von Willebrand Factor and Retinal Circulation in Early-Stage Retinopathy of Type 1 Diabetes

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OBJECTIVE — Although retinopathy is a common microvascular complication of type 1 diabetes, the mechanism for this complication is still unknown. Changes in retinal circulation have been noted before the development of overt retinal pathology. Because von Willebrand factor (vWF) is a marker for endothelial dysfunction and mediates platelet adhesion, we determined if there was an association between vWF and retinal circulation in the early stages of diabetic retinopathy.

RESEARCH DESIGN AND METHODS — Twenty subjects (aged 32.4 ± 7.8 years) with type 1 diabetes and minimal or no retinopathy were studied. The mean duration of diabetes was 4.7 ± 2.6 years. Data were collected at baseline and after 4 months of 1,800 IU vitamin E therapy or placebo. Retinal circulation was evaluated by video fluorescein angiography. Plasma vWF antigen levels were measured by enzyme-linked immunosorbent assay and fibrinogen by the Clauss method.

RESULTS — Retinal blood flow was negatively correlated with vWF levels ($r = -0.44$, $P = 0.008$), whereas retinal circulation time was positively correlated with vWF levels ($r = 0.33$, $P = 0.048$). Fibrinogen levels were not significantly associated with either retinal index. However, fibrinogen levels were positively associated with HbA_{1c} levels ($r = 0.34$, $P = 0.01$), indicating an association between poor glycemic control and higher fibrinogen levels.

CONCLUSIONS — Increased vWF was associated with a prolonged retinal circulation time and reduced retinal blood flow in early-stage retinopathy of type 1 diabetes. Reduced blood flow associated with increased vWF levels may promote stasis in the retinal circulation and lead to local hypoxemia. These changes might contribute to the microvascular complications of diabetes. Whether the vWF levels predict retinal complications deserves further investigation.

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Retinopathy is a common microvascular complication of type 1 diabetes. Changes in retinal circulation have been noted before the development of overt retinal pathology (1). In diabetic patients with minimal or no diabetic retinopathy, retinal blood flow is initially reduced (2–4). However, once diabetic retinopathy has reached nonproliferative and more advanced levels, retinal blood flow increases (2,3,5,6). The regulatory response of the retina to hyperoxia has also been shown to be significantly blunted in diabetic patients compared with nondiabetic patients (6–8).

The mechanism for the retinal blood flow changes in diabetes remains uncertain. Hyperglycemia causes capillary basement membrane thickening with nonenzymatic glycation, aldose reductase/sorbitol pathway activation, and increased intracellular levels of diacylglycerol and protein kinase C activation (9,10). Selective loss of retinal capillary pericytes and the expansion of intercapillary nonperfusion zones are early changes (11,12). Abnormalities in the response of retinal circulation to vascular endothelial growth factor have been implicated in the subsequent increase in retinal blood flow and venous dilation and the induction of neovascularization (3). These local and systemic abnormalities impair the ability of the retinal vessels to autoregulate, potentially leading to chronic retinal hypoxia and, ultimately, increased retinal vascular permeability and retinal neovascularization (7,8,13).

von Willebrand factor (vWF) is a glycoprotein that mediates platelet adhesion by binding with platelet surface receptor GP Ib/IX. This receptor is particularly important in blood vessels at high shear rates (14,15). In addition, vWF also binds with the platelet GP IIb/IIIa receptor mediating platelet aggregation. vWF is a marker for endothelial dysfunction (15,16), and qualitative and quantitative abnormalities of vWF have been reported in diabetic patients (17–21). Little is known about the relationship between vWF and retinal microcirculation. We therefore determined if there was any association between vWF and retinal circulation in early-stage retinopathy of type 1 diabetes. Because fibrinogen is an acute inflammatory marker and a major determinant of plasma viscosity and blood rheology, its relation to retinal circulation was also investigated.

RESEARCH DESIGN AND METHODS

Study design

This was a substudy of a double-masked randomized crossover clinical trial, the

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Abbreviations: ETDRS, Early Treatment Diabetic Retinopathy Study; MCT, mean circulation time; vWF, von Willebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Vitamin E levels, hemostatic factor levels, and vitamin E therapy*

	Baseline	Placebo	Vitamin E	*P*
Plasma vitamin E (μg/ml)	8.80 ± 1.95	9.44 ± 1.91	13.59 ± 5.05*	<0.0001
Erythrocyte vitamin E (μg/ml)	0.39 ± 0.14	0.37 ± 0.13	0.57 ± 0.21*	<0.0001
vWF (%)	127.3 ± 37.1	125.1 ± 53.2	132.7 ± 43.9	0.65
Fibrinogen (mg/dl)	301.7 ± 55.3	325.1 ± 56.5	311.9 ± 70.0	0.52
HbA_{1c} (%)	7.96 ± 1.29	8.14 ± 1.43	8.29 ± 1.26	0.19

