Impaired Retinal Vasoreactivity: An Early Marker of Stroke Risk in Diabetes

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ABSTRACT

Diabetes is a common cause of small vessel disease leading to stroke and vascular dementia. While the function and structure of large cerebral vessels can be easily studied, the brain's microvasculature remains difficult to assess. Previous studies have demonstrated that structural changes in the retinal vessel architecture predict stroke risk, but these changes occur at late disease stages. Our goal was to examine whether retinal vascular status can predict cerebral small vessel dysfunction during early stages of diabetes. Retinal vasoreactivity and cerebral vascular function were measured in 78 subjects (19 healthy controls, 22 subjects with prediabetics, and 37 with type-2 diabetics) using a new noninvasive retinal imaging device (Dynamic Vessel Analyzer) and transcranial Doppler studies, respectively. Cerebral blood vessel responsiveness worsened with disease progression of diabetes. Similarly, retinal vascular reactivity was significantly attenuated in subjects with prediabetics and diabetics compared to healthy controls. Subjects with prediabetics and diabetes with impaired cerebral vasoreactivity showed mainly attenuation of the retinal venous flicker response. This is the first study to

explore the relationship between retinal and cerebral vascular function in diabetes. Impairment of venous retinal responsiveness may be one of the earliest markers of vascular dysfunction in diabetes possibly indicating subsequent risk of stroke and vascular dementia.

Introduction

Diabetes is a frequent cause of retinal and cerebral vascular disease leading to blindness, stroke, and vascular dementia.[1] The examination of retinal blood vessels yields important information about small vessel architecture and function. Retinal vascular dysfunction occurs early in diabetes[2,3] and contributes to the pathogenesis of retinopathy.[4] Microcirculatory dysfunction and alterations in vascular architecture also play an important role in the development of cerebral small vessel ischemic disease. Because the retinal and cerebral microcirculation share common embryogenic, anatomical, and physiological properties, such as autoregulation of blood flow, retinal vascular changes may reflect cerebrovascular pathophysiology. Thus, the study of retinal vascular function may provide important information about predisposition to cerebrovascular disease associated with diabetes.[5] Retinal photography-based epidemiological studies demonstrate that structural alterations of retinal blood vessels are associated with increased risk for cardiovascular disease, as well as cortical and subcortical infarcts.[6-11] As these structural changes occur during advanced disease stages of diabetes, they are not ideal risk markers to assess the efficacy of therapeutic interventions or to identify patients at early disease stages when treatment is most efficacious. In contrast, measures of endothelial dysfunction that are already present at very early disease stages and therefore potentially reversible could prove to be clinically more meaningful.

Flickering light is a well-established metabolic stimulus for the retinal vasculature,[2,12] which causes vasodilation and increased blood flow in healthy individuals.[13,14] Similarly, standard hyperventilation/breath hold (HVBH) during measurement of blood flow in the middle cerebral artery (MCA) by transcranial Doppler studies (TCDs) can be used to assess cerebral autoregulation.[15] Both the retinal and cerebral vasomotor responses are attenuated in

diabetes,[2,16-19] but the relationship between an impaired retinal response, cerebral dysautoregulation, and stroke risk in diabetes has not been studied.

Research Design and Methods

Subjects

Seventy-eight subjects, age 21 years and older (35 men, 43 women) were enrolled. Based on American Diabetes Association guidelines, individuals were classified as to have type-2 diabetes based on physician diagnosis, an HbA1c of at least 6.5% (48 mmol/mol) and fasting plasma glucose of >126 mg/dL (7 mmol/L). Prediabetes was defined by an HbA1c of 5.7-6.4% (39-46 mmol/mol) and fasting plasma glucose level of 100-125 mg/dL (5.6-6.9 mmol/L).

The study was approved by the Institutional Review Board and followed the Tenets of the Declaration of Helsinki. After signing informed consent, all subjects completed a medical history, physical examination, and ocular examination. Subjects were required to have a normal eye examination with corrected acuity 20/30 or better and intraocular pressures below 21 mmHg. All subjects were free from eye diseases, morbid obesity (body mass index [BMI] > 40 kg/m^2) and were not pregnant. All subjects were nonsmokers and controls had no history of hypertension. Diabetic subjects' blood pressures had to be controlled by antihypertensive medications.

