Original Article

Plasma Visfatin Concentrations and Fat Depot–Specific mRNA Expression in Humans

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Visceral and subcutaneous adipose tissue display important metabolic differences that underlie the association of visceral obesity with obesity-related cardiovascular and metabolic alterations. Recently, visfatin was identified as an adipokine, which is predominantly secreted from visceral adipose tissue both in humans and mice. In this study, we examined whether visfatin plasma concentrations (using enzyme immunosorbent assay) and mRNA expression (using RT-PCR) in visceral and subcutaneous fat correlates with anthropometric and metabolic parameters in 189 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance. Visfatin plasma concentration correlates positively with the visceral visfatin mRNA expression (r^2 = 0.17, P < 0.0001), BMI (r^2 = 0.062, P = 0.004), percent body fat (r^2 = 0.048, P = 0.01), and negatively with subcutaneous visfatin mRNA expression (r^2 = 0.18, P < 0.0001). However, in a subgroup of 73 individuals, in which visceral fat mass was calculated from computed tomography scans, there was no correlation between plasma visfatin concentrations and visceral fat mass. We found no significant correlation between visfatin plasma concentrations and parameters of insulin sensitivity, including fasting insulin, fasting plasma glucose concentrations, and the glucose infusion rate during the steady state of an euglycemic-hyperinsulinemic clamp independent of percent body fat. Visfatin gene expression was not different between visceral and subcutaneous adipose tissue in the entire study group nor in selected subgroups. We found a significant correlation between visceral visfatin gene expression and BMI (r^2 = 0.06, P = 0.001) and percent body fat (measured using dual-energy X-ray absorptiometry) (r^2 = 0.044, P = 0.004), whereas no significant association between BMI or percent body fat and subcutaneous visfatin mRNA expression existed (both P >0.5). In conclusion, visfatin plasma concentrations and visceral visfatin mRNA expression correlated with measures of obesity but not with visceral fat mass or waist-to-hip ratio. In addition, we did not find differences in visfatin mRNA expression between visceral and subcutaneous adipose tissue in humans. *Diabetes* 54:2911–2916, 2005

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OGTT, oral glucose tolerance test; WHR, waist-to-hip ratio.

Epidemiological and animal studies reported an association between increased visceral obesity and the prevalence of insulin resistance, type 2 diabetes, and the risk of cardiovascular disease (rev. in 1–3). Moreover, differences in gene expression of adipocyte-secreted molecules suggest that there are intrinsic fat depot–specific differences in the endocrine function of adipose tissue. These differentially expressed adipokines include leptin (4,5), plasminogen activator inhibitor-1 (6), and interleukin-6 (7).

Recently, visfatin was identified as a peptide predominantly expressed in and secreted from visceral adipose tissue in both humans and mice (8). This peptide was previously described as a growth factor for early B-cells called pre–B-cell colony–enhancing factor (9). Visfatin activated the insulin receptor in various insulin-sensitive cell types in vitro and visfatin treatment of mice acutely lowered plasma glucose in vivo. Moreover, mice heterozygous for a loss-of-function mutation in the visfatin gene had higher plasma glucose levels compared with wild-type littermates. In humans, visceral fat mass estimated by computed tomography was strongly correlated with plasma visfatin levels, whereas only a weak relationship with subcutaneous fat was observed (8). Taken together, these findings suggested that visfatin could play a role in the association between visceral obesity and increased metabolic risk.

Visfatin was originally detected by a differential display method using paired samples of visceral and subcutaneous fat from only two female volunteers (8). If visfatin is a marker of visceral obesity, a correlation between visfatin plasma concentrations and/or visfatin gene expression in visceral adipose tissue and alterations associated with the metabolic syndrome should exist. We therefore studied plasma visfatin concentrations together with visfatin gene expression in adipose tissue using quantitative real-time RT-PCR in paired samples of visceral and subcutaneous adipose tissue from 189 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance. We used correlational analysis to dissect whether and how variability of these parameters are explained by the variability in visfatin plasma concentration and gene expression in different fat depots.

