Impaired Retinal Circulation in Patients with Type 2 Diabetes Mellitus: Retinal Laser Doppler Velocimetry Study

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Purpose. To evaluate the differences in retinal circulation in eyes of patients with type 2 diabetes with no or early-stage diabetic retinopathy compared with control eyes.

Methods. Seventy-five nondiabetic eyes and 194 eyes with type 2 diabetes mellitus were evaluated. The type 2 diabetic eyes were classified into two groups: 139 eyes (139 patients) without diabetic retinopathy (NDR) and 55 eyes (55 patients) with mild nonproliferative diabetic retinopathy (NPDR). The retinal circulatory parameters were measured with laser Doppler velocimetry, and the factors that affect retinal hemodynamics were determined in a cross-sectional population of patients with type 2 diabetes.

Results. The group-averaged blood velocity (*V*) and retinal blood flow (RBF) in the NDR and mild NPDR groups were significantly ($P < 0.01$) lower than in the non-DM group. The diameter and wall shear rate were also significantly ($P < 0.05$) lower in the NDR group than in the nondiabetic control eyes. Multiple regression analysis showed that the RBF was independently and negatively correlated with serum low-density lipoprotein and creatinine. HbA1c was significantly ($P < 0.05$) higher in participants in the lowest RBF quartile than in the highest quartiles.

Conclusions. The results indicate that the RBF may decrease in patients with type 2 diabetes without retinopathy and in those with mild retinopathy. (*Invest Ophthalmol Vis Sci.* 2010;51: 6729–6734) DOI:10.1167/iovs.10-5364

Although diabetic retinopathy is a leading cause of blindness in Western countries, the causes of vascular and visual diseases are not fully understood. Diabetic retinopathy is characterized clinically by multiple microvascular diseases associated with microaneurysms and hemorrhages in neovascularization. However, histopathologic findings in the retinal capillaries precede the clinical retinal signs. Therefore, early surrogate clinical markers are needed to diagnose and quantitate the preclinical lesions of diabetic retinopathy that would allow treatment to begin in the early stages of the disease.

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Supported by a Grant-in-aid for Young Scientists (B) 16791037 and (A) 19689035 (TN) from the Ministry of Education, Science, and Culture, Tokyo, Japan, the Uehara Memorial Foundation (TN), and the Takeda Foundation (TN).

Submitted for publication February 11, 2010; revised May 24 and June 22, 2010; accepted June 23, 2010.

Disclosure: **T. Nagaoka**, None; **E. Sato**, None; **A. Takahashi**, None; **H. Yokota**, None; **K. Sogawa**, None; **A. Yoshida**, None

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Measurements of retinal blood flow (RBF) in early-stage diabetic retinal disease are necessary to understand the role of alterations in retinal hemodynamics in the development of retinopathy and may eventually help to manage retinopathy. Although abnormalities of RBF have been reported in patients and animals with diabetes, the results remain controversial.[1–3] It is noteworthy that almost all clinical studies were performed in patients with type 1 diabetes mellitus only or in a combination of patients with type 1 and 2 disease. The latter is a disease of middle-aged and elderly individuals that typically develops in the context of a cluster of cardiovascular risk factors—notably, obesity, hypertension, dyslipidemia, hyperinsulinemia, and impaired insulin-stimulated glucose levels. Because type 2 diabetes accounts for 90% of all diabetes in humans and the incidence is increasing,[4] it is important to examine the retinal circulation and determine the effect of these systemic factors on the retinal microcirculation in patients with type 2 diabetes mellitus. However, few studies have evaluated the retinal hemodynamics in patients with type 2 diabetes.

Herein, we examined the changes in the retinal microcirculation in a cross-sectional population of patients with type 2 diabetes. We also determined factors that have some effect on the retinal hemodynamics.

