Plasma PTX3 protein levels inversely correlate with insulin secretion and obesity, whereas visceral adipose tissue PTX3 gene expression is increased in obesity


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ABSTRACT

O. Osorio-Conles O, Guitart M, Chacón MR, Maymo-Masip E, Moreno-Navarrete JM, Montori-Grau M, Naf S, Fernandez-Real JM, Vendrell J, Gómez-Foix AM. Plasma PTX3 protein levels inversely correlate with insulin secretion and obesity, whereas visceral adipose tissue PTX3 gene expression is increased in obesity. Am J Physiol Endocrinol Metab 301: E1254–E1261, 2011. First published September 6, 2011; doi:10.1152/ajpendo.00163.2011.—Plasma acute-phase pentraxin 3 (PTX3) concentration is dysregulated in human obesity and metabolic syndrome. Here, we explore its relationship with insulin secretion and sensitivity, obesity markers, and adipose tissue PTX3 gene expression. Plasma PTX3 protein levels were analyzed in a cohort composed of 27 lean [body mass index (BMI) ≤25 kg/m²] and 48 overweight (BMI 25–30 kg/m²) men (cohort 1). In this cohort, plasma PTX3 was negatively correlated with fasting triglyceride levels and insulin secretion after intravenous glucose administration. Plasma PTX3 protein and PTX3 gene expression in visceral (VAT) and subcutaneous (SAT) whole adipose tissue and adipocyte and stromovascular fractions were analyzed in cohort 2, which was composed of 19 lean, 28 overweight, and 15 obese subjects (BMI >30 kg/m²). An inverse association with body weight and waist/hip ratio was observed in cohort 2. In VAT depots, PTX3 mRNA levels were higher in subjects with BMI ≥25 kg/m² than in lean subjects, positively correlated with IL-1β mRNA levels, and higher in the adipocyte than stromovascular fraction. Human preadipocyte SGBS cell line was used to study PTX3 production in response to factors that obesity entails. In SGBS adipocytes, PTX3 gene expression was enhanced by IL-1 and TNFα but not IL-6 or insulin. In conclusion, the negative correlation between PTX3 and glucose-stimulated insulin secretion suggests a role for PTX3 in metabolic control. PTX3 gene expression is upregulated in VAT depots in obesity, despite lower plasma PTX3 protein, and by some proinflammatory cytokines in cultured adipocytes.

protein pentraxin 3; pentraxins; insulin sensitivity; proinflammatory cytokines

OBSERVATIONS

Obesity results in a chronic low-grade systemic inflammatory state, which is brought about by adipose tissue (AT), mainly from visceral depots. In AT, macrophage infiltration and production of proinflammatory cytokines (mostly IL-6 and TNFα) are increased (5), particularly in the nonadipose cell fraction (14). Obesity, and especially visceral adiposity, is linked to increased levels of circulating inflammatory markers, such as acute-phase pentraxin C-reactive protein (CRP), as a consequence of the high levels of IL-6 released from AT depots (5). Both the local (in AT) and systemic inflammation present in obesity have been associated with an increased risk of developing type 2 diabetes and cardiovascular disease (5, 39).

Pentraxins are key components of the humoral innate immune system (27) characterized by the presence of a structural motif in their COOH-terminal region, the pentraxin domain (16). The pentraxin family (27) is formed of short pentraxins, such as CRP, and long pentraxins, such as protein pentraxin 3 (PTX3) (9), also called TNF-stimulated gene (TSG)-14 protein (24). PTX3 is a secreted acute-phase protein that conserves the COOH-terminal pentraxin domain but has no homology in its NH2-terminal half with any other known protein (9). This has led to the suggestion that it may have differential biological functions from those of other pentraxin family members. Indeed, PTX3 has different cell-specific gene expression and ligand-binding properties from CRP (8). PTX3 is produced in response to proinflammatory signals by mononuclear phagocytes (cytes 3, 20), myeloid dendritic cells (12), fibroblasts (9, 20, 23), endothelial cells (9, 20), hepatic cells (9, myoblasts (20), synoviocytes (26), lung epithelial cells (17), renal epithelial cells (32), adipocytes (1), AT-derived mesenchymal stem cells (25), and smooth muscle cells (11). Expression of the PTX3 gene increases in human cultured myotubes compared with the skeletal muscle tissue (38). Finally, the in vivo expression of the PTX3 gene in mouse tissues is high in heart and limb muscle after intravenous injection with the immune stimulator lipopolysaccharide, whereas a weaker signal is detected in lung, ovary, and thymus (20). This diverse cellular origin is in contrast to that of CRP, which is produced almost exclusively in the liver (36).

