Pigment Epithelium-Derived Factor Declines in Response to an Oral Glucose Load and Is Correlated with Vitamin D and BMI but Not Diabetes Status in Children and Young Adults

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Keywords

Diabetes · Obesity · Adolescents · Bone · Metabolism

Abstract

Background: Pigment epithelium-derived factor (PEDF) is associated with obesity and diabetes complications in adults, yet little is known about PEDF in younger individuals. We investigated the relationship between PEDF and various metabolic biomarkers in young healthy volunteers (HV) and similar-aged patients with diabetes (type 1 and type 2). **Methods:** A fasting blood sample was collected in 48 HV, 11 patients with type 1 diabetes (T1D), and 11 patients with type 2 diabetes (T2D) 12–25 years of age. In 9 healthy subjects, PEDF was also serially measured during a frequently sampled oral glucose tolerance test (OGTT). **Results:** PEDF was positively correlated with BMI and systolic blood pressure and negatively correlated with vitamin D. Upon multivariable analysis, BMI and vitamin D were independent predictors of PEDF. Prior to adjustment, PEDF was highest in T2D patients ($7,168.9 \pm 4417.4$ ng/mL) and lowest in individuals with T1D ($2,967.7 \pm 947.1$ ng/mL) but did not differ by diag-

nosis when adjusted for BMI and vitamin D. Among volunteers who underwent an OGTT, PEDF declined by ~20% in response to an oral glucose load. **Conclusion:** PEDF was acutely regulated by a glucose load and was correlated with BMI but not with diabetes. The negative correlation with vitamin D, independent of BMI, raises the question whether PEDF plays a compensatory role in bone matrix mineralization.

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Introduction

Pigment epithelium-derived factor (PEDF) is a 50-kDa secreted glycoprotein that belongs to the noninhibitory serpin group. It is a neurotrophic factor and a natural angiogenesis inhibitor with antitumor, anti-inflammatory, immunomodulatory, and microvascular protective properties [1]. It is widely expressed in a range of human tissues including the eyes, brain, bone, and kidneys, and is one of the most highly secreted proteins from human adipocytes [1]. Surprisingly little is known about its met-

abolic regulation and functions, other than a close, positive relationship with adipose tissue mass [2, 3]. For example, PEDF expression in adipocytes and circulating PEDF levels increased with the development of obesity and insulin resistance in a rodent model [4] and weight loss resulted in decreased serum PEDF in both animal models of obesity and obese human subjects [5, 6].

Higher PEDF concentrations have been observed in patients with diabetes relative to healthy controls, especially in those with vascular complications, compared to patients without vascular disease [7–10]. It has been hypothesized that PEDF increases in order to limit microvascular damage [4, 11–13]. This is exemplified by retinopathy, where PEDF has been shown to exert antiangiogenic effects [14]. PEDF-mediated protection against reactive oxygen species has also been proposed [15].

While PEDF is associated with insulin resistance in adults [16], limited data are available in children. Tryggesstad et al. [17] reported that PEDF concentrations were similar in obese children with and without type 2 diabetes (T2D) and higher in obese compared to normal-weight children, regardless of diabetes [17]. In a separate report by the same group, PEDF was found to be positively associated with both lean mass and fat mass in children with and without T2D [18]. Taken together, these findings suggest that obesity, rather than diabetes per se, promotes increased PEDF.

Given that cardiovascular disease disproportionately affects individuals with type 1 diabetes (T1D) [19] and that certain adipokines (e.g., adiponectin) are regulated differently in this condition, we were especially interested in investigating PEDF and biomarkers of cardiometabolic health in young individuals with T1D in comparison with youth with T2D and in healthy volunteers (HV). We also tested whether PEDF responds acutely to a metabolic challenge by conducting an oral glucose tolerance test.

Methods

Study participants were recruited through the NIH Healthy Volunteer Office. Patients with diabetes were referred by their physicians and enrolled into NCT00445627. All patients enrolled in this trial were included in the present analysis, without selection bias. They underwent detailed assessment including personal and family history, physical examination, residual insulin secretion, and antibody status. Forty-eight HV aged 18.3 ± 4.1 years, 11 patients with T1D aged 17.9 ± 3.3 years, and 11 patients with T2D aged 18.3 ± 3.4 years, all without overt microvascular or macrovascular complications, provided written consent prior to enrollment. Individuals under the age of 18 years and their parents (or guard-

ians) also provided written assent and consent. All study procedures were reviewed and approved by the Institutional Review Board (IRB) at the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) at the National Institutes of Health (NIH) and in accordance with the Declaration of Helsinki.

