

Reduced Plasma Visfatin/Pre-B Cell Colony-Enhancing Factor in Obesity Is Not Related to Insulin Resistance in Humans

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Context: Visfatin was recently identified as a protein highly expressed and secreted in adipose tissue with insulin-mimetic effect and is a candidate hormone to help explain the association among adipose tissue expansion, insulin resistance, and type 2 diabetes.

Objective: The objective of the study was to assess expression of visfatin in lean and obese subjects and in sc and visceral adipose tissue and moreover to explore the role of visfatin on insulin resistance in humans.

Design: We measured circulating visfatin and its mRNA expression in sc adipose tissue (SAT) in lean and obese subjects. Furthermore, we measured visfatin mRNA in visceral adipose (VAT) and SAT by quantitative RT-PCR. Finally, plasma visfatin and its mRNA in SAT were measured under free fatty acid-induced insulin resistance in healthy subjects.

Results: Plasma visfatin and its mRNA in SAT were significantly lower in obese subjects, compared with normal-weight controls. Both circulating visfatin and SAT visfatin mRNA were negatively correlated with body mass index, whereas no correlation was found with homeostasis model assessment. Significantly higher visfatin mRNA was found in VAT of obese subjects, compared with lean controls. Interestingly, visfatin mRNA in VAT was positively correlated with BMI. Elevation of free fatty acid induced a condition of insulin resistance but did not affect either circulating visfatin or its mRNA.

Conclusions: Our findings show that, in human obesity, plasma visfatin is reduced, whereas visfatin mRNA is differentially regulated in SAT and VAT. Visfatin is not related to insulin resistance either as assessed by homeostasis model assessment or during lipid infusion. (*J Clin Endocrinol Metab* 91: 3165–3170, 2006)

THE ASSOCIATION BETWEEN accumulation of visceral adipose tissue (VAT) and insulin resistance is well established in obesity and type 2 diabetes (T2D), and both visceral fat and insulin resistance are strongly associated with increased cardiovascular risk (1–3).

Visfatin/PBEF was recently identified as a protein highly expressed in VAT, compared with sc adipose tissue (SAT) (4). Visfatin, previously known as a pre-B cell colony-enhancing factor (PBEF), also has a function in the immune system, in which it was described as a growth factor for early B cells (5). Visfatin/PBEF binds and activates the insulin receptor in different insulin-sensitive cells *in vitro*, and treating mice with recombinant visfatin/PBEF elicited insulin-like effects also *in vivo*. Plasma glucose is lowered by treatment with visfatin/PBEF, whereas heterozygous mice knockout for the visfatin/PBEF gene have plasma glucose higher than wild-type littermates (4). In 3T3-L1 adipocytes, visfatin/PBEF expression is up-regulated by differentiation and dexamethasone and is down-regulated by GH, isoproterenol, forskolin, and cholera toxin. Insulin has no effect on visfatin/PBEF

mRNA (6). Moreover, visfatin/PBEF is up-regulated by peroxisomal proliferator-activated receptor (PPAR)- α and PPAR γ agonists in obese rats in association with improved glycemic control and lipid profile, thus suggesting that PPAR α and PPAR γ agonists may act, at least in part, through the up-regulation of visfatin/PBEF expression (7). A recent study in humans in a wide population reported plasma visfatin/PBEF to be directly correlated with body mass index (BMI) and body fat content in males only and failed to find a different expression between visceral and sc fat depots (8). Because VAT is strongly associated with insulin resistance, and due to its peculiar molecular mechanism of action, visfatin/PBEF is a candidate to link visceral fat excess, insulin resistance, and the metabolic syndrome.

Free fatty acids (FFAs), which are often elevated in obese individuals (9), have been implicated as an important causative link among obesity, insulin resistance, and T2D (10). Infusion of FFAs in humans and rodents rapidly induces hyperinsulinemia and insulin resistance in both the skeletal muscle and the liver. This is believed to be due to multiple mechanisms including competition with glucose use, increase of hepatic gluconeogenesis, and glucose output (11–13). However, it is not possible to exclude that the effects of FFAs may be mediated also by hormonal mechanisms. In fact, FFAs may alter insulin secretion and insulin clearance by the liver, thus contributing to hyperinsulinemia (14). However, it is not known whether infusion of a lipid substrate and induction of insulin resistance may affect circulating levels of visfatin/PBEF and its expression in adipose tissue.