Transcranial Doppler Studies

The MCA was insonnated through the temporal bone window using pulsed wave ultrasound

(2 MHz probe, Siemens Sequoia, USA). Mean flow velocity was measured at rest and following HVBH maneuver. Pulsatility (PI) and resistency (RI) indexes were obtained as indirect measures of peripheral downstream compliance. Cerebral autoregulation was assessed following standardized HVBH maneuver.[19,20]

Eye Studies

Retinal vessel diameters within a region of interest, at one-half to one disc diameter from the optic disc were measured using a modified fundus camera (Zeiss FF450, Zeiss Jena, Germany). The diameters were combined into summary indices, including the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). From these equivalents, the summary index of all arteriolar and venular diameters was calculated and expressed as arteriole-to-venule ratio (AVR).

To assess retinal vasomotor function, vessel diameter changes following flickering light stimulation compared to resting state were measured by the Dynamic Vessel Analyzer software (DVA, Imedos Inc, Germany) following our previously described protocol.[3] The eye with the best visual acuity was dilated with tropicamide (1%) and, if needed, phenylephrine (2.5%).

Experimental Protocol

An initial room air-breathing period was used to allow stabilization of baseline parameters**.** After a rest period of 15 minutes, flickering light measurements were conducted. The subject's retinal vessels were imaged continuously as previously described.[2,3,18] After 15 minutes, rest TCD studies were conducted.

Blood pressure and heart rate were measured continuously by EKG and Finometer (model 2300, Ohmeda, Boulder, CO, confirmed by an automated sphygmomanometer, Dinamap, Critikon, Tampa, FL) and collected online at 200 Hz for subsequent Medlab analysis. Additionally, end tidal CO_2 and finger oxygen saturation were continuously measured during the HVBH maneuver using a respiratory gas monitor (RGM 5250; Ohmeda, Madison, WI) and pulse oximetry (Biox 3740, Ohmeda, Louisville, CO) to ensure sufficient change in blood gases. Fasting glucose, insulin, hemoglobin HbA1c, and lipid profile were measured using radioimmunoassays (Diagnostic Products Corp., Los Angeles, CA).

Data Analysis

For flickering light trials, resting baseline measurements were averaged from the last 15 seconds of resting conditions and peak retinal dilation was captured from the last 3-10 seconds of the flickering stimuli and the first 3 seconds after the stimulus ended. The percentage change in vessel diameter was calculated comparing baseline retinal diameter as previously described.[3,18] The overall range of change in the retinal diameter known as relative amplitude was calculated.[12]

For the HVBH trials, the last 30 seconds of resting baseline and the first 5 seconds after breath hold were measured and the percent change from baseline was calculated.

ANOVA testing was used to compare retinal vessel diameter changes and hemodynamic cerebral data between the three groups. Vasomotor responses following HVBH within each group were compared using paired *t*-tests. Merging data from all groups, correlations between retinal and cerebral vasoreactivity were assessed by Pearson's correlation coefficients and adjusted for covariates (glucose and insulin) using a significance threshold of <.05. Multiregression analyses were performed to

adjust for age, blood pressure, and hyperlipidemia on measures of retinal and cerebral vasoreactivity and on the correlation between cerebral and retinal vasoreactivity.

Results

Group Characteristics

Demographic details are summarized in Table 1. Seventy individuals completed all vascular studies (19 healthy controls, 21 with prediabetes, and 30 patients with diabetes). Most diabetic subjects received oral diabetic medications (87%), and 20% were on combination therapy with long and/or short acting insulin. The majority of diabetic patients were on antihypertensive (93%) and statin (60%) medications, indicating that they had metabolic syndrome. Across all groups, increased BMI was associated with higher hsCRP levels (P = .008), but hsCRP levels were not different between groups and were also not associated with fasting lipid levels. There were no significant differences in blood pressure values between groups.

