Plasma pigment epithelium-derived factor is positively associated with obesity in Caucasian subjects, in particular with the visceral fat depot

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Short running title: Plasma PEDF is elevated in human obesity

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Abstract

**Objective:** adipose tissue releases factors (adipokines) that influence local, peripheral as well as central processes. In the present study we determined the relationship between plasma concentration of a recently identified adipokine, pigment epithelium-derived factor (PEDF), and human obesity, particularly specific adipose tissue depots, and other features of the metabolic syndrome.

**Methods:** we examined the plasma concentration of PEDF, anthropometric parameters, abdominal subcutaneous and visceral adipose tissue, lipid, glucose, insulin and alanine aminotransferase level in a non-diabetic general Caucasian population \((n=59)\).

**Results:** plasma PEDF level in males \((6.2 \pm 2.1 \mu g/ml)\) was higher than in females \((3.1 \pm 1.4 \mu g/ml; P<0.001)\). Plasma PEDF was positively correlated with age and all features of metabolic syndrome. However, in multiple linear regression analysis with adjustment for age and gender, only visceral fat thickness \((\beta=0.361, P=0.010)\) and body mass index (BMI) \((\beta=0.288, P=0.008)\) were significant independent determinants of plasma PEDF level, together with the gender \((\beta=-0.424, P<0.001)\).

**Conclusions:** we conclude that the plasma PEDF level is strongly associated with body adiposity, in particular with the visceral fat depot in the non-diabetic general population. This association may (partly) explain the relationship between PEDF and metabolic syndrome in this population.
Keywords

Pigment epithelium-derived factor, adiposity, visceral fat, metabolic syndrome

Abbreviations

ATGL: adipose triglyceride lipase

BMI: body mass index

PEDF: pigment epithelium-derived factor

SKF: skinfold

WHR: waist to hip ratio
**Introduction**

Obesity is a major health problem worldwide. The dramatic rise in the prevalence of obesity contributes to the increase in obesity-associated diseases, such as type II diabetes, cardiovascular disease and certain types of cancer. In recent years adipose tissue has been increasingly recognized as an important secretory organ that releases factors influencing local, peripheral as well as central processes. These factors, called adipokines, are involved in glucose and lipid metabolism, vascularisation and other biological processes. Adipokines are believed to play a role in development of obesity-related diseases. Previously, we have identified pigment epithelium-derived factor (PEDF) as a novel protein secreted by preadipocytes and adipocytes.

The PEDF protein was originally identified in retinal cell culture supernatant and characterized as a neurotrophic factor. In addition, PEDF plays a role in the development of diabetic retinopathy in animals and humans, perhaps by acting antagonistically to vascular endothelial growth factor. Outside the boundaries of the eye, PEDF has antitumor effects based on its anti-angiogenesis and pro-apoptosis activities. In a recent study a lipase-linked receptor has been identified for PEDF. This PEDF receptor was previously characterised as adipose triglyceride lipase (ATGL). ATGL has been demonstrated to be critical in adipose lipid mobilization and PEDF is also able to regulate hepatocyte lipid content through ATGL.

The PEDF gene is highly expressed in adipose tissue in mice, and in adipose tissue, liver and bone marrow in humans. The PEDF protein has been detected as a secreted factor from both murine 3T3-L1 preadipocytes and adipocytes, and from human primary adipocytes. These findings suggest that adipose tissue contributes to plasma PEDF levels. Interestingly, a
study in a Japanese cohort demonstrated that serum PEDF level is associated with central obesity and other components of metabolic syndrome\textsuperscript{16}. However, not all (abdominal) fat is equal, and since abdominal subcutaneous fat, visceral fat, and also hepatic fat can all contribute to central abdominal obesity, a more accurate measurement of individual fat depots would allow better discrimination of which depot is most strongly related to circulating PEDF level. In the present study we used ultrasound to more extensively measure subcutaneous and visceral fat depots to investigate their relation with circulating PEDF in a general Caucasian population.

