Influence of Flickering Light on the Retinal Vessels in Diabetic Patients

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OBJECTIVE — Stimulation of the retina with flickering light increases retinal vessel diameters in humans. Nitric oxide is a mediator of the retinal vasodilation to flicker. The reduction of vasodilation is considered an endothelial dysfunction. We investigated the response of retinal vessels to flickering light in diabetic patients in different stages of diabetic retinopathy.

RESEARCH DESIGN AND METHODS — We studied 53 healthy volunteers, 68 type 1 diabetic patients, and 172 type 2 diabetic patients. The diameter of retinal vessels was measured continuously online with the Dynamic Vessel Analyzer (DVA). Diabetic retinopathy was classified using Early Treatment Diabetic Retinopathy Study criteria. Changes in vasodilation are expressed as percent change over baseline values.

RESULTS — After adjustments for age, sex, and antihypertensive treatment, the response of retinal arterioles to diffuse luminance flicker was significantly diminished in patients with type 1 diabetes compared with healthy volunteers. The vasodilation of retinal arterioles and venules decreased continuously with increasing stages of diabetic retinopathy. The retinal arterial diameter change was 3.6 ± 2.1% in the control group, 2.6 ± 2.5% in the no diabetic retinopathy group, 2.0 ± 2.7% in the mild nonproliferative diabetic retinopathy (NPDR) group, 1.6 ± 2.2% in the moderate NPDR group, 1.8 ± 1.9% in severe NPDR group, and 0.8 ± 1.6% in proliferative diabetic retinopathy group.

CONCLUSIONS — Flicker responses of retinal vessels are abnormally reduced in diabetic patients. This decreased response deteriorated with increasing stages of retinopathy. The response was already reduced before clinical appearance of retinopathy. The noninvasive testing of retinal autoregulation with DVA might prove to be of value in early detection of diabetic vessel pathological changes.

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Hyperglycemia, dyslipidemia, hypertension, and diabetes duration are the main risk factors for the development and progression of diabetic retinopathy (1–3). However, the exact pathogenesis of this disease remains incompletely understood. There is evidence that endothelial dysfunction may play an important role in the pathogenesis of diabetic retinopathy (4). Markers of endothelial dysfunction such as soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 are elevated in patients with diabetic retinopathy. However, the association between markers of endothelial dysfunction and diabetic retinopathy has not always been consistent, presumably because of the considerable biological variation in the measurement of such markers (5). Therefore, the use of other parameters is desirable to assess the regulation ability of retinal vessels.

Endothelial cells regulate vascular reactivity by responding to mechanical forces and neurohumoral mediators with the release of a variety of relaxing and contracting factors (6). One of the most important endothelium-derived vasodilators is nitric oxide (NO), the bioavailability of which is decreased in diabetes (7). In addition, NO appears to be a mediator of the retinal vasodilator response to flicker light (8). Several human studies showed an increase in retinal vessel diameter during stimulation with diffuse luminance flicker (8–10).

In the current study, we investigated the response of retinal arterial and venous vessels to flickering light in patients with diabetes. All retinal vessels are by definition vessels of microcirculation. For our purpose, a recently developed provocation test was used. Diffuse luminance flicker was applied, and retinal vessel diameters were measured with a Dynamic Vessel Analyzer (DVA) (Imedos, Jena, Germany). We determined the endothelium-derived vasodilation of retinal arteries in different stages of diabetic retinopathy.

RESEARCH DESIGN AND METHODS

— The study was performed on a group of healthy volunteers and type 1 and 2 diabetic patients who were being treated in a large outpatient diabetes clinic at a tertiary university hospital. A total of 53 healthy subjects, 68 patients with type 1 diabetes, and 172 patients with type 2 diabetes were investigated. All subjects were of Caucasian origin. Of the diabetic patients, 83.3% were treated with insulin, 31.6% with oral antidiabetic agents, and 80.4% with antihypertensive drugs. All examinations were performed after the patients had received oral and written information about the study and had given their consent to participate. The examinations were performed in accordance with the Declaration of Helsinki and were approved by the local ethics committee.

