**Title: Retinal vasculometry associations with cardiometabolic risk factors in the European Prospective Investigation of Cancer Norfolk study**

Authors: Christopher G Owen PhD,1 Alicja R Rudnicka PhD,1 Roshan A Welikala PhD,2 M Moazam Fraz PhD,3 Sarah A Barman PhD,2 Robert Luben MSc,4 Shabina A Hayat PhD,4 Kay-Tee Khaw FRCP,4 David P Strachan MD,1 Peter H Whincup FRCP, 1 Paul J Foster FRCOphth 5,6

1. Population Health Research Institute, St George’s, University of London, Cranmer Terrace, London, SW17 0RE, UK

2. Faculty of Science, Engineering and Computing, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK

3. School of Electrical Engineering and Computer Science, National University of Sciences and Technology, Campus H-12, Islamabad, Pakistan

4. Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge

5. Integrative Epidemiology Research Group, UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK

6. NIHR Biomedical Research Centre at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London EC1V 2PD, UK.

Words: Abstract 350 words, main text 4524 words

## SUPPLEMENTAL MATERIAL

This article contains additional online-only material. The following should appear online-only: Supplemental Figures 1, 2 and 3 and Supplementary Table 1.

## FINANCIAL SUPPORT

EPIC was funded by the Medical Research Council, UK (G0401527), and Research into Ageing, UK (262). The retinal vessel morphometry work was supported by the Medical Research Council Population and Systems Medicine Board (MR/L02005X/1) and British Heart Foundation (PG/15/101/31889). Prof Foster has received additional support from the Richard Desmond Charitable Trust (via Fight for Sight) and the Department for Health through the award made by the National Institute for Health Research to Moorfields Eye Hospital and the UCL Institute of Ophthalmology for a Biomedical Research Centre. The views expressed in this article are those of the authors and not necessarily those of the Department for Health.

## CONFLICT of interest

None.

## RUNNING HEADER

Retinal vasculometry associations with cardiometabolic risk factors

## CORRESPONDING AUTHOR AND ADDRESS FOR REPRINTS

Professor Christopher G Owen, as above, tel: +44 (0)20 87252796, fax: +44 (0)20 87253584, e-mail: cowen@sgul.ac.uk

## Abstract

Purpose: To examine associations between retinal vessel morphometry and cardiometabolic risk factors in older British men and women.

Design: Retinal imaging examination as part of the European Prospective Investigation into Cancer-Norfolk Eye study.

Participants: 7411 participants underwent retinal imaging and clinical assessment. Retinal images were analysed using a fully automated validated computerised system, which provides novel measures of vessel morphometry.

Methods: Associations between cardiometabolic risk factors, chronic disease and retinal markers were analyzed using multi-level linear regression, adjusted for age, sex and within person clustering, to provide percentage differences in tortuosity and absolute differences in width.

Main outcomes measures: Retinal arteriolar and venular tortuosity and width.

Results: 279,802 arterioles, and 285,791 venules from 5947 participants (mean age 67.6 years, SD 7.6, 57% female) were analysed. Increased venular tortuosity was associated with higher BMI (2.5%, 95% CI 1.7,3.3% per 5 kg/m²) and HbA1c (2.2%, 95%CI 1.0,3.5% per %), and with prevalent type 2 diabetes (6.5%, 95%CI 2.8,10.4%); wider venules were associated with older age (2.6m, 95%CI 2.2,2.9m per decade), higher triglycerides (0.6µm, 95%CI 0.3,0.9µm per mmol/L), BMI (0.7m, 95%CI 0.4,1.0 per 5 kg/m²) and HbA1c (0.4µm, 95%CI -0.1,0.9 per %) and being a current smoker (3.0m, 95%CI 1.7,4.3m); similarly smoking was also associated with wider arterioles (2.1m, 95%CI 1.3,2.9m). Thinner venules were associated with HDL (1.4m, 95%CI 0.7,2.2 per mmol/L). Arteriolar tortuosity increased with age (5.4%, 95%CI 3.8,7.1% per decade), higher systolic blood pressure (1.2%, 95%CI 0.5,1.9% per 10mmHg), in females (3.8, 95%CI 1.4,6.4%) and with prevalent stroke (8.3%, 95%CI -0.6,18%); no association was observed with prevalent myocardial infarction. Narrower arterioles were associated with age (0.8m, 95%CI 0.6,1.0m per decade), higher systolic blood pressure (0.5m, 95%CI 0.4,0.6m per 10mmHg), total cholesterol (0.2m, 95%CI 0.0,0.3m per mmol/L) and HDL (1.2m, 95%CI 0.7,1.6m per mmol/L).

Conclusions: Metabolic risk factors show a graded association with both tortuosity and width of retinal venules, even among people without clinical diabetes, whereas atherosclerotic risk factors correlate more closely with arteriolar width, even excluding those with hypertension and cardiovascular disease. These non-invasive microvasculature measures should be evaluated further as predictors of future cardiometabolic disease among apparently healthy individuals.