Transcranial Doppler Results

Resting mean flow velocities in the MCA were comparable between all groups. However, vasoreactivity was significantly impaired in diabetic patients compared to healthy individuals (P = .004) with a steady decrease of cerebral blood vessel responsiveness across the diabetic spectrum (see Fig 1). Individuals with prediabetes tended to have worse vasoreactivity than healthy controls, but better cerebral autoregulatory function than their diabetic counterparts. Similarly, poor cerebral vasoreactivity was associated with higher HbA1c and higher fasting glucose levels (P = .02), but not with dyslipidemia or hsCRP. Individuals with impaired

cerebral autoregulation also had higher pulsatility and resistancy indices (P = .01 and P = .006, respectively) than those with normal cerebral vasoreactivity, as well as significantly higher HbA1c (P < .001) and fasting glucose levels (P = .01). These findings remained statistical significant after adjustment for age, blood pressure, and BMI.

Retinal Vessel Characteristics

Standard measures of structural retinal blood vessel integrity including AVR, CRVE, and CRAE were not significantly different between groups. In contrast, retinal vascular reactivity following flickering light stimulation was significantly attenuated in subjects with prediabetics and in diabetic patients compared to healthy controls (Fig 2). This impairment in vasoreactivity of both retinal arterioles and venules progressed with worsening glucose regulation, and was already present in the early diabetic state (prediabetic arteriolar vascular impairment, P = .05, and prediabetic venular impairment, P = .004). Retinal venous reactivity was more severely attenuated than the arteriolar response (see Fig 2). These observations remained robust after adjustment for potential confounders (age, hypertension, dyslipidemia, statin use, and BMI). Flickering light stimulation did not significantly change resting heart rate or blood pressure. Resting retinal artery and vein diameters were similar at baseline between the three groups.

Correlation between Cerebral and Retinal Vascular Function

Measures of structural retinal vessel changes including AVR, CRVE, and CRAE were not correlated with retinal or cerebral vasoreactivity (results not shown). In contrast, subjects with prediabetes and diabetes with impaired cerebral vasoreactivity showed a significant attenuation of their retinal venous flicker response (r = .24, P = .046), but not in their retinal

arteriolar vasoreactivity (Fig 3). We also found inverse associations between RI, PI, and retinal vasoreactivity (see Fig 4). Individuals with high RI and PI had attenuated retinal venular ($r = –.3$, $P = .029$ for RI, and $P = .035$ for PI) as well as arteriolar ($r = –.3$, $P = .02$ for RI, and $P = .014$, respectively) vasomotor responses. These observations also remained robust after adjustment for potential confounders (age, hypertension, dyslipidemia, statin use, and BMI).

Discussion

We found an increase in cerebral arterial resistance across the diabetes spectrum, suggesting progression of cerebral microvascular disease as glucose regulation worsens. The decrease in vascular compliance was most pronounced in patients with poorly controlled diabetes and high HbA1c levels, but was already detectable in subjects with prediabetes. Although elevated RI and PI values are only indirect measures of small vessel disease with low specificity, these indices can be useful indicators of downstream vascular status.[19] Increased flow indices in our study most likely suggest cerebral microangiopathy as subjects with aortic valve insufficiency or increased intracranial pressure were not included, and all subjects were free from significant intra- or extracranial stenosis. Furthermore, RI and PI remained significantly elevated across the diabetic spectrum after adjusting for age and blood pressure.

Our findings of an impaired cerebral autoregulation in diabetes are consistent with previous studies that demonstrated abnormalities of cerebral hemodynamics, even in

the absence of neurological dysfunction and stroke.[21,22] Diabetes impairs endothelial function that can cause decreased cerebral perfusion, anaerobic metabolism of glucose, atherosclerosis,[23,24] and subsequent stroke and dementia.[25] The observation that impairment of cerebral vasoreactivity is already present in prediabetes, stresses the clinical importance of screening for prediabetes and tight glucose control in early disease stages when interventions are most effective to prevent vascular complications.

