Journal of Diabetes Science and Technology
Volume 6, Issue 1, January 2012

ORIGINAL ARTICLE

Pilot Study for the Evaluation of Morphological and Functional Changes in Retinal Blood Flow in Patients with Insulin Resistance and/or Type 2 Diabetes Mellitus

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Abstract

Background:

The aim of this study was to investigate early morphological and functional pathology in the retinal microcirculation in patients with insulin resistance and/or type 2 diabetes mellitus (T2DM).

Methods:

Fifty-four subjects, without features of retinopathy under ophthalmological investigation, were recruited for study participation and were classified into three study groups according to their metabolic staging: (1) Group C comprised nondiabetic, insulin-sensitive subjects with a BMI <28kg/m²; (2) Group IR comprised nondiabetic, insulin-resistant, obese subjects with a BMI ≥28 kg/m²; and (3) Group DM comprised patients with manifested T2DM.

Retinal microvascular blood flow was assessed using scanning laser doppler flowmetry (Heidelberg Retina Flowmeter) before and after flicker light stimulation (10 Hz; Photo Stimulater 750).

Results:

No significant difference was observed in retinal blood flow (RBF) among the three groups, neither at baseline nor after stimulating the retina with flicker light. The arterial wall-to-lumen ratio (WLR) tended to be smaller in Group DM compared with Group C, and was significantly lower when comparing Group IR with Group C. When the subjects were grouped according to their insulin resistance, a steady decline in RBF and WLR could be observed with increasing insulin resistance.

Conclusions:

In conclusion, laser scanner flowmetry of the retina was found to detect very early changes in microvascular blood flow. Development of insulin resistance seems to be an important component in the deterioration of RBF.

J Diabetes Sci Technol 2012;6(1):163-168

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Abbreviations: (AD) arteriole diameter, (BMI) body mass index, (DM) diabetes mellitus, (DR) diabetic neuropathy, (HOMA) homeostatis model assessment, (IR) insulin resistance, (LD) lumen diameter, (NO) nitric oxide, (RBF) retinal blood flow, (SD) standard deviation, (SLDF) scanning laser Dopper flowmetry, (T2DM) type 2 diabetes mellitus, (WLR) wall to lumen ratio

Keywords: diabetes mellitus type 2, flicker stimulation, insulin resistance, retinal blood flow, scanning laser doppler flowmetry

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Introduction

Despite improved therapeutic opportunities, microvascular complications such as retinopathy, nephropathy, or neuropathy still represent a major challenge in the health care of patients with diabetes mellitus (DM). Microvascular disease develops through endothelial dysfunction, which could be observed in association with the occurrence of insulin resistance (IR), and even before increasing blood glucose levels identify a person as having DM.[1–3]

Studies investigating retinal blood flow (RBF) have revealed conflicting results. While decreased RBF and retinal artery velocities have been reported in patients with early non-proliferative diabetic retinopathy (DR),[4,5] other studies have found an increased RBF in advanced non-proliferative and proliferative DR in comparison to nondiabetic control subjects.[6,7] Laser doppler scanning of the retina has become an established technology for the investigation of microvascular blood flow in the retina.[8–10] Using this technology, Cuypers and colleagues[11] found an increased retinal microvascular blood flow in patients with different stages of DR. In this study, patients with pronounced stages of DR revealed lower RBF compared with those with milder stages of DR. Therefore, RBF in patients with type 2 diabetes mellitus (T2DM) might be affected in different ways according to the metabolic and retinal stage of the disease.

Laser Doppler scanning of the retina before and after stimulating retinal nerve cells with flicker light has been introduced to obtain additional information on the functional integrity of RBF.[12–14] In hypertensive patients, retinal microvascular response to flicker light was found to be reduced, which could be improved by angiotensin I receptor blockade.[15]

The purpose of this pilot study was to generate explorative data on retinal microvascular blood flow and the vascular response to flicker light application in insulin-resistant, obese and/or DM patients without features of retinopathy under ophthalmological investigation compared to an age-matched, insulin-sensitive, nondiabetic control group.

