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The relationship of visfatin/pre–B-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase in adipose tissue with inflammation, insulin resistance, and plasma lipids

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Abstract

Visfatin/pre–B-cell colony-enhancing factor (PBEF)/nicotinamide phosphoribosyltransferase (Nampt) has been proposed as an insulin-mimicking adipocytokine predominantly secreted from visceral adipose tissue (VAT) and correlated with obesity. However, recent evidence challenged this proposal and instead suggested visfatin/PBEF/Nampt as a proinflammatory cytokine. The study aimed to examine whether visfatin/PBEF/Nampt was predominantly expressed in VAT and was correlated with obesity. The relationship of visfatin/PBEF/Nampt gene expression in adipose tissues with proinflammatory gene expression and metabolic phenotypes was also examined. The relative messenger RNA (mRNA) levels of visfatin/PBEF/Nampt, macrophage-specific marker CD68, and proinflammatory genes were measured in paired abdominal VAT and subcutaneous adipose tissues (SAT) and from 53 nondiabetic adults using quantitative real-time polymerase chain reaction. Fasting glucose, insulin, triglyceride, cholesterol, and uric acid levels were measured; and systemic insulin sensitivity was quantified with modified insulin suppression tests. There was no difference in visfatin/PBEF/Nampt mRNA levels between VAT and SAT, and neither was associated with measures of obesity. Visfatin/PBEF/Nampt mRNA levels were strongly correlated with proinflammatory gene expression including CD68 and tumor necrosis factor–α gene in both VAT and SAT. The VAT and SAT visfatin/PBEF/Nampt mRNA expressions were positively correlated with steady-state plasma glucose concentrations measured with modified insulin suppression tests, a direct measurement of systemic insulin resistance ($r = 0.42$, $P = .03$ and $r = 0.44$, $P = .03$, respectively). The VAT visfatin/PBEF/Nampt mRNA expression was also positively correlated with fasting triglyceride ($r = 0.42$, $P = .002$) and total cholesterol levels ($r = 0.37$, $P = .009$). Visfatin/PBEF/Nampt is not predominantly secreted from VAT and is not correlated with obesity. Our findings suggest that visfatin/PBEF/Nampt is a proinflammatory marker of adipose tissue associated with systemic insulin resistance and hyperlipidemia.

1. Introduction

Recent research in adipocyte biology has revealed that adipose tissue functions as an endocrine organ capable of producing and secreting a variety of factors including free fatty acids (FFAs), leptin, adiponectin, tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), and plasminogen activator inhibitor–1 [1]. These factors profoundly influence whole-body metabolic homeostasis and participate in the pathogenesis of insulin resistance and atherosclerosis [1]. Fukuhara et al [2] recently identified visfatin/pre–B-cell colony-enhancing factor (PBEF)/nicotinamide phosphoribosyltransferase (Nampt) as a novel adipokine predominantly expressed and released by visceral adipose tissue (VAT). Plasma visfatin/PBEF/Nampt concentration and gene expression in VAT correlated strongly with obesity [2]. Visfatin/PBEF/Nampt binds and activates insulin receptors, exerts insulin-mimetic effects, and lowers plasma glucose concentrations in mice [2]. Authors have claimed that visfatin/PBEF/Nampt is an adipocyte-derived cytokine secreted preferentially from VAT, correlated with obesity, and exerts insulin-mimicking effects in peripheral tissue.

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However, subsequent studies failed to confirm that visfatin/PBEF/Nampt was expressed predominantly in VAT [3-7]. Studies investigating the association of visfatin/PBEF/Nampt gene expression in adipose tissue with obesity also yielded inconsistent results [3-5,8]. Furthermore, methodological concerns have been raised about experiments demonstrating its insulin-mimicking action [9]. Instead, a growing body of evidence now suggests that visfatin/PBEF/Nampt is involved in inflammation and innate immunity [10-13]. Visfatin/PBEF/Nampt was found to be released predominantly from macrophages rather than from adipocytes in VAT [14]. The expression of visfatin/PBEF/Nampt was increased in the macrophages of unstable atherosclerotic plaques [10], in the synovial tissue of patients with rheumatoid arthritis [11], and in the neutrophils of septic patients [12]. Such conflicting data challenge the proposed role of visfatin/PBEF/Nampt and raise questions regarding the source of visfatin/PBEF/Nampt, its association with obesity, and its physiologic function.