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Abbreviations: DVA, Dynamic Vessel Analyzer; ETDRS, Early Treatment Diabetic Retinopathy Study; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of the study groups*

Parameter	Control group	Type 1 diabetes	Type 2 diabetes
n	53	68	172
Sex			
Male	14 (26)	30 (44)*	97 (56)*
Female	39 (74)	38 (56)	75 (44)
Age (years)	41.9 ± 16.6	47.5 ± 15.3*	61.7 ± 10.1*
Mean arterial pressure (mmHg)	87.8 ± 8.5	92.9 ± 8.2*	100.7 ± 9.9*
A1C (%)	—	7.9 ± 1.2	7.4 ± 1
Duration of diabetes (years)	—	17.7 ± 10.3	11.4 ± 7.8
Antihypertensive treatment	0 (0)	33 (49)*	156 (92)*
Missing data	2	1	2

Data are *n* (%) or means ± SD. A1C normal range: 4.6–5.9. *Significantly different compared with the control group.

In all subjects, the left eye was studied. Volunteers were taking no medication at the time of the study. The healthy participants were nonsmokers, had no previous history of arterial hypertension or metabolic or cardiovascular disease, and did not receive any medication on prescription. All subjects had no history of epilepsy or ocular disease other than diabetic retinopathy and carotid artery obstruction and were nonsmokers. Every patient had undergone measurement of intraocular pressure within 1 year before enrollment. Patients with increased intraocular pressure were excluded. All subjects were asked to refrain from use of alcohol, nicotine, and caffeine for at least 1 h before the examination.

The clinical data of the patients examined are shown in Table 1. Diabetic patients were significantly older and had higher mean arterial blood pressure compared with the healthy control group.

Study protocol

At the start of the study, fundus examination was performed after induction of mydriasis with 1% tropicamide eye drops. Diabetic retinopathy was classified using Early Treatment Diabetic Retinopathy Study (ETDRS) criteria (11) as no diabetic retinopathy (ETDRS level 10), mild nonproliferative diabetic retinopathy (NPDR) (ETDRS level ≥20), moderate NPDR (ETDRS level ≥43), severe NPDR (ETDRS level ≥53), and proliferative diabetic retinopathy (PDR) (ETDRS level ≥61).

The DVA was used for digital fundus imaging for conventional fundus examinations and for retinal vessel analysis. Retinal vessel analysis with the DVA allows noninvasive diagnosis of microvascular function by measuring the diameter of arterial and venous retinal vessels continuously 25 times/s and by using stimulation tests of vessel functions. By interrupting of the green measuring light, the DVA generates flicker light with a frequency of 12.5 Hz and with a bright-to-dark ratio of 25:1 for a stimulation test. Diameter responses can be recorded by use of flicker light periods during the vessel diameter measurements. The dilatation of vessel diameter caused by flickering light can be used as a function diagnostic parameter for the endothelium-derived vasodilation. Details of the DVA and the processes of diameter measurements and flicker stimulations are described elsewhere (12–15).

After the baseline vessel diameter was measured for 50 s, provocation with flicker light was performed for 20 s, and the response was observed for 80 s after the end of the flicker exposure. The cycle was then repeated two times. An arterial segment of ~1.5 mm was evaluated in each eye. Selection criteria for the segment were location within a circular area of two disc diameters, no crossing or bifurcation in the measuring segment, curvature of not >30°, a distance to neighboring vessels of at least one vessel diameter, and sufficient contrast to the surrounding fundus. The position of the vessel edges, the vessel course, the vessel diameter, and correction for ocular movements were calculated automatically online.

Blood pressure measurements

The mean systemic arterial blood pressures of the groups are listed in Table 1. No significant increase in blood pressure occurred during the examination. The mean arterial blood pressure (mean RR) was calculated as mean RR = RR diastole + ⅓ * (RR systole − RR diastole) mmHg, where RR systole is systolic blood pressure and RR diastole is diastolic blood pressure.

