Measurement of Retinal Vascular Caliber: Issues and Alternatives to Using the Arteriole to Venule Ratio

Gerald Liew,[1] *A. Richey Sharrett,*[2] *Richard Kronmal,*[3] *Ronald Klein,*[4] *Tien Yin Wong,*[5,6,7] *Paul Mitchell,*[1] *Annette Kifley,*[1] *and Jie Jin Wang*[1]

Purpose. The arteriole to venule ratio (AVR) is widely used in studies of the associations of retinal microvascular disease with systemic and ocular outcomes. This is a discussion of the limitations of AVR; a comparison of its predictive information with that of its components, arteriolar and venular caliber; and a description of a suggested alternative method of modeling arteriolar and venular calibers directly.

Methods. Data from the population-based Blue Mountains Eye Study were used to compare the predictive information in models using AVR with models using arteriolar and venular calibers directly. Determination was made of how the apparent relationship between vessel caliber and two systemic outcomes (blood pressure [BP] and white blood cell count [WBC]) was influenced by the choice of regression model. These findings were interpreted with reference to the known biological relationship among vessel calibers, BP, and WBC.

Results. Models using arteriolar and venular calibers directly had more predictive information than models using AVR. The apparent relationship of vessel caliber to BP and WBC differed substantially, depending on the model chosen. For example, after adjustment for age, sex, and other covariates, decreasing venular caliber was associated with *higher* systolic BP when modeled separately, but was associated with *lower* systolic BP when modeled simultaneously with arteriolar caliber.

Conclusions. The findings suggest AVR provides less information with regards to predicting systemic outcomes than its two components. Modeling arteriolar and venular calibers separately could be biased by confounding, while modeling both simultaneously appears to provide unbiased, biologically plausible results. The use of this approach is recommended in future research relating retinal vascular caliber to systemic or ocular outcomes. (*Invest Ophthalmol Vis Sci.* 2007;48:52–57) DOI:10.1167/iovs.06-0672

Changes in retinal vascular caliber may carry important information regarding the state of the microcirculation in the eye and in other vascular beds.[1,2] In particular, generalized narrowing of the retinal arterioles has long been known to be associated with chronic hypertension.[1] However, because generalized retinal arteriolar narrowing proved extremely difficult to estimate precisely from clinical examination by ophthalmoscopy, a summary measure, the retinal arteriole-to-venule ratio (AVR; ratio of the caliber of arterioles to venules) was proposed by Wagener et al.[3] as an index of the severity of generalized arteriolar narrowing. The use of the ratio implicitly assumes that in most cases, the venular diameter is relatively constant and does not change with blood pressure (BP), age, and other factors. Thus, a smaller AVR was thought to reflect narrower arterioles, relative to presumed stable venular caliber.

Recent studies in which digital imaging methods were used have allowed retinal vascular caliber to be measured more precisely from retinal photographs than from ophthalmoscopic examination. In these studies, although measurements of individual retinal arteriolar and venular calibers were made from fundus photographs, the AVR continued to be used as an index of severity of generalized arteriolar narrowing.[1,4–7]

The AVR, as a relative measure that combines information from both arms of the circulatory system, has the advantage of controlling for magnification differences from camera lenses and refractive error.[8] When a low AVR was found to be associated with aging,[9] cigarette smoking,[10] current and past BP,[4,11] open-angle glaucoma[12] and cardiovascular outcomes such as incident stroke[13,14] and coronary heart disease,[15] the associations were initially thought to reflect generalized arteriolar narrowing, rather than changes in venular calibers.

New analyses, however, have pointed out weaknesses and limitations of using the AVR.[16,17] First, there are statistical reasons why ratios such as the AVR may capture less information with respect to prediction of outcomes than would consideration of the numerator and denominator separately.[18] Second, studying the associations of arteriolar and venular calibers separately may shed light on underlying pathophysiological mechanisms. Retinal arteriolar and venular calibers themselves carry different prognostic information,[17,18] and use of a summary measure such as AVR may lead to incorrect inferences.[17,18] For example, if both arteriolar and venular calibers are associated with the study outcome in the same direction (such as narrowing of both with increasing BP), the magnitude of the association between arteriolar caliber and increasing BP may be masked substantially, as AVR will not appear to change much with BP. In contrast, if both vessel calibers are associated with the study outcome but in opposite directions, AVR may substantially exaggerate or mask the apparent magnitude of the association with arteriolar caliber, as has been shown in several recent studies.[17,19] Clearly, further understanding of appropriate methods to analyze retinal vessel