This study aimed to examine whether visfatin/PBEF/Nampt is predominantly expressed in VAT and correlates with obesity. We next examined the association of visfatin/PBEF/Nampt gene expression with macrophage-specific marker CD68 [15] and proinflammatory gene expression in human adipose tissue. The relationships of visfatin/PBEF/Nampt gene expression in adipose tissues with glucose homeostasis and lipid metabolism were also investigated.

2. Methods and subjects

2.1. Subjects

Fifty-three nondiabetic Taiwanese subjects (44 women and 9 men) aged 19 to 59 years were recruited. Biopsies were performed on abdominal VAT and subcutaneous adipose tissue (SAT) in fasting state and during an elective abdominal operation either for benign uterine myoma or for bariatric surgery. All medications were discontinued before the study. Fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, and uric acid levels were analyzed by an automatic analyzer (Hitachi 7250 special; Hitachi, Tokyo, Japan). Serum insulin levels were determined by a microparticle enzyme immunoassay using AxSYM system from Abbott Diagnostics (Abbott Laboratories, Dainabot, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from the product of the fasting insulin concentration (in microunits per liter) and plasma glucose (in millimoles per liter) divided by 22.5 [16]. The homeostasis model assessment of β-cell function (HOMA-β) was calculated as 20 × fasting insulin (in microunits per milliliter)/[fasting plasma glucose (in millimoles per liter) − 3.5] [16]. Obesity was defined as body mass index (BMI) of at least 30 kg/m^2. Informed consent was obtained from each patient. This study was approved by the ethical board of the National Taiwan University Hospital.

2.2. Modified insulin suppression test

We quantified insulin sensitivity in a subset of 24 subjects (20 women and 4 men) from the 55 study subjects by modified insulin suppression tests [17]. In brief, after overnight fasting, a venous catheter was placed in each of the subjects' arms. One arm was used for the 180-minute infusion of octreotide (0.5 μg min^{-1} preceded by a 25-μg bolus), insulin (25 mU m^2 min^{-1}), and glucose (240 mg m^2 min^{-1}). Steady-state plasma glucose concentrations (SSPGs) were measured 150 to 180 minutes after the infusion. Steady-state plasma glucose concentration provides a direct measure of the ability of insulin to mediate disposal of infused glucose [17].

2.3. Adipose tissue RNA extraction and reverse transcription

Adipose tissue was immediately placed in liquid nitrogen after resection and stored at −80°C until processed. Total RNA was extracted using REzol (Promega, Madison, WI) according to the manufacturer's instructions. Reverse transcription was performed using a reverse transcription kit (Promega) with 1 μg of total RNA and 0.5 μg of random hexamers in a final volume of 25 μL containing 200 U of Maloney murine leukemia virus reverse transcriptase, 20 nmol/L of dNTP, and 25 U of rRNasin (Promega, Medison, WI) for 1 hour at 37°C. The reaction mixture was diluted to 100 μL with double-distilled water before polymerase chain reaction (PCR) amplification.

2.4. Measurement of messenger RNA levels by real-time PCR

A 5-μL sample of diluted complementary DNA was added to a mixture of 12.55 μL 2× TaqMan Master Mix Buffer and 1.25 μL 20× probe/primer assay mix containing the predeveloped gene-specific primers and probes in a final volume of 25 μL (Applied Biosystems, Foster City, CA). The primers and probes used were Hs00154355_m1 for CD68, Hs00237184_m1 for visfatin/PBEF/Nampt, Hs00174128_m1 for TNF-α, Hs00174097_m1 for interleukin-1β (IL-1β), Hs00174131_m1 for IL-6, and Hs0018168_m1 for β-actin (Applied Biosystems).

Real-time quantitative PCR was analyzed by the ABI PRISM 7000 Sequence Detection System (TaqMan, Perkin-Elmer Applied Biosystems). The fluorescent signal from each PCR reaction was collected as a peak-normalized value plotted against the cycle number. Reactions were characterized by comparing the *threshold cycle* (Ct) value, which is a unitless number defined as the cycle number at which the normalized sample fluorescence signal passes a fixed threshold above baseline. Samples with a high starting copy number of complementary DNA showed an increase in fluorescence earlier in the PCR process, resulting in a lower

Ct number. The comparative Ct method eliminating the need for standard curves was used and was calculated as the Ct value of the target gene minus the Ct value of β-actin. Relative gene expression in relation to β-actin was calculated using the formula $2^{-\Delta Ct}$.

