ORIGINAL RESEARCH

Plasma Placental Growth Factor Concentrations Are Elevated Well in Advance of Type 2 Diabetes Mellitus Onset: Prospective Data From the WHS

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Background—Pathologic angiogenesis is a hallmark of type 2 diabetes mellitus (T2DM) microvascular complications and may modulate adipogenesis and precede the onset of clinical diabetes mellitus; however, longitudinal data are unavailable. Placental growth factor is a potent proangiogenic factor that stimulates the formation of mature and durable vessels but is understudied in human diseases.

Methods and Results—We conducted a prospective case-cohort study of baseline placental growth factor and incident T2DM within the WHS (Women's Health Study). A random sample of incident T2DM cases (n=491) occurring over a 15-year follow-up period was selected and compared with a reference subcohort (n=561). Case subjects were matched to the reference risk set on 5-year age groups and race. All subjects in this analysis were required to have a hemoglobin A_{1c} <6.5% at WHS enrollment. Median baseline levels of placental growth factor were higher in case subjects compare to the reference subcohort (18.0 pg/mL versus 17.2 pg/mL) but were only weakly correlated with glycemic measures and not associated with obesity. The risk of diabetes mellitus increased across placental growth factor quartile in the base model (hazard ratios, 1.00, 1.14, 1.46, and 2.14; *P*-trend<0.001) and in multivariable-adjusted models accounting for clinical T2DM risk factors (hazard ratios, 1.00, 1.17, 1.45, and 2.61; *P*-trend<0.001). These findings were not substantially altered by further adjustment for high-sensitivity C-reactive protein, hemoglobin A_{1c}, or fasting insulin and remained robust in sensitivity analyses excluding those diagnosed within 2 years of enrollment and those with baseline hemoglobin A_{1c} ≥6.0%.

Conclusions—Elevated placental growth factor levels are associated with future T2DM independent of traditional risk factors, measures of glycemia, insulin resistance, and high-sensitivity C-reactive protein. These prospective data suggest that pathologic angiogenesis may occur well before the clinical onset of T2DM and thus may have relevance to vascular complications of this disease.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00000479. **(*J Am Heart Assoc*. 2019;8: e012790. DOI: 10.1161/JAHA.119.012790.)**

Key Words: angiogenesis • diabetes mellitus • placenta growth factor • vascular disease • vascular growth factor

Type 2 diabetes mellitus (T2DM) has become a global epidemic[1] and is the leading cause of visual disability, blindness, and end-stage kidney disease in US adults.[2,3] Microvessel proliferation is a cardinal feature of these microvascular complications of diabetes mellitus[1,4,5] and may also contribute to impaired wound healing in diabetic foot ulcer,[6] coronary plaque progression and rupture,[7–9] and diminished collateral formation in diabetic coronary vascular disease.[10] Adipose tissue plasticity and expansion in obesity is also regulated by proangiogenic factors.[11] Yet our current

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Accompanying Tables S1 through S3 and Figures S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012790

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Clinical Perspective

What Is New?

- Among women aged 45 years or older without cardiovascular disease at baseline, elevated levels of the potent proangiogenic factor placental growth factor (PlGF) were associated with future type 2 diabetes mellitus.
- The risk of incident type 2 diabetes mellitus associated with PlGF was independent of traditional cardiovascular disease and diabetes mellitus risk factors including smoking, hypertension, hyperlipidemia, inflammation, measures of dysglycemia, and obesity.
- A 2-fold risk of diabetes mellitus in those with elevated PlGF levels was evident whether testing occurred 2 or 10 years before diabetes mellitus onset.

What Are the Clinical Implications?

- Consistent with the angiogenic paradox of type 2 diabetes mellitus, pathologic angiogenesis measured by baseline levels of PlGF may indicate that processes leading to diabetic microvascular complications occur years before the clinical onset of diabetes mellitus.
- Further studies evaluating elevated levels of PlGF and incident diabetic microvascular outcomes related to pathologic angiogenesis are warranted.
- PlGF may improve risk assessment for type 2 diabetes mellitus and its vascular complications beyond current clinical measures and may represent a potential target for therapeutic intervention.

understanding of instigators, disease modulators, and potential targets for treatment of this process remains limited. Further complicating the issue, a mixture of enhanced and suppressed angiogenesis, described as the "angiogenesis paradox" of diabetes mellitus,[12–14] is evident such that enhanced neovascularization occurs in retinopathy, nephropathy, and coronary atherosclerosis progression, whereas reduced neovascularization contributes to impaired wound healing and impaired coronary collateralization.

Neovascularization in embryogenesis and a broad range of human diseases is governed by proangiogenic factors, the most studied of which is vascular endothelial growth factor (VEGF).[15,16] Placental growth factor (PlGF) is a VEGF homologue, sharing 42% sequence homology, that enhances angiogenesis via signaling through its receptor Flt-1 on endothelial cells.[17,18] PlGF is not highly expressed in normal adult tissues,[19–21] but under pathologic conditions such as tissue hypoxia, upregulation of PlGF in endothelial cells contributes to an "angiogenic switch," resulting in angiogenesis and collateral growth.[19] While both VEGF-A and PlGF exert strong angiogenic effects, the vascular response to PlGF is less heterogeneous, leading to formation of more mature and durable vessels.[22] An emerging body of evidence suggests that PlGF may also be an important linking factor between T2DM and associated microvascular complications.[19,23,24] Specifically, elevations in PlGF levels have been observed in patients with diabetic retinopathy (DR) and diabetic nephropathy,[25,26] and interest in its use as a potential therapeutic target to modulate angiogenesis in DR has grown in recent years.[27] Angiogenic factors have also been implicated in the development of T2DM,[11,28] yet no prior epidemiologic studies have been available to prospectively evaluate this hypothesis. We conducted a case-cohort study of baseline PlGF levels and incident T2DM in a population cohort of over 38 728 apparently healthy baseline women without diabetes mellitus who participated in the WHS (Women's Health Study).

Methods

Data Availability

The data will not be made available to other researchers for purposes of reproducing the results. However, the methods in the analysis are available on request.

Study Participants and End Point Ascertainment

The study population consisted of participants enrolled in the WHS, a completed 2×2 factorial randomized, double-blind, placebo-controlled trial of aspirin and vitamin E in the prevention of cardiovascular disease and cancer among 39 876 female health professionals aged 45 years and older. Enrollment began in 1992 and the trial completed in March 2004, at which time women were invited to participate in observational follow-up. Among study participants, 19 502 (48.9%) did not have diabetes mellitus and provided fasting specimens at enrollment. These women comprised the source population for the sample analyzed in this study. All participants were continuously followed for the occurrence of incident diabetes mellitus via annual questionnaires asking whether and when they had been diagnosed with diabetes mellitus since baseline. Reported cases of diabetes mellitus were confirmed by either telephone interview (A.D.P.) or supplemental questionnaire.[29] Cases with diabetes mellitus accrued during both the initial trial period and during observational follow-up. Laboratory data were unavailable to distinguish type 1 diabetes mellitus (T1DM) or other diabetes mellitus variants. However, since the vast majority of diabetes mellitus diagnosed at age 45 years or older is of the type 2 variant, incident diabetes mellitus in the WHS cohort is considered T2DM.

Laboratory Analysis

Blood samples were stored in liquid nitrogen (−150°C to −180°C) and thawed at time of analysis. Total free PlGF was

measured using an enzyme-based electrochemiluminescence assay provided by Roche Diagnostics (Indianapolis, IN). The lower limit of detection was 3 pg/mL, and the coefficients of variation were <5% on the basis of blinded split duplicates. Hemoglobin A_{1c} was measured by immunoassay using a Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, IN). Fasting insulin concentration was analyzed using double antibody systems with <0.2% cross reactivity between insulin and its precursors (Linco Research, St Louis, MO). High-sensitivity C-reactive protein (hsCRP) concentrations were measured by a high-sensitivity immunoturbidimetric assay (Denka Seiken, Niigata, Japan). Total cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured using direct enzymatic colorimetric assays on a Hitachi 911 analyzer. Low-density lipoprotein cholesterol was measured using a homogeneous direct method with a Hitachi 917 analyzer using reagents from Roche Diagnostics. Triglycerides were measured enzymatically with correction for endogenous glycerol. Coefficients of variation were <10% for hsCRP, <2% for triglycerides, <3% for HDL and low-density lipoprotein, 7.2% for hemoglobin A_{1c} (HbA_{1c}), and 14.7% for insulin.

