Relationship Between Retinal Blood Flow and Serum Adiponectin Concentrations in Patients With Type 2 Diabetes Mellitus

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PURPOSE. To study the relationship between retinal microcirculation and serum adiponectin, an important adipocytokine secreted by adipocytes, concentrations in patients with type 2 diabetes mellitus.

METHODS. Using a laser Doppler velocimetry system, we simultaneously measured the retinal blood flow (RBF) values and retinal vessel diameter and blood velocity in 64 consecutive Japanese patients (mean age ± SD, 59.8 ± 10.4 years) with type 2 diabetes with no or mild nonproliferative diabetic retinopathy. We compared the values with the RBF and serum adiponectin concentrations in these patients. The patients were divided into two groups based on sex (33 males, 31 females).

RESULTS. The plasma adiponectin concentrations were correlated positively with the retinal vessel diameter (r = 0.480; P = 0.005), retinal blood velocity (r = 0.539; P = 0.002), and RBF (r = 0.518; P = 0.022) and correlated negatively with the retinal arterial vascular resistance (r = 0.480; P = 0.0002) in males, but not females, with type 2 diabetes with early-stage diabetic retinopathy. Multiple regression analysis showed that the plasma adiponectin level was independently and positively correlated with RBF and negatively correlated with retinal arterial vascular resistance. Our results indicated that a high concentration of serum adiponectin may be associated with increased RBE probably via the increased blood velocity and dilated vessel diameter in males with type 2 diabetes with early-phase diabetic retinopathy. Keywords: adiponectin, diabetic retinopathy, retinal circulation

The pathogenesis of type 2 diabetes mellitus (DM) is complex and involves interaction of genetic and environmental factors such as excessive caloric intake leading to obesity. Furthermore, obesity, which is the accumulation of excessive adipose tissue, is considered the most important risk factor for type 2 diabetes and cardiovascular disease in numerous epidemiologic studies. Thus, adipocytes may be implicated in the pathogenesis of vascular disorders in type 2 diabetes. Indeed, adipose tissue currently is considered not only a reservoir for energy storage but also an active endocrine tissue that produces several proinflammatory cytokines, referred to as adipocytokines, that play an important role in maintaining metabolic homeostasis. Although the evidence supporting a relationship between obesity or high body mass index (BMI) and increased risk of diabetic retinopathy (DR) is inconclusive, adipocytokines released from adipose tissue potentially may affect the development and progression of DR in patients with type 2 diabetes.

Adiponectin, an adipocytokine, is present abundantly in circulating blood and inhibits inflammation, which occurs during early-stage atherosclerosis in vitro and in vivo via activation of adiponectin receptors (AdipoR) in multiple tissues.

Although the results of previous clinical studies that have examined the relationship between plasma adiponectin concentrations and the severity of DR are inconsistent, we reported recently that adiponectin elicits endothelial-depend vasodilation of the retinal arterioles and that AdipoR is expressed in the endothelial cells of the retinal arterioles. Using a retinal laser Doppler velocimetry (LDV) system, we also reported that the retinal blood flow (RBF) decreases in patients with type 2 diabetes with and without mild DR. Therefore, it is likely that decreased plasma adiponectin concentrations may impair the RBF, leading to development and progression of DR. However, it has not been fully clarified whether the plasma adiponectin levels affect the RBF in patients with type 2 diabetes. In the current study, we investigated the relationship between the serum adiponectin concentrations and retinal circulation in type 2 diabetes. Because the circulating adiponectin concentration generally is lower in males than in females, we further divided the patients by sex to determine if changes were associated with sex differences.

