Decreased Retinal Capillary Flow Is Not a Mediator of the Protective Myopia–Diabetic Retinopathy Relationship

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Purpose. The mechanisms supporting the protective relationship between a longer axial length (AL) and a decreased risk of diabetic retinopathy (DR) remain unclear. Previous studies have demonstrated reduced retinal blood flow in axial myopia, and it has been suggested that the compromised retinal capillaries in diabetes are less likely to leak and rupture as a result of this decreased flow. In this study, we therefore investigated if reduced retinal capillary flow (RCF) is a potential mechanism underpinning this protective relationship.

Methods. Retinal capillary flow was assessed using the Heidelberg Retinal Flowmeter in 150 eyes of 85 patients with diabetes aged 18+ years from the Royal Victorian Eye and Ear Hospital and St. Vincent's Hospital (Melbourne), Australia. Axial length was measured using the Intraocular Lens Master. Diabetic retinopathy was graded from two-field retinal photographs into none, mild, moderate, and severe DR using the modified Airlie House classification system.

Results. A total of 74 out of 150 eyes (49.3%) had DR. A longer AL was associated with decreased odds of DR presence (per mm increase in AL, odds ratio [OR] 0.61, 95% confidence interval [CI] 0.41–0.91) and DR severity (OR: 0.65; 95% CI: 0.44–0.95). However, no association was found between AL and RCF (per mm increase in AL, regression coefficient [β] −1.80, 95% CI −13.50 to 9.50) or between RCF and DR (per unit increase in RCF, OR 1.00; 95% CI 0.99–1.00).

Conclusions. Our finding suggests that diminished RCF may not be a major factor underlying the protective association between axial elongation and DR.

Keywords: axial length, retinal blood flow, retinal capillary flow, myopia, diabetic retinopathy

Recent studies have reported that an increased axial length (AL) may be the main contributor to the protective relationship between myopia and a decreased risk and severity of diabetic retinopathy (DR).[1,2] However, the mechanisms underpinning this protective association remain uncertain. One plausible theory is that as the eye elongates, the blood vessels of the retina stretch and become thinner. These changes result in a reduction in retinal blood flow according to the Hagen-Pouseille law,[3] leading to a decrease in capillary hydrostatic pressure and resulting in decreased chances of leakage and rupture of compromised retinal capillaries in diabetes (Starling and Laplace's law).[4]

Increased vessel wall dilation[5–7] and retinal blood flow[8–10] have been associated with DR progression. Furthermore, a decrease in ocular blood flow has been demonstrated in healthy eyes with axial myopia.[11–13] These findings support the proposed hypothesis that decreased retinal capillary flow (RCF) is one of the mechanisms contributing to the protective influence of an increased AL on the risk of DR. However, no study has directly assessed the associations of RCF with AL and DR in patients with diabetes.

In this study, we investigated if reduced RCF is one of the mechanisms underpinning the protective influence of increased AL on DR by (1) confirming the relationship between longer AL and reduced likelihood of having DR or increasing severity of DR, (2) evaluating the relationship between AL and RCF, and (3) determining the correlation between RCF and DR in a clinical sample of diabetic patients with and without DR. We hypothesize that a longer AL is associated with a reduction in RCF, and RCF is inversely associated with a decreased likelihood of having DR.

Methods

Study Population

In this cross-sectional clinic-based study, we recruited 85 English-speaking Caucasian patients with diabetes (types 1 and 2) from the eye clinics at the Royal Victorian Eye and Ear Hospital, as well as the endocrinology clinics at St. Vincent's Hospital (Melbourne). Patients were excluded if they had intraocular

pressure (IOP) > 21 mm Hg; had presence of significant cataract or other media opacities; had any history or signs of retinal or optic nerve disease/degeneration with the exception of DR; or had undergone previous laser photocoagulation for DR. All patients were over the age of 18 years, were free from significant cognitive and hearing impairment, and were advised to refrain from consuming caffeinated products and alcohol for at least 12 hours before the study. As there is no evidence of any diurnal variations in blood flow, subjects were seen in either the morning or afternoon according to their preference.[14] The study was approved by the human ethics committee of the Royal Victorian Eye and Ear hospital (No. 11/1304H), as well as that of St. Vincent's Hospital (Melbourne) (No. 141/12), and adhered to the tenets of the Declaration of Helsinki.

