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Effects of Habitual Cigarette Smoking on Retinal Circulation in Patients With Type 2 Diabetes.

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Purpose. To examine the long-term effects of cigarette smoking on retinal circulation in patients with type 2 diabetes.

Methods. Seventy-four patients with type 2 diabetes mellitus and minimal (no or mild nonproliferative) diabetic retinopathy (DR) were evaluated. These patients with type 2 diabetes were divided into three groups based on their smoking history: current smokers ($n = 19$), past smokers ($n = 20$), and never smoked ($n = 35$). The retinal circulatory parameters were measured with laser Doppler velocimetry and were compared among the groups.

Results. There were significant decreases in the retinal blood flow (RBF; 8.9 ± 2.9 vs. 11.6 ± 3.1 μL/min, $P = 0.009$) with decreased blood velocity (V; 29.6 ± 6.8 vs. 37.8 ± 9.0 mm/s, $P = 0.003$) but no difference in the vessel diameter (D; 112.0 ± 11.9 vs. 113.7 ± 8.6 μm, $P = 0.57$) in the current smokers compared with those who never smoked. There were no differences in the RBF, blood V, and vessel D in the past smokers compared with those who never smoked and current smokers. Multiple regression analysis showed that the creatinine level was correlated negatively with the RBF and that current smoking was significantly and independent 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

Cigarette smoking has been identified as a major risk factor for atherosclerotic complications in the coronary, aortic, and cerebral circulatory systems.[1] Further, several previous reports have indicated that cigarette smoking is a significant and independent risk factor for cardiovascular diseases in patients with diabetes.[2–4] Thus, these observations indicate that cigarette smoking may be involved in 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.[5–10] In addition to basal ocular blood flow, retinal vascular reactivity to flicker stimuli,[11,12] and gas provocation[10,13–15] was shown to decrease in chronic smokers. Thus, most data regarding effects of regular smoking on the ocular circulation and retinal vascular reactivity in smokers without diabetes are available. However, few studies have examined the effects of long-term smoking on the ocular circulation in patients with diabetes.

The relationship between chronic smoking per se and the retinal circulation in patients with diabetes is unclear. Using a retinal laser Doppler velocimetry (LDV) system, we reported that the retinal blood flow (RBF) decreases in patients with type 2 diabetes without retinopathy and with mild retinopathy.[16] Although the role of smoking in diabetic microangiopathy, particular retinopathy, has not been clearly established,[17–23] impaired retinal microcirculation caused by cigarette smoking may be implicated in the pathogenesis of diabetic retinopathy (DR). Herein, we examined the effects of chronic smoking on the retinal microcirculation in patients with type 2 diabetes with early-stage of retinopathy.

Methods

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 74 consecutive native Japanese patients (42 men, 32 women; age, mean ± SD, 59.0 ± 10.2 years) with type 2 diabetes mellitus diagnosed according to the criteria of the American Diabetes Association.[24] 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.[25] 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.[26]

The urinary 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 less than ACR less than 300 mg/g

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creatinine; stage III (macroalbuminuria), 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 m^2. The serum creatinine was measured within 4 hours of fasting venous blood collection using a Hitachi 747 biochemistry 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,[29] 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 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 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 (NPDR; levels 21–37), moderate-to-severe NPDR (levels 43–53), or proliferative DR (levels 60–65).[31] Patients with moderate-to-severe NPDR and PDR and clinically relevant macular edema were excluded. The ophthalmologic exclusion criteria included a previous intraocular surgery, history of laser photocoagulation, moderate-to-severe cataract, and moderate-to-high refractive error (> ±3.0 diopters). The eye with the 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 (V_{mean}) 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 = V_{mean} × area, where V_{mean} is calculated as V_{mean} = 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 (MABP) 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(MABP) − IOP.[16] 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 Poiseuillean parabolic model of V distribution across the arterial lumen according to the formula: WSR = 8 × V_{mean}/D.[33]

Statistical Analysis

All values are expressed as the mean ± SD. Comparisons between groups were made using 1-way ANOVA (for continuous variables) and the χ^2 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, HbA_{1C}, 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.[36] 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, HbA_{1c}, 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-smoked 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

