Circulating Pigment Epithelium-Derived Factor Levels Are Associated with Insulin Resistance and Decrease after Weight Loss

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Objective: We aimed to study circulating pigment epithelium-derived factor (PEDF) *in vivo* in association with insulin resistance and *in vitro* in human adipocytes.

Methods: Circulating PEDF (ELISA) and metabolic profile were assessed in 125 Caucasian men. PEDF levels were also assessed in an independent cohort of subjects (n = 33) to study the effects of changing insulin action. *PEDF* gene expression and secretion were measured during differentiation of human preadipocytes.

Results: In all subjects, PEDF was positively associated with body mass index ($r = 0.326$; $P < 0.0001$), waist-to-hip ratio ($r = 0.380$; $P < 0.0001$), HbA_{1c}, and fasting triglycerides and negatively with insulin sensitivity ($r = -0.320$; $P < 0.0001$). PEDF levels were significantly increased in subjects with altered glucose tolerance and type 2 diabetes. Of the inflammatory markers measured, PEDF levels were positively associated with serum soluble TNF-α receptor 1 and IL-10 in obese subjects. Interestingly, weight loss led to significantly decreased PEDF concentration from 34.8 $\pm$ 19.3 to 22.5 $\pm$ 14.2 μg/ml ($P < 0.0001$). Multiple linear regression analyses revealed that insulin sensitivity contributed independently to explain 14% of the variance in PEDF levels after controlling for the effects of body mass index, age, and log fasting triglycerides. Differences in PEDF observed after weight loss were related to changes in obesity, insulin resistance, and blood pressure measures. *PEDF* gene expression and secretion increased during differentiation of human preadipocytes.

Conclusion: Circulating PEDF is associated with insulin sensitivity. The findings show, for the first time in humans, that PEDF concentrations decrease significantly after weight loss in association with blood pressure. PEDF seems to be involved in human adipocyte biology. (***J Clin Endocrinol Metab* 95: 4720–4728, 2010**)

Obesity is closely associated with several metabolic diseases, such as dyslipidemia, hypertension, insulin resistance, type 2 diabetes, and atherosclerosis (1). Adipose tissue is well known not only for its essential role as an energy storage depot but also for secreting adipokines that affect other tissues such as brain, liver, muscle, β-cells, gonads, lymphoid organs, and systemic vasculature (2, 3).

Pigment epithelium-derived factor (PEDF) is a 50-kDa protein first identified in the conditioned medium of human retinal pigment epithelial cells as a neurotrophic

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Abbreviations: AGT, Altered glucose tolerance; Ct, cycle threshold; CV, coefficient of variation; DBP, diastolic blood pressure; FBS, fetal bovine serum; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; HOMA-IR, HOMA of insulin resistance; NGT, normal glucose tolerance; PEDF, pigment epithelium-derived factor; SBP, systolic blood pressure; sTNFR1, soluble TNF-α receptor 1.

factor (4). Sequence analysis of the human *PEDF* gene showed that it is a member of the serpin (serine protease inhibitor) family (5) but lacks a serine-reactive loop and thus has no function on protease inhibition (6). PEDF has antiangiogenic, antioxidant, and antiinflammatory effects and is widely distributed in a broad range of human fetal and adult tissues including liver, testis, stomach, ovaries, prostate, eye, heart, colon, brain, and spinal cord (7). However, important PEDF proinflammatory actions have also been described. Acute PEDF administration activated the proinflammatory serine/threonine kinases c-Jun terminal kinase and ERK in both muscle and liver (8). PEDF also promoted the activation of nuclear factor-κB (9) and increased the expression of Fas ligand, activating a transduction cascade that promotes endothelial death (10).

Circulating levels of PEDF are thought to derive primarily from the liver (11). However, PEDF was also identified as forming part of the secretome in primary cultures of human sc adipose-derived stem cells [derived from four individual female donors (12) or from a 5-yr-old male donor (13)]. Recent observations showed that PEDF is up-regulated during adipogenesis and is mostly produced by mature adipocytes (8). The results in animal models suggested a causal role for PEDF in obesity-induced insulin resistance (8). Lean mice injected with recombinant PEDF exhibited reduced insulin sensitivity during hyperinsulinemic-euglycemic clamps. Acute PEDF administration activated proinflammatory signals leading to reduced insulin signal transduction. Prolonged PEDF administration stimulated adipose tissue lipolysis, resulted in ectopic lipid deposition, and reduced insulin sensitivity, whereas neutralizing PEDF in obese mice enhanced insulin sensitivity (8).

The evidence of PEDF influencing insulin resistance in human obesity is scarce. Serum levels of PEDF were associated with some components of the metabolic syndrome among 196 Japanese (14) and 59 Caucasian (15) subjects or associated with visceral adiposity in 86 Japanese subjects (16). Circulating PEDF concentration was increased in patients with type 2 diabetes (17) and associated with the homosteosasis model assessment (HOMA) value in 97 Japanese subjects with essential hypertension (18). However, fasting insulin was not independently associated with serum PEDF concentration in one study (15).

