Relationship between the Parameters of Retinal Circulation Measured by Laser Doppler Velocimetry and a Marker of Early Systemic Atherosclerosis

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Purpose. To examine the relation between intima–media thickness (IMT) in the common carotid artery, which is generally recognized as a marker of early atherosclerosis, and retinal circulatory parameters.

Methods. The vessel diameter and blood velocity of retinal arterioles were measured with laser Doppler velocimetry (LDV) in 33 patients with coronary artery disease (CAD), because they were thought to have general atherosclerosis. The retinal blood flow (RBF) and wall shear rate (WSR), an index of wall shear stress in retinal vessels, were calculated from the two parameters. The carotid IMT was evaluated with B-mode ultrasonography.

Results. The patients were divided into two groups: those with an IMT >0.70 mm and those with an IMT ≤0.70 mm. The blood velocity, WSR, and upstroke time in the retinal arterioles were significantly higher in the group with an IMT >0.70 mm compared with the group with an IMT ≤0.70 mm. There were no significant differences in vessel diameter and RBF between the groups. Positive correlations were found between the carotid IMT and blood velocity ($r = 0.51$, $P = 0.002$) and WSR ($r = 0.60$, $P = 0.0001$). Multiple regression analysis showed that the retinal WSR and blood velocity correlated independently with the IMT in patients with CAD.

Conclusions. The present findings from this pilot study suggest that the retinal circulatory parameters measured by LDV may be associated with systemic atherosclerosis. These results support recent studies showing an association between retinal microvascular changes and cardiovascular disease. (*Invest Ophthalmol Vis Sci.* 2005;46:720–725) DOI:10.1167/iovs.04-0906

Atherosclerosis remains the leading cause of morbidity and mortality in the Western world. As the prevalence of atherosclerosis increases worldwide, there is a pressing need for investigators to refine and evaluate noninvasive techniques to ensure reliable detection of atherosclerosis during the long presymptomatic phase of the disease. Atherosclerosis is a systemic process that involves several arterial beds. Abnormalities of the retinal vasculature may reflect the degree of microvascular damage due to hypertension, arteriolosclerosis, or both, which, in turn, may influence the type and severity of cerebrovascular and cardiovascular complications.[1,2]

We recently demonstrated that a retinal laser Doppler velocimetry (LDV) system is a reliable, noninvasive, and useful tool to evaluate the retinal circulation in humans.[3,4] The system we have recently described enables the simultaneous measurement of vessel diameter and blood velocity and calculates the absolute retinal blood flow (RBF) and wall shear rate (WSR). Using LDV, we have reported that flow-induced vasodilation is involved in the increased RBF during hypoxia by measuring the feline retinal WSR.[5] The present study was designed to determine the association of the retinal circulatory parameters with *systemic atherosclerosis* in humans. Therefore, the purpose of this pilot study was to examine the relation between the intima–media thickness (IMT) in the common carotid artery, which is generally recognized as a marker of early atherosclerosis,[6] and retinal circulatory parameters in patients with coronary artery disease (CAD), because they were thought to have general atherosclerosis.

Methods

Subjects

Thirty-three patients (24 men and 9 women, aged 47–79 years; mean ± SD, 61.0 ± 9.6) with CAD participated in this study. All were inpatients at the Asahikawa City Hospital (Asahikawa, Japan), and each provided written, informed consent before enrollment in the study, after having received a detailed explanation of the study design and protocol. The procedures conformed with the tenets of the Declaration of Helsinki. This study was conducted in accordance with the guidelines approved by the ethics committee at the hospital. The inclusion criteria were CAD, confirmed by coronary arteriography (50% stenosis in ≥1 coronary artery), a documented history of myocardial infarction within 5 years, or both. These patients had undergone diagnostic catheterization for the evaluation of chest pain syndromes. We excluded patients with congenital heart failure, remote prior anterior myocardial infarction or any infarction in another area within the previous 3 months. In addition, we excluded the patients who were considered to have diabetes mellitus if they were being treated with insulin or oral hypoglycemic agents or if fasting blood glucose exceeded 140 mg/dL, because the retinal circulation is impaired in patients with diabetes.[7] All subjects had corrected visual acuity better than 20/40, clear media, and no history of ocular disease or therapy.

