

# Low plasma levels of FGF-2 and PDGF-BB are associated with cardiovascular events in type II diabetes mellitus (diabetes heart study)<sup>1</sup>

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**Abstract.** *Objective:* We tested associations of the growth factors VEGF, FGF-2, HGF and PDGF-BB with coronary artery calcium scores and cardiovascular events (CVD) in type 2 diabetes mellitus (T2DM).

*Methods:* A cross-sectional study selected 40 frequency matched (by age, gender and race) subjects with T2DM from the first (0–111) and the third (> 1400) coronary artery calcium (CAC) score tertiles in the Diabetes Heart Study (DHS), in which 36 were with and 41 were without history of CVD events. Plasma levels of VEGF, FGF-2, HGF and PDGF-BB were measured in all subjects.

*Results:* None of the growth factors was significantly different between the first and third CAC score tertiles. Mean plasma FGF-2 and PDGF-BB levels were significantly higher in the group without prior CVD events compared with the group with prior CVD events [mean(95%CI): 219.20 (194.42–247.15) vs. 152.93 (135.64–172.43) pg/ml,  $p = 0.03$ ] and [mean(95%CI): 106.70 (89.12–127.74) vs. 61.56 (50.91–74.44) pg/ml,  $p = 0.03$ ], respectively. Subgroup analysis in the first CAC tertile showed a significantly higher PDGF-BB levels in those without compared with those with CVD events [mean (95%CI): 208.36 (190.57–228.15) vs. 102.93 (80.64–125.21) pg/ml,  $p = 0.004$ ].

*Conclusion:* Plasma growth factor levels were not significantly different between the extremes of CAC scores in T2DM. However, low plasma levels of PDGF-BB and FGF-2 are associated with prior cardiovascular events in T2DM. Studies are needed to confirm our results and also to establish temporality of this association.

**Keywords:** Diabetes mellitus, macrovascular complications, angiogenic factors, growth factors, cardiovascular events

## 1. Introduction

Diabetes mellitus (DM) is a major health problem in the United States and its prevalence is predicted to rise exponentially with the obesity epidemic. Vascu-

lar complications are major cause of morbidity and mortality in diabetics compared with non-diabetics [1]. Growth factors have been implicated in microvascular complications of diabetes, but their role in macrovascular complications is not well understood.

Microvascular complications such as retinopathy, nephropathy and neuropathies have all been associated with growth factors [2–4]. Elevated intraocular levels of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatic growth factor (HGF) and platelet derived growth factor-BB (PDGF-BB) have all been associated with diabetic retinopathy. Although plasma levels of PDGF-BB have not been

<sup>1</sup>This study was supported by NIH grant RO1 HL67348 (D.W.B.) and NIH T32 training grant 5T32HL076132-02.

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studied in relation to the occurrence of diabetic ulcers due to neuropathy, topical application of this growth factor has been shown to be beneficial in the treatment of such ulcers [5,6].

For reasons not clearly understood, atherosclerosis, the underlying pathology of macrovascular complications, is more common in diabetics than in nondiabetics [7–9]. There is a two- to four-fold increase in macrovascular events in diabetics compared with those in nondiabetics [10]. Macrovascular complications can be clinical or subclinical, based on either the evidence of significant atherosclerosis using noninvasive measurements (such as carotid intimal medial thickness and coronary artery calcium scores) or the presence of overt disease (such as acute myocardial infarction, stroke and peripheral arterial diseases). Although coronary artery calcium (CAC) score is a good predictor of cardiovascular events [11,12], individuals without significant CAC scores also proceed to have cardiovascular events, suggesting that coronary artery calcification and cardiovascular events can occur independently. Neither the mechanisms of vascular calcifications nor cardiovascular events is fully understood, making it difficult to determine whether the high prevalence of cardiovascular events in diabetics is due to poor glycemic control and insulin resistance [13,14] or to other unexplored markers in T2DM.

Type 2 diabetics have dysregulation of neovascularization compared with nondiabetics [15–17]. Diabetics with microvascular complications also tend to have macrovascular complications, suggesting a possible link in the mechanism of both vascular diseases. The associations of growth factors with microvascular diseases and the occurrence of the two vascular diseases together in diabetics makes growth factors a possible contributor to the high prevalence of atherosclerosis in diabetes mellitus. We assessed the associations of plasma VEGF, FGF-2, HGF and PDGF-BB concentrations with CAC scores and subsequently with cardiovascular events in T2DM.