Keywords: Retinal vessels, morphology, cardiometabolic risk factors

Cardiovascular disease (CVD), including coronary heart disease (CHD), heart failure and stroke, is responsible for a substantial burden of morbidity and disability.1 Type 2 diabetes is an increasing public health problem, affecting 1 in 10 adults globally, and a major cause of premature death and morbidities, especially CVD.2 Early detection and prevention both of CVD and Type 2 diabetes is key to limiting future morbidity and mortality.3;4 While disease risk factors for Type 2 diabetes, such as blood glucose levels and HbA1c, are yet to show good screening performance,5 established markers of early vascular disease are used in risk prediction models to estimate future risk of CVD, providing indications for medical / lifestyle interventions to alter disease trajectory.6;7 There have been a number of attempts to improve the performance of these risk prediction models, by adding other risk factors.6;7 However, the addition of novel risk factors have added little to CHD prediction.8 Recent evidence suggests that early markers for the presence of vascular disease (as opposed to additional risk factors) are needed to improve risk prediction for population screening.5;9

Detailed retinal vasculometry may offer such a marker. Growing evidence suggests that morphological features in retinal vessels, in particular vessel width, are early physiological markers of cardiometabolic risk and disease (as well as other disease processes).10-13 While strong evidence has accrued for some of these associations, particularly associations with Type 2 diabetes and CVD (and their related risk factors), other associations have remained inconsistent. For instance, wider arterioles have been associated with higher levels of blood glucose, total cholesterol, triglycerides and inflammatory markers, but not in all studies.10;12 Similarly associations of venular width with blood pressure have also been inconclusive,10 although recent evidence suggests increased width associated with hypertension.14 Wider venules also seem to be associated with diabetes, elevated glycosylated haemoglobin, lower levels of high density lipoprotein, inflammatory markers, smoking and obesity.10-12 However, some inconsistencies in the presence or absence of these associations (perhaps due to uncertainty caused by sample size) remain.11;12 Moreover, in comparison to studies examining vessel width, associations with vessel tortuosity have been little studied,15 especially in relation to metabolic markers, and may provide further insight into vasculometry changes associated with cardiometabolic risk. Large population studies are needed to resolve these uncertainties, and to allow the comparative performance of width and tortuosity associations to be gauged. However, the assessment of retinal vessel morphometry from retinal images, even with computerized assistance, has so far been heavily reliant on subjective operator involvement, which is time consuming and open to measurement error,16 limiting its use in large scale, preventative initiatives in a community setting. We have developed a fully automated system for examining retinal vessel size and tortuosity, which overcomes many of these difficulties.17-19 We have used this system to examine the associations between cardiometabolic risk factors and retinal vascular characteristics in a large prospective population study of older British men and women, to confirm associations previously reported with vessel width, but to provide novel associations with measures of vessel tortuosity.

## RESEARCH DESIGN AND Methods

**Study Population**:- The European Prospective Investigation into Cancer (EPIC) study is a European based prospective cohort study designed to investigate the aetiology of major chronic diseases.20 The UK component of the study, EPIC-Norfolk, recruited from general practices in and around the city of Norfolk, and examined 25,639 participants (99.7% white European) aged 40 to 79 at baseline, between 1993 and 1997 (response rate 33%).21;22 Study participants had a detailed examination (including anthropometry, blood pressure, urine and venous blood sampling) and questionnaire assessment at entry (including information on pre-existing cardiovascular disease, type 2 diabetes and other medical conditions), and completed periodic questionnaires about their health (with a particular focus on dietary habits). Participants have been followed up over a 13-year period for morbidity and mortality. In addition to questionnaire data, participants were invited for further clinical examinations over this period, including repeat anthropometric assessment, venous blood sampling, retinal imaging, and physiological measures.22

**Third Follow-Up**: Between 2004 and 2011, 8623 participants provided updated information on medical history and lifestyle behaviour.22 Weight and height, were measured with participants in light clothing without shoes. Weight was measured to the last 0.1 kg using regularly calibrated digital scales (Tanita TBF-300, Tanita UK Ltd, Middlesex, UK), and height to the last complete 0.1 cm using a stadiometer (Chasmors, UK). Body mass index (BMI) was calculated as weight / height squared in kg/m². Seated blood pressure was measured twice using an automated blood pressure monitor (Accutorr PlusTM, Datascope Patinet Monitoring, Huntington, UK); the mean of both measures was used. A non-fasting venous blood sample was collected; details of the analytic measures have been published previously.22 HbA1c was measured in whole blood using high performance liquid chromatography. Serum total cholesterol and HDL-cholesterol were measured using an auto-analyser (RA 1000 Technicon, Bayer Diagnostics, Basingstoke, UK). LDL-cholesterol was calculated using the Fredrickson–Friedewald equation.23

