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Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations

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Aims The retinal microvasculature may reflect pre-clinical changes in the cerebral and coronary microcirculations. We assessed whether smaller retinal arterioles and larger venules predicted coronary heart disease (CHD)- and stroke-mortality.

Methods and results We pooled data from the Beaver Dam Eye Study ($n = 4926$, aged 43–86) and the Blue Mountains Eye Study ($n = 3654$, aged 49–97). Retinal vessel diameters were measured from digitized retinal photographs. Change point models were used to assess and document the existence of threshold effects. We defined smaller arterioles as diameters within the narrowest quintile and larger venules as diameters within the widest quintile, with other quintiles as the reference. Of 8550 participants, 7494 (88%) with complete data were included, of whom 653 died from CHD and 299 from stroke over 10–12 years follow-up. After multivariable adjustment, each standard deviation (SD) increase in arteriolar diameter, or SD decrease in venular diameter, was not found to be significantly associated with either CHD-mortality or stroke-mortality. However, smaller arterioles [hazard ratio (HR) 1.34, 95% confidence interval (CI) 1.11–1.62] and larger venules (HR 1.24, CI 1.02–1.52), predicted increased risk of CHD-mortality. These associations were mainly evident among persons aged 43–69 (smaller arterioles: HR 1.70, CI 1.27–2.28; larger venules: HR 1.41, CI 1.06–1.89). Smaller arterioles (HR 1.64, CI 1.00–2.67) and larger venules (HR 1.53, CI 0.94–2.47) were also associated with an increased risk of stroke-mortality among persons aged 43–69.

Conclusion Retinal vessel diameter may predict risk of CHD and stroke deaths in middle-aged persons.

Introduction

The retina offers a readily accessible site to non-invasively evaluate the microcirculation.[1] Subtle changes in the retinal vasculature may mirror pre-clinical structural changes in the cerebral[2] and coronary microcirculations,[3] and may therefore carry prognostic information useful for predicting clinical cardiovascular events.

A number of studies have recently shown that subtle changes in retinal vessel diameter predict risk of clinical coronary heart disease (CHD),[3–6] sub-clinical and clinical stroke, and stroke mortality,[7–10] independent of traditional risk factors. Despite these data, there is an incomplete understanding of the relationship between retinal vessel diameter and cardiovascular events, with inconsistent findings in the current literature.[4,6,7,11,12] In the Rotterdam Study,[8] for example, larger venular diameter was associated with a high risk of stroke, but no similar association of arteriolar diameter was found. Data from the Beaver Dam Eye Study in WI, USA[12] showed that neither smaller arteriolar or larger venular diameter was associated with CHD and stroke mortality, although an earlier nested case–control study from that study population found an association between smaller arteriole-to-venule ratio and cardiovascular mortality[6] in participants aged 43–74. The Blue Mountains Eye Study in Australia reported that larger retinal venular diameter was associated with a higher risk of CHD in men and women aged 75 or younger, and smaller retinal arteriolar diameter was associated with an increased risk of CHD in women but not in men of similar age,[13] and no association of arteriole-to-venule ratio was found for stroke and stroke mortality.[11] Additionally, the relationship between retinal vessel diameter and cardiovascular disease in different sub-groups of the population (e.g. older and younger persons, males and females, persons with and without hypertension) has not been well examined, as most previous studies lacked adequate power for these

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analyses. Therefore, while promising, the value of an assessment of retinal vessel diameter for cardiovascular risk prediction remains uncertain.

To address these uncertainties, we pooled data from two population-based cohorts from the US and Australia to assess the relationship of retinal vessel diameter and long-term CHD and stroke mortality. In particular, we aimed to assess consistency of associations across the two different cohorts, and in subgroups stratified by age, sex, and hypertension. On the basis of pre-existing literature, we hypothesized that small retinal arteriolar diameter and larger retinal venular diameter would be related to cardiovascular deaths, and that this relationship would be stronger in younger people (i.e. age $<$70 years),[6,13–17] in women,[4,13] and in persons without a major competing cardiovascular risk factor such as hypertension.[13]

Methods

The two cohorts for this study have been previously described.[18] The baseline examination of the US cohort was conducted from 1988 to 1990 in Beaver Dam, WI, USA; 4926 (83.1%) persons, 43–86 years of age, of 5924 eligible residents participated. The baseline examination of the Australian cohort was conducted from 1992 to 1994 in the Blue Mountains region, west of Sydney, Australia; 3654 (82.4%) of 4433 eligible residents $\geq$49 years participated. Both studies, approved by their respective university Human Research Ethics Committees, were conducted in adherence with the Declaration of Helsinki. Signed informed consent was obtained from all participants.

At baseline, all participants had stereoscopic retinal photographs taken of both eyes using identical protocols and retinal cameras (Zeiss FF3, Oberkochen, Germany). Photographs were obtained of at least one eye in 98% of participants (US cohort, 4829/4926; Australian cohort, 3583/3654) and complete data were available for 7494 persons (88% of 8550 participants).

