstrated that retinal venules tend to widen rather than narrow with increasing blood pressure.[57] Contrary to this finding, when no adjustment was made for retinal arteriolar caliber, some,[51,60,72,84,172] although not all,[161,163,166] studies suggested an inverse relationship between retinal venular caliber and blood pressure. This is another example that highlights the need of controlling for the confounding effect of the two vessel components. The finding of wider venular caliber with blood pressure is in line with the longitudinal data that have shown that wider retinal venular caliber predicts incident hypertension.[54,93]

C. ASSOCIATION WITH PAST BLOOD PRESSURE

A key issue is whether retinal vascular caliber changes are markers of cumulative, long-term blood pressure damage or only reflect a transient effect of acutely raised blood pressure. Several studies addressed this question by analyzing the association of retinal vascular caliber with both current and past blood pressure levels.[87,127,162] These studies found that both lower AVR[87,127,162] and narrowed retinal arteriolar caliber[87] were independently associated with past blood pressure levels, measured up to 10 years prior to the retinal assessment, suggesting that retinal arteriolar caliber changes reflect persistent damage from long-term hypertension.

The strength of the cross-sectional association between narrower retinal arteriolar caliber and elevated blood pressure has been shown to vary with age.[51,57,166] Both the Beaver Dam Eye and the Rotterdam studies have demonstrated that the impact of blood pressure is more dominant in younger participants and in arterioles than venules. After further adjusting for the confounding effect from retinal venular caliber, data from the Blue Mountains Eye Study provide more evidence of an interaction between age and blood pressure in their effects on retinal arteriolar caliber.[57] This might reflect the age-dependent progression in rigidity and sclerosis of arterioles that restricts their ability to adequately react to blood pressure changes in the elderly.[51,57,166]

Finally, there are reports that retinal arteriolar narrowing may be related to marker of chronic hypertensive damage, such as increased aortic stiffness,[15] left ventricular hypertrophy and left ventricular remodeling.[12]

D. ASSOCIATION WITH FUTURE BLOOD PRESSURE

Longitudinal data from four population-based studies have demonstrated that smaller retinal arteriolar caliber (and smaller AVR) precedes the

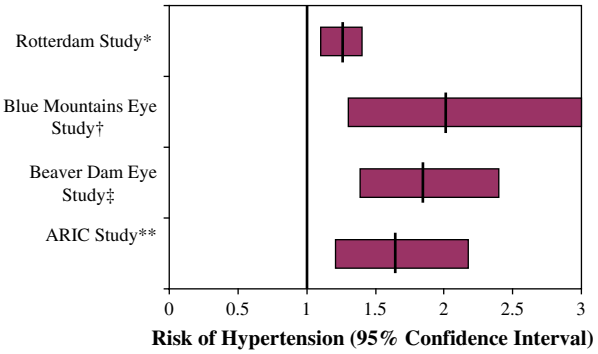

Fig 3. Retinal AVR and incidence of hypertension. *Rotterdam Study: n = 1,900, aged 55 yr or older, follow-up for 6.6 yr. †Blue Mountains Eye Study: n = 1,319, aged 54 yr or older, follow-up for 5 yr. ‡Beaver Dam Eye Study: n = 2,451, aged 43–84 yr, follow-up for 10 yr. **ARIC Study: n = 5,628, aged 49-73 yr, follow-up for 3 yr.

clinical stage of hypertension and predict the development of hypertension in initially normotensive individuals (Fig. 3).[55,132,170,183] The ARIC study first reported prospectively that normotensive participants who had lower AVR at baseline were 60% more likely to be diagnosed with hypertension within a 3-year period than participants with a high AVR.[170] The association was independent of pre-existing blood pressure levels, body mass index, and other known hypertension risk factors. Prospective data from the Beaver Dam, Blue Mountains, and Rotterdam cohorts have shown almost identical results,[55,93,132,183] providing strong evidence that smaller arteriolar caliber and AVR are pre-clinical markers of hypertension.