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Abbreviations: BMI, Body mass index; FFA, free fatty acid; HOMA, homeostasis model assessment; K_{it} , insulin sensitivity; M/F, male/female; PBEF, pre-B cell colony-enhancing factor; PPAR, peroxisomal proliferator-activated receptor; SAT, sc adipose tissue; T2D, type 2 diabetes; VAT, visceral adipose tissue.

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We therefore studied visfatin/PBEF concentration and SAT mRNA expression in lean and obese subjects and correlate them with anthropometric parameters and insulin resistance assessed by homeostasis model assessment (HOMA) index. Moreover, in a different set of subjects, we compared visfatin/PBEF mRNA expression in SAT and VAT. Furthermore, to investigate a possible role for visfatin/PBEF in FFA-induced insulin resistance, we measured plasma visfatin/PBEF and SAT expression in healthy subjects before and after a 5-h infusion of a triglyceride emulsion combined with heparin to acutely raise plasma FFAs and induce an acute condition of insulin resistance.

Taken together, our findings show that plasma visfatin/PBEF is reduced in human obesity and is not related to insulin resistance either as assessed by HOMA or during lipid infusion.

Subjects and Methods

Study 1: plasma visfatin/PBEF and expression of visfatin/PBEF mRNA in sc adipose tissue of lean and obese subjects

We studied 30 lean and 39 sex- and age-matched obese subjects whose anthropometric and biochemical parameters are illustrated in Table 1. All patients were recruited in the outpatient clinic and among the hospital staff and were on an unrestricted dietary regimen. Body weight was stable during the 3 months preceding the study. In each subject a venous blood sample was collected after overnight fasting between 0800 and 0900 h for biochemical and hormonal determinations (glucose, total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, triglycerides, insulin, visfatin/PBEF, and leptin). Plasma was immediately separated and frozen and stored at -80°C until subsequent analysis. HOMA index of insulin resistance was calculated as: $[\text{insulin (milliinternational units per liter)} \times \text{glucose (millimoles per liter)}]/22.5$. In all subjects height and body weight were measured. Waist was measured in standing position halfway between costal edge and iliac crest, whereas hip was measured as the greatest circumference around the buttocks. BMI and waist to hip ratio were calculated. Body composition was assessed by bioelectric impedance (BIA-STA equipment; Akern, Florence, Italy) and data analyzed with the software provided by the producer (Bodygraml Akern).

In a subgroup of subjects, visfatin/PBEF mRNA expression was assessed by quantitative RT-PCR in SAT obtained by percutaneous needle biopsies of the gluteal region of lean [$n = 9$, male/female (M/F) 4/5, age 32 ± 3 yr, BMI 22.2 ± 0.9 kg/m²] and obese ($n = 12$, M/F 5/7, age 32 ± 3 yr, BMI 42.2 ± 4.0) subjects. SAT was obtained under local anesthesia (lidocaine) after the purpose of the study was illustrated to patients, and written informed consent was obtained. The protocol was approved by the institutional ethics committee. Briefly, after induction

of anesthesia, a G16 needle was inserted in the SAT and a 30- to 50-mg biopsy was obtained by gentle aspiration with a syringe. Tissue was immediately washed in cold saline, rapidly frozen in liquid nitrogen, and then stored at -80°C for subsequent analysis. The procedure was well tolerated by all subjects.

Study 2: visfatin/PBEF mRNA expression in sc and visceral adipose tissue of lean and obese subjects

Biopsies of sc abdominal and omental adipose tissue (~ 300 mg) were collected from patients undergoing elective abdominal surgery for gastric banding (obese patients, $n = 8$, M/F 3/5, age 41 ± 4 yr, BMI 45.0 ± 2.1 kg/m²) or nonmalignant abdominal disease (lean patients, $n = 7$, M/F 3/4, age 46 ± 5 yr, BMI 23.9 ± 1.1 kg/m²), which included pancreatic pseudocysts and abdominal aortic aneurysm. All patients were free from malignant disease, major renal and hepatic dysfunction, diabetes, or endocrine-metabolic disorders other than obesity. The purpose of the study was illustrated to the patients, and written informed consent was obtained. The protocol was approved by the institutional ethics committee.

