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Retinal microvascular parameters are not associated with reduced renal function in a study of individuals with type 2 diabetes

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The eye provides an opportunistic “window” to view the microcirculation. There is published evidence of an association between retinal microvascular calibre and renal function measured by estimated glomerular filtration rate (eGFR) in individuals with diabetes mellitus. Beyond vascular calibre, few studies have considered other microvascular geometrical features. Here we report novel null findings for measures of vascular spread (vessel fractal dimension), tortuosity, and branching patterns and their relationship with renal function in type 2 diabetes over a mean of 3 years. We performed a nested case-control comparison of multiple retinal vascular parameters between individuals with type 2 diabetes and stable (non-progressors) versus declining (progressors) eGFR across two time points within a subset of 1072 participants from the GoDARTS study cohort. Retinal microvascular were measured using VAMPIRE 3.1 software. In unadjusted analyses and following adjustment for age, gender, systolic blood pressure, HbA1c, and diabetic retinopathy, no associations between baseline retinal vascular parameters and risk of eGFR progression were observed. Cross-sectional analysis of follow-up data showed a significant association between retinal arteriolar diameter and eGFR, but this was not maintained following adjustment. These findings are consistent with a lack of predictive capacity for progressive loss of renal function in type 2 diabetes.

Type 2 diabetes mellitus is a disease characterised by micro- and macrovascular complications in 30–50% of people with the condition 1 and is the commonest cause of end-stage renal disease (ESRD), accounting for approximately 45% of incident and 38% of prevalent ESRD in the United States 2. High rates of incident chronic kidney disease (CKD) have been reported in both type 1 and type 2 diabetes based on established renal function cut-offs (estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m²), with up to 29% of those with newly diagnosed type 2 diabetes likely to develop CKD over 15 years of follow-up 1, with similar rates also reported in type 1 diabetes 3,4.

Pathological microvascular changes that manifest themselves in diabetes are important contributory mechanisms leading to increased prevalence of CKD in those with the condition 5–8. The retina enables accessible visualisation of tissue vascularisation and quantitative evaluation of retinal microvascular parameters (RVP) and specific retinal microvascular changes have been associated with a range of vascular-related conditions, including hypertension 9, coronary heart disease 10, stroke 11 and diabetes 12. Retinal microvascular parameters have also been suggested to reflect systemic microvascular damage resulting from renal dysfunction 13, and in population-based studies, reduced eGFR has been associated with both narrower retinal arteriolar 14–21 and venular calibre 14,16,22,23, but to date the evidence has been limited and the findings reported, inconsistent.

A wide range of perturbed metabolic pathways are associated with vascular injury in diabetes. Advanced glycation end products are formed, microRNA profiles become attenuated, nitric oxide bioavailability is reduced,

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Table 1. Baseline sample characteristics. Yrs; years; eGFR: estimated glomerular filtration rate (calculated using the CKD-EPI equation); SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; SD: standard deviation. P values were calculated by independent sample t and chi squared tests for comparisons between progressors and non-progressors.

| Baseline Variables                      | Sample n = 1068 | Progressors n = 335 | Non-progressors n = 570 | p     |
|----------------------------------------|----------------|--------------------|------------------------|-------|
| Age, yrs (SD)                          | 63.0 (7.6)     | 62.5 (7.7)         | 63.1 (7.8)             | 0.21  |
| Gender, female (%)                     | 521 (49)       | 168 (50)           | 281 (49)               | 0.81  |
| eGFR, ml/min/1.73 m² (SD)              | 94.0 (17.2)    | 98.6 (21.3)        | 91.3 (14.3)            | <0.001|
| SBP, mmHg (SD)                         | 138 (13)       | 139 (14)           | 137 (13)               | 0.08  |
| DBP, mmHg (SD)                         | 77 (8)         | 76 (9)             | 77 (8)                 | 0.63  |
| HbA1c, % (SD); mmol/mol                | 7.41 (1.38); 57.5 | 7.51 (1.36); 58.6 | 7.40 (1.41); 57.4 | 0.25  |
| Diabetic retinopathy present, n (%)    | 244 (23)       | 82 (25)            | 118 (21)               | 0.19  |
| Mean follow-up period, yrs (SD)        | 3.01 (0.35)    | 3.02 (0.35)        | 3.02 (0.34)            | 0.98  |