METHODS

Subjects

The study adhered to the tenets of the Declaration of Helsinki and the guidelines approved by the ethics committee of our institution. All participants provided written informed consent. The current study included 64 consecutive native Japanese patients (33 males, 31 females; age, mean ± SD 59.8 ± 10.4 years).
years) with type 2 DM diagnosed according to the criteria of the American Diabetes Association. Patients were considered to have diabetes if they were 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 the blood pressure (BP) exceeded 140/90 mm Hg or they used antihypertensive drugs. Dyslipidemia was diagnosed in patients with low-density lipoprotein (LDL) cholesterol of 140 mg/dL or higher and/or high-density lipoprotein (HDL) cholesterol below 40 mg/dL and/or triglyceride values of 140 mg/dL or higher in subjects with a history of cholesterol-lowering therapy.

The urinal albumin excretion is presented as the albumin-to-creatinine ratio (ACR) (mg/g creatinine). Diabetic nephropathy was staged based on analysis of spot urine samples: stage I (normoalbuminuria, ACR < 30 mg/g creatinine); stage II (microalbuminuria, 30 < ACR < 300 mg/g creatinine); stage III (macroalbuminuria, ACR > 300 mg/g creatinine; or dipstick urinalysis showing 2+, 3+, or 4+), and an estimated GFR (eGFR) less than 30 mL/min/1.73 m². The serum creatinine was measured within 4 hours of fasting venous blood collection using a Hitachi 747 biochemistry analyzer (Hitachi High-Technologies Corp., Tokyo, Japan). Renal function also was evaluated based on the eGFR, which was calculated using a previously reported formula. The Modification of Diet in Renal Disease Study Group suggested the following equation compiled for Japanese individuals and the Japanese Society of Nephrology recommended: eGFR (ml/min/1.73 m²) = 194 × scr⁻¹.094 × age⁻⁰.287 × 0.739 (if female). The chronic kidney disease (CKD) stages were based on the National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines.

In the current study, we recruited patients with type 2 diabetes, no or mild DR, and no or microalbuminuria. Because the impaired renal function might be associated with decreased RBF in early-phase DR, patients with stage 3 CKD, macroalbuminuria, or proteinuria and those undergoing hemodialysis were excluded. In addition, patients with poorly controlled diabetes (hemoglobin [Hb] A1 >10.0%) uncontrolled hypertension (BP > 140/90 mm Hg), acute renal failure, chronic glomerulonephritis, and interstitial nephritis were excluded as were those with cardiovascular diseases, such as coronary artery diseases, congestive heart failure, peripheral vascular disease, and ischemic stroke. The specialists in our institution diagnosed and were masked to the information from the ocular examination.

All patients underwent a baseline ophthalmologic evaluation before the RBF measurement. All patients had a visual acuity exceeding 20/20 and IOP below 20 mm Hg. After the pupils were dilated with a 0.5% tropicamide eye drop, a well-trained ophthalmologist, masked to the status of the RBF, assessed the DR at every visit. For each eye, the maximal grade in any of the seven standard photographic fields was determined for each lesion and used to define the DR levels. The severity of the DR was determined once when the patients entered the study and categorized as none (level 10), mild nonproliferative DR (NPDR) (levels 21–37), moderate-to-severe NPDR (levels 43–53), or proliferative DR (PDR) (levels 60–65). Patients with moderate-to-severe NPDR and PDR and clinically relevant macular edema were excluded. The eye with the worse DR that met the inclusion criteria was included; if both eyes were equal, one eye was randomly chosen. The ophthalmologic exclusion criteria included a previous intraocular surgery, history of laser photocoagulation, moderate-to-severe cataract, vitreous hemorrhage, tracial retinal detachment, and a moderate-to-high refractive error (> ±3.0 diopters).

**Measurements of RBF**

The RBF was measured after the ocular examination. The subjects abstained from coffee for at least 12 hours before the measurement. A retinal LDV system (Canon Laser Blood Flowmeter, model CLBF 100; Canon, Tokyo, Japan) estimated the blood flow in the superior branch of the first-order major temporal retinal artery. The detailed system methodology was described previously.