Methods

The study included 194 consecutive patients (105 men, 89 women; mean ± SD age, 59.8 ± 10.3 years; range, 27–81) with type 2 diabetes mellitus and 75 healthy age-matched subjects (42 men, 33 women; age 59.1 ± 10.1 years; range, 34–79). The protocol was approved by the ethics committee at Asahikawa Medical College, Asahikawa, Japan, and adhered to the guidelines of the Declaration of Helsinki. All subjects were native Japanese who provided written informed consent before enrollment and after receiving a detailed explanation of the study design and protocol.

Diabetes mellitus was diagnosed based on American Diabetes Association criteria.[5] Patients were considered to have diabetes if they were being treated with insulin or oral hypoglycemic agents or if the fasting blood glucose value exceeded 140 mg/dL. Patients were considered to have hypertension if their blood pressure exceeded 140/90 mm Hg or they used antihypertensive drugs. Patients with poorly controlled diabetes (HbA1c, >10.0%) and uncontrolled hypertension (blood pressure, >140/90 mm Hg) were excluded. Patients with a plasma total cholesterol level exceeding 220 mg/dL, a plasma low-density lipoprotein (LDL) cholesterol level over 130 mg/dL, or both or those who were receiving cholesterol-lowering medication were classified as having dyslipidemia.

All patients underwent clinical and laboratory assessments, ophthalmic examinations, and RBF measurement. The red blood cell (RBC) and white blood cell (WBC) counts, hematocrit, and hemoglobin were measured by using routine methods. The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP), and

heart rate were estimated with an electronic sphygmomanometer (EP-88Si, Colin, Tokyo, Japan). The body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters.

Patients with the history of renal dysfunction and cardiovascular disease were excluded. Renal dysfunction was defined by a serum creatinine concentration below 1.5 mg/dL and a 24-hour estimated creatinine clearance rate below 30 mL/min. The diagnosis of cardiovascular disease included coronary heart disease, congestive heart failure, and ischemic stroke. These diagnoses were established by well-trained specialists at our hospital who were masked to the results of the ocular examinations.

All patients underwent a baseline ophthalmic evaluation before measurement of the RBF. All patients had good visual acuity (VA, >20/20) and normal intraocular pressure (IOP, <20 mm Hg). IOP was monitored by applanation tonometry (Haag Streit, Bern, Switzerland). After the pupils were dilated with a 0.5% tropicamide eye drop, a well-trained ophthalmologist who was masked to the information about the RBF measurements assessed the retinopathy at every visit. For each eye, the maximum grade in any of the seven standard photographic fields was determined for each lesion and used to define the retinopathy levels.[6,7] The severity of retinopathy was categorized as none (level 10), mild nonproliferative (levels 21–37), moderate to severe nonproliferative (levels 43–53), or proliferative (levels 60–65).[8] Patients were excluded who had moderate-to-severe NPDR and PDR and clinically significant macular edema.

The eye with the worse retinopathy that met the inclusion criteria was included. If both eyes had equal retinopathy, one eye was randomly assigned to the study. The ophthalmic exclusion criteria included a history of intraocular surgery, laser photocoagulation, moderate-to-severe cataract, vitreous hemorrhage, foveal deposition of hard exudates, tractional retinal detachment, previous ocular inflammation, and moderate-to-high refractive error (> ±3.0 D).

RBF Measurements

The RBF was measured after the ocular examination. The subjects abstained from drinking coffee for at least 12 hours before the test. A retinal laser Doppler velocimetry (LDV) system (Canon Laser Blood Flowmeter, model CLBF 100; Canon, Tokyo, Japan) was used to estimate the blood flow in the superior branch of the first-order major temporal retinal artery. The methodology of this system has been described in detail.[9,10] Briefly, the retinal LDV system allows noninvasive measurement of the absolute values of the RBCs flowing in the centerline of the vessel, based on the bidirectional LDV.[10] The mean retinal blood V (V_{mean}) was defined as the V of the averaged maximum speed during one cardiac cycle. The diameter (D) of the retinal artery was determined automatically by computer analysis of the signal produced by the arterial image on the array sensor, using the half-height of the transmittance profile to define the vessel edge. All steps throughout the observation of a patient's fundus were virtually the same. All data analyzed were corrected for a magnification factor calculated from the axial length and keratometry, according to the formula of Littmann.[11]

