Reduced Retinal Vessel Response to Flicker Stimulation but Not to Exogenous Nitric Oxide in Type 1 Diabetes

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PURPOSE. Various studies have shown that retinal vessels in patients with diabetes mellitus have a reduced capacity to adapt to changes in perfusion pressure and to stimulation with flickering light. Structural and functional changes in retinal vessels in diabetes could lead to a general reduction of vasoconstrictor capacity. To gain more insight into this topic, we compared the response of retinal vessel diameters to systemic glyceryl trinitrate (GTN) and stimulation with diffuse lumiance flicker in patients with diabetes and healthy control subjects.

METHODS. Twenty patients with type 1 diabetes mellitus featuring no or mild nonproliferative diabetic retinopathy and 20 healthy, age-matched subjects were included in this study. A vascular analyzer was used for measurement of diameters of retinal arteries and veins. The response of diameters was measured continuously during stimulation with flickering light, as well as immediately after sublingual application of 0.8 mg GTN.

RESULTS. The response of retinal vessels to flickering light was significantly reduced in the patients with diabetes (arteries: 2.9% in diabetes versus 7.0% in control subjects, P < 0.002; veins: 4.6% in diabetes versus 6.8% in control subjects, P = 0.020). GTN-induced vasodilatation was not different between the patients with diabetes and the healthy control subjects (P ≥ 0.70).

CONCLUSIONS. The present study confirmed reduced response of retinal vessels to stimulation with flickering light in diabetes. The response of retinal vessels to a direct NO-donor, however, was maintained. This result indicates that abnormal flicker-induced vasodilatation in diabetes is not a consequence of generally reduced retinal vascular reactivity (ClinicalTrials.gov number, NCT00432029). (Invest Ophthalmol Vis Sci. 2009;50:4029–4032) DOI:10.1167/iovs.08-3260

Blood flow in the retina is in large part regulated by the diameter of retinal vessels. Because these vessels are not supplied with autonomic innervation, their vascular tone is mostly regulated by endocrine and paracrine factors. Several studies have shown a reduced vasodilatation of retinal vessels during stimulation with flickering light in patients with diabetes mellitus.1,2 This retinal flicker response has been shown to be dependent on endogenously synthesized nitric oxide (NO).3 Hence, reduced vasodilator responses to stimulation with diffuse lumiance flicker have been interpreted as a reduced endothelium-dependent vasodilatory capacity of retinal vessels in diabetes.1 In addition, various studies have shown that retinal vessels in patients with diabetes have a reduced capacity to adapt to changes in perfusion pressure.4,5 The mechanisms underlying this abnormal autoregulatory response have not been completely identified yet. One hypothesis is that endothelial dysfunction due to abnormal endogenous release or action of NO plays a role in the development of diabetic retinopathy.6 This hypothesis is supported by the notion that endothelial dysfunction is an early feature of vascular and retinal cell damage in the pathogenesis of diabetic retinopathy.7 Alternatively, reduced flicker responses and autoregulatory capacity may also be related to a general reduction in vascular reactivity. Early changes due to diabetes, including basement membrane thickening, retinal capillary nonperfusion, capillary loss, and pericyte loss could well contribute to a reduced capacity of retinal vessels for vasodilatation and vasoconstriction.8,9 To gain more insight into the causes of reduced retinal vasodilatory capacity, we compared the response of retinal vessel diameters to stimulation with flickering light and to exogenous stimulation with the NO-donor nitroglycerin, also known as glyceryl trinitrate (GTN). Patients with diabetes and healthy control subjects were examined to compare flicker-induced vasodilatation and endothelium-independent dilatation of retinal vessels.

METHODS

Subjects

The study protocol was approved by the Ethics Committee of the Medical University of Vienna and followed the guidelines set forth in the Declaration of Helsinki. All patients signed written informed consent and passed a screening examination before the study day including physical examination, venous blood sampling for clinical chemistry including glucose and HbA1c levels, assessment of visual acuity with ETDRS charts, slit lamp biomicroscopy, funduscopy, and measurement of intraocular pressure (IOP).

Forty individuals aged over 18 years were included in this open comparative study. Twenty patients with type 1 diabetes mellitus with no sign of diabetic retinopathy or with mild nonproliferative diabetic retinopathy were included. The eyes were classified according to the Modified Arlie House Classification.10 As control subjects, 20 age- and sex-matched healthy subjects with resting blood pressure below 140/90 mm Hg, serum cholesterol levels <200 mg/dL, and normal ocular findings were included.
Exclusion criteria for all subjects were ametropia ≥3 D, other ocular abnormalities, a clinically relevant illness before the study, pregnancy or lactation, and an individual or family history of epilepsy. The participants had to abstain from beverages containing alcohol or caffeine for 12 hours before the study. Hba1c and plasma glucose levels were controlled in all subjects before the study day, and glucose levels of the patients with type 1 diabetes were controlled directly before measurements.