The Rotterdam study initially reported that retinal venular narrowing may predict incident hypertension.[55] However, after adjusting for the confounding effect of retinal arteriolar caliber, both the Blue Mountains and Rotterdam studies demonstrated that wider, instead of narrower, retinal venular caliber is associated with incident hypertension.[54,93] Results from the re-analysis of these data were biologically plausible and consistent with previous study regarding the pathophysiological changes of the microcirculation reflecting hypertensive damage.[101,124] These data suggest that narrower retinal arteriolar caliber, smaller AVR and larger venular caliber may serve as pre-clinical markers for hypertension.[178]

E. ASSOCIATION WITH HYPERGLYCEMIA AND DIABETES MELLITUS

Microvascular disease has long been suggested to be an early pathogenic features of diabetes mellitus,[46,144] and this is further supported by evidence from recent population-based studies on retinal vascular caliber. These studies show a strong association of higher fasting glucose level and diabetes with larger retinal venular caliber and, less consistently, with larger arteriolar caliber.[161] Recent analysis from MESA showed that although wider retinal venular caliber was related to diabetes and higher levels of serum glucose, larger arteriolar caliber was only associated with glucose.[163] In people with type 1 diabetes in the WESDR, higher glycosylated hemoglobin was associated with both wider retinal arteriolar and venular caliber.[71] The Blue Mountains Eye Study reported that participants with diabetes and retinopathy have wider retinal venular caliber than diabetic participants without retinopathy and non-diabetic participants.[62]

Longitudinal data from both the ARIC[173] and Beaver Dam Eye[182] studies initially reported that lower AVR was associated with risk of diabetes mellitus. Investigators in those studies suggest that this may reflect an association of narrower arteriolar caliber and risk of diabetes. Subsequently, the Rotterdam study found that both lower AVR and wider retinal venular caliber predict incident impaired fasting glucose, suggesting that the association of lower AVR with incident diabetes in other studies was largely explained by venular dilation.[52]

While the underlying mechanisms remain unclear, these findings are in keeping with clinical studies that have demonstrated that administration of intravenous dextrose can cause dilatation of retinal venules in normoglycemic patients.[33] In people with diabetes, changes in the retinal vasculature may also be due to the effect of diabetes on retinal blood flow and vascular diameters.[38,130] Moreover, reduced vascular reactivity associated with endothelial dysfunction and inflammatory processes may also play a role in the development of wider retinal venules in people with impaired glucose and diabetes.[66]

F. ASSOCIATION WITH DYSLIPIDEMIA

Elevated serum lipid is a known risk factor for diabetic retinopathy.[19,65] Data suggest dyslipidemia may also have an effect on retinal vascular caliber size in the general population. Initial studies focusing on the AVR did not find consistent patterns of associations. The ARIC Study reported that AVR was not related to either plasma total cholesterol or low-density lipoprotein (LDL) cholesterol,[74] which are strong risk factors for large vessel atherosclerosis.[24,94,117] The Rotterdam study found that lower AVR was related to lower high-density lipoprotein (HDL)-cholesterol levels,[51] but not other lipids, and the Cardiovascular Health Study[172] found no association between lower AVR and any measures of lipids.

Studies investigating individual retinal caliber provide a clearer picture that dyslipidemia is associated with larger retinal venular caliber. For

example, larger retinal venular caliber was related to higher triglyceride levels in the ARIC[161] and MESA studies,[163] and with lower HDL cholesterol levels in the Blue Mountains,[86] MESA,[163] and Rotterdam studies.[51] We also found larger retinal venular was associated with elevated LDL cholesterol level in the MESA study,[163] although this has not been confirmed in the Blue Mountains Eye study.[86] It has also been suggested that these relationships might involve inflammation factors and endothelial dysfunction.[51,66,74] These data provide further support for a potentially beneficial effect of therapeutic agent such as statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) on the retinal vasculature, possibly due to its impact on lowering LDL cholesterol levels,[82] an additional effect on prevention of inflammation and endothelial dysfunction,[36,126] increasing retinal blood flow and dilating isolated retinal arterioles.[99,102]