## 2. Methods

Our study was cross-sectional, primarily testing the association of VEGF, FGF-2, HGF and PDGF-BB with CAC tertiles (subclinical) and subsequently their association with cardiovascular event (clinical) status in 80 T2DM (mean age 61.4 years) selected from the Diabetes Heart Study (DHS) cohort. DHS is an NIH-funded single center study of genetic and environmental

contributors to CVD in diabetes mellitus. The cohort consists of about 1200 mainly Caucasians diabetics, recruited in the Winston Salem North Carolina area with extensive data on subclinical measures of atherosclerosis and cardiovascular event. CAC scores of the subjects were measured using multidetector CT scanners during enrollment into the study and data on prior cardiovascular events was obtained through interviews and the presence of significant Q wave abnormalities on enrollment EKG (Minnesota codes 1.1, 1.2, 1.3, 4.1, 4.3, 5.1 or 5.3).

The CAC scores of the subjects in the DHS cohort were divided into tertiles. Forty subjects were selected from the first tertile by random sampling. Using frequency matching for age, gender and race, forty subjects were also selected from the third tertile. The first tertiles CAC score ranged from 0–111 and that of the third tertile was > 1400 (Agatston). As shown in Table 1, 36 subjects had either a history or evidence of CVD events defined as history of myocardial infarction, revascularization, stroke and peripheral vascular disease or had evidence of CVD defined as a significant Q wave abnormality on EKG and 41 had neither history of CVD event nor significant Q wave abnormality on EKG at enrollment. The cardiovascular event status for three subjects was not available in the DHS database and hence they were not included in the CVD event status analysis.

Plasma levels of the VEGF, FGF-2, HGF and PDGF-BB were measured from thawed samples of plasma obtained at the time of enrollment into the Diabetes Heart Study. Plasma levels of these growth factors were measured by Enzyme-Linked Immunosorbent Assay (SEARCHLIGHT HUMAN ANGIOGENESIS ARRAY 2, PIERCE BIOTECHNOLOGY, INC. in Rockford, Illinois, USA) under standard conditions in the Wake Forest University School of Medicine Hypertension Core Laboratory. Samples were identified by codes and therefore laboratory technicians were blinded to either sample CVD event status or CAC tertile.

## 3. Data analysis

Sample size analysis based on plasma VEGF concentrations in a prior study of similar T2DM [18] indicated that 80 subjects would be required to provide 80% power for detecting 50% change in VEGF levels at  $\alpha = 0.05$ . Our primary goal was to detect a difference of growth factor concentration between the CAC score tertiles and the secondary goal was to de-

Table 1  
Subjects based on CAC score tertile and prior cardiovascular (CVD) event status

	First Tertile (mean CAC = 40.9)	Third Tertile (mean CAC = 5489.6)	Total
No CVD Events	GROUP A 25	GROUP C 16	41
CVD Events	GROUP B 12	GROUP D 24	36
TOTAL	37	40	77

Table 2  
Demographics of subjects with and without prior cardiovascular events (CVD)

Variables	No CVD (N = 41) Mean (sd)	CVD (N = 36) Mean (sd)	P VALUE
Age (yrs)	60.6 (8.2)	62.4 (8.4)	0.36
Gender			
Men (%)	17 (41.5)	11 (30.6)	0.45
Women (%)	24 (58.5)	25 (69.4)	
Race			
Whites (%)	33 (80.5)	33 (91.7)	0.28
Blacks (%)	8 (19.5)	3 (8.3)	
Cholesterol (mg/dl)	191.9 (39.7)	186.6 (39.8)	0.56
LDLc (mg/dl)	102.7 (29.4)	102.5 (31.1)	0.98
HDLc(mg/dl)	46.6 (14.9)	46.9 (14.5)	0.92
Duration of Diabetes(yrs)	10.4 (8.5)	11.6 (7.9)	0.53
Use of Insulin			
No (%)	30 (73.2)	22 (61.1)	0.26
Yes (%)	11 (26.8)	14 (38.9)	
Systolic Blood Pressure(mmHg)	140.9 (19.5)	140.3 (19.6)	0.89
Diastolic Blood Pressure(mmHg)	73.3 (11.1)	69.4 (11.8)	0.13
Hemoglobin A1C (%)	7.6 (1.2)	8.1 (1.9)	0.19
BMI (Kg/m <sup>2</sup> )	31.9 (5.0)	33.2 (7.1)	0.38
CAC Tertiles			
T1 (%)	25 (61.0)	12 (33.3)	0.03
T3 (%)	16 (39.0)	24 (66.7)	
Smoking			
Former (%)	19 (46.3)	13 (36.1)	0.73
Current (%)	8 (19.5)	8 (22.2)	
Never (%)	14 (34.2)	15 (41.7)	
Lipid lowering Medication			
No (%)	23 (56.1)	17 (47.2)	0.44
Yes (%)	18 (43.9)	19 (52.8)	
Blood pressure Medication			
No (%)	15 (36.6)	8 (22.2)	0.17
Yes (%)	26 (63.4)	28 (77.8)	