Our findings of a worsening retinal vasoreactivity across the diabetic spectrum are consistent with previous observations.[2,17] Our study suggests that impaired retinal venous reactivity is an earlier indicator of endothelial dysfunction in prediabetes and early stages of diabetes than retinal arterial dysfunction. This finding is in line with fundoscopy-based studies suggesting involvement of the retinal venous rather than the arterial microcirculation in early stages of diabetic retinopathy.[26,27] Previous studies have described retinal venous stasis and enlargement of retinal veins as one of the earliest and sometimes only finding in diabetic retinopathy, found in about 10% to 43% of patients with type 1 or type 2 diabetes.[28,29] These venous changes can be associated with increased vascular permeability and impaired endothelial integrity of the microvasculature,[30] and result in lower AVRs in diabetics compared to healthy individuals.[3,32]

Epidemiological studies have shown that alterations in the architecture of retinal vessels are associated with higher risk of cardiovascular disease and stroke.[6-11] Wider retinal vein diameters and decreased AVRs correlate with overall higher stroke risk, presence of lacunar infarcts, chronic cerebral ischemic white matter disease, and

vascular dementia.[34,35] These findings suggest that microvascular changes in the eye parallel the development of cerebrovascular disease, although further confirmatory studies are required.

In contrast to previous studies, we did not find significant lower AVRs in our diabetic study population compared to controls. In addition, AVRs were surprisingly not associated with impaired retinal or cerebral vasoreactivity. This could be due to population bias, in that the present study enrolled primarily patients with earlier disease stages or individuals with better diabetes control, who do not yet show structural vessel alterations, but predominantly early endothelial dysfunction. It could also be due to the relatively small size of our study population as the overall effect size of reduced AVRs may be small in early stages of diabetes.

We found a modest correlation between impaired retinal venous light flicker responses and alterations in cerebral autoregulation. This relatively modest association could also be due to the small size of the study population. However, it may also well be in line with previous observations, showing that large conduit vessels, such as the MCA, are differently affected by insulin resistance and diabetic metabolic dysfunction than the microcirculation.[35] A linear relationship between hyperglycemia and diabetic microvascular complications has been described, whereas the effects of hyperglycemia on larger vessels remain less well understood. Several clinical trials seem to indicate that intense glucose control reduces the rate of microvascular, but not necessarily macrovascular complications,[35-38] and that the efficacy of glucose control on vascular function in different vascular beds may also depend on the duration and severity of diabetes.[39]

The effects of diabetic metabolic dysfunction on vessels of different sizes may at least in part be explained by differences in vessel architecture and cellular constituents between the micro- and macrocirculation. In the retina, endothelial cells and pericytes are affected early by hyperglycemia-related oxidative stress, the production of advanced glycation end products, and epigenetic changes that cause endothelial dysfunction and inflammation that are followed by basal membrane thickening and small vessel drop out.[40]

The impairment of vascular autoregulation may initially be more pronounced in smaller than large vessels, as it has been suggested that microvascular complications precede the onset of large vessel atherosclerotic disease, although this requires further study.[40] The earlier impairment of the cerebral microvasculation may also explain why we found a stronger association between PI, RI, and impaired retinal venous and arteriolar responses to the flickering light stimulus.

There are several limitations to the present study. First, the study involves the comparison of two different measures of vascular function (ie, blood velocity in the cerebral and diameter changes in the retinal vasculature). To control for this, we presented the data as percent change from baseline. Some of our subjects with diabetes (52%) required additional pupillary dilation by phenylephrine, which theoretically could elevate mean arterial pressure through increasing sympathetic stimulation; however, our findings did not change when these individuals were excluded from the analysis. A majority of the diabetic patients were taking statins, which could affect flow responses. However, the vasoconstrictor responses were still attenuated in these individuals and statistical analysis showed no confounding of our

results by statin use. In addition, associations remained robust when adjusting for blood pressure and age. Third, the sample size of this exploratory study was relatively small and future studies need to confirm associations between retinal vasoreactivity, cerebral dysautoregulation, and more importantly the risk of stroke and dementia.

