

Correlation of Light-Flicker–Induced Retinal Vasodilation and Retinal Vascular Caliber Measurements in Diabetes

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PURPOSE. Subtle changes in retinal vascular caliber have been shown to predict diabetic retinopathy and other diabetic complications. This study was undertaken to investigate whether retinal vascular caliber correlates with light-flicker–induced retinal vasodilation, a measure of endothelial function.

METHODS. The participants were 224 persons with diabetes (85 type 1 and 139 type 2) and 103 persons without diabetes (controls). Flicker-induced retinal vasodilation (percentage increase over baseline diameter) was measured with a vessel analyzer. Retinal vascular caliber was measured from digital retinal photographs according to a standardized, validated protocol. Data from both right and left eyes were used and modeled with generalized estimating equations to account for correlation between eyes.

RESULTS. In persons with diabetes, after adjustment for age and sex, reduced flicker-induced vasodilation was associated with wider retinal vascular caliber. Eyes with the lowest tertiles of flicker-induced arteriolar dilation had wider arteriolar caliber (5.40 μm ; 95% confidence interval [CI], 1.76–9.05) and eyes with the lowest tertiles of flicker-induced venular dilation had corresponding wider venular caliber (12.4 μm ; 95% CI, 6.48–18.2), respectively, than eyes with the highest tertile of vasodilation. These associations persisted after further adjusting for diabetes duration, systolic blood pressure, fasting glucose, lipids, body mass index, current smoking, and presence of diabetic retinopathy. No associations were evident in persons without diabetes.

CONCLUSIONS. Changes in retinal vascular caliber (wider arterioles and venules) are associated with impaired flicker-induced vasodilation in persons with diabetes. Determining whether endothelial dysfunction explains the link between retinal vascular caliber and risks of diabetic microvascular complications calls for further study. (*Invest Ophthalmol Vis Sci*. 2009;50:5609–5613) DOI:10.1167/iovs.09-3442

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Supported by a grant from the Diabetes Australia Research Trust (TTN, JJW, TYW).

Submitted for publication January 21, 2009; revised June 27, 2009; accepted August 28, 2009.

Disclosure: T.T. Nguyen, None; R. Kawasaki, None; A.J. Kreis, None; J.J. Wang, None; J. Shaw, None; W. Vilser, None; T.Y. Wong, None

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Digital retinal photography and imaging techniques have allowed precise measurement of subtle retinal vascular caliber changes in large populations.¹ Recent studies have shown that changes in retinal vascular caliber are associated with the development of type 2 diabetes,^{2–4} and vascular complications of both type 1 and 2 diabetes.^{5–12} In persons with diabetes, studies suggest that wider retinal arterioles are associated with the risk of diabetic retinopathy,^{6–8,12} whereas wider retinal venules are associated with progression of retinopathy¹¹ and diabetic nephropathy.^{9,10}

However, the underlying mechanisms for these relationships and why subtle changes in the caliber of the retinal vasculature are associated with both macro- and microvascular complications in diabetes remain unclear.^{1,13} Endothelial dysfunction has been suggested as a possible pathophysiological mechanism, but the limited number of studies examining this association with indirect systemic markers of endothelial function (e.g., von Willebrand factor, soluble vascular adhesion molecule-1) have shown inconsistent findings.^{14–16}

The response of retinal vessels to diffuse luminance flicker can be measured noninvasively and may reflect endothelial function of the retinal circulation, since it has been demonstrated that nitric oxide (NO) is released in the retinal vasculature when stimulated by flickering light.^{17–21} Persons with diabetes and diabetic retinopathy have been shown to have a reduction in vasodilatory response to flickering-light stimulation.^{22,23} In the present study, we investigated whether retinal vascular caliber correlates with light-flicker–induced retinal vasodilation in persons with diabetes. This information will provide further insight into the pathophysiological relationships and mechanisms underlying the relationship of retinal vascular caliber and diabetic vascular complications.

MATERIALS AND METHODS

We conducted a hospital-based clinical study between October 2006 and April 2008, prospectively recruiting 224 participants with diabetes (85 with type 1 and 139 with type 2) from the diabetic eye clinics at International Diabetes Institute (Melbourne, Australia) and 103 control subjects (persons without diabetes or retinal disease) from the general eye clinics at the Royal Victorian Eye and Ear Hospital (Melbourne, Australia). Participants were excluded who were older than 70 years, had a history of epilepsy or glaucoma, had undergone previous vitreal surgery and/or had cataract diagnosed on examination.

