Flicker Light–Induced Retinal Vasodilation in Diabetes and Diabetic Retinopathy

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OBJECTIVE — Flicker light–induced retinal vasodilation may reflect endothelial function in the retinal circulation. We investigated flicker light–induced vasodilation in individuals with diabetes and diabetic retinopathy.

RESEARCH DESIGN AND METHODS — Participants consisted of 224 individuals with diabetes and 103 nondiabetic control subjects. Flicker light–induced retinal vasodilation (percentage increase over baseline diameter) was measured using the Dynamic Vessel Analyzer. Diabetic retinopathy was graded from retinal photographs.

RESULTS — Mean ± SD age was 56.5 ± 11.8 years for those with diabetes and 48.0 ± 16.3 years for control subjects. Mean arteriolar and venular dilation after flicker light stimulation were reduced in participants with diabetes compared with those in control subjects (1.43 ± 2.10 vs. 3.46 ± 2.36%, $P < 0.001$ for arteriolar and 2.83 ± 2.10 vs. 3.98 ± 1.84%, $P < 0.001$ for venular dilation). After adjustment for age, sex, diabetes duration, fasting glucose, cholesterol and triglyceride levels, current smoking status, systolic blood pressure, and use of antihypertensive and lipid-lowering medications, participants with reduced flicker light–induced vasodilation were more likely to have diabetes (odds ratio 19.7 [95% CI 6.5–59.1], $P < 0.001$ and 8.14 [3.1–21.4], $P < 0.001$, comparing lowest vs. highest tertile of arteriolar and venular dilation, respectively). Diabetic participants with reduced flicker light–induced vasodilation were more likely to have diabetic retinopathy (2.2 [1.2–4.0], $P = 0.01$ for arteriolar dilation and 2.5 [1.3–4.5], $P = 0.004$ for venular dilation).

CONCLUSIONS — Reduced retinal vasodilation after flicker light stimulation is independently associated with diabetes status and, in individuals with diabetes, with diabetic retinopathy. Our findings may therefore support endothelial dysfunction as a pathophysiological mechanism underlying diabetes and its microvascular manifestations.

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Diabetes affects more than 240 million individuals worldwide, and diabetic retinopathy is the leading cause of blindness in the working-age population in most developed countries (1). There is increasing recognition that early endothelial dysfunction plays a key role in the pathogenesis of diabetes (2) and the development of subsequent microvascular complications (3). In support of endothelial dysfunction in diabetic retinopathy (4) are studies showing relationships of diabetic retinopathy with cardiovascular diseases, including stroke, coronary heart disease, and heart failure, independent of traditional risk factors (5–7). Diabetic retinopathy has also been linked with subclinical manifestations of vascular diseases such as coronary artery calcification and cardiac remodeling (5). However, clinical and epidemiological studies have not found consistent associations of serum markers of endothelial dysfunction (e.g., soluble vascular adhesion molecule-1) with diabetic retinopathy, with some reporting positive associations (8,9), but others not finding any (10,11).

The response of retinal vessels to diffuse luminance flicker can be measured noninvasively (12) and may reflect endothelial function of the retinal circulation because it has been demonstrated that nitric oxide is released in the retinal vasculature when it is stimulated by flicker light (13). One recent study showed that individuals with diabetes and diabetic retinopathy have reduced flicker-induced retinal vasodilation but did not control for concomitant risk factors including hyperglycemia, hypertension, and diabetes duration (14). In our current study, we sought to clarify whether flicker light–induced vasodilation is impaired in patients with diabetes and in those with diabetic retinopathy, signs independent of major risk factors.

RESEARCH DESIGN AND METHODS

— We conducted a hospital-based clinical study between October 2006 and April 2008, prospectively recruiting 224 Caucasian/white participants with diabetes (85 with type 1 diabetes and 139 with type 2 diabetes) from the diabetic eye clinics at the International Diabetes Institute (Melbourne, VIC, Australia) and 103 white nondiabetic control subjects from the general eye clinics at the Royal Victorian Eye and Ear Hospital (Melbourne, VIC, Australia). Control subjects were consecutive patients seen at the hospital among individuals without diabetes and any retinal or eye pathological conditions. Individuals were excluded from participation if they were aged >70 years, were of nonwhite ethnic background, had a history of epilepsy or glaucoma, had previous vitreal surgery, and/or had a cataract on examination.

