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COMPARISON OF THE RETINAL MICROVASCULATURE IN EUROPEAN AND AFRICAN-CARIBBEAN PEOPLE WITH DIABETES

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Keywords: retina, microvascular, diabetes, ethnicity

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ABSTRACT

Background Diabetes aggravates the impact of elevated BP on the microcirculation and people of African ancestry with diabetes are more susceptible to microvascular damage than Europeans. We investigated possible differences in the retinal microcirculation in people of European and African Caribbean ethnicity with diabetes that might account for this.

Methods 51 subjects with type 2 diabetes (age 40-65yrs; 25 male; 29 African-Caribbean) were studied. Clinic and 24 hour ambulatory BP, fasting glucose, insulin, and lipids were measured. Digital retinal images were analysed using custom written semi-automatic software to determine: length:diameter ratio (LDR) and arteriolar:venular diameters (AVR), branching angles, vessel tortuosity and number of terminal vessel branches. Data are means (SD).

Results Arterioles were narrower in European people with diabetes than in African Caribbean people with diabetes (mean arteriolar diameter = 76 (7) vs. 82 (11) μm ; $p = 0.03$; arteriolar LDR = 28.1 (8.5) vs. 23.7 (7.0); $p = 0.046$ and AVR = 0.66 (0.21) vs. 0.90 (0.36); $p = 0.028$). Ethnic differences in arteriolar LDR, arteriolar diameter and AVR were not explained by differences in BP but were attenuated by adjustment for duration of diabetes. There was no significant relationship between BP and arteriolar narrowing in the group as a whole, although the relationship between arteriolar LDR and SBP was stronger in Europeans than African-Caribbeans ($\beta = 0.08$ (0.07) vs. $\beta = 0.03$ (0.06); $p = 0.03$).

Conclusions In the presence of diabetes, a relationship between BP and retinal arteriolar diameter is not evident and implies impaired small artery remodelling in the presence of diabetes. African Caribbean people with diabetes have wider retinal arterioles and this could contribute to enhanced microvascular damage in this ethnic group.

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INTRODUCTION

Microvascular disease is a common and important consequence of diabetes mellitus that affects many tissues, including the eye. Diabetic eye disease is the commonest complication of diabetes, and remains the main cause of blindness in people of working age [1]. Blood pressure plays a particularly important role in diabetic retinopathy. Although, classically the features of diabetic retinopathy are not identical to those of hypertensive retinopathy, there is considerable overlap [2], furthermore elevated BP predicts progression of diabetic retinopathy [1;3;4]; finally, anti-hypertensive therapy markedly reduces retinopathy risk, to an even greater extent than good glycaemic control [5].

The mechanism by which blood pressure so strongly influences diabetic retinopathy is unclear. In hypertension elevated BP results in widespread 'remodelling' of small arteries and arterioles [6], a process that probably involves both functional and structural changes [7]. Remodelling contributes to the elevated peripheral vascular resistance that is a feature of established hypertension [8], but may also protect the microvasculature downstream from the elevated systemic blood pressure [9]. In the eye microvascular 'remodelling' is manifest as a narrowing of retinal arteriolar diameters [10-12] and reduced arteriolar density or number [11;13]. It has been suggested [4;14] that concurrent diabetes mellitus may impair the ability of the resistance vasculature to protect downstream capillaries from the elevated BP in hypertension and could therefore, at least in part, account for the well recognised detrimental consequences of these 'bad companions' [15].

Diabetes and hypertension are commoner in people of African or African-Caribbean ancestry than those of European ancestry [16;17]. Previous studies have also suggested that people of African or African-Caribbean ethnicity are more vulnerable than Europeans to retinopathy [18-24] and also suffer more microvascular disease in other tissues [25-27]. In this study we investigated whether retinal microvascular changes differed between people of European and African-Caribbean ethnicity with type 2 diabetes, and explored the role of blood pressure in these differences.

METHODS

Twenty two European and 29 African-Caribbean men and women with diabetes aged 40 to 65 years were recruited from a sample of the general population stratified by age, sex and ethnicity drawn from a family practice register in North West London. The family practice register is probably the most accurate and comprehensive register of the general population in the UK and it is estimated that more than 97% of the population is registered with a practitioner [15]. Ethnicity was confirmed with the participants' own self assessment [25].