Results

Overall the sample population had a mean age of 63.0 years (standard deviation (SD) = 7.6) and 49% were female. Mean follow-up time was 3.01 years (SD = 0.35). The study sample had a mean glycated haemoglobin (HbA1c) of 7.41% (SD = 1.39), and a mean systolic blood pressure (SBP) of 138 mmHg (SD 13). A total of 570 participants met the group 1 definition and were designated non-progressors, 335 participants met the group 2 criteria and were designated progressors. There were no significant differences in blood pressure, HbA1c or diabetic retinopathy status between progressors and non-progressors at baseline. Baseline eGFR was significantly higher in progressors compared to non-progressors (98.6 ml/min/1.73 m², SD = 21.3 vs. 91.3 ml/min/1.73 m², SD = 14.3, p < 0.001) (Table 1).

Over the mean follow up time of 3.01 yrs, mean change in eGFR was −27.73 ml/min/1.73 m² (SD = 14.31 ml/min/1.73 m²) in progressors versus +2.74 ml/min/1.73 m² (SD = 10.85 ml/min/1.73 m²) in non-progressors, p < 0.001. SBP and diastolic blood pressure (DBP) fell in both groups, but decreased significantly more in progressors than in non-progressors (SBP decreased by 2.49 mmHg (16.26) and 0.23 mmHg (12.94) for progressors and non-progressors respectively, p = 0.04; DBP decreased by 2.61 mmHg (9.36) and 1.15 mmHg (7.69) respectively in progressors and non-progressors, p = 0.02). There was no significant change in HbA1c in both groups between time-points (+0.13% (1.42), and +0.04% (1.37) for progressors and non-progressors respectively, p = 0.41). In both groups a non-significant decrease in arteriolar and venular calibre of approximately 1% was observed between time-points but there was no significant difference in the rate of vascular narrowing between groups. No significant differences in the other parameters measured (fractal dimension, tortuosity, or number of first branches) were detected between progressors and non-progressors (Table 2).

In unadjusted and adjusted logistic regression models controlling for age, sex, SBP, and HbA1c, none of the baseline RVP were significantly associated with greater odds of being a progressor. For instance, per unit increase in central retinal arteriolar calibre (CRAE) the odds of being a progressor were multiplied by 1.02 (OR = 1.02, 95% CI = 0.97, 1.08) in the adjusted analysis but this was not statistically significant (p = 0.46). Similarly, per unit increase in central retinal venular calibre (CRVE), the odds of being a progressor were multiplied by 1.03 (OR = 1.03, 95% CI = 0.99, 1.07) in the adjusted analysis but this was also not statistically significant (p = 0.18). Further adjustment for diabetic retinopathy and fellow vessel calibre also failed to identify any significant associations between RVP and likelihood of decline in eGFR. No associations were identified between baseline RVP and odds of being a progressor in this type 2 diabetes cohort (Table 3).

Lower CRAE at follow up was significantly associated with follow-up eGFR in unadjusted linear regression analysis (β = −0.47, 95% CI = −0.87, −0.07, p = 0.02), with CRVE (β = −0.30, 95% CI = −0.60, 0.00, p = 0.05) and arteriolar fractal dimension (β = −18.41, 95% CI = −36.92, 0.10, p = 0.05) approaching statistical significance. After adjustment for age, gender, follow-up SBP and HbA1c, the associations between RVP and eGFR...
narrower retinal arterioles 36 and sparser vasculature37 have also been reported in association with proteinuria. to support the findings from WESDR, but do support other cross sectional studies in type 2 diabetes 42, and given previous reported associations with RVP in both type 1 and type 2 diabetes33–37,41.

However, a short time period. Inclusion of proteinuria or albuminuria measures with eGFR may prove more informative, changes in retinal vascular morphology40. Although most studies adjust for the effects of age, the potential for in eGFR resulting in increased prevalence of CKD in older populations which may be sufficient to obscure any

venular calibre reported previously in association with DN. Older age is also strongly associated with a reduction in eGFR resulting in increased prevalence of CKD in older populations which may be sufficient to obscure any

changes in retinal vascular morphology40. Although most studies adjust for the effects of age, the potential for in eGFR resulting in increased prevalence of CKD in older populations which may be sufficient to obscure any

limited time-period considered.