Statistical analyses

Changes in ocular hemodynamic parameters are expressed as the percent change over baseline values. Retinal vessel diameters were calculated as an average of the last 30 s of the baseline of each cycle. Vessel diameter during flicker was calculated as an average of the last 3 s of light stimulation and the following 3 s after stimulation. All variables obtained were described by adequate statistical measures. Differences between groups were statistically evaluated by *t* test, the Mann-Whitney *U* test, or χ^2 test as appropriate. To adjust for imbalances of age, sex, and antihypertensive treatment, ANCOVA was applied. Contrasts were defined to test differences between type 1 or type 2 diabetic patients compared with the control group and to analyze the linear trend of

Table 2—*Mean diameter change of retinal arteries and veins to flicker in healthy subjects and diabetic patients*

Parameter	Control group	Type 1 diabetes	Type 2 diabetes
Arterial vasodilation (%)	3.6 ± 2.0	2.1 ± 2.3	2.2 ± 2.5
*P**		<0.001	<0.001
Arterial vasoconstriction (%)	−1.4 ± 1.7	−1.0 ± 1.7	−0.6 ± 1.4
P		0.135	0.001
Venous diameter change (%)	4.5 ± 2.4	4.0 ± 2.3	3.5 ± 2.1
P		0.212	0.005

Data are means ± SD. **P* values from unadjusted comparison with control group.

vasodilatation depending on the severity of diabetic retinopathy. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed with SPSS (version 13.0.1; SPSS, Chicago, IL).

RESULTS — In retinal arterioles, the response to stimulation with luminance flicker was diminished in diabetic patients compared with healthy volunteers (Table 2). In healthy control subjects, flicker stimulation increased the retinal arterial diameter by 3.6 ± 2.0%, in type 1 diabetic patients by 2.1 ± 2.3%, and in type 2 diabetic patients by 2.2 ± 2.5%. The response was significantly decreased regardless of type of diabetes. The constriction of the retinal arteries, as well as the response of retinal venous diameters, was also diminished in diabetic patients compared with control subjects, but differences were significant only in type 2 diabetic patients compared with control subjects.

Association of retinal vessel flicker response with age and duration of the disease

Age and duration of diabetes were significantly associated with arterial diameter response in diabetic subjects. The vasodilation of the arteries decreased significantly with increasing age and duration of disease. With increasing age, there was a tendency toward smaller dispersion of the dilation.

The age versus arterial diameter change scatterplot shows a decreasing flicker response and increasing dispersion of the measured values in subjects of middle to advanced age. The small coefficient of correlation ($r = 0.22$) indicates a weak correlation between the two parameters (data not shown).

To account for confounding by age, by antihypertensive treatment, and probably by sex, vasodilatation was further analyzed by ANCOVA (Table 3). After adjustment, the difference between diabetic patients and the control group remained significant for the arterial diameter change in type 1 diabetic patients. The difference in venous diameter change in type 2 diabetic patients compared with control subjects was more pronounced after adjustment but failed to reach statistical significance at the global significance level.

Table 3—*Age-, antihypertensive treatment–, and sex-adjusted mean differences of diameter change comparing type 1 and type 2 diabetic patients with the control group of healthy subjects*

Group	Adjusted difference (%)*	95% CI	*P* value (global test)†
Arterial vasodilatation			
Control	Reference		0.024
Type 1 diabetes	−1.1	−2.0 to −0.2	
Type 2 diabetes	−0.3	−1.4 to 0.8	
Arterial vasoconstriction			
Control	Reference		0.823
Type 1 diabetes	0.2	−0.4 to 0.9	
Type 2 diabetes	0.2	−0.5 to 0.9	
Venous diameter change			
Control	Reference		0.063
Type 1 diabetes	−0.7	−1.6 to 0.2	
Type 2 diabetes	1.2	−2.2 to −0.2	

Groups: type 1 diabetic ($n = 68$), type 2 diabetic ($n = 170$), and control ($n = 53$). *Difference$_{(\text{type 1}-\text{control})}$ or difference$_{(\text{type2}-\text{control})}$. †*P* values from covariance analyses; deviation from symmetry of CIs because of rounding).

Association of retinal vessel flicker response with mean arterial blood pressure and A1C

There was no significant association between arterial retinal flicker response and mean arterial blood pressure or A1C in diabetic patients (multiple regression analyses). The flicker response of retinal arteries in diabetic patients deteriorated but not significantly with increasing A1C (data not shown).