All participants attended the clinic after an overnight fast having refrained from smoking or caffeine consumption for at least 12 hours and completed a detailed questionnaire on demographic characteristics, past medical history, medications, and lifestyle behaviours. Anthropometric measures (height, weight, and waist-to-hip ratio) were collected according to a standard protocol [28]. Clinic BP was measured at the right brachial artery three times after 5 minutes of rest using an automated device (Omron 705CP, Omron Healthcare Europe B.V., Hoofddorp, the Netherlands) equipped with an appropriately sized cuff. Subjects were classified as hypertensive if their BP exceeded 135/85mmHg or they had a previous diagnosis of hypertension and were taking antihypertensive medication. Antihypertensive agents used included angiotensin converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, thiazide diuretics and calcium channel blockers. Nineteen European and 18 African-Caribbean subjects also agreed to undergo 24 hour ambulatory BP monitoring using a Duolter device (Novacor, Rueil-Malmaison, France). Fasting blood samples were taken for glucose, insulin and lipid measurements [25]. Retinal photography was performed in all participants using a Zeiss FF 450 plus fundus camera (Carl Zeiss Ltd., Welwyn Garden City, UK) with a 50° field of view following mydriasis with tropicamide (1%) eye drops. Subjects did not undergo retinal assessment using the gold standard seven field stereo

photography, but on the basis of the 50° field of view none had proliferative retinopathy and the majority (53% Europeans and 47% African Caribbeans) had either no, or mild non-proliferative retinopathy ($p = 0.7$ for difference between ethnic groups by χ^2 test). The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by the local research ethics committee of the Hammersmith Hospital. Informed written consent was obtained from all participants.

Retinal image analysis

Retinal images were digitised to a resolution 2800×2400 pixels using a LS100 slide scanner (Nikon, Tokyo, Japan). Digitized images were then reduced to 1818×1593 pixels using a Gaussian filter for subsequent analysis [29]. Analysis was performed on 'red-free' monochrome images by a single trained observer masked to subject identifiers. Vascular trees were segmented automatically using Retinal Image multiScale Analysis (RISA), a program written in MATLAB 5.5 (MathWorks Inc., Natick, Massachusetts, USA). Some details of the program and reproducibility have been published elsewhere [29;30]. After segmentation trees were identified as arteriolar or venular by the operator and any defects in segmentation (such as vessel discontinuities) were corrected at this stage.

Examples of a retinal image and the segmentation of a vascular tree are shown in figure 1. A number of parameters were used to assess the arteriolar and venular networks. Absolute arteriolar and venular diameter were estimated in micrometers using the calibration approach described by Hubbard et al.,[31]. Diameters represent the mean of all diameters in the segmented trees and measurements were not corrected for refraction; this has been reported to cause only minor errors in diameter estimates [12;32]. To derive a measure of vessel narrowing that is applicable to both arterioles and venules and is unaffected by the refractive properties of the eye, the ratio of the length of a vessel segment between two branching points to its average diameter (LDR) [33;34] was also calculated and the mean value derived. In addition, we estimated the ratio of arteriolar to venular diameters (AVR), a widely used measure of arteriolar narrowing [12], on the basis of the average diameters of the arterioles and venules. The central retinal artery (CRAE) and central retinal vein (CRVE) equivalents were not calculated. Values of AVR differ depending on the method used to calculate them (e.g. average diameter vs. CRAE/CRVE), but reductions in AVR correlate with BP irrespective of the method of calculation used [35;36]. We also measured the vessel tortuosity, as the ratio of the actual length of the vessel segment to the straight-line distance between two connected branching points; the number of terminal branches (N_T) as a measure of rarefaction [13], and the branching angle (the angle between the 2 daughter vessels at a branching point) as this has been shown to be altered in hypertension [37].

Reproducibility and validation of measures

Reproducibility was studied in 10 individuals in whom segmentation and analysis were repeated by the same observer blinded to subject identifier. Data were analysed using the approaches advocated by Bland and Altman [38] and Lin [39] and this showed reproducibility of various measures to be excellent (Table 1). The validity of the measurements of diameter was assessed in a separate study by comparing the estimates from RISA with measurements of vessel diameter made using another validated tool based on the sliding linear regression filter technique [40]. These studies showed very close agreement between diameter measurements using different techniques (mean difference (SD) = 4.2 (4.8) μm ($n = 19$)).

Statistical Analysis and power calculation

Sample size estimates were based on previous [13;37] and unpublished data, these calculations indicated that 22 subjects in both groups would be required to show a difference in arteriolar LDR

of 5 units between ethnic groups with 90% power and with 5% significance. This difference corresponded to approximately half that seen between normotensive and hypertensive individuals.

Data are presented as mean (SD) or standardized beta coefficients (SE). Statistical analyses were performed using Stata 9.2 (StataCorp LP). Statistical comparisons were made using a Student's t-test, or a Mann Whitney non-parametric rank test for skewed data, and analysis of covariance (ANCOVA). All tests were two-tailed and $p < 0.05$ was considered statistically significant.