After collection adipose tissue samples were washed in cold saline, frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Study 3: plasma visfatin/PBEF and expression of visfatin/PBEF mRNA in sc adipose tissue of lean subjects under FFA-induced insulin resistance

Nine male volunteers with normal body weight (age 29 ± 2 yr; BMI 23 ± 1 kg/m²) were studied. Intralipid 20% (Pharmacia and Upjohn, Milan, Italy), a commercial emulsion of soybean oil, was infused at 1.5 ml/min (0.3 g/min) with sodium heparin (200 U in bolus and 0.4 U/min⁻¹·kg⁻¹ iv) for 5 h. Before lipid infusion and at the end of the 5-h infusion, a sample of gluteal SAT was obtained under local anesthesia as described in study 1.

An insulin tolerance test was performed at the end of the lipid infusion and on a separate day after a 5-h saline infusion as control. Briefly, recombinant regular insulin was administered iv as a bolus (0.1 IU/kg) and plasma glucose measured at baseline and after 3, 6, 9, 12, and 15 min after insulin administration. Insulin sensitivity (K_{itt}) was measured as previously described by Bonora *et al.* (15).

Quantification of visfatin/PBEF expression by quantitative RT-PCR

Total RNA was extracted with RNeasy minikit (QIAGEN GmbH, Hilden, Germany) following the supplier's instructions. One microgram of RNA was treated with DNase treatment and removal reagents (Ambion, Inc., Austin, TX) and reverse transcribed for 1 h at 37°C in a 50- μl reaction containing 1 \times reverse transcription buffer, 150 ng random hexamers, 0.5 mM deoxynucleotide triphosphates, 20 U RNAs in ribonuclease inhibitor, and 200 U Moloney murine leukemia virus reverse

TABLE 1. Anthropometric and biochemical parameters of obese subjects and controls

	Control (n = 30)	Obese (n = 39)	P value
M/F	15/15	17/22	
Age (yr)	34 \pm 15	33 \pm 18	ns
BMI (kg/m ²)	22.1 \pm 2.1	39.6 \pm 12.4	<0.001
Waist circumference (cm)	73 \pm 10	110 \pm 31	<0.001
Hip circumference (cm)	95 \pm 10	123 \pm 25	<0.001
Waist to hip ratio	0.77 \pm 0.05	0.89 \pm 0.11	<0.001
Fasting glucose (mmol/liter)	4.4 \pm 0.5	5.5 \pm 0.6	<0.001
Fasting insulin (mIU/liter)	5.6 \pm 2.2	15.4 \pm 9.3	<0.001
HOMA	1.1 \pm 0.5	3.6 \pm 1.8	<0.001
Total cholesterol (mmol/liter)	5.15 \pm 1.0	5.20 \pm 1.30	ns
High-density lipoprotein cholesterol (mmol/liter)	1.60 \pm 0.27	1.29 \pm 0.36	0.006
Low-density lipoprotein cholesterol (mmol/liter)	3.36 \pm 1.25	3.21 \pm 0.63	ns
Triglycerides (mmol/liter)	0.82 \pm 0.80	1.35 \pm 0.72	0.03
Leptin (ng/ml)	10.3 \pm 9.7	53.0 \pm 40.3	<0.001
Fat mass (% of total body mass)	21.8 \pm 5.4	40.9 \pm 11.7	<0.001

Data are expressed as mean \pm SD. ns, Not significant.

transcriptase (Promega Corp., Madison, WI). The primers were as follows: human visfatin/PBEF, 5'-CCGAGTTCAACATCCTCC-3' (forward) and 5'-GTTTCCTCATATTTACCTTCC-3' (reverse) and 18S, 5'-CGGCTACCACATCCAAGGAA-3' (forward) and 5'-GCTGGAAT-TACCGCGCT-3' (reverse). All results were normalized by 18S rRNA content.

