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Increased plasma basic fibroblast growth factor is associated with coronary heart disease in adult type 2 diabetes mellitus

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Abstract

Basic fibroblast growth factor (bFGF) is a potent endothelial and smooth muscle cell mitogen that does not normally circulate. Plasma bFGF-like bioactivity was increased in association with persistent microalbuminuria (a risk marker for cardiovascular disease) in adult type 2 diabetes mellitus. In the present study, we tested whether baseline plasma bFGF immunoreactivity (IR) predicts the occurrence of a subset of cardiovascular disease outcomes in adults with advanced type 2 diabetes mellitus from the Veterans Affairs Diabetes Trial (mean: age, 59 years; diabetes duration, 11 years; baseline hemoglobin A_{1c}, 9.5%). Plasma bFGF-IR was determined with a sensitive and specific 2-site enzyme-linked immunoassay in 399 patients at the baseline visit. These results were then evaluated as possible predictors of the occurrence of prespecified cardiovascular or coronary heart disease end points. There was a borderline-significant association ($P = .07$) between plasma bFGF-IR and the main study cardiovascular disease outcome (myocardial infarction, congestive heart failure, cerebrovascular accident, amputation, cardiovascular death, coronary, cerebrovascular or peripheral revascularization, and inoperable coronary artery disease). Plasma bFGF-IR was significantly associated with the occurrence of coronary heart disease ($P = .01$). After adjusting for clinical risk factors, bFGF (hazard ratio [HR], 1.013; 95% confidence interval [CI], 1.007-1.019; $P < .0001$), prior macrovascular event (HR, 3.55; 95% CI, 2.154-5.839; $P < .0001$), and duration of diabetes (HR, 1.041; 95% CI, 1.012-1.071; $P = .0055$) were all significantly associated with time to first postrandomization coronary heart disease occurrence. These results suggest that increased plasma bFGF-IR may be a novel risk marker for coronary heart disease occurrence in adult men with advanced type 2 diabetes mellitus.
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1. Introduction

Coronary heart disease (CHD) is the leading cause of death in adults in the United States [1], and adults with type 2 diabetes mellitus have a disproportionately high rate of CHD morbidity and mortality [2]. Dyslipidemia, hypertension, and prothrombotic and proinflammatory factors each contribute to accelerated atherosclerosis in obese type 2 diabetes mellitus [3]. Still, the pathophysiologic mechanisms accounting for a substantially increased risk for CHD in adult type 2 diabetes mellitus remain unclear.

A possible role for hyperglycemia as a mediator of cardiovascular disease (CVD) risk was recently explored in the Veterans Affairs Diabetes Trial (VADT). Seventeen hundred ninety-one older adults with advanced type 2 diabetes mellitus were randomized to standard (STD) or intensive (INT) glycemic treatment groups in which blood pressure (BP) and lipids were maintained at similar, desirable levels [4]. After an average of 6 years of treatment, CVD outcomes did not differ significantly according to original glycemic treatment group assignment [5]. The current report is from a planned secondary analysis to the VADT that investigated whether plasma basic fibroblast growth factor (bFGF), an angiogenic growth factor implicated in early atherosclerosis, may predict the occurrence of CHD in adult type 2 diabetes mellitus.

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Basic fibroblast growth factor is a potent mitogen in endothelial cells and smooth muscle cells that is released after endothelial injury [6] and is capable of inducing smooth muscle cell migration and proliferation important in neointima formation [7,8]. Plasma bFGF is low or undetectable in healthy subjects [9], but increases in microalbuminuric adult type 2 diabetes mellitus [10] and in coronary artery disease [11]. Micro- and macroalbuminuria are markers of diffuse endothelial damage [12] associated with increased CVD mortality in adult diabetes [13]. To our knowledge, however, there has been no prior report of plasma bFGF itself as a possible marker for cardiovascular or CHD risk in adult type 2 diabetes mellitus.

We now report on evidence of a significant association between baseline plasma bFGF immunoreactivity (IR) and the first postbaseline occurrence of CHD events in a subset of 399 patients undergoing VADT treatment for an average of 6 years. This is the first evidence that substantially increased plasma bFGF-IR is associated with an increased risk for CHD morbidity and mortality in middle-aged or older adults with advanced type 2 diabetes mellitus.