G. ASSOCIATION WITH OBESITY

Obesity may have profound ocular effects,[18] and appears influence changes in both arterioles and venules. An association of retinal arteriolar caliber narrowing with obesity has been reported in four adult populations,[51,161,163] and in children.[140] Both cross-sectional and prospective studies have shown that obesity is also associated with larger retinal venular caliber and lower AVR. These associations are seen in both the general population[51,156,161,163] and in persons with diabetes.[71,72] Prospective data from the Blue Mountains Eye Study demonstrated that larger retinal venular caliber at baseline predicted the incidence of obesity at the 5-year follow-up, suggesting a possible role of microvascular structural change in the pathogenesis of weight gain.[156] These findings are in keeping with data from the cohort of young healthy children,[14,140] in whom the confounding effect of systemic and ocular disease processes is of less concern. These later studies in young children suggest that the adverse effect of obesity on the long-term risk of cardiovascular diseases may begin in early life.[14,140]

H. ASSOCIATION WITH DIRECT MEASURE OF ATHEROSCLEROSIS

There are no consistent links between retinal vascular caliber and direct measures of atherosclerosis. In the ARIC study, smaller AVR was associated with carotid artery plaque and carotid arterial stiffness, but not with carotid intima-media thickness.[74,89] In contrast, the Rotterdam study found that a lower AVR was related to higher carotid intima-media thickness and increased carotid plaque score.[51] The Cardiovascular Health Study reported no association between AVR and any direct measures of atherosclerosis.[172]

The Rotterdam study described independent associations of larger venular caliber with higher carotid plaque-score and increased levels of aortic calcification.[51] Smaller arteriolar diameters were associated with increased carotid intima-media thickness in the same study. The Hoorn study reported that the relationship between wider venular caliber and carotid intima-media thickness become non-significant after controlling for cardiovascular risk factors,[149] suggesting the associations may simply reflect shared cardiovascular risk factors.

I. ASSOCIATION WITH INFLAMMATION AND ENDOTHELIAL DYSFUNCTION

Inflammation and/or endothelial dysfunction are now considered as major underlying mechanisms of both large and small vessel diseases.[121] Data from several population-based studies show that systemic inflammatory markers (e.g., C-reactive protein, white blood cell counts, interleukin 6) are associated with larger retinal venules (and, in some studies, with smaller AVR).[26,51,66,74,163] The Rotterdam study showed that larger retinal venular caliber was associated with higher levels of white blood cell counts and erythropoietin sedimentation rate.[51] The MESA found association of larger venular caliber with C-reactive protein, plasma fibrinogen, and interleukin 6, independent of age, cigarette smoking, lipids, and other factors.[163] These data add to the observations of larger retinal venular caliber with conditions related to inflammation, such as the metabolic syndrome[161] and obesity.[156]

The association of endothelial dysfunction and retinal vascular caliber is less consistent. In MESA, larger venular caliber was associated with some systemic markers of endothelial dysfunction (e.g., soluble intercellular adhesion molecule-1),[163] but this association was not apparent in the Beaver Dam, ARIC, Rotterdam, or Hoorn studies.[51,66,74,149] Differences in the distribution of cardiovascular risk factors among different study populations may explain some of the inconsistencies.

In summary, the evidence to date suggests that inflammation, and possibly endothelial dysfunction, may affect retinal venular caliber size.

J. ASSOCIATION WITH STROKE AND SUBCLINICAL CEREBROVASCULAR DISEASE

The retinal and cerebral microcirculation share similar anatomical, physiological, and embryological characteristics.[159,167] Disease of small arteries and arterioles has been hypothesized to be a major risk factor for stroke, and may also be the underlying

cause of magnetic resonance imaging (MRI)-defined white matter lesions.[37] White matter lesions, for example, are associated with risk of stroke independent of established risk factors.[169] Thus, there is a strong biological rationale for studying retinal vessels to understand cerebral microvascular diseases.