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RESULTS

Comparison between ethnic groups

Mean age was similar in the two ethnic groups. Blood pressures were higher in African Caribbeans, but this difference did not reach statistical significance and the proportion of people with hypertension, or on anti-hypertensive medication was similar. Statistically significant differences were observed for serum fasting triglycerides which were significantly higher in Europeans and duration of diabetes which was longer in African-Caribbeans (Table 2). Measures of arteriolar narrowing were significantly higher in Europeans than African-Caribbeans (i.e. mean arteriolar diameter was smaller, arteriolar LDR was higher and AVR was lower; Table 3). These differences were not affected by adjustment for systolic BP, fasting triglycerides or fasting glucose, but were attenuated by adjustment for duration of diabetes (Table 4). Bifurcation angles were narrower in African-Caribbeans, but other measures of the retinal microvasculature, including vessel segment lengths and vascular density, did not differ significantly by ethnicity.

Relationships between arteriolar narrowing, rarefaction and risk factors

No significant relationships were seen between systolic BP and measures of arteriolar narrowing in people with diabetes (β (se) = 0.043 (0.064); $p = 0.5$ for mean arteriolar diameter, β (se) = 0.036 (0.048); $p = 0.5$ for arteriolar LDR and β (se) = -0.003 (0.002); $p = 0.2$ for AVR). There was also no significant relationship between BP and arteriolar NT, a measure of microvascular density (β (se) = -0.013 (0.026); $p = 0.6$). Taking account of possible effects of antihypertensive therapy by assigning maximum systolic BP rank to subjects receiving treatment did not alter these findings. The relationship between systolic BP and arteriolar LDR was significantly weaker in African-Caribbeans (β (se) = 0.029 (0.060)) than Europeans (β (se) = 0.080 (0.077); $p = 0.028$ for comparison of slope). Arteriolar LDR was inversely related to duration of diabetes (β (se) = -0.37 (0.14); $p = 0.01$) indicating that increased duration of diabetes was associated with arteriolar widening. Similar, albeit weaker, positive relationships were seen between duration of diabetes and mean arteriolar diameter (β (se) = 0.25 (0.18); $p = 0.16$) and AVR (β (se) = 0.14 (0.01); $p = 0.08$).

DISCUSSION

This study has shown that African Caribbean people with type 2 diabetes have wider retinal arterioles than Europeans. Previous studies in the UK and USA have reported increased prevalence of both hypertensive and diabetic retinopathy in people of African or African-Caribbean ethnicity compared with Europeans [18-24]. Several reasons for this have been suggested including differences in BP, duration or onset of diabetes, and glycaemic control. A previous large population-based study [41] also reported that increased duration of diabetes was associated with arteriolar widening assessed using AVR and more recent observations from the Multi-Ethnic Study of Atherosclerosis (MESA) also showed significantly wider retinal arterioles in people with diabetes, despite high blood pressures [42]. Our observations also indicate that differences in duration of diabetes may at least partially explain ethnic differences in arteriolar narrowing in diabetes, however although differences arteriolar narrowing were no longer statistically significant after adjustment for duration of diabetes they remained substantial and we cannot exclude that other factors contribute to inter-ethnic differences in retinal arteriolar narrowing. We speculate that altered arteriolar remodelling in diabetes could be a contributory factor to ethnic differences in susceptibility to retinopathy.

The association between elevated BP and arteriolar narrowing is well recognised [43;44], and using identical methodology we have previously reported that hypertension is associated with increased arteriolar narrowing and reduced arteriolar density in subjects of European origin without diabetes [13]. Our findings therefore suggest that diabetes may affect the relationship between increased BP and arteriolar narrowing and this suggestion is supported by other studies. An inverse relationship between duration of diabetes and degree of arterial narrowing was seen in this study. In the Cardiovascular Health Study relationships between BP and generalized arteriolar narrowing were weaker in diabetic than non-diabetic individuals [45]. Also, in a large population-based study of elderly individuals Wang *et al.*, [46] failed to see a significant relationship between mean arterial pressure and quintiles of AVR in people with diabetes, although a strong relationship was evident in non-diabetic individuals in the same population. In our study a relationship between BP and arteriolar narrowing was not marked. This was particularly the case in African Caribbean individuals, suggestive of an increased attenuation of the relationship in this ethnic group. In part this could relate to the longer duration of diabetes in these individuals, but adjustment for duration of diabetes only partially attenuated differences between ethnic groups. This could imply that other mechanisms contribute, but it should be noted that our estimate of duration of diabetes is an imprecise measure and is likely to underestimate the true duration of exposure to impaired glucose tolerance leaving the question unresolved. Our findings in diabetic people contrast with previous reports of increased retinal arteriolar narrowing independent of blood pressure in African Americans compared with White Americans in a population study excluding diabetic individuals [47] and narrower retinal arterioles in African Caribbean people in the UK, although in that instance the greater narrowing may have been attributable to higher blood pressures [48]. In the latter study it was also noted that diabetes modified the relationship between arteriolar narrowing and elevated blood pressure. Recent observations from the Multi-Ethnic Study of Atherosclerosis (MESA) in the US showed wider adjusted central retinal artery equivalents in African American participants compared with White American participants across categories of normoglycaemia, impaired fasting glucose and diabetes, although a statistical comparison of this difference between ethnic groups were not reported. Interestingly, in MESA there was attenuation of the trend for wider retinal arterioles with increasing dysglycaemia in African Americans compared with White Americans [42].