PCR was carried out on DNA Engine Opticon TM 2 continuous fluorescence detection system (MJ Research, Waltham, MA), and all reactions were performed on at least two occasions. Each 30- μ l reaction contained 5 μ l first-strand cDNA (1 ng), 15 μ l 2 \times SYBR Green PCR master mix (Applied Biosystems, Foster City, CA), and the forward and reverse primers. An initial denaturation at 95 C for 10 min was followed by 40 cycles each consisting of 95 C for 30 sec, 58 C for 30 sec for visfatin/PBEF, and 60 C for 18S and 72 C for 30 sec. At the end of the reaction, the melting curve analysis was performed to verify the identity and specificity of amplification products. Standard curves were constructed using a cDNA of a positive sample serially diluted by plotting values for log cDNA quantity (in arbitrary units) vs. cycle threshold (the cycle number at which the fluorescence signal exceeds the background). Each sample was assayed in duplicate and a no-template control was included in every reaction.

Biochemical and hormonal determinations

Visfatin/PBEF was measured by enzyme immunoassay using a commercially available kit (Phoenix Pharmaceuticals, Inc., Belmont, CA). Assay sensitivity was 2 ng/ml and interassay and intraassay coefficients of variation were less than 10% and less than 5%, respectively. Insulin and leptin were assayed by RIA (Linco Research Inc., St. Charles, MO). Glucose was measured by the glucose oxidase method (Beckmann glucose analyzer II; Beckmann Inc., Palo Alto, CA). Plasma lipids were measured by enzymatic automated methods.

Statistical analysis

Results are expressed as mean \pm SEM or SD as indicated. After testing for normal distribution of variables, different groups were compared by ANOVA. Correlations among different variables were analyzed by linear regression analysis and multivariate analysis using the MDAS 2.0 (Medical Data Analysis System) software package (EsKay Software, Pittsburgh, PA). $P < 0.05$ was considered significant.

Results

Study 1: plasma visfatin/PBEF and expression of visfatin/PBEF mRNA in sc adipose tissue of lean and obese subjects

Biochemical and anthropometric characteristics of obese patients and control subjects are illustrated in Table 1. Plasma visfatin/PBEF was significantly lower in obese subjects, compared with normal-weight controls (Fig. 1A). Similarly, visfatin/PBEF mRNA expression in SAT was markedly lower in obese patients (Fig. 1B). No gender difference was found in plasma visfatin/PBEF in both lean (23.7 ± 3.6 and $24.8 \pm$

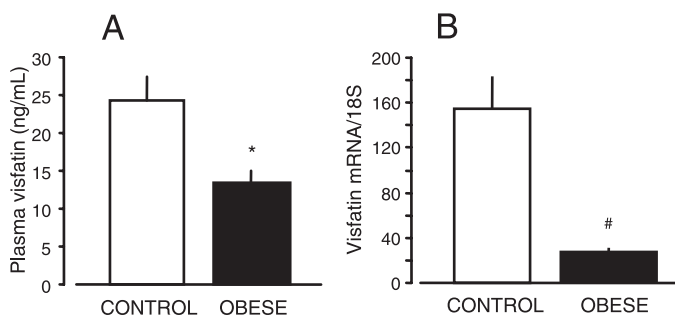


FIG. 1. Plasma visfatin (A) and visfatin mRNA expression in SAT (B) in obese and control subjects. *, $P = 0.003$ vs. control; #, $P < 0.001$ vs. control. Data represent mean \pm SEM.

5.2 ng/ml in females vs. males, $P = ns$) and obese (13.3 ± 1.4 and 13.7 ± 4.0 ng/ml in females vs. males, $P = ns$) subjects.

When correlation analysis was performed, a negative correlation was found between BMI and plasma visfatin/PBEF in obese patients only (Table 2). When other anthropometric, biochemical, and hormonal parameters were used to correlate with visfatin/PBEF, no correlation was found for any of them in either lean or obese subjects (Table 2).

To assess the contribution of SAT to circulating visfatin/PBEF, we performed a correlation analysis between visfatin/PBEF mRNA expression in SAT and plasma visfatin/PBEF and found a positive linear association ($r^2 = 0.32$, $P = 0.005$) (Fig. 2).

Finally, to clarify the relative contribution of BMI, waist circumference, and visfatin/PBEF in the determination of insulin resistance, a multiple correlation analysis was performed. In this statistical model, only waist circumference retained its significant correlation with HOMA, whereas both BMI and plasma visfatin/PBEF did not (Table 3).