Study Design

The temperature in the examination room was maintained between 22°C and 24°C. The subjects were asked to abstain from drinking coffee and smoking for at least 2 hours before the test. Each subject rested for 10 to 15 minutes in a quiet room before the test began.

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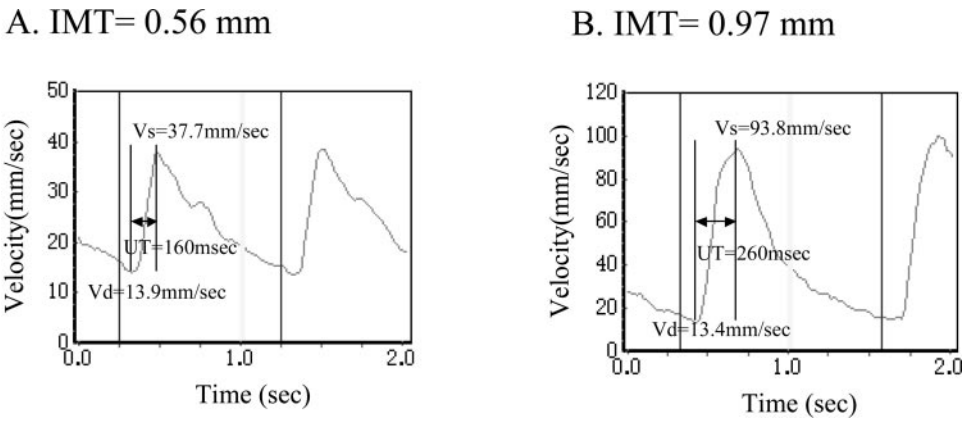

FIGURE 1. Representative data of the analysis of the retinal velocity profile in the group with an IMT ≤0.70 mm (**A**, a patient with very little atherosclerosis) and the group with an IMT >0.70 mm (**B**, a patient with much atherosclerosis). The UT was defined as the time from the minimum to the maximum retinal blood velocity. From the velocity profile analysis, V_s, V_d, and UT are calculated as 37.7 mm/sec, 13.9 mm/sec, and 160 ms, respectively, in the patient in (**A**), and 93.8 mm/sec, 13.4 mm/sec, and 260 ms in the patient in (**B**), respectively.

The systolic, diastolic, and mean arterial blood pressure and heart rate were measured by electronic sphygmomanometer (EP-88Si; Colin, Tokyo, Japan). Intraocular pressure (IOP) was monitored by applanation tonometry (Haag Streit, Bern, Switzerland). The axial length of each eye was measured by A mode ultrasound (OcuScan; Alcon Surgical, Irvine, CA) to compute the intraocular light-scattering geometry for the laser Doppler measurement. The pupils were dilated with a combination of 0.5% tropicamide and 1% phenylephrine eye drops.

A blood sample was obtained on the morning of the examination, after a 14-hour overnight fast, to measure the serum lipid profile and other biochemical parameters. Serum total cholesterol and triglyceride concentrations were measured enzymatically, and the serum high-density lipoprotein (HDL) cholesterol concentration was assayed by the heparin-Ca^{2+}-Ni^{2+} precipitation method.

Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or the use of antihypertensive drugs. Subjects with a plasma total cholesterol level >220 mg/dL, those with plasma low-density lipoprotein (LDL) cholesterol level >130 mg/dL, or those receiving cholesterol-lowering therapy were classified as having hypercholesterolemia.

RBF Measurement

In the present study, a retinal LDV system (Laser Blood Flowmeter, model CLBF 100; Canon, Tokyo, Japan) was used to estimate the blood flow in the major temporal retinal artery and adjacent vein.[3,4] A retinal LDV system allows noninvasive measurement of the absolute values of the red blood cells (RBCs) flowing in the center line of the vessel, based on the bidirectional LDV.[8] A probing red laser light (wavelength, 675 nm) is emitted by a laser source in a measuring head similar to that of a fundus camera. The red Doppler-shifted light scattered from the flowing RBCs in the retinal artery is detected simultaneously in two directions separated by a fixed angle. The signals from the two photomultiplier tube detectors undergo computer-controlled spectrum analysis, and sequential measurements of the centerline velocity of the retinal blood cells over a 2-second period are performed automatically. Therefore, we obtained the retinal velocity profile and calculated the systolic and diastolic blood velocity (V_s and V_d, respectively; Fig. 1).