Given these discordant results, we aimed to study circulating PEDF concentration using a robust measure of insulin sensitivity. In addition, we evaluated whether improvement of obesity-associated insulin resistance induced by weight loss led to parallel changes in circulating PEDF. The information regarding PEDF production by human adipose tissue is based on four individual female donors (12) and from a 5-yr-old male donor (13). For this reason, we also studied *PEDF* gene expression and protein levels in isolated preadipocytes from visceral and sc adipose tissue and during adipocyte differentiation.

Subjects and Methods

Cross-sectional study

Patient recruitment

One hundred twenty-five subjects were randomly localized from a census and were invited to participate. The participation rate was 71%. A 75-g oral glucose tolerance test according to the American Diabetes Association Criteria was performed in all subjects. All subjects with normal glucose tolerance (NGT) (n = 76) had fasting plasma glucose under 7.0 mM and 2-h postload plasma glucose under 7.8 mM after a 75-g oral glucose tolerance test. Glucose intolerance was diagnosed in 36 subjects according to the American Diabetes Association Criteria (postload glucose between 7.8 and 11.1 mM). Previously unknown type 2 diabetes was diagnosed in 13 additional subjects (postload glucose higher than 11.1 mM). Subjects with glucose intolerance and type 2 diabetes were grouped under the term altered glucose tolerance (AGT).

Inclusion criteria were 1) body mass index (BMI) less than 40 kg/m^2, 2) absence of systemic disease, and 3) absence of infection within the previous month. None of the control subjects were under medication or had evidence of metabolic disease other than obesity. Liver disease and thyroid dysfunction were specifically excluded by biochemical work-up.

All subjects gave written informed consent after the purpose of the study was explained to them. The institutional review board approved the protocol.

Measurements

Subjects were studied in the postabsorptive state. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals.

Insulin sensitivity

Insulin sensitivity was measured using the frequently sampled iv glucose tolerance test on a different day. In brief, basal blood samples were drawn at −15 and −5 min, after which glucose (300 mg/kg body weight) was injected over 1 min starting at time 0. At 20 min, regular insulin (Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark; 0.03 U/kg) was injected as a bolus. Additional samples were obtained from a contralateral antecubital vein at times 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60,70, 80, 90, 100, 120, 140, 160, and 180 min. Samples were rapidly collected via a three-way stopcock connected to a butterfly needle. Data from the frequently sampled iv glucose tolerance test were submitted to computer programs that calculate the characteristic metabolic parameters by fitting glucose and insulin to the minimal model that describes the times course of glucose and insulin concentrations. The glucose disappearance model, by accounting for the effect of insulin and glucose on glucose disappearance, provides the parameters S_I (10^{-4}) per minute per mi-

crounit per milliliter) or the insulin sensitivity index, a measure of the effect of insulin concentrations above the basal level to enhance glucose disappearance. The estimation of model parameters was performed according to the MINMOD computer program (19).

Insulin resistance was also measured by the HOMA of insulin resistance (HOMA-IR). HOMA-IR correlates well with insulin sensitivity derived from the glucose clamp technique ($r = -0.82$; $P < 0.0001$) (20).

Study of the effects of weight loss

Thirty-three Caucasian obese volunteers (16 females, 17 males) attending the Endocrinology Department at the University Clinic of Navarra were recruited. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, and comorbidity evaluation as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were nonsmokers. Patients with signs of infection were excluded. Obese patients were not receiving statins or antidiabetic medication.

Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500-1000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29; SensorMedics Corp., Yorba Linda, CA) and multiplication by 1.4 as indicated for sedentary individuals to obtain the patient's total energy expenditure. This hypocaloric regime allows a safe and steady weight loss of 0.5–1.0 kg/wk when followed and supplied 30, 54, and 16% of energy requirements in the form of fat, carbohydrates, and protein, respectively.

In this study, body weight was measured with a digital scale to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd., Crymych, UK).

The institutional review board of the participant institutions approved the protocol, so we certify that all applicable institutional regulations concerning the ethical use of information and samples from human volunteers were followed during this research.

