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Low plasma basic fibroblast growth factor is associated with laser photocoagulation treatment in adult type 2 diabetes mellitus from the Veterans Affairs Diabetes Trial

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Abstract

Basic fibroblast growth factor (bFGF) is a potent endothelial cell mitogen that does not normally circulate. Yet plasma bFGF-like bioactivity was increased in association with persistent microalbuminuria and retinopathy in adult type 2 diabetes mellitus. In the present study, we tested whether plasma bFGF immunoreactivity (IR) could predict the need for laser treatment of diabetic retinopathy in a baseline subset of advanced type 2 diabetes mellitus from the Veterans Affairs Diabetes Trial (mean: age, 59 years; diabetes duration, 11 years; baseline glycosylated hemoglobin, 9.5%). Plasma bFGF-IR was determined with a sensitive and specific 2-site enzyme-linked immunoassay in 172 patients at the baseline visit. Results were dichotomized at 4.5 pg/mL, the upper limit in healthy men. There was an unexpected significant association between low baseline plasma bFGF-IR level and the interim (4 years) need for laser treatment. First laser treatment was significantly more likely to be required in patients with low compared with high baseline bFGF (19% vs 6%, $P = .03$ for the difference). After adjusting for clinical risk factors, low vs high bFGF (hazard ratio [HR], 5.01; $P = .012$), duration of diabetes (HR, 1.05; $P = .050$), and low-density lipoprotein cholesterol concentration (HR, 0.98; $P = .027$) were all significantly associated with time to first laser occurrence. These and our prior results suggest that low plasma bFGF-IR may be a marker for the presence of anti–endothelial cell autoantibodies that may contribute to the need for laser photocoagulation treatment in adult men with advanced type 2 diabetes mellitus.
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1. Introduction

Diabetic retinopathy is a significant cause of morbidity related to visual impairment and new blindness in adults in the United States [1]. Long duration of diabetes, poor glycemic control, and albuminuria are among the clinical risk factors associated with an increased risk for diabetic retinopathy [1,2]. Angiogenic growth factors play a role in diabetic retinopathy by increasing vascular permeability and inducing new blood vessel growth [3-6]. Basic fibroblast growth factor (bFGF) is one of the most potent known angiogenesis factors [7-9]. Basic FGF does not normally circulate [10], but increases in diabetic subjects with persistent micro- or albuminuria [11]. Because of a particularly strong association between albuminuria and vision-threatening diabetic macular edema [1], in the present study, we tested for an association between baseline plasma bFGF and postbaseline laser photocoagulation occurrence in adults with long-standing type 2 diabetes mellitus from the Veterans Affairs Diabetes Trial (VADT).

Veterans Affairs Diabetes Trial is a large clinical trial in adult patients with type 2 diabetes mellitus randomized to standard or intensive glycemic control [12]. We now report a

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novel, significant association between low plasma bFGF immunoreactivity (IR) and the need for laser photocoagulation during 4 to 6 years of follow-up in a VADT subset of 172 subjects. These results suggest that low plasma bFGF-IR may be a novel risk marker for vision-threatening retinal pathology in older adult male patients with advanced type 2 diabetes mellitus.

2. Subjects and methods

2.1. Study subjects

Informed consent for the Investigational Review Board–approved substudy was obtained from 172 diabetic subjects at 5 outpatient sites who had consented to participate in the main VADT. Ethylenediaminetetraacetic acid (EDTA) plasma was then drawn in the morning after an overnight fast at each site. Plasma was aliquoted and shipped frozen (dry ice) to a central laboratory (Maveric, Boston Veterans Affairs Medical Center, Boston, MA) where it was inventoried and stored at −80°C for 1 to 2 years. Archived, coded, and frozen EDTA plasma from consecutively enrolled patients was shipped to the laboratory of Dr Zimering (Veterans Affairs New Jersey Health Care System, Lyons, NJ) where bFGF-IR assays were performed. All other assays were performed in the Central Laboratory of the VADT (Tufts University, Boston, MA).

Baseline clinical characteristics are shown in Table 1. All subjects were older than 40 years. Ninety-seven percent of patients were men.

2.2. Medications

All patients were taking antidiabetic medications at baseline including oral agents and/or insulin. Patients randomized to the standard or intensive glycemic treatment group were treated for at least 5 years (and some up to 7 years) with the same classes of medications including the thiazolidinedione rosiglitazone. Baseline antihypertensive medication use included angiotensin-converting enzyme (ACE) inhibitors in 67% of patients and angiotensin receptor blockers (ARBs) in an additional 7% of patients, indicative of a high proportion of patients with a history of persistent microalbuminuria.

