**Associations of Retinal Microvascular Diameters and Tortuosity with Blood Pressure and Arterial Stiffness: UK Biobank**

**Running Title: Retinal Microvascular Morphology and Blood Pressure**

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To examine the baseline associations of retinal vessel morphometry with blood pressure (BP) and arterial stiffness in UK Biobank. The UK Biobank included 68,550 participants aged 40-69 years who underwent non-mydriatic retinal imaging, BP and arterial stiffness measurement (ASI). A full- automated image analysis program (QUARTZ) provided measures of retinal vessel diameter and tortuosity. The associations between retinal vessel morphology and CVD risk factors / outcomes were examined using multi-level linear regression, to provide absolute differences in vessel diameter and percentage differences in tortuosity (allowing within person clustering), adjusted for age, sex, ethnicity, clinic, body mass index, smoking and deprivation index. Greater arteriolar tortuosity was associated with higher systolic BP (relative increase 1.2%;95%CI 0.9,1.4% per 10mmHg), higher mean arterial pressure (MAP, 1.3%;0.9,1.7% per 10mmHg) and higher pulse pressure (PP,1.8%;1.4,2.2% per 10mmHg). Narrower arterioles were associated with higher systolic BP (-0.9µm;-0.94,-0.87µm per 10mmHg), MAP (-1.5µm; -1.5,-1.5µm per 10mmHg), PP (-0.7µm; -0.8,-0.7µm per 10mmHg) and ASI (-0.12µm; -0.14,-0.09µm per ms/m2). Associations were in the same direction but marginally weaker for venular tortuosity and diameter. This study assessing the retinal microvasculature at scale, has shown clear associations between retinal vessel morphometry, BP and ASI. These observations further our understanding of the preclinical disease processes, and interplay between microvascular and macrovascular disease.

**Key words**: Hypertension, cardiovascular disease, microvasculature, arterial stiffness, tortuosity

Heart disease remains a leading cause of death internationally1, despite advances in the diagnosis and treatment of hypertension and cardiovascular disease (CVD). It has long been thought the microvasculature may play a pivotal role in the pathogenesis of CVD2. Optimal vascular architecture achieves the most efficient blood flow with minimum energy allowing for maximum vascular diffusion3. Therefore alterations in geometry of the retinal vasculature may reflect a state of dysfunction of the microvasculature more generally and provide a non-invasive marker of lifetime CVD risk factor load2, 4 and potentially predict disease development2, 5.

Retinal arteriolar narrowing has consistently been associated with higher blood pressure (BP)6-8 the onset of hypertension,5, 9 and with an increased risk of incident CVD events and mortality2. Evidence is emerging to suggest an association between tortuosity and an increased risk of stroke4, 10 8, and a growing body of evidence has suggested associations with arterial stiffness, indicating systemic effects11. To date, limited research has been undertaken to assess the association of retinal vessel tortuosity with BP and the direction of associations observed with vessel tortuosity have been inconsistent4, 6, 12, 13. Previous smaller studies have shown an increase in vessel tortuosity (both arteriolar and venular tortuosity) with higher BP8, while others have shown an association in the opposite direction or no association at all4, 6, 12-14

With recent advances in technology, a study by Poplin et al, using a deep learning approach, has provided some encouraging evidence on the potential value of risk prediction utilising retinal imaging at scale15. While retinal vessels appear to be key areas of interest, understanding how these models predict risk still remains a substantial problem of the deep learning approach. Retinal vessel morphometry assessment provides insight into the nature of vessel changes, furthering our understanding of potential mechanistic pathways. We examined the association of retinal vessel morphometry (using a fully automated retinal image analysis system)16 with blood pressure (BP) and arterial stiffness in UK Biobank.

**Methods**

Anonymized data will be made available by the UK Biobank on request <https://www.ukbiobank.ac.uk/scientists-3/>. The UK Biobank recruited more than 500,000 people aged 40-69 years, between 2006-2010, from across the UK. A subset of 68,550 participants, from six UK Biobank centres had retinal images captured. The Cohort profile: design and methods in the eye and vision consortium of UK Biobank has recently been published17. The eye and vision sub-study has a very similar profile to the main UK Biobank study. Non-mydriatic fundus cameras (Topcon 3D OCT-1000 Mk 2) with a 45° field-of-view were used to capture colour images. Images were saved in PNG format with a resolution of 2048 x 1536 pixels.