Detailed retinal grading methods were described previously,[19,20] and were identical between the two studies.[19,20] In brief, we used a validated computer-assisted method to measure the internal diameter of retinal arterioles and venules from all gradable digitized retinal images (*Figure 1*), which were then summarized using the Knudtson formula.[21] This formula allowed all measured vessels in an eye to be summarized as an index representing the central retinal arteriolar (CRAE) or venular (CRVE) equivalent of that eye. The intra- and inter-grader agreement was high, with quadratic weighted κ values ranging from 0.80 to 0.93 in the Australian cohort[19] and intra- and inter-grader intraclass-correlation coefficients of 0.78–0.99 in the US cohort.[6] As good correlation in the measurements between right and left eyes was reported previously,[6,20,22] we used the measurements of vessel diameters from one eye only. Generally, data from the right eye were used.

The census cut-off for cardiovascular deaths was 31 December 2002 in the US cohort and 31 December 2003 in the Australian cohort. Deaths and causes of death were obtained either from death certificates (US) or from the Australian National Death Index (NDI) (Australia). Causes of death in the NDI database were collected from death certificates and recorded using International Classification of Diseases (ICD) codes. Stroke death (thrombotic, haemorrhagic) included the following codes from ICD-9 (430.0–438.9) and ICD-10 (I60.0–I69.9). CHD death included the following codes from ICD-9 (410.0–9, 411.0–8, 412, 414.0–9) and ICD-10 (I21.0–9, I22.0–9, I23.0–8, I24.0–9, I25.0–9). The sensitivity and specificity of Australian NDI data has been estimated to be 93.7 and 100% for all-cause deaths, and 92.5 and 89.6% for cardiovascular disease deaths.[23,24] The validity of death certificates has also been reported previously,[25–27] with the sensitivities for CHD death ranging from 81 to 91%, and corresponding specificities ranging from 72 to 86%. Validity data on stroke deaths was only reported on deaths which occurred in 1970s and 1980s,[28] prior to the widespread use of computerized tomography, and the reported sensitivities and specificities at the time were 63–70% and 90–100%, respectively.

In both countries, deaths and cause(s) of death are confirmed by medical certifiers, which include the physician in attendance, coroner or medical examiner, regardless if the death occurred in a hospital or in the community. If the patient had a recent stroke or CHD event, this event is routinely included in the causes of death, although it may not be the primary cause. We used any mention of stroke or CHD events from the causes of death to include deaths from complications secondary to these events.

Unless otherwise stated, measurement techniques and cut-points were the same for the two studies. Baseline systolic and diastolic blood pressures (SBP, DBP) were recorded. Hypertension was defined as SBP $\geq$140 mmHg, DBP $\geq$90 mmHg or use of antihypertensive medications. Height and weight were measured with participants wearing no shoes and light clothing. Body mass index (BMI) was defined as weight in kilograms divided by the square of height in metres. Current smokers were defined from questionnaire responses. Diabetes was defined as a previous history of diabetes or hyperglycaemia on objective testing. Hyperglycaemia was defined as glycosylated haemoglobin $>$2 standard deviations (SDs) above the mean for the appropriate age and sex group or a casual blood glucose level $\geq$200 mg/dL (11.1 mmol/L) (US), or a fasting blood glucose $\geq$7.0 mmol/L (Australia). Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured from casual (US) or fasting blood specimens (Australia) using standard procedures. White blood cell count (WCC) count was determined using a Coulter counter method. We obtained self-reported history of angina, acute myocardial infarction (AMI), and stroke from face-to-face interviews.

Associations of retinal vessel diameter with CHD and stroke deaths were assessed initially as a continuous variable (per SD decrease in arteriolar diameter or increase in venular diameter). We also used change point (spline) models to determine whether a threshold effect is evident in the associations between retinal vascular diameter (CRAE, CRVE) and mortality (CHD, stroke),[29,30] using a macro program written in Statistical Analysis System (SAS, V9, Cary, NC). The models for CRAE assumed a constant mortality above the change point and a linear association below it. The models for CRVE assumed a constant mortality below the change point and linear association above it. The 95% confidence intervals (CIs) were determined using the bootstrap technique with 1000 replications[29,31].

We constructed two nested models, one with the linear term and the other with the change point (spline), and compared the log-likelihood ratios of these two nested models to test the threshold hypothesis. The log-likelihood ratios of the two models differed significantly ($P < 0.05$), indicating the existence of a threshold (*Table 1*).

Subsequently, retinal vessel diameter was assessed as a binary variable, with smaller retinal arterioles defined as arteriolar diameter in the lowest quintile and larger venules defined as venular diameter in the highest quintile in the combined cohort and reference groups consisted of subjects in the remaining quintiles. These quintile cut-points approximated the thresholds detected by change point models.