All participants and control subjects had a standardized clinical examination including blood pressure, measurement of blood chemistry, fasting plasma glucose and lipid levels, retinal photographs and assessment of flicker-induced vasodilation with a vessel analyzer (Dynamic Vessel Analyzer [DVA]; IMEDOS, Jena, Germany). The study complied with the tenets of the Declaration of Helsinki, institutional review board approval was granted, and written informed consent was obtained from all participants.

Measurement of Retinal Vascular Caliber

Both eyes of each participant were photographed with a 40° 6.3-megapixel digital nonmydriatic camera. Two photographic fields (optic disc and macula) were taken of each eye. The images were graded at the Centre for Eye Research Australia. The eyes were graded for diabetic retinopathy according to the Airlie House classification system.²⁴ Diabetic retinopathy was present if the level was 14 or greater.

Retinal vascular caliber was measured in a detailed protocol with a computer-based program by trained graders who were masked to participant characteristics and vessel analyzer measurements.¹¹ For each image, arterioles and venules coursing through a zone one-half to one disc diameter away from the optic disc margin were measured and summarized as the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).^{10,11} These measurements were summarized to represent the mean caliber of retinal arterioles (CRAE) and retinal venules (CRVE) of the eye.²⁵ Reproducibility of these measurements has been reported, with intra- and intergrader intraclass correlation coefficients ranging from 0.78 to 0.99.¹⁰ In this study, reproducibility of these measurements was excellent, with intra- and inter-grader intraclass correlation coefficients ranging from 0.94 to 0.99, respectively.

Flicker-Light-Induced Retinal Vasodilation

The Dynamic Vessel Analyzer (DVA; IMEDOS) measures retinal vessel dilation in response to flickering light.¹⁷ Examination was conducted in a half-lit room. The participant focused on the tip of a fixation bar within the retinal camera while the fundus was examined under green light. An arteriole and venule segment between one half and two disc diameters from the margin of the optic disc was selected. The superior temporal vessels were usually selected. The inferior temporal vessel segments were used on the rare occasions when the superior vessels could not be measured. The mean diameter of the arterial and venous vessel segments were calculated and recorded automatically. Baseline vessel diameter was measured for 50 seconds, followed by a provocation with flickering light of the same wavelength for 20 seconds, and then a nonflicker period for 80 seconds. This measurement cycle was repeated twice, with a total duration of 350 seconds per eye. When the eye blinked or moved, the system automatically stopped the measurement, and restarted once the vessel segments were automatically reidentified.

Retinal arteriolar and venular dilation in response to flickering light was calculated automatically by the DVA software. It was represented as an average increase in the vessel diameter in response to light flicker during the three measurement cycles and was defined as the percentage increase relative to the baseline diameter size.

Assessment of Diabetes

Fasting blood samples were drawn from participants at suburban pathology centers for measurement of fasting blood glucose level within 2 weeks of their eye tests. All persons with diabetes were patients recruited from diabetic eye clinics and had their disease managed with oral hypoglycemic medications and/or insulin. Control subjects (persons without diabetes) had confirmed nondiabetic status based on a lack of history of diabetes and fasting glucose levels <7.0 mmol/L (126 mg/dL).

Assessment of Other Risk Factors

A detailed questionnaire was used to obtain participant information, including medical history, current cigarette smoking, and the use of antihypertensive and lipid-lowering medications. Hypertension was defined as systolic blood pressure (SBP) \geq 140 mm Hg, diastolic blood pressure (DBP) \geq 90 mm Hg, or current use of antihypertensive medications. Dyslipidemia was defined as cholesterol >5.5 mM, or triglyceride >2.0 mM, or current use of lipid-lowering medications. Height and weight were measured to determine body mass index (BMI). Fasting (>8 hours) blood samples were obtained from participants at

suburban pathology centers for fasting blood glucose level, cholesterol, and triglyceride levels within 2 weeks of their eye tests.