2. Subjects and methods

2.1. Study subjects

The study design and clinical inclusion and exclusion criteria for the main VADT have been previously reported [4,5]. Eligible patients without renal insufficiency and without congestive heart failure were randomly assigned to STD vs INT glycemic treatment. Randomization was jointly stratified according to baseline insulin use (yes/no) and occurrence of macrovascular event before baseline (yes/no). The main VADT study end point was a pooled CVD outcome that included ischemic coronary artery disease, congestive heart failure, cerebrovascular disease, and peripheral arterial disease events. The VADT 465 B substudy prespecified CHD (cardiovascular mortality, myocardial infarction, coronary revascularization, and inoperable coronary artery disease) as the primary outcome that would encompass myocardial infarction and closely related ischemic coronary events. Informed consent for the Investigational Review Board–approved substudy was obtained at 6 outpatient sites from 399 diabetic subjects who had consented to participate in the main VADT. 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. Archived, coded frozen EDTA plasma from consecutively enrolled patients was shipped to the laboratory of Dr Zimering (VA 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, and 96% were men.

2.2. Medications

As previously reported, all patients were taking antidiabetic medications at baseline, including oral agents and/or

Table 1
Baseline and follow-up characteristics in the 399 study patients

Variable	Baseline	Follow-up[a]	*P* value
Age	59.3 ± 8.5		
Diabetes duration	11.4 ± 7.6		
Male	96%		
Prior CV event	37%		
Hypertension	70%		
Race			
Non-Hispanic white	57%		
African American	21%		
Hispanic	18%		
Current smoking	18%	12%	<.0001 (n = 397)
bFGF(pg/mL)	15.1 ± 25.8 (n = 399)	8.0 ± 16.3[b] (n = 215)	.0014 (n = 215)
HbA_{1c} (%)	9.5 ± 1.4	8.2 ± 1.6	<.0001 (n = 396)
Systolic BP (mm Hg)	130.9 ± 17.4	126.9 ± 17.3	.0002 (n = 393)
BMI (kg/m^2)	30.9 ± 4.6	32.3 ± 5.7	<.0001 (n = 397)
Weight (lb)	212.7 ± 37.3	221.5 ± 43.5	<.0001 (n = 397)
Waist to hip ratio	0.991 ± 0.07	0.998 ± 0.07	.0171 (n = 364)
Total cholesterol (mg/dL)	183 ± 44	156 ± 41	<.0001 (n = 379)
LDL cholesterol (mg/dL)	108 ± 33	83 ± 33	<.0001 (n = 369)
HDL cholesterol (mg/dL)	37 ± 10	41 ± 12	<.0001 (n = 379)
Triglycerides (mg/dL)	195 ± 175	158 ± 122	<.0001 (n = 379)
Serum creatinine (mg/dL)	0.99 ± 0.2	1.2 ± 0.5	<.0001 (n = 393)

Results for continuous variables are displayed as mean ± SD. HbA_{1c} indicates hemoglobin A_{1c}.

[a] Year 5 or last annual visit before study termination.

[b] Year 1 annual visit.

insulin [4]. Patients randomized to the STD or INT glycemic treatment group were both treated with similar classes of antidiabetic medications (but at different doses), 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. Statins were used by 62% of patients at baseline. All patients were encouraged to take aspirin 81 to 162 mg, unless otherwise contraindicated.

2.3. Study outcomes

Cardiovascular disease outcomes were adjudicated by an independent Study Endpoints Committee as previously described [5]. *Cardiovascular disease* is defined as myocardial infarction, congestive heart failure, cerebrovascular accident, amputation, cardiovascular death, coronary revascularization, cerebrovascular revascularization, peripheral revascularization, or inoperable coronary artery disease. *Coronary heart disease* is defined as myocardial infarction, coronary revascularization, inoperable coronary artery disease, or cardiovascular death. Baseline determination of plasma bFGF-IR (assayed at VA New Jersey Health Care System) was masked to the information about study end point occurrence.

The association of risk factors with time to first postbaseline cardiovascular (CVD) or CHD outcome was modeled using the 399 subjects for whom such postrandomization data were available.

2.4. Laboratory and clinical measures

Urinary microalbumin, plasma hemoglobin A_{1c}, and urine creatinine were determined by standard methods; and plasma total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were determined by standardized direct enzymatic assay methods, which have been previously described [4]. Urinary albumin to creatinine ratio was calculated as albumin concentration/creatinine concentration × 100. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation only for those samples with plasma triglyceride concentration less than 400 mg/dL. Blood pressure was recorded in the seated position after a 5-minute rest. Three consecutive readings were obtained; and the median value of the 3 consecutive determinations, computed separately for systolic and diastolic BP, was used for analysis.