Blood Chemistry

Nonfasting blood samples were collected for analysis of blood glucose, glycated hemoglobin (HbA1c), and lipids (total, high density lipoprotein, and low density lipoprotein cholesterol) and triglycerides. All blood analyses were performed at Melbourne Pathology, Melbourne, Australia, with individual results electronically delivered through a password-protected program. The laboratory is accredited according to International Standard ISO15189 (Medical Laboratories) and is certified by NATA (National Association of Testing Authorities).

Assessment of Key Covariables

Each participant underwent a comprehensive assessment, which included clinical, biochemical, and anthropometric measurements (height and weight). Standardized questionnaires were used to assess basic demographic details (age, sex), history of ocular and systemic conditions, and medication use. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were taken using the Omron Automatic Blood Pressure Monitor (model IAIB; Kyoto, Japan) before RCF measurements were taken. Intraocular pressure was assessed using Goldmann applanation tonometry after RCF assessment. Key covariables were duration of diabetes (years), anti-platelet medication use (yes/no), HbA1c level (%), SBP (mm Hg), DBP (mm Hg), body mass index (BMI; kg/m^2), cholesterol (mM), and triglycerides (mM).

Assessment of Axial Length

Axial length of both eyes of each subject was obtained using an Intraocular Lens (IOL) Master unit (version 3.02.304; Carl Zeiss Meditech AG, Jena, Germany). At least three consecutive measurements were taken to ensure consistency, and all readings were within 0.02 mm of each other, with signal-to-noise ratio of at least 2.0.

Imaging for Retinal Capillary Flow

Retinal capillary flow was assessed using a Heidelberg Retinal Flowmeter (HRF; Heidelberg Engineering, Heidelberg, Germany). Briefly, the examination was performed in a sitting position at room temperature with diffuse natural light on undilated pupils. An optic disc–centered scan was obtained, together with the regions within 1 to 1.5 disc diameters to either side of the optic disc margin. A total area of 2.56 × 0.64 mm was scanned within 2 seconds at a resolution of 256 points × 64 lines × 128 times with the default 780-nm wavelength laser head installed in the HRF camera.

During the data acquisition, the participant was asked to fixate on an adjustable mounted artificial light spot. The scans were taken from both eyes of each person.

Analysis of Capillary Flow

Image analysis was performed using the automatic full field perfusion image analyser (AFFPIA) software (version 3.3; University of Erlangen, Erlangen, Germany). Briefly, AFFPIA calculates the Doppler frequency shift of 780-nm laser light from the HRF arising from moving blood cells within each pixel of the entire image and estimates the overall flow in the form of arbitrary units (AU).[15] For a valid estimation of retinal blood flow, the software adjusts brightness to mask under- or overexposed pixels and also eliminates noise from artificial movement (saccades), avoids measuring extremely wide retinal vessels and the optic nerve head, and accounts for the heart phases (systole and diastole) by averaging the differences between the two phases. For analysis purposes, the flow readings from the temporal and nasal regions adjacent to the optic disc were averaged. A previous reliability and reproducibility study demonstrated that the HRF equipment and AFFPIA software utilized in this study yielded excellent intraobserver reliability (i.e., the ability to obtain consistent RCF values when the same image was analyzed twice by the same observer; intraclass correlation coefficient 0.98) and good intrasubject reproducibility (i.e., the ability to obtain consistent RCF measurements from scans of the same subject taken at different times by the same observer; intraclass correlation coefficient 0.74).[16] Of the 170 eyes of 85 participants, 20 were ungradable due to excessive eye movement or unstable fixation.