From the [1]Centre for Vision Research, Department of Ophthalmology, Westmead Millennium Institute, University of Sydney, Sydney, Australia; the [2]Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland; the [3]Collaborative Health Studies Coordinating Center, University of Washington, Seattle, Washington; the [4]Department of Ophthalmology and Visual Science, University of Wisconsin-Madison, Madison, Wisconsin; the [5]Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia; and the Departments of [6]Community, Occupational, and Family Medicine and [7]Ophthalmology, National University of Singapore, Singapore.

Supported by Grants 153948, 302068, 974159, and 211069 from the Australian National Health and Medical Research Council, Canberra, Australia; National Eye Institute Grant EY06594 (RK); and National Heart, Lung, and Blood Institute Grant HL59259 (RK).

Submitted for publication June 16, 2006; revised August 12, 2006; accepted October 3, 2006.

Disclosure: **G. Liew**, None; **A.R. Sharrett**, None; **R. Kronmal**, None; **R. Klein**, None; **T.Y. Wong**, None; **P. Mitchell**, None; **A. Kifley**, None; **J.J. Wang**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Jie Jin Wang, Centre for Vision Research, Department of Ophthalmology, University of Sydney, Westmead Hospital, Hawkesbury Road, Westmead, NSW Australia, 2145; jiejin_wang@wmi.usyd.edu.au.

caliber, including whether the AVR is an appropriate summary indicator, is needed.

One alternative used in the Rotterdam study and subsequently in the Multi-Ethnic Study of Atherosclerosis (MESA) was to analyze associations of retinal arteriolar and venular caliber separately.[17,20] However, neither study explored whether this approach provides more valid information than the AVR. Moreover, whether modeling arteriolar and venular caliber in separate models provides valid conclusions regarding their associations is uncertain, as arteriolar and venular caliber are highly correlated. The purpose of the present study was to explore these issues using data from the Blue Mountains Eye Study.

Materials and Methods

For this study, we used data from the Blue Mountains Eye Study, a population-based cohort of a predominantly white population, details of which are given elsewhere.[21,22] The study was conducted according to the recommendations of the Declaration of Helsinki and was approved by the Western Sydney Area Health Service Human Research Ethics Committee. Written, informed consent was obtained from all participants.

We analyzed the association of retinal vascular caliber to two outcomes: systolic BP (SBP) and white blood cell count (WBC). The association between narrower arterioles and higher BP is well established, and there is good evidence suggesting that wider venular caliber is related to systemic inflammation.[17,23]

For this study, we performed the following. First we tested whether AVR predicts outcome as well as arteriolar and venular calibers jointly. Next we constructed models assessing the relationship between vessel calibers and the two outcomes. We compared regression coefficients from models containing a single term (either arteriolar or venular caliber) compared with coefficients from models containing both terms simultaneously. We interpreted these coefficients based on prior biological knowledge of the likely relationships between vessel calibers and the two outcomes.

The Blue Mountains Eye Study has been described.[5] At the baseline examination (1992–1994), color retinal photographs (30°) of the macula and other retinal fields of both eyes were taken with a fundus camera after pupil dilation (model FF3; Carl Zeiss Meditec GmbH, Oberkochen, Germany). Detailed grading methods have been described[5] and are identical with those used in large population-based studies.[24] In brief, we used a computer-assisted method to measure the internal caliber of retinal arterioles and venules from digitized retinal images, which were then summarized with published formulas[7,25] with correction for magnification.[3,8] The formulas take into account branching patterns and allow all measured vessel calibers in an eye to be summarized as an index representing the mean arteriolar or venular caliber of that eye and AVR to be calculated from these indices. An AVR of 1.0 suggests that arteriolar calibers are, on average, the same as venular calibers in that eye, whereas a lower AVR suggests either relatively narrower arterioles compared with venules, or relatively wider venules, compared with arterioles.

We measured WBC, fasting plasma glucose, and total serum cholesterol in fasting blood samples within 4 hours of blood collection. We measured the SPB and diastolic BP (DBP) of each participant with the same mercury sphygmomanometer with appropriate adult cuff size, after seating the participant for at least 10 minutes. Body mass index (BMI) was calculated from height and weight measured at the same examination, and smoking status was determined from self-report.