Table 1
Baseline characteristics in study subjects

	Mean ± SD
Age (y)	59.2 ± 8.4
BMI (kg/m^2)	31.4 ± 4.7
Diabetes duration (y)	11.4 ± 8.1
HbA_{1c} (%)	9.5 ± 1.4
Systolic BP (mm Hg)	130.2 ± 17.9
Diastolic BP (mm Hg)	74.2 ± 10.8
ACR (mg/g)	151 ± 491
LDL cholesterol (mg/dL)	104 + 32

BP indicates blood pressure; ACR, urine albumin-creatinine ratio.

2.3. Laser photocoagulation

Information regarding laser photocoagulation for retinopathy was obtained from questionnaires administered at the baseline and each annual visit. Baseline determination of plasma bFGF-IR (at Veterans Affairs New Jersey) was masked to the information about laser photocoagulation occurrence.

The risk factors associated with time to first laser treatment were modeled in 156 subjects in whom postbaseline data about laser occurrence were available between the second and sixth postbaseline annual visits. Laser events occurring during the first year of study follow-up were censored to minimize the effect of detection bias on time to first laser occurrence.

2.4. Baseline fundus photographs

Baseline fundus photographs were obtained in all patients. The photographs were evaluated at the Central Fundus Photography Reading Center, University of Wisconsin, Madison, WI. The frequencies of no retinopathy, microaneurysms, and mild nonproliferative and severe nonproliferative and proliferative retinopathy were 29%, 18%, 29%, 17%, and 7%, respectively. Macular edema was present in 16 of 156 patients (10.3%) in whom it could be assessed from photographs.

2.5. Laboratory and clinical measures

Urinary microalbumin, plasma glycosylated hemoglobin (HbA_{1c}), and urine creatinine were determined by standard methods as previously described [12]. Urinary albumin-creatinine ratio was calculated as albumin concentration/creatinine concentration × 100. Plasma total cholesterol, triglycerides, and high-density lipoprotein cholesterol were determined by standardized direct enzymatic assay methods as previously reported [12]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedenwald equation on all samples with plasma triglyceride concentration less than 400 mg/dL. Blood pressure was recorded in the seated position after 5-minute rest. Three consecutive readings were obtained, and the median value of the 3 consecutive determinations was used for analysis.

2.6. Plasma samples

Archived, coded EDTA plasma samples were kept frozen (−40°C) for 1 to 2 years before assay for bFGF-IR. Plasma bFGF-IR and bFGF-like bioactivity were previously shown to be stable for 5 years or longer at −20°C, and for up to 3 freeze-thaw cycles [11].

2.7. Basic FGF assays

Basic FGF-IR in plasma was determined using a sensitive specific 2-site enzyme-linked immunoassay (R&D Systems, Minneapolis, MN).

The mean minimal detectable dose of FGF-2 was 0.5 pg/mL (n = 9 assays). The method was linear between

0.5 and 64 pg/mL. The average correlation coefficient for the runs was 0.99. The intraassay coefficients of variation for low- and high-dose calibration standards or human diabetic plasma samples were less than or equal to 8%; the interassay coefficient of variation(s) for patient samples or calibration standards ranged from 10% to 14%. Recovery of bFGF-IR in diluted (1:2) samples of normal human plasma ranged from 108% to 123%. The dilution curves of patient plasma samples were parallel to the standard curve. Acidic FGF, FGF-4 (hst), FGF-5, and FGF-6 did not cross-react in the assay. In prior studies that used the same bFGF-IR assay method, mean serum bFGF-IR in 15 healthy subjects (men and women, ranging from 39-74 years old) was 0.9 pg/mL (range, 0-4 pg/mL) [13].

Plasma bFGF-IR in 43 healthy male blood donors, aged 21 to 63 years, ranged from 0 to 4 pg/mL; and there was no effect of age on plasma bFGF level [14].

2.8. Cut point for "low" vs "high" bFGF-IR

We dichotomized around the value of 4.5 pg/mL, the previously reported upper limit in healthy adult men [14].

2.9. Statistics

Basic FGF-IR values were not normally distributed. The Wilcoxon rank sum test was used for group comparisons of bFGF-IR (Table 2), and the correlations reported are Spearman correlation coefficients. Cox proportional hazards regression analysis was used to model baseline risk factors associated with time to first postbaseline laser treatment. Modeling was performed with a set of clinical risk variables (age, diabetes duration, low vs high bFGF, history of hypertension, LDL cholesterol concentration, baseline HbA_{1c}) that was based upon published literature [15,16] and is known or likely to be associated with retinopathy or laser treatment. Backward elimination was used to obtain the best fit model using an α level not exceeding .05 as the cutoff for variable inclusion in the final model. Excluded variables with P values greater than .20 included age, history of hypertension, baseline HbA_{1c}, insulin use, ACE inhibitor use, and ARB use. Other excluded variables (LDL cholesterol concentration, glycemic treatment arm [standard or intensive], and duration of diabetes) had P values equal to .06.