PEDF expression during differentiation of human preadipocytes

Isolated preadipocytes from lean and obese subjects and visceral and sc adipose tissue (SP-F-1, SP-F-3, and OP-F-1; Zen-Bio Inc., Research Triangle Park, NC) were plated on T-75 cell culture flasks and cultured at 37 C and 5% CO_2 in DMEM/Nutrient Mix F-12 medium (1:1, vol/vol) supplemented with 10 U/ml penicillin/streptomycin, 10% fetal bovine serum (FBS), 1% HEPES, and 1% glutamine (all from GIBCO, Invitrogen S.A., Barcelona, Spain). One week later, the isolated and expanded human visceral and sc preadipocytes were cultured (~40,000 cells/cm^2) in 12-well plates with preadipocytes medium (Zen-Bio) composed of DMEM/Nutrient Mix F-12 medium (1:1, vol/vol), HEPES, FBS, penicillin, and streptomycin in a humidified 37 C incubator with 5% CO_2. Twenty-four hours after plating, cells were checked for complete confluence (d 0), and differentiation was induced using differentiation medium (Zen-Bio) composed of preadipocytes medium, human insulin, dexamethasone, isobutylmethylxanthine, and peroxisome proliferator-activated receptor-γ agonists (rosiglitazone). After 7 d, differentiation medium was replaced with fresh adipocyte medium (Zen-Bio) composed of DMEM/Nutrient Mix F-12 medium (1:1, vol/vol), HEPES, FBS, biotin, pantothenate, human insulin, dexamethasone, penicillin, streptomycin, and amphotericin. Negative control (nondifferentiated cell) was performed with preadipocyte medium during all differentiation processes. Eighteen days after the initiation of differentiation, cells appeared rounded with large lipid droplets apparent in the cytoplasm. Cells were then considered mature adipocytes, harvested, and stored at −80 C for RNA extraction to study *PEDF* gene expression levels after human adipocyte differentiation. When cells were checked for complete confluence (d 0), proliferation medium and cells were collected (d 0) and replaced with differentiation medium. After 7 d, differentiation medium and cells were collected (d 7) and replaced with adipocyte medium. At d 14, adipocyte medium and cells were collected for analysis.

Adipocyte-conditioned medium was stored at −80 C for the determination of circulating PEDF concentration (as above mentioned). The experiment was performed in triplicate for each sample. The differentiation was monitored with the fatty acid synthase (*FASN*, Hs00188012_m1; Applied Biosystems Inc., Madrid, Spain) and adiponectin (*Adipoq*, Hs00605917_m1; Applied Biosystems) expression.

PEDF gene expression

RNA was prepared from these samples using RNeasy Lipid Tissue Mini Kit (QIAGEN, Izasa SA, Barcelona, Spain). The integrity of each RNA sample was checked by Agilent bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant; GE Healthcare, Piscataway NJ) reverse transcribed to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturer's protocol.

Gene expression was assessed by real-time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems), using TaqMan technology suitable for relative genetic expression quantification.

The commercially available and prevalidated TaqMan primer/probe sets used were as follows: endogenous control *PPIA* (4333763, cyclophilin A; Applied Biosystems) and target gene human *PEDF* (Hs01106937_m1; Applied Biosystems). The RT-PCR TaqMan reaction was performed in a final volume of 25 μl. The cycle program consisted of an initial denaturing of 10 min at 95 C and then 40 cycles of 15 sec denaturizing phase at 95 C and 1 min annealing and extension phase at 60 C. A threshold cycle (Ct) value was obtained for each amplification curve, and a ΔCt value was first calculated by subtracting the Ct value for human cyclophilin A (*PPIA*) RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta Ct}$, so gene expression results are expressed as expression ratio relative to *PPIA* gene expression according to manufacturers' guidelines.

Analytical methods

Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Glycated hemoglobin (HbA1c) was measured by the HPLC method (Jokoh HS-10 autoanalyzer; Bio-Rad, Muenchen, Germany). Intraassay and interassay coefficients of variation (CV) were less than 4% for all these tests. High-density lipoprotein (HDL) cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured through the reaction of glycerol/phosphate/oxidase and peroxidase. Serum insulin was measured in duplicate in the same cen-

TABLE 1. Clinical characteristics of subjects in the cross-sectional study

	Clinical characteristics		
	NGT, mean ± SD	AGT, mean ± SD	*P*
n	76	49	
Age (yr)	48.9 ± 11.7	54.2 ± 11.0	0.012
BMI (kg/m^2)	27.1 ± 3.4	28.8 ± 3.9	0.009
WHR	0.9 ± 0.1	1.0 ± 0.1	0.015
SBP (mm Hg)	125.1 ± 13.8	134.3 ± 15.5	0.001
DBP (mm Hg)	79.8 ± 8.9	83.3 ± 9.8	0.044
Fasting glucose (mg/dl)	93.0 ± 7.4	106.3 ± 13.3	<0.0001
HbA_{1c} (%)	4.8 ± 0.3	5.0 ± 0.6	0.051
Total cholesterol (mg/dl)	208.5 ± 42.6	219.6 ± 37.3	0.136
HDL-cholesterol (mg/dl)	53.7 ± 13.1	51.2 ± 10.9	0.266
LDL-cholesterol (mg/dl)	134.2 ± 40.0	140.8 ± 32.3	0.333
Log 10 fasting triglycerides	1.9 ± 0.3	2.0 ± 0.3	0.032
Uric acid (mg/dl)	5.6 ± 1.5	6.0 ± 1.6	0.183
Insulin sensitivity	0.6 ± 0.2	0.3 ± 0.2	<0.0001
PEDF (μg/ml)	14.7 ± 7.3	18.7 ± 11.4	0.018

LDL, Low-density lipoprotein.