Baseline RVP and change in eGFR between two time points approximately 3 years apart was found, suggesting a lack of potential for RVP as predictive biomarkers of eGFR decline in this cohort of type 2 diabetes over the limited time-period considered.

Several studies have previously reported associations between renal disease and RVP in both type 1 and type 2 diabetes. Cross-sectional studies in type 1 diabetes found associations between narrower retinal arterioles and prevalent diabetic nephropathy (DN)33,34. In contrast, prospective studies of type 1 diabetes have reported wider venular calibre in association with albuminuria35,36 and renal insufficiency37 over 16 years of follow-up, while narrower retinal arterioles38 and sparser vasculature39 have also been reported in association with proteinuria. These previous findings in type 1 diabetes contrast to the present study which found no significant associations with eGFR decline in type 2 diabetes. Reductions in eGFR can occur in the presence or absence of albuminuria33 and may have entirely independent associations with retinal vascular morphology. Population differences between type 1 and type 2 diabetes are also worthy of further consideration. Age is a well-established confounder of RVP and type 1 diabetes populations are typically younger. The population of the Danish Cohort of Pediatric Diabetes33 had a mean age of 21 years, far younger than the 63 year average age of the population of this study. Population differences between type 1 and type 2 diabetes are also worthy of further consideration. Age is a well-established confounder of RVP and type 1 diabetes populations are typically younger. The population of the Danish Cohort of Pediatric Diabetes33 had a mean age of 21 years, far younger than the 63 year average age of the population of this study.

Table 2. Between group comparisons for progressors and non-progressors. eGFR: estimated glomerular filtration rate (calculated using the CKD-EPI equation); SD: standard deviation. P values were calculated by independent sample t test. *Tortuosity variables were log transformed before to produce normal distribution.

| Variables | Progressors n = 335 Mean change (SD) | Non-progressors n = 570 Mean change (SD) | p |
|-----------|------------------------------------|----------------------------------------|---|
| SBP, mmHg | −2.49 (16.26)                      | −0.23 (12.94)                         | 0.04 |
| DBP, mmHg | −2.61 (9.36)                       | −1.15 (7.69)                          | 0.02 |
| HbA1c, % (SD), mmol/mol | 0.13 (1.42); 1.4 | 0.04 (1.37); 0.4 | 0.38 |
| eGFR, ml/min/1.73 m² | −27.73 (14.31) | 2.74 (10.85) | <0.001 |
| Calibre | | | |
| Central retinal arteriolar equivalent | −0.46 (2.41) | −0.52 (2.55) | 0.72 |
| Central retinal venular equivalent | −0.57 (2.94) | −0.56 (3.41) | 0.95 |
| Arteriovenous ratio | 4.7 × 10⁻³ (5.0 × 10⁻³) | −1.9 × 10⁻³ (5.5 × 10⁻³) | 0.60 |
| No. of first branches in zone C | | | |
| Arteriolar | 0.01 (1.15) | −0.13 (1.19) | 0.09 |
| Venular | −0.03 (1.03) | −0.09 (0.97) | 0.32 |
| Fractal dimension | | | |
| Arteriolar | −5.5 × 10⁻³ (0.05) | −9.1 × 10⁻³ (0.06) | 0.36 |
| Venular | −6.3 × 10⁻³ (0.05) | −9.1 × 10⁻³ (0.05) | 0.45 |
| Tortuosity | | | |
| *Arteriolar | −1.1 × 10⁻² (0.25) | 3.3 × 10⁻² (0.27) | 0.44 |
| *Venular | 4.5 × 10⁻² (0.31) | 4.0 × 10⁻² (0.29) | 0.81 |

at follow-up were no longer statistically significant (Table 4). Associations were not materially altered following additional adjustment for diabetic retinopathy or fellow vessel calibre (data not shown).

Discussion

Routine investigation of retinal biomarkers has improved through advances in digital imaging systems and routine eye screening programmes, wider availability through high street opticians and software improvements capable of quantifying multiple RVP with improved accuracy.