Study Subjects

Overview of case-cohort design

We conducted a prospective case-cohort study to efficiently address the study hypothesis for multiple outcomes (Figure S1). Funding was available for ≈1500 samples to be analyzed; thus, we first randomly sampled both incident T2D (n=500) and incident cardiovascular disease cases (n=500, previously found not to be associated with PlGF[30] and not included in this analysis). By chance, 14 subjects developed both T2DM and cardiovascular disease during the follow-up period. To reduce the chance of selection bias, all subjects who developed T2DM were retained in this analysis independent of follow-up cardiovascular disease status. A reference subcohort of 564 women were then selected independently as controls and stratified by age (5-year increments) and race (white versus nonwhite) to approximately match the frequency of these characteristics in the case sample. Since this subcohort was sampled independent of follow-up case status, some subcohort subjects (n=50) were also previously selected as cases. For efficiency (to maximize power within budgetary constraints), the subcohort sample size was augmented such that at least 500 women who were not selected in the case group were included in the subcohort. After accounting for depleted or insufficient blood specimens and subcohort augmentation, the final study sample comprised 491 women with incident T2DM approximately matched by age and race to 561 women in the subcohort.

Statistical Analysis

Baseline characteristics and correlations with PlGF levels

Baseline characteristics in T2DM cases and the reference subcohort were summarized as proportions (categorical variables) and medians (continuous variables), with standardized differences computed for comparison. Baseline characteristics were then summarized across quartiles of PlGF (unweighted subcohort cut points) using logistic regression for categorical variables and median regression for continuous variables. All analyses of baseline characteristics were adjusted for matching factors and were reweighted to allow for comparison to the WHS source population. The association of PlGF with select continuous clinical risk factors and biomarkers was also assessed by correlation coefficients estimated in the reference subcohort. As the distribution of PlGF was found to be nonnormal, weighted partial Pearson correlation coefficients of log-transformed variables were calculated with adjustment for matching factors. All analyses were performed using SAS version 9.4 (Cary, NC). This study was approved by the Institutional Review Board at Brigham and Women's Hospital, and subjects provided informed consent.

Cox proportional hazards regression

Employing methods appropriate for a stratified case-cohort design,[31–33] Cox proportional hazards models were used to test the association between baseline PlGF quartile and incident T2DM weighted by stratum-specific sampling frequencies (Barlow weighting) and computed robust sandwich variance estimates. All models accounted for stratification variables (age and race) and original treatment assignment in the WHS trial (aspirin, vitamin E). The expanded adjusted model included conventional risk factors for T2DM (model 2: continuous BMI, history of hypertension, history of hyperlipidemia, current smoking status, parental history of diabetes mellitus, menopausal hormonal therapy use, and weekly exercise versus none), while additional models also accounted for baseline hsCRP (model 3), HbA_{1c}, and fasting insulin (model 4), or all 3 (model 5). Tests for trend across PlGF quartiles were performed after assigning the median PlGF value to each quartile. We also compared the relative strength of the association between PlGF and T2DM to those of other known markers of risk, including hsCRP, HbA_{1c}, and fasting insulin. We examined potential nonlinear relationships between PlGF and the risk of T2DM nonparametrically with restricted cubic splines.[34] Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. We repeated this process for hsCRP, HbA_{1c}, and fasting insulin.

In sensitivity analyses, we excluded women with a baseline HbA_{1c} ≥6.0%. We also repeated analyses after exclusion of cases diagnosed within 2 years of enrollment to exclude women with potentially undiagnosed T2DM at baseline. To assess the impact of time from blood draw (time from PlGF ascertainment), we repeated the main analysis after dividing follow-up into 3 time periods corresponding to approximate tertiles of T2DM events by years of follow-up: 0 to 5.9 years (n=160 cases), 6.0 to 9.9 years (n=175 cases), and >10.0 years (n=156 cases).

Joint effects of PlGF with fasting insulin and body mass index

To assess the joint effects of PlGF with fasting insulin, we divided the population into 4 groups based on subcohort median cut points for each biomarker. PlGF and insulin were also included in each of the models as continuous variables to account for within-group variation of each biomarker. To assess the joint effects of PlGF and obesity, we used the median cut point of PlGF and a body mass index (BMI) cut point of ≥30.0 kg/m^2 and adjusted for continuous BMI within groups. Interaction terms were also included in models to assess whether the risk of PlGF was additive to elevated insulin and BMI.

Results

Baseline Characteristics

Baseline characteristics of the incident cases of T2DM and reference subcohort are shown in Table 1. As expected, women who developed diabetes mellitus were more likely to have a higher BMI; higher systolic blood pressure; and higher baseline prevalence of hypertension, hypercholesterolemia, and parental history of diabetes mellitus. Women developing diabetes mellitus also had a generally less favorable lipid profile with higher median total cholesterol and triglyceride levels and lower median HDL-C levels. Low-density lipoprotein cholesterol levels, in contrast, did not greatly differ between groups (127.5 mg/dL versus 124.0 mg/dL, respectively). As expected,[29] the median hsCRP level in women developing diabetes mellitus was nearly double that of those in the reference subcohort (4.08 mg/L versus 2.10 mg/L, respectively). HbA_{1c} levels and fasting insulin at baseline were higher in the cases with diabetes mellitus compared with the reference risk set (5.26% versus 5.00% for HbA_{1c}; 12.4 μIU/L versus 6.83 μIU/L for fasting insulin). Baseline levels of PlGF were also higher in the case subjects compared with the reference subcohort (18.0 pg/mL versus 17.2 pg/mL). To better understand this relationship, we examined baseline characteristics in the subcohort according to quartiles of PlGF (Table 2). There were no apparent differences in BMI or hsCRP across categories, while age increased with each quartile and HDL-C decreased. HbA_{1c} and fasting insulin trended up across quartiles of PlGF, though only minimally (Q1 versus Q4: 4.98% versus 5.03%; Q1 versus Q4: 6.21 mIU/L versus 7.37 mIU/L). Other clinical risk factors for diabetes mellitus are also shown. Pearson partial correlation coefficients adjusting for matching factors are presented in Table S1. No strong correlations with measured diabetes mellitus risk factors or biomarkers were observed. PlGF level was weakly correlated with age (r=0.294, P<0.001) and fasting insulin (r=0.137, P=0.001) and weakly inversely correlated with total cholesterol (r=−0.096, P=0.024) and HDL-C (r=−0.179, P<0.001). PlGF level was not associated with systolic blood pressure, BMI, hsCRP, HbA_{1c}, or other lipid levels. Scatter plots for adjusted baseline concentrations of PlGF with select T2DM risk factors with corresponding Pearson partial correlation coefficients are shown in Figure S2.

Association of Placental Growth Factor With Incident T2DM

In the base model adjusting for age, race, and WHS treatment assignment, the risk of T2DM steadily increased across PlGF quartile (hazard ratio $[HR]_{Q1}$, 1.00 [ref]; HR_{Q2}, 1.14 [95% CI, 0.79–1.64]; HR_{Q3}, 1.46 [95% CI, 1.02–2.08]; HR_{Q4}, 2.14 [95% CI, 1.46–3.15]; P-trend<0.001). Addition of traditional risk factors for T2DM (model 2) did not alter this association; adjusted HRs were 1.0 (ref), 1.17, 1.45, and 2.61 (P-trend<0.001). This graded risk association persisted in models adjusting for baseline hsCRP (model 3), both HbA_{1c} and fasting insulin (model 4), and all 3 (model 5). In comparison to other biomarkers, the risk of T2DM among those in the highest quartile of PlGF was most comparable to risk of those in the second and third quartiles of hsCRP, HbA_{1c}, and fasting insulin. In the fully adjusted models, HbA_{1c} was the strongest predictor of incident T2DM. The results are shown in Table 3. Spline analyses for the association between PlGF concentration and incident T2DM is presented in Figure 1. The risk of T2DM steadily increased with increasing concentration of PlGF in the base model (Figure 1A) and was independent of T2DM risk factors (Figure 1B), measures of dysglycemia, and inflammation (Figure 1C). Splines for HbA_{1c}, insulin, and hsCRP using the fully adjusted models (model 5+PlGF, excluding spline variable) are shown in Figure S3.