Data from the ARIC study reported that individuals with a smaller AVR tend to have more white matter lesions,[169] MRI cerebral infarcts,[21] and an increased risk of incident clinical stroke.[165] However, the link between smaller AVR with incident stroke has not been confirmed in the Blue Mountains Eye Study.[98]

Prospective data from both the Rotterdam and Cardiovascular Health studies have now demonstrated consistently that larger retinal venular caliber is associated with an increased risk of clinical stroke.[49,164] The Rotterdam study has further shown that wider venular caliber is also associated with cerebral infarction,[49] MRI-defined white matter lesions, and lacunar infarction.[50] In contrast, no association was observed between arteriolar caliber and incident stroke in the Cardiovascular Health Study,[164] or with progression of cerebral small vessel disease in the Rotterdam study.[50] In summary, there is considerable evidence supporting an association of altered retinal vascular caliber with both clinical and sub-clinical stroke and a variety of cerebrovascular conditions.

K. ASSOCIATION WITH CORONARY HEART DISEASE AND CARDIOVASCULAR MORTALITY

There is also good evidence from prospective studies regarding an association of retinal vascular caliber with coronary heart disease. Prospective data from the ARIC study suggested that lower AVR was associated with incident coronary heart disease, which was stronger in women than men.[171] The Cardiovascular Health Study demonstrated that both smaller retinal arteriolar caliber and larger venular caliber, which contributed to a lower AVR, were independently associated with 5-year risk of coronary heart disease in elderly persons.[164]

In the Beaver Dam Eye Study, a smaller AVR was related to cardiovascular mortality only in younger (aged 43–74 years) but not older (75–84 years) participants,[168] whereas no relationship was found of lower AVR with all-cause mortality, or with vascular-disease-related and non-vascular-disease-related mortality.[176] The Blue Mountains Eye Study reported that larger retinal venular caliber was associated with risk of coronary heart disease mortality; again, this association was seen in younger (49–75 years) but not older (>75 years) participants.[154]

More recently, pooled analysis of data from participants of both the Beaver Dam and Blue Mountains Eye studies provided further support to the hypothesis that both smaller arteriole and larger venules predicted the future risk of cardiovascular death.[153] The stronger and more consistent associations found in the younger but not in the older persons in both the Beaver Dam[168] and Blue Mountains[154] Eye studies may partly reflect a higher prevalence of comorbid condition in the older people, which may lead to cause-of-death misreporting, and therefore increase the likelihood of non-differential misclassification of cardiovascular mortality in this age group. Interestingly, the Blue Mountains Eye Study also demonstrated gender difference showing strengthened association in women aged 49–75 years, with smaller AVR and smaller retinal arterioles being associated with coronary heart disease death.[154]

In individuals with type 1 diabetes, lower AVR was associated with an increased coronary heart disease death in the WESDR, but the association may have been confounded by other complications of diabetes.[64] New prospective data from the WESDR have further confirmed that both smaller retinal arterioles and larger venules predicted 22-year stroke mortality in people with type 2 diabetes.[70]

L. ASSOCIATION WITH PROTEINURIA AND RENAL DYSFUNCTION

Microvascular damage has been postulated to represent one of the earliest pathological changes and a key pathogenesis mechanism in the development of kidney dysfunction.[131]

Cross-sectional data from the ARIC study have suggested that lower AVR is associated with a greater 6-year change in serum creatinine level but not related to the development of renal insufficiency.[160] The analysis of separate components of AVR indicated that both narrowing of arteriolar and venular caliber was related to the change in serum creatinine levels, and was similar in participants with and without diabetes.[160] There have been further support on the association of smaller retinal arteriolar caliber with prevalent gross proteinuria in type 1 diabetic patients.[71] However, the cross-sectional nature of these analyses does not provide any insights regarding antecedent-consequent associations. Furthermore, selection biases, including selective mortality, may have obscured or attenuated some relevant associations.[160] In addition, such an association has not been reported consistently by other studies.[32]

There are few prospective studies evaluating the link between retinal vascular caliber and renal dysfunction.