Briefly, the retinal LDV system allows noninvasive measurement of the absolute values of the red blood cells flowing in the centerline of the vessel, based on the bidirectional LDV. The mean retinal blood velocity (Vmean) was defined as the V of the averaged maximal speed during one cardiac cycle. Computer analysis of the signal produced by the arterial image on the array sensor using the half height of the transmission profile to define the vessel edge automatically determined the retinal artery diameter (D). The patients with DM had not changed any medications for at least 6 months before the RBF measurements.

**Assay**

The plasma adiponectin concentration was measured using an ELISA kit developed by R&D Systems (Minneapolis, MN, USA).

**Calculations**

The RBF was calculated as RBF = Vmean × area, where Vmean is calculated as Vmean = V of the averaged maximal speed/2, and area is the cross-sectional area of the retinal artery at the LDV measurement site. The mean arterial BP (MABP) was determined by the formula: diastolic BP + (systolic BP – diastolic BP)/3. Ocular perfusion pressure (OPP) was determined by the formula OPP = 2/3(MABP) – IOP. Retinal arterial vascular resistance (RVR) was determined using the formula RVR = OPP/RBF. The wall shear rate (WSR) was not measured directly in this model but was calculated with a Poiseuille parabolic model of V distribution across the arterial lumen according to the formula: WSR = 8 × Vmean/D.

**Statistical Analysis**

All values are expressed as the mean ± SD. Comparisons between groups were made using the Mann-Whitney U test (for continuous variables) and the χ² test (for categorical variables). Pearson’s correlation analysis was used to study the relation between plasma adiponectin and the retinal circulatory parameters. Standardized regression coefficients from multiple regression analysis of the retinal circulatory parameters in relation to various factors, including adiponectin, were analyzed. For this analysis, based on our previous studies, clinically important variables, including age, HbA1c, duration of diabetes, plasma glucose, BMI, BP, HR, IOP, OPP, LDL, creatinine, and adiponectin were entered. Second, these variables with P less than 0.2 from Pearson’s analysis were included in the final multiple regression analysis. To avoid multicollinearity, if there was significant correlation (r > 0.7) between two variables, only one variable was selected and entered into the model; P less than 0.05 was considered significant.

**Results**

Because serum adiponectin levels varied depending on sex, we subdivided the patients with type 2 diabetes with early-stage DR by sex. Although the group-averaged values of serum creatinine were higher in males compared with females, no
significant differences in age, HbA1c, duration of diabetes, BMI, systemic BP, diastolic BP, mean BP, heart rate, IOP total cholesterol, triglyceride, HDL, LDL, or blood urea nitrogen were seen between the sexes. Serum adiponectin levels were significantly different in females than in males (Table 1). There were no significant differences in the retinal circulatory parameters between the groups (Table 2).

Pearson’s correlation analysis showed that the plasma adiponectin concentrations were positively correlated with D (r = 0.480; P = 0.005), V (r = 0.399; P = 0.02), and RBF (r = 0.518; P = 0.002), and negatively correlated with RVR (r = −0.598; P = 0.0002) in males, but not females, with type 2 diabetes with early-stage DR (Figs. A–D; Table 3). There were no differences between the WSR and plasma adiponectin concentrations in males and females with type 2 diabetes with early-stage DR.

Multiple regression analysis showed that the RBF correlated positively with serum adiponectin; there was no significant correlation between the RBF and mean BP (MBP), BMI, LDL, and duration of diabetes in males with type 2 DM with no DR and mild NPDR (Table 4). The vessel diameters and blood velocity were not correlated with any independent parameters. The RVR strongly correlated with the MBP and serum adiponectin.