The LDV also contains a system that measures vessel diameter and a vessel tracking system. The green stripe (wavelength, 544 nm) is oriented perpendicular to the axis of the vessel. During the session, a linear imaging sensor takes 15 profiles of the target vessel, which is illuminated by a green laser beam. The diameter of the retinal artery (D) is 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's edge.[9] The D is measured before and after each of two velocity measurements. The result is compensated for by the axial length of the eye, which is input into a computer, and the ocular refractive error, which is measured by the LDV system. The vessel images are also used for autovessel tracking through a beam-steering galvanometer system, which stabilizes the center of the green tracking stripe on the center of the vessel and locks the red laser onto the target vessel.

Laser Doppler measurements were obtained from a temporal retinal artery in one eye of each subject. The arteries chosen for measurement had relatively straight segments that were sufficiently distant from adjacent vessels. Measurement sites were generally one disc diameter from the disc margin in the first bifurcation. All steps throughout the observation of the patient's fundus are virtually the same.

Calculations

As previously described, the RBF was calculated as RBF = $2 \times V_{mean} \times$ area, where V_{mean} is the time average of the center line blood speed during the cardiac cycle, and area is the cross-sectional area of the retinal artery at the laser Doppler measurement site.[10] The area is calculated from the D, assuming a circular cross-section. The factor of 2 in the formula for the blood flow arises from the assumption of Poiseuille flow.[11] The WSR was not directly measured in this model but can be calculated by use of a Poiseuillean parabolic model of velocity distribution across the arterial lumen according to the formula $WSR_{mean} = 8 \times V_{mean}/D$.[12] We calculated the WSR_{max} from the V_s and D and WSR_{min} by the V_d and D.

After the measurement was completed, a velocity profile analysis of the retinal velocity profile was performed. We defined the upstroke time (UT) as the time from the minimum to the maximum retinal blood velocity (Fig. 1).

Measurement of IMT of the Carotid Artery

The carotid IMT was evaluated with high-resolution B-mode ultrasonography. The IMT measurement was performed on the same day as the RBF measurement. Ultrasound measurements of the IMT of the common carotid artery were performed by an examiner who was unaware of the subjects' clinical backgrounds. The IMT of the carotid artery was measured from high-resolution, two-dimensional ultrasound images obtained by an ultrasound machine (SSA-270A; Toshiba, Tokyo, Japan) with a 7.5-MHz linear-array transducer. The subject reclined on the examination table for 15 minutes before the initial carotid ultrasound scanning was performed. The IMT measurement of the carotid artery was performed according to the method of Salonen et al.,[13] as described previously in a quiet, temperature-controlled room. After the bifurcation of the common carotid artery was confirmed, the IMT was measured on the B-mode screen with electric calipers to within 10 mm proximal to the bifurcation. We evaluated the retinal circulation of the right eye and the IMT of the right common carotid artery in all patients.

Statistical Analysis

All data in the text, tables, and figures are expressed as the mean ± SD. Differences in continuous variables between the two groups were analyzed by Student's unpaired *t*-test. Differences in categorical variables were analyzed by the Fisher exact tests. The Pearson correlation