Sensitivity Analyses

Our results were minimally attenuated when participants with HbA_{1c} ≥6.0% were excluded; the model 2 HR for

Table 1. Baseline Characteristics of the Study Cohort, Reweighted to the Source Population

Characteristic	Incident Diabetes Mellitus (N=491) ($N_{reweighted}$=1228)	Reference Subcohort (N=561) ($N_{reweighted}$=19 277)	Std Diff
Age, y	53.5 (49.3, 59.6)	53.5 (49.2, 59.3)	0.00
Aspirin	617 (50.2%)	9312 (48.3%)	0.04
Vitamin E	631 (51.4%)	9310 (48.3%)	0.04
White race	1134 (92.3%)	18 468 (95.8%)	−0.18
Body mass index, kg/m^2	29.6 (26.5, 33.9)	25.6 (23.0, 28.3)	0.22
Obese, N (%)	585 (47.6%)	3237 (16.8%)	0.77
Systolic blood pressure, mm Hg	126.7 (117.6, 136.2)	118.2 (109.4, 129.1)	0.13
History of hypertension, %	630 (51.3%)	5225 (27.1%)	0.55
Treatment for hypertension, %	409 (33.3%)	2674 (13.9%)	0.52
History of hyperlipidemia, %	551 (44.8%)	5796 (30.1%)	0.33
Treatment for hyperlipidemia, %	78 (6.3%)	637 (3.3%)	0.16
Parental history of diabetes mellitus, %	520 (42.3%)	5178 (26.9%)	0.37
Alcohol, at least 1 serving/week, %	361 (29.4%)	7749 (40.2%)	−0.26
Exercise, at least once/week, %	399 (32.5%)	8991 (46.6%)	−0.29
Menopausal hormone therapy, N (%)	526 (42.9%)	8094 (42.1%)	0.00
Active smoker, N (%)	158 (12.9%)	1999 (10.4%)	0.07
Total cholesterol, mg/dL	213.9 (187.9, 242.8)	210.9 (185.7, 239.3)	0.03
High-density lipoprotein cholesterol, mg/dL	42.5 (36.5, 50.8)	50.7 (43.2, 61.9)	−0.17
Low-density lipoprotein cholesterol, mg/dL	127.5 (105.8, 153.6)	124.0 (104.2, 147.5)	0.03
Triglycerides, mg/dL	177.1 (124.0, 244.9)	116.5 (81.9, 172.5)	0.17
High-sensitivity C-reactive protein, mg/L	4.08 (2.24, 7.01)	2.10 (0.87, 4.52)	0.16
Hemoglobin A_{1c}, %	5.26 (5.06, 5.52)	5.00 (4.84, 5.18)	0.25
Insulin, mIU/L	12.4 (8.36, 18.8)	6.83 (4.28, 10.7)	0.23
Placental growth factor, pg/mL	18.0 (15.6, 20.6)	17.2 (15.0, 19.6)	0.05

Table entries are N (%) or median (interquartile range) and are reweighted to the WHS source population. All biomarkers were measured in the fasting state (no food or drink within 8 hours before laboratory draw). All subjects had hemoglobin A_{1c} <6.5% at baseline by study design. Std Diff=standardized difference, that is, the differences in medians or proportions divided by the pooled standard deviation.

extreme quartiles was 2.49 (95% CI, 1.51–4.12; *P*-trend<0.001) (Table S2). No material difference was noted when cases diagnosed within the first 2 years of follow-up (individuals with presumably prediabetes or undiagnosed diabetes mellitus) were excluded; the model 2 HR for extreme quartiles was 2.69 (95% CI, 1.62–4.48; *P*-trend<0.001) (Table S3). In both sensitivity analyses, inclusion of hsCRP had no appreciable impact on these results, while adjustment for HbA_{1c} and fasting insulin slightly attenuated the risk association, which remained statistically significant. Additional adjustment for HDL-C, alcohol consumption, and systolic blood pressure (in place of history of hypertension) to the most conservative model presented in Table S2 had no material impact on our results (HRs, 1.00, 0.75, 1.15, and 1.90 across quartiles of PlGF; *P*-trend=0.018).

Time to Diagnosis and Joint Effects Analyses

The overall median time to T2DM event was 8.2 years. HRs for incident T2DM did not change according to time from PlGF measurement as shown in Figure 2 (cases per 1 follow-up interval shown in Figure S4), suggesting that chronic rather than acute PlGF elevation precedes a diagnosis. In analyses examining the joint effects of fasting insulin and PlGF, the HR for T2DM was highest among women with both high fasting insulin and high PlGF (far right bar) and greater than for either marker alone (middle bars) (Figure 2B). In analyses examining the joint effects of obesity and PlGF, similar observations were made, such that HR for T2DM was highest among those with high PlGF and obesity (Figure 2C). No interaction was found between PlGF and fasting insulin (*P*=0.41) or BMI (*P*=0.69), consistent with an additive effect of high PlGF independent of insulin status or BMI status.

Table 2. Baseline Characteristics According to Quartile of Placental Growth Factor in the Reference Subcohort

Characteristic	Quartile 1 (N=139) (N_W=5564)	Quartile 2 (N=142) (N_W=5068)	Quartile 3 (N=139) (N_W=4725)	Quartile 4 (N=141) (N_W=3920)
Age, y	51.6 (0.67)	52.6 (0.95)	53.4 (0.64)	58.6 (1.22)
White race, %	95.6%	94.2%	96.2%	98.1%
Body mass index, kg/m^2	25.0 (0.49)	25.6 (0.51)	25.5 (0.61)	26.0 (0.47)
Systolic blood pressure, mm Hg	127 (1.5)	122 (1.8)	126 (1.7)	126 (2.4)
History of hypertension, %	31.2%	31.8%	39.2%	45.0%
History of hyperlipidemia, %	35.0%	35.5%	37.0%	40.8%
Parental history of diabetes mellitus, %	28.8%	32.9%	23.4%	31.0%
Alcohol, at least 1 serving/week, %	45.6%	46.6%	36.1%	31.1%
Exercise, at least once/week, %	51.3%	45.5%	44.3%	44.7%
Menopausal hormone therapy, %	45.8%	53.4%	39.7%	38.2%
Active smoker, %	11.1%	13.0%	5.8%	10.9%
Total cholesterol, mg/dL	220.2 (3.8)	218.2 (5.7)	215.8 (4.0)	205.0 (3.7)
High-density lipoprotein cholesterol, mg/dL	53.9 (1.6)	56.1 (1.6)	50.8 (1.7)	48.5 (1.6)
Low-density lipoprotein cholesterol, mg/dL	130.1 (2.7)	124.3 (3.0)	132.2 (3.5)	123.1 (2.9)
Triglycerides, mg/dL	123.7 (6.6)	123.1 (7.1)	132.9 (7.6)	111.4 (7.2)
High-sensitivity C-reactive protein, mg/L	2.17 (0.27)	2.31 (0.19)	2.38 (0.35)	1.79 (0.24)
HbA_{1c}, %	4.98 (0.02)	5.02 (0.03)	5.02 (0.02)	5.03 (0.04)
Insulin, mIU/L	6.21 (0.45)	6.51 (0.49)	7.23 (0.56)	7.37 (0.57)

Table entries are % or median (SE) adjusted for matching factors and reweighted to the WHS source population. All biomarkers were measured in the fasting state (no food or drink within 8 hours before laboratory draw). All subjects had HbA_{1c} <6.5% at baseline by study design. HbA_{1c} indicates hemoglobin A1c; N_W, weighted N.

Discussion

In this prospective study of apparently healthy women, elevated levels of PlGF were associated with incident T2DM over a median follow-up period of 8.2 years. Among those with PlGF concentration in the highest quartile, there was a >2-fold increased risk of developing T2DM that was not substantially altered by adjustment for traditional diabetes mellitus risk factors, hsCRP, HbA_{1c}, or fasting insulin. These findings persisted in our spline analyses, showing a nearly 4-fold increased risk among those with the highest PlGF concentrations (Figure 1B and 1C). Additionally, our findings of a gradient in risk remained robust in sensitivity analyses excluding women with baseline HbA_{1c} ≥6.0% and those diagnosed within the first 2 years of follow-up. The risk of diabetes mellitus in those with elevated PlGF levels also remained independent of time from baseline PlGF such that a 2-fold elevation in risk was evident whether testing occurred 2 or 10 years before diabetes mellitus onset. Consistent with prior clinical studies,[26,35–37] we found that PlGF was modestly correlated with age and, at most, weakly correlated with other diabetes mellitus risk indicators including HDL-C and fasting insulin. Additionally, as noted in prior human studies,[26,35–37] no substantive correlation was observed with HbA_{1c}, BMI, or hsCRP despite preclinical evidence linking PlGF expression to hyperglycemia, obesity, and subclinical inflammation.[38–41] The minimal attenuation of risk associations after adjustment for established diabetes mellitus risk factors, minimal correlation with fasting insulin, and absent correlation with HbA_{1c} or BMI suggests an independent biologic effect in this study population of women at relatively low risk for diabetes mellitus.