Research Design and Methods

This was a single-center, cross-sectional study. Main exclusion criteria were DR as determined by nonmydriatic fundus photography, smoking within the past 6 months, and treatment with nitrate, angiotensin-converting enzyme inhibitors, or angiontensin II-receptor blockers. Study participants were categorized into three different study groups according to their metabolic status: (1) group C comprised nondiabetic, insulin-sensitive subjects with a BMI <28kg/m²; (2) group IR comprised nondiabetic, insulin-resistant, obese subjects with a BMI ≥28 kg/m²; and (3) group DM was defined as patients with manifested T2DM. Patients in groups C and IR received an oral glucose tolerance test to exclude patients with yet undiagnosed DM. For an additional subgroup analysis, groups IR and DM were pooled and graded into tertiles according to the degree of insulin resistance.

The study was approved by the local ethics committee, and all patients gave their written informed consent.

Measurement of Retinal Capillary Blood Flow

Retinal capillary blood flow was assessed using scanning laser Doppler flowmetry (SLDF) at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering, Germany). Briefly, a retinal sample of 2.56 × 0.64 × 0.30 mm was scanned within 2 s at a resolution of 256 points × 64 lines × 128 lines. The confocal technique of the device ensured that only the capillary blood flow of the superficial retinal layer of 300 µm was measured. Measurements were performed in the juxtapapillary area of both eyes 2 to 3 mm temporally to the optic nerve; the average of three singular measurements was taken.

Analysis of perfusion images was performed offline with automatic full-field perfusion imaging analysis. This led to a perfusion map excluding vessels with a diameter of >30µm, without lines with saccades, and without pixels with inadequate reflectivity. The mean retinal capillary blood flow was calculated in the area of interest and expressed as arbitrary units. In our study retinal capillary blood flow was measured before and during flicker-light stimulation (10 Hz over 3 min; Photo Stimulator 750, Siemens-Elema AB, Solna, Sweden).

Analysis of vessel diameters was performed offline with automatic full-field perfusion imaging analysis (SLDF version 3.7).[16,17] Outer arteriole diameter (AD) was measured in reflection images, and lumen diameter (LD) was measured in perfusion images. The wall to lumen ratio (WLR) was calculated as (AD-LD)/LD.

Laser scanning records were stored electronically and sent to a central reading center (Interdisciplinary Centre for Ophthalmic Preventive Medicine and Imaging of the Friedrich-Alexander-University Erlangen-Nuernberg, Germany), for measurement of retinal microvascular blood flow and calculation of the retinal wall-to-lumen ratio (WLR). This reading center was blinded for all other study procedures.

Biochemical Measurements

Fasting blood glucose was measured electrochemically (SuperGL, Dr. Müller Gerätebau, GmbH, Freital, Germany), and hemoglobin A1c was determined by high-performance liquid chromatography (ADAMS-HA-8160, A. Menarini Diagnostics, Neuss, Germany). Plasma insulin was determined using a chemiluminescence-immunoassay (Linco Research, St. Charles, MO). Insulin resistance was calculated using the homeostatis model assessment (HOMA) method by calculating fasting plasma insulin × fasting plasma/22.5. Insulin resistance was defined as $HOMA_s$ index >2.[18,19]

Statistical Analysis

Results are presented using descriptive summary statistics. Data are expressed as mean ± standard deviation (SD). Because no previous data have been obtained on the retinal vascular response in insulin-sensitive and insulin-resistant nondiabetic and diabetic subjects, this study was designed as an exploratory study without predefined sample size calculation. The Wilcoxon rank-sum test was used to compare efficacy variables between groups. Statistical significance was defined as $p < 0.05$.

Results

Fifty-four patients participated in the study and were classified in three groups: (1) group C with nondiabetic, lean, insulin sensitive subjects; (2) group IR with non-diabetic, obese, insulin-resistant subjects; and (3) group DM consisting of patients with manifested T2DM. The clinical characteristics of these groups are given in **Table 1**. As a result of group definition, group C showed a significant lower body mass index (BMI) compared with group IR and group DM. Group DM had a significant higher hemoglobin A1c compared with groups IR and C. No difference between the groups was observed for age or blood pressure.