Table 2
Correlations of baseline risk factors with plasma bFGF-IR

	Spearman correlation coefficient	P value
Age	0.04	.61
BMI	−0.04	.63
Diabetes duration	−0.01	.86
Systolic BP	−0.01	.87
HbA_{1c}	−0.14	.07
LDL cholesterol	−0.08	.34
ACR	0.01	.88
Waist-hip ratio	0.23	.003

LDL indicates low-density lipoprotein.

2.10. Protein A affinity chromatography

Protein A affinity chromatography was carried out as previously described [17]. Four-tenths–milliliter aliquots of plasma were adjusted to pH 8.0 by adding 0.8 mL 100 mmol/L Tris (pH 8). After syringe filtration to clarify samples, 1 mL was applied to a 1-mL column of packed protein A beads (Pierce Chemical, Rockford, IL) equilibrated in 100 mmol/L Tris (pH 8.0). The column was washed and eluted as previously described [17]. The eluate fractions containing nearly all the recovered protein were pH neutralized and stored at 0°C to 4°C. Inhibitory activity in protein A eluate fractions was unchanged, appearing in the retentate fraction after dialysis (10 mmol/L phosphate, pH 7.4) and ultrafiltration on a 10-kd cutoff membrane (Centricon-10; Millipore, Bedford, MA). All fractions were sterile filtered (Millipore, 0.2 μm) before assay for growth-promoting activity.

2.11. Cell culture and growth assays

Bovine pulmonary artery endothelial cells (Clonetics, San Diego, CA) were maintained at 37°C in 5% CO_2/95% air in endothelial cell growth medium (Clonetics) plus 10% fetal bovine serum. Bovine pulmonary artery cells were passaged continuously and used between passages 4 and 10.

2.12. Colorimetric estimation of endothelial cell number

Colorimetric estimation of cell number was carried out as previously reported [17]. Confluent cells were trypsinized and plated at 10^3 to 10^4 cells per well in medium 199 plus 10% fetal calf serum in 96-well plates. After up to 4 days of incubation for cells to reach 60% to 80% confluency, test fractions (1:50 dilution of protein A eluates of plasma) were added to wells in quadruplicate. After 2 days of incubation in the presence of test fractions, cells were washed with phosphate-buffered saline and processed for the colorimetric estimation of cell number, that is, cell-associated acid phosphatase activity, as previously described [17]. There was a linear relationship between endothelial cell number and optical density at 410 nm as previously described [17]. Growth-promoting activity is expressed as a percentage of the control cell number for cells grown in the absence of test protein A eluate fractions. Each point represents the mean of quadruplicate determinations. The intra- and interassay coefficients of variation were 4% and 7% at 1:50 dilution of protein A–eluted fractions from plasma of 3 diabetic subjects (n = 3 assays in each patient).

2.13. Heparin Sepharose affinity chromatography

Heparin affinity chromatography was performed on protein A eluates from diabetic plasma that had been adjusted to pH 7.4 as previously described [18]. After applying the protein A eluate (starting material), the column was washed extensively with starting buffer containing 10 mmol/L phosphate and 0 mol/L NaCL (pH 7.4) and then eluted stepwise with 2 column volumes each of 0.1, 0.5, 1,

and 2 mol/L NaCL. The flow through (FT) and eluate fractions were assayed in quadruplicate for growth promotion in endothelial cells.

3. Results

3.1. Relation of bFGF-IR to baseline characteristics

There was no association between plasma bFGF-IR and patient age, body mass index (BMI), diabetes duration, systolic blood pressure, urine albumin-creatinine ratio, or serum LDL cholesterol concentration (Table 2). There was a marginal (P = .07) inverse association between plasma bFGF-IR and baseline HbA_{1c} (Table 2). There was a significant association between plasma bFGF and waist-hip ratio (P = .003, Table 2). High plasma bFGF was significantly associated with baseline thiazide diuretic use (P = .01, Table 3). There was no significant association between low bFGF and any other categorical risk factor shown in Table 3 including various classes of antidiabetic or antihypertensive medication use.

Table 3
Associations between low vs high bFGF and baseline categorical risk factors

Variable	bFGF ≤4.4	bFGF ≥4.5	P value[a]
Demographics			
Male	97.8	96.3	.58
Hispanic	13.3	22.0	.14
Non-Hispanic white	62.2	63.4	.87
African American	23.3	13.4	.10
Current smoker	15.6	19.5	.49
Baseline medications			
β-Blocker	13.3	8.5	.32
ACE inhibitor	68.9	67.1	.80
ARB	8.9	4.9	.30
Calcium channel antagonist	22.2	18.3	.52
Thiazide diuretic	10.0	25.6	.01
Statin	66.7	61.0	.44
Fibrate	14.4	23.2	.14
Thiazolidinedione	22.2	18.3	.52
Insulin	46.7	46.3	.97
Sulfonylurea	64.4	63.4	.89
Metformin	76.7	75.6	.87
Thyroid hormone	6.7	6.1	.88
History			
Hypertension	68.2	77.2	.19
Myocardial infarction	10.23	16.5	.23
Coronary revascularization	23.3	13.4	.10
Any macrovascular event (MI, CABG, angina, stroke, PVD)	40.9	34.2	.37
Albuminuria (ACR)			
Macro >300 mg/g	9.2	10.1	.89
Micro 30-299 mg/g	26.4	29.1	
Normo < 30 mg/g	64.4	60.8	

Results are percentage of patients. MI indicates myocardial infarction; CABG, coronary artery bypass graft; PVD, peripheral vascular disease.
[a] P values from χ^2 test.