**Ocular Examination**: Ocular assessment included measurement of vision, visual acuity (LogMAR acuity) and closed field auto-refraction (Humphrey model 500, Humphrey Instruments, San Leandro, California, USA). Macular centred 45º digital fundus photographs were taken using a TRC-NW6S non-mydriatic retinal camera and IMAGEnet Telemedicine System (Topcon Corporation, Tokyo, Japan) with a 10 megapixel Nikon D80 camera (Nikon Corporation, Tokyo, Japan) without pharmacological dilation of the pupil. Image processing was carried out using an automated computerised system (QUARTZ).17-19 The automated system distinguishes between right and left eyes (by optic disc localisation), venules and arterioles, identifies vessel segments, out-puts centreline coordinates, and measures vessel width and angular change between vessel centreline coordinates, as well as providing further measures of tortuosity.17-19;24 An ensemble classifier of bagged decision trees (with colour information) was used to classify vessels as being either venules or arterioles. Only vessels which were classified with 80% or more probability were retained, to balance the number of venules and arterioles detected, as well as maximise the number of vessels included for analyses.18 The performance of the Arteriole/Venule (A/V) detection program was manually verified in a sub-set of images, and had detection rates of 84% for arterioles and 77% for venules, and corresponding false positive rates of 23% and 16% respectively.18 An automated assessment of image quality was also made based on the segmented vasculature.18 The system obtains thousands of measures of width and tortuosity from the whole retinal image (dependent on image quality), not just concentric areas centred on the disc.10 These measures were summarised using mean width in microns and tortuosity with arbitrary units, weighted by segment length, for arterioles and venules separately for each image. In the case of multiple images per person, an automated algorithm developed to assess image quality allowed the best right eye and best left eye images to be selected for analyses. A previously validated tortuosity measure which shows good agreement with subjective assessment of vessel tortuosity, based on the mean change in chord length between successive divisions of the vessel was used.24 System performance has been outlined in detail and validated previously, and allows automated batch processing of images from large population based studies.17-19 A model eye was used to quantify the magnification characteristics of the telecentric fundus camera used (Topcon TRC-NW6S), allowing pixel dimensions of vessel width to be converted to real size.25

**Ethics, Governance and Consent**: The EPIC-Norfolk Eye Study was carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk and Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent.

**Statistical Analysis**: Statistical analyses were carried out using STATA software (version 13, StataCorp LP, College Station, TX). Segment wise weighted mean widths and tortuosity were used, to provide a measure for venules and arterioles separately, for each eye. Histograms of retinal vessel widths showed normal distributions, while measures of tortuosity were positively skewed and log-transformed. Multilevel linear regression models adjusting for age and sex were used to examine associations of cardiometabolic risk factors and prevalent disease status to retinal vessel morphometry outcomes, allowing for repeated measures of vessel indices within the same person. Regression models provided mean differences in width and percentage differences in tortuosity for venules and arterioles separately, per decade in age, females versus males, current smokers and former smokers versus never-smokers, or per unit increase in cardiometabolic risk factor (per 5 kg/m² increase in BMI, per 10 mmHg in systolic and diastolic blood pressure, per mmol/L increase in total cholesterol, high density lipoprotein, triglyceride, and per percentage rise in HbA1c). For disease outcomes, differences in vessel indices were obtained comparing those with prevalent disease present (including type 2 diabetes, MI, stroke, and known / treated hypertension) versus absent. Differences in associations between men and women were formally examined by inclusion of an interaction term between the risk factor and sex into the regression model. Risk factors found to be statistically significantly related to vascular measures at the 5% level were subsequently included in mutually adjusted models. We also examined associations after exclusion of participants with prevalent disease outcomes.

## Results

Of 18,380 individuals invited to participate in this phase of the study, 8,623 (47%) took part (mean age 67.6 years, 57% female). Supplemental Figure 1 shows a flow diagram of the numbers participating in the study. Fundus imaging and refractive assessment were carried out in 7411 individuals, of whom 5,957 participants (80%) had at least one image of sufficient quality and classified vessels as arterioles or venules with a probability set at 80% detection. It was not possible to obtain useful data from the remainder as images were miscentred, defocussed, or were obstructed by lashes and/or media opacities. A small number had missing data for height, weight or blood pressure (n=10), leaving 5947 participants with measures of vessel width and tortuosity for 565,593 vessel segments (279,802,arterioles, 285,791 venules) from 10,474 images; blood sample data were available for 5514. Participant characteristics of EPIC participants at baseline, and those who took part in the third health examination with and without useable fundus images have been described previously.26 Those attending the 3rd Health Check (3HC) were younger at baseline, of higher BMI and socioeconomic status, and were less likely to be a current smoker compared to participants not followed-up.26 Participant characteristics of EPIC participants who took part in the third health examination, and who were included in the analyses compared with those who were not (5,947 versus 2,676 participants) are summarised in Table 1. Other than those included being slightly younger (mean age 68 years vs 71 years), there was no clear evidence of a systematic difference in 3HC participant characteristics. Retinal vessel morphometry in those with useable fundus images are also summarised for arterioles and venules separately. Histograms of arteriolar and venular width and tortuosity measures (with and without log transformation) are shown in Supplemental Figure 2, and shows appreciable variation in these measures within this study population.

Differences in retinal vessel width in microns, and percentage differences in tortuosity by Type 2 diabetes and CVD risk factors and outcomes are shown by vessel type in Table 2. Arterioles were inversely associated and more tortuous with older age (0.8μm, 95%CI 0.6, 1.0μm and 5.4%, 95%CI 3.8, 7.1% per decade respectively). Wider venules were observed with older age (mean difference 2.6μm, 95%CI 2.2,2.9μm per decade), and amongst current smokers compared to never smokers (3.0μm, 95%CI 1.7, 4.3μm). Narrower arterioles (0.5μm, 95%CI 0.2,0.8) and more tortuous arterioles and venules were strongly associated with being female compared to male (3.8%, 95% CI 1.4, 6.4%; 2.2%, 95% CI 0.7, 3.6% respectively).