In this longitudinal, case-control study of individuals with type 2 diabetes, the prognostic value of a wide range RVP for the identification of those at increased risk of eGFR decline was considered. No association between baseline RVP and change in eGFR between two time points approximately 3 years apart was found, suggesting a lack of potential for RVP as predictive biomarkers of eGFR decline in this cohort of type 2 diabetes over the limited time-period considered.

Previously reported associations between renal disease and RVP in both type 1 and type 2 diabetes have not always been consistent. Data from the Wisconsin Epidemiological study of Diabetic Retinopathy (WESDR) reported an association between wider venular calibre and DN incidence over 14 years of follow-up41. In contrast, our data failed consistent. Data from the Wisconsin Epidemiological study of Diabetic Retinopathy (WESDR) reported an association between wider venular calibre and DN incidence over 14 years of follow-up41. In contrast, our data failed

to support the findings from WESDR, but do support other cross sectional studies in type 2 diabetes42, and prospective data with similar 2-year follow-up duration43 suggesting RVP may not predict change in eGFR over a short time period. Inclusion of proteinuria or albuminuria measures with eGFR may prove more informative, given previous reported associations with RVP in both type 1 and type 2 diabetes33–37,41.

The majority of previous investigations have been limited to the analysis of vessel calibre, with only a single study considering fractal dimensions with regard to albuminuria in a young type 1 diabetes population37. We report novel findings for measures of vascular spread (retinal vessel fractal dimension), tortuosity, and branching
patterns in type 2 diabetes. Fractal dimension and tortuosity were not associated with eGFR decline over the 3-year time-period in this type 2 diabetes cohort.

This study had several strengths. The prospective design allowed the predictive capacity of RVP to be examined over a 2–4 year period. Participant recruitment was clinically driven and electronic data record linkage provided an extensive range of variables on most participants. Our study provided novel data on the predictive utility of RVP and eGFR in type 2 diabetes and included wide range RVP previously under-reported in this context (i.e. fractal dimension, tortuosity, and number of first arteriolar branches). RVP were found to have no predictive value for 3-year change in eGFR in type 2 diabetes in our cohort with a mean age of 63 years. Our cohort originated from a population of type 2 diabetes from Tayside, Scotland, with healthcare record linkage which reduced the likelihood of bias. As a result, the findings are likely to be generalisable to other type 2 diabetes populations. The population was however almost entirely white and therefore, this sample is likely to be most closely generalisable to other predominantly white populations with type 2 diabetes of a similar age, given known associations of diabetes and renal disease with ethnicity. There is also evidence to suggest ethnic differences in retinal microvascular parameters47, perhaps in part due to factors associated with iris colour, retinal pigmentation and/or underlying genetic influences48.

The limitations of this study include the case-control design which increased the likelihood of regression toward the mean, although the risk of regression toward the mean was reduced through the use of median eGFR values calculated from all available measurements recorded within 6 months either side of the date of each retinal photograph. The definition of progressors as those with eGFR < 60 ml/min/1.73 m² at follow-up, or a reduction in eGFR of at least 15% between baseline and follow up, risked the inclusion of participants with limited reduction in renal function (e.g. eGFR 60 ml/min/1.73 m² at baseline, and 59 ml/min/1.73 m² at follow-up). This proved not to be an issue as only two participants had eGFR < 60 ml/min/1.73 m² at follow-up combined with reductions in eGFR smaller than −15%, and each of these had reductions in eGFR from baseline exceeding 14% (data not shown).

Another limitation was the 3-year duration between baseline and follow-up measures, which may have been insufficient to detect associations between RVP and change in eGFR. As both retinal calibre and eGFR decline over time, the age (mean age 63 years, SD 7.6) of the population may also have limited the sensitivity to detect such associations with RVP. A study with longer follow-up, including participants entering at a younger age may be required to detect such associations.

A more comprehensive assessment of renal function (such as albumin / creatinine ratio (ACR)) and/or appropriate GFR estimating equations in ‘at risk’ individuals may have improved the sensitivity of our approach and comparability with other studies. Unfortunately, proteinuria/ACR data was not available for the earlier phase of GoDARTS recruitment. Although, HbA1c was included in regression models, duration of diabetes was not, and may have a confounding influence. The suitability and size of our sample may also have limited our capacity to detect meaningful associations. Inclusion of additional individuals with baseline eGFR at the lower end of CKD stage 2 (60–70 ml/min/1.73 m²) may have provided more meaningful clinical significance as they transition from CKD stage 2 to stage 3.