To assess the relative value of vessel diameter while avoiding collinearity between arteriolar and venular diameters,[13] we adjusted arteriolar diameter for venular diameter, and venular diameter for arteriolar diameter, following the residual method suggested by Willett.[32]

Cox regression was used to estimate hazard ratios (HR) and 95% CI. We constructed two multivariable models; the first adjusting for age, gender, BMI, hypertension, diabetes, current smoking, serum total cholesterol, HDL cholesterol and WCC (multivariable adjusted Model 1); the second model further adjusted for a past history of stroke in the model assessing stroke death, and

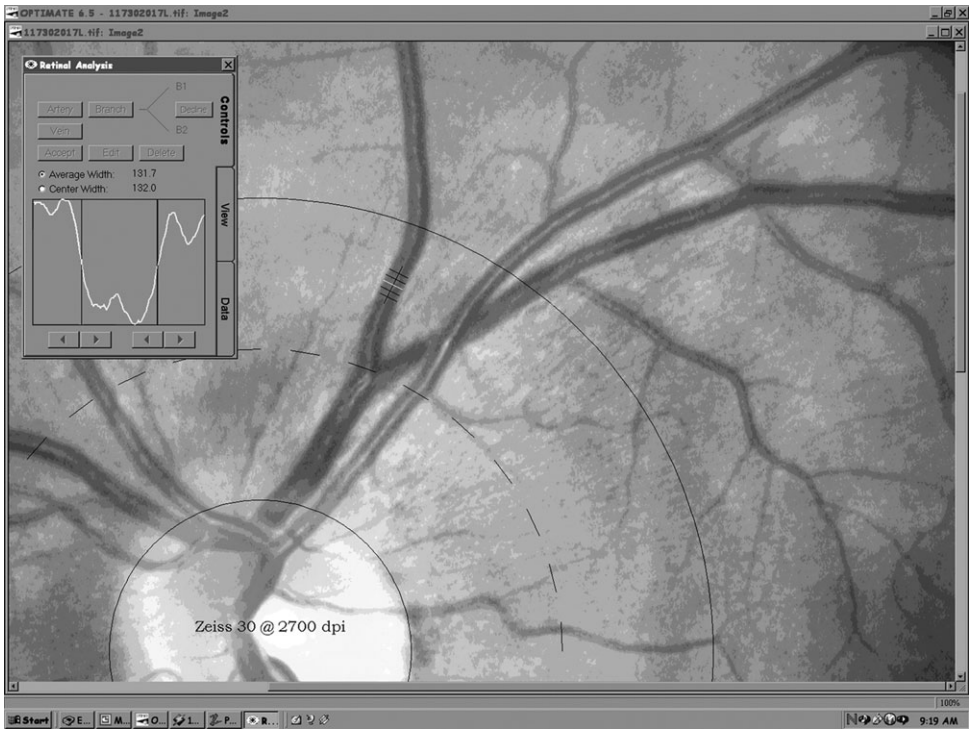

Figure 1 Digitized retinal image with grid defining the measurement areas (Zone B) 0.5–1 disc diameters from the disc margin. The five equidistant measurement markers and the pixel histogram are shown. The distance between the two vertical lines on the histogram represents the width of the vessel.

Table 1 Comparison of two nested models with Log-likelihood (LogL) ratio tests, one with the linear term and the other with the change point (spline)

	−2LogL for spline models	−2LogL for linear model	Log-likelihood ratio test	*P*-value
Coronary heart disease deaths				
CRAE	11334.48	11338.33	3.85	0.0408
CRVE	11320.05	11346.44	26.39	<0.0001
Stroke deaths				
CRAE	1416.58	1421.32	4.74	0.0245
CRVE	1410.35	1418.82	8.47	0.0036

CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent.

past history of angina and AMI in the model assessing CHD death (multivariable adjusted Model 2).

We used two methods to test the proportional hazards assumption with respect to the time period variable, namely visual inspection of the log cumulative hazard plots and adding time-dependent variables into the full Model 2. Age and study-site violated the proportional hazards assumption and hence were modelled as strata variables (in 10-year categories, and dichotomously, respectively, for age and study-site). We checked the assumption of linearity for continuous co-variables included in the model by entering the transformed variable in addition to the variable itself in the full Model 2. Natural logarithm and square transformations were used. A significant change in the −2 log-likelihood for any model was taken as a sign of non-linearity. Otherwise the linearity assumption was accepted. As all variables met the linearity assumption except for BMI, which we henceforth modelled categorically (<20, 20–25, 26–29, >= 30 kg/m^2).