We hypothesized that PlGF levels would be linked to obesity and insulin resistance as central drivers of T2DM risk. However, our data do not support this mechanistic link as an explanation for our main findings. The VEGF family of growth factors initiates a cascade of events that begins with endothelial cell proliferation, followed by directed cell migration, intercellular junction and lumen formation, organization of perivascular support, and anastomosis with existing vessels.[28] It is now well established from preclinical models that tissue hypoxia in obesity is a potent stimulus for this process and adipose tissue plasticity is tightly linked to vascular supply.[11] In mouse models of nutritional obesity, PlGF deficiency impairs fat expansion and anti-PlGF treatment reduces de novo fat pad formation,[39] an

Table 3. Incident Cases of Type 2 Diabetes Mellitus, According to Baseline Concentration of PlGF, hsCRP, HbA_{1c}, and Fasting Insulin

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* Value
PlGF, mg/dL	<15.4	15.4 to 17.6	17.7 to 20.3	≥20.4	
Incident cases (N=491)	109	114	130	138	
Incident cases, reweighted (N=1228)	274	284	324	346	
Model 1 HR (95% CI)	1	1.14 (0.79–1.64)	1.46 (1.02–2.08)	2.14 (1.46–3.15)	<0.001
Model 2 HR (95% CI)	1	1.17 (0.72–1.89)	1.45 (0.88–2.38)	2.61 (1.58–4.29)	<0.001
Model 3 HR (95% CI)	1	1.09 (0.65–1.83)	1.57 (0.96–2.56)	2.62 (1.57–4.38)	<0.001
Model 4 HR (95% CI)	1	1.03 (0.60–1.76)	1.34 (0.74–2.43)	2.35 (1.35–4.10)	0.002
Model 5 HR (95% CI)	1	0.95 (0.55–1.65)	1.35 (0.75–2.45)	2.39 (1.37–4.18)	0.001
hsCRP, mg/L	<0.94	0.94 to 2.15	2.16 to 4.61	≥4.62	
Incident cases (N=489)	37	81	157	214	
Incident cases, reweighted (N=1223)	89	201	395	538	
Model 2 HR (95% CI)	1	1.76 (1.04–2.97)	3.44 (2.07–5.73)	3.92 (2.34–6.56)	<0.001
Model 2+HbA_{1c} and insulin	1	1.68 (0.92–3.08)	3.55 (1.95–6.47)	3.40 (1.80–6.40)	0.004
Model 2+HbA_{1c} and insulin and PlGF	1	1.69 (0.93–3.08)	3.49 (1.91–6.38)	3.41 (1.81–6.41)	0.003
Fasting insulin, mIU/L	<4.35	4.35 to 6.81	6.82 to 10.6	≥10.7	
Incident cases (N=491)	20	51	121	299	
Incident cases, reweighted (N=1228)	50	127	304	747	
Model 2 HR (95% CI)	1	2.13 (1.17–3.90)	3.65 (2.02–6.59)	8.49 (4.69–15.38)	<0.001
Model 2+HbA_{1c} and hsCRP	1	2.20 (1.14–4.24)	3.07 (1.60–5.89)	5.17 (2.65–10.07)	<0.001
Model 2+HbA_{1c} and hsCRP and PlGF	1	2.39 (1.18–4.83)	3.37 (1.66–6.87)	5.54 (2.68–11.43)	<0.001
HbA_{1c}, %	<4.86	4.86 to 5.01	5.02 to 5.19	≥5.20	
Incident cases (N=490)	41	64	96	289	
Incident cases, reweighted (N=1224)	102	160	241	722	
Model 2 HR (95% CI)	1	1.87 (1.13–3.11)	2.52 (1.50–4.23)	7.39 (4.56–11.99)	<0.001
Model 2+insulin and hsCRP	1	1.83 (1.08–3.11)	2.45 (1.41–4.26)	7.02 (4.21–11.71)	<0.001
Model 2+insulin and hsCRP and PlGF	1	1.85 (1.08–3.15)	2.37 (1.35–4.16)	6.98 (4.17–11.68)	<0.001

Model 1: Adjusted for age and race, and WHS randomized treatment assignments (aspirin and vitamin E). Model 2: Adjusted for model 1 covariates plus body mass index, history of hypertension, history of hyperlipidemia, current smoking, parental history of diabetes mellitus, menopausal hormone therapy use, and exercise frequency. Model 3: Adjusted for model 2 covariates plus hsCRP. Model 4: Adjusted for model 2 covariates plus HbA_{1c} and fasting insulin. Model 5: Adjusted for model 2 covariates plus hsCRP, HbA_{1c}, and fasting insulin. HbA_{1c} indicates hemoglobin A_{1c}; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; PlGF, placental growth factor.

effect that may be cell-type specific. Specifically, PlGF deficiency reduces the fraction of brown adipocytes while stimulating white adipocyte hypertrophy in mice fed a high-fat diet, changes that promote insulin resistance and hyperinsulinemia but may not alter fat mass.[42] Consistent with prior human studies,[26,36,37,43] we found no association between circulating levels of PlGF and total body adiposity as measured by BMI, and very weak, if any, correlation with dysglycemia as measured by fasting insulin and HbA_{1c} (Table 2, Table S1, Figure S2). Whether the signal detected in our epidemiologic study indicates an independent role for PlGF in diabetogenesis cannot be determined from our analysis but warrants further investigation.

Importantly, if corroborated, our data may have potential relevance to mechanisms of microvascular disease in patients with diabetes mellitus. Our understanding of PlGF as a marker of pathologic angiogenesis has grown through data derived from animal models of DR. PlGF expression is increased in mice with diabetes mellitus with retinal neovascularization compared with nonretinopathic controls with diabetes mellitus,[25,44–46] and deletion of the *plgf* gene has been shown to prevent DR altogether.[47] As a therapeutic target, neutralization of PlGF with anti-PlGF antibody therapy in mice with diabetes mellitus provides comparable results to the anti-VEGF therapy, the gold standard for DR, with the additional benefit of reducing inflammation and fibrosis.[48] With regard to

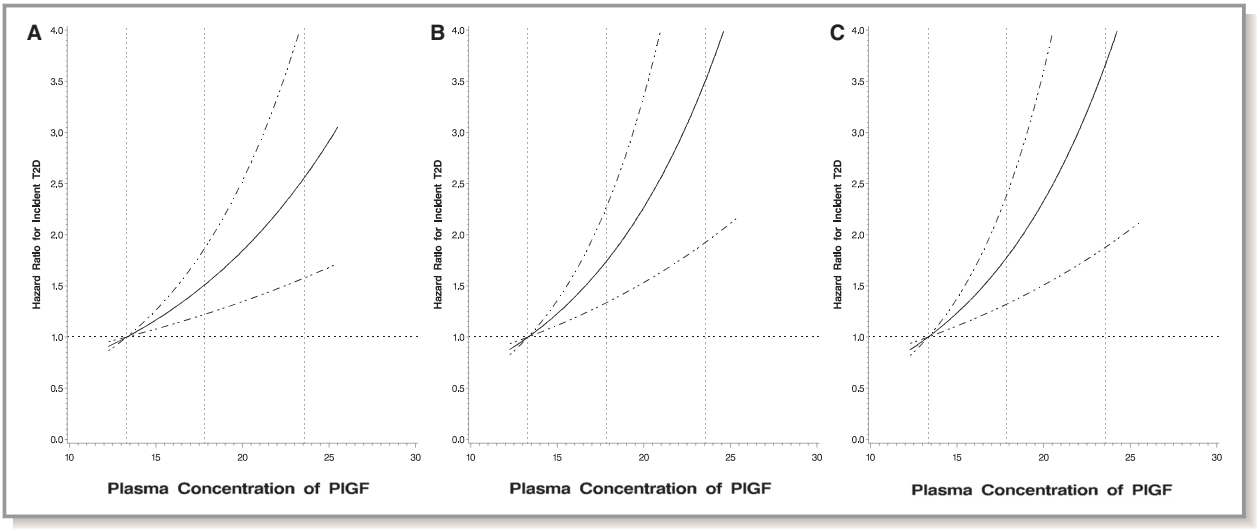

Figure 1. Spline analyses for risk associations between PlGF and incident type 2 diabetes mellitus. **A**, In the base model adjusting for WHS (Women's Health Study) treatment assignment and stratification factors, the risk of incident type 2 diabetes mellitus (T2DM) had a strongly linear association with increasing concentration of PlGF) ($P_{\text{non-linearity}}$=0.92; $P_{\text{linearity}}$<0.001). Knots for model 1 were identified at 13.1, 17.8, and 23.6 mg/dL, with the first knot set as reference. **B**, Adjustment for traditional clinical T2DM risk factors (model 2 covariates) did not significantly attenuate the linear association between PlGF and incident T2DM ($P_{\text{non-linearity}}$=0.97; $P_{\text{linearity}}$<0.001). **C**, Further adjustment for hsCRP, HbA_{1c}, and fasting insulin had no meaningful impact the association observed in (B) ($P_{\text{non-linearity}}$=0.20; $P_{\text{linearity}}$<0.001). Knots for model 2 and model 5 were identified at 13.3, 17.8, and 23.6 mg/dL, with first knot set as reference. All models exclude top and bottom fifth percentiles of data to reduce the impact of extreme data points. HbA_{1c} indicates hemoglobin A_{1c}; hsCRP, high-sensitivity C-reactive protein; PlGF, placental growth factor.

diabetic nephropathy, proteomic analysis of participants with diabetes mellitus in 2 community-based Swedish cohorts recently found that elevated PlGF levels were associated with the presence of albuminuria.[49] In patients with type 1 diabetes mellitus, PlGF levels are higher in those with diabetic nephropathy compared with those without and associate with a 2-fold excess in all-cause mortality.[26] To our knowledge, data for associations of PlGF with diabetic neuropathy have not been previously published.