Statistical Analysis

We compared flicker-induced retinal vasodilation and retinal vascular caliber in persons with diabetes and then repeated the analysis in nondiabetic control subjects. Flicker-induced arteriole/venule dilation was analyzed as percentage increase over baseline diameter and categorized into tertiles. Data from both right and left eyes were used. Multiple linear regression models were constructed, using the generalized estimating equations (GEE) to account for correlation between eyes, to determine the mean difference in CRAE and CRVE between tertiles of flicker-induced arteriolar and venular dilation. The highest tertiles of arteriolar and venular vasodilation were used as the reference groups. We constructed two models: model 1 adjusting for age and sex; and model 2 further adjusting for duration of diabetes, fasting blood glucose level (FBGL), SBP, fasting cholesterol and triglyceride levels, current smoking, use of antihypertensive and lipid-lowering medications, and the levels of diabetic retinopathy. $P < 0.05$ was considered significant (Stata, ver. 10.0; Stata Corp., College Station, TX).

RESULTS

Selected characteristics for the study sample, in persons with diabetes ($n = 224$, 85 with type 1 and 139 with type 2 diabetes) and nondiabetic control subjects ($n = 103$), are shown in Table 1. Mean age was 56.5 (\pm 11.8) years in subjects with diabetes, and 48.0 (\pm 16.3) years in the control subjects. The proportion of men was similar in participants with diabetes (59.4%) and controls (58.3%). Compared to nondiabetic controls, participants with diabetes were less likely to be current smokers but had higher BMI, more likely to have hypertension, dyslipidemia, lower DBP and lower total cholesterol levels.

In persons with diabetes, eyes with reduced flicker-induced retinal vasodilation had wider retinal vascular caliber (Table 2). Eyes with the lowest tertiles of flicker-induced arteriolar dilation had wider retinal arteriolar caliber (mean difference 5.40 μ m; 95% confidence interval [CI], 1.76–9.05) and eyes with lowest tertiles of flicker-induced venular dilation had wider venular caliber (mean difference 12.4 μ m; 95% CI, 6.48–18.2), compared with eyes with the highest tertile of arteriolar and venular dilation, respectively (Table 2). These associations persisted after further adjustment for diabetes duration, SBP, fasting glucose, cholesterol, and triglyceride levels; body mass index; current smoking; use of antihypertensive and lipid-lowering medications; and the presence of diabetic retinopathy.

In persons without diabetes (control), after adjustment for age and sex, retinal arteriolar and venular calibers were not associated with flicker-induced arteriolar and venular dilation (Table 3).

In subgroup analysis among persons with diabetes, associations were largely similar for persons with type 1 and 2 diabetes, but the associations were statistically significant only for venular caliber. In persons with type 1 diabetes, reduced flicker-induced venular dilation was significantly associated with large venular caliber (mean difference 10.1 μ m; 95% CI, 0.80–19.5; $P = 0.03$; comparing lowest versus highest tertile of venular dilation). In persons with type 2 diabetes, the corresponding mean difference in venular caliber was 9.0 μ m (95% CI, 1.40–16.6; $P = 0.02$), after adjustment for covariables in the multivariate model (data not shown).

TABLE 1. Participant Characteristics Comparing Persons with Diabetes and Normal Controls

	Persons with Diabetes (<i>n</i> = 224)		Normal Control Subjects (<i>n</i> = 103)		<i>P</i>
	<i>n</i>	%	<i>n</i>	%	
Sex (male)	133	59.4	60	58.3	0.85
Smoking					
Current	16	7.1	16	21.3	0.002
Past	88	39.3	21	28.0	—
Never	120	53.6	38	50.7	—
Hypertension	137	61.2	15	20.0	<0.001
Antihypertension medications	135	60.3	14	18.7	<0.001
Dyslipidemia	122	54.5	8	10.7	<0.001
Antihypercholesterolemia medications	119	53.1	8	10.7	<0.001

	Persons with Diabetes (<i>n</i> = 224)		Normal Control Subjects (<i>n</i> = 103)		<i>P</i>
	Mean	SD	Mean	SD	
Age, y	56.5	11.8	48.0	16.3	<0.001
Duration of diabetes, y	16.2	10.5	—	—	—
Systolic blood pressure, mm Hg	128	14.5	129.4	17.1	0.51
Diastolic blood pressure, mm Hg	76.1	8.9	80.1	9.1	<0.001
Body mass index, kg/m ²	30.4	6.3	26.2	5.2	<0.001
Hemoglobin A1c, %	7.9	1.4	5.5	0.6	<0.001
Glucose (BSL), mmol/L	9.2	3.7	5.1	0.6	<0.001
Total cholesterol, mmol/L	4.6	1.1	5.4	0.9	<0.001
Triglyceride, mmol/L	1.6	1.1	1.4	0.8	0.35
Diabetic retinopathy, <i>n</i> (%)					
No retinopathy (level <14)	79	(49.2)	—	—	—
Minimal NPDR (level 14–20)	29	(14.7)	—	—	—
Early-moderate NPDR (level 31 or 41)	53	(26.9)	—	—	—
Severe NPDR or PDR (level 51 to 80)	36	(18.3)	—	—	—

DR, diabetic retinopathy; NPDR, nonproliferative DR; PDR, proliferative DR.