Calculations

The RBF was calculated as RBF = $V_{mean} \times$ area, where the V_{mean} is calculated as $V_{mean} = V$ of the averaged maximum speed/2, and area is the cross-sectional area of the retinal artery at the laser Doppler measurement site.[10] The factor of 2 in the formula for blood flow arises from the assumption of Poiseuille flow.[10] Ocular perfusion pressure (OPP) was determined by the formula OPP = ⅔(MABP) − IOP.[12] The wall shear rate (WSR) was not measured directly in this model but was calculated with a Poiseuillean parabolic model of V distribution across the arterial lumen according to the formula[10]:

$$WSR = 8 \times V_{mean}/D$$

We also performed pulse wave analysis according to a published method.[13] The maximum and minimum blood velocities, which were associated with cardiac systole and diastole, were indicated by V_{max} and V_{min}, respectively. We calculated the WSR_{max} from the V_{max} and D and WSR_{min} from the V_{min} and D.[10] After the measurement was completed, a V profile analysis of the retinal blood V was performed. We defined the upstroke time as the time from the minimum to the maximum retinal blood V.[13,14] In addition, the resistive index, known as Pourcelot's ratio,[15] was calculated as $(V_{max} - V_{min})/V_{max}$ from the tracing of the retinal blood velocity profile.[16]

Data Analysis

All values are expressed as the mean ± SD. The assumption of data normality was assessed using the Shapiro-Wilk test. For statistical analysis, we used one-way analysis of variance followed by the Tukey-Kramer post hoc comparison. Differences in continuous variables between the two groups were analyzed with Student's unpaired t-test. Pearson's correlation analysis was used to determine the relations between the RBF and the traditional risk factors for diabetic retinopathy. Standardized regression coefficients from multiple regression analysis of the RBF in relation to various factors were analyzed. $P < 0.05$ was considered significant.

Results

The baseline clinical characteristics are shown in Table 1. The patients had not changed any medications for at least 6 months before the RBF measurements. The BMI, plasma glucose, and triglyceride values were significantly higher, and the high-density lipoprotein level was lower in patients with NDR compared with the age- and sex-matched nondiabetic control subjects. The SBP, MABP, and plasma glucose were higher in patients with mild NPDR than in the nondiabetic control subjects.

RBF measurements using the LDV system showed that the V_{mean} and RBF were significantly lower in patients with type 2 diabetes mellitus compared with the nondiabetic control subjects (Table 2). The vessel D was reduced in patients with no diabetic retinopathy compared with that in the nondiabetic control subjects. The current results showed that the WSR_{mean} decreased in patients with no retinopathy compared with that in the nondiabetic control subjects. Pulse wave analysis showed significant differences in the V_{min}, WSR_{min}, V_{max}/V_{min}, and resistive index but not in the V_{max}, WSR_{max}, pulse amplitude, and upstroke time in patients with diabetes compared with the nondiabetic control subjects.

Multivariable regression analysis showed that the RBF correlated negatively with serum creatinine and LDL; there was no significant correlation between the RBF and HbA1c, SBP, BMI, and disease duration in patients with type 2 diabetes with no retinopathy or mild NPDR (Table 3). The vessel D did not correlate with any independent parameters including the HbA1c ($r^2 = 0.004$, $P = 0.35$). In contrast, the V_{mean} strongly correlated with the MABP and serum LDL. The WSR_{mean} correlated significantly with the MABP.