Why diabetes might attenuate the relationship between retinal arteriolar narrowing and BP is uncertain. Other studies examining structural remodelling of subcutaneous resistance arteries in type 2 diabetes in man have also indicated that diabetes impairs eutrophic remodelling in hypertension [49;50]. Numerous studies (reviewed in [4;14]) have also shown that diabetes is

associated with reduced myogenic vasoconstriction in response to elevations in BP and impaired autoregulation. Vasoconstriction causes active arteriolar narrowing but it has also been proposed that prolonged vasoconstriction may result in structural remodelling [7]. Impairment of myogenic responses in diabetes could therefore account for attenuated structural remodelling in addition to reduced active vasoconstriction. In principle, impaired remodelling and reduced myogenic tone will result in increased transmission of systemic arterial pressure to the microvascular bed with consequential damage. Further studies on this question would be valuable in elucidating possible mechanisms by which diabetes and hypertension interact to accelerate microvascular damage.

This study has a number of limitations: the number of individuals studied is relatively small and it therefore has limited statistical power for some analyses. The study is cross sectional and therefore can only provide limited insight into mechanisms. The majority of participants were prescribed oral hypoglycaemic agents for their diabetes and many were also taking antihypertensive, lipid lowering and antiplatelet therapy. Since use of these agents was generally similar in both ethnic groups it is unlikely that this could explain interethnic differences, but we cannot exclude the possibility that treatment may have influenced other relationships between risk factors and retinal microvascular parameters. Another potential confounder could be differences in retinal pigmentation [51] causing errors in diameter measurements. We believe the algorithm we used is likely to be robust in the face of differences in retinal pigmentation since it depends on both detection of curvature and edges [29;30]. Moreover if there were a problem detecting the vessel edge one would also expect venular diameters to be wider in people of African Caribbean ethnicity, which was not the case. Nevertheless, this question is difficult to settle with absolute certainty since there is no independent means of validating vessel diameter in different ethnic groups and appropriate modification of image brightness or contrast is not straightforward and could itself introduce bias. Any inter-ethnic study of retinal vasculature is potentially subject to this criticism and currently there is no simple solution.

In summary, we have shown that African-Caribbean people with diabetes have relatively wider arterioles than Europeans with diabetes and that this difference is not fully accounted for by measured risk factors. In addition the predicted relationship between higher BP and arteriolar narrowing appears attenuated in diabetes.

REFERENCES

1. Fong,D.S., Aiello,L., Gardner,T.W., King,G.L., Blankenship,G., Cavallerano,J.D., Ferris,F.L., III and Klein,R. (2003) Diabetic retinopathy. *Diabetes Care* **26**, 226-229.
2. Bek,T. and Helgesen,A. (2001) The regional distribution of diabetic retinopathy lesions may reflect risk factors for progression of the disease. *Acta Ophthalmol.Scand.* **79**, 501-505.
3. Fong,D.S., Aiello,L., Gardner,T.W., King,G.L., Blankenship,G., Cavallerano,J.D., Ferris,F.L., III and Klein,R. (2004) Retinopathy in diabetes. *Diabetes Care* **27 Suppl 1**, S84-S87.
4. Kohner,E.M. (1997) Diabetic retinopathy and high blood pressure: defining the risk. *Am.J.Hypertens.* **10**, 181S-183S.
5. UK Prospective Diabetes Study Group (1998) Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* **317**, 703-713.
6. Heagerty,A.M., Aalkjaer,C., Bund,S.J., Korsgaard,N. and Mulvany,M.J. (1993) Small artery structure in hypertension. Dual processes of remodeling and growth. *Hypertension* **21**, 391-397.
7. Izzard,A.S., Rizzoni,D., Agabiti-Rosei,E. and Heagerty,A.M. (2005) Small artery structure and hypertension: adaptive changes and target organ damage. *J.Hypertens.* **23**, 247-250.
8. Folkow,B. (1982) Physiological aspects of primary hypertension. *Physiol.Rev.* **62**, 347-504.
9. James,M.A., Tullett,J., Hemsley,A.G. and Shore,A.C. (2006) Effects of aging and hypertension on the microcirculation. *Hypertension* **47**, 968-974.
10. Porta,M., Grosso,A. and Veglio,F. (2005) Hypertensive retinopathy: there's more than meets the eye. *J Hypertens.* **23**, 683-696.
11. Stanton,A.V., Mullaney,P., Mee,F., O'Brien,E.T. and O'Malley,K. (1995) A method of quantifying retinal microvascular alterations associated with blood pressure and age. *J.Hypertens.* **13**, 41-48.
12. Wong,T.Y., Wang,J.J., Rochtchina,E., Klein,R. and Mitchell,P. (2004) Does refractive error influence the association of blood pressure and retinal vessel diameters? The Blue Mountains Eye Study. *Am.J.Ophthalmol.* **137**, 1050-1055.
13. Hughes,A.D., Martinez-Perez,E., Jabbar,A.S., Hassan,A., Witt,N.W., Mistry,P.D., Chapman,N., Stanton,A.V., Beevers,G., Pedrinelli,R., Parker,K.H. and Thom,S.A. (2006) Quantification of topological changes in retinal vascular architecture in essential and malignant hypertension. *J Hypertens.* **24**, 889-894.
14. Wiernsperger,N.F. (2001) In defense of microvascular constriction in diabetes. *Clin.Hemorheol.Microcirc.* **25**, 55-62.
15. Keen,H. (2000) Therapeutic objectives and their practical achievement in type 2 diabetes. *J Diabetes Complications* **14**, 180-184.