The WESDR demonstrated that larger retinal venular caliber independently predicted 16-year incidence of proteinuria and renal impairment in individuals with type 1 diabetes.[181] New analyses from WESDR have further shown that larger retinal venular caliber precede the subsequent development of diabetic nephropathy in type 2 diabetes.[70] While the significance of the association in the general population remains to be determined, longitudinal studies suggests that in persons with diabetes, larger retinal venular caliber may be a pre-clinical marker of nephropathy and renal dysfunction.

V. Environmental Risk Factors and Retinal Vascular Caliber

A. ASSOCIATION WITH CIGARETTE SMOKING

Cigarette smoking is associated with multiple adverse ocular effects, including risk of age-related macular degeneration and cataract.[63,73] In the ARIC study, among non-diabetic middle-aged persons, lower AVR was associated with cigarette smoking.[74] More recent data from the Rotterdam Study demonstrated that both venular and arteriolar calibers were larger in current cigarette smokers and this effect was greater on venules.[51] In the WESDR of type 1 diabetic population, current cigarette smoking was also associated with both larger retinal arteriolar and venular caliber, but not with the AVR.[71] These population-based studies are in good agreement with clinical observations that shows cigarette smokers have dilated retinal venules.[56,120,133] However, the significance of this association needs future investigation from longitudinal studies.

B. ASSOCIATION WITH ALCOHOL CONSUMPTION

Microvascular changes, as represented by the variation of retinal vascular caliber, play an important role in the pathogenesis of cardiovascular diseases. Alcohol drinking has been actively investigated for its association with cardiovascular diseases, but only a few studies have examined the association of alcohol drinking with retinal vascular caliber.

Lower AVR was significantly associated with alcohol consumption in the ARIC study.[74] Cross-sectional data from the MESA indicated that current alcohol consumption was related to smaller retinal arteriolar caliber[163] but had no impact on retinal venular caliber. The Rotterdam study found that alcohol consumption did not affect retinal arteriolar or venular caliber but that the AVR was lower in those who drink over 20 grams per day.[51] The exact mechanism of the effect of alcohol on the variation of retinal vascular caliber, however, remains to be elucidated.

C. ASSOCIATION WITH MEDICATION

The impact of specific medication use on the size of the retinal vascular caliber has been the focus of interest in some population-based studies.[58,175] The Beaver Dam Eye Study reported that anti-glaucoma medications, particularly topical beta-blocker therapy, were associated with narrowing of both retinal arteriolar and venular caliber.[175] Systemic beta-blockers, however, did not appear to affect retinal vascular caliber.

The Beaver Dam Eye Study further reported that current estrogen replacement therapy in women was associated with narrower retinal arteriolar and venular caliber, independent of blood pressure and other vascular risk factors.[174] In contrast, the Blue Mountains Eye Study found that hormone replacement therapy had little effect on retinal arteriolar and venular caliber but that it was associated with a lower AVR.[85] Longitudinal data from the Blue Mountains Eye Study demonstrated a non-significant trend toward narrower retinal arteriolar and venular caliber with increasing duration of hormone-replacement therapy in mid-to long-term users.[58] Interestingly, women receiving hormone-replacement therapy for longer than 10 years did not have narrower retinal vascular diameters, which may suggest a protective effect of hormone-replacement therapy for long-term users. These observations are compatible with clinical trials showing an increased risk of cardiovascular disease in women using hormone replacement therapy but an apparent beneficial effect observed in long-term users.[48,96] The mechanisms of a possible effect of hormone replacement therapy on retinal vascular caliber are yet to be fully examined.

A possible association between the combined use of aspirin and antihypertensive agent(s) and wider retinal arteriolar caliber has also been reported from the Blue Mountains Eye Study.[90] This may reflect the anti-inflammatory effects of aspirin on the microvasculature.