To determine whether AVR contained all (or most) of the predictive information with regard to outcomes provided by arteriolar and venular calibers, we noted that use of AVR as a predictor assumes that a multiplicative model of the form below holds.

$$Y = a(A/V)^d\varepsilon, \quad (1)$$

where Y is the outcome variable, A and V are arteriolar and venular calibers respectively, a and d are regression parameters, and ε is a random error term with mean = 1. The equivalent multiplicative model using arteriolar and venular calibers instead of AVR would be

$$Y = aA^bV^c\varepsilon, \quad (2)$$

where b and c are regression parameters. For AVR (A/V) to contain all the predictive information of arteriolar (A) and venular (V) calibers, the two equations would have to be equivalent: $b = -c = d$. There is no a priori reason to assume this, but use of the AVR requires this assumption.

We then expressed equation 2 as

$$\ln(Y) = \ln(a) + b\ln(A) + c\ln(V) + \ln(\varepsilon). \quad (3)$$

Letting $Y' = \ln(Y)$, $a' = \ln(a)$, $A' = \ln(A)$, $V' = \ln(V)$ and $\varepsilon' = \ln(\varepsilon)$ gives

$$Y' = a' + bA' + cV' + \varepsilon'. \quad (4)$$

which is the form of the usual linear regression model.

In the same way, equation 1 can be expressed as

$$Y' = a' + dA' - dV' + \varepsilon'. \quad (5)$$

To test the null hypothesis that the model with AVR contains all the predictive information of the model with arteriolar and venular calibers together, we modeled equations 4 and 5 using SBP and WBC as outcomes, and computed the F statistic:

$$F = (\text{residual SS from model 5} - \text{residual SS from model 4})/\text{residual MS from model 4}. \quad (6)$$

where SS is the sum of squares, and MS is the mean sum of squares. This F statistic had 1 and $n - 3$ degrees of freedom, and we rejected the null hypothesis, if it reached statistical significance.

Next, we assessed the relationship between arteriolar and venular caliber (explanatory variables) and SBP and WBC (outcome variables) in different regression models—models 7, 8, and 9: where we adjusted for age and sex while entering AVR, arteriolar caliber, or venular caliber, respectively, into three separate models; model 10: age- and sex-adjusted, entering both arteriolar and venular caliber simultaneously into the same model; models 11, 12, and 13: the variables in models 7, 8, and 9 additionally adjusted for BMI, fasting plasma glucose, total serum cholesterol, and smoking status; and model 14: the variables in model 10 additionally adjusted for BMI, fasting plasma glucose, total serum cholesterol, and smoking status.

To explore the possibility of collinearity,[26] given the correlation between arteriolar and venular calibers in this population ($r = 0.58$; Liew G, unpublished data, 2006), we compared regression coefficients, standard errors (in the form of confidence intervals; [CIs]) and variance inflation factors (VIFs).[26] VIFs are a measure of the degree of collinearity, with high values indicating severe collinearity. If severe collinearity (VIF close to 10) were present, we would expect to find unstable regression coefficients and large CIs.

Results

The characteristics of our sample population have been reported previously.[21] Of the 3000 participants included in this analysis, 1288 (42.9%) were male, 1066 (35.5%) were ex-smokers, and 437 (14.6%) were current smokers. The average age was 65.5 (SD 9.4) years, with mean BMI 26.2 (4.6) kg/m^2, mean SBP 145.7 (21.3) mm Hg, mean DBP 83.4 (10.1) mm Hg, and mean WBC 6.52 (1.77) $\times$ 10^9/L. Age- and sex-adjusted SBP and

DBP were almost identical in the study group (146.1/83.3 mm Hg) and in the group excluded ($n = 654$), because of ungradable photographs or lack of data on refraction or WBC (146.6/83.6 mm Hg).

Table 1 shows the results of testing whether AVR provides the same predictive information as arteriolar and venular calibers together. For both outcomes of SBP and WBC, the F statistic is significant ($P < 0.0001$), indicating that models with AVR are substantially different from models with both arteriolar and venular calibers. Although the R^2s of all three models were low, those of the models with both arteriolar and venular calibers were more than twice the R^2 of the models with AVR (0.069 vs. 0.029 for SBP, and 0.015 vs. 0.007 for WBC, respectively), demonstrating that models with both arteriolar and venular calibers together predicted these two outcomes better than models using AVR.