P values for continuous variables calculated with t-test and p values for categorical variables calculated with chi- square test.

tect a difference in growth factor levels based on prior cardiovascular event status. Pearson correlations between the plasma levels of angiogenic growth factors in our sample were analyzed. Natural or inverse natural log were use to transform growth factor concentrations to approximate normal distribution prior to analysis. T-test and chi- square analyses were used to compare means of covariates between two groups. Univariate analysis was used to determine covariates which were significant predictors of the dependent variables and were adjusted for in the multivariate analysis. Least square means and standard errors were calculated in

the multivariate model and report as 95% confidence intervals after back transformation. Subgroup analysis for prior CVD events within each CAC Score tertile was also done. Statistical significance was declared at  $p \leq 0.05$  (two-tailed). Data analysis was done using SAS version 9.1(SAS institute Cary, NC).

#### 4. Results

Correlations between growth factors in T2DM.

Table 3  
Comparisons of the plasma VEGF, FGF-2, HGF and PDGF-BB concentrations in subjects with the first and the third CAC score tertiles

Growth Factors	First Cac Tertile (N = 40) Mean (95%CI)	Third Cac Tertile (N = 40) Mean (95%CI)	P Value
VEGF (pg/ml)	27.22 (22.87–33.45)	34.47 (28.50–41.80)	0.44
FGF-2 (pg/ml)	53.65 (46.81–64.50)	64.95 (54.59–77.31)	0.48
HGF (pg/ml)	436.22 (395.44–483.0)	425.52 (387.61–473.43)	0.82
PDGF-BB (pg/ml)	162.54 (148.41–188.67)	193.82 (172.43–219.20)	0.42

Adjusted for age, gender, race/ethnicity, BMI, smoking and use of lipid lowering medications.

Table 4  
Comparisons of plasma VEGF, FGF-2, HGF and PDGF-BB concentrations in subjects with and without prior CVD events

Growth Factors	No CVD Events (N = 41) Mean(95%CI)	CVD Events (N = 36) Mean(95%CI)	P Value
VEGF (pg/ml)	35.16 (29.08–42.52)	29.22 (23.10–34.47)	0.42
FGF-2 (pg/ml)	106.70 (89.12–127.74)	61.56 (50.91–74.44)	0.03
HGF (pg/ml)	411.58 (372.40–454.86)	487.85 (437.03–544.57)	0.25
PDGF-BB (pg/ml)	219.20 (194.42–247.15)	152.93 (135.64–172.43)	0.03

Adjusted for age, gender, race/ethnicity, BMI, smoking and the use of lipid lowering medications.

Pearson correlations between the angiogenic growth factors were as follows: between VEGF and PDGF-BB ( $\rho = 0.62$ ,  $p < 0.0001$ ), between VEGF and HGF ( $\rho = 0.50$ ,  $p < 0.0001$ ), between HGF and FGF-2 ( $\rho = 0.39$ ,  $p = 0.0004$ ), PDGF-BB and VEGF ( $\rho = 0.62$ ,  $p < 0.0001$ ), between PDGF-BB and FGF-2 ( $\rho = 0.74$ ,  $p < 0.0001$ ) and between PDGF-BB and HGF ( $\rho = 0.38$ ,  $p = 0.0004$ ).

#### 4.1. Association of growth factors with CAC scores and CVD status in T2DM

As illustrated in Table 2, the two groups (CVD events and no CVD events) were not significantly different with respect to age, gender, race, lipid profiles, duration of diabetes, insulin use, BMI, use of lipid lowering medication, smoking, blood pressure, use of blood pressure medication and glycohemoglobin levels. CAC scores were however significantly higher in the group with prior CVD events compared with those without prior CVD events.