We tested for heterogeneity between populations by entering interaction terms of a variable representing study-site and all variables in Models 1 and 2 in the combined data (i.e. arteriolar diameter, venular diameter, age, gender, BMI, etc.). As the interaction terms for study-site and diabetes, and study-site and cholesterol, were significant ($P < 0.05$), we adjusted for the heterogeneity from study-site in Cox regression models for combined data by the two site strata, estimating the HR from each study-site first, and then summarizing a weighted HR for the combined sample. There was no statistically significant heterogeneity between age-specific survival curves for retinal arteriolar and venular diameter in both populations.

We performed analyses in subgroups stratified by age, sex, and hypertension status. We chose an age cut-off of 70 years to ensure approximately equal numbers of stroke and CHD events in both age categories.

Results

Of the 8559 participants from the two study cohorts, there were 7494 subjects with complete data available for analysis, after excluding those with ungradable photographs ($n = 1036$) or unknown causes of death ($n = 29$). Those excluded were more likely to be older (mean age 69.8 vs. 63.0 years, $P < 0.0001$), female (59.7 vs. 55.9%, $P = 0.02$), more likely to have hypertension (68.0 vs. 58.3%, $P < 0.0001$) and diabetes (13.0 vs. 7.9%, $P < 0.0001$) and a history of angina (14.0 vs. 11.0%, $P = 0.005$), AMI (10.6 vs. 7.4%, $P = 0.0003$), and stroke (8.8 vs. 3.8%, $P < 0.0001$). Among the 7494 subjects, 653 had CHD deaths (US sample $n = 379$, Australian sample $n = 274$) and 299 had stroke deaths (US sample $n = 163$, Australian sample $n = 136$).

Table 2 shows the vascular risk factor distribution by the presence of smaller retinal arterioles or larger retinal venules at baseline of the combined cohort. Persons with smaller retinal arterioles were more likely to be older, male, have hypertension, and higher BMI. Persons with

Table 2 Baseline characteristics and retinal vessel diameter in combined Beaver Dam and Blue Mountains Eye Studies ($n = 7494$)

Baseline characteristics	Narrower retinal arterioles			Wider retinal venules		
	Present (1st quintile), $n = 1498$	Absent (2nd–5th quintiles), $n = 5996$	*P*-value	Present (5th quintile), $n = 1499$	Absent (1st–4th quintiles), $n = 5995$	*P*-value
Mean age in years ($\pm$standard deviation)	64.1 ($\pm$10.0)	62.6 ($\pm$10.5)	<0.0001	62.1 ($\pm$9.5)	63.1 ($\pm$10.6)	0.001
Mean body mass index in kg/m^2 ($\pm$standard deviation)	28.0 ($\pm$5.3)	27.6 ($\pm$5.1)	0.01	28.3 ($\pm$5.2)	27.5 ($\pm$5.2)	<0.0001
Mean white blood cell count, $\times 10^3/\mu$L ($\pm$standard deviation)	7.09 ($\pm$1.9)	7.00 ($\pm$2.0)	0.13	7.54 ($\pm$2.2)	6.88 ($\pm$1.9)	<0.0001
Total cholesterol, mmol/L ($\pm$standard deviation)	6.0 ($\pm$1.1)	6.0 ($\pm$1.1)	0.36	6.1 ($\pm$1.1)	6.0 ($\pm$1.1)	0.33
HDL cholesterol, mmol/L ($\pm$standard deviation)	1.38 ($\pm$0.46)	1.38 ($\pm$0.45)	0.35	1.33 ($\pm$0.44)	1.39 ($\pm$0.45)	<0.0001
Men (%)	793 (52.9)	2513 (41.9)	<0.0001	810 (54.0)	2496 (41.6)	<0.0001
Hypertension (%)	1090 (72.8)	3274 (54.7)	<0.0001	951 (63.6)	3413 (60.0)	<0.0001
Diabetes (%)	123 (8.2)	468 (7.8)	0.60	126 (8.4)	465 (7.8)	0.42
History of current smoking (%)	246 (16.9)	1063 (18.0)	0.33	441 (29.9)	868 (14.7)	<0.0001
Self-reported history of angina (%)	157 (10.6)	659 (11.1)	0.60	172 (11.6)	644 (10.9)	0.43
History of acute myocardial infarct (%)	133 (8.9)	422 (7.1)	0.01	144 (9.7)	411 (6.9)	0.0003
History of stroke (%)	81 (5.4)	201 (3.4)	0.0002	75 (5.0)	207 (3.5)	0.005

larger retinal venules were more likely to be older, male, have hypertension, higher BMI and WCC, and to be smokers.

Over a mean follow-up period of 10.9 years, interquartile range 10.4–13.4 years (mean 11.8 years, 11.5–13.9 years in US, and mean 9.7 years, 10.1–11.3 years in Australia), a total of 653 CHD-related deaths (379 in US, and 274 in Australia) and 299 stroke-related deaths (163 in US, and 136 in Australia) were recorded.

