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Relationship between vaspin gene expression and abdominal fat distribution of Korean women

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Abstract. Visceral adipose tissue-derived serpin (vaspin) is a novel adipokine that is thought to have insulin-sensitizing effects. We investigated vaspin mRNA expression in abdominal adipose tissue and examined how gene expression related to abdominal fat distribution and metabolic parameters in Korean women. We measured anthropometric variables, metabolic parameters, serum vaspin concentration, and vaspin mRNA expression in abdominal adipose tissue obtained from women who underwent abdominal gynecological surgery and were aged 18–67 years (n = 85). Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) area were measured in 40 subjects using computed tomography (CT). Vaspin expression was analyzed by real-time quantitative RT-PCR according to abdominal fat distribution. Vaspin mRNA expression was greater in adipocytes than in stroma/vascular cells. In the total subjects, vaspin expression was significantly higher in SAT than in VAT. Vaspin expression in SAT in subcutaneous fat type (VSR $\leq$ 0.3) was significantly higher than in visceral fat type (VSR > 0.3), although vaspin expression in VAT was similar between subcutaneous and visceral fat type. There was a significant negative correlation between vaspin expression in SAT and VAT area (r = –0.55, p = 0.001). Serum vaspin concentration was significantly correlated with fasting insulin (r = 0.30, p = 0.02), HOMA-IR (r = 0.29, p = 0.02), and the ratio of vaspin expression in VAT to vaspin expression in SAT (r = 0.41, p = 0.04). Vaspin expression in abdominal adipose tissue was adipocyte-specific and vaspin expression in SAT decreased as VAT area increased.

Key words: Vaspin expression, Vaspin concentration, Abdominal adipose tissue, Insulin resistance

ADIPOSE tissues secrete various bioactive peptides that are thought to play important roles in insulin action, energy metabolism, inflammation, and cell growth by acting *via* endocrine, paracrine, or autocrine mechanisms [1, 2]. Some adipokines may regulate local fat accumulation by modulating the growth and proliferation of adipocytes [3]. On the other hand, excess fat accumulation may induce the dysregulation of adipocyte function (e.g., *via* the oversecretion or hyposecretion of adipokines) [4]. Altered adipokine secretion may, in turn, contribute to obesity and the development of related diseases [4].

Visceral adipose tissue-derived serpin (vaspin) is a novel adipokine that was originally isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats [5, 6], an animal model of type 2 diabetes [7]. Vaspin mRNA was highly expressed in the visceral adipose tissues (VAT) of 30-week-old OLEFT rats and was barely detected in subcutaneous adipose tissue (SAT) [7]. Recombinant human vaspin improved glucose tolerance and insulin sensitivity in obese, insulin-resistant mice, and reversed the altered expression of genes relevant to insulin resistance in white adipose tissue [5]. These observations indicate that vaspin might have an insulin-sensitizing effect,

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mainly on white adipose tissue. The increased serum concentrations of vaspin are associated with obesity and impaired insulin sensitivity [8]. Vaspin is also expressed in human adipose tissues. Expression of this gene is higher in obese than non-obese subjects and is more commonly detected in patients with type 2 diabetes [9]. Vaspin mRNA is significantly more highly expressed in the omental adipose tissues of women with polycystic ovarian syndrome than in control subjects [10]. Induction of human vaspin mRNA expression in adipose tissues is regulated in a fat depot-specific manner and human vaspin mRNA has been detected in both visceral and subcutaneous white adipose tissues [9].

Few studies have examined vaspin expression in abdominal adipose tissue according to abdominal fat type. Furthermore, there is limited information about vaspin expression in Asian individuals, who experience more metabolic complications at a given body mass index than do Caucasians [11]. Therefore, we investigated vaspin mRNA expression in abdominal adipose tissue obtained from Korean women. In addition, we analyzed how gene expression related to abdominal fat distribution and various metabolic parameters.