Data are means ± SD. *P* values refer to analysis of variance. *$P < 0.05$ comparing vitamin E treatment with baseline and placebo.

details of which have been described elsewhere (4). The purpose of the main study was to evaluate the effect of vitamin E on retinal blood flow and creatinine clearance. Type 1 diabetic patients with either no retinopathy (Early Treatment Diabetic Retinopathy Study [ETDRS] retinopathy severity level 10) or minimal diabetic retinopathy (ETDRS retinopathy severity level 20) were recruited. The subjects were 18–45 years of age and had a duration of diabetes ≤10 years. Patients were randomly assigned to either 1,800 IU vitamin E per day or placebo and were crossed over to the other treatment after 4 months of therapy. There were 36 subjects in the main study. For the present analysis, blood samples were collected prospectively for hemostatic analysis after the first 16 subjects had been enrolled, i.e., in the remaining 20 subjects. Subjects with microalbuminuria, or those taking cardiac medications, anticoagulants, antihypertensive medications, or antihistamines within 1 month before study onset, were excluded. Women of childbearing age were required to use a medically accepted form of contraception for the duration of study and were excluded if they were pregnant or planning pregnancy. Patients with a history of fluorescein dye allergy, migraine, or ocular abnormalities other than diabetic retinopathy were also excluded. Eligible patients were asked to discontinue vitamin E supplementation for at least 2 months before starting the study. The study was approved by the Joslin Diabetes Center Committee on Human Studies, and all patients signed a written informed consent. The present analysis was based on the 20 subjects in whom hemostatic factors were measured, and retinal photography was performed at baseline and after 4 months of placebo and 4 months of vitamin E therapy. These 20 subjects were randomly assigned to either 1,800 IU vitamin E per day or placebo and were then crossed over to the other treatment after 4 months of therapy.

Retinal circulation

Retinal blood flow measurements were determined from video recordings of fluorescein dye passage through the retinal circulation. The antecubital vein catheter used to obtain the blood samples for hemostatic assays was used to introduce the bolus of fluorescein dye for the video fluorescein angiogram. A 0.75-ml bolus of 10% sodium fluorescein dye was rapidly injected to ensure a sharp dye front in the retinal vasculature. A fluorescein angiogram was obtained from both eyes of each patient. The instrumentation and methodology have been detailed in a prior publication (4). Briefly, a scanning laser ophthalmoscope was used to image the retina, and the video images were directly digitized and stored at 30 frames per second. The digitized video images were computer analyzed on a frame-by-frame basis. Image analysis was performed on the four major retinal artery/vein pairs exiting from the optic disc and perfusing the four retinal quadrants. Measurement of retinal vessel diameters and retinal vessel fluorescence intensities was performed at a fixed radial distance (1,500 μmol/l) from the center of the optic disc. The resulting vessel fluorescence intensity curves were fit to a log-normal distribution function, and the fitted parameters were used to calculate the mean circulation time (MCT) for each quadrant. The calculated retinal blood flow for each quadrant was proportional to the sum of the squares of the artery and vein diameters divided by the MCT for that artery/vein pair. Final values for retinal vessel diameters, MCT, and blood flow represent the averages of all four individual quadrants.

Measurement of coagulation factors and vitamin E

Blood samples were obtained during the morning to avoid circadian changes in hemostatic factors. Blood for hemostatic factors was drawn into 3.8% sodium citrate (9:1, vol:vol), except for vWF, for which blood was collected in EDTA. Plasma was separated by centrifugation for 20 min at 2,000*g* and stored at −80°C for later analysis. Fibrinogen was determined by the Clauss method. vWF antigen was measured by enzyme-linked immunosorbent assay. The intra-assay coefficients of variation in our laboratory were 2.6% for fibrinogen and 8.8% for vWF. The respective interassay coefficients of variation were 4.7 and 10.6%. Vitamin E assays in plasma and erythrocyte membranes were performed by high-performance liquid chromatography (4). Measures of fibrinolysis were also obtained, but they are not discussed here because the plasminogen activator inhibitor 1 results have been previously reported (4).

Statistical analysis

One-way repeated-measures analysis of variance was used to compare differences among hemostatic factors or vitamin E levels at baseline, with placebo administration, and with vitamin E treatment. Post hoc multiple comparisons were performed using the Student-Newman-Keuls test. Correlation between variables was performed by the Spearman rank method. For the correlation analyses of vWF and retinal blood flow, data were first analyzed separately according to whether or not the subjects were on vitamin E therapy and then combined, because the associations between vWF and retinal blood flow were similar with or without vitamin E therapy. All data are reported as means ± SD. A two-tailed *P* value <0.05 was regarded as statistically significant. Statistical analyses were performed using Sigma Stat (Jandel Scientific, San Rafael, CA).