Our data may also have biologic relevance to accelerated atherosclerosis in T2DM. As recently reviewed,[50] plaque neorevascularization and intraplaque hemorrhage play an important role in the pathogenesis of atherosclerosis. Intimal thickening is thought to induce hypoxia as a major stimulant for VEGF-induced angiogenesis with subsequent erythrocyte extravasation, free cholesterol deposition, and macrophage accumulation. Experimental models[6] have demonstrated that PlGF expression and Flt-1 activation are culprit in this maladaptive process and accumulating evidence from population studies, while not unique to patients with diabetes mellitus, demonstrate the prognostic value of elevated plasma PlGF on future cardiovascular events.[26,36,37,43,51–53]

There are several limitations to our current study. First, as our study cohort consisted of primarily middle-aged white American female health professionals, these findings may not be generalizable to other age and racial groups, men, or groups of different socioeconomic status at risk for T2DM. Second, other measures of adiposity, specifically central adiposity, were not available in the WHS. However, adjustment for BMI as a measure of total adiposity had no impact on our results. Third, undiagnosed diabetes mellitus at study entry may be a source of potential misclassification; however, our results remained robust in sensitivity analyses, excluding cases within the first 2 years and those with HbA_{1c} ≥6.0%. Fourth, laboratory data were not available to distinguish between subtypes of T2DM, thus limiting our ability to assess uniformity across diabetes mellitus variants that may be relevant to our results. Fifth, baseline standardized difference in PlGF concentration was small (Table 1), indicating <7.7% non-overlap in case and control population distributions; thus, multiple lines of study including replication in other populations in addition to studies examining precise metabolic effects of PlGF levels may provide further insight into our findings. Sixth, data related to other potential confounders such as polycystic ovarian syndrome and prior gestational diabetes mellitus were not available in our study, thus we are unable to explore potential associations with this disease states. Finally, our study used a single baseline measurement of PlGF; thus, intraindividual variation during the follow-up period could not be assessed. Stable PlGF levels in patients with and without diabetes mellitus, however, have been previously observed during at least 6 months of follow-up.[54]

In summary, in this prospective evaluation of a novel systemic proangiogenic marker in otherwise healthy women,

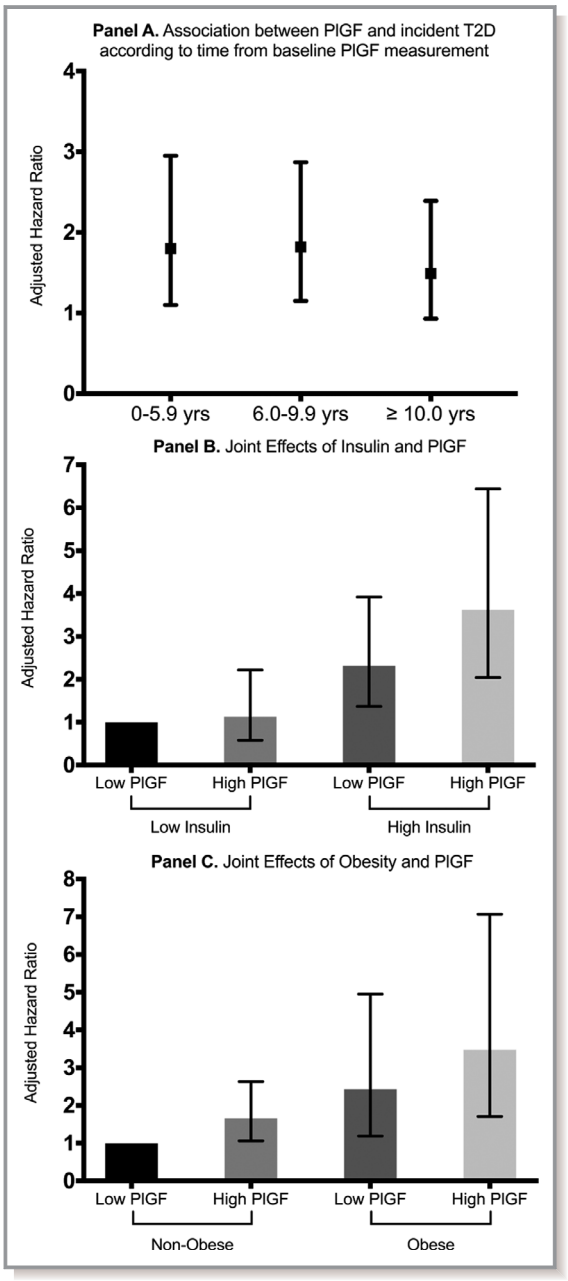

Figure 2. Stratified analyses for risk associations between placental growth factor and incident T2DM. **A**, Hazard ratios for the association between median concentration of PIGF and incident T2DM according to 3 ranges of follow-up time. As shown, time at which diabetes mellitus was diagnosis had no meaningful impact on the association with PIGF level. **B** and **C**, Joint effects of PIGF according to insulin level (B) and obesity status (C). Elevated PIGF appeared to augment the risk of incident diabetes mellitus in the presence of both high and low insulin and in those with or without obesity ($P_{interaction}$>0.05 for both). High status indicates biomarker value greater than or equal to the median concentration in the subcohort. Subjects considered obese if BMI ≥30.0 kg/m^2. BMI indicates body mass index; PIGF, placental growth factor; T2DM, type 2 diabetes mellitus.

baseline PIGF concentration was found to be strongly associated with an increased risk of incident diabetes mellitus. Our findings remained robust in multivariate models adjusting for traditional risk factors of T2DM. These epidemiologic observations cannot confirm a causal association but do demonstrate that elevations in PIGF occurs years in advance of clinical T2DM, and this association is independent of traditional diabetes mellitus risk factors, including obesity, measures of dysglycemia, and inflammation. Our data are relevant to the understanding drivers of micro- and macrovascular disease in this patient population and drug development programs to target these adverse outcomes.

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Disclosures

Dr Ridker is listed as a coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease, which have been licensed to AstraZeneca and Siemens; has received investigator research support from Kowa Research Institute, Novartis, Pfizer, and Astra-Zeneca; has served as a consultant to Jannsen, Novartis, and Sanofi-Regenerson; and serves as co-principal investigator of the PROMINENT (Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients With Diabetes) trial (NCT03071692). Dr Pradhan receives investigator-initiated research support from Kowa Research Institute and serves as co-principal investigator of the PROMINENT trial (NCT03071692). The remaining authors have no disclosures to report.

References

1. Diabetes: Facts and Figures. Available at: http://www.idf.org/about-diabetes/facts-figures. Accessed July 21, 2018.
2. Diabetic Retinopathy. Available at: https://www.nei.nih.gov/eyedata/diabetic. Accessed July 21, 2018.
3. Diabetic Kidney Disease. Available at: https://www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/diabetic-kidney-disease. Accessed July 21, 2018.
4. Crawford TN, Alfaro DV III, Kerrison JB, Jablon EP. Diabetic retinopathy and angiogenesis. *Curr Diabetes Rev*. 2009;5:8–13.
5. Moulton KS. Plaque angiogenesis and atherosclerosis. *Curr Atheroscler Rep*. 2001;3:225–233.
6. Khurana R, Moons L, Shafi S, Lutten A, Collen D, Martin J, Carmeliet P, Zachary I. Placental growth factor promotes atherosclerotic intimal thickening and macrophage accumulation. *Circulation*. 2005;111:2828–2836.
7. Nakagawa T, Kosugi T, Haneda M, Rivard CJ, Long DA. Abnormal angiogenesis in diabetic nephropathy. *Diabetes*. 2009;58:1471–1478.