RESEARCH DESIGN AND METHODS

Paired samples of visceral and subcutaneous adipose tissue were obtained from 189 Caucasian men (n = 95) and women (n = 94) who underwent open abdominal surgery for gastric banding, cholecystectomy, appendectomy, weight reduction surgery, abdominal injuries, or explorative laparotomy. In

163 of these 189 subjects, fasting blood samples were taken before the operation. The age ranged from 24 to 86 years and BMI from 20.8 to 54.1 kg/m^2. A total of 36 subjects had type 2 diabetes, and 31 subjects had impaired glucose tolerance. All subjects had a stable weight with no fluctuations of >2% of the body weight for at least 3 months before surgery. Patients with severe conditions including generalized inflammation or end-stage malignant diseases were excluded from the study. Samples of visceral and subcutaneous adipose tissue were immediately frozen in liquid nitrogen after explantation. The study was approved by the ethics committee of the University of Leipzig. All subjects gave written informed consent before taking part in the study.

Assays. Plasma visfatin concentrations were measured in triplicate with a human Visfatin (COOH-terminal) enzyme immunometric assay (Phoenix, Belmont, CA). Basal, fasting blood samples were taken after an overnight fast to determine glucose, insulin, and standard laboratory parameters. Plasma insulin was measured with a two-site chemiluminescent enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products, Los Angeles, CA).

Measures of body fat content and oral glucose tolerance test. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Waist and hip circumferences were measured and waist-to-hip ratio (WHR) was calculated. Percentage body fat was measured by dual-energy X-ray absorptiometry. In addition, in a subgroup of 73 subjects (33 male and 40 female), visceral fat area and the relative ratio of intra-abdominal visceral fat to the subcutaneous fat area using computed tomography scans at the level of L4-L5 was calculated as previously described (10). The oral glucose tolerance test (OGTT) was performed according to the criteria of the American Diabetes Association (11). Three days before the OGTT, the patients documented a high-carbohydrate diet. The OGTT was performed after an overnight fast with 75 g standardized glucose solution (Glucodex Solution 75 g; Merieux, Montreal, Canada). Venous blood samples were taken at 0, 60, and 120 min for measurements of plasma glucose concentrations.

Euglycemic-hyperinsulinemic glucose clamp. Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method (12). The cutoff for insulin resistance was arbitrarily chosen from the euglycemic-hyperinsulinemic clamp results of >120 individuals, which share the same population background and underwent the same clamp conditions as previously reported (13).

Analysis of human visfatin gene expression. Human visfatin gene expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler using the TaqMan assay, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated from paired subcutaneous and visceral adipose tissue samples using TRIzol (Life Technologies, Grand Island, NY), and 1 μg RNA was reverse transcribed with standard reagents (Life Technologies). From each RT-PCR, 2 μl was amplified in a 26-μl PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7000 sequence detector for an initial denaturation at 95°C for 10 min, followed by 40 PCR cycles, each cycle consisting of 95°C for 15 s, 60°C for 1 min, and 72°C for 1 min. The following primers were used: human Visfatin (accession no. NM_005746) 5′gacgccagcagggaattttgttac 3′ (sense) and 5′agctttttgtcaccttgc cattct 3′ (antisense) and human 36B4 (accession no. NM_001002) 5′aacat gctcaacatctcccc3′ (sense) and 5′ccgactcctccgactcttc 3′ (antisense). SYBR Green I fluorescence emissions were monitored after each cycle. Expression of human visfatin and human 36B4 mRNA were quantified by using the second derivative maximum method of the TaqMan Software (Applied Biosystems) determining the crossing points of individual samples by an algorithm that identifies the first turning point of the fluorescence curve. Human visfatin mRNA expression was calculated relative to 36B4, which was used as an internal control due to its resistance to hormonal regulation (14). Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

Statistical analysis. Data are shown as means ± SD unless stated otherwise. Before statistical analysis, non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Expression differences between visceral and subcutaneous adipose tissue were assessed using the paired Student's t test. Linear relationships were assessed by least square regression analysis. Multivariate linear relationships were assessed by a general linear model. Statistical software from the SAS Institute (Cary, NC) was used. P values <0.05 were considered to be statistically significant. Separate analyses were performed in subgroups for sex, BMI (BMI <25; n = 48, BMI 25–29.9; n = 52; BMI >30; n = 89), and WHR (<0.7 or >1.0).

TABLE 1
Linear regression analysis of visfatin plasma concentration with anthropometric and metabolic parameters

	All	Male	Female
n	163	83	80
Age	0.01	0.02	0.0
BMI	0.04*	0.11†	0.0
Body fat (%)	0.04*	0.11†	0.0
Waist	0.02	0.09†	0.0
WHR	0.01	0.01	0.01
Glucose infusion rate	0.0	0.0	0.0

Data are r^2. *$P < 0.05$; †$P < 0.01$.