*Retinal vasculometry associations with metabolic risk factors:-*

Venular width was positively associated with Type 2 diabetes risk factors, including higher BMI (0.7μm, 95%CI 0.4, 1.0μm per 5 kg/m²), and HbA1c (0.4μm, 95%CI -0.1, 0.9μm per %). Wider venules were also positively associated with elevated levels of triglycerides (0.6μm, 95%CI 0.3, 0.9μm per mmol/L). Venular tortuosity was also positively associated with Type 2 diabetes risk factors, as well as prevalent Type 2 diabetes. Venules were 2.5% more tortuous (95% CI 1.7, 3.3%) per 5 kg/m² increase in BMI, 2.2% more tortuous (95% CI 1.0, 3.5%) per percentage rise in HbA1c, and 6.5% more tortuous (95% CI 2.8, 10.4%) amongst those with Type 2 diabetes compared to those without.

*Retinal vasculometry associations with cardiovascular risk factors:-*

Arteriolar widths were inversely associated with age, systolic (0.5μm 95%CI 0.4, 0.6μm per 10mmHg rise) and diastolic blood pressure (1.0μm, 95%CI 0.9, 1.2μm per 10mmHg rise). Arteriolar tortuosity was also positively associated with systolic blood pressure (1.2%, 95% CI 0.5, 1.9% per 10mmHg respectively). Arteriolar width was inversely associated with total cholesterol (0.2μm, 95%CI 0.0, 0.3μm per mmol/L) and HDL (1.2μm, 95%CI 0.7, 1.6μm per mmol/L). Narrower venules and decreased venular tortuosity were associated with HDL cholesterol (1.4μm, 95%CI 0.7, 2.1μm, 1.8%, 95%CI -0.1, 3.7% less tortuosity per mmol/L). No associations were observed with prevalent MI, but there was a suggestion of increased arteriolar tortuosity with prevalent stroke (8.3%, 95%CI -0.6, 18%). Arterioles were narrower and more tortuous with increasing age; venular width increased with age. Both vessel types were wider amongst smokers compared with lifelong never smokers. Figure 1 shows the associations between retinal vessel indices and Type 2 diabetes and CVD risk factors by quintile; statistically significant associations appeared to be graded. These associations remained after exclusion of those with prevalent disease, including MI, stroke, and diabetes (n=466).

*Sensitivity and multiple variable analyses:-*

Sensitivity analyses examined the differences in vessel width and tortuosity associated with cardiometabolic risk factors, excluding those with clinical diabetes / cardiovascular disease, and those with known / treated hypertension (data available on request). Metabolic associations with venular width and tortuosity persist after exclusion of those with clinical diabetes, and arteriolar width associations with vascular risk factors (particularly blood pressure) remain after excluding those with cardiovascular disease and hypertension. Retinal vessel associations were similar in males and females (tests for interaction P>0.05), except for HDL, for which opposing associations with arteriolar tortuosity were apparent. Per mmol/L higher HDL, arteriolar tortuosity was 5.8% (95% CI 0.1, 11.8%) higher in men, but 4.0% (95% CI 0.0, 7.8%) lower in women (test for interaction p=0.006).

The mutual independence of these risk factor associations was also examined. Mutually adjusted risk factor associations are presented in Supplemental Table 1. Risk factors that were statistically significantly associated with retinal vasculometry in Table 2 were included in multiple variable regression models. Associations with both arteriolar morphometry measures and cardiometabolic risk factors remained remarkably stable. Consistent associations were observed between arteriolar width and age, current smoking status, blood pressure and HDL cholesterol, but there was no evidence of an independent association with total cholesterol. Similarly strong associations remained for arteriolar tortuosity with age, sex and blood pressure. Associations from mutually adjusted models for venular measures were also remarkably similar to the associations presented in Table 2. Venular width associations with age, current smoking, BMI and diastolic blood pressure were relatively unchanged, but associations with HDL cholesterol and triglycerides were attenuated towards the null. Further investigation showed that associations with lipids were primarily confounded by BMI. Venular tortuosity associations with sex and BMI were relatively unchanged. However, the association with HbA1c was attenuated (1.3%, 95%CI 0.0,2.6%, increase in venular tortuosity per % increase in HbA1c), and the association with systolic blood pressure was weakened by adjustment for BMI. Multilevel regression models adjusting for age, sex and blood pressure showed a stronger association with prevalent stroke than in Table 2, with 9.0% more tortuous arterioles amongst those who had suffered a stroke compare to those who had not (95%CI 0.1,18.8%, p<0.001), suggesting that the effect on arteriolar tortuosity is independent of systolic blood pressure. Increased venular tortuosity among those with prevalent diabetes was independent of sex, BMI and blood pressure (5.5%, 95%CI 1.4%,8.9%).

## Discussion

Our results are consistent with previously documented retinal vasculometry associations with Type 2 diabetes and CVD risk factors and outcomes,10-13 but provide further insight where uncertainties over the presence or absence of associations exist. Moreover, novel associations with vessel tortuosity provide further evidence of vasculometry changes. Findings suggest that Type 2 diabetes risk factors and prevalent Type 2 diabetes are associated with the morphology of retinal venules, both in terms of width and tortuosity, while coronary risk factors have a greater influence on arteriolar width. These associations remain after exclusion of those with prevalent diabetes, cardiovascular disease, and with known / treated hypertension, suggesting that these vessel changes may be indicative of preclinical phases of disease.