Assessment of Diabetic Retinopathy

Following the RCF assessment, pupils were dilated with tropicamide 1%. Diabetic retinopathy was then graded from two-field fundus photographs (Canon CR6-45NM; Canon, Inc., Tokyo, Japan) utilizing the modified Airlie House classification system. We categorized the severity of DR as none (Early Treatment of Diabetic Retinopathy Study [ETDRS] levels 10–15), mild nonproliferative DR (NPDR) (level 20), moderate NPDR (levels 31–43), severe NPDR (levels 53–60), and PDR (levels 61–80). For analytical purposes, the severity of DR was further categorized as no DR or mild (ETDRS = 20), moderate (ETDRS = 31–43), or severe DR (ETDRS > 43). Diabetic retinopathy grading was performed by a single trained grader from the Centre for Eye Research Australia.

Sample Size Calculations

To date, no study has investigated the relationship between AL and RCF in patients with diabetes. Therefore, an unadjusted regression coefficient of 0.33 between RCF and AL, derived from our study sample, was utilized in sample size calculations. In order to detect a statistically significant association between AL and RCF (based on $\beta = 0.33$) with 80% power at the 5% significance level, a sample size of 52 eyes is needed.

To detect a significant difference in RCF in eyes with and without DR, we referred to a previous paper that evaluated the same relationship in diabetic participants.[17] A sample size of 96 eyes (48 per group) is needed to detect a statistically significant difference of 51 AU in RCF in eyes with DR compared to eyes without DR with 80% power at the 5% significance level.

Lastly, to detect a significant difference of 0.58 mm in mean AL between eyes with and without DR as derived from our study sample, with 80% power at the 5% significance level, a sample size of 138 eyes (69 per group) is needed.

From the above calculations, a sample size of at least 138 diabetic eyes (69 with DR and 69 without DR) is needed to detect significant associations between AL, RCF, and DR with 80% power at the 5% significance level.

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Table 1. Characteristics of Participants Without and With Diabetic Retinopathy ($n = 85$)

Characteristics	No DR	DR	*P* Value
Male sex, %	48.28	51.72	0.49
Mean (SD) or median (IQR)*			
Duration of diabetes, y	10 (5.25–17)*	15 (6.5–22.5)*	<0.001
Age, y	57.66 (2.03)	55.34 (1.99)	0.42
HbA1c, %	7.38 (1.32)	8.06 (1.79)	0.02
Systolic blood pressure, mm Hg	130.72 (17.72)	135.06 (20.43)	0.17
Diastolic blood pressure, mm Hg	79.75 (9.47)	80.31 (10.82)	0.74
Intraocular pressure	14.0 (0.29)	14.4 (0.37)	0.37
Body mass index, kg/m^2	28.25 (7.03)	29.78 (5.65)	0.15
Cholesterol, mM	4.47 (1.28)	4.50 (1.11)	0.88
Triglycerides, mM	1.45 (0.99)	1.27 (0.59)	0.25
Axial length, mm	23.90 (1.35)	23.32 (1.05)	0.005
Retinal capillary flow, AU	282.27 (113.94)	279.81 (76.05)	0.89

* IQR, interquartile range.

Statistical Analysis

Analyses were performed using Intercooled STATA version 12.1 for Windows (StataCorp., College Station, TX, USA). Eye-specific data (AL, RCF, DR presence, and DR severity) from the left and right eyes of eligible subjects were used in analyses. Participants' characteristics by the presence or absence of DR were compared using the χ^2 statistic for proportions and a *t*-test or Mann-Whitney *U* test for means or medians as appropriate.