All participants and control subjects had a standardized clinical examination, measurement of blood chemistry, retinal photographs, and assessment of flicker-induced vasodilation using the Dynamic Vessel Analyzer (DVA; IMEDOS, Jena, Germany). Tenets of the Declaration of

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Helsinki were followed, institutional review board approval was granted, and written informed consent was obtained from all participants.

Flicker light–induced retinal vasodilation

The DVA measures retinal vessel dilation in response to diffuse luminance flicker (12). Examination was conducted in a half-light 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 were selected. The mean diameters of the arterial and venous vessel segments were calculated and recorded automatically. Baseline vessel diameter was measured for 50 s, followed by a provocation with flicker light of the same wavelength for 20 s and then a nonflicker period for 80 s. This measurement cycle was repeated twice, with a total duration of 350 s/eye. When the eye blinked or moved, the system automatically stopped the measurement and restarted it once the vessel segments were automatically reidentified.

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

Measurement of static retinal vessel diameter

In addition to quantifying the flicker-induced vasodilation, we assessed overall static arteriolar and venular diameter using a computer-assisted program. Details of the digital image preparation are described elsewhere (15). In brief, diameters of the largest six arterioles and venules passing through the circular zone between one-half and one disc diameter away from the optic disc margin were summarized as the central retinal arteriolar equivalent and central retinal venular equivalent using the Parr-Hubbard formula further modified by Knudtson and colleagues (15).

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 testing. All participants with diabetes were patients recruited from the diabetic eye clinics and were managed with oral hypoglycemic mediations and/or insulin. Control subjects (individuals without diabetes) had confirmed nondiabetic status based on a lack of history of diabetes and fasting glucose <7.0 mmol/l (126 mg/dl).

Assessment of diabetic retinopathy

In participants with diabetes, diabetic retinopathy was graded from fundus photographs at the Centre for Eye Research Australia, by graders masked to clinical details. For each eye, a retinopathy severity score was assigned based on modification of the Airlie House Classification system (16). For our analysis, levels 10, 11, and 12 were defined as no diabetic retinopathy, 14 to 20 as minimal nonproliferative diabetic retinopathy (NPDR), 31 and 41 as early to moderate NPDR, and 51–80 as severe NPDR (proliferative diabetic retinopathy).

Assessment of other risk factors

A detailed questionnaire was used to obtain participant information, including past medical history, current cigarette smoking, and the use of antihypertensive and lipid-lowering medications. Hypertension was defined as systolic blood pressure (SBP) >140 mmHg, diastolic blood pressure (DBP) >90 mmHg, or current use of antihypertensive medications. Dyslipidemia was defined as cholesterol >5.5 mmol/l or triglyceride >2.0 mmol/l or current use of lipid-lowering medications. Height and weight were measured to determine BMI. Fasting blood samples were drawn from participants at suburban pathology centers for fasting blood glucose level, cholesterol and triglyceride levels, and A1C within 2 weeks of their eye testing.

Statistical analysis

We compared flicker light–induced retinal vasodilation between individuals with diabetes and control subjects and in individuals with diabetes between those with and without DR. Flicker-induced arteriolar/venular dilation was analyzed as percent increase over baseline diameter, both as a continuous measure and in categories (tertiles). Data from both right and left eyes were used. Multiple logistic regression models were constructed using the generalized estimating equation models to account for correlation between the right and left eyes and to assess the odds of diabetes (vs. nondiabetic control subjects) or diabetic retinopathy (vs. no diabetic retinopathy among subjects with diabetes), comparing the lower versus upper tertiles of flicker light–induced arteriolar and venular dilation. In addition, multiple linear regression models were used to estimate the mean difference in arteriolar and venular dilation. We initially adjusted for age, sex, and fasting blood glucose level (model 1) and further adjusted for duration of diabetes (in analysis of diabetic patients), use of antihypertensive and lipid-lowering medications, current smoking status, SBP, and cholesterol and triglyceride levels (model 2). Analyses were performed in Stata (version 10.1; StataCorp, College Station, TX).

