Cigarette smoking has been identified as a major risk factor for atherosclerotic complications in the coronary, aortic, and cerebral circulatory systems. Further, several previous reports have indicated that cigarette smoking is a significant and independent risk factor for cardiovascular diseases in patients with diabetes. Thus, these observations indicate that cigarette smoking may be involved in the development of vascular disorders in diabetes mellitus via a deleterious effect on the circulation.

Most previous studies have investigated the effects of cigarette smoking on ocular blood flow parameters in nondiabetic smokers. In addition to basal ocular blood flow, retinal vascular reactivity to flicker stimuli and gas provocation was shown to decrease in chronic smokers. There were no differences in the RBF, blood velocity, and vessel diameter in the past smokers compared with those who never smoked. Multiple regression analysis showed that the creatinine level was correlated negatively with the RBF and that current smoking was significantly and independently correlated with decreased RBF.

Conclusions. Our results indicated that the blood V and RBF in the retinal arterioles may decrease in patients with type 2 diabetes who are chronic smokers, suggesting that chronic smoking may be associated with decreased RBF probably via lower blood V in the retinal arterioles in early-phase DR.

Keywords: smoking, diabetic retinopathy, retinal blood flow
creatinine; stage III (macularbuminuria), ACR greater than 300 mg/g creatinine (or dipstick urinalysis showing 2+, 3+, or 4+), and an estimated glomerular filtration rate (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 "717" biochemical assay analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Renal function also was evaluated based on the eGFR, which was calculated using a previously reported formula.27 The chronic kidney disease (CKD) stages were based on the National Kidney Foundation Disease Outcomes Quality Initiative clinical practice guidelines.28

In the current study, we recruited patients with type 2 diabetes, no or mild DR, and no or microalbuminuria. Because impaired renal function may be associated with decreased RBF in early-phase DR,39 patients with stage 3 CKD, macroalbuminuria, or proteinuria and those undergoing hemodialysis were excluded. In addition, patients with poorly control diabetes (hemoglobin [Hb] A1 >10.0%) uncontrolled hyper-tension (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 RBF status, 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.30 The severity of the DR was determined once when the patients entered the study and categorized as none (level 10), mild nonproliferative DR (levels 21–37), moderate-to-severe NPDR (levels 43–60), or proliferative DR (levels 60–65).31 Patients with worse DR that met the inclusion criteria was included; if both eyes were equal, one eye was chosen randomly.

These patients were divided into three groups: the never smoked group, the past-smoker group, and the current-smoker group. The current smoker was defined as a patient currently smoking cigarettes every day. Smokers were defined as those who smoked more than 10 cigarettes daily for more than 10 years. The past smoker was defined as a patient who had smoked regularly but had ceased for at least 1 year before entrance into the study.32

RBF Measurements

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.33

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 bidirectional LDV.33 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 transmittance profile to define the vessel edge automatically determined the retinal artery diameter (D).33 The patients with diabetes mellitus had not changed any medications for at least 6 months before the RBF measurements.

Calculations

The RBF was calculated as RBF = Vmean × x 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.33 The mean arterial BP (MAPB) was determined by the formula: diastolic BP + (systolic BP – diastolic BP)/3.33 Ocular perfusion pressure (OPP) was determined by the formula OPP = 2/3(MAPB) – IOP.36 Retinal arterial vascular resistance (RVR) was determined using the formula RVR = OPP/RBF.34 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.34

Statistical Analysis

All values are expressed as the mean ± SD. Comparisons between groups were made using 1-way ANOVA (for continuous variables) and the χ² test (for categorical variables). One-way ANOVA was followed by a post hoc comparison with the Tukey-Kramer procedure. Standardized regression coefficients from multiple regression analysis of the retinal circulatory parameters in relation to various factors including smoking status were analyzed. For this analysis, based on our previous studies,16,35 clinically important variables were entered: age, Hba1c, diabetes duration, plasma glucose, body mass index (BMI), BP, heart rate, IOP, OPP, LDL, creatinine (CRE), eGFR, and smoking (current or otherwise). Second, the variables with a P value below 0.2 from Pearson’s analysis were included in the final multiple regression analysis.35 To avoid multicollinearity, if there was a 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