Retinal vessel flicker response in different stages of diabetic retinopathy

The retinal arterial diameter changes were 3.6 ± 2.1, 2.6 ± 2.5, 2.0 ± 2.7, 1.6 ± 2.2, 1.8 ± 1.9, and 0.8 ± 1.6% in the control group ($n = 53$), no diabetic retinopathy group ($n = 145$), mild NPDR group ($n = 36$), moderate NPDR group ($n = 27$), severe NPDR group ($n = 18$), and PDR group ($n = 14$), respectively

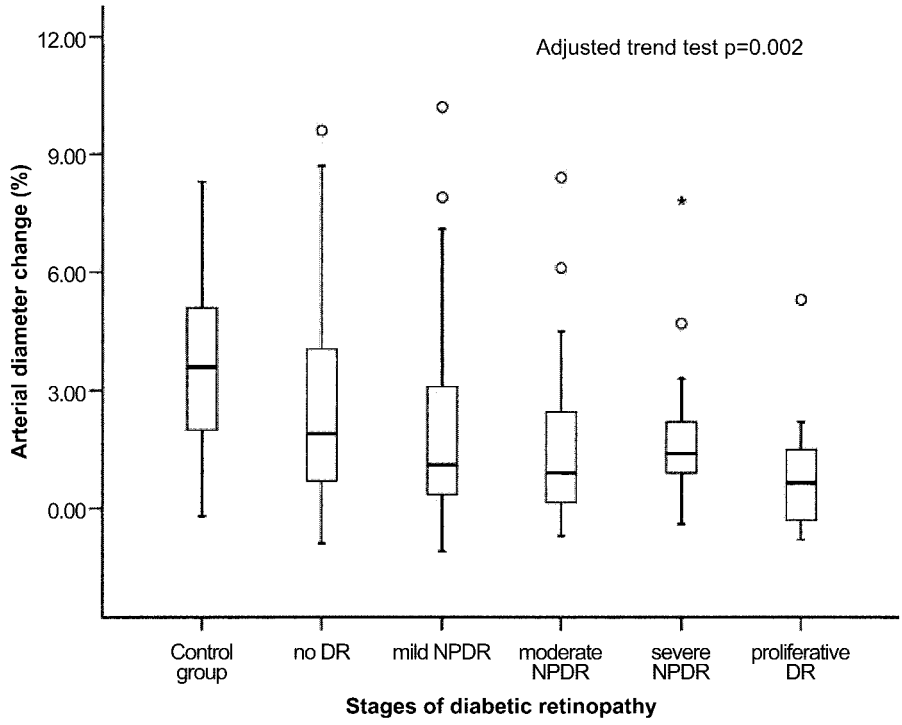

Figure 1—*Arterial diameter changes at stages of diabetic retinopathy (DR).*

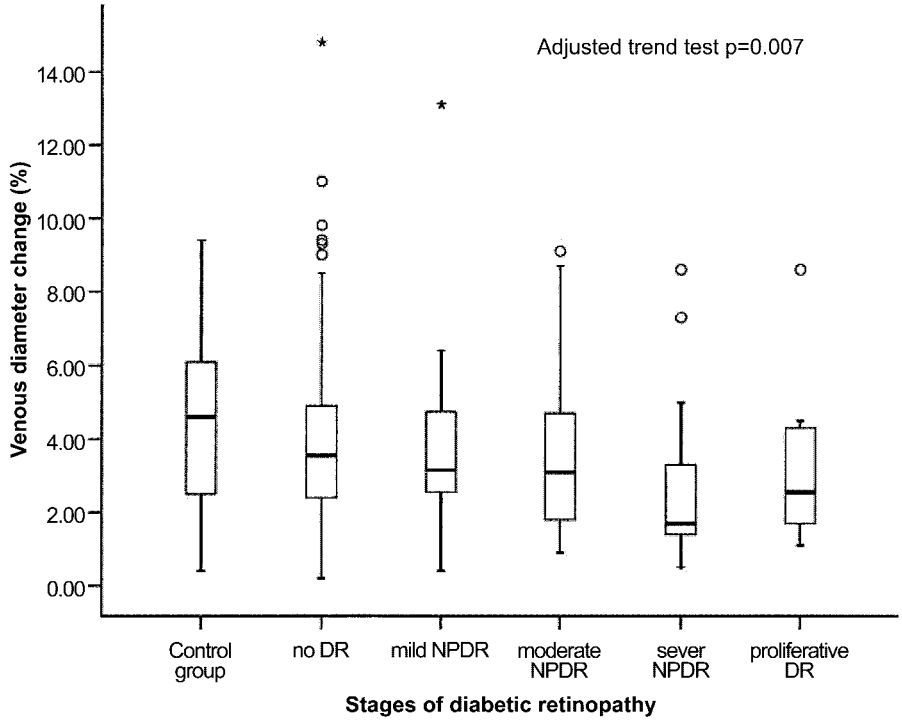

Figure 2—*Venous diameter changes at stages of diabetic retinopathy (DR).*

(Fig. 1). There was a significant trend of decreasing retinal arterial response along the groups (age-, antihypertensive treatment–, and sex-adjusted trend test $P = 0.002$). The venous diameter changes were 4.6 ± 2.4, 3.9 ± 2.3, 3.7 ± 2.2, 3.5 ± 2.1, 2.7 ± 2.2, and 3.1 ± 2.0% in the control group, no diabetic retinopathy group, mild NPDR group, moderate NPDR group, severe NPDR group, and PDR group, respectively (Fig. 2). The adjusted retinal venous response was also significantly decreased (trend test $P = 0.007$). No significant trend could be observed for constriction of the retinal arteries.

CONCLUSIONS — In this in vivo study, we compared endothelial function under physiological flow conditions and in the presence of the diabetic milieu. Noninvasive testing of the function of autoregulation of retinal arterioles is possible with the DVA (13–16). There is much evidence for abnormal autoregulation of retinal vessels in diabetic patients. Using the laser Doppler technique, Grunwald et al. (17) reported reduced retinal arterial and venous blood velocity as well as enlarged retinal veins in patients with diabetes with background retinopathy. Moreover, retinal blood flow is reduced in patients with diabetes with no diabetic retinopathy compared with patients without diabetes (18,19). There is also evidence that the early stages of diabetic retinopathy are associated with increased retinal blood flow and retinal vasodilation, abnormal retinal vascular response to hyperoxia, and abnormal retinal autoregulation (20–23). The intrinsic abnormality in diabetic retinopathy appears to be endothelial cell dysfunction (24–26). The present study focuses on the retinal diameter changes of major temporal retinal vessels of diabetic patients to diffuse luminance flicker. In humans, the flicker light–induced vasodilation is mediated by NO (27). Hence, this test could be used as an estimate of the capacity of endothelial cells of retinal vessels to release NO in response to a physiological stimulus in disease states.