### DISCUSSION

The current study found large differences between males and females regarding the relationship between serum adiponectin concentrations and retinal circulatory parameters of the retinal arterioles in patients with type 2 diabetes and minimal DR. Although definitive reasons for these disparate results between the sexes are unclear, it has been reported that AdipoR1 protein expression was higher in males than females in the vastus lateralis muscle and that males had higher AdipoR1 and AdipoR2 levels than females in twin sets. Further, our research showed that adiponectin per se caused vasodilation of isolated retinal arterioles via production of nitric oxide (NO), a powerful vasodilator of retinal arterioles, from the vascular endothelial layer of the retinal arterioles. Because it has been reported that adiponectin may have dilatory effects on the retinal arterioles, changing the resistance of the retinal arterioles. Because our recent study indicated that adiponectin elicited vasodilation of
more distal retinal arterioles, compared with the first-branch retinal arterioles measured in the current study. Adiponectin may evoke increased upstream blood flow velocity followed by decreased resistance elements with distal retinal arteriolar vasodilation in reaction to adiponectin. This was supported by a previous observation that intracoronary infusion of monomeric adiponectin caused a dose-dependent decrease in coronary vascular resistance and further resulted in a dose-dependent increase in coronary blood flow in pigs. Therefore, we speculated that adiponectin can increase the RBF by increased velocity induced by vasodilation of resistance vessels and dilated diameter of the measured retinal artery per se in males with type 2 diabetes with no and minimal DR.

Our previous clinical study showed that RBF and WSR, an index of shear stress, were significantly lower in patients with type 2 diabetes with no DR than in nondiabetic control subjects. Recently, we showed that low shear stress upregulated mRNA expression of molecule adhesion such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, which leads to leukocyte adhesion to the endothelium in the retinal vasculature in the diabetic retina, in human retinal microvascular endothelial cells. Thus, RBF also may be regulated by interaction of shear stress with molecular adhesion to the endothelium in the retinal microcirculation. Indeed, experimental reduction of blood flow by surgical manipulation, which decreased shear stress, also increased monocytic adhesion to surface endothelium. Ouchi et al. reported that adiponectin inhibited expression of adhesion molecules, including ICAM-1 and VCAM-1, in response to inflammatory stimuli, such as TNF-α in human aortic endothelial cells. In addition, deficiency of adiponectin in knockout mice exacerbated leukocytic adhesion induced by ischemia in the retinal vessels. Overall, although we did not measure the serum level of adhesion molecules in the current study, the results indicated that reduced RBF and WSR may be attributed to increased expression of adhesion molecules on the endothelium in the retinal vessels.

In contrast to the serum adiponectin level, MBP duration of diabetes, BMI, and LDL were not correlated significantly with the RBF in males with type 2 diabetes with early DR. Despite identifying creatinine and LDL as significant independent risk factors that determine the RBF in patients with type 2 diabetes with our previous findings, these traditional DR risk factors do not seem to affect the RBF in males with type 2 diabetes mellitus with early-stage DR (Table 4; Supplementary Table S1C).

In univariate analysis, but not multivariate regression analysis, the present study showed a negative correlation between serum LDL and velocity in male patients with type 2 diabetes (Supplementary Table S1B). Low-density lipoprotein changes vascular tone by increasing the intracellular free calcium concentrations in pericytes. Because pericytes participate in maintaining the microvascular integrity and play an important role in the retinal circulation in diabetes, the decreased velocity in the retinal arterioles may be caused by the constriction of the pericytes in the retinal capillaries in reaction to the increased LDL level.

In the present study, the retinal diameter negatively correlated with the DBP, MBP, and OPP by univariate analysis but not multivariate regression analysis (see Supplementary Table S1A). This result that the retinal diameter negatively correlated with MABP was consistent with our previous observation that increased systemic BP constricts the retinal arterioles to maintain RBF in healthy subjects, which is defined as autoregulation of RBF. Although it has been reported that autoregulation of RBF may be impaired in type 1 DM, systemic BP may change the retinal diameter in male patients with type 2 diabetes. The OPP is affected by changes in systemic BP and IOP. Because there is no significant correlation between the retinal diameter and IOP (see Supplementary Table S1A), elevation of BP may exert vasoconstriction of retinal arterioles with regulation of OPP.