16. Chaturvedi,N., McKeigue,P.M. and Marmot,M.G. (1994) Relationship of glucose intolerance to coronary risk in Afro-Caribbeans compared with Europeans. *Diabetologia*. **37**, 765-772.
17. Harris,M.I., Flegal,K.M., Cowie,C.C., Eberhardt,M.S., Goldstein,D.E., Little,R.R., Wiedmeyer,H.M. and Byrd-Holt,D.D. (1998) Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. *Diabetes Care* **21**, 518-524.
18. Harris,M.I., Klein,R., Cowie,C.C., Rowland,M. and Byrd-Holt,D.D. (1998) Is the risk of diabetic retinopathy greater in non-Hispanic blacks and Mexican Americans than in non-Hispanic whites with type 2 diabetes? A U.S. population study. *Diabetes Care* **21**, 1230-1235.
19. Klein,R., Sharrett,A.R., Klein,B.E., Moss,S.E., Folsom,A.R., Wong,T.Y., Brancati,F.L., Hubbard,L.D. and Couper,D. (2002) The association of atherosclerosis, vascular risk factors, and retinopathy in adults with diabetes : the atherosclerosis risk in communities study. *Ophthalmol.* **109**, 1225-1234.
20. McDonough,J.R., Garrison,G.E. and Hames,C.G. (1964) Blood pressure and hypertensive disease among Negroes and whites. *Ann.Intern.Med.* **61 suppl 6**, 208-228.
21. Schneckloth,R.E., Stuart,K.L. and Moore,F.E. (1962) Arterial pressure and hypertensive disease in a West Indian Negro population. Report of a survey in St Kitts, West Indies. *Am.Heart J.* **63**, 607-628.
22. Wong,T.Y., Klein,R., Islam,F.M., Cotch,M.F., Folsom,A.R., Klein,B.E., Sharrett,A.R. and Shea,S. (2006) Diabetic retinopathy in a multi-ethnic cohort in the United States. *Am.J.Ophthalmol.* **141**, 446-455.
23. Sharp,P.S., Chaturvedi,N., Wormald,R., McKeigue,P.M., Marmot,M.G. and Young,S.M. (1995) Hypertensive retinopathy in Afro-Caribbeans and Europeans. Prevalence and risk factor relationships. *Hypertension* **25**, 1322-1325.
24. Wong,T.Y., Klein,R., Duncan,B.B., Nieto,F.J., Klein,B.E., Couper,D.J., Hubbard,L.D. and Sharrett,A.R. (2003) Racial differences in the prevalence of hypertensive retinopathy. *Hypertension* **41**, 1086-1091.
25. Strain,W.D., Chaturvedi,N., Nihoyannopoulos,P., Bulpitt,C.J., Rajkumar,C. and Shore,A.C. (2005) Differences in the association between type 2 diabetes and impaired microvascular function among Europeans and African Caribbeans. *Diabetologia*. **48**, 2269-2277.
26. Strain,W.D., Chaturvedi,N. and Shore,A. (2005) Ethnic differences in microvascular structure and function. *J.Hypertens.* **23**, 1434-1435.
27. Strain,W.D., Chaturvedi,N., Leggetter,S., Nihoyannopoulos,P., Rajkumar,C., Bulpitt,C.J. and Shore,A.C. (2005) Ethnic differences in skin microvascular function and their relation to cardiac target-organ damage. *J.Hypertens.* **23**, 133-140.
28. Chaturvedi,N. and McKeigue,P.M. (1994) Methods for epidemiological surveys of ethnic minority groups. *J.Epidemiol.Community Health* **48**, 107-111.