Previous studies have reported that PTX3 production is dysregulated in obesity. Plasma PTX3 protein concentrations were inversely correlated with fat mass, leptin, CRP, and IL-6 in healthy subjects undergoing changes in physical activity and energy balance (7). Likewise, negative correlations between concentrations of plasma PTX3 protein and triglycerides and BMI and lower PTX3 values in individuals with metabolic syndrome were described in a large population of subjects (34, 44). However, some recent studies (30, 45) disagree with these associations, reporting a positive link between PTX3, obesity, and some cardiovascular risk factors that associates the inflammatory function of this pentraxin with a worse cardiovascular profile. There is little information about PTX3 gene expression...
in AT, although PTX3 mRNA levels are higher in the white AT of genetically obese (ob/ob) and obese diabetic (db/db) mice than in controls (1). In humans, there is only one previous study in which PTX3 gene expression was evaluated in AT from lean and obese patients (2). PTX3 gene expression tended to be higher in the VAT depot of obese subjects compared with their nonobese counterparts. After controlling for multiple variables, only HDL (inversely) and fibrinogen (directly) plasma levels were found to be unique determinants of VAT-PTX3 gene expression in the whole population.

Here, we explore the association of plasma PTX3 protein with several metabolic markers and with glucose-stimulated insulin secretion. We assess PTX3 gene expression in depth in visceral (VAT) and subcutaneous AT (SAT) in a cohort with a wide body mass index (BMI) range in both the adipocyte and stromovascular fractions. We also analyze the regulation of PTX3 gene expression and protein secretion by cultured Simpson-Golabi-Behmel syndrome (SGBS) adipocytes in response to factors that obesity entails, such as insulin, proinflammatory cytokines, promoters of reactive oxygen species (ROS), and hypoxia.

METHODS

Selection of study-participating subjects. Two cohorts were included in the study. Cohort 1, composed of 75 apparently healthy men, was selected by the “Hospital de Girona Dr. Josep Trueta” (Girona, Spain) for the study of insulin sensitivity and insulin secretion using the minimal model approach. Insulin sensitivity ($S_i$) was measured using the frequently sampled intravenous glucose tolerance test with minimal model analysis (31). Briefly, intravenous glucose (0.3 g/kg) was administered at time 0 and insulin (0.03 U/kg) at time +20 min. Insulin secretion was calculated from the frequently sampled intravenous glucose tolerance test as the incremental insulin response from 0 to 10 min after intravenous glucose [acute insulin response to glucose (AIRg)]. The time courses of serum glucose and insulin over 3 h were analyzed using the Minimal Model Program to calculate the $S_i$ index (6). On another day, an oral glucose tolerance test (OGTT) was performed, following the American Diabetes Association recommendations. After a 12-h overnight fast, 75 g of glucose had fasted overnight, for a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. All patients had fasted overnight, for ≥12 h, before surgical procedure. Blood samples were collected before surgery from the antecubital vein. VAT and SAT samples were obtained from all 62 subjects during the surgical procedure, washed in PBS, immediately frozen in liquid N$_2$, and stored at −80°C. For AT fractionation, fresh AT was finely diced, and small pieces were washed in PBS and incubated in medium 199 plus 4% BSA and 2 mg/ml collagenase type I (Sigma-Aldrich, St. Louis, MO) for 1 h at 37°C and then filtered (200 µm) and centrifuged for 5 min at 1,500 g. Mature adipocytes were removed from the top layer, and the pellet contained the stromovascular cells. Cells washed four times in PBS were stored at −80°C. The ethics committees of both Hospital de Girona Dr. Josep Trueta and University Hospital Joan XXIII approved the study, and informed consent was obtained from all participants.

Analytical methods. Glucose, cholesterol, triglyceride, and glycerol in plasma and nonesterified fatty acid in serum were determined in an ADVIA 1200 (Siemens, Munich, Germany) autoanalyzer using standard enzyme methods. HDL cholesterol was quantified after precipitation with polyethylene glycol 6000 at room temperature. Plasma insulin was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA); assay sensitivity was 2.6 mU/ml and coefficient of variation of intra- (IACV) and interassay (IRCV) 7–8%. Serum leptin levels were determined by ELISA (Assaypro, St. Charles, MO); IACV and IRCV were 4 and 7.7%, respectively, and assay sensitivity was <150 pg/ml. The index of insulin resistance was determined by the homeostatic model assessment of insulin resistance (HOMA-IR) (28).