When assessing arteriolar and venular diameters continuously (per SD decrease in arteriolar diameter or increase in venular diameter), arteriolar or venular diameter was not found to be significantly associated with either CHD-mortality (*Table 3*) or stroke-mortality (*Table 4*) in either the combined population or individual study populations. Using change point models to assess the existence of threshold effects of retinal arteriolar or venular diameter on CHD- and stroke-mortality, we found that the quintile cut-offs we used to define smaller arterioles (<152.7 μm) and wider venules (>255.5 μm) were close to the estimated threshold cut-points and fell within the 95% CI. For example, the threshold cut-point for smaller retinal arterioles on CHD mortality was 168.2 μm (95% CI 137.4–183.2 μm), while for stroke mortality the threshold cut-point was at 159.2 μm (95% CI 136.6–181.5 μm). The threshold cut-point for larger venular diameter on CHD mortality was at 243.8 μm (95% CI 235.9–269.5 μm); while for stroke mortality it was 254.4 μm (95% CI 234.3–275.4 μm).

In persons aged 43–69, each SD increase in venular diameter was associated with an increased risk of CHD mortality (HR 1.26, 95% CI 1.14–1.39, per SD) and stroke mortality (HR 1.26, 95% CI 1.02–1.57) after adjusting for age, sex, and study-site. These associations were attenuated and became non-significant after full multivariable adjustment in Model 2 (HR 1.06, 95% CI 0.92–1.22 for CHD mortality and HR 1.07, 95% CI 0.85–1.34 for stroke mortality). We did not observe any association between per SD increase in arteriolar diameter and CHD or stroke mortality in persons aged 43–69. Neither arteriolar nor venular diameter, when assessed continuously, was associated with CHD or stroke mortality in persons aged 70 and above.

However, in dichotomous analyses, both smaller retinal arterioles (HR 1.34, 95% CI 1.11–1.62) and larger retinal venules (HR 1.24, 95% CI 1.02–1.52) predicted a significantly higher risk of CHD mortality, after adjusting for all co-variables in Model 2 (*Table 3*). In Model 2 for stroke mortality (adjusting for similar risk factors and a history of stroke), neither smaller retinal arteriolar nor larger venular diameter was associated with stroke mortality (*Table 4*). Findings by individual study were generally consistent (*Tables 3* and *4*), but were only significant in the US cohort (*Table 3*).

In subgroup analyses, both smaller arterioles and larger venules predicted an increased risk of CHD and stroke mortality in persons aged 43–69 (*Table 5*). These associations were not observed in persons aged 70 or older. In each of the two cohorts, smaller arterioles and larger venules were associated with CHD deaths only in persons aged 43–69, although separately the associations were significant in the US cohort (HR 1.70, 95% CI 1.20–2.42 and HR 1.44, 95% CI 1.02–2.02 for smaller arterioles and larger venules, respectively), but not significant in the Australian cohort (HR 1.49, 95% CI 0.88–2.54 and HR 1.23, 95% CI 0.70–2.15 for smaller arterioles and larger venules, respectively). Similarly, smaller arterioles and larger venules were non-significantly associated with stroke deaths in persons aged 49–69 in each of the two cohorts (US: HR 1.45, 95% CI 0.78–2.70 and HR 1.42, 95% CI 0.78–2.57 for smaller arterioles and larger venules, respectively; Australia: HR 2.03, 95% CI 0.88–4.72 and HR 1.75, 95% CI 0.75–4.07 for smaller arterioles and larger venules, respectively).

In gender subgroups, the associations between smaller arterioles and CHD mortality were similar in women and men, but the association between larger retinal venules

Table 3 Coronary heart disease deaths by presence or absence of smaller retinal arteriolar diameter and larger retinal venular diameter

Baseline retinal vessel diameter	Numbers at risk[a]	Numbers affected[a]	Hazard ratio (95% confidence interval) Age–sex adjusted	Multivariable adjusted Model 1[b]	Multivariable adjusted Model 2[c]
Smaller arteriolar diameter					
Combined Beaver Dam and Blue Mountains Eye Studies[d]					
Per standard deviation decrease			1.04 (0.96–1.13)	1.06 (0.98–1.15)	1.08 (0.99–1.17)
Absent	5996	491	1.0	1.0	1.0
Present[e]	1498	162	1.22 (1.01–1.46)	1.29 (1.07–1.56)	1.34 (1.11–1.62)
Beaver Dam Eye Study					
Per standard deviation decrease			1.04 (0.94–1.16)	1.05 (0.94–1.17)	1.08 (0.96–1.20)
Absent	3542	299	1.0	1.0	1.0
Present[e]	684	80	1.31 (1.02–1.68)	1.32 (1.03–1.70)	1.36 (1.06-1.75)
Blue Mountains Eye Study					
Per standard deviation decrease			1.04 (0.92–1.17)	1.09 (0.95–1.24)	1.07 (0.94–1.23)
Absent	2454	192	1.0	1.0	1.0
Present[e]	814	82	1.08 (0.83–1.40)	1.19 (0.89–1.59)	1.23 (0.92–1.64)
Larger venular diameter					
Combined Beaver Dam and Blue Mountains Eye Studies[d]					
Per standard deviation increase			1.09 (1.01–1.18)	1.03 (0.95–1.12)	1.04 (0.95–1.13)
Absent	5995	503	1.0	1.0	1.0
Present[e]	1494	150	1.35 (1.12–1.62)	1.23 (1.01–1.50)	1.24 (1.02–1.52)
Beaver Dam Eye Study					
Per standard deviation increase			1.11 (1.00–1.23)	1.03 (0.92–1.14)	1.03 (0.92–1.15)
Absent	3409	288	1.0	1.0	1.0
Present[e]	817	91	1.50 (1.18–1.90)	1.34 (1.05–1.72)	1.37 (1.07–1.75)
Blue Mountains Eye Study					
Per standard deviation increase			1.07 (0.95–1.20)	1.05 (0.92–1.20)	1.06 (0.93–1.21)
Absent	2586	215	1.0	1.0	1.0
Present[e]	682	59	1.14 (0.85–1.52)	1.03 (0.74–1.44)	1.02 (0.73–1.43)

