The Relationship Between Glycemic Control and Plasma Vascular Endothelial Growth Factor and Endothelin-1 Concentration in Diabetic Patients

Hiroaki Kakizawa, Mitsuyasu Itoh, Yasutoshi Itoh, Shigeo Imamura, Yoko Ishiwata, Takashi Matsumoto, Keiko Yamamoto, Taiya Kato, Yasunaga Ono, Mutsuko Nagata, Nobuki Hayakawa, Atsushi Suzuki, Yoshie Goto, and Naohisa Oda

Understanding the causes of diabetic vascular complications has become an increasingly important issue because of the rapidly rising prevalence of diabetes. Recently discovered vasoconstrictors and angiogenesis regulators, such as endothelin (ET) and vascular endothelial growth factor (VEGF), have been intensely studied for possible pathogenic roles in diabetic vascular complications. The present study was undertaken to clarify the effect of glycemic control on serum VEGF and plasma ET-1 concentrations in diabetic patients, and to identify other factors that may cause fluctuations of these substances. Plasma VEGF and ET-1 concentrations of 45 hospitalized diabetic patients and 54 control subjects were measured by enzyme immunoassay (EIA) and radioimmunoassay (RIA), respectively. Plasma VEGF was elevated in poorly controlled diabetic patients compared with healthy subjects and plasma VEGF concentrations declined after hospitalized treatment with either insulin or oral hypoglycemic agents in combination with diet. There was a significant correlation between plasma VEGF concentration and both fasting plasma glucose (FPG) and hemoglobin A_{1c} (HbA_{1c}). Plasma ET-1 in poorly controlled diabetic patients was higher than in healthy controls, but improved glycemic control did not affect plasma ET-1 concentrations. Thus, poor glycemic control causes increased levels of plasma VEGF, which may result in hypertension and vascular complications in diabetes. Short-term treatment resulting in good glycemic control can improve levels of VEGF and may provide beneficial effects on diabetic vascular complications.

THE NUMBER of diabetic patients has dramatically increased each year, probably because of changes in lifestyle and eating habits. The resultant vascular complications, in the form of macroangiopathies like cardiovascular disease, and microangiopathies like diabetic nephropathy, can result in mortality and life-long morbidity.[1] Therefore, understanding the causes of these diabetic vascular complications is crucial in reducing the long-term adverse effects of this disease.

Recently discovered bioactive substances that control vasoconstriction, such as endothelin (ET) and vascular endothelial growth factor (VEGF), have been intensely studied for possible pathogenic roles in diabetic vascular complications.[2,3] The importance of the control of blood pressure in preventing the progression of diabetic nephropathy has been demonstrated by the United Kingdom Prospective Diabetes Study (UKPDS).[4]

VEGF has been cloned and identified as a factor that causes proliferation of endothelial cells.[5,6] Four subtypes (VEGF121, VEGF165, VEGF189, and VEGF206) have been classified by differences in splicing. Many studies have reported the pathogenetic roles of VEGF on diabetic retinopathy. Aiello et al[7] and Adamis et al[8] showed an increase in VEGF concentrations in the vitreous humor and aqueous humor of diabetic patients with hemorrhagic retinopathy, suggesting a role for VEGF in angiogenesis in the retina. VEGF administered into the vitreous humor in primates can induce changes similar to diabetic retinopathy, such as capillary aneurysm, apoplexia retinalis, increased permeability of retinal vessels, blockage, and rubeosis iridis.[9,10] Increased VEGF gene expression has also been reported in simple retinopathy, even before new blood vessels emerge.[11] There are fewer studies about the possible role of VEGF in diabetic vascular complications other than retinopathy. However, recent studies have focused on various pathogenetic roles of VEGF in these complications. VEGF is related to capillary permeability,[12] and increased concentrations of plasma VEGF have been suggested as a predisposing factor for future microalbuminuria in type 1 diabetes.[13]

ET has a strong, vasoconstrictive action on vascular smooth muscle.[14,15] It also has various physiological effects, such as growth-stimulating actions on aldosterone-producing mesenchymal cells, stimulation of aldosterone and catecholamine secretion, production of vasodilators from vascular endothelial cells, potentiation of atrial natriuretic peptide (ANP) production from heart muscle, and other pleotropic actions, such as inhibitory effects on renin secretion and on the central nervous system.[16,17] From these data it has been suggested that ET participates in the pathophysiology of hypertension and angiospasm, and that it may contribute to endothelial dysfunction in diabetes.[18] ET-1 is mainly produced by vascular endothelial cells, but many other cells, including vascular smooth muscle cells and myocardial cells also produce it, suggesting a paracrine or autocrine role.[19,20] Diabetic patients who have retinopathy showed increased levels of ET-1 in comparison to patients without retinopathy.[21] Furthermore, hyperglycemia caused production of VEGF from vascular smooth muscle cells or mesangial cells in vitro,[22,23] as well as production of ET-1 from endothelial cells.[21]

