

"Influence of flickering light on the retinal vessels in diabetic patients"

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Running title: Vasodilation and diabetic retinopathy

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Abstract

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 as an endothelial dysfunction. We investigate the response of retinal vessels to flickering light in diabetic patients in different stages of diabetic retinopathy (DR).

Research Design and Methods: We studied 53 healthy volunteers, 68 type 1 and 172 type 2 diabetic patients. The diameter of retinal vessels was measured continuously on-line with the Dynamic Vessel Analyzer (DVA). The diabetic retinopathy was classified using ETDRS-criteria. Changes in vasodilation are expressed as percentage change over baseline values.

Results: After adjusting for age, sex and antihypertensive treatment the response of retinal arterioles to diffuse luminance flicker was significantly diminished in patients with type 1 diabetes mellitus compared to healthy volunteers. The vasodilation of retinal arterioles and venules decreased continuously with increasing stages of diabetic retinopathy. The retinal arterial diameter change was $3.6\pm 2.1\%$ in control group, $2.6\pm 2.5\%$ in no DR group, $2.0\pm 2.7\%$ in mild nonproliferative DR, $1.6\pm 2.2\%$ in moderate nonproliferative DR, $1.8\pm 1.9\%$ in severe nonproliferative DR and $0.8\pm 1.6\%$ in proliferative DR 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 pathology.

Hyperglycaemia, dyslipidaemia, 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 still incompletely understood. There is evidence that endothelial dysfunction may play an important role in pathogenesis of diabetic retinopathy (4). Markers of endothelial dysfunction such as sICAM-1 and sVCAM-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 due to 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), which bioavailability is decreased in diabetes mellitus (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 on flickering light in patients with diabetes mellitus. All retinal vessels are by definition vessels of microcirculation. For this purpose, a recently developed provocation test was used. Diffuse luminance flicker was applied and retinal vessel diameter were measured with a Dynamic Vessel Analyser (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 collective of healthy volunteers and type 1 and 2 diabetic patients who were being treated in a large out-patient diabetes clinic at a tertiary university hospital. A total of 53 healthy subjects, 68 patients with type 1 and 172 patients with type 2 diabetes mellitus were investigated. All subjects were of Caucasian origin. 83.3% of diabetic patients 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 ethic committee.

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

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

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

The Dynamic Vessel Analyzer - DVA (Imedos GmbH, Jena, Germany) was used for Digital Fundus Imaging for conventional fundus examinations and for Retinal Vessel Analysis. Retinal Vessel Analysis of the DVA allows noninvasive microvascular function diagnostic by measuring the diameter of arterial and venous retinal vessels continuously 25 times per second and by using stimulation tests of vessel functions. By interrupting of the green measuring light DVA generates flicker light with frequency of 12,5 Hz and with an bright to dark ratio of 25:1 for stimulation test. Diameter responses can be recorded by use of this 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 seconds, provocation with flicker light was performed for 20 seconds, and the response was observed for 80 seconds after the end of the flicker exposure. The cycle was then repeated two times. An arterial segment of approximately 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 more than 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 on-line.

Blood pressure measurements: The mean systemic arterial blood pressure of the resultant groups is listed in Table 1. No significant increase of the blood pressure occurred during the examination. The mean arterial blood pressure (mean RR) was calculated as: $\text{Mean RR} = \text{RR diastole} + 1/3 * (\text{RR systole} - \text{RR diastole})$ mmHg with RR systole = systolic blood pressure and RR diastole = diastolic blood pressure.

Statistical analyses: Changes in ocular hemodynamic parameters were expressed as the percentage change over baseline values. Retinal vessel diameters were calculated as an average of the last 30 seconds of the baseline of each cycle. Vessel diameter during flicker was calculated as an average of the last 3 seconds of light stimulation and the following 3 seconds after stimulation. All obtained variables were described by adequate statistical measures. Differences between groups were statistically evaluated by t-test, Mann-Whitney U test, or Chi² test as appropriate. To adjust for imbalances of age, gender and antihypertensive treatment analysis of covariance was applied. Contrasts were defined in order to test differences between type 1 or type 2 diabetic patients compared to the control group and to analyse the linear trend of vasodilatation depending on the severity of diabetic retinopathy. A p-value of <0.05 was considered to be statistically significant. Statistical analysis was performed with SPSS 13.0, Version 13.0.1 (SPSS, Chicago, IL, SA).