First, we confirmed the association between longer AL and reduced odds of DR to verify our previous observation in this study sample.[2] Eye-specific data (AL and DR) and generalized estimating equation (GEE) models utilizing the exchangeable correlation matrix to account for the correlation between the two eyes were used. Two models were constructed, initially adjusting for age and sex (model 1), and additionally adjusting for age, sex, RCF, and known risk factors of DR (HbA1c and duration of diabetes) (model 2). To assess the associations of AL with the severity of DR, ordinal logistic regression models were used for both age- and sex- and multivariable-adjusted analyses.

Second, we determined the relationship between AL and RCF. Generalized estimating equation models with exchangeable correlation matrix were used to explore the unadjusted associations of RCF with AL and other key covariables (age, sex, BMI, duration of diabetes, anti-platelet medication use, HbA1c, triglycerides, cholesterol, SBP, DBP, and the presence of DR), while controlling for the correlation between the two eyes. Two multivariable-adjusted models were then constructed to assess the relationship between AL and RCF: initially including age and sex (model 1) and additionally including significant variables identified in the unadjusted analysis (model 2).

Last, we investigated the associations between RCF and DR. Eye-specific data and GEE models were utilized to assess the associations between RCF and DR in separate models, first including age and sex (model 1) and then additionally including AL and known DR risk factors (duration of diabetes and HbA1c) (model 2) while controlling for correlation between the two eyes. Ordinal logistic regression models were then utilized to assess associations of both AL and RCF with the severity levels of DR in models 1 and 2.

To better illustrate the relationship between AL and RCF with DR severity, the adjusted means of the predictors (AL and RCF) were obtained from the GEE models described above and plotted against the outcome (DR severity).

Covariables included in the models were either categorical or continuous (e.g., per unit change in age and SBP, per year for diabetes duration, and per percent for HbA1c). Eyes that were ungradable for DR were excluded from analyses.

Results

A total of 150 eyes were included in this study. Of these, 74 (49%) had DR. Among the eyes with DR, 28 (19%), 32 (21%), and 14 (9%) had mild, moderate, and severe DR, respectively. The mean (standard deviation [SD]) AL and RCF values were 23.60 (1.23 mm) and 279.67 (95.69) AU, respectively. Compared to participants without DR, those with DR had longer duration of diabetes ($P < 0.001$), higher HbA1c ($P = 0.02$), and shorter AL ($P = 0.005$, Table 1).

Associations Between AL and DR

A longer AL was associated with decreased odds of having DR in age- and sex-adjusted models (odds ratio [OR] 0.63, 95% confidence interval [CI] 0.46–0.85; Table 2, model 1). After adjusting for age, sex, RCF, duration of diabetes, and HbA1c, eyes with longer AL were less likely to have any DR (per mm increase in AL, OR 0.61; 95% CI 0.41–0.91; Table 2, model 2). Ordinal logistic regression models also demonstrated that a longer AL was associated with decreased odds of having a more

Table 2. Associations of Axial Length With Diabetic Retinopathy ($n = 150$ Eyes)

Parameters	No. of Eyes	Model 1 OR (95% CI)	*P* Value	Model 2 OR (95% CI)	*P* Value
Presence of DR and axial length					
Per mm increase	150	0.63 (0.46–0.85)	0.003	0.61 (0.41–0.91)	0.016
Severity of DR and axial length					
Per mm increase	150	0.62 (0.46–0.83)	0.001	0.65 (0.44–0.95)	0.03

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, HbA1c, duration of diabetes, and retinal capillary flow.