tralized laboratory by a monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The intraassay CV was 5.2% at a concentration of 10 mU/liter and 3.4% at 130 mU/liter. The interassay CV were 6.9 and 4.5% at 14 and 89 mU/liter, respectively. Plasma IL-6 levels were measured by human IL-6 ELISA high sensitivity (Bender MedSystems, Vienna, Austria) according to commercial kit instructions. Plasma levels of soluble TNF-α receptor 1 (sTNFR1) and sTNFR2 were determined by a solid-phase sandwich enzyme-amplified sensitivity immunoassay according to commercial kit instructions (sTNFR1-EASIA and sTNFR2-EASIA; Biosource Europe, Fleurus, Belgium). Intra- and interassay CV were less than 7 and 9%, respectively. Il-10 concentrations were measured using high-sensitivity enzyme immunoassay (human IL-10 Quantikine HS ELISA kit; R&D Systems, Minneapolis, MN). Analytical intraassay sensitivity was 0.5 pg/ml. The intra- and interassay CV were 7.8 and 11.3%, respectively.

PEDF level in serum and adipocyte-conditioned medium was measured by a commercial ELISA kit (chemokine PEDF sandwich ELISA kit; Chemicon International, Inc., Temecula, CA) according

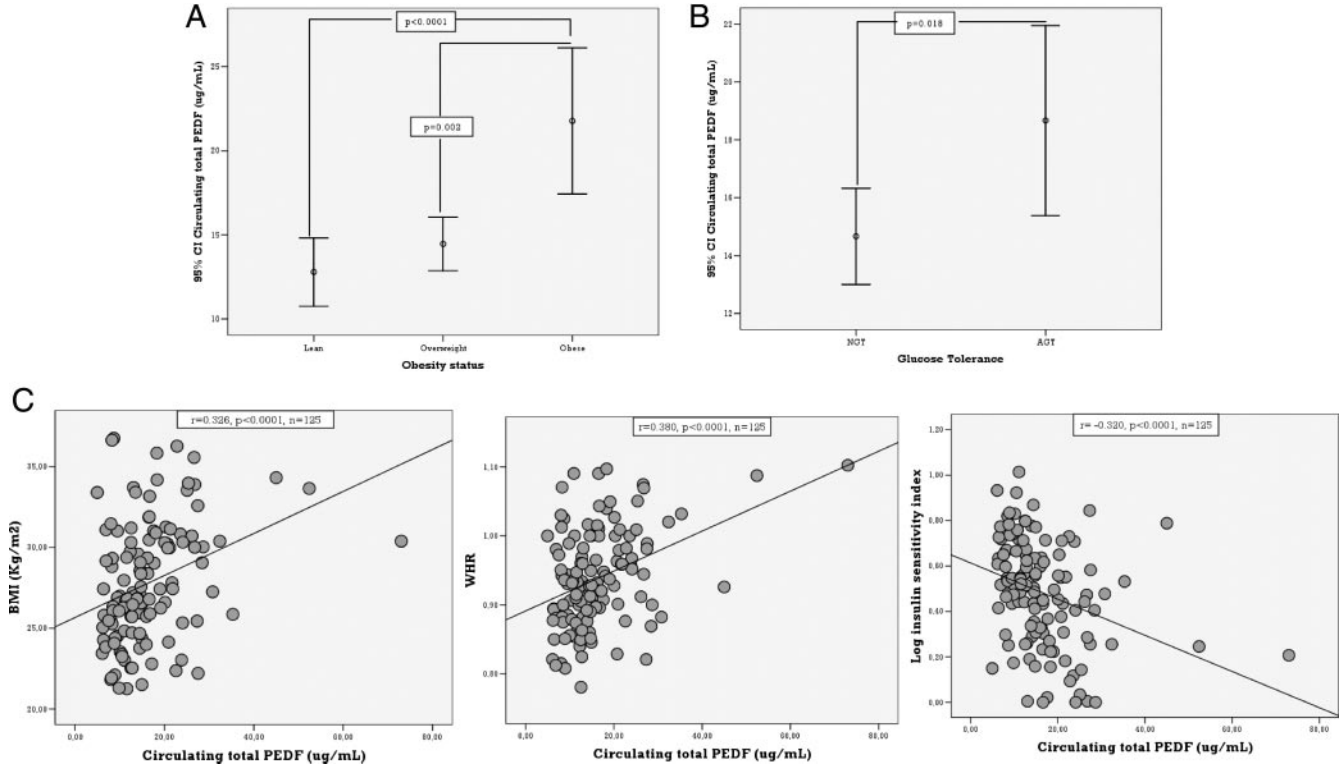

FIG. 1. A, Circulating PEDF levels according to obesity status; B, circulating total PEDF levels in NGT *vs.* AGT; C, linear relationship between circulating PEDF and BMI, waist-to-hip ratio (WHR), and log insulin sensitivity index. After the exclusion of the three outliers, the correlations among PEDF concentrations and BMI (r = 0.285; $P = 0.001$), insulin sensitivity index (r = 0.299; $P = 0.001$), and waist-to-hip ratio (r = −0.380; $P < 0.0001$) remained significant. CI, Confidence interval.

to the manufacturer's protocol. Samples were pretreated with 8 mol/liter urea to measure total PEDF. The intraassay CV was between 2 and 5%, and the interassay CV was between 2 and 10%.