8. Qi M, Zhou Q, Zeng W, Wu L, Zhao S, Chen W, Luo C, Shen M, Zhang J, Tang CE. Growth factors in the pathogenesis of diabetic foot ulcers. *Front Biosci (Landmark Ed)*. 2018;23:310–317.
9. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med*. 2001;7:425–429.
10. Marfella R, Esposito K, Nappo F, Siniscalchi M, Sasso FC, Portoghese M, Di Marino MP, Baldi A, Cuzzocrea S, Di Filippo C, Barboso G, Baldi F, Rossi F, D'Amico M, Giugliano D. Expression of angiogenic factors during acute coronary syndromes in human type 2 diabetes. *Diabetes*. 2004;53:2383–2391.
11. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab*. 2013;18:478–489.
12. Duh E, Aiello LP. Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. *Diabetes*. 1999;48:1899–1906.
13. Waltenberger J. New horizons in diabetes therapy: the angiogenesis paradox in diabetes: description of the problem and presentation of a unifying hypothesis. *Immunol Endocr Metab Agents Med Chem*. 2007;7:87–93.
14. Cheng R, Ma J. Angiogenesis in diabetes and obesity. *Rev Endocr Metab Disord*. 2015;16:67–75.
15. Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. *Semin Cancer Biol*. 1999;9:211–220.
16. Carmeliet P, Collen D. Molecular basis of angiogenesis. Role of VEGF and VE-cadherin. *Ann N Y Acad Sci*. 2000;902:249–262; discussion 262–244.
17. Tjwa M, Luttun A, Autiero M, Carmeliet P. VEGF and PlGF: two pleiotropic growth factors with distinct roles in development and homeostasis. *Cell Tissue Res*. 2003;314:5–14.
18. Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, Kroll J, Plaisance S, De Mol M, Bono F, Kliche S, Fellbrich G, Ballmer-Hofer K, Maglione D, Mayr-Beyrle U, Dewerchin M, Dombrowski S, Stanimirovic D, Van Hummelen P, Dehio C, Hicklin DJ, Persico G, Herbert JM, Communi D, Shibuya M, Collen D, Conway EM, Carmeliet P. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med*. 2003;9:936–943.
19. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, Scholz D, Acker T, DiPalma T, Dewerchin M, Noel A, Stalmans I, Barra A, Blacher S, VandenDriessche T, Ponten A, Eriksson U, Plate KH, Foidart JM, Schaper W, Charnock-Jones DS, Hicklin DJ, Herbert JM, Collen D, Persico MG. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. 2001;7:575–583.
20. Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B, Compernolle V, Daci E, Bohlen P, Dewerchin M, Herbert JM, Fava R, Matthys P, Carmeliet G, Collen D, Dvorak HF, Hicklin DJ, Carmeliet P. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med*. 2002;8:831–840.
21. Oura H, Bertoncini J, Velasco P, Brown LF, Carmeliet P, Detmar M. A critical role of placental growth factor in the induction of inflammation and edema formation. *Blood*. 2003;101:560–567.
22. Nagy JA, Dvorak AM, Dvorak HF. VEGF-A(164/165) and PlGF: roles in angiogenesis and arteriogenesis. *Trends Cardiovasc Med*. 2003;13:169–175.
23. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp Mol Med*. 2012;44:1–9.
24. Semenza GL. Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol*. 1999;15:551–578.
25. Al Kahtani E, Xu Z, Al Rashaed S, Wu L, Mahale A, Tian J, Abboud EB, Ghazi NG, Kozak I, Gupta V, Arevalo JF, Duh EJ. Vitreous levels of placental growth factor correlate with activity of proliferative diabetic retinopathy and are not influenced by bevacizumab treatment. *Eye (Lond)*. 2017;31:529–536.
26. Theilade S, Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Evaluation of placental growth factor and soluble Fms-like tyrosine kinase 1 as predictors of all-cause and cardiovascular mortality in patients with type 1 diabetes with and without diabetic nephropathy. *Diabet Med*. 2012;29:337–344.
27. Nguyen QD, De Falco S, Behar-Cohen F, Lam WC, Li X, Reichhart N, Ricci F, Pluim J, Li WW. Placental growth factor and its potential role in diabetic retinopathy and other ocular neovascular diseases. *Acta Ophthalmol*. 2018;96:e1–e9.
28. Corvera S, Gealekman O. Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. *Biochem Biophys Acta*. 2014;1842:463–472.
29. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286:327–334.
30. Pradhan AD, Cook NR, Kim E, Everett BE, Magnone MC, Bobadilla M, Rifai N, Ridker PM. Placental growth factor is associated with incident type 2 diabetes but not cardiovascular disease in otherwise healthy women. *Diabetes*. 2011;60(supplement 1):A1–A104, 39-OR.
31. Barlow WE. Robust variance estimation for the case-cohort design. *Biometrics*. 1994;50:1064–1072.
32. Langholz B, Jiao J. Computational methods for case-cohort studies. *Comput Stat Data Anal*. 2007;51:3737–3748.
33. Therneau TM, Li H. Computing the Cox model for case cohort designs. *Lifetime Data Anal*. 1999;5:99–112.
34. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8:551–561.
35. Barcelo A, Bauça JM, Yañez A, Fueyo L, Gomez C, de la Peña M, Pierola J, Rodriguez A, Sanchez-de-la-Torre M, Abad J, Mediano O, Amilibia J, Masdeu MJ, Teran J, Montserrat JM, Mayos M, Sanchez-de-la-Torre A, Barbé F; Spanish Sleep Group. Impact of obstructive sleep apnea on the levels of placental growth factor (PlGF) and their value for predicting short-term adverse outcomes in patients with acute coronary syndrome. *PLoS One*. 2016;11:e0147686.
36. Cassidy A. Potential role for plasma placental growth factor in predicting coronary heart disease risk in women. *Arterioscler Thromb Vasc Biol*. 2009;29:134–139.
37. Matsui M, Uemura S, Takeda Y, Samejima K, Matsumoto T, Hasegawa A, Tsushima H, Hoshino E, Ueda T, Morimoto K, Okamoto K, Okada S, Onoue K, Okayama S, Kawata H, Kawakami R, Maruyama N, Akai Y, Iwano M, Shiiki H, Saito Y; NARA-CKD Investigators. Placental growth factor as a predictor of cardiovascular events in patients with CKD from the NARA-CKD study. *J Am Soc Nephrol*. 2015;26:2871–2881.
38. Lijnen HR, Christiaens V, Scroyen I, Voros G, Tjwa M, Carmeliet P, Collen D. Impaired adipose tissue development in mice with inactivation of placental growth factor function. *Diabetes*. 2006;55:2698–2704.
39. Christiaens V, Voros G, Scroyen I, Lijnen HR. On the role of placental growth factor in murine adipogenesis. *Thromb Res*. 2007;120:399–405.
40. Kang M, Jeong J, Lee J, Park S, Sung Y, Choi M, Kwon W, Jang S, Choi KS, Choo YS, Yoon D, Kim MO, Ryoo ZY. Placental growth factor (PlGF) is linked to inflammation and metabolic disorders in mice with diet-induced obesity. *Endocr J*. 2018;65:437–447.
41. Zhao B, Cai J, Boulton M. Expression of placenta growth factor is regulated by both VEGF and hyperglycaemia via VEGFR-2. *Microvasc Res*. 2004;68:239–246.
42. Hemmeryckx B, van Bree R, Van Hoef B, Vercruysse L, Lijnen HR, Verhaeghe J. Adverse adipose phenotype and hyperinsulinemia in gravid mice deficient in placental growth factor. *Endocrinology*. 2008;149:2176–2183.
43. Matsui M, Samejima K, Takeda Y, Tanabe K, Morimoto K, Okamoto K, Tagawa M, Onoue K, Okayama S, Kawata H, Kawakami R, Akai Y, Saito Y. Prognostic impact of placental growth factor on mortality and cardiovascular events in dialysis patients. *Am J Nephrol*. 2015;42:117–125.
44. Khaliq A, Foreman D, Ahmed A, Weich H, Gregor Z, McLeod D, Boulton M. Increased expression of placenta growth factor in proliferative diabetic retinopathy. *Lab Invest*. 1998;78:109–116.
45. Klaassen I, de Vries EW, Vogels IMC, van Kampen AHC, Bosscha MI, Steel DHW, Van Noorden CJF, Lesnik-Oberstein SY, Schlingemann RO. Identification of proteins associated with clinical and pathological features of proliferative diabetic retinopathy in vitreous and fibrovascular membranes. *PLoS One*. 2017;12:e0187304.
46. Yu Y, Zhang J, Zhu R, Zhao R, Chen J, Jin J, Tian Y, Su SB. The profile of angiogenic factors in vitreous humor of the patients with proliferative diabetic retinopathy. *Curr Mol Med*. 2017;17:280–286.
47. Huang H, He J, Johnson D, Wei Y, Liu Y, Wang S, Lutty GA, Duh EJ, Semba RD. Deletion of placental growth factor prevents diabetic retinopathy and is associated with Akt activation and HIF1alpha-VEGF pathway inhibition. *Diabetes*. 2015;64:200–212.
48. Van Bergen T, Hu TT, Etienne I, Reyns GE, Moons L, Feyen JHM. Neutralization of placental growth factor as a novel treatment option in diabetic retinopathy. *Exp Eye Res*. 2017;165:136–150.
49. Carlsson AC, Sundström J, Carrero JJ, Gustafsson S, Stenemo M, Larsson A, Lind L, Ärnlöv J. Use of a proximity extension assay proteomics chip to discover new biomarkers associated with albuminuria. *Eur J Prev Cardiol*. 2017;24:340–348.
50. Guo L, Harari E, Virmani R, Finn AV. Linking hemorrhage, angiogenesis, macrophages, and iron metabolism in atherosclerotic vascular diseases. *Arterioscler Thromb Vasc Biol*. 2017;37:e33–e39.
51. Heeschen C, Dimmeler S, Fichtlscherer S, Hamm CW, Berger J, Simoons ML, Zeiher AM; CAPTURE Investigators. Prognostic value of placental growth factor in patients with acute chest pain. *JAMA*. 2004;291:435–441.