*Physical examination*

Age, gender, and other sociodemographic characteristics (at a postcode level, neighborhood deprivation was expressed in terms of the the Townsend deprivation index, a higher Townsend index score implies a greater degree of deprivation) were collected by questionnaire. Height was measured to the nearest centimeter (cm) using a Seca 202 height measure, and a Tanita BC-418 body composition analyser was used to measure weight to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m2). Two BP and heart rate measurements were taken with an Omron 705 IT electronic BP monitor, while seated at least 1-minute apart18 and the average of the measures used in the analyses. A question on medication for BP was used to define antihypertensive medication usage. Hypertension was defined as having systolic BP ≥140 mmHg, or diastolic BP ≥90 mmHg, or self-reported use of antihypertensive medication. Mean arterial pressure (MAP) was calculated as [(2 x diastolic BP) + systolic BP] / 3. Mean pulse pressure (PP) was calculated as systolic BP minus diastolic BP. History of myocardial infarction, stroke and diabetes were determined by self-report. The HbA1c assay was performed using five Bio-Rad Variant II Turbo analysers. Analysis of serum biomarkers utilised 10 immunoassay analysers (6x DiaSorin Liaison XL & 4x Beckman Coulter DXI 800 and 4 clinical chemistry analysers (2x Beckman Coulter AU5800 & 2x Siemens Advia 1800).

*Retinal imaging and processing*

Retinal images were processed using an automated computerised system (QUARTZ)16. In brief, the automated system obtained thousands of measures of diameter and tortuosity from the whole retinal image16, 19, 20. These measures were summarised using mean diameter and tortuosity, weighted by vessel segment length, for arterioles and venules separately for each image. The following image processing modules were all validated on a subset of 4692 retinal images from a random sample of 2346 UK Biobank participants: including vessel segmentation, image quality score, optic disc detection, vessel width measurement, tortuosity measurement, arteriolar venular recognition16, 20, 21. The performance of the Arteriole/Venule (A/V) recognition had detection rates of up to 96% for arterioles and 98% for venules when the automated probability of arteriole or venule was set to a cut-off of 0.8. An automated assessment of image quality was also made based on the segmented vasculature21. The algorithm achieved a sensitivity of 95.3% and a specificity of 91.1% for the detection of inadequate images20. A model eye was used to quantify the magnification characteristics of the Topcon 3D OCT-1000 Mk 2 fundus camera, allowing pixel dimensions of vessel diameter to be converted to real size22.

*Arterial stiffness*

Arterial stiffness was measured non-invasively using the pulse waveform obtained at the finger with an infra-red sensor (Pulse Trace PCA2, CareFusion, USA)23. The time between peaks of the waveform (the peak-to-peak time; PPT) was divided by the person’s height to obtain the Arterial Stiffness Index (ASI).

The UK Biobank study was approved by the Northwest Region NHS research ethics committee.

**Statistical Analysis**

Stata 15.0 IC (Stata Corp LP, College Station, TX, USA) was used to analyse the data. Retinal vessel diameters were normal distributed, measures of tortuosity were positively skewed and therefore log-transformed. Multilevel linear regression models adjusting for age, sex, ethnicity and UK Biobank centre as fixed effects, with a random effect for person to allow for repeated measures of vessel indices within the same person (model 1), were used to examine associations of cardiometabolic risk factors with retinal vessel outcomes. Model 2 extended model 1 with further adjustment for smoking, Townsend deprivation index and BMI. Model 3 included the same factors as model 2 with additional adjustment for total cholesterol, triglycerides and HbA1c and exclusion participants with a history of myocardial infarction, stroke, diabetes, on treatment for hypertension or who answered unknown or declined to answer. Data missing on categorical variables were included as an additional category for each variable, to minimise data loss. Associations with the log-transformed tortuosity were exponentiated to give percentage differences in tortuosity and absolute differences (in microns) in vessel width per specified increase with CVD risk markers. Associations between retinal markers and each CVD risk factor were examined for interactions with sex; interactions were considered statistically significant if the p values for interaction were <0.01 given the large sample size of UK Biobank.

**Results**

A total of 54,714 participants (79.8% of UKBB participants who underwent fundus imaging) were included in these analyses (Figure 1). Population characteristics are shown in Table 1. Figure 2 shows adjusted mean retinal vessel measures (adjusted for age, sex and random effect for person), by risk factor deciles.