**Subjects and methods**

**Subjects**

A general non-diabetic Caucasian population was composed of 59 genetically independent males and females with a continuous body mass index (BMI) range of 19 to 35 kg/m\textsuperscript{2}. They were extracted from the spouse database of the Familial Combined Hyperlipidemia study performed in Maastricht\textsuperscript{17, 18}. The subjects were extensively characterized for body fat distribution with specific attention for the subcutaneous and visceral depots. The study protocol has been described in detail elsewhere\textsuperscript{18}. In brief, all subjects visited the Maastricht research clinic between 2003 and 2005 where the plasma samples were collected. The participants were asked to come to the laboratory after an overnight fast and to refrain from smoking, drinking alcohol and doing strenuous exercise for a period of 24 h prior to the study. Any lipid-lowering medication had been withdrawn during the last 2 weeks before blood sampling. Subject characteristics are summarized in Table 1. The study protocol was approved by the Medical Ethics Committee of Maastricht University Hospital and the clinical
investigations were performed according to the Declaration of Helsinki. All subjects gave informed consent.

**Anthropometric measurements**

Body weight, height, waist circumference, hip circumference, BMI and waist to hip ratio (WHR) were measured in fasting state as described previously\(^{19}\). The skinfold thickness of biceps, triceps, subscapular and suprailiac regions was measured, and body fat percentage was derived from the sum of the four skinfold (SKF) measurements using the method of Durnin and Womersley\(^{20}\).

**Ultrasound measurements**

The size of abdominal subcutaneous adipose tissue and visceral adipose tissue were measured with an ultrasound method as described in detail previously\(^{18}\), which has been validated\(^{21}\). In brief, both visceral adipose tissue thickness and subcutaneous adipose tissue thickness were determined at the same level as waist circumference.

**Biochemical analysis**

Fasting venous blood samples were collected in pre-cooled EDTA vacutainer tubes and immediately processed. EDTA-plasma aliquots were stored at -80°C until analysis. Triglycerides, total cholesterol, free fatty acids, glycerol, glucose and insulin levels were measured as described previously\(^{19,22}\). The amount of hepatic fat was assessed by a surrogate plasma alanine aminotransferase level\(^{23}\). PEDF was quantified by ELISA (Chemikon, Temecula, CA, USA). According to the manufacturer, the assay sensitivity is 0.9 ng/ml; range of detection is 0.9 ng/ml to 62.5 ng/ml, intra-assay variation is 5.3% and inter-assay variation is 16.0%. Since PEDF protein strongly associates with other circulating proteins, which may
interfere with quantification of total PEDF protein in plasma, we performed pre-treatment for plasma as recommended by the manufacturer, with urea (8 mol/l final concentration) for 60 min on ice, diluted 400 times in dilution buffer then applied to the ELISA plate. Samples were measured in duplicate and the average was used in the data analysis.

Statistical analysis
Statistical analyses were performed with SPSS for Windows version 12 (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation, or medians (interquartile range) when variables significantly deviate from normal distribution. The latter ones were natural logarithmically (ln) transformed for use in later analyses. For comparing the males and females, an independent-samples t-test was used. The bivariate relationships among the parameters were assessed by Pearson correlation analysis within the groups, and by gender-adjusted analysis in the pooled subjects. Subsequently, for parameters correlated with plasma PEDF level at a significance level $P<0.05$ in the pool, backward multiple linear regression analysis was performed to assess their independent relation with PEDF. Statistical calculations were performed two-tailed and $P<0.05$ was considered statistically significant.

Results

Determination of plasma PEDF levels in male and female subjects
Plasma PEDF levels were normal distributed inside the study population, with 6.2 ± 2.1 and 3.1 ± 1.4 µg/ml for males and females, respectively. A significant difference between genders was observed ($P<0.001$).