DISCUSSION

Our study demonstrated a correlation of the dynamic response of retinal circulation to flickering light and measurements of retinal vascular caliber in persons with diabetes. We found that eyes with reduced flicker-induced arteriolar and venular dilation had wider retinal arterioles and venules, respectively, independent of age, sex, diabetes duration, glycemia levels, and other risk factors, and this was not seen in the control subjects without diabetes.

In a previous study, Polak et al.²¹ found an association at 8 Hz in retinal veins but not at 16 Hz. The flicker used in our study was 12.5 Hz. No association was found in our nondiabetic control. We are unclear whether this is due to differences in frequencies used, sample size, or the way we calculated the vessel diameter (by combining the biggest six arterioles and venules passing through the circular zone between 0.5 and 1 disc diameter away from the optic disc margin were summarized as the CRAE and CRVE, instead of a single vessel, as per Polak et al.²¹).

TABLE 2. Relationships between Dynamic Flicker-Induced Vasodilation and Retinal Vascular Caliber in Persons with Diabetes

Number of Eyes*	Flicker-Induced Vasodilation		Retinal Vascular Caliber				
	Tertiles	Range, %	Mean (SD), μ m	Mean Difference (95% CI), Model 1†	<i>P</i>	Mean Difference (95% CI), Model 2‡	<i>P</i>
Arteriolar							
120	Lowest	≤0.3	149.9 (15.5)	5.40 (1.76 to 9.05)	0.004	4.94 (1.00 to 8.90)	0.014
127	Middle	0.4 to 1.8	150.1 (16.6)	4.55 (0.97 to 8.13)	0.01	3.86 (0.08 to 7.64)	0.046
127	Highest	≥1.9	146.5 (16.2)	1.00 (reference)		1.00 (reference)	
Venule							
120	Lowest	≤1.7	225.7 (27.4)	12.4 (6.48 to 18.2)	<0.001	9.81 (3.76 to 15.9)	0.001
122	Middle	1.8 to 3.3	220.2 (21.2)	5.91 (0.07 to 11.8)	0.047	3.33 (−2.51 to 9.16)	0.28
133	Highest	≥3.4	215.1 (25.9)	1.00 (reference)		1.00 (reference)	

n = 224. Bold data represent statistically significant results.

* Number of eyes.

† Model 1: age- and sex-adjusted.

‡ Model 2: model 1 + duration of diabetes, FBGL, SBP, fasting cholesterol and triglyceride levels, current smoking, BMI, presence of hypertension and dyslipidemia, and levels of diabetic retinopathy.

TABLE 3. Relationships between Dynamic Flicker-Induced Vasodilation and Retinal Vascular Caliber in Normal Control Subjects

Number*	Flicker-Induced Vasodilation		Retinal Vascular Caliber		P
	Tertiles	Range, %	Mean (SD), μm	Mean Difference (95% CI), Model 1†	
Arteriolar					
51	Lowest	≤ 2.3	149.9 (14.2)	5.78 (−0.03 to 11.6)	0.05
54	Middle	2.4 to 4.2	150.3 (16.6)	5.82 (0.12 to 11.5)	0.05
53	Highest	≥ 4.3	145.2 (16.7)	1.00 (Reference)	
Venule					
51	Lowest	≤ 2.9	221.1 (27.2)	−6.79 (−16.1 to 2.51)	0.15
54	Middle	3.0 to 4.3	221.0 (25.2)	−4.49 (−13.9 to 4.88)	0.35
56	Highest	≥ 4.4	228.3 (22.6)	1.00 (Reference)	

Normal control subjects ($n = 103$).

* Number of eyes.

† Model 1: age- and sex-adjusted.