Table 4
Cumulative first laser treatment for low- and high-bFGF group

Maximum years of follow-up	Total first laser event		P value[a]
	Low bFGF	High bFGF	
4	16 (19)	4 (6)	.03
5	18 (21)	6 (8)	.055
6	18 (21)	7 (10)	.056

Results are number (percentage) of patients affected.
[a] Log-rank test.

3.2. Time to first laser occurrence

Over 4 years of study treatment, first laser treatment was significantly more likely to be required in patients with low compared with high baseline bFGF (19% vs 6%, P = .03 for the difference, Table 4). Extending the possible follow-up time to 5 years, first laser treatment was marginally significantly more likely to be needed in patients with low compared with high baseline bFGF (21% vs 8%, P = .055, Table 4). The best fit model of risk factors associated with the time to first laser treatment during 4 years of follow-up included as significant predictors the following: bFGF (low vs high) (hazard ratio [HR], 5.01; P = .012), duration of diabetes (HR, 1.05; P = .050), and LDL cholesterol concentration (HR, 0.98; P = .027) (Table 5). The same variables, bFGF (low vs high) (HR, 3.49; P = .016), duration of diabetes (HR, 1.06; P = .009), and LDL cholesterol concentration (HR, 0.98; P = .023) (Table 5), also were significantly associated with time to first laser after up to 5 years of study treatment. In Cox proportional hazards regression models that adjusted for diabetes treatment group, ACE inhibitor, ARB use, or insulin treatment, bFGF (low vs high) (HR, 4.08; P = .025) was the only variable significantly associated with time to first laser after 4 years of study treatment.

3.3. Lack of association between plasma bFGF and baseline retinopathy stage

There was no significant association between low baseline bFGF and baseline retinopathy stage or the baseline presence or absence of macular edema (Table 6). In Cox proportional hazards regression models that adjusted for baseline

Table 5
Cox proportional hazard regression models of time to first laser occurrence

Variable	HR	95% CI	P value
4 y postbaseline			
Plasma bFGF-IR (low vs high)	5.01	1.43-17.46	.012
Diabetes duration	1.05	1.00-2.72	.050
LDL cholesterol	0.98	0.97-1.00	.027
5 y postbaseline			
Plasma bFGF-IR (low vs high)	3.49	1.26-9.58	.016
Diabetes duration	1.06	1.01-1.10	.009
LDL cholesterol	0.98	0.97-1.00	.023

n = 156 subjects. Results nearly identical to those after 5 years of follow-up were obtained after extending the possible follow-up time to 6 years. CI indicates confidence intervals.

Table 6
Association between bFGF and baseline ophthalmologic results in 172 patients

Variable	bFGF ≤4.4	bFGF ≥4.5	*P* value[a]
No or minimal retinopathy	35	38	.78
Mild-moderate retinopathy	24	22	.87
Severe nonproliferative retinopathy	14	12	.94
Macular edema	11	5	.19
Proliferative retinopathy	7	4	.47

Results are number of affected subjects.
[a] *P* value from *t* test.

indicators for laser treatment, proliferative retinopathy (HR, 29.11; $P = .0002$) and macular edema (HR, 8.17; $P = .0036$), but not basic FGF (low vs high) (HR, 3.44; $P = .10$), were significantly associated with time to first laser treatment.

3.4. Endothelial cell inhibitory autoantibodies in plasma from low or undetectable bFGF

We compared inhibitory bioactivity in endothelial cells in a 1/50th dilution of the protein A eluate fraction from plasma of diabetic subjects with macular edema and those without significant retinopathy who did not differ significantly in their baseline clinical characteristics (Table 7). Average inhibitory growth-promoting activity in the protein A eluates from diabetic maculopathy plasmas (n = 7) (72% ± 20%) significantly exceeded average growth-promoting activity in the protein A eluates from plasma of diabetic subjects with no or minimal retinopathy (n = 7) (101% ± 8%, $P = .004$ for the difference) (Table 7). The protein A eluates from plasma were subjected to heparin Sepharose (HS) affinity chromatography (Pharmacia Biotech, Uppsala, Sweden). In the protein A eluate of plasma from a representative diabetic patient with macular edema, significant inhibitory activity in endothelial cells eluted at 0.5, 1, and/or 2 mol/L NaCL from an HS column (eg, Fig. 1A). Average peak inhibitory endothelial cell activity in the protein A eluates of plasma from diabetic macular edema (n = 6) significantly exceeded average peak inhibitory activity in the protein A eluates of plasma from diabetics with no or minimal retinopathy (n = 6, $P = .0001$, Fig. 1D). There was no difference in the average FT activity from HS columns in protein A eluates from diabetic subjects with macular edema and those without retinopathy (Fig. 1D). The protein A eluate of plasma from a diabetic subject with proliferative retinopathy displayed significant inhibitory activity eluting at 0.5 mol/L NaCL and significant stimulatory activity in endothelial cells eluting at 1 mol/L NaCL from an HS column (Fig. 1B).