2.5. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Data that were not distributed normally including BMI, waist circumference, fasting plasma triglyceride, glucose, insulin concentrations, HOMA-IR, HOMA-β, and the relative messenger RNA (mRNA) levels were logarithmically transformed to approximate normal distribution before analysis. Differences between VAT and SAT were assessed using paired *t* test. Pearson correlations were used to examine the correlations between gene expression and metabolic phenotypic variables. Statistical analyses were performed using STATA 10 (Stata, College Station, TX) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA). A null hypothesis was rejected if the *P* value was < .05.

3. Results

3.1. Difference in visfatin/PBEF/Nampt mRNA levels between VAT and SAT

The characteristics of study participants according to the obesity status and sex are summarized in Table 1. There was no difference in visfatin/PBEF/Nampt mRNA levels between VAT and SAT (*P* = .25, Fig. 1A). The VAT or SAT visfatin/PBEF/Nampt mRNA levels were not different between nonobese and obese subjects (*P* = .74 and .84, Fig. 1B). These results were similar in the female subgroup (data not shown). We found no difference in visfatin/PBEF/Nampt mRNA levels between male and female subjects in either VAT (*P* = .31) or SAT (*P* = .72) (Fig. 1C).

Table 1
Characteristics of the study participants stratified by obesity status and sex

Characteristics	Nonobese subjects	Obese subjects	
Age (y)	47.74 ± 5.96	27.2 ± 5.90	31.02 ± 8.31
n, sex (male/female)	15, female	9, male	29, female
BMI (kg/m^2)	23.42 ± 2.69	42.01 ± 10.6	38.89 ± 4.90
Waist circumference (cm)	91.5 ± 4.75	121.9 ± 13.16	114.8 ± 13.03
Triglyceride (mmol/L)	1.39 ± 1.98	2.65 ± 0.79	1.73 ± 0.88
Total cholesterol (mmol/L)	4.47 ± 1.49	5.09 ± 0.78	5.06 ± 0.75
LDL-C (mmol/L)	2.09 ± 0.33	2.77 ± 1.16	3.06 ± 0.69
HDL-C (mmol/L)	1.27 ± 0.28	1.03 ± 0.22	1.25 ± 0.34
Uric acid (μmol/L)	425.3 ± 197.7	532.0 ± 117.4	414.2 ± 86.11
Fasting insulin (pmol/L)	96.32 ± 99.70	236.3 ± 81.87	148.5 ± 102.7
Fasting glucose (mmol/L)	5.82 ± 1.44	6.09 ± 1.16	5.50 ± 0.90
HOMA-IR	3.27 ± 3.56	7.40 ± 2.89	5.05 ± 3.70
HOMA-β	141.1 ± 145.1	387.4 ± 93.73	235.2 ± 164.4
SSPG (mmol/L)	25.03	16.69 ± 1.85	15.71 ± 2.66
log VAT visfatin mRNA levels	−3.51 ± 2.66	−2.60 ± 1.94	−3.48 ± 2.32
log SAT visfatin mRNA levels	−2.67 ± 4.91	−3.11 ± 1.64	−2.93 ± 2.25

Data are presented as means± SD.

3.2. Correlations between visfatin/PBEF/Nampt and proinflammatory gene expression in human adipose tissue

Visfatin/PBEF/Nampt mRNA level was strongly correlated with macrophage-specific marker CD68 and TNF-α mRNA levels in both VAT and SAT (Fig. 2A-D). However, the correlation between visfatin/PBEF/Nampt and IL-6 mRNA levels was only marginally significant in VAT (*r* = 0.47, *P* = .05, Fig. 2E) and was not significant in SAT (*r* = 0.32, *P* = .20, Fig. 2F). The correlation between visfatin/PBEF/Nampt and IL-1β mRNA levels was also not significant in either VAT (*r*= 0.33, *P* = .20) or SAT (*r* = 0.15, *P* = .57). These results were similar in the female subgroup except for the insignificant correlation between visfatin/PBEF/Nampt and IL-6 gene expression in VAT (data not shown).