*Retinal Tortuosity, BP and ASI measures*

Increased arteriolar tortuosity was associated with higher systolic BP, diastolic BP, MAP and PP, after adjustment (table 2, model 1). For example, a 10mmHg higher systolic BP was associated with a 1.2% (95%CI 0.9,1.4%;p=6.4E-20) rise in arteriolar tortuosity. Associations observed were unaffected by additional adjustments in model 2 or after removal of participants with history of CVD and with adjustment for total cholesterol, triglycerides and HbA1c (model 3 – table S1). Increased retinal venular tortuosity was associated with higher systolic BP, diastolic BP, MAP, PP and ASI (model 1). For example, a 10mmHg rise in PP was associated with a 1.1% (0.8,1.3%) rise in venular tortuosity. Adjustment for factors in model 2 marginally attenuated the association of venular tortuosity with systolic BP and PP, but each association remained highly statistically significant. However, associations were no longer significant for MAP or ASI. Removal of participants with history of CVD (model 3, table S1) and adjustment for total cholesterol, triglycerides and hbA1c did not alter these associations but the association with diastolic BP was no longer statistically significant. The association of increased tortuosity (both arteriolar and venular tortuosity) with higher systolic BP remained after adjustment for retinal diameters.

*Retinal Diameters BP and ASI measures*

Narrower arterioles were associated with higher systolic BP, diastolic BP, MAP, PP, heart rate and ASI (Table 3, model 1). For example, a unit increase in ASI was associated with a -0.11µm (-0.13,-0.09µm;P=8.59E-23) decrease in arteriolar diameter which would equate approximately to 0.33µm narrowing per SD increase in ASI. Adjustments in model 2, had no impact on the association of narrower arterioles with higher systolic BP, diastolic BP, MAP, PP, heart rate and ASI. Additional adjustment for systolic BP had little impact on the association of ASI with arteriolar diameter. The associations with CVD risk factors were unaffected by removal of those with history of CVD or adjustment for total cholesterol, triglycerides and HbA1c (model 3, table S2). Narrower venules were also associated with higher systolic BP, diastolic BP, MAP and PP. Wider venules were associated with increased ASI and HR (model 1, table 3). Adjustment for additional confounding (model 2) did not alter the associations (with diameters), except for ASI which was no longer significantly associated with wider venules. Associations were unaffected by removal of event history or adjustment for total cholesterol, triglycerides and HbA1c (model 3 table S2).

Although females exhibited narrower and more tortuous vessels, formal tests of interaction of all associations with sex were not statistically significant. Out of 36 tests for interaction performed, 6 were statistically significant at the 1% level. However, the absolute differences in slopes were minimal and all of the associations were in the same direction in men and women (data available on request). None of the associations were in opposing directions and there results are presented for men and women combined.

**Discussion**

In this study, the first to examine the retinal microvasculature at scale using fully automated software (with over 3.5 million vessel segments from over 50,000 participants), we have shown novel associations between retinal microvascular tortuosity with BP and ASI and confirmatory associations with diameters. Each significant risk factor expressed in deciles (figure 2), showed strong graded associations with retinal vessel morphometry measures. The associations held after adjustment for confounding factors and removal of those with self-reported diabetes and CVD morbidity. Importantly these morphometric associations may be indicative of preclinical disease processes, suggesting a role in CVD risk prediction.

*Retinal Tortuosity and CVD Risk Factors*

The key findings from the present study were that increased retinal tortuosity (arteriolar and venular) showed strong graded associations with higher BP, MAP and PP, with p-values as small as 1×10-300. The retinal microvasculature abnormalities observed with increased BP have not been so clear until now (figure 2). The European Prospective Investigation into Cancer-Norfolk Eye study of 5947 participants (which used an identical methodology), showed remarkably similar increased tortuosity with systolic BP in older adults (arteriolar tortuosity 1.2%;95%CI;0.5,1.9% per 10mmHg and venular tortuosity 0.5%;0.02,0.88% per 10mmHg)8. However, evidence from other smaller scale studies have been less supportive. A nested case-control study by Witt et al. among 682 adults, showed an increase in arteriolar tortuosity was weakly associated with higher systolic BP, with no evidence of an association with venular tortuosity4. In contrast, a cross-sectional study by Cheung et al showed a decrease in arteriolar tortuosity with higher BP and MAP and an increase in venular tortuosity with higher BP and MAP, in an Asian population of adults (n=2915)14. Disagreements between studies may be due to smaller sample sizes together with measurement error (particularly with methods relying on manual measurement), where there is less certainty over the presence or absence of underlying associations.