52. Lenderink T, Heeschen C, Fichtlscherer S, Dimmeler S, Hamm CW, Zeiher AM, Simoons ML, Boersma E; CAPTURE Investigators. Elevated placental growth factor levels are associated with adverse outcomes at four-year follow-up in patients with acute coronary syndromes. *J Am Coll Cardiol*. 2006;47:307–311.

53. Glaser R, Peacock WF, Wu AH, Muller R, Mockel M, Apple FS. Placental growth factor and B-type natriuretic peptide as independent predictors of risk from a multibiomarker panel in suspected acute coronary syndrome (Acute Risk and Related Outcomes Assessed With Cardiac Biomarkers [ARROW]) study. *Am J Cardiol*. 2011;107:821–826.

54. Hanefeld M, Engelmann K, Appelt D, Sandner D, Weigmann I, Ganz X, Pistrosch F, Köhler C, Gasparic A, Birkenfeld AL. Intra-individual variability and circadian rhythm of vascular endothelial growth factors in subjects with normal glucose tolerance and type 2 diabetes. *PLoS One*. 2017;12:e0184234.

Supplemental Material

Table S1. Weighted Pearson Partial Correlation Coefficients of Placental Growth Factor with Risk Factors and Biomarkers in the Reference Subcohort.

	Age*	SBP	BMI	HbA1c	Insulin	hsCRP	TC	HDL-C	LDL-C	TG
Pearson Partial Correlation Coefficient	0.294	-0.002	0.049	0.057	0.137	-0.069	-0.096	-0.179	-0.012	0.004
P – value	<0.001	0.95	0.25	0.18	0.001	0.10	0.024	<0.001	0.79	0.92

All entries adjusted for age and race unless otherwise noted. * = bivariate correlation with placental growth factor, adjusted for race. All variables were natural-transformed and reweighted to the reference cohort. SBP = baseline systolic blood pressure, BMI = body mass index, HbA1c = Hemoglobin A1c, hsCRP = high sensitivity C-reactive protein, TC = total cholesterol, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol TG = Triglycerides.

Table S2. Sensitivity Analysis Excluding Participants with Baseline HbA1c ≥ 6.0%.

	Quartile of Placental Growth Factor, pg/mL				
	Q1	Q2	Q3	Q4	
	< 15.4	15.4-17.6	17.7-20.3	≥ 20.4	*P* for Trend
Cases (N=461)	105	109	116	131	
Subcohort (N=554)	137	141	136	140	
Model 1	1	1.13 (0.78 - 1.64)	1.37 (0.95 - 1.98)	2.14 (1.44 - 3.17)	< 0.001
Model 2	1	1.09 (0.67 - 1.78)	1.27 (0.76 - 2.12)	2.49 (1.51 - 4.12)	< 0.001
Model 3	1	1.01 (0.59 - 1.72)	1.36 (0.82 - 2.27)	2.49 (1.48 - 4.17)	< 0.001
Model 4	1	0.74 (0.44 - 1.25)	1.08 (0.60-1.95)	1.85 (1.08 - 3.19)	0.018

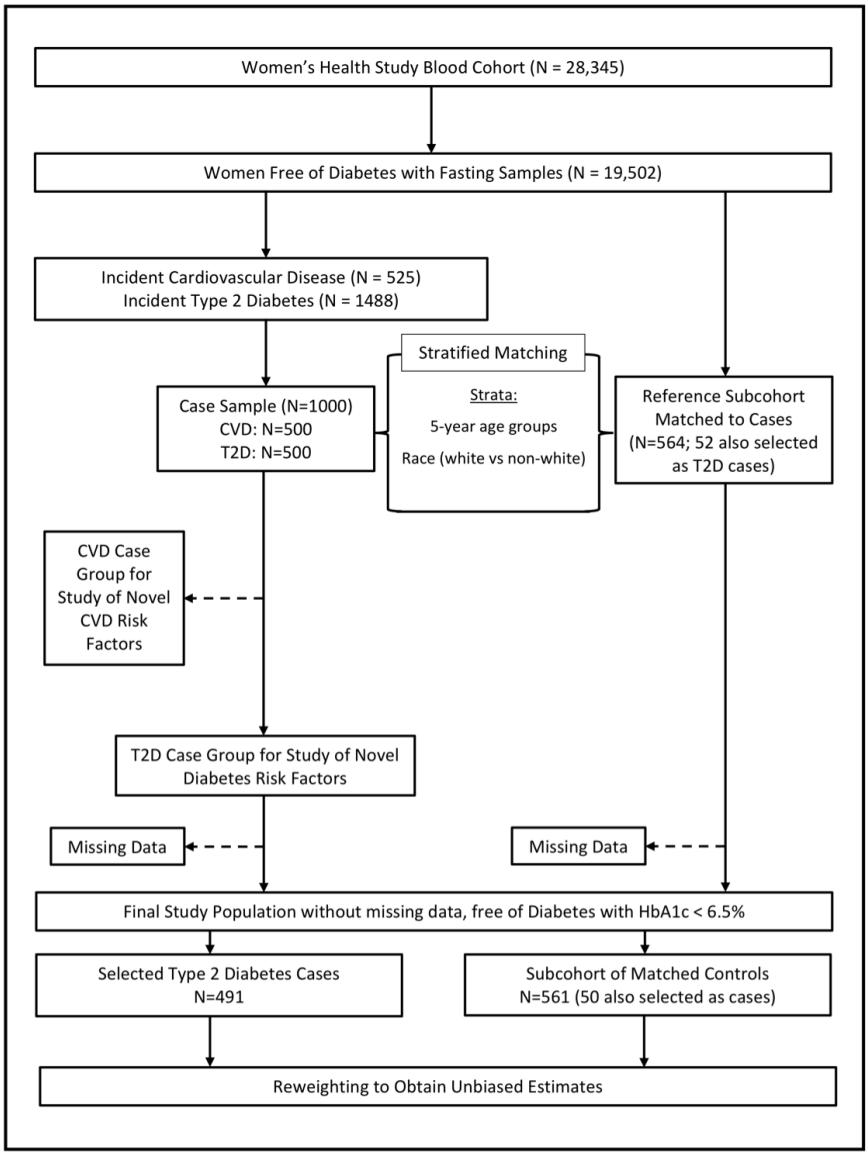
Model 1: Adjusted for age and race, and WHS randomized treatment assignments (aspirin and vitamin E)
Model 2: Adjusted for model 1 covariates plus BMI, history of hypertension, history of hyperlipidemia, current smoking, parental history of diabetes, MHT use, and exercise frequency (weekly vs. none).
Model 3: Adjusted for model 2 covariates plus hsCRP
Model 4: Adjusted for model 2 covariates plus HbA1c and insulin

Table S3. Sensitivity Analysis Excluding Cases Diagnosed Within First 2 Years of Follow Up.

	Quartile of Placental Growth Factor, pg/mL				
	Q1	Q2	Q3	Q4	
	< 15.4	15.4-17.6	17.7-20.3	≥ 20.4	*P* for Trend
Cases (N=458)	101	109	121	127	
Subcohort (N=551)	137	139	139	136	
Model 1	1	1.18 (0.81 - 1.71)	1.47 (1.02 - 2.12)	2.18 (1.47 - 3.24)	< 0.001
Model 2	1	1.21 (0.74 - 1.98)	1.48 (0.89 - 2.46)	2.69 (1.62 - 4.48)	< 0.001
Model 3	1	1.14 (0.67 - 1.92)	1.59 (0.96 - 2.63)	2.71 (1.61 - 4.56)	< 0.001
Model 4	1	1.08 (0.63 - 1.85)	1.40 (0.77 - 2.54)	2.45 (1.40 - 4.30)	0.002