As shown in Table 3, the mean plasma concentration of VEGF, FGF-2, HGF and PDGF-BB were not significantly different between the first and the third CAC tertile groups. Subsequent analysis however showed PDGF-BB and FGF-2 concentrations significantly higher in the group without prior CVD events compared with those with prior CVD events [mean(95%CI): 219.20 (194.42–247.15) vs 152.93

(135.64–172.43) pg/ml,  $p = 0.03$ ] and [mean(95%CI): 106.70 (89.12–127.74) vs 61.56 (50.91–74.44) pg/ml,  $p = 0.03$ ] respectively (Table 4) after adjusting for confounders such as age gender, BMI, race/ethnicity, smoking and use of lipid lowering therapy. Plasma mean VEGF and HGF concentrations were not significantly different between prior CVD and no prior CVD event groups. Subgroup analysis showed significantly higher mean levels of PDGF-BB in those in the first tertile without CVD events (group A) compared with those with events (group B) [mean (95%CI): 208.36 (190.57–228.15) vs 102.93 (80.64–125.21) pg/ml,  $p = 0.004$ ]. FGF-2 was not significantly different in the subgroup analysis. Subgroup analysis within the third CAC tertile was also not significant (data not shown).

## 5. Discussion

Our study found significantly lower concentrations of PDGF-BB and FGF-2 in T2DM with prior CVD events compared with T2DM without prior CVD events. There was however no significant difference in all the four growth factor levels between the extremes of CAC scores in T2DM.

Prior studies have shown no significant difference between plasma VEGF in diabetics with CAD compared with diabetics without CAD [15,19,20]. Data on the association of plasma PDGF-BB, HGF and FGF-2

and either CAC scores or CVD events however are lacking. Our study suggests that angiogenic growth factors (PDGF-BB and FGF-2) may play a significant role in macrovascular complications, similar to that described for microvascular complications in diabetes mellitus.

The significantly high CAC scores in the T2DM with CVD events indicate a higher atheroma burden compared with those without CVD events. However the plasma levels of the PDGF-BB and FGF-2 were not significantly different between the first and third tertiles of CAC scores implying that the lower levels are not necessarily associated with atheroma burden but with cardiovascular events. This inference suggests that growth factors, PDGF-BB and FGF-2, may have a different mechanism of protection against cardiovascular events in T2DM.

Although it would be premature to say whether this association is a cause-effect, effect-effect or an effect-cause relationship, possible mechanisms can be proposed. The basic role of these vascular smooth muscle cell mitogens has been well studied in vitro. They stimulate the proliferation and migration of vascular smooth muscle cells and hence play a major role in the progression of atherosclerosis and subsequent occlusion of blood vessels [21,22]. However research has shown that cardiovascular events are mainly due to plaque rupture with subsequent occlusion of the vessel by thrombosis as opposed to the mere progression of atheroma [23,24]. PDGF-BB and FGF-2 may protect against events by stimulating the proliferation and migration of vascular smooth muscle cells which contributes to a thicker fibrous cap, making the atheroma less vulnerable to rupture [25]. Vascular endothelial growth factor and hepatic growth factor on the other hand are angiogenic factors which may have no effect on the formation and thickening of the fibrous cap in the pathogenesis of atherosclerosis [26].

Our study has the following limitations. Our pilot study has a sample size of eighty participants. Although from all indications this sample size should be enough for such a study, we are uncertain that our findings would have been the same if we had a much larger population of T2DM. Due to lack of prior studies in this area, we only found data to support the lack of association of plasma VEGF and cardiovascular events. We found no such association studies on plasma PDGF-BB, FGF-2 and HGF with macrovascular complications in T2DM.

Our study was cross sectional and therefore could not establish the temporal relationship between these angiogenic growth factors and macrovascular compli-

cations in T2DM. Additional research is needed in this area to determine the role and mechanisms by which these growth factors influence CVD events in vivo in T2DM. Replication of the findings of this study is also needed in order to verify our positive findings and to re-assess our negative results in a larger sample with greater statistical power.

**CONCLUSION:** Our study showed no significant difference between VEGF, FGF-2, HGF and PDGF-BB between the extremes of CAC scores in T2DM. Our study also suggests that low plasma levels of PDGF-BB and FGF-2 may be associated with cardiovascular events in T2DM. More studies are needed to confirm our findings and also to establish temporality of the association between PDGF-BB and FGF-2 and CVD events in T2DM.

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