Results

Main patient characteristics are given in Table 1. In retinal arterioles, the response to stimulation with luminance flicker was diminished in diabetic patients compared to healthy volunteers (Table 2). In healthy controls, 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 to controls but differences were significant only in type 2 diabetic patients compared to controls.

The 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 the 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, antihypertensive treatment and probably by gender vasodilatation was further analyzed by analysis of covariance (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 of venous diameter change in type 2 diabetic patients compared to controls was more pronounced after adjustment but failed statistical significance at the global significance level.

The association of retinal vessel flicker response with mean arterial blood pressure and glycated haemoglobin: There was no significant association between arterial retinal flicker response and mean arterial blood pressure or glycated haemoglobin in diabetic patients (multiple regression analyses). The flicker response of retinal arteries in diabetic patients deteriorated not significantly with increasing glycated hemoglobin A1 (data not shown).

The retinal vessel flicker response in different stages of diabetic retinopathy: The retinal arterial diameter change was 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 DR group (n=145), mild NPDR group (n=36), moderate NPDR (n=27), severe NPDR (n=18) and PDR group (n=14), respectively (Figure 1). There was a significant trend of decreasing retinal arterial response along the groups (age, antihypertensive treatment and gender adjusted trend test p=0.002). The venous diameter change was 4.6±2.4, 3.9±2.3, 3.7±2.2, 3.5±2.1, 2.7±2.2, 3.1±2.0 % in the control group, no DR group, mild NPDR group, moderate NPDR group, severe NPDR and PDR group, respectively (Figure 2). The adjusted retinal venous response was also significantly decreased (trend test p=0.007). No significant trend could be observed regarding constriction of the retinal arteries.

Conclusions

In this in vivo study we compare the 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 Dynamic Vessel Analyzer (13-16). There is much evidence for an abnormal autoregulation of retinal vessels in diabetic patients. Using the laser Doppler technique, Grunwald et al. reported reduced retinal arterial and venous blood velocity as well as enlarged retinal veins in patients with diabetes with background retinopathy (17). Moreover, retinal blood flow is reduced in patients with diabetes mellitus with no diabetic retinopathy compared to patients without diabetes (18-19). There is also evidence that 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 the nitric oxide in response to a physiological stimulus in diseased states.

We demonstrated that retinal vessel flicker response is diminished in diabetic patients compared with normal control participants. This finding is in agreement with the previous report indicating reduced flicker response in IDDM patients (28). We have also reported the 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 found to be abnormal yet 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 in comparison to 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 report (30). For example, Jeppesen et al. reported significantly reduced diameter response in normal persons above the age of 40 years (31).

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 suggests that diabetes mellitus has a deteriorating effect per se on the flow regulation in response to flicker stimulation.

In conclusion, this study demonstrated a decreased retinal vessel flicker response in patients with diabetes mellitus. 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 to detect the diabetic patients at risk for the development of diabetic retinopathy needs to be tested with long-term observational studies.

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Table 1. Characteristics of the Study Groups

Parameter	Control group n=53	Diabetes type 1 n=68	Diabetes type 2 n=172
Gender			
Male (%)	14 (26)	30 (44) ^a	97 (56) ^a
Female (%)	39 (74)	38 (56)	75 (44)
Age (y)	41.9±16.6	47.5±15.3 ^a	61.7±10.1 ^a
MAP (mmHg)	87.8±8.5	92.9±8.2 ^a	100.7±9.9 ^a
HbA1c %	-	7.9±1.2	7.4±1
Duration of diabetes (y)	-	17.7±10.3	11.4±7.8
Antihypertensive treatment (%)	0 (0)	33 (49) ^a	156 (92) ^a
(missing)	(2)	(1)	(2)

Data are expressed as the mean ± SD

^a Significant different compared to control group

Table 2. Mean diameter change of retinal arteries and veins to flicker in healthy subjects and diabetic patients (p-values from unadjusted comparison with control group)

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

Table 3: Age, antihypertensive treatment and gender adjusted mean differences of diameter change comparing type 1 (n=68) and type 2 (n=170) diabetic patients to the control group (n=53) of healthy subjects (p-values from covariance analyses; deviation from symmetry of confidence intervals due to rounding)