4. Discussion

The present data suggest a novel association between low baseline plasma bFGF-IR and the need for first laser photocoagulation in patients with long-standing type 2 diabetes mellitus. The increased requirement for laser treatment in patients with low baseline plasma bFGF persisted for up to 5 years after initiation of study treatment despite the known strong influence of duration of diabetes (Table 5). Low plasma bFGF was still significantly associated with the need for laser treatment after adjusting for standard vs intensive glycemic treatment arm and for antihypertensive medications (ACE inhibitors, ARBs) shown to lower bFGF [11] and possibly slow the progression of retinopathy [19-21]. Our data are consistent with the possibility that low plasma bFGF may signify the presence of an additional risk factor or factors, for example, autoantibodies inhibitory in endothelial cells, which may contribute to the need for laser treatment.

Basic FGF did not correlate significantly with several baseline risk factors associated with retinopathy. A marginal inverse association between baseline HbA_{1c} and plasma bFGF (Table 2) suggests that baseline poor glycemic control may contribute to low bFGF. Glycosylated hemoglobin was inversely associated with plasma vascular endothelial growth factor (VEGF) level in a population of older Japanese adults with advanced type 2 diabetes mellitus [22]. It is possible that shared or different mechanisms may account for the unexpected low levels of the 2 heparin-binding angiogenesis factors bFGF and VEGF in subsets of advanced type 2 diabetes mellitus.

In prior studies, plasma bFGF was increased in association with activation in the renin-angiotensin system, such as occurs in subsets of type 2 diabetes mellitus with persistent micro- or albuminuria [11] and/or essential hypertension [23]. The present finding of significant associations between high plasma bFGF and waist-hip ratio or thiazide diuretic use is consistent with visceral adipose tissue [24] or renin-angiotensin system activation [25] as sources for increased plasma bFGF. Waist-hip ratio was significantly associated with an increased need for laser treatment in a recent large study of less advanced type 2 diabetes mellitus [26]. We cannot exclude the possibility that high plasma bFGF, a

Table 7
Baseline characteristics in 14 representative subjects with macular edema or no retinopathy with low or undetectable plasma bFGF

Subject group	Age (y)	Duration of diabetes (y)	HbA_{1c} (%)	ACR (mg/g)	bFGF (pg/mL)	Growth activity (%)[a]
Macular edema (n = 7)	64 ± 5	13 ± 4	8.3 ± 1.4	322 ± 381	0 ± 0	72 ± 20
No retinopathy (n = 7)	59 ± 11	8 ± 6	9.7 ± 1.2	45 ± 49	0.7 ± 1.3	101 ± 8
P value	.27	.11	.08	.08	.16	.004

P value from *t* tests.
[a] Percentage of basal endothelial cell number after 48 hours of incubation with a 1/50th dilution (30 μg/mL) of the protein A eluate fraction from plasma.