Male ratio in sex distribution and the plasma glucose value in the current- and past-smoker group were significantly higher compared with never-smoked group. Although the group-average triglyceride values were significantly lower and the group-average CRE values were significantly higher in the past-smoker group compared with the other groups, there were no significantly differences in age, Hba1c, diabetes duration, BMI, systemic and diastolic BP, mean BP, heart rate, IOP, total cholesterol, HDL, LDL, blood urea nitrogen, or eGFR among the groups (Table 1). We found significant decreases in the blood V (P = 0.003), RBF (P = 0.009), and WSR (P = 0.01) in the current-smoker group compared with the group that never smoked; however, there were no differences in the vessel D among the groups. We found no significant differences in blood V, RBF, and WSR in the past-smoker group compared with the group that never smoked and current-smoker group. Moreover, our results showed a modest, but not significant, elevation of RVR in the current-smoker group compared with the never-smoker group (Table 2). In univariate analysis, the CRE remained negatively correlated with the RBF (Table 3). Multiple regression analysis was performed to determine whether smoking was an independent variable related to the RBF in patients with type 2 diabetes, based on our previous findings. Variables with a P value lower than 0.20 in univariate
Medications

<table>
<thead>
<tr>
<th>Dyslipidemia, no. (%)</th>
<th>23 (66)</th>
<th>8 (40)</th>
<th>14 (74)</th>
<th>0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension, no. (%)</td>
<td>16 (46)</td>
<td>10 (50)</td>
<td>6 (30)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Never Smoked, n = 35</th>
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<th>Current Smoker, n = 19</th>
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<tbody>
<tr>
<td>Mean age, years</td>
<td>59.8 ± 11.0</td>
<td>60.5 ± 10.2</td>
<td>56.1 ± 8.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Men/women</td>
<td>15/22</td>
<td>16/4</td>
<td>15/6</td>
<td>0.004</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>6.9 ± 0.8</td>
<td>7.0 ± 0.9</td>
<td>7.2 ± 1.0</td>
<td>0.48</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>9.2 ± 7.9</td>
<td>11.1 ± 7.1</td>
<td>10.8 ± 8.3</td>
<td>0.63</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>133.7 ± 8.6</td>
<td>1216 ± 299</td>
<td>1065 ± 249*</td>
<td>0.01</td>
</tr>
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</tbody>
</table>

**DISCUSSION**

The current study found, for the first time, that the blood V and RBF in the retinal arterioles decreased in current smokers with type 2 diabetes, suggesting that chronic smoking may be associated with decreased retinal circulation in early-stage DR in type 2 diabetes. Indeed, some previous studies of diabetes have reported abnormalities in the retinal vessel parameters. 

Several previous studies have focused on determining the effect of chronic smoking on the ocular blood flow. Acute smoking increases the tissue blood V in the optic nerve and choroid of habitual smokers. In keeping with that, a study using the blue-field entoptic technique showed increased leukocyte V in the perimacular region in chronic smokers. 

**TABLE 1. Characteristics of Patients With Type 2 Diabetes With Early-Stage Retinopathy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Never Smoked, n = 35</th>
<th>Past Smoker, n = 20</th>
<th>Current Smoker, n = 19</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel diameter, μm</td>
<td>113.7 ± 8.6</td>
<td>110.6 ± 12.6</td>
<td>1120 ± 11.9</td>
<td>0.57</td>
</tr>
<tr>
<td>Blood velocity, mm/s</td>
<td>37.8 ± 9.0</td>
<td>33.3 ± 8.1</td>
<td>29.6 ± 6.8*</td>
<td>0.003</td>
</tr>
<tr>
<td>RBF, μL/min</td>
<td>11.6 ± 3.1</td>
<td>9.8 ± 3.4</td>
<td>8.9 ± 2.9*</td>
<td>0.009</td>
</tr>
<tr>
<td>RVR, mm Hg min/μL</td>
<td>4.6 ± 1.4</td>
<td>5.5 ± 2.1</td>
<td>5.8 ± 2.8</td>
<td>0.07</td>
</tr>
<tr>
<td>WSR, s⁻¹</td>
<td>1337 ± 340</td>
<td>1216 ± 299</td>
<td>1065 ± 249*</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with the group that never smoked.
reported to be lower in smokers than in nonsmokers. Moreover, Morgado et al. reported that acute smoking reduced the RBF in habitual smokers. Not only the basal ocular blood flow but also the retinal vascular reactivity to flicker-light stimulation and gas provocation were shown previously to be reduced in chronic smokers. Although the reason for these contradicting results is unknown, these findings suggested alterations in ocular perfusion in chronic smokers without diabetes.

