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Clinical Correlates of Serum Pigment Epithelium-Derived Factor in Type 2 Diabetes Patients

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Abstract

Aim—To determine if serum pigment epithelium-derived factor (PEDF) levels in Type 2 diabetes are related to vascular risk factors and renal function.

Methods—PEDF was quantified by ELISA in a cross-sectional study of 857 male Veterans Affairs Diabetes Trial (VADT) subjects, and associations with cardiovascular risk factors and

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renal function were determined. In a subset (n=246) in whom serum was obtained early in the VADT (2.0 ± 0.3 years post-randomization), PEDF was related to longitudinal changes in renal function over 3.1 years.

Results—Cross-sectional study: In multivariate regression models, PEDF was positively associated with serum triglycerides, waist-to-hip ratio, serum creatinine, use of ACE inhibitors or angiotensin receptor blockers, and use of lipid-lowering agents; it was negatively associated with HDL-C (all $p < 0.05$).

Longitudinal study—PEDF was not associated with changes in renal function over 3.1 yrs ($p > 0.09$)

Conclusions—Serum PEDF in Type 2 diabetic men was cross-sectionally associated with dyslipidemia, body habitus, use of common drugs for blood pressure and dyslipidemia, and indices of renal function; however, PEDF was not associated with renal decline over 3.1 years.

Keywords

PEDF; type 2 diabetes; cardio-vascular risk factors

Introduction

Pigment epithelium-derived factor (PEDF), an adipokine, is a secreted glycoprotein belonging to the superfamily of serine protease inhibitors (serpins). Although first described in the eye [1], the major sources of circulating PEDF are thought to be liver and adipose tissue [2]. PEDF has potent anti-angiogenic, anti-inflammatory, anti-oxidant, and neuroprotective properties [3, 4], and has been associated with insulin resistance [5–8], diabetes mellitus, and diabetic vascular complications, including nephropathy [9–12]. PEDF has been shown to inhibit the secretion of angiogenic and pro-fibrotic factors [10], and to suppress vascular endothelial cell proliferation [13], microvascular cell apoptosis [14] and renal fibrosis [15]. In relatively small cross-sectional studies, we previously reported elevated serum PEDF levels in Type 2 diabetic vs. non-diabetic subjects [16], and in Type 1 diabetic subjects with vs. without microvascular complications [11]. We found associations of PEDF with body mass index (BMI), lipid levels, and renal and vascular dysfunction [11, 16]. Altered levels of PEDF and growth factors such as TGF β and VEGF have been associated with, and mechanistically implicated in, diabetic nephropathy [10], diabetic retinopathy [17], and atherosclerosis [18], and PEDF has been found to be independently associated with coronary artery disease [19]. In animal and cell culture models of diabetic microvascular damage, PEDF has exhibited protective effects [20–25].

The Veterans Affairs Diabetes Trial (VADT) was a prospective, longitudinal study of 1,791 subjects with Type 2 diabetes (T2DM), of whom 97% were male. Participants were randomized to receive either intensive or standard glycemic management, with the purpose of assessing the effect of intensive management on major cardiovascular disease (CVD) events (primary end-point) and microvascular complications (secondary end-points) [26]. Six months after randomization, mean glycated hemoglobin (HbA1c) levels in the intensive and standard groups were 6.9% and 8.4% respectively [26]. As intended, lipid and blood pressure levels, as defined by ADA-recommended targets [27], were well controlled in both

treatment groups. The study did not demonstrate any favorable effects of intensive glucose control on CVD events, neuropathy, or retinopathy. Intensive control was however associated with diminished progression of albuminuria [26], but despite efforts to manage hyperglycemia, hypertension, and dyslipidemia, renal function still declined in 8.8% of VADT participants during the 5-year trial (defined as doubling of serum creatinine level within the study time-frame), regardless of treatment assignment [26].