While retinal signs of diabetic eye disease are well described,27 there have been some uncertainties about the association between diabetes, particularly risk factors for Type 2 diabetes, and retinal vessel morphometry, with inconsistencies between cross-sectional and longitudinal findings.28 However, a recent meta-analysis showed that wider venules, but not arterioles, were associated with diabetes;29 consistent with cross-sectional observations suggesting that wider venules are associated with increasing levels of fasting glucose and HbA1c levels.28 Findings from the present study are consistent with these risk factor observations, not only replicating the associations between increased venular width and glycosylated haemoglobin (although not formally statistically significant), but also showing coherent associations with other metabolic risk factors, including BMI, as well as novel associations with levels of triglyceride; associations which were absent with arteriolar width. The present study also showed that narrow venules were associated with increased HDL, which when considered in relation to levels of triglyceride, might be considered as a further indicator of insulin resistance.30 However, venular width associations with HDL and triglycerides were weakened after multivariable adjustment, and HDL-tortuosity associations differed in males and females. Reasons for these sex differences are unclear, but may relate to sex differences observed in retinal width-CHD associations, where associations are evident in women not men.13;31 Moreover, this study was novel in showing consistent metabolic associations with retinal vessel tortuosity, whereby increased venular tortuosity was associated with Type 2 diabetes risk factors (including levels of BMI and HbA1c), in addition to showing a strong association with prevalent Type 2 diabetes. These associations persist after mutual adjustment, and exclusion of those with clinical diabetes, suggesting that these associations may be independent early markers of the disease process. Associations observed in this study appear to contrast with those observed with overt disease, whereby arteriolar (not venular) tortuosity has been related to the duration of diabetes.32 Associations with Type 2 diabetes risk markers (including levels of BMI and HbA1c), as well as other cardiovascular risk factors (systolic blood pressure and blood cholesterol) were not observed amongst this diseased group.32 This may suggest differences in retinal vessel morphometry associations between disease development and overt disease.

Cross sectional and longitudinal associations between retinal vasculometry and CVD outcomes have been studied, including coronary heart disease (CHD), stroke and cardiovascular mortality.13;33-35 However, more recent evidence from prospective studies has raised some inconsistencies. In particular, retinal vessel calibre changes are only associated with CHD events in women not men,13;31 and in some studies vessel width associations with stroke appear only apparent in venules, which appears to contradict the perceived disease process.36 In the present study, we observed no association between retinal vascular width measures and prevalent CHD, although there was the suggestion of a positive association between arteriolar tortuosity and prevalent stroke, which was stronger after adjustment for age, sex and blood pressure. An association between narrower arterioles and high blood pressure has been well documented.10;11;14;37 The present study confirms these findings, showing decreased arteriolar width associated with both increased systolic and diastolic blood pressure.

Evidence examining associations between venular width and blood pressure have been less consistent,10 although a recent meta-analysis suggested increased width associated with hypertension.14 Our study showed a small but statistically significant decrease in venular width with increasing diastolic blood pressure, which remained after multivariable adjustment, although the magnitude of association was less than the association observed with arterioles. This association was no longer statistically significant when those with prevalent cardiovascular disease and known / treated hypertension were excluded, but associations with systolic blood pressure remained. The observation of an association between vessel width and systolic blood pressure amongst non-hypertensives, strengthens the potential additional use of retinal vessel morphometry assessment in routine health checks. Of particular note were the different associations with vessel tortuosity, where increased arteriolar and venular tortuosity was associated with greater systolic blood pressure (but not diastolic blood pressure), while decreased venular tortuosity was associated with higher HDL. The apparent different direction of associations with these cardiovascular risk factors are potentially consistent, and replicate findings observed in one other large population based study.15

By far the strongest associations observed were those with age and smoking, where per decade rise in age there was arteriolar narrowing and increased tortuosity, and with current smoking appreciable arteriolar and venular dilation. There was also the suggestion of smaller arterioles and markedly greater tortuosity (both arteriolar and venular) in females compared to males. However, sex differences in width were largely explained and differences in tortuosity partially explained by height (data not presented). While differences in CVD risk between males and females may have contributed to these associations, explanations for potential sex differences in retinal vessel morphometry remain uncertain. The effect of age was independent of blood pressure, as well as other cardiometabolic risk factors, but smaller compared to a body of literature suggesting a 2 to 5µm decrease in arteriolar width per decade in age (although these later effect sizes were seen in relation to central retinal vessel equivalent sizes, which are 2-3 times larger as they are scaled-up from retinal measures taken within 0.5 to 1.5 disc diameters from the disc).10;38 Nevertheless, these observations demonstrate the well-known association between narrower more tortuous arterioles and older age.39 The vasodilatory effects of smoking have also been widely reported in venules, less so in arterioles.10 Increased carbon monoxide levels amongst smokers may well provide a biological explanation for these findings.40

Computerised assessment of vessels from retinal images have so far been heavily reliant on operator involvement, which is subjective, open to measurement error and time consuming,16 limiting its use in large population based studies. The EPIC Eye study is such a study, which is richly phenotyped, allowing examination of multiple CVD risk factors within the same cohort. Our fully automated system provides a rapid, detailed quantification of retinal vasculature in this population, for both arterioles and venules separately, since they show some opposing patterns of association with risk markers and disease states.41 The system has been extensively validated, and was successful in obtaining vessel measures in 4 out of five who underwent retinal imaging. It was not possible to obtain useful data from the remainder, as image quality was graded as insufficient (with the AV detection program unable to distinguish arterioles from venules), with images being decentred, defocussed, or obstructed by media opacities or lashes; an inevitable consequence of non-mydriasis, especially in this older age group. This did not appear to reflect a selection bias, as there was no evidence of a marked differences in other phenotypes between those with and without vessel measures. While those participating in the 3HC did appear to be select (being significantly younger, with higher BMI and of more privileged socioeconomic status compared to participants at baseline), this is unlikely to invalidate retinal vessel morphometry and cardiometabolic risk factor associations.42