[a]Over a mean follow-up period of 10.9 years (interquartile range 10.4–13.4 years) for the combined populations, 11.8 (11.5–13.9) years for Beaver Dam, and 9.7 (10.1–11.3) for Blue Mountains.

[b]Model 1, adjusted for age (as a strata variable—see text), sex, body mass index, presence of hypertension, diabetes, serum total cholesterol, high density lipoprotein cholesterol, white blood cell count, and current smoking status.

[c]Model 2, adjusted for all factors in Model 1 plus history of angina or acute myocardial infarct.

[d]Additional adjusting for study-site.

[e]Smaller arterioles defined as measurements within the narrowest quintile; larger venules as measurements within the widest quintile, with other quintiles as the reference group.

and CHD mortality was only evident in men but not in women (*Table 6*). The associations between smaller retinal arterioles and CHD mortality were similar for persons with and without hypertension (*Table 6*). The association between larger retinal venules and CHD mortality was only evident among persons with hypertension but not among persons without hypertension (*Table 6*).

Discussion

In pooled data from two population-based cohorts, we found a relatively consistent pattern of association between retinal vessel diameter and cardiovascular mortality. In persons aged 43–69, both smaller retinal arterioles (with a threshold at the smallest 20% of the population) and larger retinal venules (with a threshold at the largest 20% of the population) were significantly associated with a greater risk of CHD mortality, and were also marginally significantly associated with an increased risk of stroke mortality, independent of age, gender, and other vascular risk factors. These associations were not observed in persons aged 70 or older at baseline in both cohorts. We did not find a linear association of either vessel diameter with the mortality outcomes.

There are biologically plausible mechanisms supporting our study hypotheses.[33] Retinal arteriolar narrowing is a marker of chronic damage from elevated blood pressure, and is associated with current,[34–36] past, and future development of hypertension.[37–41] A consistent gradient of association between elevated blood pressure (BP) and retinal arteriolar narrowing has been shown in numerous studies.[34,36,37,42,43] Wider retinal venules have been associated with obesity,[44] the metabolic syndrome,[43,45] and other cardiovascular risk factors including systemic inflammatory markers and dyslipidaemia.[43] A recent study on genetic determinants of retinal vessel diameter showed that retinal arteriolar and venular diameter is linked to multiple gene loci which overlap with regions associated with essential hypertension, endothelial dysfunction, or vasculogenesis.[46,47] Our finding of independent associations between retinal vessel diameter and CHD death support the concept that retinal vessel diameter may reflect lifetime cumulative effects of various vascular processes on the microvasculature, and thus may be a novel biomarker for CHD risk.

Table 4 Stroke deaths by presence or absence of smaller retinal arteriolar diameter and larger retinal venular diameter