Further analysis was performed in patients with diabetes grouped by RBF quartiles (Table 4). The HbA1c was significantly higher in patients in the lowest quartile than in the highest. The vessel D and V_{mean} gradually increased with increasing RBF quartiles. Pulse wave analysis revealed that the V_{max}, V_{min}, and pulse amplitude increased with increasing quartiles of RBF. WSR_{mean} and WSR_{max}, but not WSR_{min}, were significantly higher in the highest RBF quartile than in the lowest RBF quartile.

Discussion

The present study showed for the first time that the RBF was significantly lower in patients with type 2 diabetes with

TABLE 1. Clinical and Physical Characteristics in the Study Groups

	Nondiabetic Control	No Retinopathy	Mild NPDR	*P*
Patients, *n* (men:women)	75 (42:33)	139 (75:64)	55 (30:25)	0.96
Age, years	59.2 ± 10.1	58.8 ± 10.7	62.4 ± 8.8	0.09
BMI	23.4 ± 3.4	25.1 ± 4.9*	24.6 ± 4.4	0.045
SBP, mm Hg	124.1 ± 11.8	130.1 ± 16.3	139.9 ± 15.7*†	<0.0001
DBP, mm Hg	74.3 ± 9.3	73.8 ± 10.6	75.8 ± 8.4	0.44
Mean blood pressure, mm Hg	90.9 ± 9.0	92.6 ± 11.4	97.2 ± 9.4*†	0.003
IOP, mm Hg	14.6 ± 2.4	15.1 ± 2.9	15.9 ± 2.7	0.06
OPP, mm Hg	46.0 ± 6.9	46.6 ± 7.7	48.9 ± 6.3	0.06
Heart rate, beats/min	67.1 ± 10.9	70.5 ± 11.7	71.5 ± 13.2	0.09
Duration of diabetes, y	—	7.8 ± 7.8	13.4 ± 8.1†	<0.0001
HbA1c, %	—	7.7 ± 2.0	7.8 ± 1.3	0.71
Plasma glucose, mg/dL	107.5 ± 23.1	167.6 ± 67.9*	200.5 ± 80.2*†	<0.0001
Blood urea nitrogen, mg/dL	14.8 ± 4.0	15.3 ± 4.7	15.8 ± 4.8	0.71
Creatinine, mg/dL	0.66 ± 0.12	0.68 ± 0.22	0.70 ± 0.32	0.64
Total cholesterol, mg/dL	209.6 ± 32.2	198.7 ± 40.6	199.7 ± 39.1	0.17
Triglycerides, mg/dL	112.4 ± 80.0	145.7 ± 66.9*	135.8 ± 70.3	0.01
HDL, mg/dL	61.0 ± 13.4	53.8 ± 15.7*	59.1 ± 14.4	0.006
LDL, mg/dL	126.2 ± 31.5	118.0 ± 35.0	114.8 ± 33.7	0.2
WBC count ($10^3/\mu L$)	5.7 ± 1.5	6.2 ± 1.7	5.8 ± 1.2	0.1
RBC count ($10^6/\mu L$)	4.5 ± 0.5	4.6 ± 0.4	4.5 ± 0.5	0.18
Hemoglobin (g/dL)	13.9 ± 1.4	14.0 ± 1.5	13.7 ± 1.5	0.61
Hematocrit (%)	41.8 ± 3.7	41.9 ± 4.0	40.9 ± 3.9	0.45
Insulin use, *n* (%)	—	27 (19)	20 (36)	0.01
Oral antidiabetic drugs, *n* (%)	—	86 (62)	41 (75)	0.09
Hypertension, *n* (%)	—	72 (52)	27 (49)	0.4
Hyperlipidemia, *n* (%)	—	70 (50)	25 (45)	0.38
Medications				
β-Antagonist, *n* (%)		13 (9)	6 (11)	0.74
Angiotensin converting enzyme inhibitor, *n* (%)	—	13 (9)	6 (11)	0.74
Angiotensin II type 1 receptor blocker, *n* (%)	—	26 (19)	15 (27)	0.19
Calcium channel antagonist, *n* (%)	—	31 (22)	21 (38)	0.02
Diuretic, *n* (%)	—	10 (7)	6 (11)	0.4
Statin, *n* (%)	—	52 (37)	16 (29)	0.27

Values are expressed as the mean ± SD.
* Significant ($P < 0.05$) vs. nondiabetic control subjects.
† Significant ($P < 0.05$) vs. no retinopathy.