Subjects underwent a routine medical evaluation, which included medical history and a physical examination. Anthropometric measurements were performed, and BMI was calculated. Blood pressure was measured using standard procedures with an appropriate cuff size. A fasting blood sample was collected for determination of complete blood count, lipid profile (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), blood glucose, HbA1c, leptin, adiponectin, vitamin D (total 25-hydroxyvitamin D2 and D3), high-sensitivity C-reactive protein, free fatty acids, liver enzymes (alanine transaminase, aspartate transaminase), and PEDF.

PEDF was measured using a sandwich ELISA kit (sensitivity: 0.9 ng/mL; range of detection: 0.9–62.5 ng/mL; intra-assay variation: $\pm 5.3\%$ (7.5 ng/mL); inter-assay variation $\pm 16.0\%$ (7.5 ng/mL); CHEMICON International, Inc.). All samples were run using the same assay. Leptin was measured in serum using the Human Leptin ELISA kit from Millipore (Billerica, MA, USA). The minimum detectable concentration was 0.5 ng/mL (IntraCV: 3.7% and InterCV: 4.0%). Adiponectin was measured in serum by ELISA (Human Total Adiponectin Quantikine kit; R&D Systems, Minneapolis, MN, USA; IntraCV: 3.5% and InterCV: 6.5%). The minimum detectable concentration was 0.25 ng/mL. All other laboratory measurements were conducted using standard clinical procedures in the NIH Clinical Center Laboratory.

In a subset of subjects (9 HV) who underwent an oral glucose tolerance test (OGTT), PEDF was measured prior to (–10, 0 min) oral ingestion of 75 g glucose and then serially (10, 20, 30, 60, 90, 120, 150, and 180 min) following the glucose load.

Statistical Analysis

Statistical analyses were performed using SAS Enterprise Guide version 9.3 for Windows (SAS Institute Inc.). All continuous data are expressed as mean $\pm$ SD. All categorical data are reported as frequencies (%). Univariate analyses were performed to evaluate associations between serum PEDF and metabolic biomarkers. Multivariable regression was then performed using PROC REG, with adjustment for relevant covariates. Statistical significance was defined as $p < 0.05$. As this was an exploratory study, a correction for multiple comparisons was not performed.

Results

Clinical and biochemical characteristics of the study subjects are shown in Table 1. In univariate analyses, PEDF was significantly higher in patients with T2D compared to those with T1D or HV ($7,168.9 \pm 4,417.4$ ng/mL [T2D], $2,967.7 \pm 947.1$ ng/mL [T1D], and $5,229.9 \pm 2,274.7$ [HV], $p = 0.0015$) (Fig. 1).

Bivariate analyses revealed significant positive correlations of PEDF with BMI ($r = 0.540$, $p < 0.0001$) and systolic blood pressure ($r = 0.352$, $p = 0.003$) and a negative