Conclusions

Impairment of venous retinal responsiveness to flickering light is an early and sensitive marker of vascular complications in diabetes and could be easily detected by adding this measurement to routine eye examinations in clinical practice. Retinal and cerebral endothelial dysfunctions are already present in prediabetes and early stages of diabetes stressing the importance for early screening and intervention to prevent cerebrovascular disease.

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Table1. Demographics: Population Characteristics of Study Cohorts

	Healthy	Prediabetics	Diabetics	*P*-Value
Subjects	13	22	37	
Men/Women	7/12	10/12	18/19	.709
Age (years)	52.47 ± 8.93	59.86 ± 9.86	57.57 ± 10.47	.44
BMI	28.45 ± 5.03	29.03 ± 5.53	30.16 ± 5.01	.464
HbA1c	5.279% ± .26 (34 mmol/mol)	6.04% ± .28 (43 mmol/mol)	7.47% ± 1.60 (58 mmol/mol)	.001
hCRP	3.36 ± 4.72	3.13 ± 6.14	2.24 ± 2.60	.624
Total cholesterol	199.58 ± 33.96	206.05 ± 34.16	174.27 ± 38.11	.003
LDL	125.37 ± 8.03	128.27 ± 30.17	100.86 ± 33.93	.002
HDL	55.42 ± 18.67	53.91 ± 15.55	44.97 ± 12.60	.022

Healthy controls, prediabetic, and diabetic patients were matched regarding age, gender, and BMI and had similar hCRP values. A majority of diabetics were on statin drugs and had significantly lower levels of total cholesterol and LDL, but also the lowest LDL levels compared to the other cohorts. BMI = body mass index; HbA1c = glycated hemoglobin; hCRP = high-sensitivity c-reactive protein; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Fig 1. Cerebral vasoreactivity. Both subjects with prediabetes and type 2 diabetes showed an attenuated cerebral vasoreactivity of the middle cerebral artery following hyperventilation breath hold maneuver compared to controls (P = .04 for diabetics vs. controls; nonsignificant attenuation for prediabetics vs. controls and diabetics, respectively). After adjustment for age, statin use, and hypertension, this attenuation remained robust. * = significance <.05.

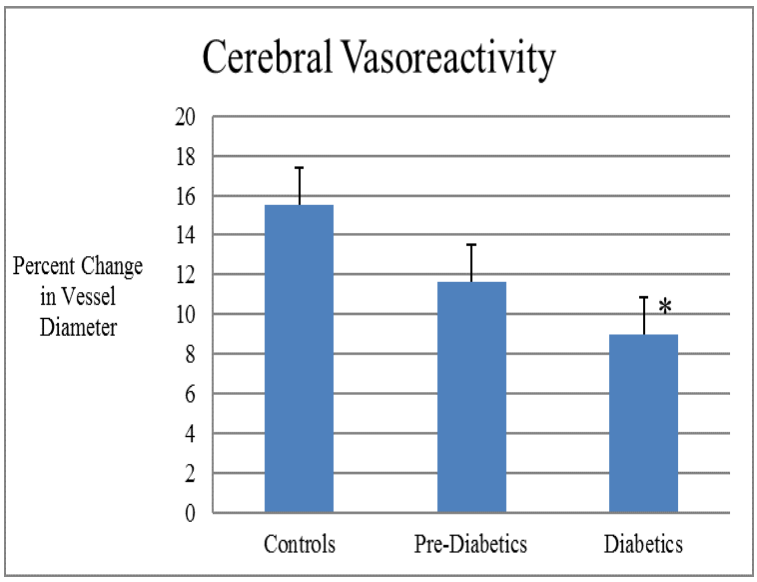

Fig 2. Retinal vasoreactivity. Subjects with type 2 diabetes and prediabetes compared to healthy controls had attenuated retinal vasomotor responses (P = .003 for venous and P = .04 for arteriolar flicker response, respectively) across all groups. Patients with type 2 diabetes exhibited a significantly attenuated arteriolar (P = .003) and venous (P = .012) vasodilation response to the flickering light stimulus compared to controls. Prediabetic subjects also exhibited significantly attenuated venous (.004), and to a lesser degree arteriolar (P = .52) vasodilation in response to the flickering light stimulus compared to controls. Overall, the retinal venous flicker response was attenuated more than the arteriolar response.