Statistical methods

Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and SD for Gaussian variables. Parameters that did not fulfill normal distribution were logarithmically transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Pearson's test), partial correlation, and multiple regression analyses. Unpaired and paired *t* tests were used to compare NGT and AGT subjects and the effects of weight loss, respectively. Levels of statistical significance were set at $P < 0.05$.

Results

Cross-sectional study

Clinical and biochemical variables of the study subjects are summarized in Table 1. Circulating PEDF levels were significantly increased in subjects with AGT (18.67 ± 11.43 *vs.* 14.66 ± 7.27 μg/ml, $P = 0.018$) (Table 1 and Fig. 1).

In all subjects, considered as a group, circulating PEDF concentration was significantly and positively associated with BMI (Fig. 1), waist-to-hip ratio, HbA_{1c}, log fasting triglycerides, and uric acid and negatively with insulin sensitivity (Table 2 and Fig. 1). In men with NGT, similar relationships with circulating total PEDF and BMI, waist-to-hip ratio, fasting glucose, log fasting triglycerides, uric acid, and insulin sensitivity were observed. In AGT subjects, only the associations with BMI and HbA_{1c} remained significant (Table 2). BMI adjustment changed little these observations (Table 2).

Multiple linear regression analyses to predict PEDF levels revealed that insulin sensitivity contributed independently to explain 14% ($P < 0.0001$) of the variance in circulating PEDF levels after controlling for the effects of BMI, age, and log fasting triglycerides.

In another model to predict insulin sensitivity, BMI ($P < 0.0001$), log fasting triglycerides ($P < 0.0001$), and PEDF levels ($P = 0.02$) contributed independently to explain 37, 11, and 2%, respectively, of the variance in insulin sensitivity, after controlling for age.

We found no associations between circulating PEDF and several inflammatory markers in all subjects as a whole (sTNFR1, sTNFR2, IL-6, or IL-10). However, circulating PEDF concentration was associated with serum IL-10 levels ($r = 0.255$; $P = 0.039$; $n = 66$) and with circulating sTNFR1 concentration ($r = 0.46$; $P = 0.02$; $n = 25$) in a subsample of obese subjects.

Weight loss study

Clinical characteristics of subjects are shown in the Supplemental Table (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). In these subjects, the associations of circulating PEDF levels with BMI ($r = 0.550$; $P < 0.0001$) and waist-to-hip ratio ($r = 0.348$; $P = 0.030$) were replicated. Weight loss led to significantly decreased circulating

TABLE 2. Correlations with total PEDF concentrations before and after adjustment for BMI in the cross-sectional study

	Total PEDF						Total PEDF (BMI-adjusted)					
	All subjects, n = 125		NGT, n = 76		AGT, n = 49		All subjects, n = 125		NGT, n = 76		AGT, n = 49	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Age (yr)	−0.006	0.947	−0.029	0.800	−0.088	0.549	−0.045	0.625	0.007	0.955	−0.187	0.208
BMI (kg/m^2)	0.326	<0.0001	0.368	0.001	0.460	0.001						
WHR	0.380	<0.0001	0.261	0.023	0.231	0.110	0.271	0.003	0.130	0.273	0.432	0.002
SBP (mmHg)	0.066	0.466	−0.114	0.326	0.107	0.463	−0.038	0.676	−0.17	0.2	0.062	0.678
DBP (mm Hg)	0.102	0.259	−0.062	0.597	0.183	0.208	0.006	0.944	−0.194	0.101	0.149	0.318
Fasting glucose (mg/dl)	0.118	0.190	0.290	0.011	−0.155	0.289	0.066	0.470	0.206	0.080	−0.136	0.363
HbA_{1c} (%)	0.237	0.008	0.069	0.552	0.283	0.049	0.209	0.021	0.029	0.804	0.290	0.048
Total cholesterol (mg/dl)	0.131	0.144	0.139	0.229	0.079	0.590	0.079	0.386	0.100	0.400	0.027	0.859
HDL-cholesterol (mg/dl)	−0.052	0.568	−0.145	0.210	0.101	0.489	−0.017	0.850	−0.091	0.443	0.089	0.553
LDL-cholesterol (mg/dl)	0.020	0.824	0.090	0.441	−0.105	0.474	−0.020	0.832	0.048	0.689	−0.133	0.374
Log 10 fasting triglycerides	0.187	0.037	0.252	0.028	0.065	0.658	0.152	0.096	0.269	0.022	0.017	0.911
Uric acid (mg/dl)	0.229	0.010	0.310	0.006	0.124	0.397	0.192	0.034	0.277	0.018	0.102	0.494
Insulin sensitivity	−0.320	<0.0001	−0.261	0.024	−0.263	0.067	−0.184	0.043	−0.094	0.428	−0.194	0.191

Results are presented as mean ± SD. WHR, Waist-to-hip ratio.