Therefore, VEGF and ET-1 may be involved in the progression of various types of diabetic microangiopathies, and the

From the Division of Endocrinology and Metabolism, Department of Internal Medicine, Fujita Health University, School of Medicine, Aichi, Japan; and the Department of Internal Medicine, Enshu General Hospital, Shizuoka, Japan.

Submitted February 10, 2003; accepted December 29, 2003.

Supported by grants from Fujita Health University.

Address reprint requests to Mitsuyasu Itoh, MD, Division of Endocrinology and Metabolism, Department of Internal Medicine, Fujita Health University, School of Medicine, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi 470-1192, Japan.

0026-0495/04/5305-0009$30.00/0

doi:10.1016/j.metabol.2003.12.002

Table 1. Patient Characteristics

Patient No.	Sex/Age (yr)	BMI	HbA_{1c} (%)	Pretreatment	Patient No.	Sex/Age (yr)	BMI	HbA_{1c} (%)	Pretreatment
1	M/61	19.4	8.6	SU	24	M/55	21.5	12.6	None
2	M/33	25.6	7.2	None	25	M/51	29.4	9.0	SU
3	M/55	22.6	11.3	SU + GI	26	F/55	25.3	12.1	Insulin
4	F/52	31.2	11.1	Insulin	27	M/67	24.3	11.9	SU + GI
5	M/55	19.7	9.3	Insulin	28	F/31	27.2	10.5	None
6	M/62	22.2	8.1	None	29	F/39	26.4	10.5	None
7	M/57	26.5	9.8	Insulin	30	F/46	17.0	8.7	SU + Met
8	F/57	29.2	13.0	SU	31	M/30	28.6	6.6	None
9	M/50	31.2	11.7	SU	32	M/36	30.8	7.6	SU + GI + Pio
10	M/33	27.2	11.9	None	33	M/37	36.0	8.6	None
11	F/60	26.6	10.2	Insulin	34	M/33	41.4	7.9	None
12	F/59	22.9	11.2	Insulin	35	M/65	21.1	8.9	None
13	F/26	28.8	8.6	SU	36	F/76	14.0	10.4	SU + GI
14	M/52	23.8	11.4	None	37	F/79	21.4	8.6	None
15	M/40	17.8	10.3	GI	38	M/71	23.5	10.1	None
16	F/58	25.1	10.9	SU	39	F/72	31.3	8.6	Insulin
17	M/56	22.7	11.5	None	40	M/31	17.1	13.6	None
18	M/65	21.8	8.0	Insulin	41	F/33	33.3	11.1	Insulin
19	F/61	27.5	7.8	None	42	F/29	18.3	12.5	None
20	F/57	29.1	9.8	SU + GI	43	M/33	22.0	12.9	None
21	F/47	30.2	9.6	Insulin	44	M/54	21.0	13.3	None
22	F/55	22.2	9.4	None	45	F/55	19.2	7.2	None
23	F/62	24.6	9.6	Insulin					

Abbreviations: SU, sulfonylurea; GI, α-glucosidase inhibitor; Met, metformin; Pio, pioglitasone.

interaction between these 2 factors in the hyperglycemic condition may contribute to accelerate the progression of vascular complication in diabetes. The present study was undertaken to clarify the effect of glycemic control on serum VEGF and plasma ET-1 concentrations in diabetic patients, and to identify factors that may relate to fluctuations of these substances.