**Dynamic Vessel Analyzer**

After instillation of 1 drop of tropicamide into the study eye and after a resting period of 15 minutes the diameters of one temporal retinal artery and vein were measured in mydriasis with a vessel analyzer (Dynamic Vessel Analyzer; [DVA], IMEDOS GmbH, Jena, Germany). The DVA comprises a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany), a digital video camera, a monitor, and a personal computer running analysis software for the determination of retinal vessel diameters, which are analyzed in real time in digitized images. The system provides excellent reproducibility and sensitivity. After selection of the measurement location the system is able to follow the vessels during movements within the measurement window. After 1 minute of baseline measurements, the response of vessel diameters to 1 minute of stimulation with full-field flickering light was assessed by square wave pattern moduation of the fundus camera illumination with a frequency of 12.5 Hz. After another resting period of 15 minutes, the retinal vessel diameters were measured again for 1 minute under baseline conditions. Then, a single sublingual dose of 0.8 mg GTN was applied, and measurements were continued immediately for 5 minutes. Flickering-light-induced vasodilatation was defined as the average values of the last 20 seconds of the flicker stimulation over the average values of the 60 seconds baseline recording. The effect of GTN was assessed as the mean of minutes 4 to 5 after administration compared with baseline values. Both responses were expressed as the percentage change over baseline.

**Statistical Analysis**

Baseline data were compared by ANOVA. Changes in retinal vessel diameters were expressed as the percent change over baseline ± SD. Flicker-induced and GTN-induced changes in the percentage of retinal vessel diameters were selected as the main outcome variables. The retinal vessel response and its difference between the two groups were compared by a covariance model (ANCOVA). In this model, the baseline diameter was introduced as a covariable, the percentage vessel response as the dependent variable, and the group (healthy versus diabetes) as the categorical predictor. P < 0.05 was considered as the level of significance.

**RESULTS**

The baseline characteristics of both groups are presented in Table 1. Systemic blood pressure and IOP levels were comparable between the two groups. Hba1c plasma levels were significantly higher in patients with diabetes than in healthy control subjects. Serum cholesterol levels were slightly but not significantly higher in the patients with diabetes. Mean capillary blood glucose levels of the patients on the study day were 8.4 ± 3.4 mM. Mean baseline retinal artery diameters were significantly higher in the patients (P = 0.029, ANOVA). The baseline diameters of retinal veins, on the other hand, were slightly but not significantly larger.

There was a significant response to flicker stimulation in both groups. Retinal arteries showed a mean dilatation of 2.9% ± 2.8% in the patients with diabetes and 7.0% ± 2.3% in the healthy control subjects (Fig. 1, P < 0.001, ANOVA). Similarly, retinal vein diameters increased by 4.6% ± 2.0% in the patients and by 6.8% ± 3.4% in the healthy control subjects (P < 0.001, ANOVA). ANCOVA between the groups showed a highly significant smaller response of retinal vessel diameters to stimulation with flickering light in the patients with type 1 diabetes (arteries: P < 0.002; veins: P = 0.020).

After oral GTN application there was a mean increase in retinal vessel diameters in both groups (Fig. 2, P = 0.003). In the arteries of the patients with diabetes this increase was 1.6% ± 3.5%, whereas it was 1.7% ± 2.8% in the healthy control subjects. The retinal venous response was 1.6% ± 3.2% in the patients and 1.6% ± 2.5% in the healthy control subjects. Neither the GTN-induced vasodilatation in retinal arteries or veins was significantly different between the two groups (arteries: P = 0.70, veins: P = 0.92, ANCOVA). Systemic blood pressure was reduced in both groups 5 minutes after GTN was administered. MAP was reduced by 10.8% ± 8.5% in the patients and by 11.1% ± 5.3% in the healthy control subjects (P > 0.90 between groups). No change in IOP was observed (data not shown).

**Measurement of IOP and Systemic Hemodynamics**

IOP was measured before and after vessel measurements with a slit lamp–mounted Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). Before each measurement, 2 drops of oxybuprocaine hydrochloride combined with sodium fluorescein were instilled for local anesthesia.

Systolic, diastolic, and mean arterial blood pressures (SBP, DBP, MAP) were repeatedly measured before and after vessel measurements on the upper arm by an automated oscillometric device (BP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded by the same unit from a finger pulse oximeter.
The results of the present study indicate that the reactivity of retinal vessels to a stimulus that tests endothelium-independent vasodilatation is unaltered in early-stage diabetic retinopathy. The endothelium-dependent response to flicker stimulation was larger than the response to GTN in both the healthy control subjects and the patients with diabetes. The low rate of dilatation after GTN may represent a limitation in the study, because we cannot entirely exclude that vasodilatation persists in diabetic patients at low levels, but this would not be true of more pronounced dilatation. We deem this possibility unlikely, however, because the response to flicker stimulation in retinal arteries (2.9%) was only slightly larger than the response to GTN (1.6%) in the patients.