Our image analysis system has improved performance or is similar to earlier approaches,43-46 obtaining measures from the whole retinal image, not just concentric areas centred on the disc.10 Earlier studies have considered effect sizes in relation to central retinal artery and central retinal vein equivalents (CRAE, CRVE).10 It was not possible to directly compare measures with CRAE and CRVE, as the number of measures of width were considerably more and located over the entire image. Reducing the measurement area, typically between 0.5 to 1.5 disc diameters, to provide these measures would result in a huge data reduction, which might exclude vessel changes occurring elsewhere in the retina. Moreover, poor agreement between different systems has been highlighted, making direct comparisons in retinal calibre measures between systems problematic.47 Despite this we report similar effect sizes (e.g., the change in vessel width associated with smoking) in relation to a narrower mean width indicative of a far greater measurement area. Vessel density is not uniform across the retina.48 Supplemental Figure 3 shows the extent of vessel measures in a typical image. While the measures are not constrained to concentric areas close to the disc, as used in comparable systems,47 this was not perceived as a weakness given that our system is fully automated and does not allow for measurement areas to be selected. Moreover, consistent inclusivity of measures across the whole image was observed in all images that were automatically selected as being of sufficient quality for inclusion, limiting any potential selection effects.19 Our approach is further supported by the first paper examining use of artificial intelligence (AI) in detecting cardiovascular disease, which appears to show that retinal vessels over their entire length are key areas of interest in estimating cardiovascular risk factors, such as age, blood pressure and HbA1c.49 While it is difficult at present to get precise information on how AI algorithms arrive at decisions, these findings suggest that retinal vasculometry studies, such as ours, are key to understanding processes associated with cardiometabolic disease.

We have condensed these measures to provide an overall summary of mean width, but it is possible that relative changes in vessel indices over time and perhaps variations in measures along the length of a vessel may be stronger predictors of vascular health than absolute size, although this remains to be established. The presence of differential retinal vasculometry associations with cardiometabolc risk factors underline the importance of making separate arteriolar and venular width and tortuosity measures, calling into question the validity of arteriolar / venular ratio measures for cardiovascular risk profiling.

The modest vasculometry association with prevalent stroke and the absence of associations with prevalent MI does not necessarily mean that retinal vasculometry measures are unlikely to have a role in CVD risk prediction. Prevalent cases are likely to be very different to premorbid incident cases, with established cases often receiving vasoactive medications, which might have a modifying effect on vascular morphometry. It is also possible that there was insufficient power to determine change in these dichotomous outcomes, given the small number of prevalent events within this study population. However, retinal vessel associations with Type 2 diabetes risk markers and diabetes mellitus were observed, even after exclusion of those with prevalent outcome, suggesting that pre-clinical vasculometry changes are apparent. This is commensurate with recent longitudinal evidence, raising the possibility that retinal vasculometry may have a role in risk prediction),50 as well as surveillance and disease management. Power to determine change in continuous outcomes was greater, replicating previous observations and yielding a number of novel associations, particularly those with vessel tortuosity, as well as metabolic markers. However, given the cross-sectional nature of data collection, these associations between cardiometabolic risk factors and retinal vessel abnormalities do not of themselves allow the potential role of retinal vessel quantification in disease risk prediction to be formally ascertained; future follow-up of this and other large cohorts with high quality retinal imaging data will allow this issue to be investigated.

## ACKNOWLEDGEMENTS

Dr Aachal Kotecha (UCL Institute of Ophthalmology) provided assistance with magnification correction of ophthalmic images using the model eye. Ms Connie Tang (University of Cambridge) helped with data preparation.

## Contribution STATEMENT

All Authors contributed to this manuscript. CGO,ARR,SAB,DPS,PHW,PJF designed the present study and raised funding; RL,SAH,NJW,KTK,PJF for the EPIC Eye study. RL,SAH,SAB,RAW,ARR collected data for the study and undertook data management. RAW,SAB,ARR analysed the data. CGO wrote the first draft of the report, to which all authors contributed. CGO is responsible for data integrity and will act as guarantor.

## REFERENCES

 (1) World Health Organization. Global status report on noncommunicable diseases 2014. <http://www.who.int/nmh/publications/ncd-status-report-2014/en/> [Accessed June 2016]; 2014.

 (2) World Health Organization. Global report on diabetes. Available from <http://www.who.int/diabetes/global-report/en/> [Accessed March 2017]; 2016.

 (3) Law MR, Watt HC, Wald NJ. The underlying risk of death after myocardial infarction in the absence of treatment. *Arch Intern Med* 2002; 162(21):2405-2410.

 (4) Herman WH, Ye W, Griffin SJ, Simmons RK, Davies MJ, Khunti K et al. Early Detection and Treatment of Type 2 Diabetes Reduce Cardiovascular Morbidity and Mortality: A Simulation of the Results of the Anglo-Danish-Dutch Study of Intensive Treatment in People With Screen-Detected Diabetes in Primary Care (ADDITION-Europe). *Diabetes Care* 2015; 38(8):1449-1455.