Study 2: visfatin/PBEF mRNA expression in sc and visceral adipose tissue of lean and obese subjects

Visfatin/PBEF mRNA expression was measured in abdominal SAT and intraabdominal omental VAT samples. Visfatin/PBEF mRNA was similarly expressed in the SAT of lean and obese subjects, whereas it was significantly higher in VAT of obese compared with lean subjects (Fig. 3). When correlation analysis was performed between visfatin/PBEF mRNA expression in VAT and BMI, a positive correlation was found ($r^2 = 0.35$, $P < 0.05$).

Study 3: plasma visfatin/PBEF and expression of visfatin/PBEF mRNA in sc adipose tissue of lean subjects under FFA-induced insulin resistance

Increasing FFA by exogenous infusion of a lipid emulsion plus heparin determined a condition of insulin resistance. Insulin tolerance test after a 5-h infusion showed a clear reduction of K_{itt} , compared with saline infusion, confirming that insulin resistance had developed (Table 4). As shown in Table 4, elevation of FFA did not produce changes of either circulating visfatin/PBEF or visfatin/PBEF mRNA expression in gluteal SAT.

TABLE 2. Regression analysis between plasma visfatin concentration (dependent variable) and anthropometric and biochemical and hormonal parameters in lean and obese subjects

Independent variable	Lean		Obese	
	r	P	r	P
Age (yr)	-0.07	ns	0.18	ns
BMI (kg/m ²)	-0.03	ns	-0.37	0.02
Waist circumference (cm)	-0.14	ns	-0.23	ns
Fat mass (% of total body mass)	-0.04	ns	-0.03	ns
Fasting glucose (mmol/liter)	-0.06	ns	-0.05	ns
Fasting insulin (mIU/liter)	-0.04	ns	-0.14	ns
HOMA	-0.14	ns	-0.10	ns
Leptin (ng/ml)	0.10	ns	-0.04	ns

Statistical analysis was performed by linear regression with Bonferroni correction. r, Coefficient of regression; ns, not significant.

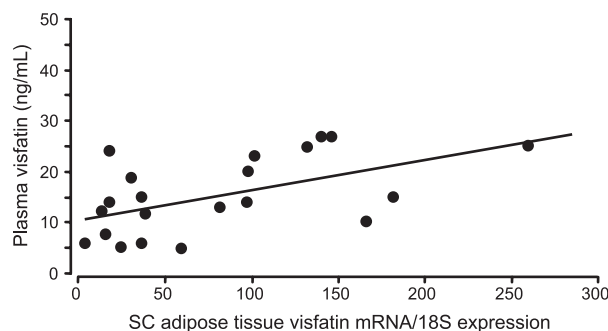


FIG. 2. Linear regression analysis between plasma visfatin and visfatin mRNA expression in SAT measured by quantitative RT-PCR. Plasma visfatin = $0.06 (\text{visfatin mRNA}) + 10.11$, $r^2 = 0.32$, $r = 0.57$, $P = 0.005$.

Discussion

Obesity and insulin resistance are core components of the metabolic syndrome. However, the precise mechanisms linking obesity to its complications (T2D, atherosclerosis, dyslipidemia, hypertension) have not been clearly elucidated. Recently Fukuhara *et al.* (4) identified visfatin/PBEF as a secreted protein expressed and regulated in adipose tissue with a predominant abundance in visceral fat depot. Moreover, an insulin-like action was reported both *in vivo* and *in vitro* due to a specific binding of visfatin/PBEF with the insulin receptor. It was consequently hypothesized that visfatin/PBEF could play a role in the regulation of insulin sensitivity in humans and possibly link obesity to its complications.

We studied plasma visfatin/PBEF concentration and mRNA expression in SAT and VAT in lean and obese subjects and during FFA-induced insulin resistance in healthy subjects. Our results essentially show that plasma visfatin/PBEF is reduced in obese subjects due to a lower expression in SAT. On correlation analysis, SAT was revealed to be a determinant of circulating visfatin/PBEF. A negative correlation was found between plasma visfatin/PBEF and BMI in obese subjects only. It should be emphasized that these associations were weak, and only 14% of variability of plasma visfatin/PBEF could be explained by BMI in obese patients. Moreover, visfatin/PBEF mRNA expression in SAT accounts for only about 30% of the variability of circulating visfatin/PBEF, thus suggesting a role for other sources of circulating visfatin/PBEF. In fact, visfatin/PBEF is expressed also in skeletal muscle, liver, and immune cells (5), and it is also likely that these tissues, whose function is altered in obesity, may be responsible for lower levels of circulating visfatin/PBEF in obesity.