In brachial and femoral arteries, endothelium-independent vasodilatation has been found to be significantly reduced in type 1 diabetes and type 2 diabetes. These results suggest that the vascular smooth muscle in diabetes is less sensitive to exogenous NO. Our present results indicate that this is not the case in retinal vessels. The systemic GTN doses used in these previous studies in larger arteries, however, were lower or equal to those in our experiment. The results of the present study therefore indicate that reactivity of the considerably smaller retinal vessels is different from that of larger vessels.

As we did not measure retinal blood flow in this study, we cannot speculate about differences in retinal perfusion between the patients and control subjects after dilatation with GTN. Given that both retinal and venous responses were almost equal in the two study groups a major difference in blood flow is unlikely. Increased blood glucose levels in patients with diabetes may represent another limitation of this study, because reduced flicker-response and reduced response to hyperoxia have been found in healthy subjects during hyperglycemia. However, the flicker response in healthy subjects has been shown to be maintained up to levels of 11 mM, which is far above the values seen in diabetic subjects in the present study. Larger retinal vessel diameters in patients with diabetes may also represent a limiting factor to this study. Increased arterial and venous diameters have been found previously in long-standing diabetes. However, given that our measures were made on only a single artery and vein and not in all visible vessels, our data do not represent increased total cross-sectional retinal vessel diameters in diabetes. Since GTN-induced vasodilatation was preserved in the predilated vessels of patients with diabetes, we deem it unlikely that the reduced

**DISCUSSION**

Changes in retinal vessel characteristics have been shown to be associated with the incidence of cardiovascular events and mortality in type 1 diabetes, indicating that microvascular disturbances precede severe macrovascular complications. Hence, there is much interest in new techniques for assessing retinal microvasculature and its regulation in diabetes. Measurement of retinal vessel response to diffuse luminance flicker may be an easily applicable technique to study abnormalities in retinal vascular response, even in large population-based studies. In the present study, the flicker response of retinal vessels was markedly reduced in diabetes, but the reduction was not related to a generally reduced capacity of these vessels to react to a vasodilator stimulus.

In the healthy retina NO from the vascular endothelium contributes to the control of normal vascular tone of large and small vessels. It mediates vasodilatation and blood flow increase in retinal vessels and capillaries. Endothelial dysfunction due to an abnormal release or action of NO has been implicated as an early feature in the pathogenesis of diabetic vascular disease, and patients with diabetes have a high risk of vascular complications in the micro- and macrovasculature, including sight-threatening retinopathy. Several abnormalities in ocular blood flow have been observed in diabetic retinopathy. Although studies so far have not identified the exact nature of blood flow disturbances in the development of diabetic retinopathy consistently, early alterations in vascular reactivity have been shown by different functional tests in diabetes with no or mild retinopathy. In a recent cross-sectional study Guan et al. found an increasing reduction in compliance of retinal arteries with progression of diabetic retinopathy, which was interpreted as an increase in vascular rigidity. As mentioned before, retinal vessels in patients with diabetes mellitus have been found to have a reduced capacity to adapt to changes of perfusion pressure. In addition, a reduced retinal vascular response to experimental hyperoxia has been found in diabetes. Finally, in accordance with the results of the present study, others have also found a reduced vascular reactivity during stimulation with flickering light in diabetes patients with no or only mild retinopathy. Taken together, these findings suggest a generally reduced vascular reactivity in retinal vessels of patients before the development of nonproliferative retinopathy.

![Flicker induced vasodilatation](image1)

**Figure 1.** Retinal vessel response to stimulation with flickering light. Data are group mean ± SD. *Significant difference (P ≤ 0.02, ANCOVA).

![GTN induced vasodilatation](image2)

**Figure 2.** Retinal vessel response to oral application of 0.8 mg GTN. Data are group mean ± SD; no significant difference (P ≥ 0.70, ANCOVA).
flicker response is related to the different baseline vessel diameter. This conclusion is reflected in the results of the covariance analysis, taking the baseline diameter into consideration as a covariable.

In conclusion, the present study indicates that in patients with type 1 diabetes who have no or mild nonproliferative diabetic retinopathy the vasodilatory response of retinal vessels to a direct NO-donor is maintained. This indicates that neither the reduced vasodilator response to flicker stimulation nor abnormal retinal autoregulation, as observed previously, is the consequence of a generally reduced vascular reactivity of retinal vessels in this disease.

References