While tortuosity (arteriolar and venular) was higher overall among women compared to men, in the present study, the association between CVD risk markers and tortuosity was evident in both sexes (and independent of further adjustment for height). Overall the association of increased tortuosity (both arteriolar and venular tortuosity) with higher BP in the present study was independent of retinal diameters. This suggests that tortuosity reflects different structural vascular changes from those shown for diameters and may provide additional value to CVD risk prediction tools beyond the current testing of diameters24.

The association of retinal tortuosity with stroke has not been well studied, though two studies have shown a more tortuous retinal microvascular network with prevalent stroke8, 10. In UK Biobank there was no evidence of an association between self-reported stroke and arteriolar tortuosity. This difference may be related to differences in age (UK Biobank participants are relatively younger) and case ascertainment of stroke. In UK Biobank presence of stroke was based on self-report, while in the other studies it was based on clinical information from health records8, 10. Conversely in the present study increased venular tortuosity was strongly associated with stroke for which some evidence has been reported previously8, 10. The discrepancies overall in findings from earlier studies with tortuosity may relate to the limited sample sizes, characteristics of the population (i.e., age and risk factor profile, differences in diabetes duration and type) or the time placement of the study. For example treatment has improved over time and therefor it may be harder to observe changes associated with a history of events in more recent studies compared to studies from 10 to 20 years ago. Given the strong graded association of CVD risk factors with tortuosity (both arteriolar and venular) in the present study of over 50,000 participants and the replication of the findings observed in EPIC8, the associations between tortuosity and CVD risk factors are now very clear warranting verification in a large longitudinal follow-up study, to confirm causality.

*Retinal Diameters and CVD Risk Factors*

Retinal arteriolar narrowing has consistently been associated with elevated BP in epidemiological studies6-8, 25 and meta-analysis9, supporting the finding of the present study (figure 2b), where each 10mmHg increase in systolic BP was associated with a 0.9µm narrowing in arteriolar diameter. The weaker association observed for venular diameter in the present study and no association observed in smaller previous studies6, 7 suggests that the impact of BP on arterioles is more prominent than on venules. In general the literature is in agreement that arterioles and venules are differentially associated with cardiometabolic risk factors; narrower arterioles are associated with higher BP8, and wider venules with inflammation and higher BMI / obesity8, 26. This most likely relates to the effect of different mechanistic pathways, which are yet to be fully understood.

We identified a strong association between older age and venular widening (figure 2a) and arteriolar narrowing. The ageing process has been linked to decreased retinal vessel density, reduced inner retinal layer thickness, and retinal blood flow velocity, particularly in venules27. The gender difference, with females having narrower arteriolar and venular diameters compared to males would need to be considered in the development of CVD risk prediction tools.

*Future Direction – Fully Automated CVD Risk Prediction*

The work by Poplin et al using a convolutional neural network (CNN) was able to predict CVD risk factors and outcomes from a retinal image alone as accurately as available CVD risk prediction tools15. In support of growing evidence, the present study has shown that narrower arteriolar diameters are associated with increased BP, ASI and PP very strongly and provide an indication of systemic microvascular and macrovascular changes11. Understanding how models predict risk remains a substantial problem of the deep learning, which is very much a black box approach, therefore assessment of vessel morphology coupled with CNN deep learning approaches may help further inform potential pathways of disease processes and prediction.

The strength of this study includes its large sample size of over 50,000 participants. The QUARTZ software is fully automated, incorporates CNN technology and utilizes information from all vessels extracted within an image, providing precise measurement. The QUARTZ software is fully automated, incorporates CNN technology and utilizes information from all vessels extracted within an image, providing precise measurement. A potential limitation is the use of the entire retinal image, compared to a section of the image used by other grading systems, however given our findings are consistent with previous literature this is unlikely to be a major issue. The study limitations include the cross-sectional study design, meaning the issue of causality cannot be resolved until further follow-up. Although the UKBB is not a representative sample of the UK population, which is an issue if one were attempting to determine prevalence of outcomes or genetic associations in particular28. However, this study was focused on assessing cross-sectional phenotypic associations and Pizzi et al29 showed that using restricted sampling for a cohort study is unlikely to appreciably bias estimates of exposure-disease associations, under a range of potential scenarios. In support of this, our findings show very similar patterns of association to those observed in an entirely independent UK based cohort study of similar and older age (the EPIC-Norfolk study)8. The impact of hypertension treatment duration could not be assessed so we are unsure of the impact of treatment duration on the association between retinal measures and BP, though we did note that removing those on BP treatment did not alter the associations observed. The PWV device used a non-reference method to estimate PWV, which is not as accurate as other approaches and may have led to an underestimation of the association, particularly given that pulse pressure shows stronger associations than PWV in this study. However, reassuringly, the association is in the expected direction and a p value of 2.9E-24 is highly unlikely to be a chance finding. This lends weight to the null association observed between PWV and vessel tortuosity