Identification of novel biomarkers and mechanisms implicated in diabetic microvascular damage may facilitate early identification and treatment of people at risk. In the present work, we studied subsets of the VADT cohort to assess the significance of serum PEDF levels, defining its cross-sectional associations with clinical factors, including CVD risk factors and renal function. In a smaller and more rigorously defined cohort, we also assessed whether PEDF is associated with subsequent decline in renal function. Renal function was assessed by serum creatinine (sCr), urine albumin-to-creatinine ratio (ACR), and estimated glomerular filtration rate (eGFR).

Subjects

The VADT was conducted according to the principles of the Declaration of Helsinki and was approved by Human Ethics Committees at all participating institutions. Each participant gave written informed consent. Our sub-study received Ethics Committee approval at the Medical University of South Carolina and the University of Oklahoma Health Sciences Center. Details of design and clinical and routine biochemical procedures of VADT have been reported previously [26, 27]. In brief, participants with T2DM (n=1,791, of whom 1,739 were male) were enrolled and randomized to intensive vs. standard glucose control between December 1, 2000 and May 30, 2003, and followed for five years. The treatment and initial follow-up period ended on May 30, 2008, and an additional observational follow-up is underway.

In our ancillary study of 857 VADT men ('Group A'), serum for quantification of PEDF was collected on a single occasion from each participant. PEDF values were analysed against clinical data based on the nearest annual study visit. For blood pressure and body mass index (BMI), the serum sample was matched to data from the nearest visit within six months. For medication effects, the serum data were matched to data from the nearest visit prior to sample collection to ensure that the data reflected actual medication exposure.

In order to standardize exposure to the VADT treatment assignment, a further subset of 246 men from Group A (referred as 'Group B') was chosen for a longitudinal analysis (and additional cross-sectional analysis). This subset included only those subjects in whom the PEDF sample was obtained during a narrow time frame centred on the two-year post-randomization visit (range 1.51 to 2.49 years post-randomization), and in whom complete PEDF, renal function, and covariate data were available. The sampling criteria for the longitudinal cohort were more restrictive to ensure that, by design, the study participants were standardized in terms of their exposure to the VADT treatment assignment when investigating the association between PEDF and subsequent renal function. Restriction was chosen as the preferred method to adjust for confounding and effect modification due to

time on randomized therapy, which may impact both the PEDF measure and renal outcomes, as opposed to post-hoc statistical adjustment through regression modelling.

Materials and Methods

Biochemistry

HbA1c, serum lipid profiles, and renal function tests were performed by VADT laboratories as previously described [27]. Renal function was reflected by serum creatinine (sCr; $\mu\text{mol/L}$), urinary albumin to creatinine ratio (ACR; mg/g creatinine), and estimated glomerular filtration rate (eGFR; MDRD formula; mL/min/1.73m^2). Renal dysfunction was defined as sCr $\geq 176.8 \mu\text{mol/L}$ and/or eGFR $< 60 \text{ mL/min/1.73m}^2$. Categories of albuminuria were defined as ‘no albuminuria’ (ACR from 0–29 mg/g creatinine), microalbuminuria (30–300 mg/g creatinine), and macroalbuminuria ($> 300 \text{ mg/g creatinine}$).

Serum PEDF was quantified by ELISA (Chemicon Int., Inc., Temecula, CA) as previously described [16], with intra- and inter-assay coefficients of variation of 3.4% and 12.0% respectively. The mean of duplicate measures was used in data analyses.

Statistics

Data analysis was limited to male subjects, because the number of females in VADT was so low. Spearman’s rank correlation coefficient was used to quantify the strength of the linear association between pairs of continuous measures. Linear regression modelling was used to investigate the association between PEDF levels (independent factor of interest) and renal outcome measures (dependent variables) with and without adjustment for age, T2DM duration, race/ethnicity, lipid-lowering therapy, VADT treatment assignment, hypertension, BMI, and waist-to-hip ratio (WHR). For the longitudinal analyses, a mixed effects modelling approach was used to account for the correlation among repeated annual renal function measures (outcome variable) for each subject. The regression models included a random intercept for each subject and utilized a linear link for continuous outcome measures and a cumulative logistic link for ordered categorical measures. The interaction between study time point and the PEDF measure was investigated to indicate whether the changes in renal function over the course of the follow-up, considered as repeated continuous or categorical measures, were significantly associated with the 2-year PEDF measure (Group B). Cox proportional hazards regression modelling was used to model the association between the hazard of renal disease progression, based on a categorical definition of a clinical disease progression event, and PEDF measures. Data were analysed using SAS (SAS System for Windows, ver. 9.1, SAS Institute Inc., Cary, NC) and statistical significance was defined as $p < 0.05$.