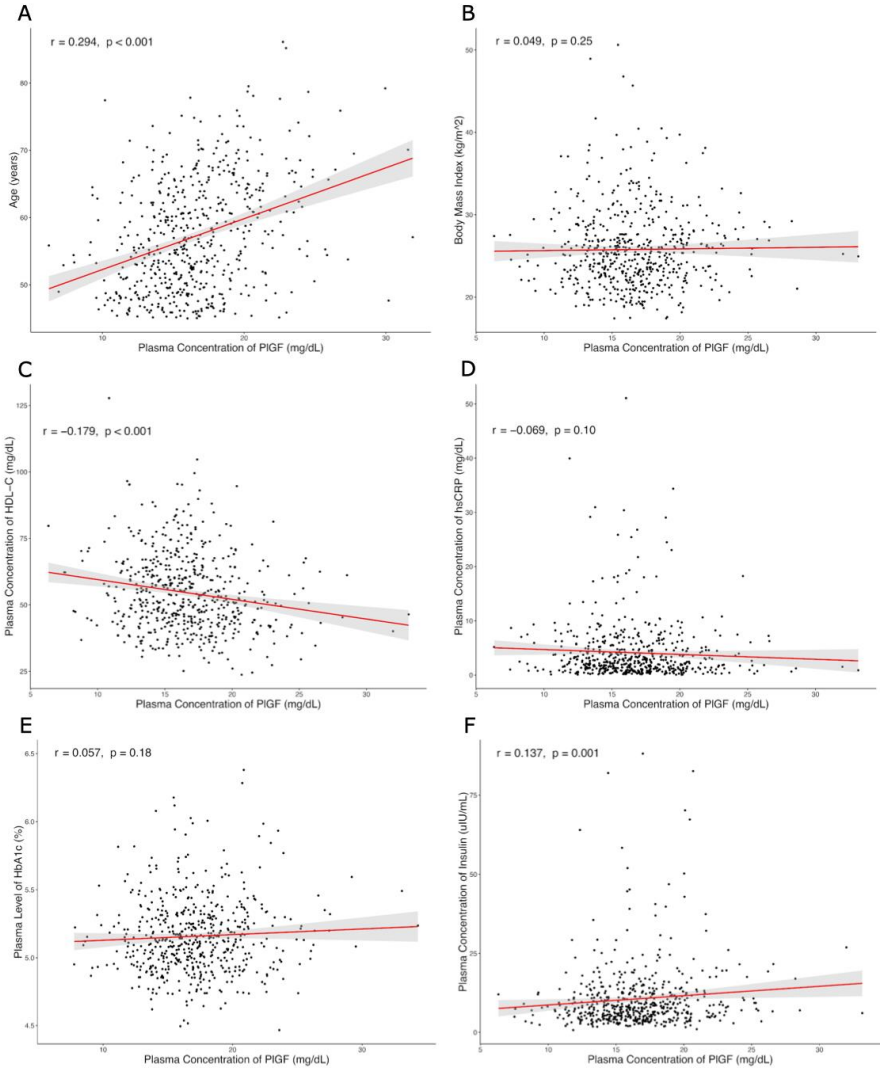
Model 1: Adjusted for age and race, and WHS randomized treatment assignments (aspirin and vitamin E)
Model 2: Adjusted for model 1 covariates plus BMI, history of hypertension, history of hyperlipidemia, current smoking, parental history of diabetes, MHT use, and exercise frequency (weekly vs. none).
Model 3: Adjusted for model 2 covariates plus hsCRP
Model 4: Adjusted for model 2 covariates plus HbA1c and insulin

Figure S1. Overview of Case-Cohort Study Design.



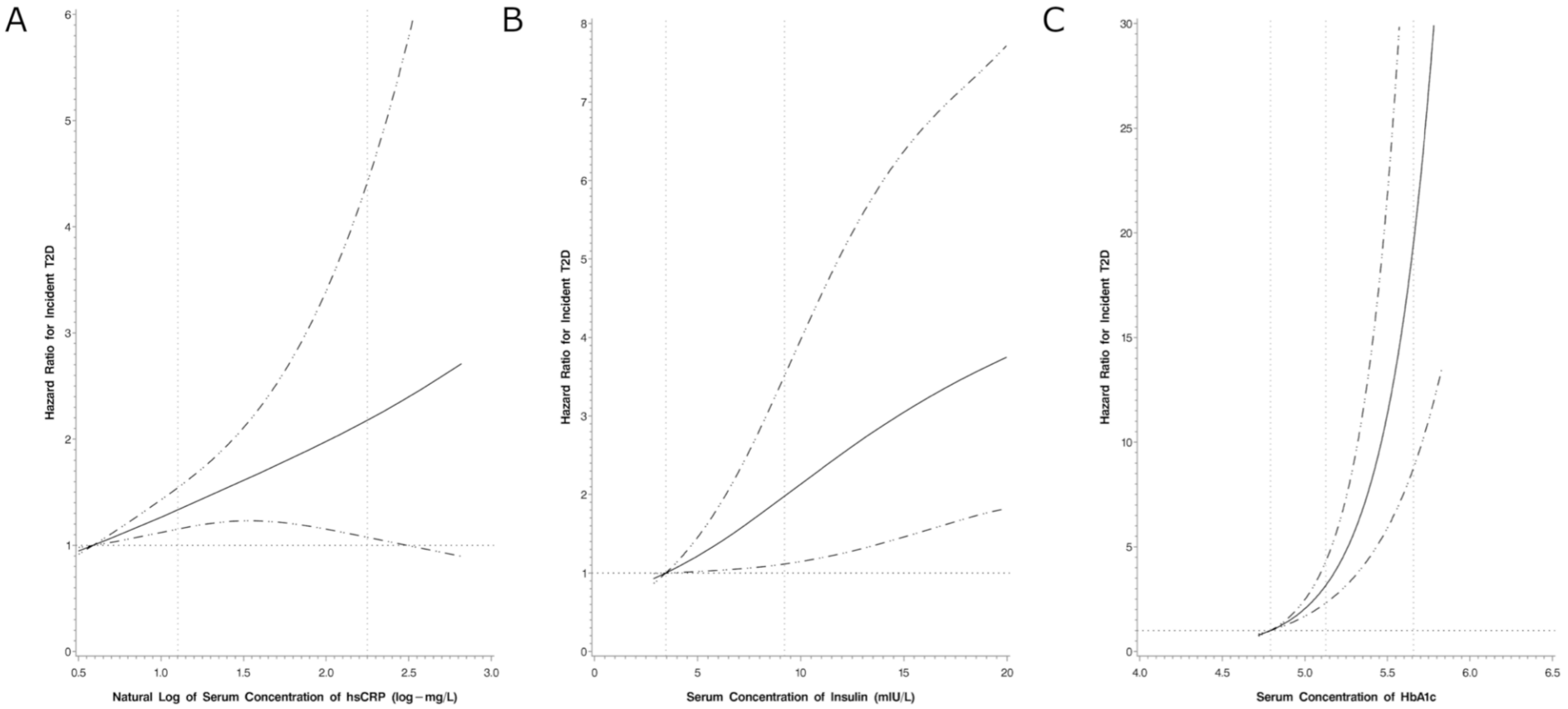
Flow chart representing the creation of case-cohort population used in current analyses. Cases and subcohort were derived from the Women's Health Study (WHS) Blood Cohort of over 28,000 healthy women. The subcohort was sampled independent of follow up case status, thus a portion of subjects in the subcohort were also selected as cases and reflect the proportion of those at risk of the outcome of interest. Stratified matching was performed based on 5-year age groups and race (white vs non-white). Sample of incident cardiovascular disease (CVD) cases was also sample for use in analyses examining novel risk factors of CVD was also obtained but not used in the current analysis. After accounting for missing data of key exposures and restricting to those with Hemoglobin A1c (HbA1c) concentrations not consistent with a diagnosis of Type 2 Diabetes (T2D; HbA1c < 6.5%), the final study population consisted for 491 cases and a subcohort of 561 subjects (50 of whom were also selected as cases).

Figure S2. Association between Placental Growth Factor and Select Biomarkers.



Scatter plots and pearson correlation coefficients for placental growth factor (PlGF) with selected biomarkers are shown. As seen in panel ***A***, age was the only biomarker with any meaningful association with PlGF. Remaining biomarkers had either very weak or no association with PlGF (panels ***B***-***E***). All data points represent age and race adjusted concentrations obtained from linear regression. All pearson correlation analyses were reweighted to better reflect associations in the Women's Health Study (WHS) source population.

Figure S3. Spline Analyses for Risk Associations Between hsCRP, Insulin, and HbA1c with Incident Type 2 Diabetes.



A. The association between baseline high-sensitivity C-reactive protein (hsCRP) and the risk of incident Type 2 Diabetes (T2D) is shown. Due to extreme right-skewed distribution, hsCRP was long transformed. In the fully adjusted model, log-hsCRP had a linear association with incident T2D ($p_{non\text{-}linearity} = 0.32$; $p_{linearity} < 0.001$). Knots for model 1 were identified the equivalent of 0.62 mg/L, 3.01 mg/dL, and 9.49 mg/L, with the first knot set as reference. ***B.*** The association between fasting insulin and incident T2D was found to have a non-linear association. ($p_{non\text{-}linearity} = 0.019$). Knots were identified at 3.46 mIU/L, 9.21 mIU/L, and 23.66 mIU/L with the first knot set as the reference. ***C.*** The relationship between Hemoglobcin A1c (HbA1c) and incident T2D appeared to be nearly asymptotic and found to be non-linear in nature. ($p_{non\text{-}linearity} < 0.001$). Knots were identified at 4.79%, 5.13%, and 5.66%, with first knot set as reference. All models included model 5 covariates and placental growth factor (excluding the spline variable), and excluded top and bottom 5th percentiles of data to reduce the impact of extreme data points.

Figure S4. Distribution of Incident T2D Cases Over Time.