Parameter	Group	Adjusted difference (%) ^a	95% Confidence Interval	p-Value (global test)
Arterial vasodilatation	Control	Reference		0.024
	Typ 1 diabetes	-1.1	(-2.0, -0.2)	
	Typ 2 diabetes	-0.3	(-1.4, 0.8)	
Arterial vasoconstriction	Control	Reference		0.823
	Typ 1 diabetes	0.2	(-0.4, 0.9)	
	Typ 2 diabetes	0.2	(-0.5, 0.9)	
Venous diameter change	Control	Reference		0.063
	Typ 1 diabetes	-0.7	(-1.6, 0.2)	
	Typ 2 diabetes	-1.2	(-2.2, -0.2)	

^a Difference (Typ1-Control) or difference (Typ 2-Control)

Figure 1

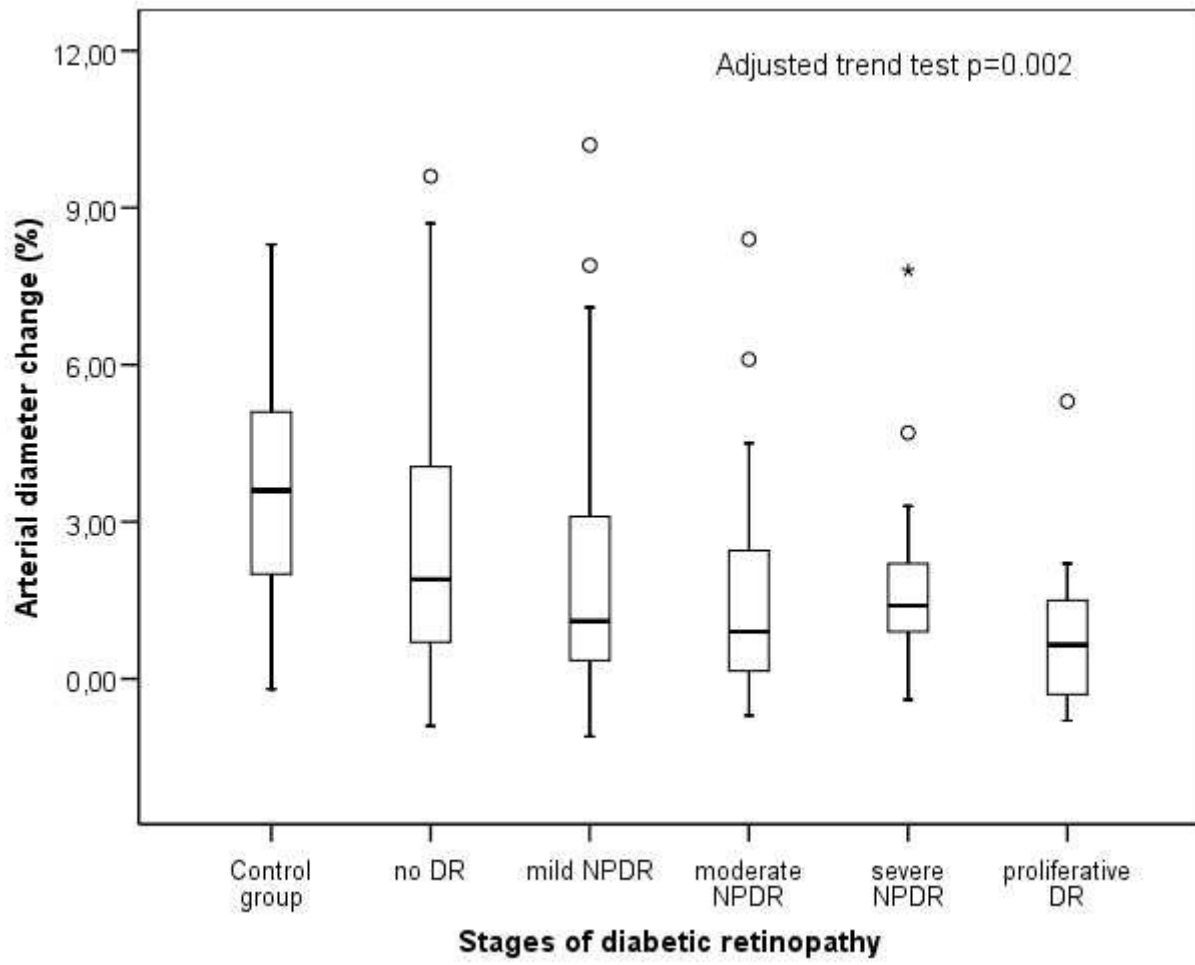


Figure 2