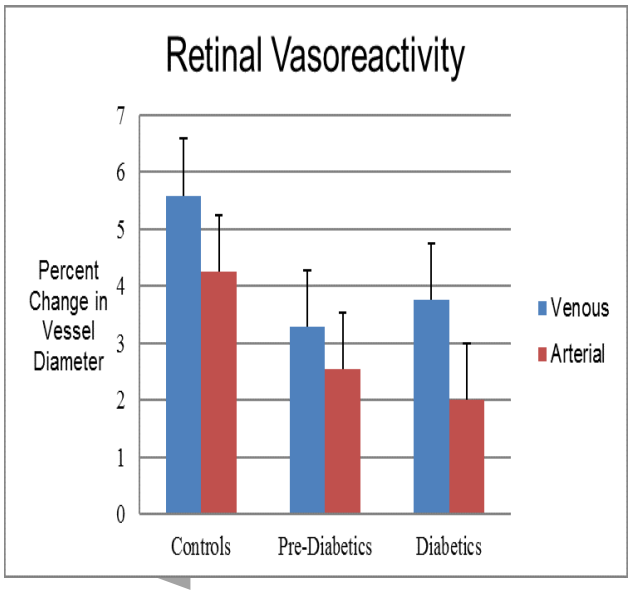

Fig 3. Relationship between cerebral and retinal vasoreactivity. Middle cerebral artery (MCA) reactivity following hyperventilation-breath hold maneuver versus retinal vasodilation response to flicker light stimulus across all groups. There was a significant modest correlation between reduced MCA reactivity and attenuated retinal venous vasodilation response to flicker light stimulus (r = .24, P = .046), but not retinal arterial vasodilation response (not shown).

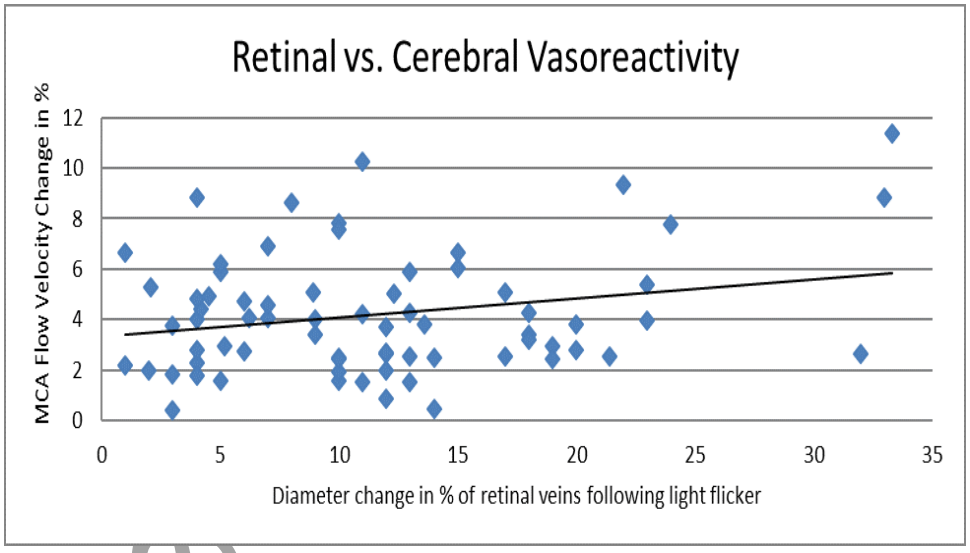

Fig 4. Correlation between retinal vasoreactivity and cerebral vascular compliance. Middle cerebral artery pulsatility index versus retinal vasodilation response to flicker light stimulus across groups. (A) There was a significant correlation between the venous retinal vasodilation response to flicker light stimulus and the pulsatility index (PI) (r = –.33, P = .005). (B) There was also a significant correlation between retinal arteriolar vasodilation response to flicker light stimulus and the PI (r = –.3, P = .011). Individuals with impaired retinal vasoreactivity had higher indexes indicating worse cerebral microvascular compliance.