RESULTS

Baseline characteristics

The mean age of the subjects was 32.4 ± 7.8 years with duration of type 1 diabetes of 4.7 ± 2.6 years. Of the 20 subjects, 13 (65%) were male. At baseline, the mean systolic and diastolic blood pressures were 117 ± 10 and 75 ± 6 mmHg, respectively; the mean blood glucose and HbA_{1c} levels were 198 ± 94 mg/dl and 8.0 ± 1.3%, respectively; and the mean total cholesterol, LDL cholesterol, and HDL cholesterol levels were 200 ± 34, 130 ± 30, and 51 ± 15 mg/dl, respectively. The baseline vitamin E level was 8.8 ± 2.0 μg/ml in plasma and 0.39 ± 0.14 μg/ml in the red cell membrane.

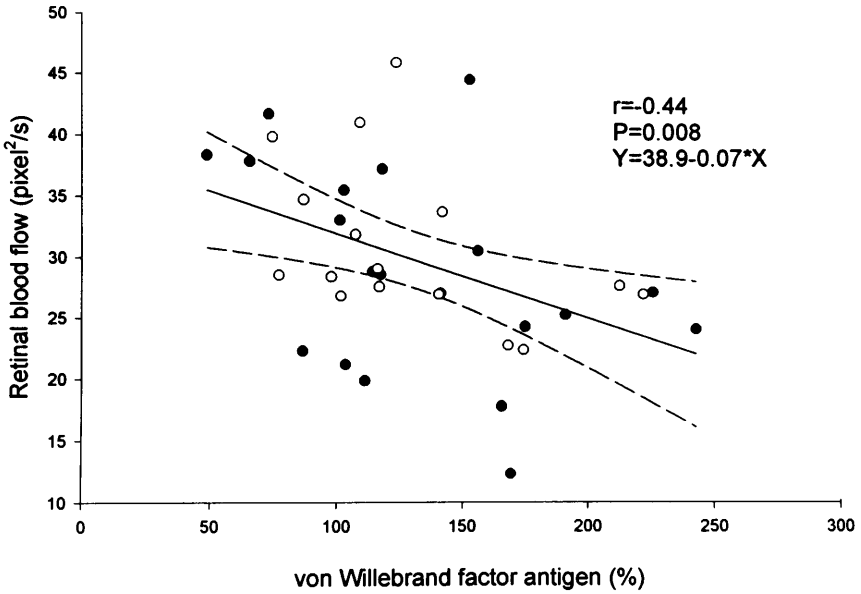

Figure 1—*vWF levels were inversely correlated with retinal blood flow. The correlation coefficient was −0.44,* P *= 0.008.* ●*, Baseline or placebo administration;* ○*, vitamin E therapy.*

Effect of vitamin E on hemostatic factor levels

Although the 4 months of vitamin E treatment significantly increased plasma and erythrocyte vitamin E levels, vitamin E did not significantly change vWF or fibrinogen (Table 1). The HbA_{1c} levels did not change significantly during the course of the study.

Vitamin E therapy and retinal blood flow

The mean circulation time was 6.15 ± 2.74 s at baseline, 5.43 ± 0.96 s with placebo administration, and 5.00 ± 0.91 s with vitamin E therapy ($P = 0.27$). The respective retinal blood flow was 28.8 ± 9.21, 30.8 ± 6.36, and 31.7 ± 8.52 pixel²/s ($P = 0.64$).

Hemostatic factor levels and retinal circulation

Data were first analyzed separately according to whether or not the subjects were on vitamin E treatment. Because similar associations between vWF and retinal blood flow were found with or without vitamin E therapy (in Fig. 1, data are shown with different symbols), results are presented here using combined data. Lower retinal blood flow was associated with higher vWF levels ($r = −0.44$, $P = 0.008$ [Fig. 1]), whereas mean retinal circulation time was positively associated with vWF levels ($r = 0.33$, $P = 0.048$). Fibrinogen levels were not significantly associated with either retinal index (Table 2). HbA_{1c} levels were not significantly associated with retinal circulatory parameters. The correlation coefficients between HbA_{1c} and retinal blood flow and between HbA_{1c} and circulation time were 0.04 ($P = 0.82$) and 0.02 ($P = 0.89$), respectively.

Hemostatic factor levels and HbA_{1c}

Fibrinogen levels were positively associated with HbA_{1c} levels ($r = 0.34$, $P = 0.01$), indicating an association between poor glycemic control and higher fibrinogen levels. vWF levels were not statistically significantly associated with HbA_{1c} levels ($r = 0.21$, $P = 0.10$).