VI. Genetic Determinants of Retinal Vascular Caliber

A. FAMILIAL AGGREGATION STUDY

Familial aggregation is a key feature of almost all human traits and diseases, including retinal vascular caliber. In the Beaver Dam Eye Study, retinal arteriolar and venular caliber were more correlated in relatives than in unrelated individuals.[83] The sibling, parent–child, avuncular, cousin, and spousal

correlations for retinal venular caliber were 0.23, 0.24, 0.13, 0.08, and 0.03, respectively. The observed correlation patterns were similar for AVR in this study, which were consistent with a genetic influence showing similar parent–child and sibling correlations (who share 50% of their genes), about half the parent–child correlations for avuncular correlations (25% of their genes) and about half again for the cousin correlations (12.5% of their genes).

This study assumed that all the variability of retinal vascular caliber was due to genes and unshared effects, and that there were no gene–gene interactions. Under these assumptions, heritability was estimated by doubling the parent–child correlations and reflected the proportion of the overall population variation that can be attributable to genetic differences. The heritability of the retinal arteriolar caliber, venular caliber, and the AVR were 0.48, 0.54, and 0.32, respectively.[83]

Higher sibling correlation than parent–child correlation and similar cousin and avuncular correlations were observed for retinal arteriolar caliber. This supports population-based studies that show different systemic and environmental factors may influence retinal arteriolar and venular caliber. Generation effects may also cause sibling and cousin correlations to be higher or lower than expected.

In the Beaver Dam Eye study, the correlation pattern was similar after adjustment for age, sex, blood pressure, diabetes, refractive error, and smoking status. However, the effect of shared but unmeasured environmental factors, which were not considered in this study, may still contribute to the trait variability. Therefore, similarity of retinal vascular caliber observed between relatives in this study could be from genetic influences, but could also be due to shared environment.

B. TWIN STUDY

A twin study provides a stronger way to examine the relative influence of genetic and environmental risk factors on the variation of retinal vascular caliber than a family study. Genetic predisposition is confounded in studies of familial correlations of natural families due to the difficulty in distinguishing between genetic and shared familial environmental influences on the trait. Additional advantages of twin studies include the ability to assess the relative influence of genetics and environment as well as a lower susceptibility to cohort effects. Some twin studies have the limitation, however, that they are restricted to healthy subjects and have little variation in parameters of interest in relation to disease, notably blood pressure and blood glucose.

Monozygotic and dizygotic twin pairs share environment to the same degree, and therefore any greater phenotypic similarity among monozygotic than dizygotic twins is solely due to the twofold greater genetic similarities.[143] Under the assumption of equal environment influence, the classic twin study becomes a powerful tool to establish the role of genetic and environmental influence on the retinal vascular caliber variability.

A twin study of retinal vascular caliber from the Danish twin registry provides further support for a genetic influence on the development of retinal vascular caliber.[138] This twin study reported that the heritabilities (95% confidence interval) of retinal arteriolar and venular calibers were 70% (54–80%) and 83% (73–89%), respectively. Results from the twin study also suggested that retinal vascular caliber and its associated variations with the development of systemic diseases may be primarily determined by genetic influence rather than a reflection of the variation in blood pressure. Mean arterial blood pressure was also found to be governed mainly by genetic factors, with a heritability of 61%, when controlling for sex and age, in a study population of healthy subjects.[138] The sample size may have been smaller than in some other twin studies. Nevertheless, the narrow confidence intervals supported that the study was adequately powered.

C. GENOME-WIDE LINKAGE STUDY

Linkage studies have the advantage of searching the whole genome in an unbiased manner without presupposing the involvement of particular genes. Linkage is the tendency of two gene markers to be inherited together within families as a consequence of their physical proximity on the same chromosome. Such cosegregating haplotypes are broken up by the process of recombination. The probability of a recombination between two loci becomes less likely if they are very close; conversely, recombination occurs more frequently for genes that are at a larger distance from each other. The aim of linkage studies is to determinate whether or not a genetic marker of known location is in close proximity to the hypothesized gene that is associated with the susceptibility of the trait variability.[189]