Soluble tumour necrosis factor receptor 2 was determined by enzyme IA (DiaSource, Nivelles, Belgium); the limit of detection was 0.1 ng/ml, and IACV and IRCV were <7 and <9%, respectively. IL-6 was determined by enzyme IA (DiaSource), with assay sensitivity of 0.039 pg/ml and IACV and IRCV <9.8 and <11.2%, respectively. PTX3 protein was measured in 10 µl of plasma and SGBS-conditioned medium samples by ELISA (Cosmo-Bio-Co, Tokyo, Japan), with limit of detection 0.1 ng/ml and IACV and IRCV <4.1 and <4.3%, respectively.

Cell culture. The SGBS human preadipocyte cell line is derived from an infant with SGBS (42). Confluent SGBS preadipocytes were induced to differentiate to mature adipocytes, as described (10). Six hours before the addition of effectors, mature adipocytes were incubated with serum-depleted DMEM, and then the medium was replaced by 3 ml of fresh medium, which was added with the effectors studied, and incubation prolonged for 16 h at 37°C in a humidified atmosphere of 5% CO$_2$ and 95% air. We used TNFα (BioNova, Madrid, Spain), IL-1β, IL-6, antymycin A, insulin, and H$_2$O$_2$ (Sigma-Aldrich). For hypoxia induction, after 6 h of preincubation with serum-depleted DMEM, the medium was replaced by 3 ml of fresh medium, and cells were incubated for 16 h at 37°C in a humidified atmosphere of 5% CO$_2$ and 1% oxygen balanced with nitrogen. Serum-free conditioned media were collected at the end of the experiments, filtered (0.22 µm) to remove cells and debris, and stored at −20°C. Cell monolayers were rinsed in PBS, frozen in liquid N$_2$, and stored at −80°C.

Gene expression analysis. Total RNA was extracted from AT samples using the RNeasy lipid tissue midi kit and treated with 550Nase-free deoxyribonuclease (Qiagen, Hilden, Germany). The RNeasy Mini Kit (Qiagen) was used to extract total RNA from SGBS cells and AT fractions. Extracts were homogenized using a Polytron homogenizer.

RNA was retrotranscribed with TaqMan reagents from Applied Biosystems (Carlsbad, CA), using random hexamers and RNase inhibitor. Real-time PCR was performed using either an ABI Prism 7700 sequence detection system or a 7900HT fast real-time PCR system with the TaqMan universal PCR master mix and Gene Expression Assays. Probes for 18S rRNA (Hs99999901_s1), PTX3 (Hs00173615_m1), and IL-1β (Hs00174097_m1) were used. SDS software 2.3 and RQ Manager 1.2 (Applied Biosystems) or the relative expression software tool program (37) were used to estimate the gene expression fold change by the $2^{-ΔΔCt}$ method. For AT and fractionated AT samples, data were expressed as an n-fold difference.
The values of 2

systolic blood pressure; DSP, diastolic blood pressure; PTX3, protein pentraxin 3.

1 mM phenylmethylsulfonyl fluoride, 1 mM NaF, 1 mM Na3VO4, composed of 50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 10% SDS-PAGE. Antibodies against PTX3 (WH0005806M2; Sigma-Aldrich) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 2118; Cell Signaling Technology, Beverly, MA) were used. Horseradish peroxidase-conjugated secondary antibodies were used, and membranes were developed with ECL Plus (GE Healthcare, Buckinghamshire, UK). Protein bands were revealed and quantified using a LAS-3000 luminescent image analyzer (FujiFilm, Tokyo, Japan).

Statistical analysis. We used the SPSS/PC+ statistical package (version 16; SPSS, Chicago, IL). For clinical and anthropometrical variables, normally distributed data are expressed as means ± SD or SE, and for variables with a non-Gaussian distribution, values are expressed as the median (75th percentile) to provide an easy measurement of the dispersion of the variable. For statistical analysis of expression variables, values that did not have a Gaussian distribution were logarithmically transformed or analyzed by nonparametrical tests. Comparisons between groups were performed by one-way ANOVA with a post hoc Bonferroni correction or by a Kruskal-Wallis nonparametric test when appropriate. Associations between quantitative variables were evaluated by Pearson correlation analysis or Spearman correlation for nonnormally distributed variables. The independence of the associations was evaluated by linear regression analysis. Statistical significance occurred if a computed two-tailed probability value was <0.050. For cell culture experiments a general linear model repeated-measures test was used for the statistical analysis, and a two-tailed probability value <0.050 was considered as statistically significant.

RESULTS

Circulating plasma PTX3 protein. Plasma PTX3 protein concentration was assessed. Clinical and laboratory data for the participants in cohort 1 are summarized in Table 1. This cohort was composed of 27 lean (BMI ≤ 25 kg/m²) and 48 overweight (BMI 25–30 kg/m²) subjects. No differences in the PTX3 relative to a calibrator (a mix of different depot samples). For SGBS cells, the 18S rRNA gene was used as the control to normalize the crossing point (Cp) for each probe assay. Data were expressed as mean values of 2−ΔΔCt for upregulated and 1/2−ΔΔCt for downregulated mRNA.