RESULTS — Selected characteristics of normal control subjects ($n = 103$), participants with diabetes ($n = 224$, 85 with type 1 and 139 with type 2 diabetes), and those with ($n = 144$) and without ($n = 80$) diabetic retinopathy are shown in Table 1. Mean ± age was 56.5 ± 11.8 years in subjects with diabetes and 48.0 ± 16.3 years in control subjects. The proportion of men was similar for participants with diabetes (41.6%) and control subjects (39.4%). Compared with nondiabetic control subjects, participants with diabetes were less likely to be current smokers but had higher BMI and were more likely to have hypertension, dyslipidemia, lower DBP, and lower total cholesterol levels. Compared with those with type 1 diabetes, individuals with type 2 diabetes were older, had greater BMI, but a shorter duration of diabetes, and were more likely to have hypertension and dyslipidemia (data not shown). In participants with diabetes, those with diabetic retinopathy had a longer duration of diabetes, had higher SBP, and were more likely to have hypertension. In addition, participants with diabetes had wider static arteriolar diameter than nondiabetic control subjects, whereas those with diabetic retinopathy had wider retinal venules than those without (Table 1).

Flicker light–induced retinal vasodilation was reduced in participants with diabetes compared with that in control subjects (Table 2). Flicker light–induced arteriolar dilation was 1.43 ± 2.10% in participants with diabetes and 3.46 ± 2.36% in normal control subjects ($P <$ 0.001 after adjustment for age, sex, fasting glucose, cholesterol and triglyceride levels, use of antihypertensive and lipid-lowering medications, and current smok-

Table 1—*Participant characteristics (age-adjusted means and proportions) comparing participants with diabetes and normal control subjects, and, among participants with diabetes, those with and without diabetic retinopathy*

	Control subjects	Diabetic subjects	*P**	Diabetic retinopathy	No retinopathy	*P*†
n	103	224		144	80	
Sex (% male)	39.4	41.6	0.74	37.5	49.2	0.09
Current smoking (%)	20.7	7.3	0.002	10.2	2.4	0.05
Hypertension (% present)	14.2	56.3	<0.001	63.8	42.1	0.006
Dyslipidemia (% present)	7.0	48.1	<0.001	49.9	44.1	0.46
Age (years)	48.0	56.5	<0.001	57.4	54.8	0.08
Diabetes duration (years)	—	16.2		18.7	11.9	<0.001
SBP (mmHg)	129.5	127.1	0.22	128.5	124.4	0.03
DBP (mmHg)	80.2	75.9	<0.001	75.7	76.4	0.55
BMI (kg/m^2)	26.3	30.3	<0.001	30.5	29.7	0.38
A1C (%)	5.5	7.9	<0.001	8.0	7.7	0.27
Glucose (mmol/l)	5.0	9.2	<0.001	9.5	8.5	0.07
Cholesterol (mmol/l)	5.4	4.6	<0.001	4.6	4.7	0.28
Triglyceride (mmol/l)		1.6	0.25	1.5	1.6	0.45
Dynamic retinal vessel dilation						
Maximal arteriolar dilation	3.33	1.50	<0.001	1.21	1.81	0.006
Maximal venular dilation	3.91	2.88	<0.001	2.57	3.21	0.003
Static retinal vessel diameter						
CRAE (μm)	146.0	149.8	0.007	150.8	149.4	0.35
CRVE (μm)	220.7	222.2	0.52	225.8	218.9	0.006