Table 2 shows the relationship between SBP and retinal vessel indices, as determined according to different models. In models 7, 8, and 9, where single-vessel caliber terms were entered, decreasing arteriolar caliber, decreasing venular caliber, and decreasing AVR were strongly associated with higher SBP. However, in model 10 where both vessel calibers were simultaneously entered, decreasing arteriolar caliber was strongly associated with *higher* SBP, but decreasing venular caliber was strongly associated with *lower* SBP, demonstrating a reversal in the direction of the association with venular calibers. Additional adjustment for BMI, fasting plasma glucose, total serum cholesterol, and smoking status attenuated the magnitude of the changes in SBP, with changes in arteriolar or venular caliber, but the directions of the associations remained as in the age- and sex-adjusted models. We found a similar reversal in the direction of the associations when DBP instead of SBP was modeled as the outcome variable (data not shown). The change in direction of the association between venular caliber and BP is unlikely to be due to collinearity between these two correlated variables, as the CIs for both terms remained narrow and the VIFs for both terms were low (approximately 1.5).

Table 3 shows similar changes in coefficients, with WBC as the outcome variable. In models 7, 8, and 9, increasing arteriolar caliber and venular caliber were strongly associated with higher WBC, whereas increasing AVR was strongly associated with lower WBC. In model 10, arteriolar caliber was no longer associated with WBC, whereas the association with venular caliber remained strong. These results remained similar after additional adjustment for BMI, fasting plasma glucose, total serum cholesterol, and smoking.

Discussion

In this article, we have highlighted several issues in analyzing retinal vessel caliber measurements. First, in our study, we showed that using the AVR in regression analyses for a given endpoint involves making the a priori assumption that the coefficient of arteriolar caliber for that endpoint is equal in magnitude, but opposite in sign, to the coefficient of venular caliber. Using data from a large population sample, we found that this assumption is incorrect both for BP and WBC (although it may be correct for other endpoints). Further, we demonstrated that AVR was not a particularly informative summary of retinal vessel measurement, as it conveyed less information (lower R^2) regarding these two endpoints than that provided by individual arteriolar and venular calibers. A major reason for the initial use of AVR, to correct for magnification differences, may not be as important as previously thought. Refractive error, when available, can be used to control for magnification,[8] whereas, in its absence, bias from magnification differences is not profound in most eyes within the refractive power range of ±3 D.[8] Further, refractive errors are usually not associated with many outcomes of interest and thus are unlikely to confound the associations assessed. Hence, analyses for BP, WBC, and other similar systemic (or ocular) endpoints, should ideally be performed using individual vessel calibers.

Second, as an alternative to the AVR, one could either model these variables separately, or include them simultaneously in the same model. It is unclear which method provides less biased results. On the one hand, modeling vessel calibers separately may give estimates confounded by the effects of the fellow component variable. The main determinant of venular caliber is in fact arteriolar caliber, with arteriolar caliber explaining approximately 30% of the variability in venular caliber (Liew G, unpublished data, 2006), presumably due to their shared genetic and environmental determinants (e.g., diet, health, and growth) as well as magnification artifacts.[24] On the other hand, including both components in the same model would provide an adjustment that would avoid this confounding effect, but this approach has not been used previously, and there are no data on its merits. Thus, currently there is no consensus as to the most appropriate approach for analyzing the relationship of retinal vessel calibers and systemic outcomes: Should one continue to use the AVR on its own, analyze vessel calibers separately, or include both vessel calibers simultaneously in the same model?

In the examples we used in this report, when arteriolar or venular caliber terms were entered *separately* into different

Table 1. Results from Models 4, 5 and 6

Outcome	Test Statistic	Model with ln(A), ln(V)	Model with ln(A/V)	Regression Coefficients b	c	d	F Statistic	df	*P* Value
Systolic BP	Residual SS	116.23	121.16	−0.354	0.033	−0.256	127.1	1,2997	<0.0001
	Residual MS	0.0388	0.0404						
	R^2	0.069	0.029						
WBC	Residual SS	403.71	407.23	−0.14	0.412	−0.223	26.1	1,2997	<0.0001
	Residual MS	0.1348	0.1359						
	R^2	0.015	0.007						

BP indicates blood pressure; SS, sum of squares; MS, mean square; WBC, white blood cell count.