TABLE 1. Clinical and Metabolic Characteristics in Both Groups

	Low IMT Group	High IMT Group	*P*
Patients, *n* (M:F)	16 (11:5)	17 (13:4)	0.62
IMT (mm)	0.62 ± 0.06	0.82 ± 0.09	—
Age (y)	58.9 ± 10.9	63.0 ± 8.1	0.11
Systolic BP (mm Hg)	129.0 ± 16.0	124.4 ± 15.5	0.41
Diastolic BP (mmHg)	80.8 ± 15.4	72.6 ± 8.8	0.07
Mean BP (mmHg)	96.8 ± 14.8	89.9 ± 9.5	0.12
Heart rate (beats/min)	75.8 ± 13.6	69.9 ± 12.2	0.19
Total cholesterol (mg/dL)	192.1 ± 36.2	176.2 ± 31.2	0.18
Triglycerides (mg/dL)	117.3 ± 48.3	119.0 ± 49.6	0.91
HDL (mg/dL)	46.7 ± 11.2	28.6 ± 11.0	0.046*
LDL (mg/dL)	123.8 ± 29.0	115.8 ± 24.8	0.4
White blood cell count (%)	6.39 ± 1.36	6.23 ± 1.60	0.77
Red blood cell count (%)	4.29 ± 0.59	4.38 ± 0.59	0.66
Hemoglobin (%)	13.3 ± 2.1	13.1 ± 2.4	0.86
Hematocrit (%)	38.9 ± 5.3	39.0 ± 1.5	0.97
Hypertension, *n,* (%)	10 (63)	12 (71)	0.62
Hypercholesterolemia, *n,* (%)	11 (69)	12 (71)	0.91
Current smoking, *n,* (%)	12 (75)	15 (88)	0.14
Medications			
β-Antagonist, *n,* (%)	2 (13)	2 (12)	—
ACE inhibitor, *n,* (%)	6 (38)	4 (24)	0.71
ATI inhibitor, *n,* (%)	2 (13)	2 (12)	—
Calcium channel antagonist, *n,* (%)	10 (63)	11 (65)	—
Diuretic, *n,* (%)	1 (6)	0 (0)	—
Statins, *n,* (%)	7 (44)	12 (71)	0.17
Nitrates, *n,* (%)	11 (69)	14 (82)	0.44

Data are expressed as means ± SD.
* Significant ($P < 0.05$).

analysis was used to study the relations between the IMT and retinal circulatory parameters. Standardized regression coefficients from multiple regression analysis of the IMT in relation to various factors were analyzed. $P < 0.05$ was considered statistically significant.

RESULTS

Comparison with Parameters Obtained from the Two IMT Groups

After the IMT was measured, we divided the patients into two groups based on an IMT >0.70 mm (severe atherosclerosis group) or an IMT of ≤0.70 mm (mild atherosclerosis group; Table 1) because the median IMT in our population was 0.70 mm.

Clinical Characteristics

Ten (30%) patients were taking angiotensin-converting enzyme inhibitors, 4 (12%) angiotensin-one receptor antagonist, 4 (12%) beta antagonists, 21 (64%) calcium antagonists, 1 (3%) a diuretic, and 19 (58%) 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). Twenty-five (76%) patients regularly took nitroglycerin. There were no significant differences in the medications between the two IMT groups (Table 1).

Systemic Parameters

The group-averaged values of age, systemic arterial blood pressure, and serum cholesterol level are shown in Table 1. The only significant difference between the low and high IMT groups ($P = 0.046$) was in the HDL level. Other parameters were not significantly different between the two groups. The prevalences of hypertension, hyperlipidemia, and current smoking are presented in Table 1. None of the differences reached statistical significance.

Retinal Parameters

The averaged coefficients of variation of D, V_{mean}, and RBF in a retinal arteriole of five baseline measurements obtained from all patients in this study were 4.7% ± 0.9%, 12.1% ± 1.1%, and 16.3% ± 1.5%, respectively. The group-averaged V_s, V_{mean}, WSR_{max}, WSR_{mean}, and UT in retinal arterioles were significantly higher in the group with an IMT >0.70 mm than in the group with an IMT ≤0.70 mm (Table 2). In contrast, there were no significant differences in V_d, RBF, and WSR_{min} between the groups.

Relation between the IMT of the Common Carotid Artery and Retinal Circulatory Parameters

Table 3 shows the results of the a Pearson correlation analysis between the IMT and retinal circulatory parameters. As shown in Figure 2, the IMT was positively and strongly related with the retinal WSR_{mean} (Fig. 2A) and V_{mean} (Fig. 2B) by this analysis. In contrast, neither the IMT nor the retinal circulatory