 (5) Barry E, Roberts S, Oke J, Vijayaraghavan S, Normansell R, Greenhalgh T. Efficacy and effectiveness of screen and treat policies in prevention of type 2 diabetes: systematic review and meta-analysis of screening tests and interventions. *BMJ* 2017; 356:i6538.

 (6) Collins GS, Altman DG. An independent and external validation of QRISK2 cardiovascular disease risk score: a prospective open cohort study. *BMJ* 2010; 340:c2442.

 (7) Hippisley-Cox J, Coupland C, Robson J, Brindle P. Derivation, validation, and evaluation of a new QRISK model to estimate lifetime risk of cardiovascular disease: cohort study using QResearch database. *BMJ* 2010; 341:c6624.

 (8) Kaptoge S, Di AE, Pennells L, Wood AM, White IR, Gao P et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med* 2012; 367(14):1310-1320.

 (9) Wannamethee SG, Welsh P, Lowe GD, Gudnason V, Di AE, Lennon L et al. N-terminal pro-brain natriuretic Peptide is a more useful predictor of cardiovascular disease risk than C-reactive protein in older men with and without pre-existing cardiovascular disease. *J Am Coll Cardiol* 2011; 58(1):56-64.

 (10) Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol* 2009; 54(1):74-95.

 (11) Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. *Ophthalmologica* 2013; 229(3):125-136.

 (12) Cheung CY, Ikram MK, Klein R, Wong TY. The clinical implications of recent studies on the structure and function of the retinal microvasculature in diabetes. *Diabetologia* 2015; 58(5):871-885.

 (13) Seidelmann SB, Claggett B, Bravo PE, Gupta A, Farhad H, Klein BE et al. Retinal Vessel Calibers in Predicting Long-Term Cardiovascular Outcomes: The Atherosclerosis Risk in Communities Study. *Circulation* 2016; 134(18):1328-1338.

 (14) Ding J, Wai KL, McGeechan K, Ikram MK, Kawasaki R, Xie J et al. Retinal vascular caliber and the development of hypertension: a meta-analysis of individual participant data. *J Hypertens* 2014; 32(2):207-215.

 (15) Cheung CY, Zheng Y, Hsu W, Lee ML, Lau QP, Mitchell P et al. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. *Ophthalmology* 2011; 118(5):812-818.

 (16) Trucco E, Ruggeri A, Karnowski T, Giancardo L, Chaum E, Hubschman JP et al. Validating retinal fundus image analysis algorithms: issues and a proposal. *Invest Ophthalmol Vis Sci* 2013; 54(5):3546-3559.

 (17) Fraz MM, Welikala RA, Rudnicka AR, Owen CG, Strachan DP, Barman SA. QUARTZ: Quantitative Analysis of Retinal Vessel Topology and size - An automated system for quantification of retinal vessels morphology. *Expert Systems with Applications* 2015; 42(20):7221-7234.

 (18) Welikala RA, Fraz MM, Hayat S, Rudnicka AR, Foster PJ, Whincup P et al. Automated retinal vessel recognition and measurements on large datasets. *EMBC* 2015;5239-5242.

 (19) Welikala RA, Fraz MM, Foster PJ, Whincup PH, Rudnicka AR, Owen CG et al. Automated retinal image quality assessment on the UK Biobank dataset for epidemiological studies. *Comput Biol Med* 2016; 71:67-76.

 (20) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997; 26 Suppl 1:S6-14.

 (21) Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br J Cancer* 1999; 80 Suppl 1:95-103.

 (22) Hayat SA, Luben R, Keevil VL, Moore S, Dalzell N, Bhaniani A et al. Cohort profile: A prospective cohort study of objective physical and cognitive capability and visual health in an ageing population of men and women in Norfolk (EPIC-Norfolk 3). *Int J Epidemiol* 2014; 43(4):1063-1072.

 (23) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18(6):499-502.

 (24) Owen CG, Rudnicka AR, Nightingale CM, Mullen R, Barman SA, Sattar N et al. Retinal arteriolar tortuosity and cardiovascular risk factors in a multi-ethnic population study of 10-year-old children; the Child Heart and Health Study in England (CHASE). *Arterioscler Thromb Vasc Biol* 2011; 31(8):1933-1938.

 (25) Bennett AG, Rudnicka AR, Edgar DF. Improvements on Littmann's method of determining the size of retinal features by fundus photography. *Graefes Arch Clin Exp Ophthalmol* 1994; 232(6):361-367.

 (26) Khawaja AP, Chan MP, Hayat S, Broadway DC, Luben R, Garway-Heath DF et al. The EPIC-Norfolk Eye Study: rationale, methods and a cross-sectional analysis of visual impairment in a population-based cohort. *BMJ Open* 2013; 3(3):e002684.

 (27) Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL, III et al. Diabetic retinopathy. *Diabetes Care* 1998; 21(1):143-156.

 (28) Nguyen TT, Wang JJ, Sharrett AR, Islam FM, Klein R, Klein BE et al. Relationship of retinal vascular caliber with diabetes and retinopathy: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2008; 31(3):544-549.