RESULTS

Plasma visfatin concentrations. In the entire study population, plasma visfatin concentrations were not different between men (14.2 ± 2.1 ng/ml) and women (15.4 ± 1.7 ng/ml). There was a significant correlation between plasma visfatin concentrations, BMI, and percent body fat (Table 1), which was essentially accounted for by men (Fig. 1). We did not find a correlation between plasma visfatin concentration, age, and WHR (Table 1).

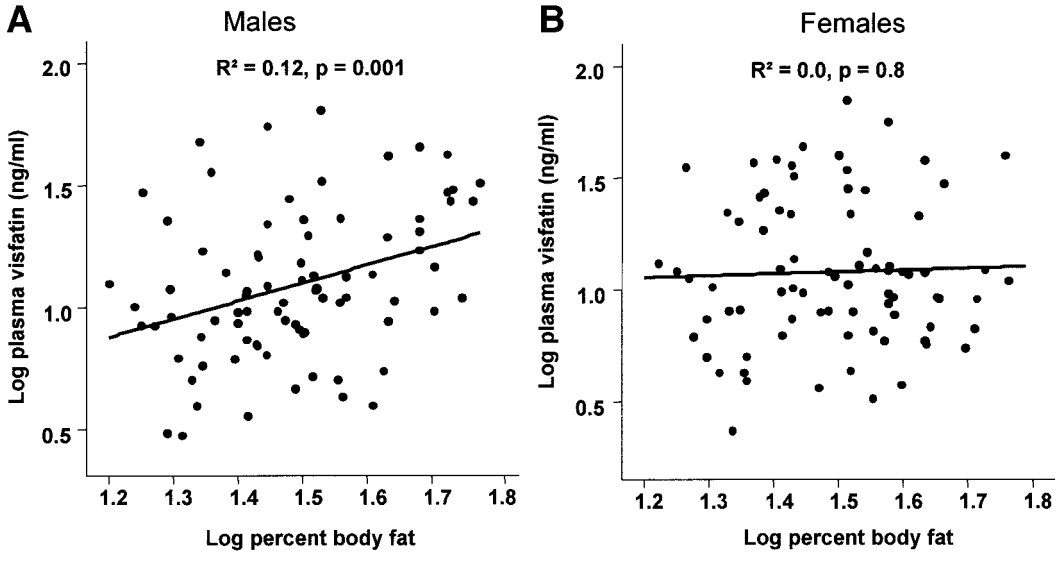

FIG. 1. Correlation between plasma visfatin concentrations and % body fat in men (*A*) and women (*B*). Data were log transformed to achieve normal distribution.

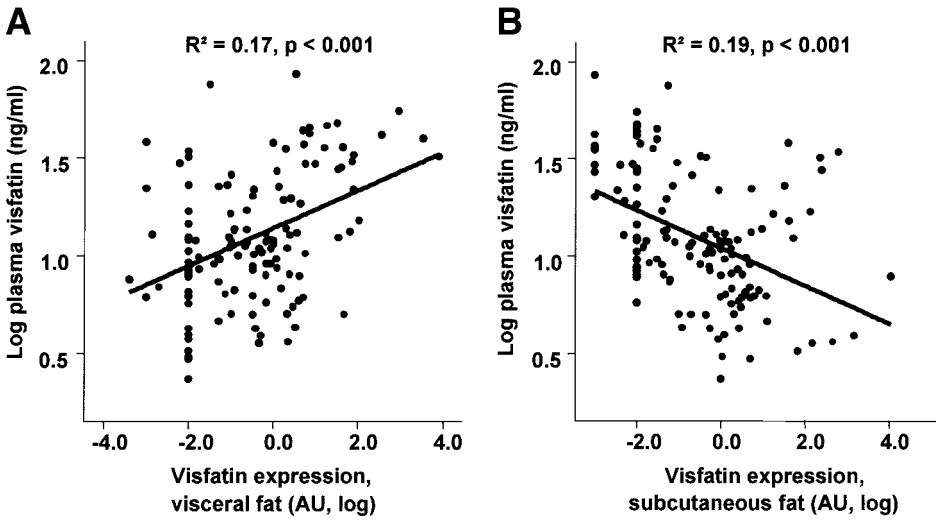

FIG. 2. Correlation between plasma visfatin concentration and visfatin mRNA expression in visceral (*A*) or subcutaneous (*B*) fat depots (n = 163). Data were log transformed to achieve normal distribution.