29. Martinez-Perez,M.E., Hughes,A.D., Stanton,A.V., Thom,S.A., Chapman,N., Bharath,A.A. and Parker,K.H. (2002) Retinal vascular tree morphology: a semi-automatic quantification. *IEEE Trans.Biomed.Eng.* **49**, 912-917.
30. Martinez-Perez, M. E. Computer Analysis of the Geometry of the Retinal Vasculature. 2001. PhD Thesis, Imperial College London.
31. Hubbard,L.D., Brothers,R.J., King,W.N., Clegg,L.X., Klein,R., Cooper,L.S., Sharrett,A.R., Davis,M.D. and Cai,J. (1999) Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmol.* **106**, 2269-2280.
32. Wong,T.Y., Knudtson,M.D., Klein,R., Klein,B.E., Meuer,S.M. and Hubbard,L.D. (2004) Computer-assisted measurement of retinal vessel diameters in the Beaver Dam Eye Study: methodology, correlation between eyes, and effect of refractive errors. *Ophthalmol.* **111**, 1183-1190.
33. King,L.A., Stanton,A.V., Sever,P.S., Thom,S.A. and Hughes,A.D. (1996) Arteriolar length-diameter (L:D) ratio: a geometric parameter of the retinal vasculature diagnostic of hypertension. *J Hum.Hypertens.* **10**, 417-418.
34. Witt,N., Wong,T.Y., Hughes,A.D., Chaturvedi,N., Klein,B.E., Evans,R., McNamara,M., Thom,S.A. and Klein,R. (2006) Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. *Hypertension* **47**, 975-981.
35. Pose-Reino,A., Gomez-Ulla,F., Hayik,B., Rodriguez-Fernandez,M., Carreira-Nouche,M.J., Mosquera-Gonzalez,A., Gonzalez-Penedo,M. and Gude,F. (2005) Computerized measurement of retinal blood vessel calibre: description, validation and use to determine the influence of ageing and hypertension. *J Hypertens.* **23**, 843-850.
36. Hemminki,V., Kahonen,M., Tuomisto,M.T., Turjanmaa,V. and Uusitalo,H. (2007) Determination of retinal blood vessel diameters and arteriovenous ratios in systemic hypertension: comparison of different calculation formulae. *Graefes Arch Clin.Exp.Ophthalmol* **245**, 8-17.
37. Stanton,A.V., Wasan,B., Cerutti,A., Ford,S., Marsh,R., Sever,P.P., Thom,S.A. and Hughes,A.D. (1995) Vascular network changes in the retina with age and hypertension. *J.Hypertens.* **13**, 1724-1728.
38. Bland,J.M. and Altman,D.G. (1999) Measuring agreement in method comparison studies. *Stat.Methods Med.Res.* **8**, 135-160.
39. Lin,L.I. (1989) A concordance correlation coefficient to evaluate reproducibility. *Biometrics* **45**, 255-268.
40. Chapman,N., Witt,N., Gao,X., Bharath,A.A., Stanton,A.V., Thom,S.A. and Hughes,A.D. (2001) Computer algorithms for the automated measurement of retinal arteriolar diameters. *Br.J.Ophthalmol.* **85**, 74-79.
41. Klein,R., Klein,B.E., Moss,S.E., Wong,T.Y., Hubbard,L., Cruickshanks,K.J. and Palta,M. (2003) Retinal vascular abnormalities in persons with type 1 diabetes: the Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVIII. *Ophthalmol.* **110**, 2118-2125.