MATERIALS AND METHODS

The study subjects consisted of 45 diabetic patients (24 men and 21 women; mean age, 50.9 ± 13.9 years), who were hospitalized for treatment of their hyperglycemia. The subjects' body mass index (BMI), hemoglobin A_{1c} (HbA_{1c}), and previous treatment are listed in Table 1. The mean (±SD) duration of diabetes was 8.8 ± 7.3 years. Blood was drawn from these patients before and after their hyperglycemia was improved. Before admission, 26.7% of the diabetic patients had been treated with oral hypoglycemic agents and 26.7% had been treated with insulin in combination with outpatient-based instruction for diet, while 46.7% of the patients were not treated at all. On recruitment into this study, the patients' glycemic control had not reached satisfactory levels, that is, repeated measurements of HbA_{1c} remained greater than 8.0% in spite of treatment, or no previous treatment was done (Table 1). Informed consent was obtained from each subject, and the study was approved by the institutional review board. The control subjects consisted of 54 healthy volunteers (22 men and 32 women; mean age, 46.8 ± 11.5 years; mean BMI, 22.3 ± 2.3; mean HbA_{1c}, 5.1% ± 0.3%), whose ages and sex were matched to those of the diabetic patients. Blood pressure (systolic, 128.3 ± 13.4 mm Hg; diastolic, 76.2 ± 9.5 mm Hg), HbA_{1c}, and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of control subjects were all within the normal range; the subjects showed no glucosuria or proteinuria, and they had no family history of diabetes. Forty-one patients completed treatment with a calorie-controlled diet regimen (30 kcal/kg of ideal body weight; 50% to 55% carbohydrate, 25% to 30% fat, and 18% to 20% protein) plus oral hypoglycemic agents (28%) and/or insulin (79%) to reduce hyperglycemia. Glycemic control was monitored by comparing blood glucose before each meal and at bedtime (9 PM) during their hospitalization, and HbA_{1c} before and after the treatment.

VEGF was measured by enzyme immonoassay (EIA) kit (Quantikine, R&D Systems, Minneapolis, MN), and ET-1 was measured by radioimmunoassay (RIA; Sumikin Bio-Science, Sagamihara, Japan). Recombinant human VEGF165 was used as a standard, and the cross-reactivity between this assay and VEGF121 is 100%. The minimal detectable VEGF concentration was 31 pg/mL. Intra- and interassay coefficients of variation were less than 7% and 6%, respectively. The minimal detectable ET-1 concentration was 3 pg/mL, and intra- and interassay coefficients of variation were less then 10% each. Blood glucose was measured by the glucose-oxidation method and HbA_{1c} was measured by high-performance liquid chromatography (HPLC) with a reference range of 4.2% to 5.7%.

Statistical Analysis

Data are presented as means ± SD. Differences between data sets were evaluated by analysis of variance (ANOVA) and the Scheffé test was used as a post hoc test. Paired data were compared by paired *t* test. A simple linear regression analysis was used to evaluate the correlation between the 2 parameters. All statistical analyses were performed with the StatView 4.5 software program (Abacus, Berkeley, CA) for Macintosh. $P < .05$ was considered statistically significant.

RESULTS

Plasma VEGF was elevated in the 45 uncontrolled diabetic patients compared with 54 healthy subjects (345.0 ± 175.0 *v* 234.2 ± 179.1 pg/mL; $P < .01$) (Fig 1). After 33.0 ± 21.2 days of hospitalization and medical treatment, the diabetic patients' blood glucose values before each meal and at bedtime changed from 215.1 ± 63.9 mg/dL (before breakfast), 266.1 ± 71.7

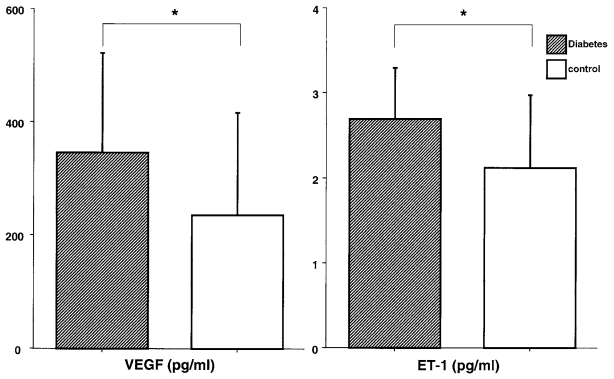

Fig 1. Plasma VEGF and ET-1 concentrations in diabetic patients (n = 45) and in control subjects (n = 54). *$P < .05$

mg/dL (before lunch), 206.1 ± 76.0 mg/dl (before dinner), and 247.9 ± 81.1 mg/dL (9 PM) to 107.8 ± 23.2, 118.0 ± 31.9, 116.7 ± 31.1, and 155.1 ± 45.5 mg/dL, respectively ($P < .05$). Glycosylated Hb A_{1c} changed after 1 month from 10.0% ± 1.8 % to 8.2% ± 1.2 % ($P < .0001$). After diet and pharmacological treatment, plasma VEGF levels in the 41 patients whose glycemic control had improved from 339.0 ± 179.0 pg/mL to 273.2 ± 142.2 pg/mL, were not different from the controls (Fig 2). There was a significant correlation between plasma VEGF concentration and both FPG and HbA_{1c} in subjects who did not have good glycemic control (Fig 3; $P < .05$). There was also a significant correlation between plasma VEGF concentration and HbA_{1c} after good glycemic control was obtained (Fig 4; $P < .05$).