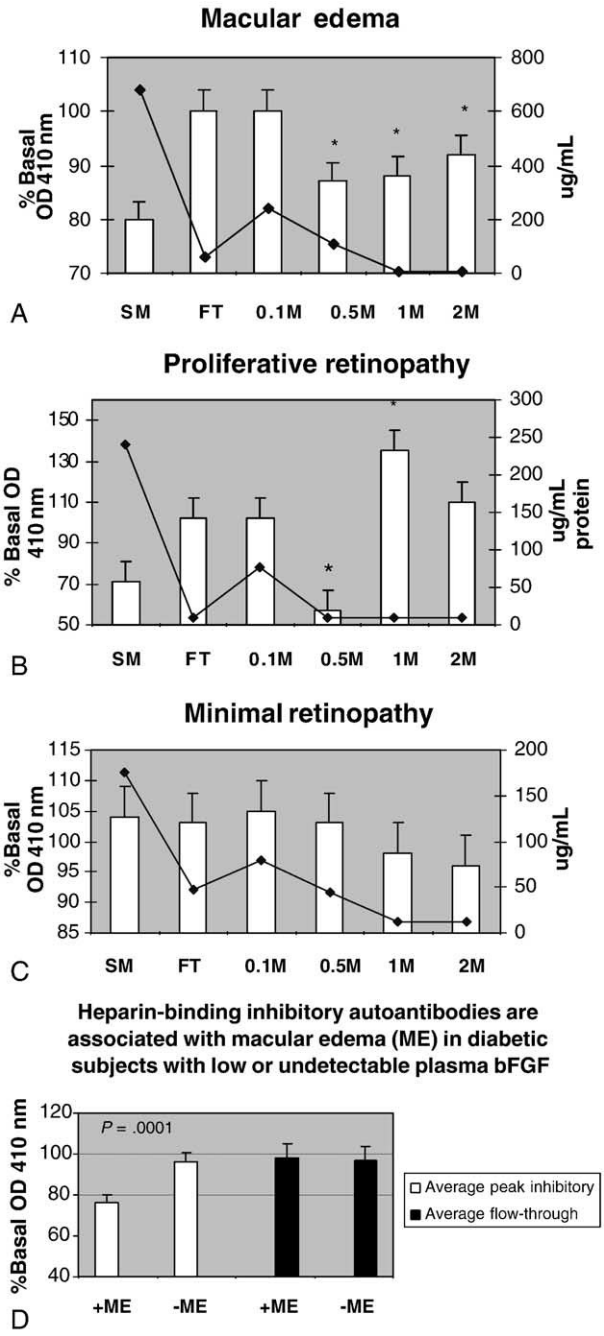

Fig. 1. Heparin Sepharose affinity chromatography of protein A eluate fractions from plasma of representative diabetic subjects with (A) macular edema, (B) proliferative retinopathy, and (C) minimal or no retinopathy. D, Average results in 12 patients with low or undetectable plasma bFGF: 6 with macular edema (ME) (A) or 6 with minimal retinopathy (-ME) (C). Heparin Sepharose chromatography was carried out as described in "Subjects and methods." Growth-promoting activity was assessed as change in cell number as described in "Subjects and methods." Peak inhibitory activity represents results (percentage basal OD_{410}), with the fraction eluting from HS showing the most inhibitory activity on the growth of endothelial cells (eg, 0.5 mol/L, A). Flow through activity represents results with the fraction not retained on the HS column (eg, FT). *$P < .05$ compared to basal cell number.

potent angiogenesis factor, or other adipocytokines may contribute to the mechanism for increased laser risk in earlier stages of type 2 diabetes mellitus. In our VADT substudy group, decreases in high plasma bFGF in association with aggressive treatment of blood pressure and glycemia may have accounted in part for the lack of a direct association between baseline high plasma bFGF and the subsequent need for laser photocoagulation. Yet randomization to the intensive VADT treatment arm itself was associated with only a marginally significant lower subsequent risk for laser treatment ($P = .06$).

Basic FGF does not normally circulate [10], but it increases in plasma from a variety of cancers [27]. In prior work, we described potent endothelial cell inhibitory autoantibodies in plasma from subsets of advanced cancer [17]. Because many of the same cancer subjects had low or undetectable plasma bFGF, in the current study, we tested plasma from advanced type 2 diabetes mellitus with low plasma bFGF for endothelial cell inhibitory autoantibodies. We found a significant association ($P < .0001$) between baseline plasma bFGF-IR and inhibitory endothelial cell activity in the protein A–eluted (immunoglobulin G) fractions from diabetic plasma [28]. The endothelial cell inhibitory autoantibodies displayed increased affinity for heparin, suggesting that they may bind to and modulate the bioavailability of the heparin-binding growth factor bFGF. Heparan sulfate proteoglycans are low-affinity receptors for bFGF that are abundant in endothelial cells [29]. Heparan sulfate proteoglycans are also known targets for autoimmunity [30,31]. The elaboration of heparan sulfate proteoglycans [32] from the renal glomerulus is thought to contribute to generalized microvascular damage in diabetes [33,34].

Endothelial cell–binding antibodies were reported with increased frequency in subsets of type 1 diabetes mellitus and proliferative retinopathy [35]. In a recent study in diabetic pregnancy subjects, low serum VEGF level was reported in association with proliferative diabetic retinopathy; but the mechanism for this association was not clear [36]. Our preliminary data suggest a possible dual role for heparin-binding endothelial cell inhibitory autoantibodies and high-affinity heparin-binding growth factors in the mechanism for proliferative retinopathy in some patients with advanced type 2 diabetes mellitus (eg, Fig. 1B). Aggressive treatment with insulin resulted in increased plasma bFGF (5.5 pg/mL) in a patient with low baseline bFGF (0.2 pg/mL) who later required laser treatment of proliferative retinopathy (Fig. 1B). The increased bFGF plasma contained both heparin-binding endothelial cell inhibitory antibodies of moderate affinity (eluted with 0.5 mol/L NaCL, Fig. 1B) and stimulatory endothelial cell bioactivity in the protein A eluate fractions eluting with 1 or 2 mol/L NaCL from HS (Fig. 1B). Vascular endothelial growth factor characteristically elutes with 0.6 to 1.2 mol/L NaCL [37], whereas bFGF requires 2 mol/L NaCL to be dissociated from HS [7]. We cannot exclude the possibility that VEGF, or synergistic interactions between

bFGF and VEGF [38], may have contributed to neovascularization or other worsening retinal pathology requiring laser in a subset of patients.