42. Nguyen,T.T., Wang,J.J., Sharrett,A.R., Islam,F.M., Klein,R., Klein,B.E., Cotch,M.F. and Wong,T.Y. (2008) Relationship of retinal vascular caliber with diabetes and retinopathy: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* **31**, 544-549.
43. Scheie,H.G. (1953) Evaluation of ophthalmoscopic changes of hypertension and arteriolar sclerosis. *Arch.Ophthalmol.* **49**, 117-138.
44. Wong,T.Y., Klein,R., Klein,B.E., Tielsch,J.M., Hubbard,L. and Nieto,F.J. (2001) Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Surv.Ophthalmol.* **46**, 59-80.
45. Wong,T.Y., Hubbard,L.D., Klein,R., Marino,E.K., Kronmal,R., Sharrett,A.R., Siscovick,D.S., Burke,G. and Tielsch,J.M. (2002) Retinal microvascular abnormalities and blood pressure in older people: the Cardiovascular Health Study. *Br.J.Ophthalmol.* **86**, 1007-1013.
46. Wang,J.J., Mitchell,P., Leung,H., Rochtchina,E., Wong,T.Y. and Klein,R. (2003) Hypertensive retinal vessel wall signs in a general older population: the Blue Mountains Eye Study. *Hypertension* **42**, 534-541.
47. Wong,T.Y., Klein,R., Sharrett,A.R., Manolio,T.A., Hubbard,L.D., Marino,E.K., Kuller,L., Burke,G., Tracy,R.P., Polak,J.F., Gottdiener,J.S. and Siscovick,D.S. (2003) The prevalence and risk factors of retinal microvascular abnormalities in older persons: The Cardiovascular Health Study. *Ophthalmol.* **110**, 658-666.
48. Tillin,T., Evans,R.M., Witt,N.W., Sharp,P.S., McKeigue,P.M., Chaturvedi,N. and Hughes,A.D. (2008) Ethnic differences in retinal microvascular structure. *Diabetologia* **51**, 1719-1722.
49. Rizzoni,D., Porteri,E., Guelfi,D., Muiesan,M.L., Valentini,U., Cimino,A., Girelli,A., Rodella,L., Bianchi,R., Sleiman,I. and Rosei,E.A. (2001) Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non-insulin-dependent diabetes mellitus. *Circulation* **103**, 1238-1244.
50. Schofield,I., Malik,R., Izzard,A., Austin,C. and Heagerty,A. (2002) Vascular structural and functional changes in type 2 diabetes mellitus: Evidence for the roles of abnormal myogenic responsiveness and dyslipidemia. *Circulation* **106**, 3037-3043.
51. Rochtchina,E., Wang,J.J., Taylor,B., Wong,T.Y. and Mitchell,P. (2008) Ethnic variability in retinal vessel caliber: a potential source of measurement error from ocular pigmentation?-- the Sydney Childhood Eye Study. *Invest Ophthalmol Vis.Sci* **49**, 1362-1366.

TABLE 1. Reproducibility of retinal vascular measures

	mean difference	SD _{diff}	CV, %	ρ_c	n
Arteriolar diameter, μm	0.4	2.0	2.3	0.95	10
Arteriolar length, μm	-12.0	51.9	2.9	0.99	10
Venular diameter, μm	0.5	1.4	1.2	0.98	10
Venular length, μm	13.4	39.6	2.2	>0.99	10
Arteriolar tortuosity	-0.001	0.004	0.4	0.96	10
Arteriolar LDR	-0.09	0.87	3.9	0.98	10
Venular LDR	0.04	0.37	2.3	>0.99	10
AVR	0.00	0.02	2.1	0.98	10

Abbreviations AVR = arteriolar venular ratio, CV = within observer coefficient of variation, LDR = length diameter ratio, ρ_c = Lin's concordance coefficient, SD_{diff} = standard deviation of the difference, n = number of subjects.

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TABLE 2. Characteristics of the population studied.

	Europeans (n = 22)	African-Caribbeans (n = 29)	P value
Age, yrs	56 (7)	55 (8)	0.6
Male, n (%)	13 (59)	12 (41)	0.2
Body mass index, kg/m ²	32.0 (5.6)	31.9 (6.0)	0.9
Waist Hip Ratio	0.95 (0.07)	0.92 (0.08)	0.3
Systolic BP, mmHg	146 (24)	153 (22)	0.3
Diastolic BP, mmHg	84 (10)	87 (11)	0.3
Heart rate, beats/min	71 (2)	70 (2)	0.8
24h systolic BP, mmHg [†]	114 (11)	117 (13)	0.5
24h diastolic BP, mmHg [†]	73 (8)	73 (12)	>0.9
Glucose, mmol/l	8.2 (2.5)	8.9 (3.6)	0.5
Insulin, pmol/l	12.5 (7.6)	15.7 (15.8)	0.4
HbA _{1c} , %	7.9 (1.8)	7.7 (1.9)	0.8
Total cholesterol, mmol/l	4.5 (1.5)	4.7 (0.8)	0.6
HDL cholesterol, mmol/l	1.1 (0.9, 1.3)	1.2 (1.1, 1.5)	0.1
Triglycerides, mmol/l	1.6 (1.0, 2.2)	0.8 (0.7, 1.2)	0.02
Smoking (never/current/ex), n (%)	3(17) / 10(56) / 5(28)	2(8) / 9(38) / 13(54)	0.2
Hypertension, n (%)	17 (81)	26 (90)	0.4
Antihypertensive treatment, n (%)	13 (59)	17 (59)	>0.9
Lipid lowering treatment, n (%)	10 (45)	6 (21)	0.06
Aspirin treatment, n (%)	11 (50)	11 (38)	0.4
Oral hypoglycaemic treatment, n (%)	14 (63)	18 (62)	0.9
Insulin treatment, n (%)	3 (14)	5 (17)	0.7
Duration of diabetes, years	3.0 (1.5, 6.1)	7.0 (4.0, 10.4)	0.02

Abbreviations BP = blood pressure, HDL = high density lipoprotein. Continuous data are shown as means (SD) or medians (interquartile range) for skewed data. Categorical data are n (%). [†] n = 19 and 18 for Europeans and African-Caribbeans respectively. Statistical comparisons were made using a Student's t-test, a Mann Whitney rank test for skewed data, or a Chi² test for categorical data.