Plasma ET-1 in poorly controlled diabetic patients was higher than in healthy controls (2.69 ± 0.61 pg/mL *v* 2.11 ± 0.85 pg/mL, $P < .005$) (Fig 1). However, improved glycemic control did not affect plasma ET-1 concentration (2.81 ± 0.57 pg/mL *v* 2.61 ± 0.84 pg/mL; Fig 5) and no correlation was found between plasma ET-1 concentration and HbA_{1c} or FPG. Plasma ET-1 levels increased in some patients and decreased in other patients after good glycemic control was obtained. Therefore, several factors were compared by dividing the patients into 2 groups: those with levels of ET-1 that increased and those with levels that decreased. There were no significant difference between the 2 groups with regard to age (48.0 ± 17.5 *v* 50.4 ± 15.0 years), HbA_{1c} (10.1% ± 2.2% *v* 9.9% ± 1.5%), BMI (24.2 ± 5.4 *v* 24.5 ± 5.5 kg/m^2), systolic blood pressure (127.4 ± 18.7 *v* 128.3 ± 17.5 mm Hg), total cholesterol (214.3 ± 46.4 *v* 214.1 ± 36.2 mg/dL), or FPG (224.1 ± 60.6 *v* 250.5 ± 61.5 mg/dL). The group that had a decreased ET-1 response showed higher triglyceride levels (191.7 ± 113.5 *v* 77.6 ± 23.0 mg/dL, $P < .05$), and slightly higher VEGF levels (397.5 ± 215.1 *v* 238.3 ± 125.0 pg/mL, $P = .066$) and diastolic blood pressure (75.3 ± 12.0 *v* 65.4 ± 5.5 mm Hg, $P = .051$), although the difference for diastolic blood pressure was not statistically significant. As a whole, serum total cholesterol and triglyceride levels in poorly controlled diabetic patients were higher than those in normal controls (total cholesterol, 221.8 ± 44.0 mg/dL *v* 203.4 ± 28.9 mg/dL, $P < .05$; total triglycerides, 156.0 ± 99.6 mg/dL *v* 104.6 ± 62.9 mg/dL, $P < .05$). Treatment improved serum total cholesterol from 221.8 ± 44.0 mg/dL to 205.5 ± 39.0 mg/dL ($P < .005$), and triglyceride from 153.7 ± 101.2 mg/dL to 133.6 ± 107.1 mg/dL ($P < .01$), and changed BMI from 24.4 ± 4.7 g/m^2 to 22.3 ± 2.3 kg/m^2 ($P < .0001$). There was no difference in systolic or diastolic blood pressure between poorly controlled diabetic (systolic, 129.4 ± 18.7 mm Hg; diastolic 74.6 ± 13.8 mm Hg) and post-controlled diabetic patients (systolic, 126.3 ± 15.0 mm Hg; diastolic, 72.6 ± 11.0 mm Hg) or controls (systolic, 128.3 ± 13.4 mm Hg; diastolic, 76.2 ± 9.5 mm Hg). No correlation was found between plasma VEGF or ET-1 concentration and the levels of total cholesterol or triglycerides, or BMI. There was also no correlation between diabetes duration (years), hypertension, diabetic nephropathy, or neuropathy and plasma VEGF or ET-1 concentration (Fig 6). However, plasma VEGF concentrations in patients with proliferative retinopathy was higher than that in those with simple retinopathy (429.9 ± 62.4 pg/mL *v* 246.5 ± 154.8 pg/mL, $P < .05$), but was not statistically different from those without retinopathy (Fig 6).

DISCUSSION

The present study showed that plasma VEGF concentration was higher in diabetic patients who were hospitalized because of poor glycemic control than in healthy subjects. The increased plasma VEGF concentration declined along with decreases in FPG and HbA_{1c} as a result of treatment during hospitalization of 33.0 ± 21.2 days. There was a significant correlation between plasma VEGF concentration and HbA_{1c}, suggesting that chronic hyperglycemia may increase plasma levels of VEGF, and that reduction of high levels of VEGF may be possible by improvement of glycemic control. Prolonged hyperglycemia activates the sorbitol pathway and induces intracellular anaerobic conditions, and hemodynamic change.[24-26] These conditions may facilitate the production of VEGF,[22,27] which is a growth factor for vascular endothelial cells that may contribute to diabetic vascular complications and arteriosclerosis.[3] This increased production of VEGF was reversed by the reduction of hyperglycemia. Our in vivo data are supported by