Results

Subject characteristics

Clinical characteristics at the time of serum collection for PEDF measurement are shown in Table 1: there were no significant differences between Groups A and B. As shown in Supplemental Table 1, compared to all non-participating VADT subjects ($n=882$), Group A

subjects were more likely to be non-Hispanic White, and to use angiotensin receptor blockers (ARB) and aspirin. They also had higher systolic blood pressures and lower LDL-cholesterol (LDL-C) levels than non-participants. The distribution of the numbers of anti-hyperglycemic medication (from 0 to 4 agents) taken also differed slightly between included and non-included groups.

As only males were studied, the effects of sex on PEDF levels and relationships with vascular risk factors and renal function could not be evaluated.

Correlates of serum PEDF in Type 2 diabetes

Cross-sectional univariate analyses (Table 2)—Based on the maximum number of available samples for each measure, factors independently associated with PEDF in the cross-sectional univariate study included: Non-Hispanic White background; body habitus (BMI and WHR); renal function in general (sCr, eGFR), and dysfunction in particular (as defined above); serum lipids (TC, HDL-C, and TG); use of anti-hypertensive medications in general and ARBs in particular; use of lipid-lowering agents (any of: statins, fibrates and nicotinic acid); and (inversely) use of metformin ($p<0.05$, Table 2). Specifically concerning medications, PEDF (mean (SD)) was significantly higher for patients taking vs. not taking a statin (10.8 (4.1) vs. 9.9 (3.6) mcg/mL), fibrates (12.0 (4.1) vs. 10.2 (3.9) mcg/mL), or an ARB (11.6 (3.5) vs. 10.4 (4.0) mcg/mL), but significantly lower for patients taking vs. not taking metformin (10.4 (3.9) vs. 11.1 (4.1) mcg/mL).

The regression modelling was repeated for the smaller subset of subjects with PEDF sampled at roughly the two-year visit (Group B). Broadly, the conclusions were similar, as also shown in Table 2. Univariate independent associations with PEDF included Non-Hispanic White background; body habitus (BMI and WHR); renal function in general (sCr, eGFR), renal dysfunction; serum lipids (TG, HDL-C) and the use of lipid-lowering agents (statins or fibrates) as well as (inversely) the use of metformin or rosiglitazone ($p<0.05$).

Cross-sectional multivariate analyses—In a multivariate linear regression model, considering effect modification by race (Non-Hispanic White vs. all others) and renal dysfunction, factors independently associated with PEDF included: serum triglycerides, WHR, and sCr (positive associations, $p<0.006$); HDL-C (negative association, $p<0.0001$); and use of ACE inhibitors or lipid-lowering agents (positive associations $p<0.01$) (Table 3). The positive association with the use of ACE inhibitors was only observed in Non-Hispanic Whites ($\beta=1.05$, $p=0.0045$): it was not observed in ‘all others’ ($\beta= -0.25$, $p=0.63$). The association with use of fibrates or niacin was significantly modified by renal impairment (test of interaction: $p=0.026$) where there was a negative association with fibrate or niacin use among subjects without renal impairment but a positive association among those with renal impairment ($\beta= -0.61$, $p=0.087$ and $\beta= 0.95$, $p=0.013$ respectively); however, the stratified comparisons were not significant.