NDR and mild NPDR compared with the nondiabetic control subjects (Table 2). The current findings suggested that the RBF in the retinal arterioles decreases in patients with type 2 diabetes at least in early-stage diabetic retinopathy. Previous clinical studies have reported that the RBF is impaired in patients with type 1 diabetes who are approximately 30 years of age, but the results were inconsistent.[1–3] Although some studies reported that blood flow in the retinal venules increased[17] or was unchanged[3] in all grades of untreated diabetic retinopathy in patients with type 1 diabetes, Konno et al.[1] reported an initial decrease in blood flow in the retinal arterioles and then an increase with longer disease duration in prospective patients with type 1 disease. In addition, other studies using video fluorescein angiography also reported decreased RBF in patients with type 1 diabetes with no diabetic retinopathy.[2,18,19]

TABLE 2. Values of Retinal Circulatory Parameters in the Study Groups

	Nondiabetic Control	No Retinopathy	Mild NPDR	*P*
D, μm	111.5 ± 10.8	107.6 ± 12.6*	107.2 ± 11.2	0.04
V_{mean}, mm/s	38.7 ± 6.7	33.7 ± 7.8†	35.2 ± 7.3†	<0.0001
RBF, μL/min	11.4 ± 2.8	9.3 ± 3.0†	9.6 ± 2.9†	<0.0001
WSR_{mean}, s^{-1}	1403 ± 295	1272 ± 337*	1326 ± 307	0.02
V_{max}, mm/s	64.2 ± 12.0	61.3 ± 16.8	65.1 ± 13.4	0.18
V_{min}, mm/s	20.0 ± 5.3	16.7 ± 5.4†	16.2 ± 5.7†	<0.0001
WSR_{max}, s^{-1}	2350 ± 501	2306 ± 685	2454 ± 573	0.32
WSR_{min}, s^{-1}	728 ± 220	631 ± 237*	615 ± 230*	0.006
Pulse amplitude, mm/sec	44.8 ± 10.8	44.7 ± 15.2	48.9 ± 12.6	0.11
V_{max}/V_{min}	3.4 ± 0.9	3.9 ± 1.4*	4.6 ± 2.5†‡	0.0001
Resistive index	0.69 ± 0.08	0.72 ± 0.08*	0.75 ± 0.10†	0.0003
Upstroke time, msec	220.7 ± 47.7	204.9 ± 46.4	212.8 ± 65.0	0.56

Values are expressed as the mean ± SD. UT, upstroke time.
* Significant ($P < 0.05$).
† Significant ($P < 0.01$) vs. nondiabetic control subjects.
‡ Significant ($P < 0.05$) vs. no retinopathy.

TABLE 3. Standardized Regression Coefficients from Multiple Linear Regression Analysis of Retinal Circulatory Parameters in Relation to Independent Variables in Patients with Type 2 Diabetes

Independent Variable	RBF	*D*	V_{mean}	WSR_{mean}
Serum creatinine	−0.165 (0.03)	−0.140 (0.08)	−0.075 (0.32)	0.010 (0.90)
LDL	−0.163 (0.04)	−0.037 (0.64)	−0.176 (0.02)	−0.008 (0.78)
MABP	0.128 (0.09)	−0.073 (0.35)	0.259 (0.0007)	0.266 (0.0006)
HbA1c	−0.127 (0.10)	−0.108 (0.17)	−0.076 (0.32)	−0.030 (0.68)
Duration of diabetes	0.115 (0.15)	0.111 (0.17)	0.046 (0.56)	−0.020 (0.78)
BMI	0.109 (0.16)	0.082 (0.31)	0.043 (0.58)	−0.008 (0.92)
	$r = 0.31$, $P = 0.009$	$r = 0.20$, $P = 0.32$	$r = 0.34$, $P = 0.003$	$r = 0.30$, $P = 0.017$

$n = 194$.