We confirm the previous finding of no gender difference and the lack of association between visfatin/PBEF and in-

TABLE 3. Multivariate regression analysis between HOMA index (dependent variable) and plasma visfatin concentration, BMI, and waist (independent variables)

Independent variable	B	SE	P value
BMI (kg/m^2)	-0.0109	0.0353	ns
Visfatin (ng/ml)	-0.0003	0.0114	ns
Waist circumference (cm)	0.0583	0.0171	0.001

ns, Not significant.

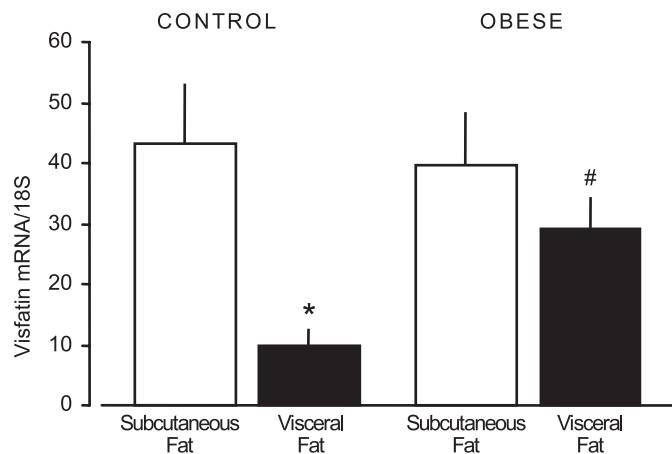


FIG. 3. Expression of visfatin mRNA in sc (white bars) and visceral (black bars) adipose tissue in lean and obese subjects. *, $P < 0.05$ vs. visceral adipose tissue; #, $P = 0.02$ vs. control. Data represent mean \pm SEM.

sulin resistance (8, 16). Our results are partly in contrast to previous reports in which plasma visfatin/PBEF and mRNA expression in SAT were positively correlated with adiposity (8), and a markedly higher visfatin/PBEF expression was present in visceral fat, compared with SAT (4). It is, however, possible that the different finding obtained by Fukuhara *et al.* (4) may be due to the limited observations (only two subjects) and the high variability of visfatin/PBEF expression in adipose tissue, which was recently reported (8).

The negative association we found in obese patients between visfatin/PBEF and adiposity is not in agreement with data previously reported by Berndt *et al.* (8). A possible explanation of this discrepancy could be the heterogeneity of subjects from whom biopsies were taken and maybe the different depots of SAT. In our study 1, biopsies were taken from the sc fat of the gluteal region, and a clear reduction of visfatin/PBEF mRNA was found in obese subjects with a negative correlation between visfatin/PBEF and BMI. In study 2, SAT was collected from the anterior abdominal wall, and in these samples we failed to find any correlation between SAT visfatin/PBEF expression and BMI (data not shown). Therefore, it is possible that SAT from different depots may behave differently in terms of visfatin/PBEF expression. Data reported by Berndt *et al.* (8) do not specify the precise site of adipose tissue samples; however, from the type of surgery reported, it is likely that their samples were taken from the anterior abdominal sc fat, and this may possibly explain the discrepancy. The different regulation of

TABLE 4. Metabolic parameters, insulin sensitivity measured by insulin tolerance test (K_{itt}), and plasma and gluteal SAT mRNA of visfatin measured in basal conditions and at the end of the lipid infusion in nine lean healthy subjects

	Saline (n = 9)	FFA (n = 9)	P value
Glucose (mmol/liter)	4.6 ± 0.3	4.3 ± 0.3	ns
Insulin (mIU/liter)	4.4 ± 2.4	3.7 ± 1.8	ns
FFA (mmol/liter)	371 ± 111	2983 ± 912	0.001
K_{itt} (%/min)	4.83 ± 0.78	3.12 ± 0.90	0.01
Plasma visfatin (ng/ml)	23.7 ± 13.2	19.2 ± 10.5	ns
Visfatin mRNA/18S	9.4 ± 5.1	11.1 ± 9.6	ns