Results were similar for multivariate analyses of the smaller cross-sectional sample (Group B). After adjustment for race (Non-Hispanic Whites vs. all others) and renal dysfunction (as defined above), factors independently associated with PEDF included: serum triglycerides, WHR, sCr, and use of lipid lowering medications and specifically fibrates (positive

correlation, $p < 0.01$); age at sampling date, ARB use, and rosiglitazone use (negative correlation, $p < 0.01$). A negative association was seen with ARB use among those with renal impairment ($\beta = -3.62$, $p = 0.0073$) and as in the larger group, the positive association with fibrate use was only observed in Non-Hispanic Whites ($\beta = 2.01$, $p = 0.0015$).

Longitudinal analysis—In Group B, a longitudinal analysis related serum PEDF (taken at time 0) to subsequent renal function (median follow-up (range): 3.1 (0.6 to 5.2) years). When considering clinical disease progression, among 216 subjects with available ACR data, 27 (13% (95% CI: 9% to 18%)) demonstrated a worsening of albuminuria, defined as any increase in the stage of albuminuria among the categories of normoalbuminuria, micro-albuminuria, and macroalbuminuria. To meet criteria, this increase had to be consistent between at least two successive yearly visits without reversion to an improved level. Regarding eGFR, 58 (24%) demonstrated a worsening of eGFR assessed by a change in category (>90 , 60–89, 30–59, 15–29 and < 15 mL/min/1.73 m²). Again, the category change had to be consistent as defined above.

By these criteria, serum PEDF levels were not significantly associated with changes in renal function over time ($p > 0.5$ for models with and without adjustment for confounding factors). Specifically, categories of PEDF (tertiles of distribution at Time 0), were not significantly associated with increased hazard of a worsening of albuminuria (Hazard Ratio (HR) 0.99 [95% CI: 0.35 – 2.82]; HR 2.00 [95% CI 0.81 – 4.98], for middle and highest vs. lowest PEDF tertiles respectively). Similarly, PEDF tertiles were not significantly associated with increased hazard of chronic kidney disease progression (HR 0.54 [95% CI: 0.27 to 1.08]; HR 0.57 [95% CI: 0.29 to 1.13] for middle and highest vs. lowest PEDF tertiles respectively)

Discussion

Our study explored whether circulating PEDF levels are associated with vascular risk factors and with renal function. In a large cross-sectional study of 857 men with T2DM from the VADT, we demonstrated significant correlations of PEDF with body habitus, renal dysfunction, dyslipidemia, use of anti-hypertensive drugs (specifically ARBs) and lipid-lowering agents (statins and fibrates) and, inversely, with insulin sensitizers (rosiglitazone and metformin). In a longitudinal analysis of a smaller subset of 246 VADT men, PEDF was not associated with changes in renal function over a median follow-up period of 3.1 years.

PEDF, body habitus, insulin resistance, and cardiovascular risk

In this cross-sectional study of men with T2DM, serum PEDF levels were positively correlated with body habitus as reflected by both BMI and WHR, while WHR was an independent predictor of serum PEDF levels. These results are in keeping with our previous cross-sectional studies in T1DM and T2DM [11, 16], and with studies involving insulin resistant, pre-diabetic, and obese T2DM subjects [5–8, 28–30]. A direct link between body fat and PEDF levels in humans is further supported by ex vivo studies of PEDF production by cultured adipocytes [2, 31], subsequent macrophage activation [31], and the observation that reductions in circulating PEDF follow weight loss [6]. PEDF may also modulate insulin resistance as suggested by studies in mice [8] and in man [6, 32–35].

Given the role of PEDF as an anti-oxidant and anti-inflammatory factor, there has been speculation that the elevation of PEDF in obesity and insulin resistance may be an adaptive, counter-regulatory mechanism aimed at protecting tissues from vascular damage and inflammation [36]. Indeed, in our univariate analysis, serum PEDF was lower in subjects using insulin sensitizers (metformin or rosiglitazone), in keeping with a possible reduction of PEDF in response to increased insulin sensitivity, but this association was not retained in multivariate analyses which included adjustment for WHR. Quantification of serum PEDF before and after the introduction of insulin-sensitizer therapy, and during insulin clamp studies would help clarify the observed associations between PEDF and factors associated with insulin resistance. In a small (n=34) prospective study, serum PEDF levels increased in Type 2 diabetic subjects taking metformin, though the change in PEDF levels was not significantly correlated with changes in body fat, glycemia, or measures of insulin resistance [35].