**Perspectives**

This study assessing the retinal microvasculature at scale, has shown clear associations between retinal vessel morphometry, BP and ASI. These observations further our understanding of the preclinical disease processes, and interplay between microvascular and macrovascular disease.

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**Disclosers**

There are no disclosures to declare.

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**1) What Is New**

* This is the first study to examine the retinal microvasculature at scale using fully automated software (with over 3.5 million vessel segments from over 50,000 participants), taking retinal morphology assessment from just a research tool to having potential use within clinical practice.
* The observations from this study further our understanding of the preclinical disease processes, and relationship between microvascular and macrovascular changes.

**2) What Is Relevant?**

* There are clear linear associations between retinal vessel morphometry measures and blood pressure (BP) and arterial stiffness index (ASI).
* Associations held after adjustment for confounding factors and removal of those with self-reported diabetes and cardiovascular disease (CVD) morbidity.

**3) Summary**

* This study assessing the retinal microvasculature at scale, has shown clear associations between retinal vessel morphometry, BP and ASI.
* Importantly these morphometric associations may be indicative of preclinical disease processes, suggesting a role in CVD risk prediction.

**Figure legends**

**Figure 1. Flow of participants by exclusion from the UK Biobank retinal imaging study**

**Figure 2. Adjusted mean retinal vessel width and tortuosity by deciles of CVD risk factors. Adjusted means (solid square symbols), 95% confidence intervals(solid vertical error bars) and regression line (dotted line) are from a multilevel model allowing for age, sex and CVD risk maker decile as fixed effects and repeated retinal vessel measures within each person.**