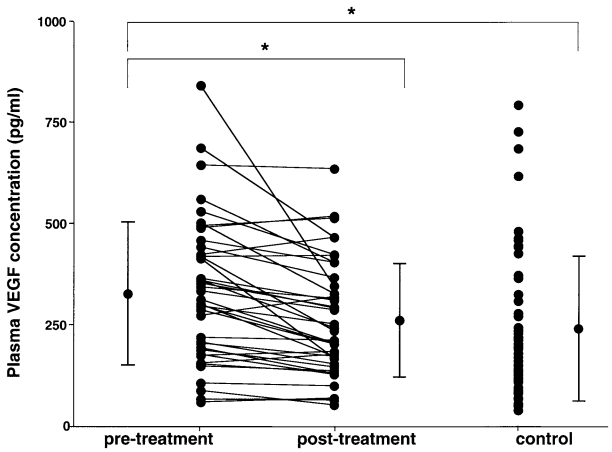

Fig 2. Changes of plasma VEGF after treatment (n = 41). *$P < .05$

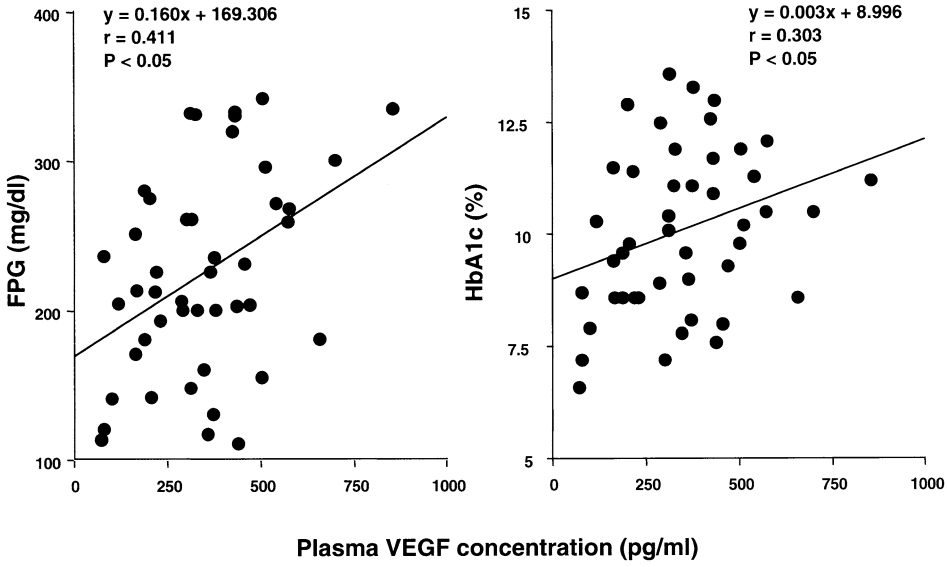

Fig 3. Correlation between plasma VEGF concentration and FPG or HbA_{1c} in poorly controlled diabetic patients (n = 45).

other in vitro studies, which showed increased production of VEGF when vascular smooth muscle cells or renal mesangial cells were cultured under high-glucose conditions.[22,23] Ischemia and hypoxia have also been shown to induce expression of the VEGF gene.[28,29] Advanced glycation endproducts (AGEs) also induce expression of VEGF, and there is a correlation between AGE deposition and expression of VEGF in the retina of diabetic retinopathy.[30] Our data suggesting a positive correlation between plasma VEGF and HbA_{1c} levels may reflect, at least in part, the clinical situation of AGE-induced VEGF overproduction.

Hypoxia activates the sorbitol pathway under high-glucose conditions,[29,30] and VEGF may be involved in the promotion of lumen formation, random migration of endothelial cells, release of tissue factors from endothelial cells, production of coagulation and fibrinolytic proteins such as plasminogen activator, expression of cell adhesion molecules in small vein neovasculation, and macrophage migration in the arteriosclerotic plaque.[31,32] Valabhji et al[33] showed that high levels of serum VEGF in type 1 diabetic patients correlated with diminished carotid artery distensibility (mean percentage increase in carotid luminal diameter measured by ultrasound), and Santilli et al[13] suggested increased serum VEGF levels as a predicting risk factor for developing persistent microalbuminuria in young type 1 diabetic patients. VEGF mRNA and urinary excretion of VEGF are increased in diabetic nephropathy.[27]