In contrast to the inconsistent results from previous clinical data, evidence from animal studies supports the presence of decreased RBF in early-stage diabetes. The RBF decreased in streptozotocin-treated rats with activation of endothelin-1,[20] which appeared to be the primary mediator in reducing RBF after the blood glucose became acutely elevated.[21,22] Overexpression of vasoconstrictors has been associated with progression of diabetic retinopathy and results in increased vascular resistance and decreased RBF. Taken together, the decreased RBF in elderly patients with type 2 diabetes obtained in our cross-sectional study may support the hypothesis that decreased RBF may be a common feature in early-stage diabetic retinopathy in patients with type 1 and in those with type 2 diabetes mellitus.

The present study showed for the first time that the WSR, an index of shear stress, was significantly lower in patients with type 2 diabetes with no retinopathy than in nondiabetic control subjects (Table 2). Decreased wall shear stress in the common carotid artery and the brachial artery were reported in patients with diabetes,[23] whereas no study has measured wall shear stress in the retinal microcirculation in these patients. Investigators in other studies have reported that the vessel *D* increased and blood *V* decreased in the retinal arterioles[24] and venules[3] in patients with type 1 diabetes, when measured by LDV, whereas the RBF decreased in the retinal arterioles[24] but increased in the venules.[3] Although the WSR, which is proportional to the ratio of the blood *V* to the vessel *D*, was not calculated, it probably decreased in patients with type 1 diabetes in those studies. All the evidence combined shows that it is likely that reduced WSR is a common feature in early-stage diabetic retinopathy in both type 1 and 2 diabetes.

Vascular endothelial dysfunction contributes to the etiology of diabetic complications including retinopathy.[25,26] We recently showed that acute hyperglycemia caused endothelial dysfunction in the retinal arterioles in cats.[27] In addition, one clinical study reported that the RBF correlated negatively with the serum level of von Willebrand factor, a marker for endothelial dysfunction, in patients with early-stage retinopathy in type 1 diabetes,[28] suggesting that decreased RBF may be associated with systemic endothelial dysfunction in early-stage diabetic retinopathy. Although it is not possible to examine endothelial function in the retinal microcirculation in humans, the decreased RBF and WSR in type 2 diabetes with no or minimal retinopathy observed in the present study is likely to be associated with impaired endothelial function, since we showed that both parameters should be constant at physiologic conditions in the retinal microcirculation.[29,30] Moreover, noninvasive measurement of WSR using LDV may be a valuable index in evaluating vascular function in the retinal microcirculation in patients with diabetes.

It is necessary to determine the importance of decreased wall shear stress in the pathogenesis of diabetic retinopathy. In diabetic retinopathy, the expression of intercellular adhesion molecule (ICAM)-1 and vascular cellular adhesion molecule (VCAM)-1 on the vascular endothelial cells is upregulated[31–33] and leads to leukocyte adhesion to the vascular endothelium and accumulation of leukocytes in the retina.[31] Several investigators have reported an inverse relationship between VCAM-1 expression and shear stress, suggesting that leukocyte binding should increase with low shear stress.[34–36] Experimental reductions of blood flow also enhanced endothelial adhesiveness to monocytes.[37,38] All these results show that it is likely that the decreased WSR is associated with the early-stage diabetic retinopathy.