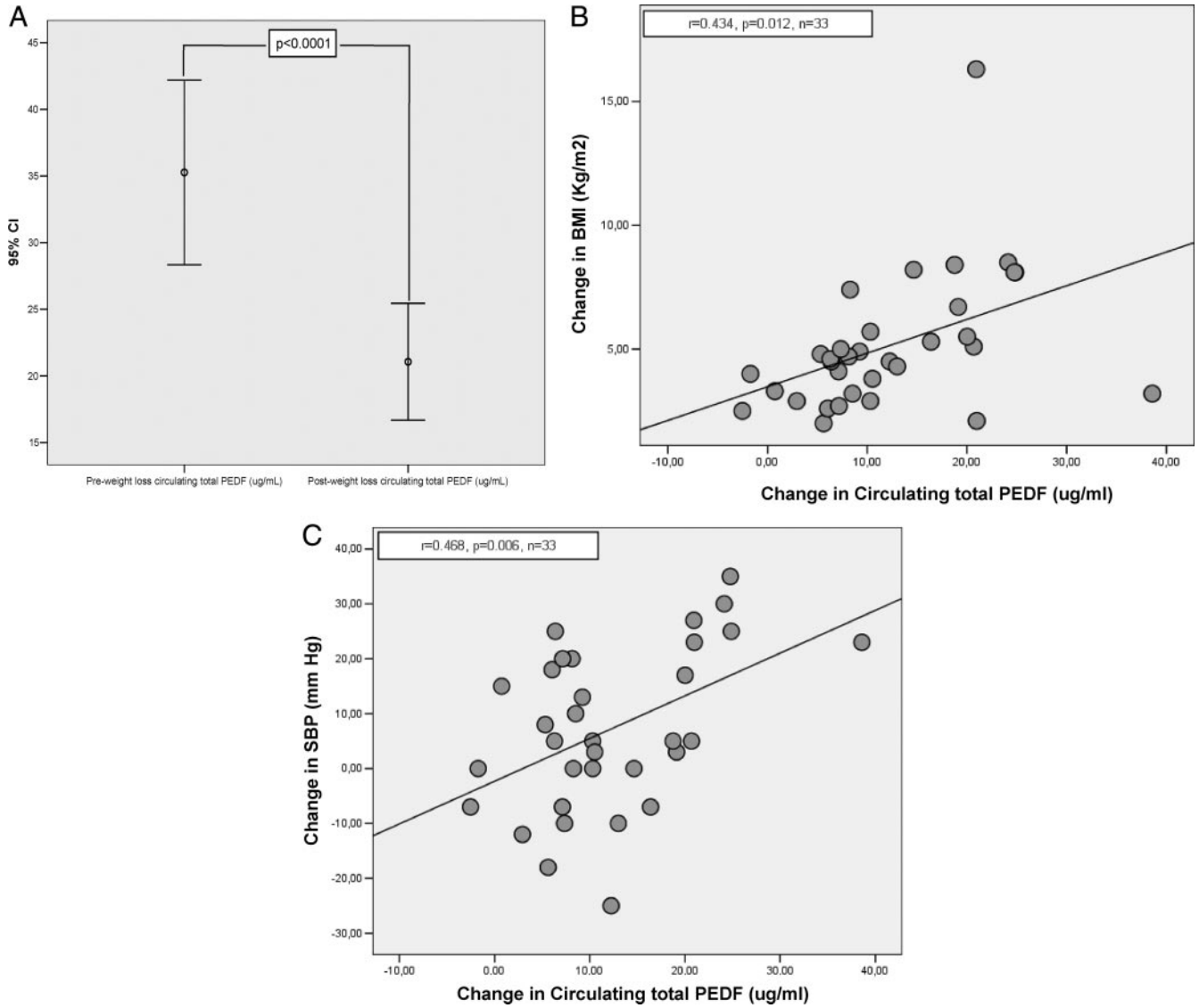

FIG. 2. A, Circulating PEDF levels before and after weight loss; B, linear relationship between the changes in circulating PEDF and the changes in BMI after weight loss; C, linear relationship between the changes in circulating PEDF and the changes in SBP after weight loss.

PEDF concentration, from 34.8 ± 19.3 to 22.5 ± 14.2 μg/ml ($P < 0.0001$) (Fig. 2).