3.3. Correlations between visfatin/PBEF/Nampt mRNA levels in human adipose tissue and metabolic phenotypes

The correlations between visfatin/PBEF/Nampt mRNA level in human adipose tissue and metabolic phenotypes are summarized in Table 2. Both VAT and SAT visfatin/PBEF/Nampt mRNA levels were not correlated with measurements of obesity including BMI and waist circumference (Table 2). Visfatin/PBEF/Nampt mRNA levels were positively correlated with SSPG measured with modified insulin suppression test, a direct measurement of insulin resistance in both VAT (*r*= 0.42, *P* = .03, Table 2, Fig. 3A) and SAT (*r* = 0.44, *P* = .03, Table 2, Fig 3B). The VAT visfatin/PBEF/Nampt mRNA level was also positively associated with fasting plasma triglyceride (*r* = 0.42, *P* = .002, Table 2, Fig. 3C) and total cholesterol levels (*r* = 0.37, *P* = .009, Table 2, Fig. 3D). These results were similar in the female subgroup (Supplementary Table 1).

4. Discussion

Fukuhara et al [2] initially identified visfatin/PBEF/Nampt as a novel adipocytokine expressed at a much higher level in VAT than SAT and strongly correlated with obesity in humans. However, no difference in visfatin/PBEF/Nampt gene expression between SAT and VAT was found in this study, which is consistent with most previous reports [3-6]. Furthermore, visfatin/PBEF/Nampt expression was not correlated with measures of obesity in either SAT or VAT. Consistent with our findings, Berndt et al [4] found no association between visfatin/PBEF/Nampt expression and BMI in women. Only a weak correlation between VAT visfatin/PBEF/Nampt expression and BMI (r^2 = 0.09) was observed in men [4]. However, Pagano et al [3] reported an

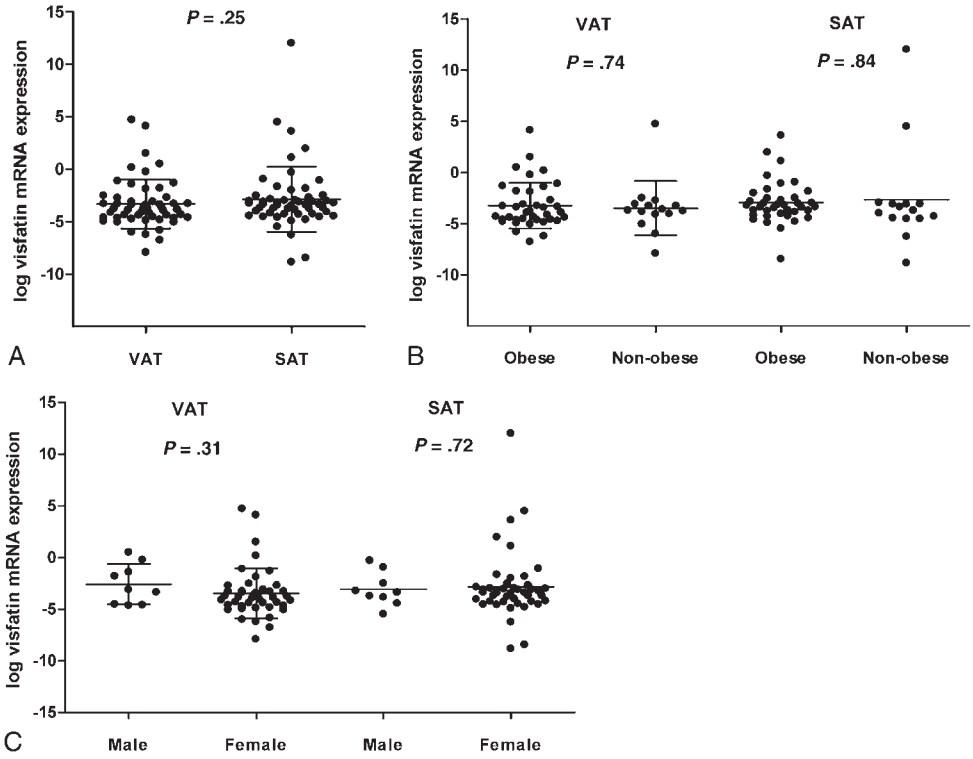

Fig. 1. A, Comparison of visfatin mRNA level between VAT and SAT in all subjects. B, Comparison of VAT and SAT visfatin mRNA level between nonobese and obese subjects. C, Comparison of VAT and SAT visfatin mRNA level between male and female subjects. Data are presented as means ± SD.