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| **Table 1. Characteristics of the population by inclusion and exclusion criteria** |
| **Characteristics** | **Total** | **Included** | **Excluded** |
| N | 68,535 | 54,714 | 13,821 |
| Age (years) | 56.8 (8.1) | 56.1 (8.2) | 59.3 (7.5) |
| Gender (% Female) | 54 | 55 | 50 |
| Ethnicity (%) |   |   |   |
|  *White* | 89.9 | 90.9 | 86.0 |
|  *Black* | 3.2 | 2.9 | 4.7 |
|  *Asian* | 3.2 | 2.8 | 4.8 |
|  *Other* | 2.9 | 2.8 | 3.5 |
|  Unknown/missing | 0.7 | 0.7 | 1.0 |
| Smoking (%) |   |   |   |
|  *Never smoker* | 55.2 | 55.9 | 52.2 |
|   *Occasionally* | 2.9 | 2.9 | 2.7 |
|  *Ex-smoker* | 34.5 | 33.9 | 36.9 |
|  *Current smoker* | 6.8 | 6.7 | 7.3 |
|  *Prefer not to say/missing* | 0.7 | 0.6 | 0.9 |
| Quartiles of Townsend deprivation index (%) |   |   |
|  *<-3.4* | 25.0 | 25.1 | 24.5 |
|  *-3.4 to -1.6* | 25.0 | 25.3 | 23.8 |
|  *-1.7 to 0.8* | 25.0 | 25.0 | 24.7 |
|  *>0.8* | 25.0 | 24.5 | 26.9 |
|  *Missing* | 0.1 | 0.1 | 0.1 |
| Salt intake (%) |   |   |   |
|  *Never salt* | 56.2 | 56.6 | 54.6 |
|  *Sometimes salt* | 27.8 | 27.6 | 28.5 |
|  *Usually salt* | 11.2 | 11.1 | 11.7 |
|  *Always salt* | 4.5 | 4.4 | 4.8 |
|  *Prefer not to say/missing* | 0.3 | 0.3 | 0.5 |
| Medication for BP (%) | 9.9 | 9.4 | 12.0 |
| Systolic BP (mmHg) | 137.1 (18.4) | 136.5 (18.3) | 139.6 (18.7) |
| Diastolic BP (mmHg) | 81.8 (10.0) | 81.7 (10.0) | 82.1 (10.1) |
| Mean arterial pressure (mmHg) | 100.2 (11.8) | 100.0 (11.8) | 101.2 (11.8) |
| Mean pulse pressure (mmHg) | 55.4 (13.5) | 54.8 (13.3) | 57.5 (14.2) |
| Heart rate (bpm) | 68.3 (11.1) | 68.3 (11.0) | 68.5 (11.3) |
| Pulse wave ASI (m/s) | 9.4 (3.0) | 9.4 (2.9) | 9.7 (3.0) |
| BMI (kg/m²) | 27.4 (4.8) | 27.3 (4.7) | 27.8 (4.9) |
| Arteriolar width (µm) | \* | 88.0 (7.8) | \* |
| Venular width (µm) | \* | 103.9 (13.1) | \* |
| Arteriolar tortuosity (x1000) | \* | 4.4 (1.6) | \* |
| Venular tortuosity (x1000) | \* | 3.2 (1.4) | \* |
| Image quality | \* | 0.9 (0.1) | \* |
| History of heart attack (%) | 2.0 | 1.8 | 2.8 |
| History of stroke (%) | 1.2 | 1.1 | 1.4 |
| Diabetes (%) | 5.6 | 4.9 | 8.2 |
| *Mean (SD) or %. Data missing on categorical variables have been included as an additional category for each variable, to minimise data loss. \* data only on those included in the study available. Data were missing on the following continuous variable: Pulse wave ASI (n=523).* |
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| **Table 2. Percentage differences in arteriolar and venular tortuosity associated with CVD risk factors** |
|   | **Percentage Difference in Arteriolar Tortuosity (95%CI)** |   | **Percentage Difference in Venular Tortuosity (95%CI)** |
|  | **Model 1** | **Model 2** |  | **Model 1** | **Model 2** |
| **Risk marker** |  | **P value** |  | **P value** |  |  | **P value** |   | **P value** |
|  |  |  |  |  |  |  |  |   |  |
| Age per decade | 2.39 (1.87, 2.92) | 1.1E-19 | 2.45 (1.93, 2.99) | 4.7E-20 |  | 2.46 (2.14, 2.77) |  <1.0E-300 | 2.41 (2.09, 2.73) |  <1.0E-300 |
| Sex (female) | 4.23 (3.37, 5.10) | 1.3E-22 | 4.40 (3.52, 5.27) | 8.5E-24 |   | 1.37 (0.87, 1.88) | 8.0E-08 | 1.83 (1.32, 2.34) | 1.5E-12 |
| SBP per 10mmHg | 1.15 (0.90, 1.39) | 6.4E-20 | 1.18 (0.93, 1.43) | 2.2E-20 |   | 0.63 (0.48, 0.77) | 7.3E-17 | 0.42 (0.27, 0.57) | 4.0E-08 |
| DBP per 10mmHg | 0.76 (0.34, 1.19) | 3.9E-04 | 0.79 (0.35, 1.23) | 3.9E-04 |   | 0.30 (0.05, 0.55) | 0.019 | -0.32 (-0.58, -0.06) | 0.016 |
| MAP per 10 mmHg | 1.24 (0.87, 1.61) | 4.1E-11 | 1.30 (0.92, 1.68) | 1.9E-11 |   | 0.62 (0.40, 0.84) | 3.6E-08 | 0.16 (-0.07, 0.38) | 0.166 |