The changes of circulating total PEDF concentration after weight loss were positively associated with the changes in BMI (r = 0.788; $P < 0.0001$), waist circumference (r = 0.681; $P < 0.0001$), hip circumference (r = 0.569; $P < 0.0001$), systolic blood pressure (SBP) (r = 0.468; $P = 0.006$) and diastolic blood pressure (DBP) (r = 0.402; $P = 0.020$). Interestingly, the change in circulating PEDF concentration was also associated with the change in fasting insulin concentration (r = 0.812; $P < 0.0001$) and HOMA-IR (r = 0.817; $P < 0.0001$) and negatively associated with the change in HDL-cholesterol (r = −0.472; $P = 0.004$) (Table 3 and Fig. 2). After adjustment for BMI changes, however, only the relation between the changes in PEDF concentration after weight loss and the changes in SBP remained significant (Table 3).

PEDF in human preadipocytes and during adipocyte differentiation

As expected, fatty-acid synthase and adiponectin gene expression increased during the differentiation process in parallel with the accumulation of lipid droplets in the cytoplasm (Fig. 3). *PEDF* gene expression was higher in differentiated adipocytes than in nondifferentiated adipocytes and increased during the differentiation process (Fig. 3). The concentration of PEDF secreted into the adipocyte-conditioned medium increased during differentiation and was higher than in nondifferentiated cells (Fig. 3).

Discussion

Dual pro- and antiinflammatory actions have been described for PEDF. Current findings showing positive as-

TABLE 3. Correlations with changes of total PEDF concentrations before and after adjustment for BMI in the weight loss study

	Changes in total PEDF		Changes in total PEDF (BMI-adjusted)	
	r	*P*	r	*P*
Change in weight (kg)	0.797	<0.0001		
Change in BMI (kg/m^2)	0.788	<0.0001		
Change in fat mass (kg)	0.653	<0.0001		
Change in waist (cm)	0.681	<0.0001		
Change in hip (cm)	0.569	<0.0001		
Change in SBP (mm Hg)	0.468	0.006	0.382	0.031
Change in DBP (mm Hg)	0.402	0.020	0.282	0.117
Change in fasting glucose (mg/dl)	0.250	0.183	−0.035	0.855
Change in insulin (mU/liter)	0.812	<0.0001	0.133	0.555
Change in total cholesterol (mg/dl)	−0.016	0.929	−0.118	0.507
Change in HDL-cholesterol (mg/dl)	−0.472	0.004	−0.186	0.292
Change in LDL-cholesterol (mg/dl)	0.103	0.554	−0.081	0.650
Change in log 10 fasting triglycerides	0.121	0.489	0.006	0.974
Change in HOMA-IR	0.817	<0.0001	0.174	0.450

Results are presented as mean ± SD. LDL, Low-density lipoprotein.

sociations of circulating PEDF with body fatness, insulin resistance and blood pressure suggest that PEDF is primarily or secondarily involved in these important metabolic phenotypes. The association of circulating PEDF with a robust measure of insulin sensitivity was observed in both univariate and multivariate analyses. Importantly, PEDF was directly associated not only with BMI and waist-to-hip ratio but also with HbA_{1c}, log fasting triglycerides, and serum uric acid concentration.

Circulating PEDF concentration decreased after weight loss-induced improvement of insulin sensitivity; this is the first study, to our knowledge, measuring circulating PEDF levels after weight loss-induced changes in insulin action. The change in circulating total PEDF observed after