A indicates arteriolar caliber; V, venular caliber; A/V, arteriole to venule ratio; df, degrees of freedom; a, b, c, and d are regression coefficients described in the text.

Model 4: $\ln(Y) = \ln(a) + b\ln(A) + c\ln(V) + \ln(\varepsilon)$ where Y is the outcome variable, and ε is a random error term.

Model 5: $\ln(Y) = \ln(a) + d\ln(A) - d\ln(V) + \ln(\varepsilon)$.

In Model 6, the F statistic is calculated as F = (Residual SS from model 5 − Residual SS from model 4)/Residual MS from model 4.

TABLE 2. Retinal Vessel Indices and Systolic Blood Pressure

	Models 7, 8, 9* Single Variable Entry			Model 10** Simultaneous Entry				Models 11, 12, 13† Single Variable Entry			Model 14‡ Simultaneous Entry			
Retinal Vessel Index	Change in SBP (mmHg)	95% CI	*P* Value	Change in SBP (mmHg)	95% CI	*P* Value	VIF	Change in SBP (mmHg)	95% CI	*P* Value	Change in SBP (mmHg)	95% CI	*P* Value	VIF
Per SD decrease in arteriolar caliber	4.3	3.6, 5.0	<0.0001	5.0	4.2, 5.9	<0.0001	1.54	2.1	1.8, 2.5	<0.0001	2.6	2.2, 3.0	<0.0001	1.57
Per SD decrease in venular caliber	1.2	0.4, 1.9	0.0015	−1.4	−2.3, −0.6	0.0006	1.49	0.5	0.08, 0.83	0.02	−0.9	−1.3, −0.5	<0.0001	1.56
Per SD decrease in AVR	3.4	2.7, 4.0	<0.0001	NA	NA	NA	NA	1.8	1.5, 2.2	<0.0001	NA	NA	NA	NA

SBP indicates systolic blood pressure; CI, confidence intervals; VIF, variance inflation factor; SD, standard deviation; AVR, arteriole to venule ratio;
* Models 7, 8, 9 adjusted for age, sex and either arteriolar caliber, venular caliber or AVR respectively.
** Model 10 adjusted for age, sex and both arteriolar caliber and venular caliber simultaneously in the same model
† Models 11, 12, 13 adjusted for variables in models 7, 8, 9 plus body mass index, fasting plasma glucose, total serum cholesterol and smoking (current, ex, never).
‡ Model 14 adjusted for variables in model 10 plus body mass index, fasting plasma glucose, total serum cholesterol and smoking (current, ex, never)

TABLE 3. Retinal Vessel Indices and White Blood Cell Count (WBC)

	Models 7, 8, 9* Single Variable Entry			Model 10** Simultaneous Entry				Models 11, 12, 13† Single Variable Entry			Model 14‡ Simultaneous Entry			
Retinal Vessel Index	Change in WBC ($\times 10^9$/L)	95% CI	*P* Value	Change in WBC ($\times 10^9$/L)	95% CI	*P* Value	VIF	Change in WBC ($\times 10^9$/L)	95% CI	*P* Value	Change in WBC ($\times 10^9$/L)	95% CI	*P* Value	VIF
Per SD increase in arteriolar caliber	0.10	0.04, 0.17	0.002	−0.03	−0.11, 0.04	0.41	1.54	0.04	−0.03, 0.10	0.25	−0.04	−0.12, 0.03	0.27	1.57
Per SD increase in venular caliber	0.24	0.17, 0.30	<0.0001	0.26	0.18, 0.33	<0.0001	1.49	0.13	0.07, 0.19	<0.0001	0.15	0.08, 0.23	<0.0001	1.56
Per SD increase in AVR	−0.1	−0.17, −0.04	0.001	NA	NA	NA	NA	−0.07	−0.13, −0.009	0.02	NA	NA	NA	NA