Materials and Methods

Study subjects

The study population included 85 female subjects who ranged in age from 18 years to 67 years, and who underwent elective abdominal surgery for benign diseases (uterine myoma, adenomyosis, endometriosis, and cystadenoma) in the Department of Gynecology at Asan Medical Center (Seoul, Korea) from January 2009 to September 2009. Written informed consent was obtained from each study participant upon enrollment. We measured anthropometric and biochemical variables in the subjects, as well as serum vaspin concentrations. Samples of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were removed during surgery. Abdominal fat computerized tomography (CT) was performed on 40 subjects who agreed to undertake fat CT to assess the distribution of abdominal fat 1 day prior to the surgery. We excluded individuals with secondary causes of obesity, as well as pregnant or lactating women, those with polycystic ovarian syndrome and diabetes, and subjects with evidence of malignancy and severe hepatic or renal diseases. Subjects taking medications that might affect weight or glucose metabolism (e.g., anti-obesity drugs, oral hypoglycemic agents, insulin, hormones) were also eliminated from the study. The study protocol was approved by the institutional review board of Asan Medical Center. We certify that all applicable institutional regulations regarding the ethical use of human volunteers were followed during this research.

Blood pressure and anthropometric measurements

Blood pressure was measured in the morning. Each patient was seated in a quiet room for 10 min; after this resting period, blood pressure was measured using a standard mercury sphygmomanometer on the patient's arm. Anthropometric measurements were taken while the subjects were dressed in light clothing, but without shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured using an automatic height-weight scale. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of the height (in meters).

Estimation of abdominal fat distribution

The distribution of abdominal fat was assessed by computed tomography (CT) on a Siemens Somatom Scanner (Erlangen, Germany), as previously described [12, 13]. Subjects were placed in a supine position. A cross-sectional scan of 10 mm thickness, centered at the L4-L5 vertebral disc space, was obtained using a skeletal radiograph to establish the position of the scans to the nearest millimeter. The area of total adipose tissue (TAT) in the abdomen was measured by computation of the adipose tissue area using an attenuation range of -190 to 30 Hounsfield units (Syngo, Siemens, Erlangen, Germany). The area of VAT was measured within the muscle wall surrounding the abdominal cavity, and the area of SAT was calculated by subtracting the VAT area from the TAT area. In addition, the ratio of the VAT area to the SAT area (VSR) was calculated.

Measurements of metabolic variables

Plasma glucose concentration was measured using the glucose oxidase method, and insulin concentration was measured using a human insulin radioimmunoassay kit (TFB, Tokyo, Japan). The homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated according to the following formula, as previously described: fasting serum insulin (μU/mL) x fasting serum glucose (mg/dL) ÷ 405 [14]. Concentrations of serum adiponectin (R&D systems, Abingdon, Oxfordshire, UK) and vaspin (Adipogen,

Seoul, Korea) were measured using an enzyme-linked immunosorbent assay kit, according to the manufacturer's protocol.

Adipose tissue sampling

During abdominal gynecological surgery, 1–3 g of SAT and VAT were removed. The SAT was obtained from the site of surgical incision (i.e., the lower abdomen), and the VAT was removed from the distal portion of the greater omentum (i.e., the epiploon). The collected samples were immediately carried to the laboratory in ice-cold 0.9% saline, frozen in liquid nitrogen, and stored at –80°C for subsequent analyses. In 10 subjects, a portion of fresh VAT sample was used to separate into adipocytes and stroma/vascular (SV) cells fractions, and the remaining tissue was frozen.

Fractionation of adipocytes and SV cells

We examined vaspin mRNA expression in adipocyte and SV cell fractions of adipose tissue (n = 10). For this purpose, we used VAT because this tissue was available in amounts sufficient to separate into the two fractions. Freshly removed VAT was washed with Krebs Ringer Henseleit (KRH) buffer to remove blood, then digested using 1 mg/mL collagenase (Worthington, Freehold, NJ) in KRH buffer containing 1% BSA for 40 minutes to 60 minutes at 37°C. The collagenase-digest was separated from undigested tissues by filtration through 100 μm nylon mesh (Falcon, Franklin Lakes, NJ). The floating adipocyte fraction was collected and washed three times with KRH buffer. Non-floating cells isolated from the collagenase digest were centrifuged for 15 minutes at 1000 rpm, and the pellet (i.e., the SV cell fraction) was collected. The adipocyte and SV cell fractions were snap-frozen in liquid nitrogen and kept at –80°C for later RNA extraction.