A genome-wide linkage study for retinal vascular caliber using data from the Beaver Dam Eye Study included 1,762 individuals from 486 families consisting of 812 sib-pairs.[186] This investigation reported the heritability for covariate-adjusted retinal arteriolar and venular caliber as 0.51 and 0.48, respectively. In this study, retinal arteriolar and venular caliber was linked not only to several shared susceptibility loci but also to specific genetic loci,

providing strong evidence that shared susceptibility loci may predispose the development of arteriolar and venular caliber and some distinct gene(s) are likely to determine the size of arteriolar and venular caliber in part. These data further confirmed the notion that retinal arteriolar and venular caliber might be influenced by different systemic conditions and environmental risk factors. The study also found linkage regions for retinal vascular caliber overlapped with regions that have been previously associated with essential hypertension, the eNOS-related pathway, coronary heart disease, and vasculogenesis[186]

Association studies are more powerful than linkage studies for the identification of genes contributing to a complex trait such as retinal vascular caliber.[118] Unrelated cases and controls are genotyped for a high number of genetic markers across the entire genome (genome-wide approach) or in particular genes of interest (candidate gene approach). However, the exact gene(s) associated with retinal vascular caliber have not been identified.[157]

Few studies have investigated the association between retinal vascular caliber and the potential candidate genes. Both the ARIC[91] and Cardiovascular Health Study[135] provide little evidence of an association between the polymorphisms of apolipoprotein E (APOE) gene and retinal vascular caliber. This is consistent with the genome-wide linkage analysis which did not detect a link between the APOE gene and retinal vascular caliber.[186] New data from the Rotterdam study suggested that complement factor H gene polymorphism, which plays a key genetic role in age-related macular degeneration, had no effect on retinal vascular caliber.[25]

Although significant linkage of several genetic loci to retinal vascular caliber from the genome-wide linkage study in Beaver Dam have been found,[186] specific genes have yet to be confirmed in other studies. Additional genetic association studies are clearly needed to elucidate the important genetic contribution to the variation of retinal vascular caliber. It will further the understanding of genetic influences and effects on both retinal diseases (e.g., diabetic retinopathy,[62] age-related macular degeneration[53]) and cardiovascular diseases and its risk factors (e.g., hypertension). It may also reveal whether retinal vascular calibers are intermediate phenotypes or biomarkers of some systemic conditions. Given the extensive recent success of genome-wide association studies in identifying known and novel genes associated with complex diseases,[142] we can expect to see genes associated with retinal vascular caliber in the near future.

VII. Conclusions and Further Research

This review demonstrates new association of retinal vascular caliber changes and the research value and potential clinical importance of retinal imaging techniques in advancing knowledge of the complex genetic-environmental interaction pathway involved in the pathogenesis of a range of vascular diseases. Data from new studies suggest the following.

First, retinal arteriolar and venular calibers seem to reflect different pathophysiological processes. Smaller arteriolar caliber (and/or AVR) is strongly associated with current, past, and future blood pressure levels, and even blood pressure levels in childhood. In contrast, wider retinal venular caliber is associated with hyperglycemia and measures of obesity and inflammation, and has been further shown to predict incident coronary heart disease and incident hypertension, independent of cardiovascular risk factors.

Second, both arteriolar and venular calibers are associated with clinical outcomes such as stroke and coronary heart disease, independent of blood pressure and other cardiovascular risk factors. Smaller arteriolar caliber and smaller AVR predict clinical stroke and, in people with diabetes, coronary heart disease mortality. Wider retinal venular caliber and smaller AVR are associated with incident coronary heart disease. Wider retinal venular caliber in diabetic individuals may also predict incidence of gross proteinuria and renal impairment. Hence, retinal vascular caliber has great potential for risk prediction of coronary heart disease and stroke. However, the clinical utility of using retinal arteriolar and venular caliber in cardiovascular risk prediction requires further evaluation. Studies that examine the incremental predictive value of incorporating retinal vascular caliber measures into traditional cardiovascular prediction models (e.g., Framingham risk models) are clearly needed. Using such models to assess the predictive value of the retinal calibers by evaluating their effect on treatment strategies, as well as changes in calibration of the risk prediction models as recommended by Cook recently,[20] researchers can determine whether any increase in risk predicted by retinal vascular caliber changes would lead to a change in the recommended treatment strategy for cardiovascular diseases.