PEDF and lipids

In our cross-sectional analyses, serum PEDF was correlated positively with fasting TG levels and negatively with HDL-C levels, but did not correlate with LDL-C levels. TG and HDL-C levels were independent determinants of serum PEDF levels in multivariate models. Such relationships have been observed in other cross-sectional studies in T2DM and insulin-resistant subjects [28], as well as in subjects with coronary artery disease in whom PEDF was strongly correlated with TG levels [19]. A recent paper has demonstrated correlations between fasting serum levels of PEDF and ApoB48, found in chylomicrons, i.e. triglyceride rich lipoproteins that rise post-prandially [32]. Our present results, in fasted Type 2 diabetic subjects, differ from our previous smaller cross-sectional T2DM study in which circulating PEDF levels correlated only with LDL-C [16]. The discrepancy may be related to statistical power and confounding effects of lipid-lowering treatments.

PEDF binds to adipose triglyceride lipase (ATGL), a highly conserved TG lipase that is involved in lipid and glucose homeostasis. It acts in liver [37], skeletal muscle, and adipose tissue, mobilizing fatty acids from TG stores, reducing fatty acid oxidation in skeletal muscle, and increasing hepatic triacylglycerol hydrolase activity, in line with its putative protective effects [38]. Intriguingly, in the present cross-sectional study, while PEDF levels were positively associated with dyslipidemia (high TG and low HDL-C levels), they were also higher in study subjects who were taking lipid-lowering (statin and fibrate) therapies. In addition, the use of a fibrate, i.e. a PPAR α agonist, was an independent determinant of serum PEDF in Non-Hispanic Whites, while its effect in other ethnicities remained neutral. In cultured liver cells, PEDF has been shown to bind to and up-regulate PPAR α transcription [39]. We are not aware of any mechanistic studies of PEDF and statins. More prospective studies of the effects lipid-lowering drugs on PEDF levels and cell responses are merited to clarify these findings.

PEDF, blood pressure, and anti-hypertensive agents

PEDF may modulate vascular tone and blood pressure, and serum levels may be lowered by blood pressure reduction associated with weight loss [6]. In the present study, serum PEDF did not correlate with concurrent blood pressure, and did not differ according to

hypertension status. On the other hand, use of anti-hypertensive agents in general, and ARBs in particular, was associated with higher PEDF levels. In addition, ACE inhibitor and ARB use were independent determinants of PEDF levels, suggesting, as with statins and fibrates, a possible role of these drugs in modulating PEDF levels. These data contrast with our smaller cross-sectional studies in men and women with T1DM and T2DM in which PEDF correlated with blood pressure, pulse pressure, and inversely with small artery elasticity [11, 16]. In the Hong Kong Cardiovascular Risk Factor Prevalence Study of non-diabetic subjects, baseline plasma PEDF was associated with blood pressure, being higher in those with vs. without hypertension, and was identified an independent predictor of later blood pressure levels and incident hypertension [40]. PEDF effects may modulate vascular tone and blood pressure by JNK activation and reduced insulin-dependent activation of IRS-1 and Akt [8, 41]. Longitudinal studies of PEDF levels before and after ACE inhibitor and ARB use would help resolve some of the contrasting evidence, and would clarify possible roles for these drugs in modulating PEDF levels, and indirectly, exerting vasoprotective effects.

PEDF and renal function

In diabetes, tissue renal [10] and retinal [17, 42] PEDF levels are decreased and, perhaps as a compensatory protective effect, circulating PEDF levels are increased in the presence of microvascular [11] and macrovascular damage [18]. In addition, based on its retinal and renal protective effects in cultured cells and animal models [20, 22, 23], PEDF may have tissue-specific therapeutic potential. Indeed, in diabetic nephropathy, PEDF can prevent inflammation (via inhibition of NF κ B), reduce fibrosis (via inhibition of TGF β 1 and Connective Tissue Growth Factor), reduce vascular leakage and proteinuria (partly via VEGF suppression), and protect against renal cell apoptosis and damage (via PPAR- γ activation and inhibition of the AGE-Receptor for AGEs (RAGE) axis) [14, 20, 22, 43, 44]. In our cross-sectional study, serum PEDF correlated with renal function as reflected by positive associations with sCr, urinary ACR, and negative association with eGFR. These observations agree with our previous study [11] and with other cross-sectional clinical studies in diabetes [45] and in end-stage renal disease [46].