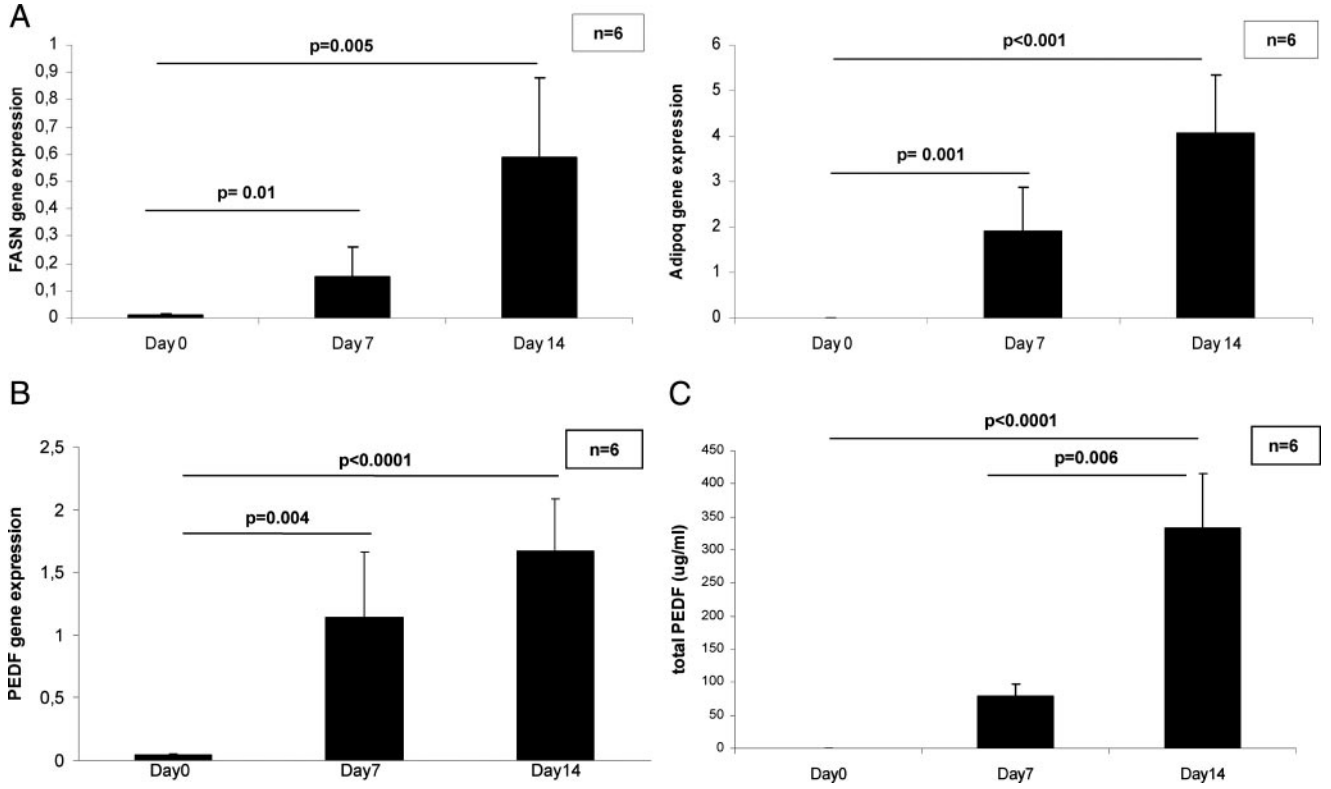

FIG. 3. A, *FASN* and adiponectin gene expression during preadipocyte differentiation process; B, *PEDF* gene expression during preadipocyte differentiation process; C, PEDF secretion in adipocyte-conditioned medium during the preadipocyte differentiation process.

weight loss was strongly associated with parameters of body fatness, insulin action, and blood pressure. The present findings agree with the previous findings, where the percent changes in serum PEDF levels during 1-yr observational period were positively correlated with those of BMI in a population of 86 Japanese patients with type 2 diabetes (16). In several rodent models of obesity, increased adipocyte *PEDF* expression and serum levels were reduced upon weight loss and insulin sensitization (8).

Interestingly, the changes in circulating PEDF concentration were positively associated with the change in systolic and diastolic blood pressure. After controlling for the change in BMI, however, only the association with SBP remained significant (Table 3). This suggests that body fatness and the vascular tone, and not insulin resistance, contributes to circulating PEDF levels, at least in obese subjects. Circulating PEDF was not associated with blood pressure in the cross-sectional study (Table 2). The association of PEDF with blood pressure has been observed in subjects with high vascular risk: obese subjects (current findings) and patients with type 1 diabetes (21). In the latter study, PEDF and inflammatory factor levels correlated, in parallel to the currently observed associations of PEDF with sTNFR1 in obese subjects. Most of the functions of TNF-α are mediated by its soluble receptors, sTNFR1 and sTNFR2, which are expressed in human adipose tissue and are present in the circulation (22, 23), and closely linked with blood pressure (24). Interestingly, in previous studies, PEDF not only promoted the activation of nuclear factor-κB (9) but also increased the expression of Fas ligand, activating a transduction cascade that promotes endothelial death (10). Fas/CD95 is a member of the TNF superfamily of receptors whose activation transmits apoptotic signals (10). PEDF was not associated with measures of oxidative stress (21).

The association of PEDF with IL-10 fits well with the previously observed increased circulating IL-10 levels in obese subjects (reviewed in Ref. 25).

PEDF gene expression and protein levels increased during differentiation of human preadipocytes. There are only two studies in humans analyzing the secretome from undifferentiated and adipocyte differentiated human adipose-derived stem cells. Zvonic *et al.* (12) observed that PEDF protein and *PEDF* gene expression were significantly induced during adipogenesis (d 0 and 9) and Chiellini *et al.* (13) confirmed that *PEDF* was highly expressed in adipocytes at d 3 compared with d 0. We have analyzed the *PEDF* gene expression and protein levels during differentiation of human preadipocytes (d 0, preadipocytes; d 7, early adipocyte differentiation; and d 14, mature adipocytes), and we have observed a gradual increase in *PEDF* gene expression and PEDF secretion during the differentiation process. In contrast to this, some studies showed that *PEDF* gene expression was decreased in mature adipocytes differentiated from 3T3-L1 preadipocytes, and PEDF was detected as a protein secreted by preadipocytes but not by adipocytes (26).

In summary, these findings suggest that adipose tissue-derived circulating PEDF might contribute to obesity-related metabolic disturbances, such as insulin resistance, hypertriglyceridemia and hypertension. In obese subjects, the change in blood pressure was the main factor associated with circulating PEDF. Future studies will be necessary to evaluate the production and regulation of circulating total PEDF levels by the adipose tissue and the vascular tree.

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