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

Variable	Never Smoked, $n = 35$	Past Smoker, $n = 20$	Current Smoker, $n = 19$	*P* Value
Mean age, years	59.8 ± 11.0	60.5 ± 10.2	56.1 ± 8.5	0.34
Men/women	13/22	16/4	13/6	0.004
HbA_{1c}, %	6.9 ± 0.8	7.0 ± 0.9	7.2 ± 1.0	0.48
Duration of diabetes, y	9.2 ± 7.9	11.1 ± 7.1	10.8 ± 8.3	0.63
Plasma glucose, mg/dL	146.1 ± 39.1	153.9 ± 41.1*	181.5 ± 87.6*	<0.0001
BMI	26.0 ± 5.8	26.1 ± 5.9	26.1 ± 4.9	0.99
Systolic BP, mm Hg	135.2 ± 17.7	130.5 ± 10.6	124.7 ± 17.4	0.07
Diastolic BP, mm Hg	75.7 ± 10.5	75.2 ± 10.1	74.0 ± 9.5	0.84
Mean BP, mm Hg	95.6 ± 11.1	93.6 ± 8.6	90.9 ± 11.4	0.30
Heart rate, beats/min	70.5 ± 11.4	69.5 ± 11.2	72.7 ± 9.4	0.64
IOP, mm Hg	14.4 ± 3.0	14.2 ± 3.1	14.9 ± 2.6	0.70
OPP, mm Hg	49.3 ± 7.4	48.2 ± 6.1	45.6 ± 8.3	0.51
Total cholesterol, mg/dL	174.5 ± 23.9	173.8 ± 23.6	177.6 ± 23.6	0.86
Triglycerides, mg/dL	127.1 ± 55.8	99.4 ± 54.6**	149.3 ± 55.9	0.03
HDL, mg/dL	52.6 ± 10.9	51.6 ± 13.5	48.9 ± 10.9	0.52
LDL, mg/dL	103.3 ± 23.0	105.8 ± 31.0	110.3 ± 22.1	0.50
Blood urea nitrogen, mg/dL	14.5 ± 3.8	14.6 ± 3.7	14.1 ± 3.3	0.90
Creatinine, mg/dL	0.63 ± 0.13	0.73 ± 0.12*	0.64 ± 0.15	0.04
eGFR, mL/min/1.73 m^2	86.1 ± 17.1	82.4 ± 13.4	94.9 ± 20.6	0.70
Insulin use, no. (%)	6 (17)	8 (40)	3 (16)	0.11
Oral antidiabetic drug, no. (%)	29 (89)	16 (80)	16 (84)	0.68
Hypertension, no. (%)	16 (46)	10 (50)	6 (32)	0.47
Dyslipidemia, no. (%)	23 (66)	8 (40)	14 (74)	0.07
Medications				
β-antagonist, no. (%)	1 (3)	0 (0)	0 (0)	0.57
Angiotensin-converting-enzyme inhibitor, no. (%)	2 (6)	1 (5)	0 (0)	0.58
Angiotensin II type 1 receptor blocker, no. (%)	10 (29)	4 (20)	2 (11)	0.30
Calcium channel antagonist, no. (%)	6 (17)	6 (30)	2 (11)	0.28
Diuretic, no. (%)	0 (0)	1 (5)	0 (0)	0.25
Statin, no. (%)	23 (66)	7 (35)	12 (63)	0.07

P values were obtained by 1-way ANOVA or the χ^2 test.
* $P < 0.05$ compared with the group that never smoked.
** $P < 0.05$ compared with the group of current smoker.

analysis were the diastolic BP, mean BP, and CRE (Table 3). Because of the high correlation between the mean BP and diastolic BP ($r = 0.896$; data not shown), the mean BP was selected instead of the diastolic BP. In addition to smoking status, the CRE and mean BP were included in the multiple regression analysis. The multiple regression analysis showed that current smoking was a significant and independent variable associated with the RBF in these patients in addition to the serum CRE (Table 4).

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.[37–40] Moreover, we previously reported that the RBF with decreased blood V was significantly lower in patients with type 2 diabetes with early-phase DR compared with in nondiabetic control subjects.[16] Taken together, we speculated that alterations in the retinal microcirculation caused by chronic smoking may be implicated in the pathogenesis of DR.

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.[6] In keeping with that, a study using the blue-field entoptic technique showed increased leukocyte V in the perimacular region in chronic smokers.[5] These results have been confirmed by another report showing increased blood flow V in the ophthalmic artery and lateral posterior ciliary artery in smokers compared with nonsmoking subjects.[7] In contrast, the blood V in the ophthalmic artery,[8] central retinal artery, and posterior ciliary artery[9] has been

Table 2. Retinal Circulatory Parameters in the Study Groups

Variable	Never Smoked, $n = 35$	Past Smoker, $n = 20$	Current Smoker, $n = 19$	*P* Value
Vessel diameter, μm	113.7 ± 8.6	110.6 ± 12.6	112.0 ± 11.9	0.57
Blood velocity, mm/s	37.8 ± 9.0	33.3 ± 8.1	29.6 ± 6.8*	0.003
RBF, μL/min	11.6 ± 3.1	9.8 ± 3.4	8.9 ± 2.9*	0.009
RVR, mm Hg min/μL	4.6 ± 1.4	5.5 ± 2.1	5.8 ± 2.8	0.07
WSR, s^{-1}	1337 ± 340	1216 ± 299	1065 ± 249*	0.01

* $P < 0.05$ compared with the group that never smoked.