Visceral and subcutaneous visfatin mRNA expression. Plasma visfatin concentration was significantly positively correlated with visfatin mRNA expression in visceral fat, whereas a significantly negative correlation was found between circulating visfatin and subcutaneous visfatin mRNA expression (Fig. 2).

In the entire study population, visfatin gene expression was not different between subcutaneous and visceral adipose tissue (Fig. 3*A*). Since our entire study population is very heterogeneous, we selected two subgroups of lean (BMI <25 kg/m^2) men (n = 21) and women (n = 27) with normal glucose tolerance. As clearly shown in Fig. 3*B*, visfatin mRNA expression was not different between visceral and subcutaneous adipose tissue even in these homogeneous subgroups. In the entire study population, no significant correlation between the subcutaneous and visceral visfatin gene expression was detected (r^2 = 0.001, P = 0.056).

There was no difference either in subcutaneous or visceral visfatin gene expression between men and women (data not shown). Results of univariate regression analysis in the entire study population and male and female subgroups are shown in Table 2. Parameters of obesity (BMI, percent body fat, and waist circumference) were significantly correlated with visceral but not with subcutaneous visfatin gene expression. However, this relationship was only present in women (Table 2). There was no correlation between visfatin gene expression and age, WHR, and fasting plasma glucose (Table 2). To express visfatin expression in visceral relative to that in subcutaneous fat, we calculated the visceral-to-subcutaneous visfatin mRNA ratio. Again, BMI (r^2 = 0.04, P = 0.009), percent body fat (r^2 = 0.03, P = 0.02), and waist circumference (r^2 = 0.02, P = 0.049) were significantly correlated with the log visceral-to-subcutaneous visfatin mRNA ratio, while the WHR was not (Fig. 4).

Plasma visfatin concentrations and visceral fat mass. To determine visceral intra-abdominal and subcutaneous abdominal fat areas, computed tomography scans from 73 individuals (33 males, 40 females) were evaluated at the level of L4-L5 with an attenuation range of −30 to −190 Hounsfield units. In addition to the visceral fat area, the ratio of intra-abdominal visceral fat to the subcutaneous fat area was calculated as previously described (10). In this subgroup, plasma visfatin concentrations and visceral visfatin mRNA expression were also correlated (r^2 = 0.26, P = 0.0001). However, we did not find a correlation between visfatin plasma concentrations and visceral fat area or the relative ratio of visceral-to-subcutaneous fat (Fig. 5). Moreover, no correlation was found between visceral visfatin mRNA expression and visceral fat area (r^2 = 0.0, P = 0.31).

Plasma visfatin concentrations and expression levels and insulin sensitivity. There was no significant correlation between plasma visfatin and fasting plasma insulin concentrations (Fig. 6*A*). There was also no correlation between plasma visfatin concentrations and glucose infusion rate during steady state of a euglycemic-hyperinsulinemic clamp (Table 1), fasting plasma glucose concen-