Real-time quantitative RT-PCR

Total RNA was extracted from adipose tissue samples using TRIzol (Invitrogen Carlsbad, CA, 1 mL/100 mg tissue), according to the manufacturer's instructions. The purity of the extracted RNA was assessed using a NanoDrop spectrophotometer. The RNA was reverse transcribed into cDNA by Superscript III reverse transcriptase (Invitrogen) and oligo-dT primers. The mRNA was quantified using a Roche Light Cycler system (Roche Molecular Biochemicals, Mannheim, Germany). Each reverse transcriptase reaction was amplified in a 25 μL PCR mixture using the SYBR Green QPCR master mix (Bio-Rad, Hercules, CA). The following primers were used: human vaspin 5′–aggcagaacatggacttagg–3′ (forward) and 5′–gtcagctcgtggatgatgta–3′ (reverse); beta-actin 5′–gacggggtcacccacac–3′ (forward) and 5′–gtggtggtgaagctgtagcc–3′ (reverse). Expression of human vaspin and beta-actin mRNA was quantified by the second derivative maximum method, which determines the crossing points of individual samples using an algorithm that identifies the first turning point of the fluorescence curve. Vaspin mRNA expression was calculated relative to the expression of beta-actin using the $\Delta\Delta C_T$ method, normalizing the C_t values of the vaspin mRNA to the C_t values of β-actin relative to a control sample in arbitrary unit. Amplification of specific transcripts was confirmed *via* melting curve profiles.

Statistical analyses

Data are presented as means ± S.E. We divided the study subjects into two groups using a VSR of 0.30 (i.e., a median value among the study subjects) as a cut-off, with subcutaneous fat type defined as a VSR ≤ 0.3 and visceral fat type defined as a VSR > 0.3. Metabolic parameters and vaspin expression in subcutaneous and visceral adipose tissue were compared using a student t-test. Vaspin expression in the VAT and SAT of each subcutaneous and visceral fat type, respectively, was compared using a paired t-test. A Spearman's test was employed to assess the correlation between vaspin mRNA expression, serum vaspin concentration, metabolic parameters, and abdominal fat distribution. For all tests, a *p*-value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS (version 12.0 for Windows; SPSS, Chicago, IL).

Results

Clinical characteristics of the study subjects

The clinical characteristics of the study subjects, including CT measurements of abdominal fat, are shown in Table 1. The mean age was 43.3 ± 1.0 years and the mean BMI was 24.1 ± 0.4 kg/m^2. The VSR of subjects whose abdominal fat was measured by CT was 0.35 ± 0.02. Table 1 also showed the metabolic parameters and serum vaspin concentrations in subcutaneous and visceral fat type. Age and fasting plasma glucose levels were significantly higher in visceral fat type than in subcutaneous fat type.

Table 1 Metabolic parameters and serum vaspin concentrations according to abdominal fat type

	Total subjects (n = 85)	Subjects measured abdominal fat CT (n = 40)	Subcutaneous fat type VSR ≤ 0.3 (n = 20)	Visceral fat type VSR > 0.3 (n = 20)	*p*-value*
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	
Age (yr)	43.33 ± 1.00	42.93 ± 1.16	40.20 ± 1.23	45.65 ± 1.81	0.01
BMI (kg/m^2)	24.12 ± 0.35	23.89 ± 0.46	23.76 ± 0.81	24.03 ± 0.48	0.77
SBP (mmHg)	116.44 ± 1.47	115.47 ± 1.89	112.72 ± 2.38	117.95 ± 2.83	0.16
DBP (mmHg)	73.84 ± 1.04	74.03 ± 1.31	72.89 ± 1.83	75.05 ± 1.87	0.41
FPG (mmol/L)	5.60 ± 0.11	5.62 ± 0.14	5.33 ± 0.15	5.90 ± 0.22	0.03
Fasting insulin (IU/L)	5.59 ± 0.52	4.41 ± 0.66	4.00 ± 1.02	4.83 ± 0.86	0.57
HOMA-IR	1.44 ± 0.14	1.11 ± 0.16	0.95 ± 0.24	1.27 ± 0.21	0.39
Serum adiponectin (μg/mL)	4.86 ± 0.61	3.48 ± 0.50	4.26 ± 0.75	2.70 ± 0.63	0.08
Serum vaspin (ng/mL)	0.29 ± 0.04	0.22 ± 0.05	0.26 ± 0.06	0.18 ± 0.08	0.13
TAT area (cm^2)		365.93 ± 27.2	382.41 ± 42.8	349.45 ± 34.4	0.55
VAT area (cm^2)		90.54 ± 7.44	73.02 ± 8.46	108.07 ± 11.11	0.03
SAT area (cm^2)		275.39 ± 21.68	309.40 ± 34.75	241.38 ± 24.49	0.11
VSR		0.35 ± 0.02	0.23 ± 0.01	0.46 ± 0.02	<0.001

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, visceral/subcutaneous adipose tissue ratio. * *p*-values reflect the comparison between subcutaneous and visceral fat types.