Table 3. Characteristics of Patients With Type 2 Diabetes With Early-Stage DR and Correlations Between RBF and Systemic and Ocular Parameters

Variable	Patients, $n = 74$	r	P Value
RBF, μL/min	10.4 ± 3.3		
Age, y	59.0 ± 10.2	−0.017	0.88
HbA_{1C}, %	7.0 ± 0.9	0.078	0.51
Duration of diabetes, y	10.1 ± 7.7	−0.004	0.97
Plasma glucose, mg/dL	156.7 ± 58.1	−0.081	0.49
BMI	26.1 ± 5.5	0.064	0.59
Systolic BP, mm Hg	131.2 ± 16.4	0.120	0.31
Diastolic BP, mm Hg	75.3 ± 10.0	0.153	0.19
Mean BP, mm Hg	93.8 ± 10.6	0.159	0.17
Heart rate, beats/min	70.8 ± 10.8	0.070	0.55
IOP, mm Hg	14.5 ± 2.9	0.018	0.88
OPP, mm Hg	48.0 ± 7.4	0.144	0.22
LDL, mg/dL	105.7 ± 23.6	−0.034	0.77
Creatinine, mg/dL	0.66 ± 0.14	−0.327	0.004
eGFR, mL/min/1.73m^2	87.3 ± 17.6	−0.150	0.21

Table 4. Multiple Regression Analysis of RBF in 74 Patients With Type 2 Diabetes

Variable	Regression Coefficient	P Value
Current smoker	−0.283	0.01
Creatinine	−0.337	0.003
Mean BP	0.062	0.59

$r^2 = 0.196$, $P = 0.002$.

reported to be lower in smokers than in nonsmokers. Moreover, Morgado et al.[10] 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[11,12] and gas provocation[10,13–15] 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 (Table 2). 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),[41] in various vessels.[42–44] 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.[45] Because NO is a well-known powerful vasodilator in the retinal vessels,[34] 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). Indeed, conscious rats exposed to high-nicotine cigarette smoke showed an enhanced and significant increase in total peripheral resistance.[46] We speculate that chronic smoking may reduce the RBF via vasoconstriction of the resistance vessels, which are smaller than the measured large artery (first branch), in patients with type 2 diabetes.

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,[47] in human retinal microvascular endothelial cells.[48] Experimentally reduced blood flow by surgical manipulation, which decreased shear stress, also increased monocytic adhesion to the surface endothelium of the common carotid arteries in rabbits.[49] 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.[50] 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 vasoconstrictor, 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[51] and nonsmoking rats,[52] 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.[53,54] 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.[55] Moreover, ET-1 production by human umbilical endothelial cells treated with serum from nonsmokers, light smokers, and heavy smokers did not differ between the three groups.[56] Thus, the effect of chronic smoking on plasma ET-1 levels is inconclusive. Recently, the expressions of mRNA and protein for endothelin type A (ET_A) and endothelin type B (ET_B) receptors in the coronary arteries of smoked-exposed rats were higher than that of animals exposed to fresh air.[57] It also has been reported that blockade of ET_A and ET_B receptors attenuates augmented vascular contractility to phenylephrine in aortas and carotid arteries of rats exposed to cigarette smoke.[52] Indeed, a previous study showed the expression of ET_A and ET_B receptor mRNA and protein 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 type 2 diabetes.

Our observation that chronic smoking reduced retinal blood V 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.[13] However, acute cigarette smoking induced a marked decrease in RBF, more pronounced in diabetics than in healthy controls.[10] Moreover, insulin upregulates the ET_A receptor in cultured rat aortic smooth muscle cells.[59] 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.[60] 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 the canine basilar artery and NO synthesis in cultured human endothelial cells,[67] suggesting that nicotine may play a critical role in altered vasoreactivity. Although nicotine tested in a glaucoma group showed significant changes in blood V in the ophthalmic artery compared with the placebo-tested group,[68] it is uncertain whether nicotine can affect retinal vasomotion in patients with type 2 diabetes. Additional investigations are needed to clarify the molecular mechanisms underlying the deleterious effect of cigarette smoking on the retinal circulation. Second, it remains unclear whether there is a linear dose effect of smoking on the retinal circulatory parameters. In the current study, no significant association was seen between the RBF and pack-years in the current-smoker patients with type 2 diabetes (Pearson's coefficient of correlation, $r = -0.13$, $P = 0.60$; data not shown). Indeed, an experimental study showed that incubation of 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.[56] 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 circulatory 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 circulatory 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 constricts 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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