TABLE 2. Retinal Circulatory Parameters in Both Groups

	Low-IMT Group	High-IMT Group	*P*
Diameter (μm)	107.4 ± 13.6	101.4 ± 15.1	0.24
V_s (mm/s)	55.1 ± 11.8	68.7 ± 15.4	0.008*
V_d (mm/s)	17.5 ± 4.2	19.5 ± 6.8	0.33
V_{mean} (mm/s)	32.2 ± 6.8	39.5 ± 6.6	0.004*
RBF (μL/min)	9.0 ± 3.1	9.8 ± 3.5	0.49
WSR_{max} (1/s)	2283 ± 453	2935 ± 782	0.003*
WSR_{min} (1/s)	727 ± 175	830 ± 329	0.17
WSR_{mean} (1/s)	1219 ± 261	1604 ± 349	0.001*
UT (ms)	169.5 ± 34.7	195.6 ± 33.5	0.036*

Data are expressed as the mean ± SD.
* Significant ($P < 0.05$).

TABLE 3. Pearson's Correlation between the IMT and Retinal Circulatory Parameters in All Patients

	r	*P*
Diameter (μm)	−0.32	0.07
V_s (mm/s)	0.51	0.002*
V_d (mm/s)	0.14	0.44
V_{mean} (mm/s)	0.51	0.002*
RBF (μL/min)	0.04	0.81
WSR_{max} (1/s)	0.59	0.0002*
WSR_{min} (1/s)	0.14	0.44
WSR_{mean} (1/s)	0.60	0.0001*
UT (ms)	0.42	0.02*

Data are expressed as means ± SD
* Significant ($P < 0.05$).

parameters were significantly correlated with age, systemic blood pressure, heart rate, blood cell counts, or serum cholesterol level in our patients, by this analysis (data not shown).

Multiple regression analysis was performed to investigate whether the WSR_{mean} was an independent variable related to the IMT. Table 4 shows the multiple regression analysis of IMT in relation to retinal WSR_{mean}, hematocrit, age, mean blood pressure, serum total cholesterol, serum HDL cholesterol, and serum triglyceride. This analysis confirmed that the retinal WSR_{mean} was an independent variable related to IMT (Fig. 2A; Table 3).

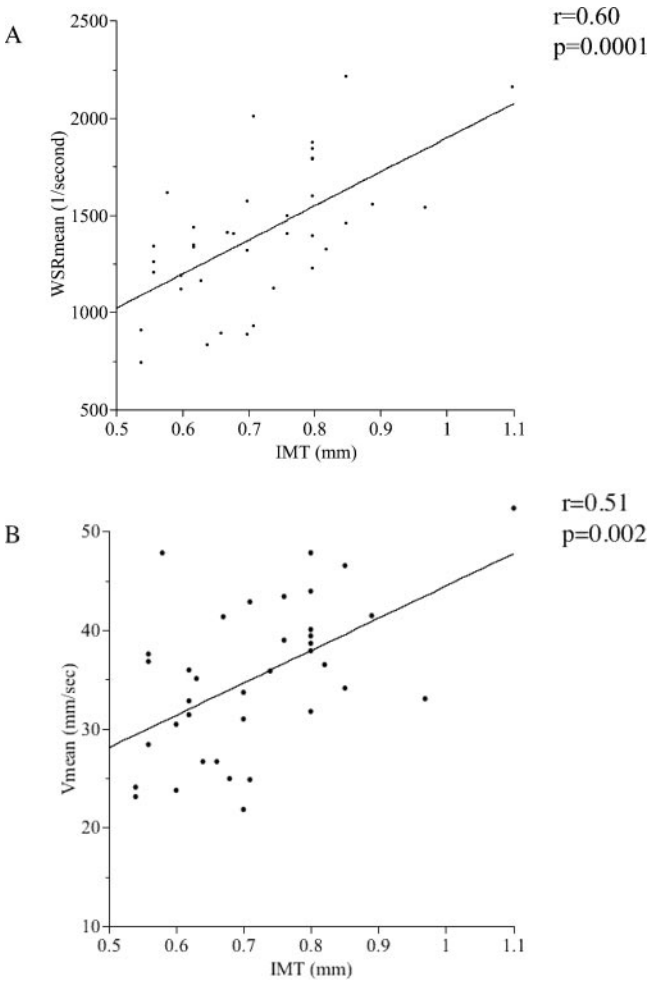

FIGURE 2. The correlation between both the WSR_{mean} (**A**) and V_{mean} (**B**) and the IMT in all patients.