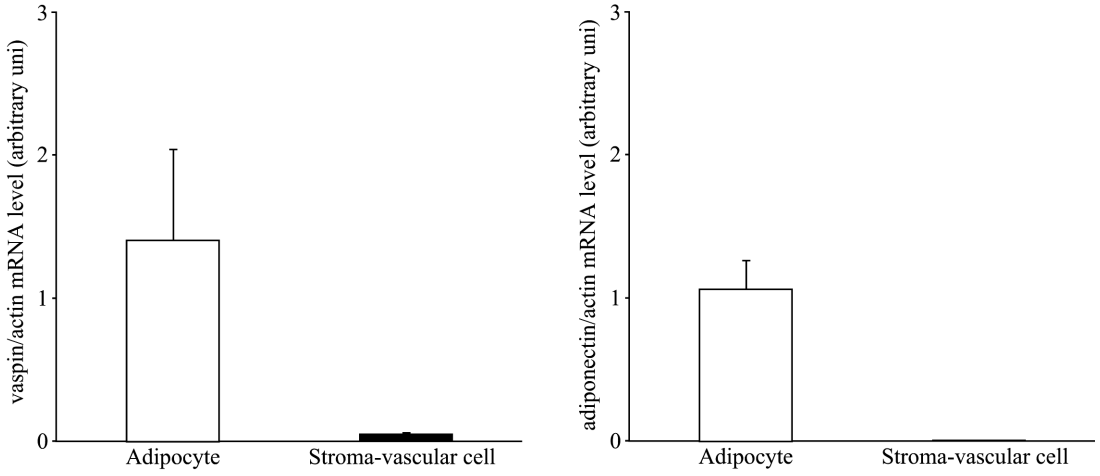

Fig. 1 Vaspin and adiponectin mRNA expression levels (arbitrary unit) in adipocyte and stroma-vascular cell fractions obtained from the visceral adipose tissue (n = 10). As expected, adiponectin was expressed exclusively in adipocytes (right side). Vaspin expression was significantly greater in adipocytes than in stroma-vascular cells (left side).

Vaspin mRNA expression of abdominal adipose tissue

Vaspin and adiponectin mRNA expression in adipocytes and in the SV cell fraction of VAT is shown in Fig. 1. As expected, adiponectin was expressed exclusively in adipocytes, which verified the integrity of the technique we used to separate adipocytes from SV cell fractions. Vaspin expression was significantly greater in adipocytes than in SV cells. Fig. 2 shows the amount of vaspin expression in the SAT and VAT in all subjects, as well as revealing the subcutaneous and visceral fat types. Vaspin expression was approximately 1.5 times higher in SAT than in VAT in total subjects. Among subjects with subcutaneous fat type, vaspin expression was significantly higher in SAT than in VAT. However, among subjects with visceral fat type, vaspin expression in SAT was similar to that in VAT. Although vaspin expression in VAT was not significantly different between subcutaneous and visceral fat type, vaspin expression in SAT was significantly higher in subcutaneous fat type than in visceral fat type.

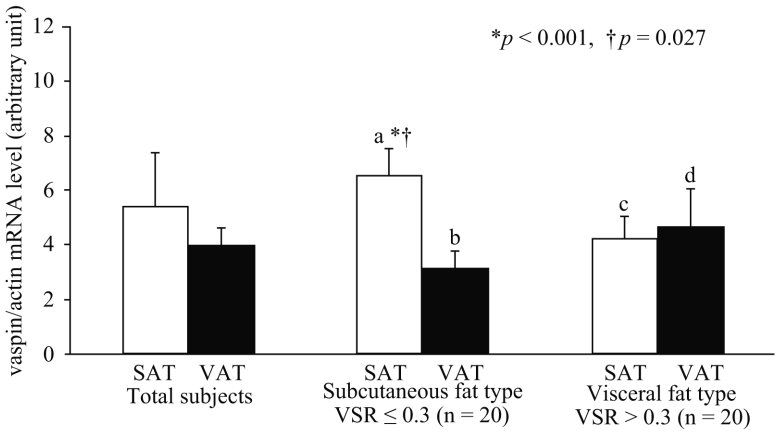

Fig. 2 Vaspin mRNA expression in visceral and subcutaneous adipose tissue according to abdominal fat type. SAT; subcutaneous adipose tissue, VAT; visceral adipose tissue, VSR; VAT/SAT ratio. * *p*-value between a and b, †*p*-value between a and c. Among subjects with subcutaneous fat type, vaspin expression was significantly higher in SAT than in VAT. However, among subjects with visceral fat type, vaspin expression in SAT was similar to that in VAT. Although vaspin expression in VAT was not significantly different between subcutaneous and visceral fat type, vaspin expression in SAT was significantly higher in subcutaneous fat type than in visceral fat type.