As shown in **Table 2**, no significant differences were observed in RBF among the three groups, neither at baseline nor after stimulating the retina with flicker light.

Table 1.
Clinical Characteristics of the Investigated Groups (mean ± SD)

	Group C	Group IR	Group DM
Gender (male/female)	7/11	7/11	12/6
Age (years)	54.3 ± 9.9	54.9 ± 8.7	55.8 ± 9.6
BMI (kg/m²)	25.1 ± 1.6	34.4 ± 3.9[a]	31.4 ± 4.6[a]
Systolic respiratory rate (mm Hg)	122 ± 15	127 ± 15	124 ± 13
Diastolic respiratory rate (mm Hg)	71 ± 7	78 ± 7	76 ± 7
Hemoglobin A1c (%)	5.4 ± 0.6	5.7 ± 0.4	6.3 ± 0.6[a]
$HOMA_S$ (μU×mmol/ml²)	5.9 ± 7.4	1.2 ± 0.3	3.5 ± 1.3

[a] $p < 0.05$ vs. group C

Table 2.
Retinal Blood Flow and Arterial Wall-to-Lumen Ratio in the Investigated Groups (Mean ± SD)

	Group C	Group IR	Group DM
RBF baseline (AU)	221 ± 41	238 ± 61	208 ± 52
RBF stimulated (AU)	231 ± 50	257 ± 69	222 ± 51
Δ RBF (AU)	10 ± 41	19 ± 46	16 ± 38
WLR	0.45 ± 0.09	0.40 ± 0.09[a]	0.41 ± 0.09

[a] $p < 0.05$ vs. control group

In addition, no differences in the absolute or relative increase in RBF could be observed among the three groups. The arterial WLR tended to be lower in patients with T2DM (group DM) compared with insulin-sensitive non-diabetic subjects (group C), and was significantly lower when comparing insulin-resistant nondiabetic subjects (group IR) with group C.

When insulin-resistant subjects (groups IR and DM) were divided into tertiles according to their degree of insulin resistance, a steady decline in baseline and flicker stimulated RBF could be observed (**Figure 1**). The RBF response to flicker light declined from 12 ± 23% to 5 ± 18% with increasing tertiles of insulin resistance. The WLR declined from 0.41 ± 0.09 in tertile I to 0.40 ± 0.09 in tertile II, and to 0.39 ± 0.03 ($p < 0.05$) in tertile III.

Discussion

Our study revealed no significant differences in baseline or flicker-stimulated RBF between diabetic and nondiabetic subjects. In contrast, a reduced baseline and flicker-

stimulated RBF were observed in those subjects presenting with increasing IR. Even though our study was done as an exploratory study including a limited number of subjects, our results suggest that IR affects RBF in those patients without morphological evidence of DR.

Retinal blood flow regulation and the vasodilatatory response of retinal microvascular blood flow to flicker light are not fully understood at present. The effects of flicker light on retinal capillary blood flow and retinal vascular diameter have been repeatedly suggested to be mediated by nitric oxide (NO). In a study with T1DM patients, a reduced retinal vessel response to flicker stimulation was observed, while retinal vascular reactivity after exogenous NO was not altered.[20] In a study by Dorner and colleagues,[13] it was found that approximately 50% of the flicker light-induced increase in retinal arteriolar and venular vasodilatation can be blocked by L-NG-monomethyl arginine citrate infusion. Two studies have shown that the retinal microvascular response to flicker light is impaired under certain pathological conditions such DM[21,22] or essential hypertension.[15,23] It is suggested that in case of DM or hypertension, endothelial dysfunction and the restricted capability of the endothelial cell to secrete NO might cause a disturbed microvascular blood flow in several tissues prone to the development of microvascular complications. In T2DM and in essential hypertension, endothelial dysfunction was found in association with IR.[1,2,24,25] Therefore, our results of an impaired RBF in subjects with IR underlines the role of IR as an important pathogenetic factor in the development of vascular complications such as retinopathy, nephropathy, neuropathy, myocardial infarction, or cerebrovascular events.[26–32]