2.5. Plasma samples

2.5.1. Basic fibroblast growth factor assays

Basic fibroblast growth factor IR in plasma was determined using a sensitive and specific 2-site enzyme-linked immunoassay (R and D Systems, Minneapolis, MN). Details about the assay performance characteristics have been previously reported [14]. Plasma bFGF-IR and bFGF-like bioactivity have previously been shown to be stable for 5 years or longer at −20°C and for up to 3 freeze-thaw cycles [15]. Among 43 healthy male blood donors aged 21 to 63 years, plasma bFGF-IR was shown to range between 0 and 4 pg/mL; and there was no effect of age on plasma bFGF level [16].

2.6. Statistics

The VADT substudy estimated that a sample size of 400 subjects would have 90% power for detecting a 50% reduction in the event rates for either the CVD or CHD primary end points, which is equivalent to a risk ratio of 2.43 for high vs low bFGF-IR. The VADT was conducted using the intention-to-treat principle [5]. Both randomization stratification variables (baseline insulin use and baseline cardiovascular event) and glycemic treatment group were included as covariates in the model when testing for a bFGF effect.

Cox proportional hazards regression analysis was used to model the association between baseline risk factors and time to first postbaseline CHD occurrence. In univariate regression analysis, age, baseline HDL cholesterol concentration, baseline nephropathy, baseline creatinine concentration, and duration of diabetes were each significantly associated ($P < .0001$) with time to first CHD occurrence. These and other risk variables (non-Hispanic white race, baseline fibrinogen concentration) significantly associated ($P < .05$) with time to first CHD event were included as covariates in models that tested for a bFGF effect. Backward elimination was used to obtain the best fit model using an α level of less than .05 as the cutoff for variable retention in the final model. Baseline ACE inhibitor and ARB use each had a borderline-significant association ($P \leq .2$) with time to first CHD event and were included as covariates in models that tested for a bFGF effect. Baseline glycosylated hemoglobin ($P = .98$), current cigarette smoking ($P = .87$), and baseline plasminogen activator inhibitor–1 (PAI-1) concentration ($P = .57$) were not significantly associated with time to first postbaseline CHD occurrence in univariate regression analysis and so were not included as covariates in models that tested for a bFGF effect.

3. Results

3.1. Baseline and follow-up characteristics in the study patients

Our subject group included 57% non-Hispanic white, 21% African American, and 18% Hispanic patients. Thirty-seven percent of our study subjects reported a prior macrovascular event at study entry (Table 1). Study treatment was associated with significant decreases in glycosylated hemoglobin, systolic BP, plasma total cholesterol, LDL cholesterol, triglycerides, and current cigarette smoking after 5 years compared with baseline levels (Table 1, $P < .001$). Body weight, body mass index (BMI), waist to hip

Table 2
First postrandomization CVD event in 399 study patients

Individual events	n = 110
Congestive heart failure	16
Amputation	3
Peripheral revascularization	1
Cerebrovascular accident	7
Cerebrovascular revascularization	5
Myocardial infarction	28
Coronary revascularization	44
Inoperable coronary artery disease	3
Cardiovascular death	3

Coronary heart disease (myocardial infarction, n = 28; coronary revascularization, n = 49; inoperable coronary artery disease, n = 4; or cardiovascular death, n = 3) was the first postrandomization event in 84 subjects.

ratio, plasma HDL cholesterol, and serum creatinine concentration all increased significantly after 5 years compared with baseline levels (Table 1).

3.2. Frequency of occurrence of pooled end points

Eighty-four first CHD events occurred in 84 patients during an average of 6 years of VADT study treatment (Table 2). One hundred ten "first" CVD events occurred in 110 patients over the same period including 16 cases of congestive heart failure, 12 cases of cerebrovascular disease, and 4 cases of peripheral arterial disease (Table 2). There was a borderline-significant association (P = .07) between baseline plasma bFGF and time to first postbaseline CVD occurrence, and the strength of this association was not substantially affected by adjustment for prior cardiovascular event or glycemic treatment.