We demonstrated that the retinal vessel flicker response is diminished in diabetic patients compared with that in normal control participants. This finding is in agreement with a previous report indicating reduced flicker response in type 1 diabetic patients (28). We have also reported abnormal autoregulation in patients with type 2 diabetes.

The present study demonstrates that the vasodilation of retinal arteries and veins under the flickering light decreases continuously with increasing stages of diabetic retinopathy. Furthermore, autoregulation was also found to be abnormal in diabetic patients without retinopathy and deteriorated continuously in patients with retinopathy, suggesting that the disturbance is involved in the disease pathogenesis. The venous retinal response was reduced in diabetic patients without any visible signs of diabetic retinopathy compared with the control group. This finding is in agreement with several previous reports indicating impairment of blood flow regulation in the retina before the clinical appearance of retinopathy (21, 29). In our study we showed an association between flicker response and age; however, the coefficient of correlation was weak, which is in agreement with previous reports (30). For example, Jeppesen et al. (31) reported significantly reduced diameter response in normal individuals aged >40 years.

Most of the diabetic patients were receiving antihypertensive treatment at the time of testing. To rule out the possible confounding effect of drugs, we adjusted the data for imbalances of antihypertensive medication. The adjusted response of retinal vessels to flickering light decreased significantly with increasing stages of diabetic retinopathy. This finding suggests that diabetes has a deteriorating effect per se on the flow regulation in response to flicker stimulation.

In summary, this study demonstrated a decreased retinal vessel flicker response in patients with diabetes. This decreased response deteriorated with increasing stages of diabetic retinopathy. Indeed, the response was already low before the clinical appearance of retinopathy. The predictive value of this method for detecting diabetic patients at risk for the development of diabetic retinopathy needs to be tested with long-term observational studies.

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