In the present study, pulse wave analysis revealed that decreased V_{mean} and WSR_{mean} were caused primarily by the decrease in V_{min} and WSR_{min}, not V_{max} and WSR_{max}, in patients with type 2 diabetes (Table 2). These results may not result from the systemic blood pressure, because there were no significant changes in SBP, DBP, and MABP among the three groups. Therefore, it is likely that the increased vascular resistance distal to the measurement site is responsible for the decreased V_{min}. In addition to the decreased V_{min} and WSR_{min}, the retinal circulatory indices V_{max}/V_{min} and resistive index increase in patients with type 2 diabetes. It was reported that the V_{max}/V_{min} increased with increasing risk of diabetic mac-

TABLE 4. Analysis of Ocular and Systemic Parameters Grouped by RBF Quartiles

	Quartiles				
	1st	2nd	3rd	4th	*P*
Range of RBF, μL/min	4.2–7.1	7.2–8.8	8.9–11.1	11.2–17.4	
Age, y	58.9 ± 12.0	60.3 ± 8.7	60.1 ± 9.3	59.5 ± 10.9	0.92
HbA1c, %	8.3 ± 1.9	7.6 ± 2.6	7.6 ± 1.8	7.3 ± 1.5*	0.047
Duration of diabetes, y	8.1 ± 8.7	8.0 ± 6.9	10.9 ± 8.5	9.8 ± 7.4	0.20
SBP, mm Hg	129.5 ± 17.4	134.4 ± 17.9	133.3 ± 16.1	134.6 ± 15.2	0.38
DBP, mm Hg	72.7 ± 9.6	75.9 ± 10.2	73.6 ± 9.5	75.6 ± 10.8	0.33
MABP, mm Hg	91.6 ± 11.3	95.4 ± 11.4	93.5 ± 9.9	95.2 ± 11.3	0.28

Data are expressed as the meany ± SD. $P < 0.05$ vs. 1st quartile.

ular edema probably via the increased vascular rigidity of the retinal arterioles.[39] In addition to the increased resistive index, the current findings suggest that the rigidity of the retinal arterioles increases with progressing diabetic retinopathy in type 2 diabetes. Although the present study did not include patients with diabetic macular edema, the decreased V_{max}/V_{min} may be involved in the pathogenesis of not only diabetic macular edema and but also retinopathy.

Multivariate regression analysis including serum creatinine, LDL, MABP, HbA1c, disease duration, and BMI identified serum creatinine and LDL as the significant independent factors determining RBF in our patients with type 2 diabetes ($P < 0.05$; Table 3). That there is no report of a relationship between LDL and RBF is not surprising, because LDL constricted rat aortic rings in vitro, suggesting that elevated LDL contributes to regulation of microvascular tone.[40–42] Moreover, LDL changes vascular tone by increasing the intracellular free calcium concentration, which leads to impaired blood flow in the microvasculature in retinal pericytes.[43] Because pericytes participate in maintaining the microvascular integrity and play an important role in the retinal circulation in diabetes,[44,45] the decreased blood flow in the retinal arterioles may be caused by the constriction of the pericytes in the retinal capillaries in response to the increased LDL level.

Our multivariate regression analysis showed a negative correlation between serum creatinine and RBF in patients with type 2 diabetes. In contrast to our results, Guan et al.[39] did not find a significant correlation between serum creatinine and retinal circulatory parameters. This discrepancy may have resulted from the inclusion of patients with diabetic macular edema in their study but not in the present study. The negative correlation between serum creatinine and RBF may indicate the involvement of renal function in the changes in RBF in patients with type 2 diabetes. To our knowledge, there is no report of a relation between renal function and retinal microcirculation in patients with diabetes. Further study is needed in this area in patients with type 2 diabetes mellitus.

We showed in earlier work that increased systemic blood pressure causes vasoconstriction of the retinal arterioles to maintain the RBF in healthy volunteers, which is defined as autoregulation of RBF.[29] It also has been reported that autoregulation of RBF, measured by LDV, may be impaired in type 1 diabetes mellitus.[46] In the present study, it was noteworthy that the V_{mean} correlated with the MABP by multiple regression analysis, which seems consistent with the previous study.[39] Because there was no significant correlation between retinal circulatory parameters and systemic blood pressure in age-matched nondiabetic subjects ($r^2 = 0.02$, $P = 0.27$), the correlation between blood V and MABP may reflect a disease-associated vascular dysfunction in patients with type 2 diabetes.