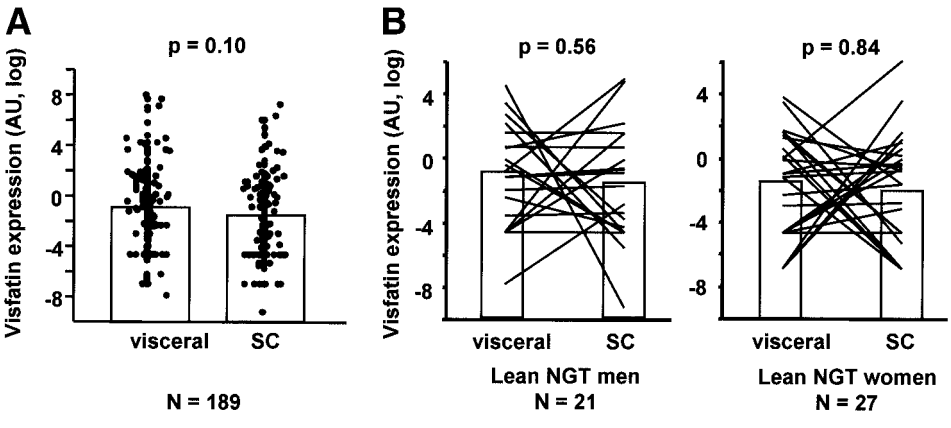

FIG. 3. Visfatin mRNA expression in human visceral and subcutaneous adipose tissue. Data were log transformed to achieve normal distribution (1 AU = 1ag/100 ng total RNA). *A*: Total RNA of paired samples from visceral and subcutaneous (SC) adipose tissue obtained from 189 Caucasian men (n = 95) and women (n = 94) was subjected to real-time RT-PCR. Mean visfatin mRNA expression was not significantly different between SC and visceral adipose tissue (P = 0.1). *B*: Visfatin mRNA expression in two homogeneous subgroups of lean (BMI <25 kg/m^2) men (n = 21) and women (n = 27) with normal glucose tolerance (NGT).

TABLE 2
Linear regression analysis of visceral and subcutaneous visfatin gene expression with anthropometric and metabolic parameters

	All (n = 189)		Male (n = 95)		Female (n = 94)	
	Subcutaneous	Visceral	Subcutaneous	Visceral	Subcutaneous	Visceral
Age	0	0.001	0.03	0.004	0	0.02
BMI	0.002	0.06*	0.02	0.02	0.002	0.09†
Body fat (%)	0.001	0.04†	0.006	0.03	0	0.06‡
Waist	0	0.03*	0.004	0.03	0	0.04‡
WHR	0	0.01	0	0.02	0.002	0.02
Glucose infusion rate	0.001	0.02	0.002	0.03	0.001	0.01

Data are r^2. *$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$.

tration (Fig. 6*B*), and the 2-h OGTT glucose concentration ($r^2 = 0.06$, $P = 0.53$).

There was a significant correlation between visceral visfatin expression and fasting plasma insulin concentrations ($r^2 = 0.02$, $P = 0.05$), but not fasting or 2-h OGTT (both $r^2 = 0, P > 0.5$) plasma glucose concentrations ($r^2 = 0.01$, $P = 0.2$). The correlation between visceral visfatin expression and fasting plasma insulin concentrations became nonsignificant upon adjusting for BMI ($P = 0.7$). There was no significant correlation between subcutaneous visfatin gene expression and fasting plasma insulin ($r^2 = 0$, $P = 0.9$) or fasting ($r^2 = 0$, $P = 0.9$) or 2-h OGTT plasma glucose ($r^2 = 0$, $P = 0.5$) concentrations. There was also no correlation between either visceral ($r^2 = 0.01$, $P = 0.3$) or subcutaneous ($r^2 = 0.03$, $P = 0.8$) visfatin mRNA expression and the glucose infusion rate during steady state of a euglycemic-hyperinsulinemic clamp (Table 2).

Subgroup analyses. To identify possible relationships pertaining only to specific subgroups, we performed a series of subanalyses for BMI (BMI <25, $n = 52$; BMI 25–29.9, $n = 54$; BMI >30, $n = 91$), WHR (<0.7 or >1.0), and type 2 diabetes. Neither in the BMI nor the WHR subgroups did we detect statistical relationships beyond those reported for the entire study population. Taking into account presence or absence of type 2 diabetes did not have any effect on the results.

DISCUSSION

Fukuhara et al. (8) identified visfatin as a peptide predominantly expressed in and secreted from visceral adipose tissue and demonstrated in 101 male and female human subjects a correlation between plasma visfatin concentrations and the amount of visceral fat.

We studied plasma visfatin concentrations in 163 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance. We found a significant correlation between visfatin plasma concentrations and measures of obesity, i.e., BMI and body fat content, but not with waist circumference or WHR. Moreover, we found a significant positive relationship between BMI, percent body fat, and waist circumference and visceral visfatin expression. No similar relationship existed for subcutaneous visfatin gene expression. These findings are at least in part compatible with the hypothesis that obesity is associated with increased visceral visfatin expression. It is unclear whether visceral visfatin gene expression is upregulated directly in response to obesity or whether this relationship reflects an epiphenomenon of some obesity-related mechanism, e.g., hyperinsulinemia, hyperleptinemia, or others. The between-sex heterogeneity is notable but cannot easily be explained. Hormonal regulation of visfatin plasma concentrations or gene expression have not been investigated.