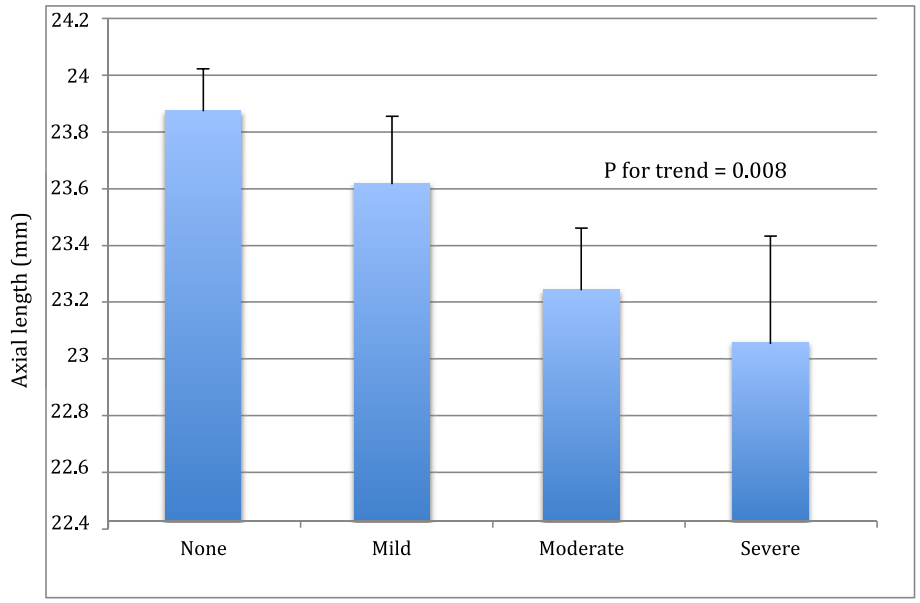

Figure 1. Bar chart of multivariable (*asterisk*) adjusted means of axial length plotted against severity of diabetic retinopathy.

severe category of DR (per mm increase in AL, OR 0.65; 95% CI 0.44–0.95; Table 2, model 2). A bar chart of the multivariable adjusted means of AL against the severity of DR is shown in Figure 1, which confirms the decreasing trend of AL as DR progresses in severity ($P = 0.008$).

Associations Between Participant Characteristics and RCF

Table 3 shows the unadjusted associations between clinical and demographic characteristics with RCF in this sample. Only age and SBP were significantly and positively associated with RCF (per year increase in age, β: 1.15, 95% CI 0.19–2.11; per mm Hg increase in SBP, β: 0.99, 95% CI 0.30–1.72, respectively). No association was found between AL and RCF (per mm increase in AL, β: 0.33, 95% CI −13.52 to 14.19).

Associations Between AL and RCF

Multivariable adjustments for age, sex, the presence of DR, and other significant characteristics associated with RCF, derived from unadjusted analyses, showed no significant associations between AL and RCF in our study sample (per mm increase in AL, β: −1.80, 95% CI −13.10 to 9.50; Table 4, model 2). A graphical representation of this relationship is shown in Figure 2.

Associations Between RCF and DR

After adjusting for age and sex, RCF was not associated with DR (OR: 1.00; 95% CI: 0.99–1.00; Table 5, model 1). In multivariable models adjusted for age, sex, AL, duration of diabetes, and HbA1c, there was no association between RCF and odds of having any DR (per unit increase in RCF, OR: 1.00; 95% CI: 0.99–1.00; Table 5, model 2). Ordinal logistic regression models also established no significant association between RCF and the severity of DR (per unit increase in RCF, OR: 1.00; 95% CI: 1.00–1.01; Table 5, model 2). The bar chart of the multivariable adjusted means of RCF plotted against DR severity showed no significant trend of an increase in RCF as DR progresses in severity ($P = 0.92$) (Fig. 3).