Table 2 Correlations of vaspin expression, serum vaspin concentration, metabolic parameters, and abdominal fat distribution

	Vaspin expression in VAT		Vaspin expression in SAT		Vaspin expression in VAT / Vaspin expression in SAT		Serum vaspin concentrations	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
In total subjects (n = 85)								
Age	0.25	0.06	0.29	0.05	0.11	0.41	0.08	0.51
BMI	–0.02	0.83	0.05	0.67	0.03	0.78	–0.01	0.90
SBP	–0.05	0.70	0.03	0.79	–0.01	0.89	0.07	0.56
DBP	0.13	0.33	0.05	0.68	0.14	0.29	0.05	0.67
FPG	0.11	0.40	0.07	0.56	0.05	0.69	–0.009	0.94
Fasting insulin	0.09	0.54	0.11	0.47	0.02	0.85	0.30	0.02
HOMA-IR	0.11	0.45	0.12	0.44	0.04	0.80	0.29	0.02
Serum adiponectin	0.01	0.91	0.14	0.34	–0.07	0.63	0.10	0.41
In subjects measured abdominal fat CT (n = 40)								
VAT area	–0.11	0.50	–0.55	0.001	0.31	0.07	–0.23	0.22
SAT area	0.07	0.67	–0.19	0.27	0.30	0.07	–0.07	0.71
VSR	–0.16	0.33	–0.29	0.09	0.07	0.67	–0.29	0.13
Serum vaspin	0.03	0.83	–0.23	0.26	0.41	0.04		

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. *p*-values were derived from Spearman correlation analysis.

Correlations between vaspin expression, serum vaspin concentrations, metabolic parameters, and abdominal fat distribution

Table 2 shows the correlations between vaspin expression in VAT and SAT, serum vaspin concentrations, metabolic parameters, and abdominal fat distribution. We identified a significant negative correlation between vaspin expression in SAT and VAT area (r = –0.55, $p = 0.001$). However, vaspin expression levels in adipose tissues were not significantly correlated with serum vaspin concentrations or metabolic parameters. There were significant positive correlations between serum vaspin concentration and fasting insulin (r = 0.30, $p = 0.02$), HOMA-IR (r = 0.29, $p = 0.02$) and the ratio of vaspin expression in VAT to vaspin expression in SAT (r = 0.41, $p = 0.04$).

Discussion

Our findings demonstrate that vaspin expression in abdominal adipose tissue was adipocyte-specific and that vaspin expression in SAT decreased as VAT area increased. However, we did not find any significant correlations between vaspin expression in abdominal adipose tissue and serum vaspin concentrations. Vaspin expression was significantly higher in the SAT in subcutaneous fat type than in the visceral fat type.

Vaspin mRNA was rarely detected in the SAT of OLEFT rats during the entire life period, whereas it was highly expressed in the VAT in 30-week-old OLEFT rats. However, in humans, vaspin mRNA expression was not restricted to VAT [9]. One study reported that the induction of vaspin expression in human adipose tissue could represent a compensatory mechanism associated with severe insulin resistance [9]. We could not find any correlation between vaspin expression in VAT or SAT and insulin resistance or metabolic parameters. However, serum vaspin concentrations had significant positive associations with fasting insulin as well as HOMA-IR. These findings are consistent with those of previous studies [8, 15]. In addition, serum vaspin concentrations positively correlated with the ratio of vaspin expression in VAT to the vaspin expression in SAT in our study.

Our study demonstrated that vaspin expression was greater in adipocytes than in the SV cells of abdominal VAT. These findings are consistent with those of a previous study conducted in OLEFT rats, which showed that vaspin mRNA and protein were expressed in mature adipocytes, as determined by Western- and Northern-blot analyses [7]. Immunohistochemistry was used to confirm the expression of vaspin in adipocytes and to show that vaspin is localized to the cytoplasm of adipocytes and is absent from SV cells [7]. We examined the site-specific expression of vaspin, in an effort to compare it to adiponectin expression (i.e., because adiponectin is expressed primarily in adipose tissue). Similar to adiponectin, vaspin expression in adipose tissue was adipocyte-specific. However, no correlation between serum vaspin concentrations and serum adiponectin concentrations was found.