ET has been identified as a vasoconstrictive factor consisting of 21 amino acid residues.[14] Three isoforms are subclassified as ET-1, -2, and -3. The ET receptor has 2 kinds of G-protein–coupled domains, termed ET-A and ET-B, depending on the affinity for the 3 isoforms.[17] ET-1 is produced in vascular endothelial cells, and is involved in circulation dynamics.[2]

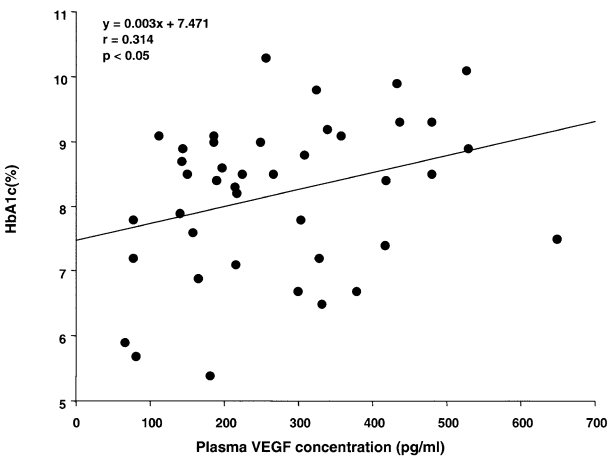

Fig 4. Correlation between plasma VEGF concentration and HbA_{1c} in well-controlled diabetic patients (n = 41).

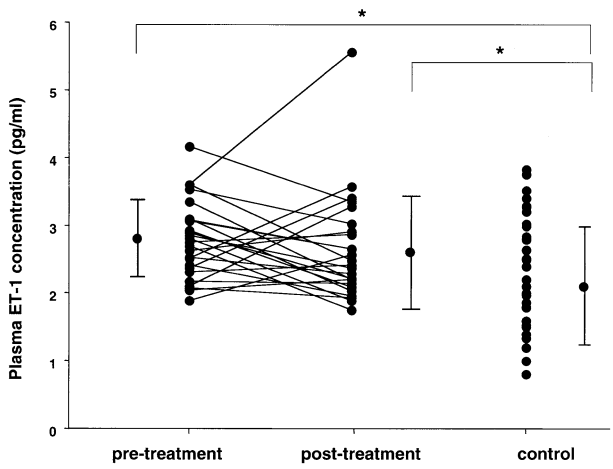

Fig 5. Plasma ET-1 concentration in diabetic patients before and after treatment (n = 25). *$P < .05$.