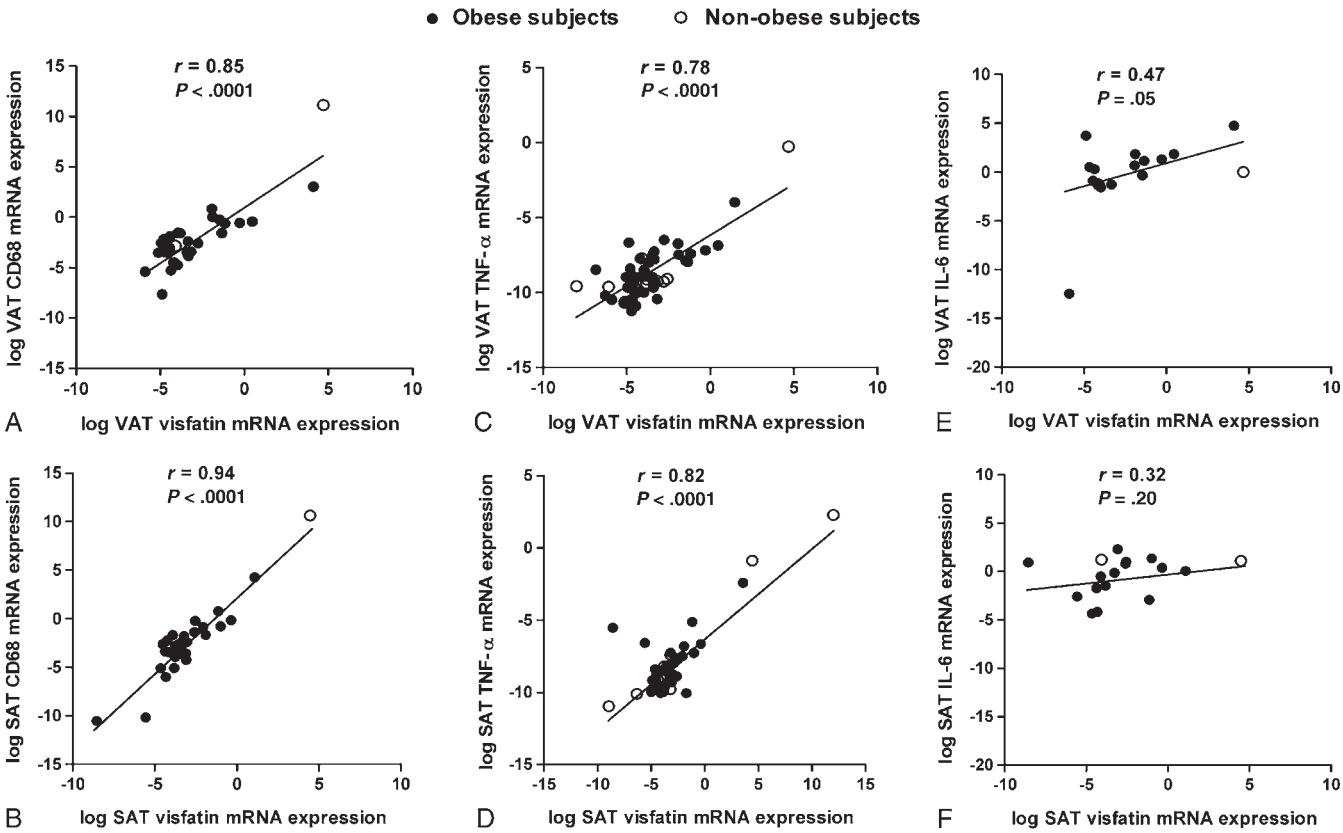

Fig. 2. Correlations between visfatin and CD 68 mRNA levels in (A) VAT and (B) SAT. Correlations between visfatin and TNF-α mRNA levels in (C) VAT and (D) SAT. Correlations between visfatin and IL-6 mRNA levels in (E) VAT and (F) SAT.