Table 3. Unadjusted Associations Between Participant Characteristics and Retinal Capillary Flow ($n = 150$ Eyes)

Outcome: Capillary Blood Flow	Unadjusted Regression Coefficient, β	95% Confidence Interval	*P* Value
Age, y	1.15	0.19 to 2.11	0.02*
Female sex	19.58	−14.53 to 53.68	0.26
Axial length, mm	0.33	−13.52 to 14.19	0.96
HbA1c, %	4.14	−5.59 to 13.87	0.40
Duration of diabetes, y	−0.39	−2.00 to 1.22	0.63
Anti-platelet medication use, yes	−1.05	−36.26 to 34.15	0.90
Body mass index, kg/m²	−0.99	−3.79 to 1.80	0.49
Cholesterol, mmol/L	−3.04	−18.41 to 12.33	0.70
Triglycerides, mmol/L	5.58	−23.15 to 34.30	0.70
Presence of diabetic retinopathy	−2.45	−37.09 to 32.19	0.89
Intraocular pressure	1.78	−4.46 to 8.03	0.58
Systolic blood pressure, mm Hg	0.99	0.30 to 1.72	0.008*
Diastolic blood pressure, mm Hg	0.89	−0.49 to 2.27	0.21

* Significant variables.

Table 4. Associations Between Axial Length and Retinal Capillary Flow ($n = 150$ Eyes)

Outcome: Retinal Capillary Flow	No. of Eyes	Model 1 β (95% CI)	P Value	Model 2 β (95% CI)	P Value
Per mm increase in axial length	150	3.64 (−10.03 to 17.31)	0.60	−1.80 (−13.10 to 9.50)	0.77

Model 1: Includes age and sex. Model 2: Includes age, sex, and systolic blood pressure.

Discussion

In this study, we found no significant association between AL and RCF, which fails to support the original hypothesis of the study that RCF was reduced as the eye elongates. We also showed that there was no association between RCF and the likelihood or severity of DR as was previously observed.[17] Furthermore, we confirmed previous observations that a longer AL was associated with a lower risk and severity of DR in these patients. Our results hence suggest that the protective relationship between axial myopia and DR is unlikely to be due to a decrease in RCF in eyes with myopic axial elongation as was originally proposed.

Theories on the mechanisms underpinning the protective myopia–DR relationship have mostly centered on changes brought about by axial elongation of the ocular axis. Recent data from Lim and colleagues,[1] as well as from our group in Australia,[2] have confirmed that axial elongation is indeed the major contributor to this protective relationship. This is further corroborated by data from the present study, in which a longer AL was associated with a lower risk of having any DR after multivariate adjustments.

Studies have shown an increase in retinal vessel diameter[5–7] and retinal blood flow[8–10] as DR progresses, as likely responses to hyperglycemia and hypoxia. According to the laws of Starling and Laplace,[4] this would result in an increase in capillary hydrostatic pressure, leading to microvascular complications such as dilatation (microaneurysms), leakage (edema), and rupture (hemorrhage). An increased RCF may therefore be one of the mechanisms underlying the pathogenesis of DR.

In support of this theory, Cuypers and colleagues[17] assessed RCF in three separate areas: the zone nasal to the optic disc, the papillomacular area, and the perifoveal region. They found that an increased RCF in the nasal and perifoveal regions, but not the papillomacular region, was associated with increased likelihood and severity of DR. In contrast, we showed that the RCF was not associated with DR in our study.

This disparity in results could be due to the difference in the areas where the RCF was assessed. In contrast to Cuypers et al.,[17] we averaged the RCF in a small area 1.5 disc diameters adjacent to the optic disc in order to minimize the impact from the choroidal circulation, as well as from nonperfused zones resulting from diabetes-induced capillary dropout and nonperfusion[18] (owing to the abundance of retinal capillaries proximal to the central retinal artery).[19] Heidelberg Retinal Flowmeter measurements taken from the perifoveal area and region nasal to the optic disc, as was the case in the study by Cuypers et al.,[17] are inherently more vulnerable to influence from the faster-flowing choroidal circulation underlying the retina,[20] by virtue of decreased capillary density in areas proximal to the foveal avascular zone and peripheral retina, respectively.[19] The reduced capillary density makes it easier for the HRF to pick up light scattered from moving red blood cells in the underlying choriocapillaries.[20] As DR develops and worsens, so too do areas of capillary dropout and nonperfusion,[21] which may have exacerbated the problem by allowing more light from the HRF to hit and scatter off moving red blood cells in the choriocapillaries instead of retinal capillaries, hence skewing results.