Retinal vasculature is morphologically and functionally related to cerebral vessels because of their common origin from the internal carotid artery. An increased arterial retinal WLR has been described in patients with a history of cerebrovascular events.[33,34] In addition, an increased retinal WLR was found to be associated with an increased albumin excretion rate.[35] An increase in retinal arterial WLR can be a consequence of wall thickening, narrowing of the lumen, or a combination of both. It is well known that the thickening of basement membrane is a hallmark of DR, and is a main contributor to capillary narrowing. In our study, only very minor differences in the retinal arterial WLR could be detected among nondiabetic subjects, insulin-resistant subjects, and patients with T2DM. Even though the difference was very small, and the clinical significance appears questionable, it was somewhat unexpected to find a reduced WLR in insulin-resistant and T2DM subjects. The reason for this finding seems unclear and might be driven by the exclusion of any patient with manifested retinopathy in our study. Therefore, it seems conceivable that the WLR might increase in a later stage during the development of DR.

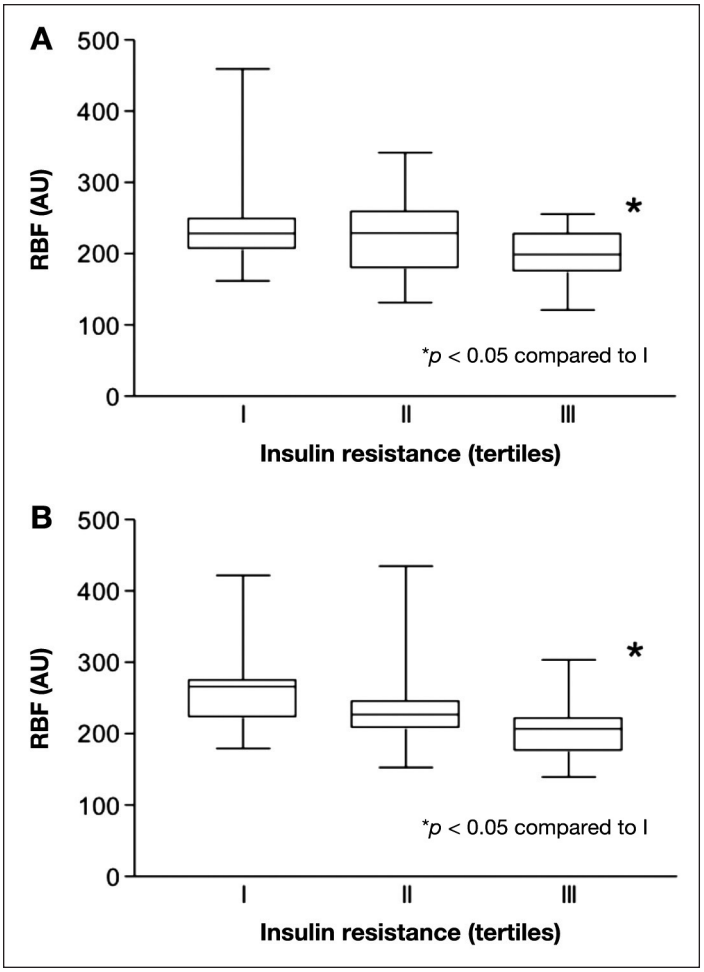

Figure 1. (A) Baseline retinal blood flow in insulin-resistant, obese subjects and in T2DM subjects according to their degree of IR and **(B)** Retinal blood flow after flicker light stimulation in insulin-resistant, obese subjects and in T2DM subjects according to their degree of IR.

Conclusion

Even before the development of clinical DR, laser scanning flowmetry of the retina and the retinal microvascular response to flicker light was found to detect very early functional and morphological changes in retinal microcirculation. Our results suggest that the development of IR might be an important component in the development of retinal microvascular damage. It should be emphasized that this study was performed as an exploratory mechanistic study to be repeated in a larger, confirmatory trial.

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