WBC indicates white blood cell count; CI, confidence intervals; VIF, variance inflation factor; SD, standard deviation; AVR, arteriole to venule ratio;
* Models 7, 8, 9 adjusted for age, sex and either arteriolar caliber, venular caliber or AVR respectively.
** Model 10 adjusted for age, sex and both arteriolar caliber and venular caliber simultaneously in the same model
† Models 11, 12, 13 adjusted for variables in models 7, 8, 9 plus body mass index, fasting plasma glucose, total serum cholesterol and smoking (current, ex, never).
‡ Model 14 adjusted for variables in model 10 plus body mass index, fasting plasma glucose, total serum cholesterol and smoking (current, ex, never)

regression models, we found that both caliber terms were strongly and negatively associated with higher systolic and DBP, after adjusting for age, sex, and other covariates. In contrast, when both arteriolar and venular caliber terms were entered *simultaneously* into the same model, decreasing arteriolar caliber, but *increasing* venular caliber, was associated with higher systolic and DBP. Similarly, when modeled separately, both increasing arteriolar and venular caliber terms were strongly associated with higher WBC, but when modeled simultaneously, venular caliber remained strongly and positively associated with higher WBC, whereas the positive association with arteriolar caliber attenuated considerably and became nonsignificant.

The apparent negative association of narrower venules with higher BP when single variables are entered into separate models is most likely a consequence of the correlation between arteriolar and venular caliber (a person with narrowed arterioles is also likely to have relatively narrowed venules). When we examined the independent effects of each variable by including both in the same model, we found that wider, not narrower, venular caliber was associated with higher SBP. The inverse association of venular caliber with BP is consistent with evidence indicating that wider venular caliber may be partly a response to chronic retinal hypoxia or hypoperfusion[27,28] from chronic hypertensive damage to the microcirculation. Hence, the apparent relationship between narrower venules and higher SBP when venules were modeled separately is probably a biased finding resulting from confounding by arteriolar caliber, which is strongly associated with both BP and venular caliber. Similarly, the apparent positive association between arteriolar caliber and WBC in the age-sex adjusted model without venular diameter is likely to be a result of confounding by venular caliber.

It should be noted that computer-assisted measurement of retinal vessel calibers from retinal photographs measures only the width of the reflective erythrocyte column and underestimates the true internal vessel caliber, as it does not measure the surrounding clear plasma zone (<10% of the internal diameter). However, we believe erythrocyte column width is a good surrogate measure of internal vessel caliber as it appears to be proportional to internal retinal vessel caliber in animal studies, given that the ratio of the width of the plasma layer to internal vessel caliber for both arterioles and venules stays constant.[29]

The potential clinical application of this method to evaluate retinal vessel calibers, and the contribution from the assessment of these vessels to cardiovascular risk stratification, is promising,[1,30] though much work is still needed before it can be implemented in routine clinical practice.

In conclusion, using examples from our population-based data, we proposed several issues that deserve attention when using ratios in analysis. Arbitrary functions of multiple variables, such as AVR, should not be used in regression modeling unless it can be shown that there is little or no loss of information in using them compared with the inclusion in the model of the component variables that make up the arbitrary function.[18] Based on prior biological knowledge of the likely associations between BP and arteriolar and venular calibers, we believe that there is a possibility of confounding by the other fellow component variable, when analyzing the two correlated vessel calibers in separate models. We thus recommend that models including both arteriolar and venular caliber terms together should be considered in situations where possible confounding from the other vessel caliber is likely to exist—for example, when assessing the relationship of vessel calibers to systemic or ocular outcomes. This approach would adjust for possible confounding from the other correlated variable, and confirm the robustness of findings from models that include vessel calibers separately. Our propositions must be explored and confirmed in other datasets.