| MPP per 10 mmHg | 1.78 (1.43, 2.13) | 1.4E-23 | 1.80 (1.44, 2.15) | 6.9E-24 |   | 1.05 (0.84, 1.26) | 4.9E-23 | 1.02 (0.81, 1.23) | 9.2E-22 |
| HR per 10 bpm | -0.35 (-0.73, 0.02) | 0.065 | -0.43 (-0.81, -0.04) | 0.030 |   | -0.19 (-0.42, 0.04) | 0.098 | -0.60 (-0.83, -0.37) | 2.6E-07 |
| PW ASI per m/s | 0.12 (-0.03, 0.27) | 0.111 | 0.10 (-0.05, 0.25) | 0.203 |   | 0.11 (0.03, 0.20) | 0.012 | 0.02 (-0.07, 0.11) | 0.633 |
| Percentage differences in retinal vessel tortuosity are from a multilevel model allowing for repeated images from the same person (random effect for person). Confidence interval (CI). Model 1 adjusting each factor for age, sex, ethnicity and UKBB centre as fixed effects (n=54,714). Model 2 adjusting each factor for model 1 plus body mass index, smoking and the Townsend deprivation index, as fixed effects (n=54,714). Note, a p value of <0.00001 would be 1.0E-5, hence 1.0E-300 is an extremely small probability. SBP – systolic blood pressure, DBP diastolic BP, PW ASI – pulse wave arterial stiffness index, HR – heart rate, MAP mean arterial pressure, MPP – mean pulse pressure. |
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| **Table 3. Mean difference in arteriolar and venular diameter (µm) associated with CVD risk factors** |
|   | **Absolute Difference in Arteriolar Diameter (95%CI) in µm** | **Absolute Difference in Venular Diameter (95%CI) in µm** |
|  | **Model 1** | **Model 2** | **Model 1** | **Model 2** |
| **Risk marker** |  | **P value** |  | **P value** |  | **P value** |   | **P value** |
|  |  |  |  |  |  |  |   |  |
| Age per decade | -0.56 (-0.63, -0.48) | <1.0E-300 | -0.51 (-0.59, -0.43) | <1.0E-300 | 1.00 (0.87, 1.13) | <1.0E-300 | 1.02 (0.89, 1.16) | <1.0E-300 |
| Sex (female) | -0.18 (-0.31, -0.05) | 5.1E-03 | -0.18 (-0.31, -0.05) | 5.5E-03 | -0.72 (-0.93, -0.50) | 7.1E-11 | -0.53 (-0.74, -0.31) | 1.90E-06 |
| SBP per 10mmHg | -0.91 (-0.94, -0.87) | <1.0E-300 | -0.90 (-0.94, -0.87) | <1.0E-300 | -0.25 (-0.32, -0.19) | 5.8E-15 | -0.31 (-0.37, -0.25) | 2.29E-21 |
| DBP per 10mmHg | -1.62 (-1.68, -1.55) | <1.0E-300 | -1.66 (-1.72, -1.59) | <1.0E-300 | -0.30 (-0.41, -0.19) | 7.7E-08 | -0.47 (-0.58, -0.36) | 2.08E-16 |
| MAP per 10 mmHg | -1.49 (-1.54, -1.43) | <1.0E-300 | -1.51 (-1.57, -1.46) | <1.0E-300 | -0.34 (-0.43, -0.24) | 2.6E-12 | -0.47 (-0.57, -0.37) | 1.66E-21 |
| MPP per 10 mmHg | -0.72 (-0.77, -0.67) | <1.0E-300 | -0.71 (-0.76, -0.66) | <1.0E-300 | -0.30 (-0.39, -0.21) | 3.6E-11 | -0.31 (-0.39, -0.22) | 2.1E-11 |
| HR per 10 bpm | -0.14 (-0.20, -0.08) | 1.9E-06 | -0.13 (-0.19, -0.07) | 1.6E-05 | 0.59 (0.50, 0.69) | 6.4E-33 | 0.47 (0.37, 0.57) | 1.2E-20 |
| PW ASI per m/s | -0.11 (-0.13, -0.09) | 8.6E-23 | -0.12 (-0.14, -0.09) | 2.9E-24 | 0.08 (0.04, 0.11) | 1.2E-04 | 0.03 (-0.01, 0.07) | 0.090 |
| Absolute difference in retinal vessel diameter are from a multilevel model allowing for repeated images from the same person (random effect for person). Confidence interval (CI). Model 1 adjusting each factor for age, sex, ethnicity and UKBB centre as fixed effects (n=54,714). Model 2 adjusting each factor for model 1 plus body mass index, smoking and the Townsend deprivation index, as fixed effects (n=54,714). Note a p value of <0.00001 would be 1.0E-5 hence 1.0E-300 is an extremely small probability. SBP – systolic blood pressure, DBP diastolic BP, PW ASI – pulse wave arterial stiffness index, HR – heart rate, MAP mean arterial pressure, MPP – mean pulse pressure. |
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**Mean Pulse Pressure (mmHg) Arterial Stiffness Index (m/s)**