In the current study, the decreased RBF resulted primarily from decreased V, because the vessel D did not change in current smoker patients compared with nonsmoker patients with type 2 diabetes. It is likely that the decreased in blood V without a change in vessel D indicates vasoconstriction of the smaller arterioles that are located downstream from the measured point (first branch retinal artery). Most clinical and experimental investigations have reported that chronic cigarette smoking impaired endothelial dependent vasodilation, which may be related to decreased endothelial production or increased degradation of nitric oxide (NO) in various vessels. The immunohistochemical expression of endothelial NO synthase (eNOS) in the pulmonary arterial endothelium and the eNOS protein in lung tissue were lower in smokers than nonsmokers. Because NO is a well-known powerful vasodilator in the retinal vessels, the reduced NO production caused by chronic smoking can evoke decreased upstream blood flow V followed by increased resistance elements with vasoconstriction of the distal retinal arterioles and/or capillaries. Moreover, this was supported by our observation that the RVR increased modestly but not significantly in the current smoker patients compared with nonsmoker patients with type 2 diabetes (Table 2).

The current study showed that RBF and WSR, indices of shear stress, were lower in current smoker patients than in nonsmoker patients with type 2 diabetes (Table 2). Our recent in vitro study reported that low shear stress upregulated mRNA expression of molecular adhesion such as vascular cellular 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. Experimentally reduced blood flow by surgical manipulation, which decreased shear stress, also increased mononuclear adhesion to the surface endothelium of the common carotid arteries in rabbits. Thus, we speculate that the RBF may be regulated by interaction of low shear stress with molecular adhesion to the endothelium in the retinal microcirculation in type 2 diabetic patients with smoking habits. In addition, serum VCAM-1 and ICAM-1 concentrations were elevated in smoking hypertensive patients compared with nonsmoking hypertensive patients. Overall, although we did not measure the serum level of adhesion molecules, the results indicated that reduced RBF and WSR may be attributed to increased expression of adhesion molecules on the endothelium in the retinal vessels.

Several reports have implicated endothelin-1 (ET-1), a potent retinal vasconstrictor, activation in the control of vascular tone as a result of cigarette smoking. Plasma ET-1 levels in patients undergoing coronary angiography who were smokers and in rats exposed to cigarette smoke for 16 weeks were higher than those of nonsmoking patients and nonsmoking rats, respectively. However, two studies of human volunteers reported that within the first 10 minutes of active smoking there is a rise in plasma ET-1 level, which is followed by a decline over time. Similarly, acute cigarette smoke exposure (30 minutes) increased ET-1 mRNA expression in rat hearts and lungs, while chronic exposure (6 months) did not alter ET-1 mRNA expression. Moreover, ET-1 production on plasma ET-1 levels is inconclusive. Recently, the ET receptors of ET-1 have been shown to be increased in the retinal arterioles.58 Although we did not measure the serum level of ET-1, the ET system can contribute to the retinal circulatory changes caused by chronic smoking in age, hypertension, and diabetes.

Our observation that chronic smoking reduced retinal blood flow and RBF in patients with type 2 diabetes with early-phase DR is inconsistent with the previous finding that no significant difference was observed in the retinal circulatory parameters between nondiabetic smokers and healthy nonsmoking subjects. However, acute cigarette smoking induced a marked decrease in RBF, more pronounced in diabetics than in healthy controls. Moreover, insulin upregulates the ET receptor in cultured rat aortic smooth muscle cells. Thus, the ET receptor can be upregulated in patients with type 2 diabetes, characterized by a state of hyperinsulinemia. Therefore, the deleterious effect of chronic cigarette smoking on
retinal vascular reactivity in type 2 diabetes can be attributed to increased sensitivity to cigarette smoking.

Cigarette smoke contains high concentrations of free radicals and oxidants.\(^{66}\) Indeed, a short exposure (30 minutes) of bovine pulmonary artery endothelial cells to cigarette smoke extracts resulted in a large increase in superoxide anion production.\(^{61}\) In diabetic patients, the antioxidant capacity is decreased,\(^{62}\) finally resulting in an increased susceptibility to oxidative stress. Therefore, it seems that the difference between diabetic and nondiabetic subjects can be attributed to the sensitivity of the retinal vasculature to oxidative stress caused by cigarette smoke.