References

1. Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med*. 2004;351:2310–2317.
2. Wong TY, McIntosh R. Systemic associations of retinal microvascular signs: a review of recent population-based studies. *Ophthalmic Physiol Opt*. 2005;25:195–204.
3. Wagener HP, Clay GE, Gipner JF. Classification of retinal lesions in the presence of vascular hypertension. *Trans Am Ophthalmol Soc*. 1947;45:57–73.
4. Sharrett AR, Hubbard LD, Cooper LS, et al. Retinal arteriolar diameters and elevated blood pressure: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol*. 1999;150:263–270.
5. Wang JJ, Mitchell P, Leung H, Rochtchina E, Wong TY, Klein R. Hypertensive retinal vessel wall signs in a general older population: the Blue Mountains Eye Study. *Hypertension*. 2003; 42:534–541.
6. Duncan BB, Wong TY, Tyroler HA, Davis CE, Fuchs FD. Hypertensive retinopathy and incident coronary heart disease in high risk men. *Br J Ophthalmol*. 2002;86:1002–1006.
7. Leung H, Wang JJ, Rochtchina E, et al. Relationships between age, blood pressure and retinal vessel diameters in an older population. *Invest Ophthalmol Vis Sci*. 2003;44:2900–2904.
8. Wong TY, Wang JJ, Rochtchina E, Klein R, Mitchell P. Does refractive error influence the association of blood pressure and retinal vessel diameters? The Blue Mountains Eye Study. *Am J Ophthalmol*. 2004;137:1050–1055.
9. Wong TY, Klein R, Klein BE, Meuer SM, Hubbard LD. Retinal vessel diameters and their associations with age and blood pressure. *Invest Ophthalmol Vis Sci*. 2003;44:4644–4650.
10. Klein R, Sharrett AR, Klein BE, et al. Are retinal arteriolar abnormalities related to atherosclerosis?—The Atherosclerosis Risk in Communities Study. *Arterioscler Thromb Vasc Biol*. 2000;20: 1644–1650.
11. Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R, Mitchell P. Impact of current and past blood pressure on retinal arteriolar diameter in an older population. *J Hypertens*. 2004;22:1543–1549.
12. Mitchell P, Leung H, Wang JJ, et al. Retinal vessel diameter and open-angle glaucoma: the Blue Mountains Eye Study. *Ophthalmology*. 2005;112:245–250.
13. Mitchell P, Wang JJ, Wong TY, Smith W, Klein R, Leeder SR. Retinal microvascular signs and risk of stroke and stroke mortality. *Neurology*. 2005;65:1005–1009.
14. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet*. 2001;358:1134–1140.
15. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA*. 2002;287:1153–1159.
16. Patton N, Aslam T, MacGillivray T, Dhillon B, Constable I. Asymmetry of retinal arteriolar branch widths at junctions affects ability of formulae to predict trunk arteriolar widths. *Invest Ophthalmol Vis Sci*. 2006;47:1329–1333.
17. Ikram MK, de Jong FJ, Vingerling JR, et al. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2004; 45:2129–2134.
18. Kronmal RA. Spurious correlation and the fallacy of the ratio standard revisited. *J R Statist Soc A*. 1993;156:379–392.
19. Ikram MK, Witteman JC, Vingerling JR, Breteler MM, Hofman A, de Jong PT. Retinal vessel diameters and risk of hypertension. The Rotterdam Study. *Hypertension.* 2005.
20. Wong TY, Islam FM, Klein R, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci*. 2006;47: 2341–2350.
21. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1995;102:1450–1460.

22. Cumming RG, Mitchell P, Leeder SR. Use of inhaled corticosteroids and the risk of cataracts [see comments]. *N Engl J Med*. 1997;337:8–14.
23. Wong TY, Duncan BB, Golden SH, et al. Associations between the metabolic syndrome and retinal microvascular signs: the Atherosclerosis Risk In Communities study. *Invest Ophthalmol Vis Sci*. 2004;45:2949–2954.
24. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology*. 1999;106:2269–2280.
25. Sherry LM, Wang JJ, Rochtchina E, et al. Reliability of computer-assisted retinal vessel measurement in a population. *Clin Exp Ophthalmol*. 2002;30:179–182.
26. Elmstahl S, Gullberg B. Bias in diet assessment methods: consequences of collinearity and measurement errors on power and observed relative risks. *Int J Epidemiol*. 1997;26:1071–1079.
27. Saldivar E, Cabrales P, Tsai AG, Intaglietta M. Microcirculatory changes during chronic adaptation to hypoxia. *Am J Physiol*. 2003;285:H2064–H2071.
28. Klijn CJ, Kappelle LJ, van Schooneveld MJ, et al. Venous stasis retinopathy in symptomatic carotid artery occlusion: prevalence, cause, and outcome. *Stroke*. 2002;33:695–701.
29. Bulpitt CJ, Dollery CT, Kohner EM. The marginal plasma zone in the retinal microcirculation. *Cardiovasc Res*. 1970;4:207–212.
30. Wong TY, McIntosh R, Wong TY, McIntosh R. Hypertensive retinopathy signs as risk indicators of cardiovascular morbidity and mortality. *Br Med Bull.* 2005;73–74:57–70.