Diabetic macular edema is the leading cause of visual impairment in type 2 diabetes mellitus [1]. It may go unrecognized for substantial periods in type 2 diabetes mellitus [39]. Proliferative diabetic retinopathy requires immediate intervention, as it is associated with a high risk for visual loss. The baseline prevalences of proliferative diabetic retinopathy (5.2%) and macular edema (10.3%) are consistent with the prevalences reported in older adults with advanced type 2 diabetes mellitus [39,40]. Our heterogeneous study group included 60% non-Hispanic white patients who had a higher baseline prevalence of cardiovascular disease and significantly lower mean baseline LDL cholesterol concentration compared with the cardiovascular disease prevalence and baseline LDL levels among African American and Hispanic subjects. The significant inverse association between LDL cholesterol concentration and the need for laser treatment in our study group (Table 5) is likely to reflect confounding by one or more factors associated with non-Hispanic white race.

The limitation of our study is that it is small and the results are only applicable to men with long-standing diabetes. Still, the present data of an association between high baseline HbA_{1c} and low bFGF may be consistent with findings from the Diabetes Control and Complication Trial or the Fenofibrate Intervention and Event Lowering in Diabetes study, respectively, that a baseline parameter, for example, prior poor glycemic level, can significantly affect the risk for progression of retinopathy [41] or the need for laser treatment [26].

In summary, we have provided evidence that low baseline plasma bFGF, although not a specific marker, may yet indicate the presence of heparin-binding endothelial cell inhibitory autoantibodies in plasma from adults with advanced, poorly controlled type 2 diabetes mellitus.

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References

[1] Girach A, Lund-Andersen H. Diabetic macular oedema: a clinical overview. Int J Clin Pract 2007;61:88-97.

[2] Aroca PR, Salvat M, Fernandez J, Mendez I. Risk factor for diffuse and focal macular edema. J Diabet Complications 2004;18:211-5.

[3] Grant MB, Afzal A, Spoerri P, Pan H, Shaw LC, Mames RN. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opin Investig Drugs 2004:1275-9.

[4] Aiello LP, Wong JS. Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int Suppl 2000;77:S113-9.

[5] Nguyen QD, Tatlipinar S, Shah SM, Haller JA, Quinlan E, Sung J, et al. Vascular endothelial growth factor is a critical stimulus for diabetic macular edema. Am J Ophthalmol 2006;142:961-9.

[6] Boulton M, Gregor Z, McLeod D, Charteris D, Jarvis-Evans J, Moriarty P, et al. Intravitreal growth factors in proliferative diabetic retinopathy: correlation with neovascular activity and glycaemic management. Br J Ophthalmol 1997;81:228-33.

[7] Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. Endocr Rev 1987;8:95-114.

[8] Schweigerer L, Neufeld G, Friedman J, Abraham JA, Fiddes JC, Gospodarowicz D. Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature 1987; 325:257-9.

[9] Folkman J, Klagsbrun M. Angiogenic factors. Science 1987;235: 442-7.

[10] Esch F, Baird A, Ling N, Ueno N, Hill F, Denoroy L, et al. Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. Proc Nat Acad Sci U S A 1985;19:6507-11.

[11] Zimering MB, Eng J. Increased basic fibroblast growth factor–like substance in plasma from a subset of middle-aged or elderly male diabetic patients with microalbuminuria or proteinuria. J Clin Endo Metab 1996;81:4446-52.

[12] Abraira C, Duckworth W, McCarren M, Emanuele N, Arca D, Reda D, et al. Design of the cooperative study of glycemic control and complications in diabetes mellitus type 2. J Diabet Complications 2003;17:314-22.

[13] Zimering MB. Effect of intravenous bisphosphonates on release of basic fibroblast growth factor in serum of patients with cancer-associated hypercalcemia. Life Sci 2002;70:1-14.

[14] Larsson A, Skoldenberg E, Ericson H. Serum and plasma levels of FGF-2 and VEGF in healthy blood donors. Angiogenesis 2002;5:107-10.

[15] Higgins GT, Khan J, Pearce IA. Glycaemic control and control of risk factors in diabetes patients in an ophthalmology clinic: what lessons have we learned from the UKPDS and DCCT studies? Acta Ophthalmol Scand 2007;85:772-6.

[16] Miljanovic B, Glynn RJ, Nathan DM, Manson JE, Schaumberg DA. A prospective study of serum lipids and risk of diabetic macular edema in type 1 diabetes. Diabetes 2004;53:2883-92.

[17] Zimering MB, Thakker-Varia S. Increased fibroblast growth factor–like autoantibodies in serum from a subset of patients with cancer-associated hypercalcemia. Life Sci 2002;71:2939-59.