**The relation between plasma PEDF level, adiposity, plasma lipid profile, blood pressure and insulin resistance**

Positive correlations were found between plasma PEDF level and total body adiposity (BMI, body fat percentage) as well as abdominal adiposity (waist circumference, WHR, abdominal visceral fat thickness) for both male and female subjects (Table 2), although the relation with body fat percentage in men was only borderline significant. However, abdominal subcutaneous fat depot was significantly associated with PEDF level only in females.

Plasma alanine transferase level, which is fairly correlated with hepatic fat as measured by magnetic resonance spectroscopy\textsuperscript{24}, was used as a plasma surrogate marker of hepatic fat accumulation. It was positively associated with PEDF in males and pooled subjects.

We also analysed the relation between PEDF and other metabolic syndrome features including blood lipid profile, blood pressure and glucose metabolism. Only triglyceride levels were consistently positively correlated with PEDF levels in both males and females. After pooling the subjects, plasma PEDF also correlated positively with diastolic blood pressure, fasting glucose and insulin concentrations (with adjustment for gender).

We observed that plasma PEDF level was correlated with age of subjects in bivariate analysis. Because the features of metabolic syndrome are inter-related, and are also related with age, we performed multiple linear regression analysis to assess their independent relation with plasma PEDF level. Since the correlations were similar in both males and females, we pooled
the data in multiple linear regression analysis to increase the power with more subjects. Age, gender, and the significantly correlated parameters (BMI, waist, WHR, body fat percentage, visceral fat, triglyceride (ln), alanine transferase (ln), diastolic blood pressure, glucose and insulin (ln)) were included as independent variables in a linear regression model, in which PEDF level was the dependent variable. Subsequently, the variables that did not show an independent significant contribution to PEDF were excluded via stepwise-backward-elimination, while adjustment for age and gender was maintained. This multiple linear regression analysis showed that thickness of the visceral fat depot and BMI were independently correlated to plasma PEDF (with gender effect). Age did not show a significant independent contribution to the PEDF level (Table 3).

**Discussion**

Using ultrasound, we were able to determine the relations of circulating PEDF and the different fat depots. Our work provides the first evidence that circulating PEDF levels are closely related to visceral fat, but not subcutaneous fat. Our data showed that in a general non-diabetic Caucasian population, circulating PEDF level was positively related to aspects of the metabolic syndrome, including obesity, triglyceride levels, diastolic blood pressure, glucose and insulin levels, consistent with the findings of Yamagishi and coworkers in a general Japanese population. Furthermore, multiple linear regression analysis indicated that the strong correlation between PEDF level and adiposity may explain the association between PEDF and multiple features of metabolic syndrome.

In this study the size of the cohort was limited, and cross-sectional data could not provide details on mechanism. However, recent studies on the function of PEDF support our finding that PEDF is related to fat. Adipose triglyceride lipase (ATGL), which catalyses the initial
step in triglyceride hydrolysis and together with hormone-sensitive lipase co-ordinately
catabolises triglycerides stored in adipose tissue, is the receptor for PEDF. In humans,
ATGL is expressed in various tissues, most abundantly in adipocytes, as shown in the NCBI
Unigene database and by a tissue-specific transcriptomics study. The high expression
level of both PEDF and ATGL in adipocytes strongly implies that PEDF may act as an
autocrine factor in adipose tissue lipid metabolism. ATGL protein level and lipase activity are
reduced in the subcutaneous fat depot of obese compared to lean subjects, but not in the
visceral fat depot. This suggests that ATGL is more active in visceral fat and that PEDF
signalling through ATGL is more relevant for visceral fat, especially in obesity. The fact that
we did not observe a clear link between circulating PEDF level and an indicator of whole
body lipolysis (glycerol), is in line with a localised activity of PEDF in the visceral fat depot.