While our cross-sectional data confirmed the relationship of serum PEDF levels with renal dysfunction, and there are identified mechanisms of renal protection, as discussed above, our longitudinal sub-study showed no statistically significant correlations between PEDF levels and subsequent changes in renal function. It may be that follow-up was not long enough, and/or that the changes in renal function were not large enough. Urinary PEDF, which has been suggested as a marker of diabetic nephropathy [47], was not quantified in our study, and serum PEDF levels were not repeated, so we are unable to investigate the association between changing serum PEDF levels and progression of renal disease. As renal disease is associated with increased cardiovascular risk in T2DM patients and serum PEDF levels have been associated with both vascular dysfunction [11, 16] and increased carotid intima media thickness [18], the role of PEDF in the linkage between renal and cardiovascular damage is of interest.

PEDF, sex and ethnicity

In other cross-sectional studies, PEDF levels have been reported as higher in men than women [48]. In our study, only men were included because the number of female participants in the VADT was so low, and therefore this question cannot be addressed.

Interactions of PEDF levels with factors such as fibrate use differed by ethnicity in men with T2DM, being significant only in Non-Hispanic Whites. In addition, Non-Hispanic White participants had a higher mean PEDF level. To the best of our knowledge, there are no other reports of differences in PEDF levels by ethnicity or race. The prevalence of T2DM, its vascular complications, and hypertension are known to differ by ethnicity [49, 50], but it is as yet unknown whether differences in PEDF expression might contribute to such effects.

Study strengths and limitations

The strengths of our study include use of the very large, well characterized, multi-ethnic VADT cohort. The VADT was successful in achieving ADA-recommended blood pressure, lipid, and glycemic targets [26]. Our findings support the impact of clinical factors and commonly used drugs (for blood pressure and lipid control) on circulating levels of a potent anti-angiogenic factor with possible protective properties against diabetic vascular complications. Serum PEDF levels may therefore have a future role in algorithms to predict risk for vascular complications of diabetes, as well as potential therapeutic utility. Indeed, our clinical data, combined with laboratory data showing that PEDF can modulate vascular structure and function [9–12], may underpin the future development of new treatments and preventive measures. Anti-VEGF drugs are now used to treat proliferative diabetic retinopathy, and by extension, agents that mimic PEDF might find future clinical use. Indeed PEDF eye-drops show protection against retinal damage in diabetic animal models [25] and over-expression of PEDF inhibits retinal inflammation and neovascularization in mice [51].

Study limitations include the absence of females, concern regarding selection bias (not all VADT study subjects volunteered for our sub-study), and external validity. Also, the full cross-sectional male cohort could not be included in the longitudinal study: our serum specimens were not sampled until after VADT randomization, and we considered that exposure time to the VADT intervention needed to be standardized (given the importance of glucose control and the potential role that glucose-modulating drugs play in vascular health and angiogenesis). Another limitation is our measurement of PEDF at only a single time point.