Table 2
Correlation of VAT and SAT visfatin mRNA expression with metabolic phenotypes

Metabolic phenotypes	n	VAT visfatin mRNA		SAT visfatin mRNA	
		R	*P*	*r*	*P*
Age	53	−0.14	.32	0.05	.75
BMI	53	0.09	.51	−0.06	.66
Waist circumference	36	−0.16	.34	−0.23	.17
Triglyceride	51	0.42	.002*	0.007	.96
Total cholesterol	51	0.37	.009*	0.18	.22
LDL-C	36	0.18	.31	0.13	.46
HDL-C	36	−0.31	.07	−0.15	.40
Uric acid	38	0.15	.38	0.14	.40
Fasting glucose	51	0.21	.14	0.15	.29
Fasting insulin	38	0.02	.89	0.32	.05
HOMA-IR	36	0.09	.59	0.37	.02*
HOMA-β	36	−0.15	.38	0.14	.42
SSPG	24	0.42	.03*	0.44	.03*

Correlation coefficients (*r*) and *P* values are shown. Data are presented as means ± SD.

* $P < .05$.

inverse relationship between gluteal SAT visfatin/PBEF/Nampt expression and BMI, no correlation between abdominal SAT visfatin/PBEF/Nampt expression and BMI, and a positive correlation between abdominal VAT visfatin/PBEF/Nampt expression and BMI. Varma et al [5] reported that VAT visfatin/PBEF/Nampt expression was positively associated with BMI, whereas SAT visfatin/PBEF/Nampt expression was negatively associated with BMI. These controversies might be partly explained by the relatively small study populations and heterogeneous sample sources. Taken together, current evidence does not support the notion that visfatin/PBEF/Nampt is predominantly expressed in VAT. The association of visfatin/PBEF/Nampt gene expression with obesity remains controversial.

A major finding of this study is the strong correlation between visfatin/PBEF/Nampt and macrophage-specific CD68 and TNF-α gene expressions in human adipose tissues. Supporting these findings, Varma et al [5] demonstrated that visfatin/PBEF/Nampt expression in the stromal vascular fraction was higher than that in the adipocyte fraction of adipose tissue. A recent work also demonstrated that visfatin/PBEF/Nampt was released predominantly from macrophages rather than from adipocytes in VATs [14]. Visfatin/PBEF/Nampt increased inflammatory gene (TNF-α, interleukin-8, IL-6) expression in monocytes [10,13]. Pharmacologic inhibition of visfatin/PBEF/Nampt reduced inflammatory cytokine secretion such as TNF-α, IL-6, and IL-1β both in vitro and in vivo [18]. These findings suggest that visfatin/PBEF/Nampt is a proinflammatory marker of resident macrophages in human adipose tissue.

We found significant association of visfatin/PBEF/Nampt mRNA levels in SAT and VAT with systemic insulin resistance. Consistent with our findings, circulating visfatin/