The RBF in past-smoker patients was decreased but not significantly compared with nonsmoking patients with type 2 diabetes (Table 2), suggesting that smoking cessation can improve retinal circulation impaired by chronic cigarette smoking in patients with type 2 diabetes. Significant improvement of flow-mediated dilation, which is a well-established and widely used method of evaluating the vascular endothelial function, of the branchial artery in current smokers 1 year after cessation has been reported.\(^{63}\) Indeed, reduction in resting cerebral blood flow caused by chronic exposure to smoking improved within 1 year after smoking cessation,\(^{64}\) and several years following smoking cessation are required for improvement of the decreased cerebral blood flow in long-term smokers.\(^{65}\) Although further investigations of whether smoking cessation can improve impaired RBF caused by chronic smoking are required, chronic cigarette smoking may be associated with potentially reversible impairment of the retinal microcirculation in patients with type 2 diabetes.

In the current study, the multivariate regression model included serum CRE and MABP (Table 3). In addition to current smoking, our multivariate regression analysis showed a negative correlation between serum CRE and RBF in patients with type 2 diabetes (Table 4), suggesting that the serum CRE and current smoking are independent risk factors for RBF in our patients.

Our multivariate regression analysis showed a negative correlation between serum CRE and RBF in patients with type 2 diabetes, suggesting that impaired renal function may be associated with reduced RBF in patients with type 2 diabetes. Indeed, NO production decreases in renal disease due to impaired endothelial function and renal NO production.\(^{66}\) Moreover, we recently reported that RBF decreased in patients with type 2 diabetes with stage 3 CKD.\(^ {29}\) Thus, impaired renal function may be associated with decreased RBF in early-phase DR.

The current study had some limitations. First, we could not determine the specific components of cigarette smoke responsible for reduced RBF. Nicotine has been one of the most widely studied among the more than 4000 chemicals in cigarettes. Nicotine significantly attenuated endothelial-dependent vasodilation in isolated rabbit aortas with cigarette smoke extract inhibited endothelial-dependent relaxation in a dose-dependent manner.\(^ {69}\) In contrast, the clinical data regarding the dose-dependent effects of smoking on endothelial-dependent vasodilation are inconclusive. Celermajer et al.\(^ {70}\) reported a dose-dependent reduction of endothelial-dependent vasodilation related to pack-years of active smoke exposure, but another study reported that both the active light (with smoking $\leq 1$ pack/week) and heavy (with smoking $\geq 1$ pack/day) smokers had a similar reduction in branchial artery endothelial-dependent vasodilation compared with nonsmokers.\(^ {60}\) Because of variations in the amount of nicotine per cigarette, the number of cigarettes consumed daily, and the amount of smoke actually inhaled, it may be difficult to examine in detail the dose-dependent effect of smoking on the retinal microcirculatory parameters in a clinical investigation. Third, there was a significant difference in sex among the three study groups. Because generally, more men than women smoke,\(^ {71}\) the current distribution of male patients was greater than that of female patients with type 2 diabetes in the past and current smoker groups. However, similar results were obtained in male-only patients (data not shown). In contrast, the number of female smoking patients with type 2 diabetes was uneven and the differences in the retinal microcirculatory parameters among the three groups were not significant (data not shown). Further study of the effects of sex differences on the retinal circulation affected by smoking is required. Fourth, we could not evaluate the effect of changes in the systemic BP caused by smoking on the retinal vessel parameters. Acute smoking caused a significant increase in the systemic BP in habitual smokers.\(^ {72}\) Furthermore, our previous report indicated that increased systemic BP constrains the retinal arterioles to maintain the RBF in healthy subjects.\(^ {73}\) Although we found no differences in the systemic BP among the groups (Table 1), a future study should examine the effect of changes in the systemic BP caused by smoking on the retinal vessel parameters.

In conclusion, the current findings showed that the RBF decreased in current smoker patients with type 2 diabetes, suggesting that chronic cigarette smoking may affect the RBF in early-phase DR. These results indicated that impaired retinal microcirculation resulting from chronic smoking may be implicated in the pathogenesis of DR.

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