In summary, in the largest and therefore best-powered cross-sectional and longitudinal study of PEDF in diabetes, we demonstrated that serum levels are correlated with adiposity, dyslipidemia, use of blood pressure and lipid modifying drugs, and with renal dysfunction. In a smaller defined subset, PEDF did not predict deterioration of renal function an average of three years' follow-up. Our study, although exploratory, adds to the increasing evidence that PEDF is associated with, and may exert potential therapeutic benefits in, the micro and macro-vascular complications of diabetes, a finding which merits further exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACE	Angiotensin Converting Enzyme
ACR	Albumin to Creatinine Ratio
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CrCl	Calculated Creatinine Clearance
CVD	Cardiovascular Disease
ELISA	Enzyme Linked Immunoassay
eGFR	Estimated Glomerular Filtration Rate
HbA1c	Glycated Hemoglobin
HDL-C	High Density Lipoprotein Cholesterol
LDL-C	Low Density Lipoprotein Cholesterol
PEDF	Pigment Epithelium-Derived Factor
sCr	Serum Creatinine
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglycerides
TGFβ	Transforming Growth Factor β
TZD	Thiazolidinedione
VADT	Veterans Affairs Diabetes Trial
VEGF	Vascular Endothelial Growth Factor
WHR	Waist to hip ratio

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Table 1

Sampling visit characteristics of the large cross-sectional (Group A; n=857) and the two-year (Group B; n=246) subset. Summary statistics are reported as mean (standard deviation) for continuous measures or column percentages for categorical measures.

	At sampling clinical visit	
Characteristic	Group A n=857	Group B n=246
Age (yr)	62.0 (8.6)	62.6 (8.7)
Known diabetes duration (yr)	13.2 (7.4)	12.9 (7.1)
Ethnic group		
Non Hispanic White	64%	68%
Hispanic White	14%	14%
Non-Hispanic Black	16%	13%
All other	5%	6%
Treatment assignment		
Standard therapy	51%	49%
Intensive therapy	49%	51%
Time since randomization (years)	1.8 (1.0)	2.0 (0.3)
HbA1c (%)	8.0 (1.6)	7.8 (1.4)
BMI (kg/m²)	32.4 (5.0)	32.5 (4.8)
WHR	1.0 (0.1)	1.0 (0.1)
Hypertension	90%	89%
SBP (mmHg)	128(15)	126(14)
DBP (mmHg)	73 (10)	72 (9)
Neuropathy	31%	29%
ETDRS score	4.6 (3.6)	4.7 (3.9)
Renal function		
sCreatinine (μmol/L)	97.2 (26.5)	97.2 (26.5)
eGFR (mL/min/1.73 m ²)	78.7 (22.9)	78.5 (23.3)
Lipid levels		
Total Cholesterol (mmol/L)	4.5 (1.1)	4.4 (1.1)
Triglycerides (mmol/L)	2.1 (2.2)	1.9 (1.2)
LDL-C (mmol/L)	2.5 (0.7)	2.5 (0.7)
HDL-C (mmol/L)	0.9 (0.2)	0.9 (0.2)
Anti-Hypertensive Medications	89%	88%
ACE Inhibitor/ARB categories		
Neither ACE inhibitor nor ARB	22%	23%
ARB only	10%	12%
ACE inhibitor only	67%	64%
Both ARB and ACE inhibitor	1%	1%
Lipid lowering Medications		

	At sampling clinical visit	
Characteristic	Group A n=857	Group B n=246
Statins	76%	78%
Fibrates	20%	19%
Nicotinic acid	14%	18%
Resins	2%	3%
Ezetimibe	3%	2%
Aspirin use	91%	93%
Number of anti-hyperglycemic agents		
0	4%	3%
1	14%	18%
2	67%	64%
3	14%	15%
4	1%	<1%
Metformin use	73%	74%
Thiazolidinedione use	79%	74%
Rosiglitazone	79%	74%
PEDF Value ($\mu\text{g/mL}$)	10.6 (4.0)	10.7 (4.1)

Table 2

Factors univariately associated with PEDF levels using cross-sectional data in the large cohort of all available subjects (Group A) and the smaller subset with PEDF sampling performed at roughly the 2-year post-randomization visit (Group B).