Immunoblotting analysis. Cell extracts were homogenized in buffer composed of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF; 1 mM Na3VO4, 2 mg/ml benzamidine, 2 mg/ml leupeptin, 1% (vol/vol) Nonidet P-40, and 1 mM dithiothreitol. Lysates were then gently rocked for 60 min at 4°C and stored at −80°C until analysis. Protein was resolved in 10% SDS-PAGE. Antibodies against PTX3 (WH0005806M2; Sigma-Aldrich) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 2118; Cell Signaling Technology, Beverly, MA) were used. Horseradish peroxidase-conjugated secondary antibodies were used, and membranes were developed with ECL Plus (GE Healthcare, Buckinghamshire, UK). Protein bands were revealed and quantified using a LAS-3000 luminescent image analyzer (FujiFilm, Tokyo, Japan).

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Table 1. Anthropometric and analytical characteristics of the healthy lean and overweight men cohort

<table>
<thead>
<tr>
<th>Anthropometric and Analytical Characteristics</th>
<th>Healthy Men Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>50.7 ± 11.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.8 ± 8.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8 ± 2.5</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>88.1 ± 6.9</td>
</tr>
<tr>
<td>Waist/hip ratio, cm</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123.7 ± 13.1</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78.8 ± 7.6</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>5.27 ± 0.58</td>
</tr>
<tr>
<td>Insulin, µIU/ml</td>
<td>8.1 ± 3.9</td>
</tr>
<tr>
<td>Log insulin sensitivity (10−4 min−1), µIU/l</td>
<td>0.58 ± 0.18</td>
</tr>
<tr>
<td>Log acute insulin response, min−1</td>
<td>2.48 ± 0.36</td>
</tr>
<tr>
<td>Fasting triglyceride, mM</td>
<td>1.18 ± 0.84</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>5.38 ± 1.04</td>
</tr>
<tr>
<td>HDL cholesterol, mM</td>
<td>1.38 ± 0.36</td>
</tr>
<tr>
<td>PTX3, ng/ml</td>
<td>2.37 ± 1.09</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 75. BMI, body mass index; SBP, systolic blood pressure; DSP, diastolic blood pressure; PTX3, protein pentraxin 3.
levels between lean and overweight subjects were observed. A bivariate correlation analysis of the whole population (Fig. 1) showed that plasma PTX3 was negatively correlated with total fasting triglyceride levels and insulin secretion after both intravenous (AIRg) and oral glucose administration (measured as serum insulin at 30 min in the OGTT). S,

\[ r = -0.03, P = 0.7. \]

On univariate analysis, fasting triglyceride was associated with insulin secretion \( (r = 0.30, P = 0.01). \) On multivariate analysis, fasting triglyceride was associated with plasma PTX3 concentration, and this association remained significant after adjustments were made for BMI, age, and insulin secretion \( (B = -1.625, P = 0.008), \) contributing to 11.4% of the variance in plasma PTX3. The association with insulin secretion remained significant after controlling for BMI, waist diameter, and age \( (P = 0.03). \)

Anthropometric and analytical characteristics of the participants in the cohort 2 are summarized in Table 2. This cohort was composed of 19 lean \( \left( \text{BMI} \leq 25 \text{ kg/m}^2 \right), \) 28 overweight \( \left( \text{BMI} 25–30 \text{ kg/m}^2 \right), \) and 15 obese \( \left( \text{BMI} > 30 \text{ kg/m}^2 \right) \) subjects. Systolic and diastolic blood pressure, fasting glucose, insulin levels, HOMA-IR indexes, and leptin levels were increased in the obese group compared with controls. Among the inflammatory markers, IL-6 concentration was higher in the obese group than in the overweight group. Plasma PTX3 protein showed a nonsignificant trend toward lower levels in the obese subjects, which remained nonsignificant when we gathered both overweight and obese subjects. Nevertheless, when we considered the whole cohort for the bivariate analysis, negative correlations of plasma PTX3 to both body weight \( (r = -0.32, P = 0.016) \) and waist/hip ratio \( (r = -0.37, P = 0.006) \) were observed. To strengthen the independence of these associations as predictors of plasma PTX3 levels, a multiple regression analysis model was constructed, including the above-mentioned bivariate correlations, adjusting for age, sex, and smoking habits. The results presented a multiple correlation coefficient \((r = 0.475, \) and plasma PTX3 levels were predicted mainly by age and waist circumference \( (B = 0.073, P = 0.0048), \) and \( B = -0.129, P < 0.001, \) respectively). When we constructed a second regression model, including as independent variables some parameters associated with this pentraxin in previous studies, such as plasma triglyceride, insulin, and HDL cholesterol levels, the results did not show the dependence mentioned above.