In univariate analysis, RVR positively correlated with the BP and OPP in male patients with type 2 diabetes (see Supplementary Table S1D). Moreover, our multivariate regression analysis showed a negative correlation between MBP and RVR in male patients with type 2 diabetes (Table 4). Recently, we reported that RVR decreased in response to reduction of OPP induced by decreased systemic BP beyond the range for flow autoregulation in cats. Because autoregulation of RBF may be impaired in type 1 diabetes, the result that RVR inversely correlated with the MBP and OPP may reflect a disease-associated vascular dysfunction in male patients with type 2 diabetes.

A pronounced reduction in plasma glucose levels induced by administration of insulin leads to a significant reduction in RBF in type 2 diabetic patients. In addition, reduced RBF after glucose reduction was found in patients with type 1 diabetes with no DR. Recently, reduction of plasma glucose using a glucose clamp induced reduced RBF in type 1 patients with no or mild DR. Thus, plasma blood glucose and insulin levels in diabetic patients can affect RBF. Although we did not measure plasma insulin levels in the current study, plasma glucose was not correlated significantly with RBF in males with type 2 diabetes with early DR (see Supplementary Table S1C). This inconsistency may be based on differences in methodology and type of diabetes.

The current study had some limitations. First, this study was cross-sectional. Further prospective studies should assess the relationship between serum adiponectin concentrations and retinal vessel parameters of DR in type 2 diabetes. Second, we could not evaluate the effects of systemic medications on the retinal vessel parameters. Experimental and clinical studies have found that peroxisome proliferator-activated receptor (PPAR)-γ agonists, the thiazolidinediones, and PPAR-α agonists augment circulating levels of adiponectin. In addition to increasing the circulating levels of adiponectin, the PPAR-γ and PPAR-α agonists increase AdipoR2 in macrophages. Thus, further studies of the effects of systemic medications, which

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<th>RVR</th>
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<td>0.438 (0.008)</td>
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<td>−0.146 (0.33)</td>
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<td>LDL</td>
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can increase the plasma adiponectin concentrations and the expression of AdipoR, on the retinal vessel parameter are required. Third, we did not assess the exact mechanism of the effect of adiponectin on the retinal circulation. Although the concentrations of endothelin-1 (ET-1), a potent retinal vasoconstrictor,57 were not investigated in the current study, a previous study showed an inverse correlation between ET-1 and adiponectin in pediatric subjects.58 Further, in experimental studies, co-infusion of adiponectin inhibited the increased perfusion pressure by ET-1 in the pump-perfused rat hindlimb59 and blockade of ET-1 receptors increased adiponectin-mediated vasodilation in isolated mesenteric arteries.58 Overall, because these results imply that ET-1 may counteract adiponectin-regulated retinal circulation, further investigation is required of the association between ET-1 and adiponectin in pediatric subjects.58 In conclusion, the current findings showed that the plasma adiponectin concentration is correlated positively with RBF in males with type 2 diabetes, suggesting that adiponectin may affect the RBF in early-phase DR. Because the RBF is impaired in early-stage DR in patients with type 2 DM, elevated plasma adiponectin levels resulting from either pharmacologic or lifestyle intervention may represent a novel therapeutic strategy for DR.

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References


Figure. Relation between serum adiponectin concentrations and retinal circulation parameters in male patients with type 2 diabetes with early-stage retinopathy. Serum adiponectin concentrations is positively correlated with (A) vessel diameter ($r = 0.480; P = 0.005; 95\%$ confidence interval $[CI] 0.163–0.707$), (B) blood velocity ($r = 0.399; P = 0.02; 95\% CI 0.065–0.652$), and (C) retinal blood flow ($r = 0.518; P = 0.002; 95\% CI 0.212–0.731$), and negatively correlated with (D) RVR ($r = -0.598; P = 0.0002; 95\% CI -0.781$ to $-0.321$).