Our study provides support to the concept that variations in retinal vascular caliber, as measured from fundus photographs, may reflect underlying endothelial dysfunction in persons with diabetes. If so, this provides an explanation of why changes in retinal vascular caliber (wider arteriolar caliber and wider venular caliber) are associated with risk of diabetic retinopathy^{6–8,12} and other diabetic complications.¹⁶

The evidence that the retinal circulatory response to diffuse flickering light is related to endothelial function²² is based on the documented role of NO in flickering light-induced vasodilation.^{18–21} In a study by Dorner et al.,¹⁸ *N*^G-monomethyl-L-arginine (LNMMMA), an inhibitor of NO synthase, blunted this flicker-induced vasodilation in healthy individuals. Furthermore, impaired response to light-flicker stimulation in persons with hypertension could be restored by angiotensin-II subtype 1 receptor blockade,²⁶ similar to a recent study in which improvement was found in the retinal arteriolar architecture with successful treatment of hypertension.²⁷ However, these were documented only in persons without diabetes, and the mechanisms may differ. For example, it has been demonstrated that systemic administration of valsartan (angiotensin II type 1 receptor blockers) has little effect on the retinal blood flow in healthy humans,²⁸ whereas treatment with either an angiotensin-converting enzyme inhibitor or an angiotensin II type 1 receptor blocker normalized retinal blood flow in diabetic rats.²⁹ In addition, there may be an endothelium-independent component to this flicker response, as the impaired vasodilatory response of the microcirculation that has been demonstrated in other vascular beds is both endothelium-dependent and -independent.³⁰ In addition, it is becoming increasingly clear that neuronal cells of the retina are also affected by diabetes, resulting in dysfunction and degeneration.³¹ As retinal blood flow is coupled with neuronal activity,³² reduced flicker-induced vasodilation can thus reflect neurodegeneration as well.^{23,33} Therefore, in persons with diabetes, reduced flicker-induced vasodilation may reflect damage to both the neural tissues and the microcirculation in diabetes.

A major obstacle to clinical research on endothelial dysfunction is the difficulty in assessing its function level in vivo. Most measurements of endothelial function are time consuming and require highly specialized personnel and equipment. In contrast, the DVA does not require much training, and the measurement can be performed noninvasively in less than 15 minutes. As DVA measurement can also reflect neural function,^{23,33} correlation with retinal neuronal testing such as electroretinography is necessary to quantify neuronal functional damage. In addition, further corre-

lation with other markers of retinal endothelial function to confirm the role of endothelial function in flicker-induced vasodilation in persons with diabetes is needed, such as (1) retinal blood flow recovery after hyperoxia (which has been shown to be influenced by NO)^{34,35} and (2) administration of simvastatin, a lipid-lowering agent, as this has been shown to prevent the inhibitory effect of inflammation (which has been considered to be a pathogenic factor in the development and progression of diabetic retinopathy^{36,37}) on endothelial function.³⁸

One alternative explanation of our findings is that retinal vessels with a larger caliber are less likely to dilate further after stimulation by light-flicker.³⁹ However, in our nondiabetic group, eyes within the highest tertile of retinal venodilation in response to light-flicker stimulation had the largest mean venular caliber. Hence, these findings in nondiabetic subjects argue against the possibility of limited dilation potential in vessels that are already relatively large.

The strengths of this study include independently measured retinal vascular caliber in a well-validated protocol, and all DVA measurements performed by one person (TTN) to measure flicker-induced vasodilation. Limitations of this study include the fact that the cross-sectional nature of the study provides no temporal information on the associations reported. Second, most participants in our study were white/Caucasian, and therefore the results did not shed light on the ethnic/racial differences in retinal vasculature noted in other studies.^{16,40} Further longitudinal studies are needed to ascertain clinical use of this measurement of retinal vessel function and correlation with retinal neuronal testing such as electroretinography to quantify neuronal damage. Of importance, the underlying mechanism of endothelial dysfunction in larger retinal vessels is still speculative at present and requires further research to substantiate the finding.

In conclusion, we have demonstrated a correlation between reduced light-flicker-induced vasodilation and measurements of retinal vascular caliber in persons with diabetes. Endothelial dysfunction may be an underlying mechanism linking changes in retinal vascular caliber with diabetic retinopathy and other diabetic vascular complications. However, further study is needed to substantiate our findings and investigate the likely mechanisms. Nonetheless, our study further supports the use of quantitatively measured retinal caliber from fundus photographs in epidemiologic and clinical studies as a potential marker for major diabetic complications including retinopathy, nephropathy, and cardiovascular disease.

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