**PTX3 gene expression profile in SAT and VAT deports.** Paired SAT and VAT samples were analyzed for PTX3 mRNA gene expression in the wide-ranging BMI cohort 2. No differences were observed between the three groups studied (Table 2). However, when we compared PTX3 mRNA levels in lean subjects \( (\text{BMI} \leq 25 \text{ kg/m}^2) \) with those of the combined obese and overweight subjects \( (\text{BMI} > 25 \text{ kg/m}^2) \), we found significantly higher VAT PTX3 transcript levels in subjects with BMI > 25 kg/m² \( (127.42 \pm 86.26 \text{ vs. } 226.01 \pm 27.16, \text{ respectively, } P = 0.039) \). No association between circulating plasma PTX3 levels and PTX3 gene expression in AT was observed.

PTX3 mRNA levels in isolated mature adipocytes and the stromovascular fraction of the VAT and SAT deports showed no differences related to obesity. When we considered all of the samples together, the PTX3 gene was expressed in the SAT depot at a similar level in both the adipocyte and stromovascular fractions (Fig. 2). In the VAT depot, PTX3 transcript levels were higher in mature adipocyte than in the stromovascular fraction. When we compared mature adipocyte from

Table 2. Anthropometric and analytical characteristics of the lean, overweight, and obese cohort according to BMI classification

<table>
<thead>
<tr>
<th>Anthropometric and Analytical Characteristics</th>
<th>Lean ((n = 19))</th>
<th>Overweight ((n = 28))</th>
<th>Obese ((n = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>51.68 ± 15.96</td>
<td>57.14 ± 14.96</td>
<td>57.40 ± 12.75</td>
</tr>
<tr>
<td><strong>Females/males ((n))</strong></td>
<td>6/13</td>
<td>12/16</td>
<td>6/9</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>64.09 ± 7.26</td>
<td>75.02 ± 8.81§</td>
<td>91.60 ± 13.82§</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>23.11 ± 1.59</td>
<td>27.29 ± 1.24§</td>
<td>32.55 ± 2.38§</td>
</tr>
<tr>
<td><strong>Waist, cm</strong></td>
<td>83.76 ± 7.90</td>
<td>94.36 ± 11.38§</td>
<td>109.14 ± 12.28§</td>
</tr>
<tr>
<td><strong>Waist/hip ratio, cm</strong></td>
<td>0.90 ± 0.06</td>
<td>0.94 ± 0.12</td>
<td>0.94 ± 0.11</td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>121.47 ± 11.77</td>
<td>128.68 ± 14.20</td>
<td>146.00 ± 12.29†‡</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>68.47 ± 9.90</td>
<td>72.00 ± 9.18</td>
<td>82.00 ± 13.16*</td>
</tr>
<tr>
<td><strong>Fasting glucose, mM</strong></td>
<td>5.07 ± 0.57</td>
<td>5.56 ± 0.83</td>
<td>5.75 ± 0.53*</td>
</tr>
<tr>
<td><strong>Insulin, μIU/ml</strong></td>
<td>4.51 ± 3.40</td>
<td>5.15 ± 3.40</td>
<td>9.08 ± 6.03*♀</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>1.11 ± 0.84</td>
<td>1.31 ± 0.91</td>
<td>2.36 ± 1.63*♀</td>
</tr>
<tr>
<td><strong>Triglyceride, mM</strong></td>
<td>0.95 (1.57)</td>
<td>1.11 (1.47)</td>
<td>1.00 (1.30)</td>
</tr>
<tr>
<td><strong>Cholesterol, mM</strong></td>
<td>5.19 ± 1.18</td>
<td>4.91 ± 0.99</td>
<td>5.20 ± 0.81</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mM</strong></td>
<td>1.46 ± 0.51</td>
<td>1.30 ± 0.29</td>
<td>1.36 ± 0.26</td>
</tr>
<tr>
<td><strong>NEFA, mM</strong></td>
<td>0.99 ± 0.71</td>
<td>0.74 ± 0.25</td>
<td>0.79 ± 0.96</td>
</tr>
<tr>
<td><strong>Glycerol, μM</strong></td>
<td>162.58 (299.45)</td>
<td>135.36 (205.01)</td>
<td>158.24 (286.42)</td>
</tr>
<tr>
<td><strong>Leptin, ng/ml</strong></td>
<td>2.16 (29.42)</td>
<td>9.90 (18.09)</td>
<td>27.59 (92.26)*♀</td>
</tr>
<tr>
<td><strong>IL-6, pg/ml</strong></td>
<td>1.38 (2.52)</td>
<td>1.04 (2.23)</td>
<td>2.53 (5.18)*♀</td>
</tr>
<tr>
<td><strong>sTNFR2, ng/ml</strong></td>
<td>3.54 (4.95)</td>
<td>3.19 (4.48)</td>
<td>3.73 (4.04)</td>
</tr>
<tr>
<td><strong>PTX3, ng/ml</strong></td>
<td>3.15 (4.40)</td>
<td>2.84 (3.43)</td>
<td>2.68 (3.38)</td>
</tr>
</tbody>
</table>