We also established that there was no association between AL and RCF in diabetic eyes with or without DR. Previous studies in healthy eyes have shown that as AL increases, ocular

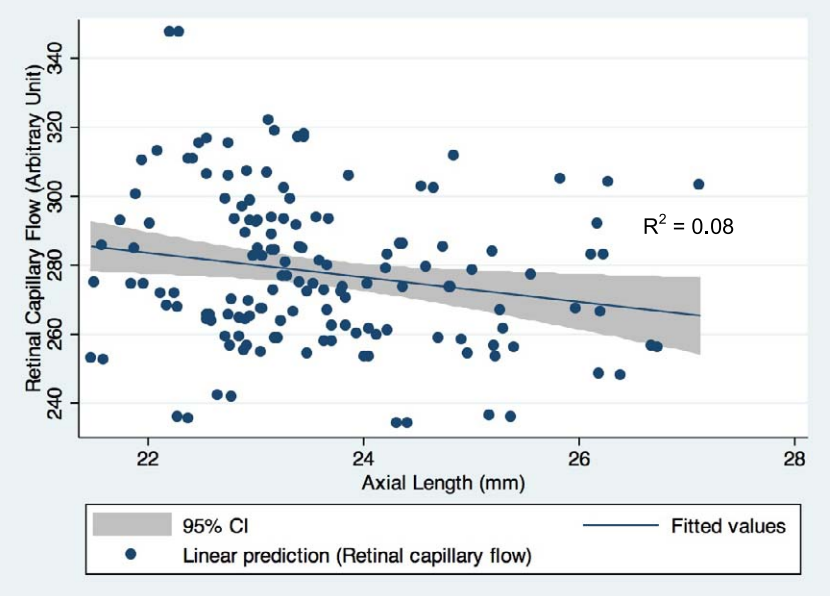

Figure 2. Scatterplot of retinal capillary flow (*asterisk*) versus axial length.

Table 5. Associations of Retinal Capillary Flow With Diabetic Retinopathy

Parameters	No. of Eyes	Model 1 OR (95% CI)	*P* Value	Model 2 OR (95% CI)	*P* Value
Presence of DR and capillary flow					
Per unit increase	150	1.00 (0.99–1.00)	0.90	1.00 (0.99–1.00)	0.88
Severity of DR and capillary flow					
Per unit increase	150	1.00 (1.00–1.00)	0.70	1.00 (1.00–1.01)	0.43

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, HbA1c, duration of diabetes, and axial length.

blood flow decreases.[11–13] This has been proposed to be due to a reduction in vessel diameter and concurrent increase in vessel length[22] as the eye lengthens. According to the Hagen-Pouseille law,[3] flow is proportional to the diameter (to the fourth power) and inversely correlated to the length of the vessel. These changes would therefore result in a decrease in retinal blood flow. Even though we did not measure blood flow in retinal arterioles and venules directly, we assessed capillary blood flow, which should be correlated with retinal arteriolar blood flow as blood drains directly from the arterioles to the capillaries. However, we acknowledge that the RCF constitutes only a portion of the retinal microcirculation, and our results may not be entirely representative of the pathological changes in the retinal microcirculation due to diabetes. A previous study has shown concurrent retinal autoregulatory dysfunction of arterioles and venules with blood flow changes in diabetes and DR.[23] Further studies are therefore needed to investigate the relationship of dynamic vascular response (e.g., to flickering light using the dynamic vessel analyzer) with increasing AL.

Our results are, however, very similar to those of the only study to correlate AL with RCF in healthy subjects. Benevente-Pérez et al.[24] found that RCF, measured using the HRF, did not decrease with increasing AL. However, their results may not be accurate, largely as a result of their not having access to the AFFPIA software, which they have also acknowledged as a major limitation in their article. The clinical reliability of RCF readings analyzed using the default HRF software has been questioned,[25] as unlike the AFFPIA program, the default software does not adjust for over- or underexposed pixels, nor does it account for saccades or the possible effect of the cardiac cycle on the RCF.