When we performed collagenase digestion to separate abdominal adipose tissue into adipocytes and the SV cell fraction, the adipocyte fraction was greater in SAT than in VAT. However, the SV cell fraction was more prominent in VAT than in SAT. We postulated that vaspin expression in SAT decreased as VAT area increased in our study subjects. The reason why vaspin mRNA expression in SAT significantly and negatively correlated with VAT area might be that the relative SAT area decrease accompanied by VAT area increase could eventually decreased vaspin mRNA expression in SAT. These results are consistent with previous findings that subcutaneous vaspin mRNA expression is negatively correlated with waist–hip ratio (WHR) [9], a simple proxy marker of abdominal obesity.

We investigated vaspin expression in the SAT and VAT of subcutaneous and visceral fat type (i.e., as assessed by the VSR) as a reflection of metabolic complications [16-18]. Vaspin expression in SAT was significantly higher in subcutaneous fat type than in visceral fat type. In subcutaneous fat type, vaspin expression was significantly higher in SAT than in VAT. The relative decrease of SAT in visceral fat type may explain why subcutaneous vaspin expression decreases significantly in visceral fat type. Because the amount of adipocyte fraction in SAT decreased with the relative decrease of SAT, subcutaneous vaspin expression might be lower in visceral fat type.

Vaspin expression was highly expressed in 30-week-old OLEFT rats, corresponding to a peak in their body and fat weights, whereas expression was absent at the age of 50 weeks [7]. In our study, visceral fat type was significantly older than subcutaneous fat type; therefore, vaspin expression was lower in the SAT of visceral fat type than in subcutaneous fat type. Long-term administration of thiazolidinediones and insulin therapy notably increased vaspin expression in SAT at 30 weeks of age and the levels of expression were maintained until 50 weeks, whereas expression was reduced in the VAT of OLEFT rats [7]. The increased adipogenesis of SAT, with improved insulin resistance, seems to correlate with an upregulation in vaspin expression in SAT. These findings may explain why we observed higher gene expression in the SAT in subcutaneous fat type than in visceral fat type.

A previous study reported that visceral vaspin mRNA expression was significantly correlated with BMI and plasma glucose levels [9]. We did not observe a correlation between visceral vaspin expression and BMI, abdominal fat area, or metabolic parameters. Furthermore, we observed similar visceral vaspin expression in subcutaneous and visceral fat type. A previous study reported that vaspin mRNA and protein expression was significantly higher in the omental

adipose tissue of women with polycystic ovary syndrome than in a control group [10]. Although we cannot fully explain our negative findings, it is possible that our small sample size may account for the lack of any significant associations.

Our study had some limitations. First, our findings may not be applicable to other populations because we included only Korean women. The previous studies found that serum vaspin levels were significantly higher in women and that gender was an independent predictor of circulating vaspin [19]. Second, we could not measure the percentages of body fat and waist circumference. We measured abdominal fat CT as a more accurate tool for assessment of abdominal fat distribution. However, only half of the patients received the fat CT examination. Third, insulin resistance was measured by the HOMA-IR method instead of the euglycemic-hyperinsulinemic clamp, a gold-standard technique for the determination of insulin resistance. However, it has been demonstrated that measurements obtained by the homeostasis model correlate significantly with those obtained by the glucose clamp technique ($r = 0.83$, $p < 0.01$) [14]. Our measurement tool has been used in other previous studies [14, 20]. In addition, we defined subcutaneous and visceral fat type using an arbitrary criterion. Some previous study used a cut-off value as VSR of 0.40 to divide the abdominal fat type into subcutaneous and visceral fat type [16]. However, only eight subjects were included into visceral fat type when we used VSR 0.40 as a cut-off value in this study subjects. Therefore, it seems more reliable to use the median value of VSR in our subjects, VSR of 0.30, to divide into two groups.

In conclusion, our findings demonstrate that vaspin expression is adipocyte-specific in human abdominal adipose tissue. We noted a significant positive association between serum vaspin concentration and insulin resistance. Vaspin expression in SAT was significantly higher in subcutaneous fat type than in visceral fat type, and vaspin expression in SAT decreased as VAT area increased. Further studies with larger sample sizes are needed to investigate the role of vaspin in human physiology.

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Disclosure Statement

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