| Relative mRNA levels (arbitrary units)       |                  |                  |                  |
| PTX3 SAT                                    | 154.80 ± 71.82   | 227.50 ± 24.03  | 134.90 ± 66.72   |
| PTX3 VAT                                    | 127.42 ± 86.26   | 217.90 ± 30.25  | 241.10 ± 21.10   |

Value data are presented as means ± SD for normally distributed data or median (75th percentile values are in parentheses) for nonnormally distributed variables. HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, nonesterified fatty acids; sTNFR2, soluble TNFα receptor 2; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. The significance of differences is as follows: *P < 0.05, §P < 0.001, †P = 0.01, and +P = 0.05 compared with lean subjects; ‡P = 0.006 and ††P < 0.05 compared with overweight subjects. Gene expression data are means ± SD of paired SAT and VAT samples from the 19 lean, 28 overweight, and 15 obese subjects.
both depots, PTX3 mRNA levels were higher in adipocytes isolated from VAT than from SAT depot.

Regulation of PTX3 gene expression and protein content in SGBS adipocytes. To explore PTX3 production in adipocytes, under conditions that occur in obesity such as upregulated proinflammatory cytokines, promoters of ROS (the oxidant H$_2$O$_2$ or the mitochondrial complex II inhibitor antimycin A), hypoxia, and insulin, we used the human preadipocyte SGBS cell line.

The mRNA levels of PTX3 were strongly upregulated by both IL-1$\beta$ (3.5-fold) and TNF$\alpha$ (2.8-fold) in SGBS adipocytes (Fig. 3). Small changes (2-fold) in PTX3 transcript levels were observed in response to hypoxia (+1.2-fold), antimycin A (+1.7-fold), or H$_2$O$_2$ (−1.3-fold) treatment. Finally, IL-6 and insulin had no effect under the conditions studied here.

Since insulin may inhibit protein breakdown in adipose tissue (41), we examined whether insulin or the tested cytokines could regulate adipocyte PTX3 protein content (Fig. 4). We found that insulin did not modify PTX3 protein level relative to GAPDH protein, although a reduction tendency was observed. IL-1$\beta$ and TNF$\alpha$ increased PTX3 protein content relative to GAPDH protein (53 and 35%, respectively), as they did with PTX3 gene expression. IL-6 caused a smaller increment (27%) in the relative level of PTX3 protein, which does not correspond to the lack of effect on gene expression.

Then we assessed whether the upregulating effect of cytokines on PTX3 gene expression was coupled with altered PTX3 protein secretion (Fig. 5). IL-1$\beta$ exerted a strong stimulatory effect (14.6-fold) on the secretion of PTX3 protein by SGBS adipocytes, whereas the increment for TNF$\alpha$ was smaller (5.3-fold) in concordance with the less powerful effect observed on gene expression. IL-6 did not alter PTX3 protein secretion, although a tendency to increase was observed, which may be related to the increase in PTX3 protein content.

Correlation between PTX3 and IL-1$\beta$ gene expression in adipose tissue. Since IL-1$\beta$ powerfully induced PTX3 gene expression, protein content, and secretion in cultured adipocytes, we examined the possible association between IL-1$\beta$ and PTX3 gene expression in SAT and VAT samples from cohort 2. A positive correlation between IL-1$\beta$ and PTX3 gene expression in VAT depot was observed ($r = 0.287$, $P = 0.025$), whereas no significant correlation was found in SAT depot.

DISCUSSION

PTX3 is a secreted acute-phase protein. Its plasma levels have been linked to obesity (7, 34, 44), the circulating lipid profile (34, 44, 45), atherosclerosis (33), and inflammation (27). Here, we show that in a cohort of healthy lean and overweight men, plasma PTX3 protein levels display a negative correlation with fasting triglyceride levels, a result that has been found consistently in previous studies (34, 44). Interestingly, we found a negative correlation between plasma PTX3 and insulin secretion after both intravenous and oral glucose administration. To the best of our knowledge, this is the first evidence of this association. In agreement with these results, plasma PTX3 was shown to be lower in subjects with metabolic syndrome (34, 44), a condition associated with insulin resistance. To explain decreased levels of circulating PTX3 in subjects with hypertriglyceridemia, increased compensatory hyperinsulinemia, or metabolic syndrome, two different hypotheses arise; PTX3 might constitute a factor that provides protection from developing metabolic disease, or PTX3 levels may decrease as a consequence of metabolic derangement.