TABLE 4. Standardized Regression Coefficients from Multiple Linear Regression Analysis of IMT in Relation to Independent Variables in All Patients

Independent Variable	Standardized Coefficient	*P*
WSR_{mean}	0.657	0.0001*
Hematocrit	0.228	0.14
Age	0.225	0.17
HDL	−0.227	0.21
MAP	−0.180	0.24
Triglyceride	−0.171	0.38
Total cholesterol	0.099	0.58

$r^2 = 0.563$.
* Significant ($P < 0.05$).

DISCUSSION

The results of this pilot study demonstrated for the first time that the retinal WSR, blood velocity, and UT were significantly higher in the group with an IMT >0.70 mm compared with those in the group with an IMT ≤0.70 mm in patients with CAD and that these retinal circulatory parameters were positively correlated with the common carotid IMT independently of age, hematocrit, systemic blood pressure, and serum cholesterol level. Because the IMT is an indicator of the extent of systemic atherosclerosis, these results suggest that these retinal circulatory parameters may be associated with generalized atherosclerosis. Abnormalities of the retinal vasculature may reflect the type and severity of cerebrovascular and cardiovascular complications.[1,2] In addition, a recent population-based study revealed that retinal microvascular abnormalities predicted a 3-year incidence of coronary heart disease[14] and was associated with 10-year cardiovascular mortality.[15] However, there have been few reports that investigated the relation between this retinal arteriolar change and systemic atherosclerosis. By measuring the diameter of the retinal arterioles, Klein et al.[16] suggested that retinal arteriolar abnormalities are distinct from atherosclerosis. In the present study, there were no significant differences in the group-averaged values of the retinal vessel diameter between the two groups, suggesting that the retinal vessel diameters may be a poor surrogate of early changes of atherosclerosis. The present results seem to be consistent with their findings showing that retinal vessel diameters do not appear to be related to measures of atherosclerosis. Although the number of subjects in their study was much greater than in the present study, the major differences between their study and ours were the methods used to evaluate the retinal microvascular abnormalities. Whereas Klein et al. evaluated only the vessel diameter, we measured diameter, blood velocity, RBF, WSR, and UT, with the LDV system. A linear regression model showed a significant correlations between carotid IMT and some retinal circulatory parameters: V_s, V_{mean}, WSR_{max}, WSR_{mean}, and UT (Table 3). Therefore, we believe that the LDV system may be an appropriate instrument for detecting the early changes in atherosclerosis because of the simultaneous and noninvasive measurement of both the vessel diameter and the blood velocity.

In the present study, we compared, for the first time, the retinal WSR and the carotid IMT and found a positive correlation between the two. In the carotid artery, Gnasso et al.[17] compared the IMT and wall shear stress in the common carotid arteries in healthy male subjects using high-resolution echo Doppler and reported that peak shear stress was inversely related to the IMT. Our results obtained from the retinal arterioles are the opposite of their results from the carotid artery. These findings in primarily elastic arteries are not necessarily

representative of the situation in purely muscular arteries because of the differences in function and structure between the elastic and muscular arteries, perhaps because of the differences between the characteristics of the arterioles in the retina and the large carotid artery. Further studies are needed to document variations in wall shear stress in different vascular beds at rest and during exercise, to gain a better understanding of the early atherosclerotic process.

The present results showing that the WSR_{mean} was independently and positively associated with the carotid IMT in patients with CAD (Fig. 2) indicate that the increased retinal WSR may be associated with systemic atherosclerosis. However, the IMT should be evaluated carefully, because it is affected by many systemic parameters, such as blood pressure and age, that were reported to be associated with the IMT. In our small analysis, we did not observe significant differences in systemic blood pressure, age, the presence of hypertension and hypercholesterolemia, and the cholesterol level, with the exception of HDL cholesterol (Table 1).

In the present study, we obtained the UT of the retinal velocity wave form (Fig. 1) and found that the UT significantly increased in the group with an IMT >0.70 mm compared with the group with an IMT ≤ 0.70 mm or less (Table 2). Although the exact interpretation of the increased UT in the retinal arterial velocity profile is still unclear, the present results indicate that the UT obtained from the retinal velocity profile also may be associated with systemic atherosclerosis.