It was suggested that WHR and waist circumference are useful variables for the crude assessment of visceral fat accumulation, since 90% of the variation in waist girth could be explained by differences in total body fatness and in visceral adipose tissue accumulation both in men and women (3,15). The lack of an association between WHR or waist circumference and plasma visfatin concentrations in our study suggests that circulating plasma visfatin is not associated with visceral fat accumulation but might be related to increased total fat mass. Therefore, we determined abdominal subcutaneous and visceral fat area using computed tomography scans between the fourth and fifth lumbar vertebrae in a subgroup of 73 individuals. In contrast to the previous report (8), we did not find a

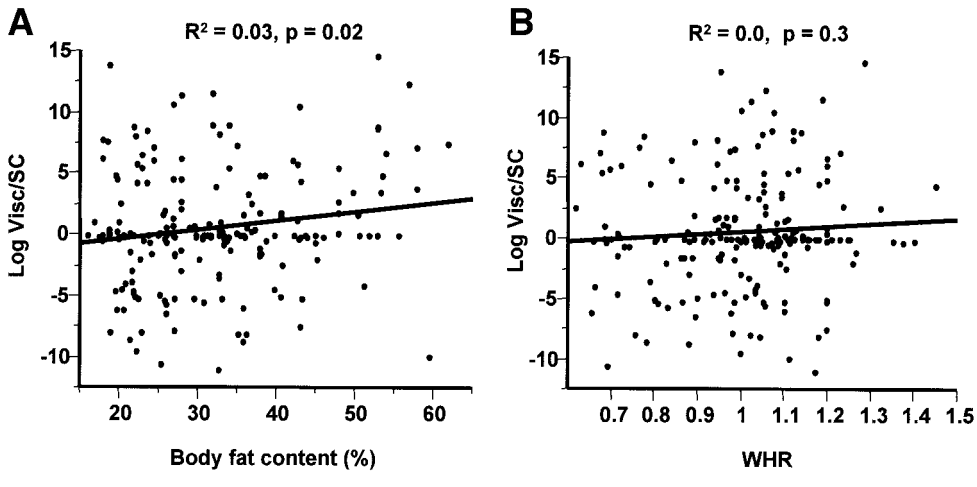

FIG. 4. Correlation of the ratio of visceral/subcutaneous (SC) visfatin mRNA expression with body fat content estimated from dual-energy X-ray absorptiometry scan analysis (*A*), but not with WHR in paired samples from visceral and SC adipose tissue (*B*) (n = 189). Data were log transformed to achieve normal distribution.

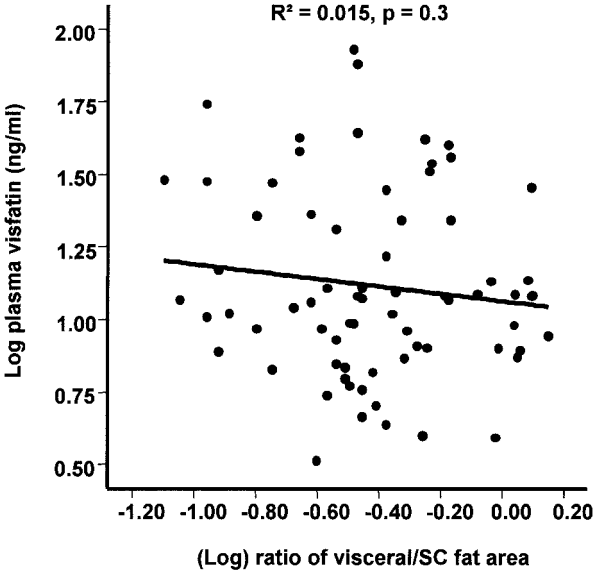

FIG. 5. Correlation between plasma visfatin concentrations and the ratio of intra-abdominal visceral fat to the subcutaneous (SC) fat area (n = 73). Data were log transformed to achieve normal distribution.

correlation between visfatin plasma concentrations and abdominal visceral fat area, despite the presence of the correlation between visceral visfatin mRNA expression and plasma visfatin concentrations.

We found a correlation of plasma visfatin concentrations with visceral visfatin mRNA expression. Surprisingly, there was a significant negative correlation between plasma visfatin levels and subcutaneous visfatin expression. This phenomenon could be the result of a reciprocal regulatory mechanism of subcutaneous visfatin gene expression by increased plasma visfatin concentration.