Although multiple regression analysis did not show a significant linear correlation between HbA1c and RBF, it is noteworthy that the HbA1c was significantly higher in patients with diabetes with the lowest RBF quartiles (Table 4), suggesting that the reduction in RBF is associated with poor glycemic control. In a clinical study, decreased RBF and a negative association between HbA1c and RBF were reported in patients with type 1 diabetes with no retinopathy compared with control patients.[18] The results showed that the poorer the level of glycemic control, the greater the reduction in RBF, which supports our findings obtained in patients with type 2 diabetes. Bertram et al.[47] also reported that patients with diabetes with good glycemic control (HbA1c, <8.0%) had shorter arteriovenous passage time, when measured by video fluorescein angiography, than did patients with poor glycemic control (HbA1c, >9.5%), suggesting that patients with type 2 diabetes with poor glycemic control may have advanced impairment of the retinal circulation. Because we excluded patients with poor glycemic control (HbA1c, >10.0%), we did not find a linear correlation between HbA1c and RBF.

In the present study, we found a slight but significant decrease in the retinal arterial D in patients with type 2 diabetes mellitus compared with that in healthy subjects. These findings were somewhat unexpected and in contrast to the findings of large-scale epidemiologic studies reporting that diabetes is associated with larger retinal arteriolar diameters.[48,49] In the Australian Diabetes, Obesity, and Lifestyle study,[49] the association between wider retinal diameter and diabetes was evident in white patients only when the study population was divided into four ethnic groups (whites, black, Hispanic, and Chinese). Although an explanation for this discrepancy was not readily available, the current findings of narrower retinal arterioles may be related to Japanese ethnicity, since the present study was the first to examine the retinal vessel D in Japanese patients with type 2 diabetes mellitus.

The present study had some limitations. First, more than half of the patients with diabetes had hypertension and/or dyslipidemia. Although we found no significant difference in the RBF between those taking and not taking medications among our patients (data not shown), the effect of systemic medications on the retinal microcirculation in patients with type 2 diabetes should be studied. Second, the current findings were from a cross-sectional study. A prospective study should elucidate the changes in retinal microcirculation in relation to the pathogenesis of diabetic retinopathy. Third, we measured only one retinal arteriole, suggesting that the results may not reflect changes in the "total" RBF in a cross-sectional study. Unfortunately, it is very difficult to measure the total RBF by collecting data from all major vessels in both the nasal and temporal regions using a LDV system, because the LDV device is unsuitable for measuring narrow retinal vessels (less than ~70 μm) close to the adjacent vessels, especially in the nasal region. Further study is needed to elucidate whether single-vessel measurement reflects the total retinal microvasculature in patients with diabetes mellitus, using a novel technique to overcome the limitations of a LDV, as mentioned previously. A previous study showed that measurement of single retinal vessels by LDV provides a rough estimation of the total blood flow by defining a scaling factor as the quotient of the total venous cross-section and the cross-section of the measured vessel and multiplying the flow in the measured vessel by this scaling factor.[50] Although these results were obtained from retinal venules in contrast to arterioles measured in the present study, we believe that it is reliable, practical, and less time-consuming to measure single retinal vessels in hospital-based clinical studies.

In conclusion, our results indicated that the RBF may decrease in patients with type 2 diabetes with no and mild retinopathy. If decreased RBF is associated with development and progression of diabetic retinopathy in type 2 diabetes, the improvement of the retinal microcirculation may be a novel target for treating diabetic retinopathy in patients with type 2 diabetes mellitus and early-stage diabetic retinopathy.

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