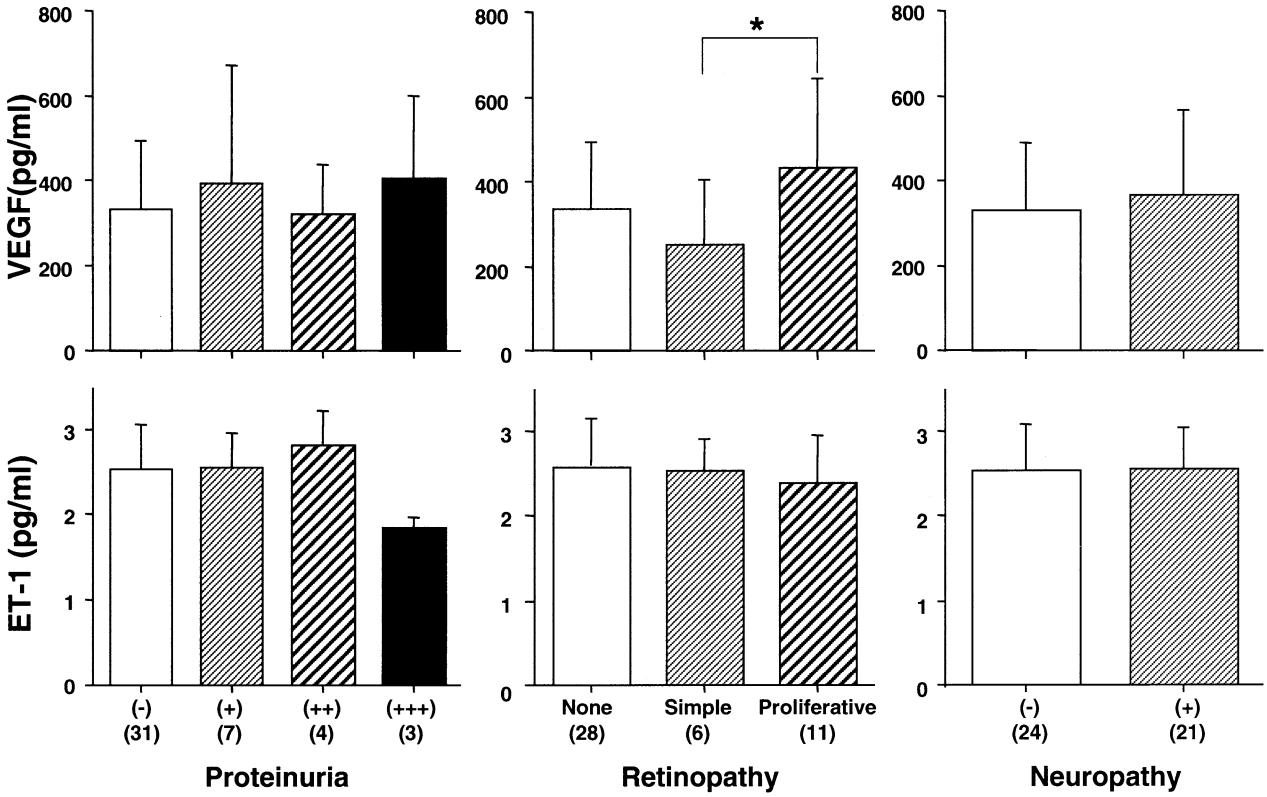

Fig 6. Plasma levels of VEGF and ET-1 in diabetic patients with or without diabetic complications. *$P < .05$.

Recent research has employed receptor inhibitors to determine whether ET-1 regulates vascular tone physiologically.[34,35] Plasma levels of ET-1 have been repeatedly shown to be high in diabetic patients[21] and our results confirmed these findings. However, improvement of blood glucose by treatment did not affect plasma ET-1 concentrations in our study. On the other hand, it may be possible to predict a decreased response of high levels of plasma ET-1 by amelioration of hyperglycemia in the case of patients with high level of triglycerides and plasma VEGF and high diastolic blood pressure, since this group showed a decreased response of ET-1 after good glycemic control was obtained. ET-1's potential role in diabetic complications requires further investigation. It is possible that ET-1 may need a certain period of time to diminish, or that once its increased production is initiated, it may not be able to return to normal levels due to other disrupted feedback mechanisms. One limit of the present study is that the number of patients necessary was not calculated for each group according to the results of a preliminary study. Further studies with enough power will be required to test the hypothesis that amelioration of increased levels of VEGF and ET-1 by glycemic control reduces diabetic complications.

In summary, poor glycemic control is associated with increased levels of plasma VEGF and ET-1 in diabetic patients. Short-term treatment leading to good glycemic control is associated with reduced VEGF levels, but not reduced ET-1 levels. Improved levels of VEGF may reduce the risk of diabetic vascular complications.

ACKNOWLEDGMENT

The authors appreciate the secretarial assistance of N. Takekawa who prepared the manuscript.

REFERENCES

1. Eastman RC, Javitt JC, Herman WH, et al: Prevention strategies for type 2 diabetes mellitus—A health and economic perspective, in LeRoith D, Taylor SI, Olefsky JM, (eds): Diabetes Mellitus—A Fundamental and Clinical Text. ed 2. Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 746-757

2. Hopfner RL, Gopalakrishnan V: Endothelin: Emerging role in diabetic vascular complications. Diabetologia 42:1383-1394, 1999

3. Aiello LP, Wong JS: Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int 58:S113-119, 2000 (suppl 77)

4. UK Prospective Diabetes Study Group: Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. Br Med J 317:713-720, 1998

5. Ferrara N, Henzel WJ: Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 161:851-858, 1989

6. Shibuya M: Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct Funct 26:25-35, 2001

7. Aiello LP, Avery RL, Arrigg PG, et al: Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331:1480-1487, 1994

8. Adamis AP, Miller JW, Bernal MT, et al: Increased vascular

endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 118:445-450, 1994

9. Tolentino MJ, Miller JW, Gragoudas ES, et al: Intravitreous injections of vascular endothelial growth factor produce retinal ischemia and microangiopathy in an adult primate. Ophthalmology 103: 1820-1828, 1996

10. Tolentino MJ, Miller JW, Gragoudas ES, et al: Vascular endothelial growth factor is sufficient to produce iris neovascularization and neovascular glaucoma in a nonhuman primate. Arch Ophthalmol 114: 964-970, 1996