Data are means unless stated otherwise. Means and proportions are adjusted for age (set to mean age of 53.8 years old), except for age. *Comparing those with diabetic subjects and normal control subjects, adjusted for age. †Comparing those with and without diabetic retinopathy in those with diabetes, adjusted for age. CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent.

ing status). Retinal arteriolar dilation was not significantly different by type of diabetes: 1.57% in those with type 1 and 1.24% in those with type 2 diabetes ($P = 0.98$). Flicker light–induced venular dilation was 2.83 ± 2.10% in individuals with diabetes and 3.98 ± 1.84% in normal control subjects ($P < 0.001$ after multivariable adjustment) and again was not significantly different by type of diabetes: 2.84% in those with type 1 and 2.83% in those with type 2 diabetes ($P = 0.99$).

Table 2—*Mean differences in flicker light–induced vasodilation between participants with diabetes and normal control subjects and by grades of diabetic retinopathy severity in participants with diabetes*

			Age- and sex-adjusted		Multivariable adjusted*	
Groups	*n*	Mean dilation	Mean difference (95% CI)	*P*	Mean difference (95% CI)	*P*
Arteriolar						
Control subjects	103	3.46	(Reference)		(Reference)	
Diabetic patients	224	1.43	−1.87 (−1.43 to −2.31)	<0.001	−1.58 (−1.05 to −2.11)	<0.001
Type 1 diabetes		1.57	−1.99 (−1.46 to −2.52)	<0.001	−1.71 (−1.10 to −2.32)	<0.001
Type 2 diabetes		1.24	−1.78 (−1.29 to −2.27)	<0.001	−1.48 (−0.90 to −2.07)	<0.001
Diabetic retinopathy severity						
None		1.76	(Reference)		(Reference)	
Minimal NPDR		1.39	−0.37 (0.29 to −1.03)	0.28	−0.53 (0.16 to −1.22)	0.13
Early-moderate NPDR		1.01	−0.75 (−0.23 to −1.28)	0.005	−0.85 (−0.29 to −1.40)	0.003
Severe NPDR		1.24	−0.51 (0.15 to −1.16)	0.13	−0.58 (0.12 to −1.28)	0.10
Venular						
Control subjects		3.98	(Reference)		(Reference)	
Diabetic patients		2.83	−0.98 (−0.57 to −1.39)	<0.001	−1.07 (−0.56 to −1.57)	<0.001
Type 1 diabetes		2.84	−1.11 (−0.62 to −1.61)	<0.001	−1.36 (−0.78 to −1.94)	<0.001
Type 2 diabetes		2.83	−0.88 (−0.42 to −1.34)	<0.001	−0.83 (−0.27 to −1.38)	0.004
Diabetic retinopathy severity						
None		3.19	(Reference)		(Reference)	
Minimal NPDR		2.94	−0.49 (0.10 to −1.09)	0.10	−0.10 (0.60 to −0.79)	0.78
Early-moderate NPDR		2.40	−1.11 (−0.63 to −1.58)	<0.001	−0.67 (−0.11 to −1.23)	0.02
Severe NPDR		2.32	−1.16 (−0.55 to −1.77)	<0.001	−0.65 (0.06 to −1.35)	0.07

Data are %. *Adjustment for age, sex, fasting cholesterol and triglyceride levels, use of antihypertensive and lipid-lowering medications, current smoking status, and fasting glucose (for analysis of diabetic retinopathy severity).

Table 3—*Associations between reduced flicker-induced arteriolar and venular dilation and diabetes*

Dynamic flicker-induced dilation			Diabetes					
*n**	Tertiles	Range (%)	%	Model 1	*P*	Model 2	*P*	Model 3	*P*
Arteriolar									
173	Lowest	≤0.6	89.0	12.6 (5.54–28.7)	<0.001	19.7 (6.53–59.1)	<0.001	19.5 (6.30–60.2)	<0.001
182	Middle	0.7–2.9	76.9	7.76 (3.75–16.1)	<0.001	11.2 (4.29–29.3)	<0.001	11.16 (4.22–29.5)	<0.001
178	Highest	≥3.0	45.5	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Venular									
178	Lowest	≤2.1	86.5	4.67 (2.19–9.96)	<0.001	8.14 (3.09–21.4)	<0.001	8.03 (3.05–21.2)	<0.001
177	Middle	2.2–3.7	67.8	1.17 (0.64–2.14)	0.62	1.43 (0.66–3.13)	0.37	1.40 (0.64–3.08)	0.40
182	Highest	≥3.8	56.0	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—