The algorithms used by the retinal vessel measurement platform have been validated against the “gold standard” method of manual vessel tracing45,46. However, establishing the ground truth for validation of retinal vessel measurements is challenging because of the time intensive nature of the manual work and relatively poor inter-grader reliability that arises from manual assessment through expert disagreement on challenging issues related to defining vessel boundaries47,48. In contrast, the semi-automatic vessel assessment platform used in this study shows excellent inter-operator reliability49. Nevertheless, results may vary from associations based on...
The median serum creatinine values, HbA1c, SBP and DBP were calculated from all available measurements within 6 months of each retinal image. Retinal fundus images were analysed using semi-automated software, Vessel Assessment and Measurement Platform for Images of the REtina (VAMPIRE; VAMPIRE group, University of Dundee, Dundee, Scotland) version 3.153,54, by trained graders blinded to participant data. VAMPIRE 3.1 measures RVP within predefined annular zones: CRAE, CRVE, arteriovenous ratio (AVR), number of first vessel branches within a pre-defined zone C, fractal dimension and vessel tortuosity (Fig. 1). Intrarater reliability of retinal vascular measurements was measured using the intraclass correlation coefficient, assessed in four sessions of 20 retinal images at regular intervals over the course of the measurement period. Mean intraclass correlation coefficient for these sessions was calculated as 0.936 for CRAE and 0.950 for CRVE, respectively, indicating excellent operator alignment. Diabetic retinopathy status (presence/absence) was determined by physicians and participants provided informed consent and agreed to electronic healthcare record linkage. All electronic medical record data was processed and provided in an anonymised form for research with similar demographics but without type 2 diabetes at the time of recruitment. The study was granted approval from the Tayside Research Ethics Committees in Scotland and was carried out in accordance with the Declaration of Helsinki, and has been described elsewhere51. Briefly, participants in GoDARTS were identified from a central database of patients registered with a general practitioner from the Tayside region of Scotland. Diagnosis of type 2 diabetes was made by physicians and participants provided informed consent and agreed to electronic healthcare record linkage. All electronic medical record data was processed and provided in an anonymised form for research through robust information governance procedures approved by local NHS Caldicott Guardians through the Health Informatics Centre at the University of Dundee.

For inclusion in the present study, GoDARTS participants had to meet the following inclusion criteria: presence of type 2 diabetes, eGFR >60 ml/min/1.73 m² at baseline, available digital retinal fundus images of sufficient quality for analysis at two time points (2–4 years apart) with corresponding clinical serum creatinine measurements within 6 months of each retinal image. Serum creatinine measurements were obtained from centralised Blood Sciences Laboratory records and retinal fundus images were obtained through routine diabetic retinopathy screening52. The earliest available digital fundus image of suitable quality for analysis was selected for the right eye with a follow-up image captured 2–4 years later from the same eye. Retinal fundus images were analysed using semi-automated software, Vessel Assessment and Measurement Platform for Images of the REtina (VAMPIRE; VAMPIRE group, University of Dundee, Dundee, Scotland) version 3.153,54, by trained graders blinded to participant data. VAMPIRE 3.1 measures RVP within predefined annular zones: CRAE, CRVE, arteriovenous ratio (AVR), number of first vessel branches within a pre-defined zone C, fractal dimension and vessel tortuosity (Fig. 1). Intrarater reliability of retinal vascular measurements was measured using the intraclass correlation coefficient, assessed in four sessions of 20 retinal images at regular intervals over the course of the measurement period. Mean intraclass correlation coefficient for these sessions was calculated as 0.936 for CRAE and 0.950 for CRVE, respectively, indicating excellent operator alignment. Diabetic retinopathy status (presence/absence) was obtained from medical records.

The median serum creatinine values, HbA1c, SBP and DBP were calculated from all available measurements recorded within 6 months either side of the date of each retinal photograph. eGFR values were calculated from median serum creatinine values using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation55.