In contrast to the previous report (8), we were not only unable to reproduce the visceral predominance of visfatin expression, but could not even detect any statistical difference, neither in the whole study group nor in any of the subgroups we had analyzed. Figure 3 shows that visfatin expression in visceral fat relative to that in subcutaneous fat varies greatly among individuals. For example, in our lean, nondiabetic female subgroup there are seven subjects in whom subcutaneous visfatin expression is about four orders of magnitudes lower than visceral visfatin expression. However, there are just as many subjects in whom visceral visfatin expression is several orders of magnitudes lower than subcutaneous visfatin expression. Thus, it is possible that Fukuhara et al. (8) had coincidentally studied two women with very low or practically absent subcutaneous visfatin expression. Another potential explanation for these different results may be that ethnic differences exist between the originally examined Japanese population and our Caucasian study group.

Visfatin was reported to have insulin-like activity and to bind to the insulin receptor, thereby lowering blood glucose levels (8,16). Like insulin, visfatin was shown to stimulate glucose uptake into adipocytes and muscle cells and to suppress glucose release from hepatocytes in vitro (8). Moreover, Fukuhara et al. (8) showed that visfatin induced phosporylation of signal transduction proteins downstream of the insulin receptor. If insulin-like activity of visfatin was of physiological importance, and given the observation that visfatin binds to the insulin receptor without competing with insulin, one could speculate that visfatin plasma concentrations and visceral visfatin gene expression correlate with plasma parameters of glucose metabolism and insulin sensitivity. In our study population, we were unable to find a relationship between visfatin plasma concentrations and parameters of insulin sensitivity or glucose homeostasis including fasting plasma insulin and glucose concentrations, whole-body glucose uptake during the steady state of an euglycemic-hyperinsulinemic clamp, and 2-h OGTT plasma glucose. However, visceral visfatin expression correlated with fasting insulin concentrations, but this was a simple function of obesity and disappeared upon adjustment for BMI. Moreover, we did not find an association between visceral visfatin mRNA expression and the glucose infusion rate during a euglycemic-hyperinsulinemic clamp. Compatible with this lack of any association between visceral visfatin mRNA expression and parameters of insulin sensitivity, we also did not find a correlation with plasma glucose concentrations. Thus, no obvious effect of circulating visfatin on insulin sensitivity exists. Elucidation of any such effect in humans will clearly require more sophisticated methods and study designs.

In conclusion, visfatin plasma concentrations were positively correlated with visceral and negatively correlated with subcutaneous visfatin mRNA expression. Plasma visfatin concentrations and visceral visfatin mRNA expres-

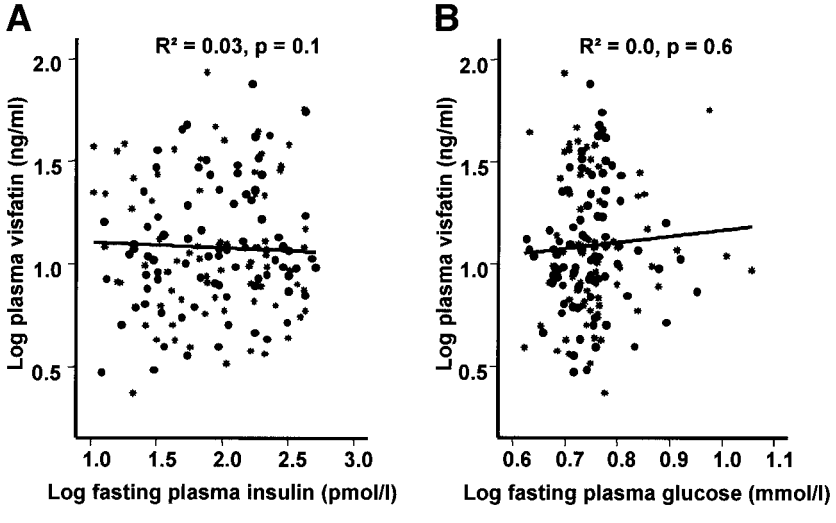

FIG. 6. Correlation between plasma visfatin concentration, fasting plasma insulin concentrations (A), and fasting plasma glucose concentrations (B) (n = 163). Data were log transformed to achieve normal distribution.

sion correlated with measures of obesity, but not with visceral fat mass or WHR. In addition, we did not find differences in visfatin mRNA expression between visceral and subcutaneous adipose tissue in humans.

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