Data are ORs (95% CI) unless indicated otherwise. Model 1: adjusted for age, sex, and fasting blood glucose level. Model 2: adjusted for covariates in model 1 plus diabetes duration, use of antihypertensive and lipid-lowering medications, current smoking status, SBP, and fasting cholesterol and triglyceride levels. Model 3: adjusted for covariates in model 2 plus static retinal arteriolar or venular diameter. **n* indicates number of eyes.

Table 3 shows that after multivariable adjustment, individuals with reduced flicker light–induced vasodilation were more likely to have diabetes (odds ratios [ORs] 19.7 and 8.1, comparing the lowest versus the highest tertile of arteriolar and venular dilation, respectively). Among participants with diabetes, those with reduced flicker induced–dilation were more likely to have diabetic retinopathy (ORs 2.2 and 2.5, respectively, for arteriolar and venular dilation) (Table 4). These associations persisted after further adjustment for static arteriolar/venular diameters (Tables 3 and 4, model 3).

The distribution of diabetic retinopathy severity was not significantly different between those with type 1 and type 2 diabetes ($P = 0.57$, data not shown). However, the association of reduced flicker light–induced vasodilation with diabetic retinopathy was stronger in participants with type 1 diabetes (arteriolar dilation OR 3.1 [95% CI 1.1–8.5]; venular dilation OR 3.8 [95% CI 1.4–10.0]) compared with those with type 2 diabetes (arteriolar dilation OR 1.8 [95% CI 0.8–4.0]; venular dilation OR 1.3 [95% CI 0.6–3.1]), although the interaction with type of diabetes was not statistically significant (*P* value for interaction term: $P = 0.50$ for arteriolar dilation and $P = 0.09$ for venular dilation).

CONCLUSIONS — In this study, we demonstrated a reduction in flicker light–induced retinal arteriolar and venular dilation in individuals with diabetes compared with nondiabetic control subjects and, among individuals with diabetes, in those with retinopathy signs. Importantly, we showed that these associations were independent of major risk factors for either diabetes or diabetic retinopathy and independent of static measurements of retinal arterioles and venular diameters.

There have been two previous studies for comparison (14,17). Garhofer et al. (17) examined 26 healthy control subjects and 26 individuals with type 1 diabetes who had none or minimal NPDR and were not receiving antihypertensive treatment, whereas Mandecka et al. (14) examined 240 individuals with diabetes (68 with type 1 and 172 with type 2 diabetes) and 58 control subjects. Both showed reduced flicker light vasodilation in those with diabetes (compared with those without diabetes). Furthermore, Mandecka et al. also demonstrated a reduction in flicker light vasodilation with increasing diabetic retinopathy severity, while controlling only for age, sex, and use of antihypertensive medications. We have now shown that the relationship of flicker light–induced vasodilation and both diabetes and diabetic retinopathy are independent of major confounders and risk factors for diabetic retinopathy, including duration of diabetes and glycemic control.

Retinal neuronal stimulation by flicker light results in retinal vessel dilation. This response probably reflects endothelial function (14), given the documented role of nitric oxide in this flickering light–induced vasodilation (13,18,19). In a study by Dorner et al. (13), N^G-monomethyl-L-arginine, an inhibitor of nitric oxide synthase, blunted

Table 4—*Associations between reduced flicker-induced arteriolar and venular dilation and diabetic retinopathy*