There are several possible explanations for the contrast between our results and those of previous studies. First, there has been evidence showing pathogenic retinal blood flow redistribution to the more superficial layer of capillary vessels in animal models of diabetes,[26] which may have affected results, as the AFFPIA software is unable to break down the RCF measurements layer by layer. Second, even though attempts were made to minimize the influence of capillary nonperfusion on RCF readings, there would still inevitably be some impact especially as the severity of DR (and the extent and size of capillary nonperfusion and dropout zones) increases,[18] which may have led to increased variability in RCF readings and affected the results. Third, the baseline range of values for RCF in patients with diabetes appears to be quite large, as evident from the sizable SD in subjects with no DR. This may also have confounded the associations under investigation. Lastly, we assessed RCF only in an area 1.5 disc diameters adjacent to the optic disc. This might not give an accurate representation of the changes in RCF of other areas of the retina, particularly the peripheral retina, which is affected in DR; however, to the best of our knowledge, no technology exists to capture retinal-wide RCF.

Strengths of this study include quantitative measures of RCF; assessment of DR from retinal photographs using standardized grading protocols; and performance of all RCF, AL, and fundus imaging measures by one researcher (REKM).

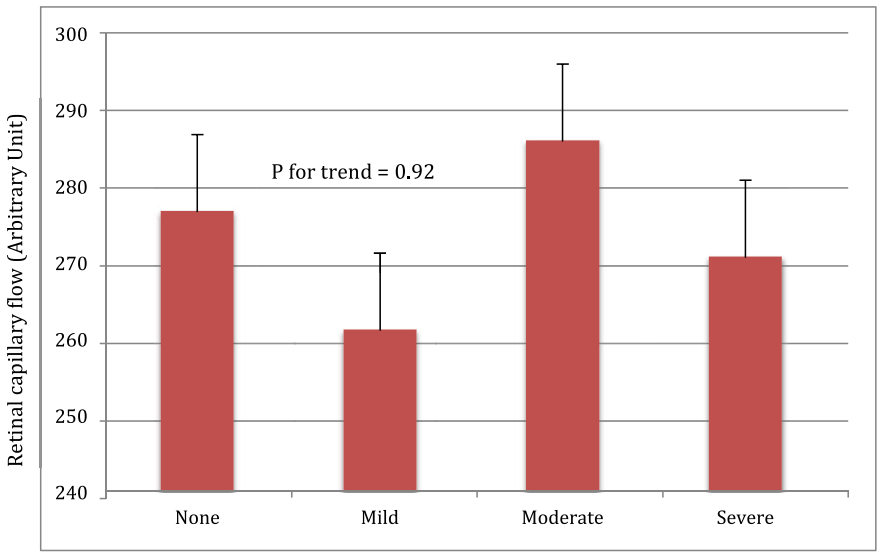

Figure 3. Bar chart of multivariable (*asterisk*) adjusted means of retinal capillary flow plotted against severity of diabetic retinopathy.

Limitations of this study should also be noted. First, we were not able to assess RCF across the whole retina, as noted above. Second, our sample size for subjects with severe DR was small, which may have led to an increased variance in our results.

In summary, we have demonstrated that RCF is not associated with AL or the likelihood of, and severity of, DR. These results suggest that the hypothesized reduction in RCF in longer eyes may not be as relevant to the protective myopia–DR relationship as originally proposed. Owing to technological advancements in recent years, assessing RCF in individual capillaries using adaptive optics,[27] as well as optical microangiography combined with en face technology,[28] to image the capillary flow layer by layer is now possible. Future studies using these techniques to confirm the veracity of our findings in different areas of the retina may therefore be warranted.

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