We found that plasma alanine transferase level, a surrogate marker of hepatic fat
accumulation, was also positively correlated with plasma PEDF level, even though most of
the subjects did not have fatty liver. The significant association between PEDF and hepatic fat
accumulation may reflect that the hepatic fat content is also closely associated with abdominal
obesity. In this cohort, plasma alanine transferase level was positive associated with liver
steatosis stage measured by ultrasound (data not shown). Interestingly, a study in rodents
suggests that PEDF deficiency induces steatosis, while in our present human study, hepatic
fat accumulation is associated with high circulating level of PEDF. Elevated circulating PEDF
in humans has been suggested as a counter action for increased triglycerides. It is tempting
to speculate that in the human obese situation PEDF resistance exists in the liver.

It is well known that obesity, in particular central/visceral obesity, is associated with insulin
resistance. These two components, together with hyperlipidemia and hypertension, are
clustered in the so-called metabolic syndrome, because of their close correlations\textsuperscript{29,30}. It is not unexpected that the plasma PEDF level, which is strongly related to obesity, particularly to visceral obesity, correlated with other components of this syndrome. The positive relation of plasma PEDF level with these components has been observed in diabetic populations as well\textsuperscript{31,32}. However, in those studies obesity was either not considered or not identified as a significant independent determinant. This could reflect the difference between diabetic and non-diabetic conditions. In diabetic subjects the relation of PEDF with obesity might be masked by other determinants.

Although plasma PEDF level is associated with BMI and visceral fat with the same strength in both genders, we observed a very significant gender difference in our study population for the absolute circulating PEDF level. This is in contrast to Jenkin’s work\textsuperscript{31}, but in line with the work by others\textsuperscript{16,32}. Since the visceral fat depot is much larger in males than in females, and visceral fat could be a major contributor to the PEDF level, a higher plasma PEDF level in males compared to females seems reasonable. However, in multiple linear regression analysis we showed that gender has its own independent effect, beyond the visceral fat. We suspect that sex hormones, which were not investigated here, may play a role in the regulation of circulating PEDF level.

In conclusion, plasma PEDF level is strongly associated with body adiposity, particularly with the visceral fat depot in the Caucasian non-diabetic general population. This association may partly explain the relationship between plasma PEDF level and features of the metabolic syndrome in this population.
Acknowledgements

The authors declare no conflict of interest.

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References


Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th>parameters</th>
<th>male (n=32)</th>
<th>female (n=27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (yr)</td>
<td>54.2 ± 12.9</td>
<td>47.5 ± 8.9</td>
<td>0.02</td>
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<td>systolic blood pressure (mmHg)</td>
<td>137.7 ± 17.6</td>
<td>120.0 ± 15.2</td>
<td>&lt;0.001</td>
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<td>diastolic blood pressure (mmHg)</td>
<td>84.6 ± 10.1</td>
<td>79.9 ± 9.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 3.0</td>
<td>24.6 ± 3.9</td>
<td>n.s.</td>
</tr>
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<td>waist circumference (cm)</td>
<td>95.8 ± 8.2</td>
<td>84.9 ± 11.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.96 ± 0.06</td>
<td>0.84 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>body fat (% SKF method)</td>
<td>27.5 ± 6.1</td>
<td>36.0 ± 5.2</td>
<td>&lt;0.001</td>
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<td>subcutaneous fat thickness (cm)</td>
<td>2.0 ± 0.7</td>
<td>2.8 ± 1.1</td>
<td>0.004</td>
</tr>
<tr>
<td>visceral fat thickness (cm)</td>
<td>9.0 ± 2.6</td>
<td>6.2 ± 1.6</td>
<td>&lt;0.001</td>
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<tr>
<td>plasma alanine transferase (U/l)</td>
<td>17.0 (14.7 – 23.4)</td>
<td>15.5 (12.3 – 18.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>total cholesterol (mmol/l)</td>
<td>5.5 ± 0.9</td>
<td>5.4 ± 1.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>triglycerides (mmol/l)</td>
<td>1.4 (1.0 – 1.9)</td>
<td>1.0 (0.77 – 1.6)</td>
<td>n.s.</td>
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<td>free fatty acid (mmol/l)</td>
<td>0.26 (0.22 – 0.42)</td>
<td>0.39 (0.28 – 0.48)</td>
<td>n.s.</td>
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<td>glycerol (µmol/l)</td>
<td>54.9 (42.4 – 68.3)</td>
<td>61.5 (52.8 – 98.5)</td>
<td>n.s.</td>
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<td>fasting glucose (mmol/l)</td>
<td>5.1 ± 0.5</td>
<td>4.9 ± 0.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>insulin (mU/l)</td>
<td>4.6 (2.0 – 7.4)</td>
<td>4.1 (2.0 – 8.2)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for normal distributed parameter, and median (interquartile range) for not-normal distributed parameters. P-value is from the comparison of male vs. female. n.s. not significant.
Table 2. The bivariate correlation coefficients between plasma PEDF level and other parameters