 (29) Sabanayagam C, Lye WK, Klein R, Klein BE, Cotch MF, Wang JJ et al. Retinal microvascular calibre and risk of diabetes mellitus: a systematic review and participant-level meta-analysis. *Diabetologia* 2015; 58(11):2476-2485.

 (30) McLaughlin T, Reaven G, Abbasi F, Lamendola C, Saad M, Waters D et al. Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? *Am J Cardiol* 2005; 96(3):399-404.

 (31) McGeechan K, Liew G, Macaskill P, Irwig L, Klein R, Klein BE et al. Meta-analysis: retinal vessel caliber and risk for coronary heart disease. *Ann Intern Med* 2009; 151(6):404-413.

 (32) Sasongko MB, Wong TY, Nguyen TT, Cheung CY, Shaw JE, Kawasaki R et al. Retinal Vessel Tortuosity and Its Relation to Traditional and Novel Vascular Risk Markers in Persons with Diabetes. *Curr Eye Res* 2016; 41(4):551-557.

 (33) Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet* 2001; 358(9288):1134-1140.

 (34) Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM et al. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA* 2002; 287(9):1153-1159.

 (35) Wong TY, Klein R, Nieto FJ, Klein BE, Sharrett AR, Meuer SM et al. Retinal microvascular abnormalities and 10-year cardiovascular mortality: a population-based case-control study. *Ophthalmology* 2003; 110(5):933-940.

 (36) McGeechan K, Liew G, Macaskill P, Irwig L, Klein R, Klein BE et al. Prediction of incident stroke events based on retinal vessel caliber: a systematic review and individual-participant meta-analysis. *Am J Epidemiol* 2009; 170(11):1323-1332.

 (37) Chew SK, Xie J, Wang JJ. Retinal arteriolar diameter and the prevalence and incidence of hypertension: a systematic review and meta-analysis of their association. *Curr Hypertens Rep* 2012; 14(2):144-151.

 (38) Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res* 2003; 27(3):143-149.

 (39) Kotliar KE, Mucke B, Vilser W, Schilling R, Lanzl IM. Effect of aging on retinal artery blood column diameter measured along the vessel axis. *Invest Ophthalmol Vis Sci* 2008; 49(5):2094-2102.

 (40) Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. *Arterioscler Thromb Vasc Biol* 2014; 34(3):509-515.

 (41) Fraz MM, Rudnicka AR, Owen CG, Strachan DP, Barman SA. Automated arteriole and venule recognition in retinal images using ensemble classification. *VISAPP* 2014; 3:194-202.

 (42) Pizzi C, De SB, Merletti F, Bellocco R, dos SS, I, Pearce N et al. Sample selection and validity of exposure-disease association estimates in cohort studies. *J Epidemiol Community Health* 2011; 65(5):407-411.

 (43) Dashtbozorg B, Mendonca AM, Campilho A. An Automatic Graph-based Approach for Artery/Vein Classification in Retinal Images. *IEEE Trans Image Process* 2013.

 (44) Huang Y, Zhang J, Huang Y. An automated computational framework for retinal vascular network labeling and branching order analysis. *Microvasc Res* 2012; 84(2):169-177.

 (45) Niemeijer M, Xu X, Dumitrescu AV, Gupta P, van GB, Folk JC et al. Automated measurement of the arteriolar-to-venular width ratio in digital color fundus photographs. *IEEE Trans Med Imaging* 2011; 30(11):1941-1950.

 (46) Saez M, Gonzalez-Vazquez S, Gonzalez-Penedo M, Barcelo MA, Pena-Seijo M, Coll de TG et al. Development of an automated system to classify retinal vessels into arteries and veins. *Comput Methods Programs Biomed* 2012; 108(1):367-376.

 (47) Yip W, Tham YC, Hsu W, Lee ML, Klein R, Klein B et al. Comparison of Common Retinal Vessel Caliber Measurement Software and a Conversion Algorithm. *Transl Vis Sci Technol* 2016; 5(5):11.

 (48) Henkind P. Radial peripapillary capillaries of the retina. I. Anatomy: human and comparative. *Br J Ophthalmol* 1967; 51(2):115-123.

 (49) Poplin R, Varadarajan AV, Blumer K, Liu Y, McConnell MV, Corrado GS et al. Prediction of cardiovascular risk factors from retinal fundus photographs via deep learning. *Nature Biomedical Engineering* 2018; 2:158-164.

 (50) Cheung CY, Sabanayagam C, Law AK, Kumari N, Ting DS, Tan G et al. Retinal vascular geometry and 6 year incidence and progression of diabetic retinopathy. *Diabetologia* 2017; 60(9):1770-1781.

## FIGURE LEGENDS

Figure 1: Adjusted mean vessel width and tortuosity by quintiles of cardiovascular and Type 2 diabetes risk factors, for venues and arterioles. Adjusted means (solid square symbols), 95% CIs (error bars), regression lines (solid line) and associated 95% CIs (dashed lines) are from a multilevel model allowing for age, sex and repeated measure of vessel indices within person.

Supplemental Figure 1: Flow diagram of participant recruitment for different phases of the European Prospective Investigation of Cancer in Norfolk study, and in particular the third follow-up which included an eye examination.

Supplemental Figure 2: Histogram of arteriolar and venular width and tortuosity measures (including with and without log transformation for tortuosity measures).

Supplemental Figure 3: Automated arteriolar (red) and venular (blue) width measures recorded in one EPIC Eye image.