We show in a second cohort composed of subjects with wide-ranging BMI that plasma PTX3 protein levels have a

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Fig. 2. Regulation of PTX3 gene expression in cultured human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes. Cells were incubated for 16 h in serum-depleted DMEM without (controls) or with 20 ng/ml TNF$\alpha$, 20 ng/ml IL-1$\beta$, 10 ng/ml IL-6, hypoxia (1% O$_2$), 100 nM antimycin A, 100 nM insulin, or 1 mM H$_2$O$_2$ as indicated. The mRNA levels of PTX3 were quantified relative to the reference 18S rRNA gene. Data are means ± SD of 2−$\Delta\Delta$C$_{\text{t}}$ for upregulated genes and 1−$\Delta\Delta$C$_{\text{t}}$ for downregulated genes; the former are + and the latter − from 6 experiments performed in duplicate. The significance of the differences with respect to control cells is #P < 0.001.

Fig. 3. Regulation of PTX3 gene expression in isolated mature adipocyte (ADI) and stromovascular fractions (SVF) of subcutaneous (SAT) and visceral adipose tissue (VAT) depots from the lean, overweight, and obese cohort. Value data are presented as means ± SD from a group of 15 samples (8 males and 7 females) with a mean age of 59.5 ± 10 yr; 14 samples for SAT (ADI and SVF) and 13 for VAT (ADI and SVF). The significance of differences is as follows: *P = 0.028, between the same depot; #P = 0.034, between different depots.
significant negative correlation with obesity indexes, specifically body weight and waist circumference. These results agree with the previous reports of inverse correlation between plasma PTX3 and fat mass (7), BMI (34, 44), body weight, and waist circumference (34). They are in contrast to other studies (30, 45) that report a positive link between PTX3, obesity, and some cardiovascular risk factors; i.e., higher circulating PTX3 was found in patients with metabolic syndrome in association with subclinical atherosclerosis (45), in obese men coexisting with decreased arterial distensibility (30), and in acute myocardial infarction patients in correlation with abdominal visceral obesity (40). There is no clear explanation for these discrepancies, and all of these results come from observational studies. Unfortunately, there are no mechanistic studies to better interpret this apparently anomalous behavior of plasma PTX3 arising from different inflammatory pathologies. It is tempting to hypothesize that the multiple sources of PTX3 may serve different functions according the tissue or the cellular component where it is produced.

Along these lines, an issue that we addressed here is the relationship between PTX3 gene expression in AT and plasma PTX3 protein in the cohort with wide-ranging BMI. We evaluated PTX3 mRNA levels in paired samples of SAT and VAT depots. We found higher PTX3 mRNA levels in the VAT depot from subjects with a BMI of >25 kg/m² than in lean subjects, with no differences in the SAT depot. A previous work (2) showed a tendency toward higher PTX3 gene expression only in isolated adipocytes derived from VAT depots. Moreover, PTX3 mRNA levels are higher in adipocytes isolated from VAT than from SAT depots. A previous study (2) assessed PTX3 gene expression only in isolated adipocytes derived from VAT and SAT from human subjects and reported no differences between depots. Our data differ from those reported in mice, in which PTX3 mRNA levels are high and low in the stromovascular fraction of the VAT depot, whereas no differences between fractions are observed in the SAT depot. Furthermore, PTX3 mRNA levels are higher in adipocytes isolated from VAT than from SAT depots. A previous study (2) assessed PTX3 gene expression only in isolated adipocytes derived from VAT and SAT from human subjects and reported no differences between depots. Our data differ from those reported in mice, in which PTX3 mRNA levels are high and low in the stromovascular and adipocyte fractions, respectively, in mouse white and brown ATs (1).

Obesity in humans is associated with enhanced release of cytokines (14) and oxidative stress (15) and hypoxia (18) in the VAT depot of obese subjects compared with normal-weight subjects. These results are in agreement with previous data from mice models, where genetically obese (ob/ob) and obese diabetic (db/db) mice show higher levels of PTX3 mRNA in white AT than their lean counterparts (1). However, PTX3 AT gene expression data do not explain lower plasma PTX3 protein levels in subjects whose BMI is >25 kg/m² in our cohort. This may indicate different PTX3 functions at the paracrine/autocrine levels in AT and at the systemic level in obesity. Such a finding would not be surprising, because PTX3 is expressed in different tissues and cell types. According to the BioGPS database (43), in humans PTX3 mRNA is more abundant in smooth muscle, adipocyte, cardiac myocytes, and fetal lung. PTX3 mRNA is also present in all of the different compartments of the adult human kidney (32). Further work will be necessary to ascertain the contribution of each of the high-expression PTX3 tissues to the plasma PTX3 protein levels and possible differential regulation. In addition, it should be considered that obesity is associated with increased proteolysis (21), and this could affect PTX3 protein production.