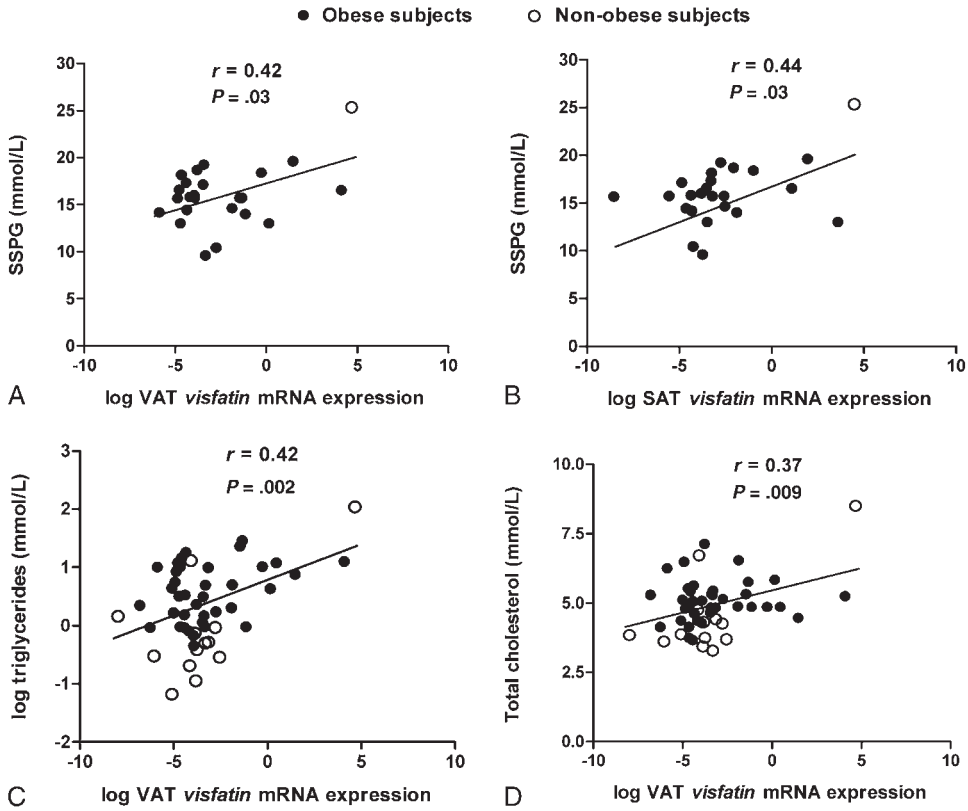

Fig. 3. Correlation between visfatin mRNA levels with SSPG during modified insulin suppression test in VAT (A) and SAT (B). Correlation between VAT visfatin mRNA levels with fasting triglyceride levels (C) and total cholesterol levels (D).

PBEF/Nampt levels were found to be elevated in patients with type 2 diabetes mellitus [19-21], women with polycystic ovary syndrome [22-24] or gestational diabetes [25], and patients with metabolic syndrome [26]. Insulin resistance has been recognized as a state of low-grade inflammation associated with macrophage infiltration in adipose tissue [27,28]. Therefore, the positive associations of visfatin/PBEF/Nampt with insulin resistance might be mediated through increased adipose tissue inflammation.

Furthermore, we found a positive association between VAT visfatin/PBEF/Nampt mRNA level and plasma triglyceride and total cholesterol levels. Consistent with this finding, circulating visfatin/PBEF/Nampt concentration was found to be positively associated with plasma triglyceride level in obese children [29] and young healthy men [30] and with LDL-C levels in patients with metabolic syndrome [26]. The molecular mechanism underlying these associations is currently not known. It is possible that increased visfatin/PBEF/Nampt expression induces adipose tissues inflammation, which increases lipolysis in adipose tissue and FFA flux to the liver. In the liver, increased FFA flux drives very low-density lipoprotein production, leading to elevated plasma triglyceride and total cholesterol. The reason why the association between visfatin/PBEF/Nampt mRNA level and elevated lipids levels was observed only in VAT is not clear. However, VAT has been demonstrated to be more lipolytically active than SAT; and the FFA released from VAT is directly delivered to the portal vein [31]. In epidemiologic studies, VAT was more strongly correlated with abnormal lipoprotein metabolism than SAT [32]. These data provide a possible explanation for the closer association of VAT visfatin/PBEF/Nampt mRNA levels with hyperlipidemia.

This study has several limitations. First, this is a cross-sectional observational study. Further functional studies are required to clarify the underlying mechanism. Second, the study population was recruited from patients undergoing an operation for benign uterine myoma or bariatric surgery and was composed of mainly obese subjects. Thus, the result could not be directly extrapolated to the general population. Third, we did not measure the concentrations of plasma visfatin/PBEF/Nampt or other proinflammatory cytokines; and their relationship with visfatin/PBEF/Nampt gene expression in adipose tissue and metabolic phenotypes could not be further explored. Lastly, we did not directly measure visfatin/PBEF/Nampt gene expression in the adipocellular and stromal vascular fractions of human adipose tissue. Therefore, direct evidence regarding the source of visfatin/PBEF/Nampt expression in adipose tissue was not available.

In summary, this study demonstrated that visfatin/PBEF/Nampt was not predominantly expressed in VAT and was not correlated with obesity. Visfatin/PBEF/Nampt gene expression was strongly and positively correlated with the expression of CD68, a macrophage-specific marker and proinflammatory gene expression in human adipose tissue. Visfatin/PBEF/Nampt gene expression in adipose tissue was associated with increased systemic insulin resistance and elevated plasma triglyceride and total cholesterol levels. These findings suggest visfatin/PBEF/Nampt as a proinflammatory marker of adipose tissue associated with insulin resistance and hyperlipidemia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.07.011.

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