Baseline retinal vessel diameter	Numbers at risk[a]	Numbers affected[a]	Hazard ratio (95% confidence interval)		
			Age–sex adjusted	Multivariable adjusted Model 1[b]	Multivariable adjusted Model 2[c]
Smaller arteriolar diameter					
Combined Beaver Dam and Blue Mountains Eye Studies[d]					
Per standard deviation decrease			0.97 (0.86–1.09)	0.94 (0.83–1.06)	0.93 (0.82–1.05)
Absent	5996	228	1.0	1.0	1.0
Present[e]	1498	71	1.12 (0.86–1.48)	1.09 (0.82–1.47)	1.10 (0.82–1.47)
Beaver Dam Eye Study					
Per standard deviation decrease			0.88 (0.76–1.03)	0.89 (0.76–1.04)	0.89 (0.76–1.04)
Absent	3542	134	1.0	1.0	1.0
Present[e]	684	29	1.08 (0.72–1.62)	1.07 (0.71–1.61)	1.03 (0.85–1.26)
Blue Mountains Eye Study					
Per standard deviation decrease			1.09 (0.92–1.30)	1.05 (0.86–1.27)	0.97 (0.80–1.18)
Absent	2454	94	1.0	1.0	1.0
Present[e]	814	42	1.17 (0.81–1.70)	1.19 (0.78–1.81)	1.18 (0.77–1.80)
Larger venular diameter					
Combined Beaver Dam and Blue Mountains Eye Studies[d]					
Per standard deviation increase			0.99 (0.88–1.11)	0.94 (0.83–1.06)	0.92 (0.81–1.04)
Absent	5995	241	1.0	1.0	1.0
Present[e]	1499	58	1.13 (0.85–1.51)	1.09 (0.79–1.48)	1.09 (0.80–1.49)
Beaver Dam Eye Study					
Per standard deviation increase			0.95 (0.81–1.12)	0.93 (0.79–1.10)	0.92 (0.78–1.09)
Absent	3409	131	1.0	1.0	1.0
Present[e]	817	32	1.23 (0.83–1.81)	1.21 (0.81–1.82)	1.19 (0.80–1.78)
Blue Mountains Eye Study					
Per standard deviation increase			1.02 (0.86–1.20)	0.96 (0.80–1.16)	0.94 (0.77–1.14)
Absent	2586	110	1.0	1.0	1.0
Present[e]	682	26	1.03 (0.67–1.58)	0.98 (0.60–1.60)	0.97 (0.59–1.60)

[a]Over a mean follow-up period of 10.9 years (interquartile range 10.4–13.4 years) for the combined populations, 11.8 (11.5–13.9) years for Beaver Dam, and 9.7 (10.1–11.3) for Blue Mountains.

[b]Model 1, adjusted for age, sex, body mass index, presence of hypertension, diabetes, serum total cholesterol, high density lipoprotein cholesterol, white blood cell count, and current smoking status.

[c]Model 2, adjusted for all factors in Model 1 plus history of stroke.

[d]Additional adjusting for study-site.

[e]Smaller arterioles defined as measurements within the narrowest quintile; larger venules as measurements within the widest quintile, with other quintiles as the reference group.

The stronger association of retinal vessel diameter and risk of cardiovascular disease in younger, middle-aged people in the two cohorts is consistent with the general epidemiology of cardiovascular risk factors. For example, the predictive value of blood pressure and cholesterol for cardiovascular disease appears to be weaker in older when compared with younger people.[14–17] The lack of association of retinal vessel diameter and cardiovascular mortality in persons older than 70 years at baseline may reflect this phenomenon, which could be due to a number of factors. Firstly, increased arteriosclerosis and other age-related changes might reduce the ability of retinal vessel diameters to respond to blood pressure and other vascular risk factors. Secondly, other cardiovascular risk factors in older age might dominate vascular disease processes. Thirdly, poorer retinal image quality (due to lens opacity) in older persons, and the resulting measurement errors, could have contributed to the negative findings in the older group. Finally, selective survival could have also contributed to the negative findings in the older age group.

We show that larger retinal venular diameter was associated with CHD mortality in men but not in women. This contrasts with previous reports from the ARIC Study[4] that the ratio of the two diameters predicted stronger associations with CHD in women. Findings from our study are not directly comparable with the ARIC Study findings, however, as the ARIC Study investigated the association between the ratio of the two vessel calibres but not for individual calibres with CHD mortality. We also showed that the association of larger retinal venules and CHD mortality was only evident in subjects with hypertension. Larger venular diameter has been linked to several traditional CHD risk factors, namely smoking, systemic inflammation, higher total serum cholesterol, measures of atherosclerosis, and obesity.[43,44,48] It is possible that larger venules are a component of microvascular damage from various CHD risk factors. The increased risk of CHD mortality associated with smaller arterioles appeared to be similar in persons with and without hypertension. Small vessel disease from processes such as hypertension and diabetes contributes to a significant proportion of stroke events (e.g. lacunar infarction), and it is speculated that small vessel disease may also contribute to a proportion of CHD events.[49,50] Our findings appear to support such speculation.

Table 5 Coronary heart disease and stroke mortality and retinal vessel diameter at baseline, stratified by age for combined Beaver Dam and Blue Mountains Eye Studies

Baseline retinal vessel diameter	Hazard ratio (95% confidence interval)					
	Age <70 years			Age ≥70 years		
	Numbers at risk[a]	Numbers affected[a]	Multivariable adjusted Model 2[b]	Numbers at risk[a]	Numbers affected[a]	Multivariable adjusted Model 2[b]
Coronary heart disease mortality						
Smaller arteriolar diameter						
Absent	4351	163	1.0	1645	328	1.0
Present[c]	1031	74	1.70 (1.27–2.28)	467	88	1.11 (0.86–1.43)
Larger venular diameter						
Absent	4224	154	1.0	1771	349	1.0
Present[c]	1158	83	1.41 (1.06–1.89)	341	67	1.06 (0.80–1.41)
Stroke mortality						
Smaller arteriolar diameter						
Absent	4351	57	1.0	1645	171	1.0
Present[c]	1031	26	1.64 (1.00–2.67)	467	45	0.88 (0.61–1.29)
Larger venular diameter						
Absent	4224	55	1.0	1771	186	1.0
Present[c]	1158	28	1.53 (0.94–2.47)	341	30	0.79 (0.51–1.22)