In the coronary arteries, atherosclerosis is associated with progressive impairment of endothelial function.[18,19] Wall shear stress is an important determination of the release of vasoactive compounds from endothelial cells. Several vasoactive molecules stimulate the expression of adhesion molecules and chemokines involved in intima–media thickening. Endothelium-derived nitric oxide especially is thought to be necessary to maintain an adequate vascular tone. In vitro and in vivo experiments have demonstrated that vessels tend to maintain constant shear stress in response to flow changes, which is called flow-mediated regulation.[20–22] Flow-mediated brachial-artery reactivity, in which endothelium-derived nitric oxide plays an important role, is impaired in persons with overt atherosclerosis and in asymptomatic persons with risk factors for coronary disease.[23–25] Although we did not examine the endothelial function in our patients, our findings suggest that the correlation between the WSR in the retinal arterioles and the IMT in the common carotid artery may be associated with impairment of the endothelial function in the retinal arterioles in patients with CAD. This speculation is supported by the study by Klein et al.[16] that showed associations between retinal changes with markers of endothelial dysfunction (von Willebrand factor and factor VIII). Further investigation is needed to evaluate the endothelial function in retinal arterioles to test this hypothesis.

Despite the widespread interest in WSR and wall shear stress, relatively little is known about the values of these mechanical entities in vivo, probably because the reliable assessment of shear stress is quite difficult. The first step in estimating wall shear stress is measurement of velocity. Because retinal circulation is a parabolic and lamellar flow,[11] it is possible to estimate the retinal WSR from vessel diameter and blood velocity.[5] In addition, we observed an increased WSR in hypertensive subjects (Nagaoka T, unpublished observation, 2004) in the recent study. We therefore believe that the noninvasive evaluation of the retinal WSR with LDV may be useful to obtain clinically significant information in patients with cardiovascular disorders.

The present study had some limitations. First, we could not actually measure the wall shear stress in retinal vessels because we did not measure the blood viscosity. Because the relation between the hematocrit and plasma viscosity is linear when the hematocrit is in normal range (~45%),[26] the present finding that there were no differences in the hematocrit between the groups (Table 1) and the relationship between the carotid IMT and WSR was not dependent on hematocrit (Table 4) suggest that the viscosity in our patients with CAD may have little influence on the present results. Second, we could not exclude the effect of medication on the retinal circulatory parameters. All patients were taking a variety of medications. There is a possibility that some drugs may have affected the measurement of the retinal circulatory parameters. However, there was no significant difference in medications between the two IMT groups, and the presence of medications did not affect the present results concerning the relation between the IMT and retinal parameters (data not shown). Third, there is a possibility that selection bias is associated with our results because we chose a high-risk population without diabetes for inclusion in the study. The small sample size may explain why the carotid IMT is not related to many traditional risk factors associated with atherosclerosis (Table 1) and some retinal parameters: V_d, RBF, and WSR_{min} (Table 2). Further clinical study with a large sample size is needed to examine whether the relationship between retinal circulatory parameters and IMT may be appropriate for patients with other diseases, such as diabetes mellitus and hypertension.

In conclusion, the results of this pilot study demonstrate for the first time that the retinal circulatory parameters (blood velocity and WSR) correlate positively with the carotid IMT, which is generally recognized as a marker of early atherosclerosis. The results also suggest that noninvasive measurement of retinal circulatory parameters using LDV may be useful for the evaluation of systemic atherosclerosis. Further prospective study is needed to assess whether this noninvasive measurements of retinal circulation has predictive power with respect to subsequent cardiovascular events.