CONCLUSIONS — In the present study of diabetic subjects with minimal or no retinopathy, higher levels of vWF were associated with a more prolonged retinal circulation time and reduced retinal blood flow. Reduced blood flow associated with increased vWF levels may promote blood stasis in the retinal circulation and lead to local hypoxemia. These changes might contribute to the microvascular complications of type 1 diabetes.

vWF is a glycoprotein synthesized by megakaryocytes and endothelial cells and is found in platelet α-granules, plasma, and the subendothelium (14). vWF is released when endothelial cells are damaged (16). vWF levels are higher in diabetic patients than in control subjects (18,21–23), and the levels are even higher among diabetic patients with clinical nephropathy or retinopathy (17–20, 23). Qualitative abnormalities of vWF antigen and changes in the proportion of high–molecular weight multimers have also been reported in diabetes (17–21). Increased plasma vWF is associated with a breakdown of the blood-retinal barrier in early minimal diabetic retinopathy (24). In vitro study has shown that cultured endothelial cells grown in media supplemented with supraphysiological concentrations of glucose produced increased amounts of vWF (25). Our finding that vWF levels tended to be positively associated with HbA_{1c} levels supports the relation between hyperglycemia and vWF levels (25).

A correlation between circulating vWF levels and cardiovascular hemodynamics has been described by Penny et al. (26), who found that higher vWF levels are associated with elevated pulmonary vascular resistance, higher pulmonary artery pressure, and lower cardiac output in subjects with valvular and nonvalvular heart disease. However, the relation between vWF and retinal circulation is unknown. In the present study, we found that increased vWF was associated with a prolonged retinal circulation time and reduced retinal blood flow. Bursell et al. (1) reported previously that the transit time through the retinal capillary bed was longer in diabetic patients with minimal retinopathy than in nondiabetic subjects. Because no consistent correlation has been found between vWF and hemorheological properties, such as whole blood viscosity, plasma viscosity, erythrocyte rigidity, or erythrocyte aggregability (17), our finding that vWF levels were associated with a

Table 2—*Correlation between hemostatic factor, HbA_{1c}, and retinal circulation*

	Retinal blood flow		Mean circulation time	
	r	*P*	*r*	*P*
vWF	−0.44	0.008	0.33	0.048
Fibrinogen	0.04	0.80	0.04	0.81
HbA_{1c}	0.04	0.82	0.02	0.89

r, correlation coefficient.

reduced retinal blood flow is unlikely to be explained by a hemorheological effect of vWF. The reduced retinal blood flow and the prolonged circulation time, with a reduced blood velocity in retinal artery and capillary (11) and with no significant change in arterial diameter in other studies (2,5,27), are more likely caused by underlining endothelium damage, as reflected by increased vWF in plasma (16) and impaired dilation of small resistant arterioles in type 1 diabetic patients. This hypothesis is further supported by the observation that plasma vWF levels have been positively correlated with endothelin, which promotes vasoconstriction and stimulates the proliferation of vascular smooth muscle cells (19).

vWF has been shown to be a predictor of acute coronary events and death in patients with coronary artery disease (28,29). vWF is also a risk factor for macrovascular mortality in patients with type 2 diabetes (30). Stehouwer et al. (31) reported that baseline levels of vWF and changes in vWF during follow-up were strongly related to the development of microalbuminuria in type 2 diabetes. However, little is known about the predictive value of vWF on retinal complications in diabetic patients. Exploring further the relationship between vWF and retinopathy is important because it would increase our understanding of the pathogenesis of retinopathy and may allow for early recognition of high-risk patients. Interestingly, a small study suggested that increased vWF was associated with the development of microalbuminuria in type 1 diabetes but not with the development of retinopathy (32). However, vWF levels have been reported to be higher in diabetic patients with retinopathy compared with control subjects or diabetic patients without retinopathy (19,23).

Although plasma fibrinogen has been shown to be a major determinant of plasma viscosity and erythrocyte aggregation (17), no correlation was found in the present study between fibrinogen levels and retinal blood flow. However, we found that fibrinogen levels were correlated with HbA_{1c}, indicating an association between poor glycemic control and higher fibrinogen levels. Our results support earlier observations made by Coller et al. (18).

Study limitations

The sample size of our current analyses was small. In this substudy of 20 type 1 diabetic subjects, vitamin E therapy tended to normalize retinal blood flow, which was directionally consistent with the main study (4), although the changes did not reach statistical significance. A power calculation showed that our substudy had a power of only 0.15 to detect such a difference. Because multiple analyses were performed, a correlation *P* value of 0.048 between vWF and mean retinal circulation time may not be significant. These findings need to be confirmed by larger studies.

In conclusion, increased vWF was associated with a prolonged retinal circulation time and reduced retinal blood flow in diabetic subjects with minimal or no diabetic retinopathy. Reduced blood flow may promote blood stasis in the retinal circulation and lead to local hypoxemia. Further prospective studies are needed to evaluate the predictive value of these hemostatic factors on microvascular complications in subjects with type 1 diabetes.

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