Table 1. Characteristics of the study subjects

	Healthy volunteers (<i>n</i> = 48)	Type 1 DM (<i>n</i> = 11)	Type 2 DM (<i>n</i> = 11)	<i>p</i>
Gender (M:F), <i>n</i>	24:24	4:7	2:9	0.14
Age, years	18.3±4.1	17.9±3.3	18.3±3.4	0.99
Race/ethnicity, %				0.37
White	44	64	36	
Black	39	9	36	
Other	17	27	28	
Hispanic	15	18	9	0.83
DM duration, years	na	7.7±4.9	1.7±1.6	na
BMI	26.3±6.5	21.5±2.8	35.4±6.5	<0.0001
Systolic BP, mm Hg	120.9±11.9	110.9±12.0	127.5±8.5	0.004
Diastolic BP, mm Hg	68.7±7.3	68.2±9.6	72.6±9.2	0.43
Pulse, beat/min	71.3±10.6	79.5±11.5	80.4±9.9	0.01
ALT, U/L	22.8±15.2	16.7±3.4	43.8±28.5	0.0021
AST, U/L	26.8±31.1	22.9±8.9	25.0±9.1	0.64
WBC, K/ μ L	5.9±1.4	6.4±1.4	8.0±2.2	0.004
Total cholesterol, mg/dL	150.3±25.9	155.6±39.3	171.4±40.4	0.31
HDL-C, mg/dL	48.3±10.4	55.5±15.4	38.2±8.1	0.0015
LDL, mg/dL	91.1±27.7	85.8±34.1	113.5±41.3	0.11
Triglycerides, mg/dL	67.0±31.1	68.3±42.8	150.3±87.1	0.0003
Free fatty acids, μ Eq/L	582.3±235.8	647.0±444.0	682.1±329.2	0.82
25-OH-vitamin D, ng/mL	16.6±7.8	26.0±7.0	15.6±9.2	0.002
Vitamin D status, % optimal ^a	24	90	27	0.001
1-25-OH2-vitamin D, pg/mL				
Normal range (22--67)	50.1±14.4	39.4±5.9	46.8±22.1	0.07
HbA1c, %	5.2±0.4	7.9±1.8	7.7±2.0	<0.0001
Glucose, mg/dL	82.4±6.3	115.7±33.1	131.6±57.8	<0.0001
Insulin, μ U/mL	7.2±5.5	NA	16.0±7.9	0.0004
C-peptide, ng/mL	1.8±0.8	0.3±0.7	2.9±1.1	<0.0001
HOMA-IR	1.5±1.2	na	4.9±3.3	<0.0001
Creatinine, mg/dL	0.79±0.15	0.73±0.09	0.64±0.08	0.005
hs-CRP, mg/L				
Normal range (<3)	1.27±2.3	2.3±3.93	6.87±9.1	0.001
Leptin, ng/mL	26.6±24.3	17.8±10	45±34.9	0.02
Adiponectin, ng/mL	5,815.5±3,745.3	7,594.4±3,006.5	2,581.9±856.8	<0.0001

Unless otherwise indicated, data are given as means $\pm$ standard deviation for all continuous variables. Group differences in continuous variables were examined using Kruskal-Wallis tests (>2 groups) and Wilcoxon rank sum tests (2 groups) for all variables, except for systolic blood pressure, pulse and 25-OH-vitamin D, where parametric tests (ANOVA) were used. χ^2 tests of (no) association were used to examine categorical variables. Inferences were made based on a Bonferroni-adjusted probability cutoff of 0.002 (0.05/*n* where *n* = 29). DM, diabetes mellitus; BMI, body mass index; BP, blood pressure; ALT, alanine transaminase; AST, aspartate transaminase; WBC, white blood cells; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein. ^aOptimal vitamin D status is defined as a 25(OH)D concentration >20 ng/mL. *p* values in bold indicate statistical significance (*p* < 0.05).

correlation with vitamin D (-0.408 , $p = 0.0006$) when all 70 subjects were analyzed together. When analyzed separately by diagnosis, vitamin D was inversely correlated with PEDF only among HV, and BMI was positively correlated in both HV ($n = 48$) and in those with T2D ($n =$

11). No significant correlations between PEDF and any biomarker analyzed were observed among patients with T1D ($n = 11$).

In a multivariable linear regression model driven by the correlation matrix, only BMI ($p = 0.05$) and vitamin

Fig. 1. Pigment epithelium-derived factor (PEDF) levels stratified by diabetes diagnosis. Fasted PEDF levels were higher in patients with type 2 diabetes (T2D) compared to those with type 1 diabetes (T1D) or healthy volunteers (HV) in unadjusted analyses, but did not differ following adjustment for BMI and vitamin D.

D ($p = 0.048$) were found to be marginally significant predictors of PEDF in the pooled sample. Importantly, diagnosis was not a significant predictor of PEDF in this model ($p = 0.21$). A final reduced model was fit, in which BMI ($p < 0.0001$) and vitamin D ($p = 0.014$) explained 34% of the variance in PEDF within the pooled sample (29.3% of variance explained by BMI and 6.4% of variance explained by vitamin D). While we explored introducing other variables such as race/ethnicity and gender into the model, none were statistically significant, and thus, only BMI (parameter estimate $\pm$ SE: 182.3 ± 41.8 , $p < 0.0001$) and vitamin D (parameter estimate $\pm$ SE: -86.5 ± 34.2 , $p < 0.014$) were retained. Because diagnosis was not a significant predictor and due to limited sample sizes of patients with diabetes, we did not stratify this model by diagnosis for further examination.

Because vitamin D deficiency was prevalent in both HV and T2D patients (Table 1), we determined whether vitamin D remained a significant predictor when levels were “normalized” by conducting a combined analysis of HV and T1D patients. We also explored whether this relationship was maintained when vitamin D deficiency was “exacerbated”, by conducting a combined analysis of HV and T2D patients. In both cases, BMI ($p = 0.005$ in T1D and HV combined, $p = 0.0004$ in T2D and HV combined) and vitamin D ($p = 0.032$ in T1D and HV combined, $p = 0.026$ in T2D and HV combined) remained significant predictors of PEDF.