TABLE 3. Comparison of retinal parameters between ethnic groups.

	Europeans (n = 22)	African-Caribbeans (n = 29)	P value
Arteriolar diameter, μm	76 (7)	82 (11)	0.03
Arteriolar length, mm	2.09 (0.67)	1.92 (0.55)	0.3
Arteriolar LDR	28.1 (8.5)	23.7 (7.0)	0.046
Arteriolar N_T	5.9 (3.4)	7.0 (4.5)	0.3
Arteriolar tortuosity	1.086 (0.028)	1.010 (0.034)	0.1
Arteriolar angle, $^\circ$	86.5 (21.4)	73.2 (16.8)	0.03
Venular LDR	21.1 (5.9)	22.1 (9.2)	0.7
Venular diameter, μm	125 (40)	109 (10)	0.3
Venular length, mm	2.48 (1.10)	2.46 (1.76)	0.9
Venular N_T	6.6 (3.6)	7.7 (4.0)	0.4
Venular tortuosity	1.087 (0.029)	1.088 (0.044)	0.9
Venular angle, $^\circ$	72.9 (18.4)	70.4 (15.8)	0.6
AVR	0.66 (0.21)	0.90 (0.36)	0.028

Data are means (SD), statistical comparisons were made using a Student's t-test.

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TABLE 4. Ethnic differences in measures of arteriolar narrowing after adjustment for various covariates

	Europeans (n = 22)	African-Caribbeans (n = 29)	P value
<i>Unadjusted</i>			
Arteriolar diameter	76 (2)	82 (2)	0.03
Arteriolar LDR	28.1 (1.8)	23.7 (1.3)	0.046
AVR	0.66 (0.05)	0.90 (0.08)	0.028
<i>Adjusted for SBP</i>			
Arteriolar diameter	76 (2)	82(2)	0.04
Arteriolar LDR	28.3 (1.6)	23.5 (1.4)	0.03
AVR	0.66 (0.08)	0.91 (0.07)	0.02
<i>Adjusted for fasting triglycerides</i>			
Arteriolar diameter	76 (2)	82 (2)	0.09
Arteriolar LDR	28.7 (1.7)	23.2 (1.4)	0.02
AVR	0.67 (0.08)	0.90 (0.07)	0.049
<i>Adjusted for duration of diabetes</i>			
Arteriolar diameter	75 (2)	80 (2)	0.06
Arteriolar LDR	26.8 (1.7)	24.9 (1.5)	0.4
AVR	0.68 (0.09)	0.89 (0.08)	0.1
<i>Adjusted for HbA1c</i>			
Arteriolar diameter	76 (2)	83 (2)	0.02
Arteriolar LDR	27.6 (1.7)	23.8 (1.4)	0.09
AVR	0.65 (0.09)	0.91 (0.07)	0.03
<i>Adjusted for fasting glucose</i>			
Arteriolar diameter	75 (2)	82 (2)	0.03
Arteriolar LDR	28.1 (1.7)	23.7 (1.4)	0.05
AVR	0.65 (0.08)	0.90 (0.07)	0.02

Data are adjusted means (standard error of the prediction) and p values were calculated by analysis of covariance (ANCOVA).

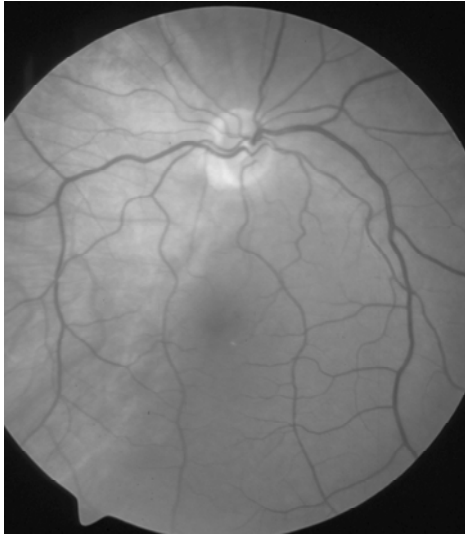
FIGURE LEGENDS

Figure 1. An example showing a digitized red-free image (A) and the segmented retinal vascular tree (B) after processing with the RISA program.

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A



B



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FIGURE 1