LABEL	Group A (n=857)			Group B (n=246)		
	Regression Coefficient Estimates	Standard Error	p-value	Regression Coefficient Estimates	Standard Error	p-value
Age (ys)	-0.024	0.016	0.13	-0.052	0.030	0.083
Duration of DM (ys)	-0.007	0.019	0.72	-0.013	0.037	0.73
Non-Hispanic White	0.823	0.283	0.0037	1.262	0.557	0.024
HTN	1.034	0.454	0.023	1.214	0.840	0.15
SBP (mmHg)	0.003	0.009	0.74	-0.020	0.019	0.31
DBP (mmHg)	-0.007	0.014	0.58	-0.006	0.028	0.82
WHR	11.103	1.867	<0.0001	11.064	3.368	0.0012
BMI (kg/m ²)	0.168	0.026	<0.0001	0.171	0.054	0.0018
Intensive Therapy	-0.286	0.272	0.29	0.685	0.526	0.19
Neuropathy	-0.917	0.313	0.0035	-0.350	0.584	0.55
Retinopathy Severity (ETDRS scale)	0.062	0.051	0.22	-0.019	0.084	0.82
sCreatinine (μmol/L)	4.344	0.493	<0.0001	5.620	0.870	<0.0001
eGFR (mL/min/1.73 m ²)	-0.051	0.006	<0.0001	-0.063	0.011	<0.0001
sCr>176.8 μmol/L or GFR < 60 mL/min/1.73 m ²	1.583	0.288	<0.0001	3.102	0.601	<0.0001
TC (mmol/L)	0.008	0.003	0.017	0.006	0.006	0.35
TG (mmol/L)	0.004	0.001	<0.0001	0.011	0.002	<0.0001
LDL-C (mmol/L)	-0.002	0.005	0.68	-0.009	0.009	0.30
HDL-C (mmol/L)	-0.119	0.013	<0.0001	-0.114	0.026	<0.0001
Anti-Hypertensives	1.052	0.432	0.015	1.300	0.813	0.11
ACE Inhibitors	0.201	0.293	0.49	0.888	0.550	0.11
ARBs	1.182	0.428	0.0059	0.221	0.784	0.78
Aspirin	-0.791	0.481	0.10	-1.384	1.011	0.17
Rosiglitazone	-0.578	0.335	0.084	-1.264	0.596	0.035
Metformin	-0.676	0.304	0.026	-1.624	0.595	0.0068

LABEL	Group A (n=857)			Group B (n=246)		
	Regression Coefficient Estimates	Standard Error	p-value	Regression Coefficient Estimates	Standard Error	p-value
Lipid Lowering Drugs (any)	1.591	0.385	<0.0001	2.540	0.756	0.0009
Nicotinic acid	0.264	0.391	0.50	1.045	0.691	0.13
Resins	0.452	0.949	0.63	1.206	1.485	0.42
Statin	0.917	0.318	0.004	1.827	0.622	0.0036
Fibrates	1.738	0.335	<0.0001	2.187	0.656	0.001
Ezetimibe	1.112	0.860	0.20	-0.186	1.709	0.91

Table 3

Factors independently associated with PEDF levels based on multivariate linear regression models utilizing cross-sectional data in the large cohort of all available subjects and considering effect modification by race (Non-Hispanic White vs. all others) and renal dysfunction (composite of sCr $\geq 176.8 \mu\text{mol/L}$ and/or or eGFR $<60 \text{ ml/min/1.73m}^2$).

Parameter	Regression Coefficient Estimate	Standard Error	P-value
Intercept	-3.565	2.290	0.12
Non-Hispanic White (NHW)	-0.609	0.521	0.24
Waist-to-hip Ratio	5.349	1.923	0.0056
Serum Creatinine	6.804	0.929	<0.0001
Renal impairment *	-1.264	0.584	0.031
TG	0.005	0.001	<0.0001
HDL-C	-0.069	0.015	<0.0001
ACE inhibitor use	-0.247	0.510	0.63
Fibrates or niacin use	-0.614	0.358	0.087
Any Lipid-lowering drug	1.549	0.433	0.0004
Interaction: NHW x ACE Inhibitor Use	1.295	0.628	0.040
Interaction: Fibrate or Niacin Use x Renal Impairment *	1.564	0.701	0.026

* sCr $\geq 176.8 \mu\text{mol/L}$ and/or or eGFR $<60 \text{ ml/min/1.73m}^2$