Fig. 2. Pigment epithelium-derived factor (PEDF) response to oral glucose load in 9 healthy volunteers. At 60, 120, 150 and 180 min, PEDF levels were significantly decreased compared to baseline ($p = 0.0402$, $p = 0.0302$, $p = 0.0145$, and $p = 0.0127$, respectively).

In the subset of HV with serial measurements of PEDF during an OGTT, PEDF decreased gradually following glucose ingestion relative to baseline (Fig. 2, $p = 0.0009$).

Discussion

Our results demonstrate that PEDF concentrations are regulated acutely by an oral glucose load inducing a moderate decline and are associated with BMI and vitamin D. We observed lower PEDF concentrations in adolescents and young adults with T1D compared to HV and those with T2D, but this difference was no longer significant after adjustment for BMI. This result is consistent with prior studies in healthy children and children with T2D [5, 7, 10, 17, 20, 21]. While some adipokines are differently regulated in T1D (e.g., disproportionately high adiponectin concentrations) [22], we did not find disease-specific alterations of PEDF.

Several mechanisms have been proposed to explain the relationship between PEDF and BMI. A direct role of adipose tissue in its regulation has been suggested and is supported by the observation that weight loss decreased PEDF in both obese humans and in animal models of obesity [5, 6]. In addition, animal studies demonstrate that expression of PEDF in adipocytes and serum PEDF levels are greater in obese versus lean mice [6]. Even in neonates, a positive relationship has been shown be-

tween anthropometrics (birth weight) and cord blood PEDF [23].

A novel observation in our study was the inverse relationship between vitamin D and PEDF, which remained statistically significant after adjustment for BMI. While vitamin D was not as strong a predictor of PEDF as BMI, this observation requires further investigation, especially in light of the inverse association of vitamin D with numerous cardiometabolic risk factors, including insulin resistance [24] and obesity [25].

In addition, PEDF may play a compensatory role to maintain bone health. Osteoblasts, and, to a lesser extent, osteoclasts express PEDF, and thus, the inverse correlation between PEDF and vitamin D may be mediated by bone matrix activity [26]. PEDF is known to inhibit adipogenesis and promote osteogenesis in mesenchymal stem cells, as PEDF knockout mice demonstrate increased adiposity and decreased bone mineral density [27]. Mutations in the gene encoding PEDF (*SERPINF1*) have been identified in autosomal recessive osteogenesis imperfecta type VI [28] and familial osteosclerosis [29]. Affected patients have not been described to have overt metabolic abnormalities, but no detailed studies are available. Several other adipokines have been investigated for their roles in bone metabolism. For example, leptin has been found to have no [30] or a positive [31] association with bone mineralization in adolescents. In contrast, higher adiponectin was associated with lower bone mass before and during puberty [31]. However, study results vary considerably and the significance of these interactions remains to be clarified.

The observed relationship between vitamin D and PEDF should be interpreted with caution, as vitamin D deficiency was prevalent among the HV and patients with T2D in our study. In addition, correlations were assessed within a small sample size of individuals with diabetes. It is also noteworthy that our HV were of a wide range of

body mass indices and many were overweight ($n = 14$) or obese ($n = 11$). However, vitamin D deficiency is highly prevalent in the general population [32], and thus, the correlation observed in this sample may be in fact generalizable. We thus speculate that PEDF may have increased in response to low vitamin D concentrations.

In addition to our cross-sectional analysis, we also demonstrated that PEDF declined moderately ($\sim 20\%$) following ingestion of a glucose load during an OGTT. Similar effects were observed in cultured human umbilical vein endothelial cells, during which exposure to high glucose media resulted in a decreased PEDF release [33]. In humans, however, data have been mixed regarding a relationship between PEDF and fasting glucose, with some studies showing a positive correlation [5, 9, 34], while others have not [3, 5]. The observed gradual glucose-stimulated decline in PEDF is consistent with most other adipokines, which either remain stable or respond nondramatically to a glucose load (e.g., leptin, resistin, or omentin) [35].

In conclusion, our study is the first to compare PEDF specifically in young individuals with T1D or T2D, as well as similar-aged HV, before and after adjustment for BMI and vitamin D. These results support a link between adipose tissue and bone physiology that requires further experiments to investigate the underlying mechanisms and clinical implications.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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