**Online Supplement**

**Associations of Retinal Microvascular Diameters and Tortuosity with Blood Pressure and Arterial Stiffness: UK Biobank**

**Running Title: Retinal Microvascular Morphology and Blood Pressure**

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| **Table S1. Percentage differences in arteriolar and venular tortuosity associated with CVD risk factors**   |
|   | **Percentage Difference in Arteriolar Tortuosity (95%CI)** |   | **Percentage Difference in Venular Tortuosity (95%CI)** |
|  | **Model 3** |  | **Model 3** |
| **Risk marker** |  | **P value** |  |   | **P value** |
|  |  |  |  |   |   |
| Age per decade | 2.21 (1.58, 2.84) | 3.1E-12 |  | 2.41 (2.04, 2.78) | 3.6E-38 |
| Sex (female) | 4.29 (3.25, 5.34) | 2.0E-16 |   | 1.52 (0.92, 2.12) | 5.5E-07 |
| SBP per 10mmHg | 1.31 (1.01, 1.62) | 3.2E-17 |   | 0.46 (0.28, 0.64) | 5.3E-07 |
| DBP per 10mmHg | 0.92 (0.38, 1.46) | 8.0E-04 |   | -0.33 (-0.64, -0.01) | 0.042 |
| MAP per 10 mmHg | 1.46 (1.00, 1.93) | 6.0E-10 |   | 0.19 (-0.08, 0.46) | 0.171 |
| MPP per 10 mmHg | 2.00 (1.57, 2.43) | 5.6E-20 |   | 1.11 (0.86, 1.36) | 5.3E-18 |
| HR per 10 bpm | -0.58 (-1.06, -0.11) | 0.017 |   | -0.80 (-1.08, -0.52) | 2.4E-08 |
| PW ASI per m/s | 0.08 (-0.10, 0.26) | 0.392 |   | 0.02 (-0.08, 0.13) | 0.688 |
| Percentage differences in retinal vessel tortuosity are from a multilevel model allowing for repeated images from the same person (random effect for person). Confidence interval (CI). Model 3 adjusting for each factor for model 2 plus HbA1c, total cholesterol and triglycerides and excluding those with diabetes, heart attack, stroke, on medication for BP or who answered unknown or declined to answer (n=38,487). NB A p value of <0.00001 would be 1.0E-5 hence 1.0E-300 is an extremely small probability. SBP – systolic blood pressure, DBP diastolic BP, PW ASI – pulse wave arterial stiffness index, HR – heart rate, MAP mean arterial pressure, MPP – mean pulse pressure. |
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| **Table S2. Mean difference in arteriolar and venular diameter (µm) associated with CVD risk factors**  |
|   | **Absolute Difference in Arteriolar Diameter (95%CI) in µm** |   | **Absolute Difference in Venular Diameter (95%CI) in µm** |
|  | **Model 3** |  | **Model 3** |
| **Risk marker** |  | **P value** |  |   | **P value** |
|  |  |  |  |   |   |
| Age per decade | -0.56 (-0.65, -0.47) | 5.0E-33 |  | 0.96 (0.80, 1.12) | 2.5E-33 |
| Sex (female) | -0.13 (-0.28, 0.02) | 0.082 |   | -0.49 (-0.75, -0.24) | 1.5E-04 |
| SBP per 10mmHg | -0.94 (-0.98, -0.90) | <1.0E-300 |   | -0.31 (-0.39, -0.23) | 3.6E-15 |
| DBP per 10mmHg | -1.74 (-1.81, -1.66) | <1.0E-300 |   | -0.47 (-0.61, -0.34) | 1.0E-11 |
| MAP per 10 mmHg | -1.58 (-1.65, -1.51) | <1.0E-300 |   | -0.47 (-0.59, -0.35) | 3.2E-15 |
| MPP per 10 mmHg | -0.75 (-0.81, -0.68) | <1.0E-300 |   | -0.31 (-0.42, -0.20) | 2.0E-08 |
| HR per 10 bpm | -0.13 (-0.21, -0.06) | 2.7E-04 |   | 0.49 (0.37, 0.61) | 4.6E-15 |
| PW ASI per m/s | -0.11 (-0.14, -0.08) | 7.6E-16 |   | 0.03 (-0.02, 0.07) | 0.268 |
| Absolute difference in retinal vessel diameter are from a multilevel model allowing for repeated images from the same person (random effect for person). Confidence interval (CI). Model 3 adjusting for each factor for model 2 plus HbA1c, total cholesterol and triglycerides and excluding those with diabetes, heart attack, stroke, on medication for BP or who answered unknown or declined to answer (n=38,487). NB A p value of <0.00001 would be 1.0E-5 hence 1.0E-300 is an extremely small probability. SBP – systolic blood pressure, DBP - diastolic BP, PW ASI – pulse wave arterial stiffness index, HR – heart rate, MAP - mean arterial pressure, MPP – mean pulse pressure.  |
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