Third, findings from family, twin, and linkage studies investigating the genetic basis of retinal vascular caliber are promising. These initial data suggest that retinal vascular caliber may also be used as an intermediate phenotype for studying complex heterogenetic diseases such as hypertension, diabetes, and cardiovascular disease, given that retinal vascular caliber is independently associated with these conditions.

Intermediate phenotypes have the advantage of helping stratify the population into more homogeneous subsets, thereby increasing the power of genetic analysis. Furthermore, using retinal vascular caliber as an intermediate phenotype may provide insight into the biological function of genes.[1] In this regard, future studies should not only investigate genetic markers, but also more carefully evaluate gene-environment interaction. As previously emphasized,[157] developing maps by genome-wide scan in other study populations, fine mapping of candidate genes, and understanding specific biological function of the gene(s) will help in further elucidating the genetic basis of retinal vascular caliber.

In summary, a range of systemic and environmental risk factors, notably age, race/ethnicity, elevated blood pressure, elevated blood glucose levels, and cigarette smoking, have profound effects on the variation of the retinal vascular caliber size. However, a large proportion of the variation of retinal vascular caliber is still not explained by these established risk factors. Emerging evidence from family and twin studies suggests that inherited factors are also important sources of variation. Therefore, it is likely that genetic, systemic, and environmental risk factors may interact to contribute to variations in retinal vascular caliber. Determining these specific influences may allow greater understanding and possible prevention of complex polygenic human diseases.

Method of Literature Search

A systematic MEDLINE search on the ISI Web of Knowledge, Web of Science, and Google Scholar with coverage up to 28 July 2007 was performed initially using the following keywords: *retinal vascular caliber (75), retinal vessel diameters (169), retinal arteriolar caliber (36), retinal venular caliber (21), generalized retinal arteriolar narrowing (41), retinal arteriolar narrowing (132), retinal venular dilatation (7), retinal microvascular signs (131), retinal microvascular abnormalities (166), retinal arteriole (721), retinal venule (269), retinal arteriolar changes (142), arteriovenous ratio (98), retinal microcirculation (801).* A further search was then conducted using a combination of the keywords *pathophysiology (251), genetics (62),* and *aging (27)* with the various keywords initially used. The search concentrated on literature appearing from 1990 to 2007. From the list of abstracts identified, the original investigations and review articles were retrieved and reviewed. Bibliography of the retrieved articles was examined for additional relevant articles. All English articles were read, and for the relevant non-English articles, the English abstracts were reviewed.

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Outline

I. Introduction
II. Methodological issues in measuring retinal vascular caliber
III. Pathophysiology of retinal vascular caliber changes
  A. Mechanisms of retinal arteriolar caliber changes
  B. Mechanisms of retinal venular caliber changes
IV. Systemic factors and retinal vascular caliber
  A. Association with age, sex, and race/ethnicity
  B. Association with current blood pressure
  C. Association with past blood pressure
  D. Association with future blood pressure
  E. Association with hyperglycemia and diabetes mellitus
  F. Association with dyslipidemia
  G. Association with obesity
  H. Association with direct measure of atherosclerosis
  I. Association with inflammation and endothelial dysfunction
  J. Association with stroke and sub-clinical cerebrovascular disease
  K. Association with coronary heart disease and cardiovascular mortality
  L. Association with proteinuria and renal dysfunction
V. Environmental risk factors and retinal vascular caliber
  A. Association with cigarette smoking
  B. Association with alcohol consumption
  C. Association with medication
VI. Genetic determinants of retinal vascular caliber
  A. Familial aggregation study
  B. Twin study
  C. Genome-wide linkage study
VII. Conclusions and further research