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
<th>pooled</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>age</td>
<td>0.321</td>
<td>0.074</td>
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<tr>
<td>BMI</td>
<td>0.671</td>
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<td>0.690</td>
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<td>waist circumference</td>
<td>0.563</td>
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<td>0.694</td>
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<tr>
<td>WHR</td>
<td>0.439</td>
<td>0.015</td>
<td>0.595</td>
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<tr>
<td>body fat percentage</td>
<td>0.345</td>
<td>0.053</td>
<td>0.588</td>
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<tr>
<td>abdominal subcutaneous</td>
<td>-0.017</td>
<td>0.926</td>
<td>0.457</td>
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<tr>
<td>fat thickness</td>
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<tr>
<td>abdominal visceral fat</td>
<td>0.582</td>
<td>&lt;0.001</td>
<td>0.599</td>
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<tr>
<td>thickness</td>
<td></td>
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<tr>
<td>alanine transferase</td>
<td>0.380</td>
<td>0.038</td>
<td>0.185</td>
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<tr>
<td>total cholesterol</td>
<td>-0.211</td>
<td>0.255</td>
<td>0.370</td>
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<tr>
<td>triglycerides</td>
<td>0.461</td>
<td>0.009</td>
<td>0.535</td>
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<tr>
<td>free fatty acid</td>
<td>0.215</td>
<td>0.282</td>
<td>0.326</td>
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<td>glycerol</td>
<td>0.035</td>
<td>0.856</td>
<td>0.386</td>
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<td>systolic blood pressure</td>
<td>0.103</td>
<td>0.588</td>
<td>0.487</td>
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<tr>
<td>diastolic blood pressure</td>
<td>0.336</td>
<td>0.069</td>
<td>0.369</td>
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<tr>
<td>fasting glucose</td>
<td>0.251</td>
<td>0.174</td>
<td>0.581</td>
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<tr>
<td>insulin</td>
<td>0.358</td>
<td>0.056</td>
<td>0.635</td>
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* Partial correlation adjusted for gender
Table 3. Multiple linear regression analysis on plasma PEDF level

<table>
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<tr>
<th>Model</th>
<th>Model</th>
<th>Variables</th>
<th>Coefficient [95% confidence interval]</th>
<th>Standardized Coefficient</th>
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<tbody>
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<td>R²</td>
<td>P</td>
<td></td>
<td>B</td>
<td>β</td>
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<tr>
<td>0.737</td>
<td>&lt;0.001</td>
<td>gender*</td>
<td>-2026 [-2945 – -1108]</td>
<td>-0.424</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>age</td>
<td>5.9 [-36 – 47]</td>
<td>0.028</td>
<td>0.777</td>
</tr>
<tr>
<td></td>
<td></td>
<td>visceral fat thickness</td>
<td>342 [88 – 595]</td>
<td>0.361</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BMI</td>
<td>201 [55 – 347]</td>
<td>0.288</td>
<td>0.008</td>
</tr>
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</table>

*male =0, female =1