Data are expressed as mean \pm SD. ns, Not significant.

visfatin/PBEF in different sc fat depots could possibly explain the discrepant results we obtained in studies 1 and 2 (reduced mRNA expression in obese group in study 1, no difference in study 2). Furthermore, it was reported that different mRNA species of visfatin/PBEF are present in tissue extracts (5), and two different isoforms of visfatin/PBEF (PBEF1 isoforms a and b) were submitted to the GenBank (accession no. U02020 and BC020691). Isoform a is considered the full-length transcript, whereas isoform b is shorter by three exons. In our experiments PCR primers were designed to amplify both isoforms, whereas data reported by Berndt *et al.* (8) in human adipose tissue were obtained with PCR primers, which amplified the full-length mRNA only. Therefore, amplification of different transcripts in adipose tissue may be a possible explanation for the different expression levels found in the different studies.

However, this does not explain the different correlation we found between plasma visfatin/PBEF and BMI. A possible reason could be the selection of patients. All obese patients from study 1 were otherwise healthy. All patients were free from diabetes and inflammatory, kidney, hepatic, neoplastic, and endocrine disorders and were homogeneous for age. This may explain the lower variability we found in plasma visfatin/PBEF and possibly the different relationships we found between visfatin/PBEF and BMI. Also ethnic factors could play a role. Moreover, it was recently reported that circulating visfatin/PBEF is increased in T2D patients (17, 18), and no association was reported between visfatin/PBEF and BMI, whereas only waist to hip ratio was found to be positively associated with visfatin/PBEF levels in T2D patients (17).

We did not find any change of plasma visfatin/PBEF and mRNA expression in SAT under lipid infusion in healthy subjects. Lipid infusion, by increasing circulating FFAs, largely increases the availability of lipid substrates, which are preferentially channeled to adipose fat stores driven by hyperinsulinemia and up-regulation of PPAR γ expression as previously reported by our laboratory (19, 20). Apart from channeling FFAs to triglyceride stores, high FFAs determine a condition of muscle and liver insulin resistance as previously reported both in humans and rats (19–21). Increased FFA disposal by adipose tissue up-regulates leptin expression and secretion and PPAR γ expression (19, 21). Therefore, we explored the possibility that, in this condition, visfatin/PBEF expression also might be altered as a consequence of increased triglyceride synthesis and PPAR γ expression. We did not find any change of both plasma visfatin/PBEF and its mRNA in adipose tissue. These findings failed to demonstrate a role for FFAs in the regulation of visfatin/PBEF expression in adipose tissue and do not support a role for visfatin/PBEF in FFA-induced insulin resistance. However, insulin resistance induced by FFAs in skeletal muscle is mainly based on substrate competition and intracellular biochemical pathways (glucose phosphorylation, regulation of pyruvate dehydrogenase complex, *etc.*). Therefore, it is not possible to exclude that visfatin/PBEF may exert a role in the development of insulin resistance in other conditions such as diabetes, glucocorticoid excess, *etc.*

Recently several studies reported a negative effect of IL-6 and TNF α on visfatin/PBEF expression in the murine adi-

pose cell line 3T3-L1 (6, 22). Because both IL-6 and TNF α are up-regulated in adipose tissue of obese subjects and both have autocrine/paracrine actions (23–26), it is possible to hypothesize that the reduced expression of visfatin/PBEF in adipose tissue of obese patients may be due to the inhibitory effect of these cytokines.

However, it should be pointed out that a limitation of this study was the cross-sectional design of the experiment, which suggests caution in the interpretation of the results. Dynamic and longitudinal studies are needed to get further insight in the regulation of visfatin/PBEF in obesity and its real physiological and pathophysiological relevance.

In conclusion, our study reported that both plasma visfatin/PBEF and expression in gluteal SAT are down-regulated in human obesity. However, higher expression of visfatin/PBEF mRNA was found in visceral fat of obese subjects, thus suggesting a divergent regulation of this adipokine in different fat depots. Finally, these data do not support a role for visfatin/PBEF in the development of insulin resistance. It will be of interest to follow future developments in our knowledge about the regulation of visfatin/PBEF and its possible implications in the pathophysiology of insulin resistance and T2D.

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