3.3. Association between plasma bFGF and first postbaseline occurrence of CHD

Plasma bFGF was significantly associated with time to first postbaseline CHD occurrence (P = .01). The best fit model of risk factors associated with the time to first CHD occurrence during up to 7.5 years of follow-up had the following as significant predictors: baseline plasma bFGF (hazard ratio [HR], 1.013; P < .0001), prior cardiovascular event (HR, 3.547; P < .0001), and duration of diabetes (HR, 1.041; P = .0055) (Table 3). There was no significant interaction between bFGF and either diabetes duration or glycemic treatment.

Table 3
Cox proportional hazard regression: time to first postbaseline CHD event

Variable	HR	95% CI	P value
Baseline bFGF	1.013	1.007-1.019	<.0001
Prior CV event	3.547	2.154-5.839	<.0001
Duration of diabetes	1.041	1.012-1.071	.0055

n = 375 subjects.

3.4. Comparison of plasma bFGF level effect on CHD event occurrence

A bFGF concentration greater than 50 pg/mL induces more than half-maximal stimulation of proliferation in endothelial cells and is required to cause significant migration and proliferation in smooth muscle cells in vitro [6]. We compared the survival curves for time to occurrence of a CHD event in groups of patients with baseline bFGF of 0 to 50 pg/mL vs bFGF greater than 50 pg/mL (Fig. 1). There was a statistically significant between-group difference (P = .03) in the time to occurrence of CHD (Fig. 1). At a bFGF level greater than 50 pg/mL, roughly two thirds of the total events had occurred after 2.5 years of study treatment; and no further events occurred after 4 years of follow-up (Fig. 1). At bFGF levels less than 50 pg/mL, events continued to occur up to 6 years after initiation of study treatment. The survival curves for the comparison of the effect of the 2 bFGF levels separate after approximately 2 years and reach their maximal separation after about 4 years of study treatment (Fig. 1).

3.5. Effect of 1-year study treatment on plasma bFGF

We were able to obtain measurement of plasma bFGF 1 year after initiation of study treatment in a subgroup of 215 consecutively enrolled subjects including nearly equal numbers of patients randomized to either STD or INT glycemic treatment. Baseline bFGF did not differ significantly for patients in the STD vs INT groups (Table 4). Mean plasma bFGF decreased significantly after 1 year in both the INT and STD treated patients (−32% and −46%), and the 1-year bFGF change was not statistically significantly different for INT vs STD treatment (Table 4).

3.6. Association between study treatment effect on bFGF and CHD occurrence

The 1-year bFGF average was not significantly associated with the time to occurrence of CHD (P = .157). There was no significant association between the 1-year difference in bFGF and CHD occurrence even after adjusting for prior cardiovascular event or glycemic treatment.

4. Discussion

The present data are the first to suggest an association between increased baseline plasma bFGF and the occurrence of CHD in adults with long-standing type 2 diabetes mellitus. The increased risk for CHD in patients with high baseline bFGF persisted during 5 to 7.5 years of study treatment despite substantial improvements in the levels of most traditional cardiovascular risk factors; and this significant association with CHD remained after adjusting for STD vs INT glycemic treatment, ACE inhibitor or ARB medication use, presence of a baseline cardiovascular event, and diabetes duration. Our data are consistent with the possibility