References

1. Michelson EL, Morganroth J, Nichols CW, MacVaugh H. Retinal arteriolar changes as an indicator of coronary artery disease. *Arch Intern Med*. 1979;139:1139–1141.
2. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet*. 2001;358:1134–1140.
3. Yoshida A, Feke GT, Mori F, et al. Reproducibility and clinical application of a newly developed stabilized retinal laser Doppler instrument. *Am J Ophthalmol*. 2003;135:356–361.
4. Nagaoka T, Mori F, Yoshida A. Retinal artery response to acute systemic blood pressure increase during cold pressor test in humans. *Invest Ophthalmol Vis Sci*. 2002;43:1941–1945.
5. Nagaoka T, Sakamoto T, Mori F, et al. The effect of nitric oxide on retinal blood flow during hypoxia in cats. *Invest Ophthalmol Vis Sci*. 2002;43:3037–3044.
6. Simons PC, Algra A, Bots ML, et al. Common carotid intima-media thickness in patients with peripheral arterial disease or abdominal aortic aneurysm: the SMART study. Second Manifestations of Arterial Disease. *Atherosclerosis*. 1999;146:243–248.
7. Konno S, Feke GT, Yoshida A, et al. Retinal blood flow changes in type I diabetes: a long-term follow-up study. *Invest Ophthalmol Vis Sci*. 1996;37:1140–8.
8. Riva CE, Feke GT, Eberli B. Bidirectional LDV system for absolute measurement of blood speed in retinal vessels. *Appl Opt*. 1979;18:2301–2306.
9. Delori FC, Fitch KA, Feke GT, et al. Evaluation of micrometric and microdensitometric methods for measuring the width of retinal vessel images on fundus photographs. *Graefes Arch Clin Exp Ophthalmol*. 1988;226:393–399.
10. Feke GT, Tagawa H, Deupree DM, et al. Blood flow in the normal human retina. *Invest Ophthalmol Vis Sci*. 1989;30:58–65.
11. Feke GT, Riva CE. Laser Doppler measurements of blood velocity in human retinal vessels. *J Opt Soc Am*. 1978;68:526–531.

12. Cabel M, Smiesko V, Johnson PC. Attenuation of blood flow-induced dilation in arterioles after muscle contraction. *Am J Physiol*. 1994;266:H2114–H2121.
13. Salonen R, Haapanen A, Salonen JT. Measurement of intima-media thickness of common carotid arteries with high-resolution B-mode ultrasonography: inter- and intra-observer variability. *Ultrasound Med Biol*. 1991;17:225–230.
14. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of coronary heart disease in men and women: the Atherosclerosis Risk in Communities Study. *JAMA*. 2002;287:1153–1159.
15. Wong TY, Klein R, Nieto FJ, et al. Retinal microvascular abnormalities and 10-year cardiovascular mortality: a population-based case-control study. *Ophthalmology*. 2003;110:933–940.
16. Klein R, Sharrett AR, Klein BE, et al. Are retinal arteriolar abnormalities related to atherosclerosis?—The Atherosclerosis Risk in Communities Study. *Arterioscler Thromb Vasc Biol*. 2000;20:1644–1650.
17. Gnasso A, Carallo C, Irace C, et al. Association between wall shear stress and flow-mediated vasodilation in healthy men. *Atherosclerosis*. 2001;156:171–176.
18. Hambrecht R, Wolf A, Gielen S, et al. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med*. 2000;342:454–460.
19. Hashimoto M, Eto M, Akishita M, et al. Correlation between flow-mediated vasodilatation of the brachial artery and intima-media thickness in the carotid artery in men. *Arterioscler Thromb Vasc Biol*. 1999;19:2795–2800.
20. Koller A, Huang A, Sun D, et al. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ Res*. 1995;76:544–550.
21. Girerd X, London G, Boutouyrie P, et al. Remodeling of the radial artery in response to a chronic increase in shear stress. *Hypertension*. 1996;27:799–803.
22. Ben Driss A, Benessiano J, Poitevin P, et al. Arterial expansive remodeling induced by high flow rates. *Am J Physiol*. 1997;272:H851–H858.
23. Neunteufl T, Katzenschlager R, Hassan A, et al. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis*. 1997;129:111–118.
24. Yataco AR, Corretti MC, Gardner AW, et al. Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *Am J Cardiol*. 1999;83:754–758.
25. Celermajer DS, Sorensen KE, Bull C, et al. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24:1468–1474.
26. Whittaker S, Winton F. The apparent viscosity of blood flowing in the isolated hindlimb of the dog and its variation with corpuscular concentration. *J Physiol* (*Lond*). 1933;78:339–369.