Participants were divided into two groups based on change in eGFR between both time points. Group 1, “non-progressors”, included participants with stable renal function or a reduction in eGFR <10% between both

### Table 4. Linear regression models testing cross-sectional associations between follow-up eGFR and follow-up RVP. Follow-up eGFR and follow-up RVP, adjusted for age, gender, systolic blood pressure at follow-up, and HbA1c at follow-up. 95% CI: 95% confidence interval. *Tortuosity variables were log transformed before linear regression to produce normal distribution.

| Retinal microvascular parameter (per unit increase) | Unadjusted β eGFR (95% CI) | P | Adjusted β eGFR (95% CI) | P |
|---------------------------------------------------|-----------------------------|---|--------------------------|---|
| Calibre                                           |                             |   |                          |   |
| Central retinal arteriolar equivalent             | -0.47 (−0.87, −0.07)       | 0.02 | -0.38 (−0.80, 0.05)     | 0.08 |
| Central retinal venular equivalent                | -0.30 (−0.60, 0.00)        | 0.05 | -0.27 (−0.58, 0.05)     | 0.10 |
| Arteriovenous ratio                               | -3.32 (−21.81, 15.16)      | 0.72 | -0.52 (−19.64, 18.60)   | 0.96 |
| Fractal dimension                                 |                            |   |                          |   |
| Arteriolar                                        | -18.41 (−36.92, 0.10)      | 0.05 | -17.64 (−36.71, 1.44)   | 0.07 |
| Venular                                           | -3.74 (−22.79, 15.31)      | 0.70 | -3.46 (−23.36, 16.43)   | 0.73 |
| No. of First branches in zone C                  |                            |   |                          |   |
| Arteriolar                                        | -0.67 (−1.63, 0.30)        | 0.17 | -0.50 (−1.50, 0.49)     | 0.32 |
| Venular                                           | 0.66 (−0.43, 1.75)         | 0.24 | 0.82 (−0.31, 1.95)      | 0.15 |
| Tortuosity                                        |                            |   |                          |   |
| Arteriolar                                        | -0.01 (−2.66, 2.65)        | 1.00 | -0.01 (−2.75, 2.73)     | 0.99 |
| Venular                                           | -3.20 (−6.73, 0.32)        | 0.08 | -2.22 (−5.86, 1.43)     | 0.23 |
time points. Group 2, “progressors”, included participants with an eGFR of < 60 ml/min/1.73 m² at follow-up or a reduction in eGFR of at least 15% between baseline and follow-up.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Statistical analyses were performed using IBM SPSS v24 (Chicago, Illinois, USA). Participants with missing data were excluded from the analyses. Continuous variables were reported using means and SDs. Categorical variables were reported as percentages. Between and within-group comparisons were made using t-tests (two-sided significance reported) for continuous variables, and Chi-squared tests for proportions. The relationship between RVP and eGFR was assessed using logistic and linear regression models. Logistic regression models were used to test for association between baseline RVP and progression of renal disease. The independent variables were RVP and progressor/non-progressor designation as a binary dependent variable. Logistic regression models were carried out unadjusted, and adjusted for important known confounding variables: age, gender, baseline SBP, and baseline HbA₁c. Additional models were used to further adjust for covariates related to RVP; diabetic retinopathy and fellow vessel calibre (for models including retinal vascular calibre as the independent variable). Associations involving continuous outcome variables were assessed using multiple linear regression. Linear regression models were used to test for cross-sectional association between renal function and RVP at follow-up. The independent variables were RVP, the dependent variable was eGFR. Linear regression models controlled for age, gender, follow-up SBP and HbA₁c. Additional models adjusted for diabetic retinopathy and fellow vessel calibre (where retinal vascular calibre was the independent variable). Tortuosity variables were log transformed before linear regression to produce normal distributions and to conform to the assumptions of the analysis. All significance values reported are two-sided.

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Author Contributions
Gareth J. McKay, Alexander P. Maxwell: obtained funding, undertook data analysis, drafted manuscript. Thomas J. MacGillivray, Emanuele Trucco, Alexander S. Doney: extracted data, undertook data analysis, drafted manuscript. Euan N. Paterson, Christopher C. Cardwell: undertook data analysis and drafted manuscript. Ruixuan Wang, Stephen Hogg: undertook retinal measurements and drafted manuscript.

Additional Information
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