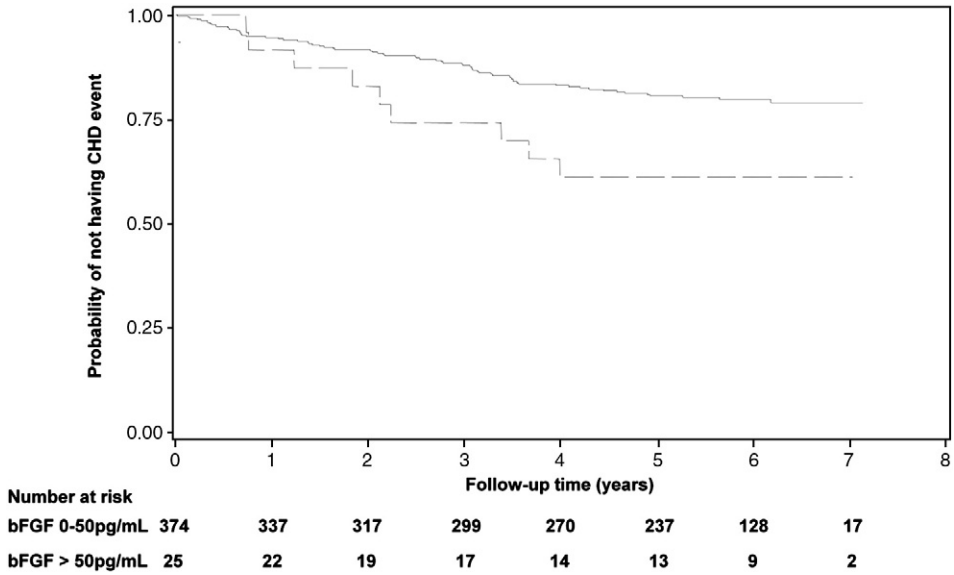

Fig. 1. Occurrence of CHD events by bFGF level. The difference in time to occurrence of first CHD event for bFGF groups was statistically significant; $P = .03$. Dashed line indicates group with plasma bFGF greater than 50 pg/mL; solid line, 0 to 50 pg/mL.

that substantially increased plasma bFGF may have a long-lasting effect on important mechanisms underlying CHD risk in older adults with advanced type 2 diabetes mellitus.

Basic FGF is one of the most potent known angiogenic factors [17]. However, lacking an amino terminal signal sequence necessary for efficient release from cells [9], bFGF does not normally circulate. Basic FGF is present in endothelial cells as well as in the extracellular matrix of vascular tissues where it is bound to heparan sulfate proteoglycan. After arterial vascular injury, bFGF is released locally and is thought to stimulate smooth muscle cell migration and proliferation important for neointima formation [8].

Plasma bFGF increases in a number of different cancers including renal cancer [18], consistent with kidney as a source of bFGF that can promote local tumor cell proliferation and angiogenesis [6]. Substantially increased plasma bFGF was also reported in children having HIV with nephropathy or hemolytic uremic syndrome [19]. In the latter syndrome, very high bFGF levels were postulated to reflect widespread endothelial injury and thrombosis [19]. Mean concentrations of plasma bFGF in the current study group are near the level reported to cause half-maximal proliferation in endothelial cells in vitro [6]. Substantially higher concentrations of plasma bFGF (>50 pg/mL) are consistent with doses reported to cause significant proliferation in smooth muscle cells [6]. Taken together, these data suggest that markedly increased bFGF, a locally acting mitogen normally involved in wound healing, may be capable of acting via the systemic circulation to promote cell proliferation and cell migration in atheromatous tissue that expresses FGF receptor [20].

Table 4
One-year change in plasma bFGF by treatment in 215 study patients

	Mean plasma bFGF			
Treatment	Baseline	Year 1	% Change (95% CI)	*P* value
STD (108)	15.0	8.1	−46 (−8 to −84)	.35
INT (107)	11.8	8.0	−32 (−5 to −60)	–

Number of subjects indicated in parentheses. $P = .35$ for comparison of mean change in bFGF, comparing STD vs INT.

The tissue sources of increased plasma bFGF in obese type 2 diabetes mellitus are not known. We reported a significant association between increased plasma bFGF and waist to hip ratio in the present VADT subset [14]. Human omentum is highly vascular; it contains substantial concentrations (2 μg/g) of a highly bioactive bFGF-like protein [21]. In addition, omental preadipocytes express high levels of bFGF messenger RNA [22]. Whether bFGF is released into the general circulation from omentum and what the mechanisms governing its release in viscerally obese type 2 diabetes mellitus are remain unanswered. Macrophages abundant in visceral fat secrete proteases capable of liberating bFGF from the extracellular matrix [23]. Proinflammatory visceral adipocytokines (tumor necrosis factor–α, interleukin [IL]-1, and interferon-γ) can substantially increase bFGF release from microvascular endothelial cells in vitro [24]. The release of proinflammatory adipocytokines is known to be regulated by angiotensin II [25], which itself increases bFGF expression in vascular smooth muscle cells [26]. Thus, angiotensin II–adipocytokine interactions may play a role in the amplification of plasma bFGF release from one or more injured microvascular beds in obese type 2 diabetes mellitus patients.