Dynamic flicker-induced dilation			Diabetic retinopathy					
*n**	Tertiles	Range (%)	%	Model 1	*P*	Model 2	*P*	Model 3	*P*
Arteriolar									
121	Lowest	≤0.3	60.3	2.52 (1.46–4.36)	0.001	2.19 (1.19–4.03)	0.01	2.02 (1.09, 3.74)	0.03
127	Middle	0.4–1.8	55.9	1.92 (1.13–3.27)	0.02	1.88 (1.04–3.41)	0.04	1.81 (0.99, 3.31)	0.05
127	Highest	≥1.9	43.3	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Venular									
120	Lowest	≤1.7	67.5	3.14 (1.82–5.45)	<0.001	2.45 (1.33–4.49)	0.004	2.41 (1.30, 4.67)	0.005
122	Middle	1.8–3.3	53.3	1.54 (0.91–2.60)	0.11	1.33 (0.75–2.38)	0.33	1.36 (0.75, 2.44)	0.31
134	Highest	≥3.4	39.6	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—

Data are ORs (95% CI) unless indicated otherwise. Model 1: adjusted for age, sex, and fasting blood glucose level. Model 2: adjusted for covariates in model 1 plus diabetes duration, use of antihypertensive and lipid-lowering medications, current smoking status, SBP, and fasting cholesterol and triglyceride levels. Model 3: adjusted for covariates in model 2 plus static retinal arteriolar or venular diameter. **n* indicates number of eyes.

this flicker-induced vasodilation in healthy individuals. In addition, impaired response to flicker light stimulation in individuals with hypertension could be restored by angiotensin II subtype 1 receptor blockade (20). However, this finding has been documented only in individuals without diabetes. It was hypothesized previously that the decreased endothelial dysfunction in subjects with diabetes is associated with impaired nitric oxide action because of its inactivation resulting from increased oxidative stress and that abnormal nitric oxide metabolism is related to advanced diabetic microvascular complications (21). This hypothesis is supported by recent data demonstrating similar retinal arteriolar and venular dilation after a single sublingual dose of 0.8 mg nitroglycerin between 20 patients with insulin-treated diabetes with no or only mild NPDR and 20 healthy age-matched control subjects (22). However, it is becoming increasingly clear that neuronal cells of the retina are also affected by diabetes, resulting in dysfunction and degeneration (23), and diabetic retinopathy is a disease of both retinal neurons and microcirculation (24). Because retinal blood flow is coupled with neuronal activity (25), reduced flicker light–induced vasodilation can thus also reflect neurodegeneration (17,24).

In our study, significantly reduced flicker light–induced vasodilation was observed in diabetic subjects with diabetic retinopathy compared with those without diabetic retinopathy. This relationship appeared to be stronger among individuals with type 1 diabetes than among those with type 2 diabetes, given the similar distribution of diabetic retinopathy severity between the two groups. This observation could be due to longer diabetes duration in those with type 1 diabetes (mean 22.1 years for type 2 diabetes vs. 12.6 years for type 2 diabetes), resulting in possibly a greater level of impairment of retinal vascular autoregulation (26), endothelial damage (26), or neurodegeneration (17,24). Alternatively, the underlying mechanisms of diabetic retinopathy may be different in type 1 and type 2 diabetes.

The strengths of this study include quantitative measures of retinal vasodilation after flicker light stimulation, assessment of diabetic retinopathy from fundus photographs using standardized grading protocols, and one researcher (T.T.N.) performing all DVA measurements. Limitations of this study should also be noted. First, the cross-sectional nature of the study provides no temporal information on the associations reported. Second, our findings are only applicable to individuals with diabetes who are aged ≤70 years. Third, we have no measurement of retinal neuronal function. Thus, further longitudinal studies are needed to ascertain cause and effect and to correlate flicker-induced vasodilation with retinal neuronal functions using tests such as electroretinography.

In summary, we demonstrated a reduction in flicker light–induced retinal vasodilation in individuals with diabetes and, among those with diabetes, in those with retinopathy signs. These findings further support the concept that early endothelial dysfunction is a likely key pathophysiological mechanism that underlies diabetes and its microvascular complications.

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