Our data highlight the importance of mature adipocytes in PTX3 production by AT, at least at the local level. We show that PTX3 mRNA is more abundant in the mature adipocyte than in the stromovascular fraction of the VAT depot, whereas no differences between fractions are observed in the SAT depot. Moreover, PTX3 mRNA levels are higher in adipocytes isolated from VAT than from SAT depots. A previous study (2) assessed PTX3 gene expression only in isolated adipocytes derived from VAT and SAT from human subjects and reported no differences between depots. Our data differ from those reported in mice, in which PTX3 mRNA levels are high and low in the stromovascular and adipocyte fractions, respectively, in mouse white and brown ATs (1).
AT, in parallel with hyperinsulinemia (13). Therefore, in cultured SGBS human adipocytes, we studied whether these different stimuli could regulate PTX3 production. We show that ROS promoters, such as H₂O₂ or the mitochondrial complex II inhibitor antimycin, have small (<2-fold) downregulatory and upregulatory effects, respectively, on PTX3 gene expression. Hypoxia is slightly stimulatory, whereas insulin has no effect. Regarding proinflammatory cytokines, both IL-1β and TNFα strongly induce PTX3 gene expression, and IL-6 has no effect. In fact, a positive effect of both IL-1 and TNFα has been observed previously in a wide range of cells: endothelial (9, 20) and hepatic cells (9), fibroblasts (9, 20, 23), myoblasts (20), mononuclear phagocytes (3, 20), and renal epithelial cells (32). Furthermore, IL-1 was effective in dendritic (12) and smooth muscle cells (11) and TNFα in 3T3-F442A adipocytes (1), synoviocytes (26), and lung epithelial cells (17). Similarly, IL-6 did not regulate PTX3 mRNA in endothelial, hepatic, or fibroblastic cells (9), mononuclear phagocytes (3), renal epithelial cells (32), or synoviocytes (26). Then, PTX3 gene expression is consistently responsive to IL-1β and TNFα in diverse cultured human and rodent cell types, whereas it is unresponsive to IL-6. This may be explained by the presence in the human PTX3 proximal promoter of an NF-κB site that mediates responsiveness to IL-1β and TNFα, whereas it does not respond to IL-6, in fibroblasts (4). We also report that IL-1β and TNFα clearly increase adipocyte PTX3 protein content, as they do with PTX3 gene expression. IL-6 has a smaller positive effect on adipocyte PTX3 protein, not associated with enhancement of PTX3 gene expression, that may be related to its reported capacity to stimulate protein synthesis in cultured sensory neurons (29). Furthermore, we corroborate that marked upregulation of PTX3 gene expression and protein content by IL-1β and TNFα in cultured SGBS adipocytes is parallel to increased protein secretion. Indeed, secretion of PTX3 protein from different stimulated cell types has been demonstrated, e.g., in rodent 3T3-F442A adipocytes (1), AT-derived mesenchymal stem cells (25), monocytes, endothelial cells and fibroblasts (3, 20), myoblasts (20), FS4 fibroblasts, and Hep3B hepatocytes (23). In conclusion, our data in cultured adipocytes suggest that some proinflammatory factors elevated in AT in obesity such as IL-1β (22) and TNFα (19) may contribute to the upregulation of PTX3 production in the VAT depots of obese subjects, whereas other obesity-related factors such as AT oxidative stress and hypoxia or hyperinsulinemia do not substantially regulate PTX3 gene expression in adipocytes. In favor of this proposal, we report a positive correlation between IL-1β and PTX3 gene expression in the VAT depot of the wide-ranging BMI cohort that constitutes evidence of the mechanism of obesity-regulated PTX3.

In summary, in this study we show a negative correlation between plasma PTX3 protein, fasting triglyceride, and insulin secretion in response to glucose in human subjects, which suggests that PTX3 is at the crossroads of metabolic disease. However, the negative association of plasma PTX3 protein with obesity indexes is concurrent with higher PTX3 gene expression in VAT depots. Some proinflammatory factors upregulated in obesity, such as IL-1β, constitute possible stimulating factors of adipocyte PTX3 production. Finally, upregulation of VAT PTX3 in obesity may participate in the local AT equilibrium between inflammatory and anti-inflammatory response.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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