[a]Over a mean follow-up period of 10.9 years (interquartile range 10.4–13.4 years) for the combined populations.
[b]Adjusted for age, sex, body mass index, presence of hypertension, diabetes, serum total cholesterol, high density lipoprotein cholesterol, white blood cell count, current smoking status, study-site (as a strata variable—see text) and either history of angina and acute myocardial infarct (for coronary heart disease mortality) or stroke (for stroke mortality).
[c]Smaller arterioles defined as measurements within the narrowest quintile; larger venules as measurements within the widest quintile, with other quintiles as the reference group.

Table 6 Coronary heart disease mortality and retinal vessel diameter at baseline, stratified by gender and hypertension status for combined Beaver Dam and Blue Mountains Eye Studies

Baseline retinal vessel diameter	Hazard ratio (95% confidence interval)					
	Numbers at risk[a]	Numbers affected[a]	Multivariable adjusted Model 2[b]	Numbers at risk[a]	Numbers affected[a]	Multivariable adjusted Model 2[b]
	Women			**Men**		
Smaller arteriolar diameter						
Absent	3483	232	1.0	2513	259	1.0
Present[c]	689	62	1.39 (1.03–1.89)	793	100	1.27 (1.00–1.63)
Larger venular diameter						
Absent	3499	252	1.0	2496	251	1.0
Present[c]	689	42	0.93 (0.65–1.34)	810	108	1.43 (1.12–1.83)
	Hypertension			**No hypertension**		
Smaller arteriolar diameter						
Absent	3274	344	1.0	2714	147	1.0
Present[c]	1090	126	1.27 (1.02–1.59)	407	36	1.55 (1.05–2.29)
Larger venular diameter						
Absent	3413	357	1.0	2576	146	1.0
Present[c]	951	113	1.29 (1.02–1.63)	545	37	1.12 (0.75–1.67)

[a]Over a mean follow-up period of 10.9 years (interquartile range 10.4–13.4 years) for the combined populations.
[b]Adjusted for age, sex, body mass index, presence of diabetes, serum total cholesterol, high density lipoprotein cholesterol, white blood cell count, current smoking status, study-site (as a strata variable—see text), and either history of angina and acute myocardial infarct (for coronary heart disease mortality) or stroke (for stroke mortality).
[c]Smaller arterioles defined as measurements within the narrowest quintile; larger venules as measurements within the widest quintile, with other quintiles as the reference group.

We have no explanation of why the effect of retinal vessel diameter on vascular mortality is not linear but appears to have a threshold effect. We can speculate, however, that within the normal range of vessel diameter, there may be a threshold beyond which the microcirculation cannot compensate, and that the microvascular structure is less than optimal. It remains to be clarified whether similar calibre characteristics in small vessels of the brain and heart are

involved in the pathogenesis of cardiovascular events, and whether retinal vessel diameter correlates to microvascular changes in the brain and heart. Although we show that smaller retinal arterioles and larger venules appear to carry additional information on cardiovascular risk prediction, the clinical significance of these associations will need to be verified.

Strengths of this study include the population-based nature of the two cohorts, their large sample sizes, use of similar masked protocols by the two studies to measure retinal vessel diameter, and reliably defined CHD deaths. As there have been no recent validation studies of stroke deaths, we cannot exclude the possibility of misclassification in defining these. Self-reported past histories of stroke, angina, or AMI were used without validation. This could have potentially contributed further misclassification to definition of these co-variables. Undifferentiated misclassification, however, would only bias the associations towards the null, resulting in an underestimation of the strength of the associations observed. We have not controlled for the use of medications by study participants, and certain medications may influence retinal vessel calibre in unknown directions. We believe, however, the number of participants who were using a specific type of medication is likely to be small, and thus an influence from medications is likely to be minimum.

In summary, pooled data from two population-based cohorts from the US and Australia demonstrate that in persons aged 43–69, the presence of either smaller retinal arterioles or larger venules predicted an increased risk of CHD mortality, and were also likely to be associated with an increased risk of stroke mortality, independent of age, gender, and other cardiovascular risk factors. These associations were not observed in people aged 70 or older. Further studies to explore the clinical significance of these subtle differences in retinal vessel diameter are warranted. Our findings, if confirmed in other populations, suggest that retinal vessel diameter could be a prognostic marker of death from cardiovascular events in middle-aged persons.

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