Poor glycemic control has been associated with several markers of inflammation, endothelial dysfunction, and/or oxidative stress, including PAI-1, C-reactive protein, tumor necrosis factor–α, IL-6, adhesion molecules, and reactive oxygen species [27]. Plasma and urinary levels of another broad-spectrum growth regulator, that is, transforming growth factor–β, have been shown to be associated with poor glucose control, elevated PAI-1, increased BMI, and diabetic nephropathy [28]. Existing data, although scant, suggest at least 4 reasons why it is unlikely that the present association between increased plasma bFGF and CHD occurrence is due to confounding by an association between bFGF and a marker of systemic inflammation. First, log baseline bFGF was significantly inversely associated with baseline glycosylated hemoglobin in the current study group [14]. Second, the procoagulant, proinflammatory protein fibrinogen was modestly associated with CHD risk in univariate regression analysis, but had no significant effect on the association between bFGF and CHD. Third, Larsson et al [29] reported no significant association (in human donor serum) between serum bFGF and either high-sensitivity C-reactive protein or serum amyloid protein, 2 inflammatory markers of cardiovascular risk. Finally, C-reactive protein, IL-6, and several other inflammatory markers of CHD risk are positively associated with BMI [27], whereas there was no significant association between bFGF and BMI [14].

Our results are consistent with a prior report that plasma bFGF was increased in patients with early coronary artery disease [11]. Prior evidence from a number of sources suggests that FGF expression may play a unifying role in mediating diverse hemodynamic, dyslipidemic, angiogenic, or prothrombotic effects on atherosclerotic vascular intimal proliferation. First, FGF expression in smooth muscle cells was increased by cholesterol esters [30] and decreased by HDL [31]. Second, hemodynamic stress induces vascular smooth muscle cell expression of bFGF [32]. Third, bFGF may act as a local angiogenesis factor to promote plaque neovascularization [33]. Fourth, bFGF induces the endothelial cell synthesis of PAI-1 [34], a major inhibitor of fibrinolysis and a risk marker for myocardial infarction [35]. Fifth, bFGF inhibits smooth muscle cell type 1 collagen synthesis and stimulates collagenase production effects that may contribute to plaque instability [36]. Basic FGF expression in certain vascular tissues activates coordinated gene expression that normally functions to permit local tissue remodeling [36,37]. The present data taken together with the known diverse effects of bFGF in the vessel wall lead us to suggest the novel hypothesis that substantial concentrations of systemically bioavailable bFGF can promote atherosclerosis, thrombosis, plaque neovascularization, and/or plaque instability.

The lack of association between glycemic treatment and CHD risk in our substudy group is consistent with findings from the main VADT that intensive glycemic treatment per se was not associated with a lower overall risk for CVD [5]. Despite a prior significant association between increased plasma bFGF and persistent microalbuminuria [10], neither nephropathy nor elevated serum creatinine was significantly associated with CHD occurrence after adjusting for duration of diabetes. These data are consistent with the possibility that a threshold level of endothelial cell injury underlying microalbuminuria may be required to increase plasma bFGF [10], but CHD risk associated with substantially increased bFGF differs from the risk associated with nephropathy.

The VADT treatment resulted in substantial improvements in BP, lipid levels, and glycemia in the majority of patients randomized to either glycemic treatment arm. This may have contributed to the lack of an association between 1-year bFGF change and glycemic treatment assignment. Significant weight gain resulted from improved glycemic control in the VADT [5]. Weight gain, fluid retention, or a specific treatment effect may have contributed to cases of congestive heart failure, possibly weakening an underlying significant association between plasma bFGF and CVD. Our model suggests that a 50-pg/mL increase in baseline plasma bFGF is associated with a 1.9-fold increase in the HR for CHD. Whether the 1-year average bFGF can predict the longer-term (10-12 years) postbaseline occurrence of CHD will be studied in the ongoing VADT Follow-up Study.

The incidence of obesity and type 2 diabetes mellitus in the United States and worldwide is increasing at an alarming rate [38], and ethnic minorities in the United States have the highest rates of diabetes [39]. A strength of our study is that it included a substantial proportion of African American and Hispanic adults who have an increased prevalence of type 2 diabetes mellitus. A limitation of our study is that the findings may only reflect the experience of middle-aged and older obese men with long-standing, poorly controlled type 2 diabetes mellitus. More study in women and young adults with type 2 diabetes mellitus is needed to determine whether plasma bFGF may indicate an increased risk for first CHD events among patients who may be at substantially lower risk for CHD than was seen in those subjects in our high-risk study group.

In summary, the current findings suggest that increased plasma bFGF was associated with a substantially increased 5-year risk for CHD occurrence in older men with advanced type 2 diabetes mellitus. Plasma bFGF is a potentially useful marker of increased risk for CHD in adult type 2 diabetes mellitus. Although the association demonstrated here does not prove causality, it suggests a novel mechanism in which bFGF may act via the general circulation to contribute to a substantially increased risk for CHD, the leading cause of death in adults with type 2 diabetes mellitus.

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