11. Amin RH, Frank RN, Kennedy A, et al: Vascular endothelial growth factor is present in glial cells of the retina and optic nerve of human subjects with nonproliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 38:36-47, 1997

12. Brausewetter F, Jehle PM, Jung MF, et al: Microvascular permeability is increased in both types of diabetes and correlates differentially with serum levels of insulin-like growth factor I (IGF-I) and vascular endothelial growth factor (VEGF). Horm Metab Res 33:713-720, 2001

13. Santilli F, Spagnoli A, Mohn A, et al: Increased vascular endothelial growth factor serum concentrations may help to identify patients with onset of type 1 diabetes during childhood at risk for developing persistent microalbuminuria. J Clin Endocrinol Metab 86:3871-3876, 2001

14. Yanagisawa M, Kurihara H, Kimura S, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411-415, 1988

15. Masaki T: Possible role of endothelin in endothelial regulation of vascular tone. Ann Rev Pharmacol Toxicol 35:235-255, 1995

16. Yanagisawa M, Masaki T: Molecular biology and biochemistry of the endothelins. Trends Pharmacol Sci 10:374-378, 1989

17. Sakurai T, Yanagisawa M, Masaki T: Molecular characterization of endothelin receptors. Trends Pharmacol Sci 13:103-108, 1992

18. Mather KJ, Mirzamohammadi B, Lteif A, et al: Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes. Diabetes 51:3517-3523, 2002

19. Sawamura T, Kimura S, Shinmi O, et al: Analysis of endothelin related peptides in culture supernatant of porcine aortic endothelial cells: Evidence for biosynthetic pathway of endothelin-1. Biochem Biophys Res Commun 162:1287-1294, 1989

20. Levin ER: Endothelins. N Engl J Med 333:356-363, 1995

21. Takahashi K, Ghatei MA, Lam HC, et al: Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia 33:306-310, 1990

22. Natarajan R, Bai W, Lanting L, et al: Effect of high glucose on vascular endothelial growth factor expression in vascular smooth muscle cells. Am J Physiol 273:H2224-2231, 1997

23. Cha DR, Kim NH, Yoon JW, et al: Role of vascular endothelial growth factor in diabetic nephropathy. Kidney Int 58:104-112, 2000 (suppl 77)

24. Stevens MJ, Obrosova I, Feldman EL, et al: The sorbitol-osmotic and sorbitol-redox hypotheses, in LeRoith D, Taylor SI, Olefsky JM, (eds): Diabetes Mellitus—A Fundamental and Clinical Text. ed 2. Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 972-983

25. Tsilibary EC: Microvascular basement membranes in diabetes mellitus. J Pathol 200:537-546, 2003

26. Candido R, Allen TJ: Haemodynamics in microvascular complications in type 1 diabetes. Diabetes Metab Res Rev 18:286-304, 2002

27. Murata T, Nagai R, Ishibashi T, et al: The relationship between accumulation of advanced glycation end products and expression of vascular endothelial growth factor in human diabetic retinas. Diabetologia 40:764-769, 1997

28. Ozaki H, Yu AY, Della N, et al: Hypoxia inducible factor-1alpha is increased in ischemic retina: Temporal and spatial correlation with VEGF expression. Invest. Ophthalmol Vis Sci 40:182-189, 1999

29. Hata Y, Nakagawa K, Ishibashi T, et al: Hypoxia-induced expression of vascular endothelial growth factor by retinal glial cells promotes in vitro angiogenesis. Virchows Arch 426:479-486, 1995

30. Lu M, Kuroki M, Amano S, et al: Advanced glycation end products increase retinal vascular endothelial growth factor expression. J Clin Invest 101:1219-1224, 1998

31. Shalaby F, Rossant J, Yamaguchi TP, et al: Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62-66, 1995

32. Jeltsch M, Kaipainen A, Joukov V, et al: Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 276:1423-1425, 1997

33. Valabhji J, Dhanjil S, Nicolaides AN, et al: Correlation between carotid artery distensibility and serum vascular endothelial growth factor concentrations in type 1 diabetic subjects and normal subjects. Metabolism 50:825-829, 2001

34. Cediel E, Vazquez-Cruz B, Navarro-Cid J, et al: Role of endothelin-1 and thromboxane A2 in renal vasocontriction induced by angiotensin II in diabetes and hypertension. Kidney Int 82:2-7, 2002 (suppl)

35. Cardillo C, Campia U, Bryant MB, et al: Increased activity of endogenous endothelin in patients with type II diabetes mellitus. Circulation 106:1783-1787, 2002