[18] Zimering MB, Brandi ML, deGrange DA, Marx SJ, Streeten E, Katsumata N, et al. Circulating fibroblast growth factor–like substance in familial multiple endocrine neoplasia type 1. J Clin Endocrinol Metab 1990;70:149-54.

[19] Chaturvedi N, Sjolie AK, Stephenson JM, Abrahamian H, Keipes M, Castellarin A, et al. Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes. The EUCLID Study Group. EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus. Lancet 1998;351:28-31.

[20] Sjølie AK. Prospects for angiotensin receptor blockers in diabetic retinopathy. Diabetes Res Clin Pract 2007;76(Suppl 1):S31-9.

[21] Chaturvedi N, Fuller JH, Pokras F, Rottiers R, Papazoglou N, Aiello LP, et al. Circulating plasma vascular endothelial growth factor and microvascular complications of type 1 diabetes mellitus: the influence of ACE inhibition. Diabet Med 2001:288-94.

[22] Shimada K, Baba T, Neugebauer S, Onozaki A, Yamada D, Midorikawa S, et al. Plasma vascular endothelial growth factor in

Japanese type 2 diabetic patients with and without nephropathy. J Diabetes Complications 2002;16:386-90.
[23] Cottone S, Vadalà A, Mangano MT, Riccobene R, Vella MC, Neri AL, et al. Endothelium-derived factors in microalbuminuric and nonmicroalbuminuric essential hypertensives. Am J Hypertens 2000;13:172-6.
[24] Teichert-Kuliszewska K, Hamilton BS, Deitel M, Roncari DA. Augmented production of heparin-binding mitogenic proteins by preadipocytes from massively obese persons. J Clin Invest 1992;90: 1226-31.
[25] Lijnen P, Fagard R, Staessen J, Amery A. Effect of chronic diuretic treatment on the plasma renin-angiotensin-aldosterone system in essential hypertension. Br J Clin Pharmacol 1981;12:387-92.
[26] Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, et al. Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. Lancet 2007; 370:1687-97.
[27] Dirix LY, Vermeulen PB, Pawinski A, Prové A, Benoy I, De Pooter C, et al. Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients. Br J Cancer 1997;76:238-43.
[28] Zimering MB, Anderson RJ, Ge L, Moritz T, Pardun J, the VADT Substudy Group. Association between endothelial cell inhibitory autoantibodies and laser treatment for retinopathy in a baseline subset from the Veterans Affairs Diabetes Trial. Endocr Soc 2008;OR50-4:163.
[29] Vlodavsky I, Miao HQ, Medalion B, Danagher P, Ron D. Involvement of heparan sulfate and related molecules in sequestration and growth promoting activity of fibroblast growth factor. Cancer Metastasis Rev 1996;15:177-86.
[30] Fillit H, Lahita R. Antibodies to vascular heparan sulfate proteoglycan in patients with systemic lupus erythematosus. Autoimmunity 1991;9: 159-64.
[31] Fillit H, Mulvihill M. Association of autoimmunity to vascular heparan sulfate proteoglycan and vascular disease in the aged. Gerontology 1993;39:177-82.
[32] Reddi AS, Ramamurthi R, Miller M, Dhuper S, Lasker N. Enalapril improves albuminuria by preventing glomerular loss of heparan sulfate in diabetic rats. Biochem Med Metab Biol 1991;45:119-31.
[33] Jensen T. Pathogenesis of diabetic vascular disease: evidence for the role of reduced heparan sulfate proteoglycan. Diabetes 1997;46 (Suppl 2):S98-S100.
[34] Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage: the Steno hypothesis. Diabetologia 1989;32:219-26.
[35] Jones DB, Wallace R, Frier BM. Vascular endothelial cell antibodies in diabetic patients. Association with diabetic retinopathy. Diabetes Care 1992;15:552-5.
[36] Weiss AG, Chacko DM, Lane PH, Margalit E, Thompson AF, Mack-Shipman LR, et al. Vascular endothelial growth factor, soluble vascular endothelial growth factor receptor–1, and progression of diabetic retinopathy in pregnant patients with type 1 diabetes. Endocr Soc 2007: P3-P160.
[37] Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989;161:851-8.
[38] Pepper MS, Ferrara N, Orci L, Montesano R. Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro. Biochem Biophys Res Commun 1992;189:824-31.
[39] Hove MN, Kristensen JK, Lauritzen T, Bek T. The prevalence of retinopathy in an unselected population of type 2 diabetes patients from Arhus County, Denmark. Acta Ophthalmol Scand 2004;82:443-8.
[40] Kempen JH, O'Colmain BJ, Leske MC, Haffner SM, Klein R, Moss SE, et al. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol 2004;122:552-63.
[41] Zhang L, Krzentowski G, Albert A, Lefebvre PJ. Risk of developing retinopathy in Diabetes Control and Complications Trial type 1 diabetic patients with good or poor metabolic control. Diabetes Care 2001;24:1275-9.