Associations of Serum Vascular Endothelial Growth Factor and Abdominal Fat Distributions in Obese Korean Women

Kyung Eun Yun*, Hye Soon Park(1), Chan Keum Park(2), Young Sook Song(2), Yeon Jin Jang(2)

Health Screening Center, Kangbuk Samsung Hospital, Sungkyunkwan University, School of Medicine; Department of Family Medicine, University of Ulsan College of Medicine(1); and Department of Physiology, University of Ulsan College of Medicine(2)

ABSTRACT

Background: Vascular endothelial growth factor (VEGF) is a very potent angiogenic factor expressed and secreted from adipocytes, and VEGF-mediated angiogenesis is necessary for the adipose tissue expansion associated with human obesity. This study investigates the associations of serum VEGF concentration and abdominal fat distributions in obese Korean women.

Methods: A cross-sectional study was conducted in 37 obese women (BMI $\geq$ 30 kg/m^2) who visited the obesity clinic in a tertiary health care center (Seoul, Korea). Anthropometric indices were measured and abdominal fat distribution was assessed by computed tomography. Fasting plasma glucose, insulin, lipid profiles, and serum VEGF concentrations were measured.

Results: Serum VEGF concentration significantly correlated with BMI (r = 0.478, *P* = 0.024) and visceral fat area (r = 0.449, *P* = 0.036) after adjustment for age. In multivariate linear regression analysis, visceral fat area was independently associated with serum VEGF concentration in the study subjects.

Conclusion: The present study shows that visceral fat accumulation is independently associated with elevation of serum VEGF concentrations in obese Korean women. This suggests the intriguing possibility that anti-angiogenic components may reduce visceral fat in obese subjects.

Key words: VEGF, BMI, Visceral fat accumulation, Obesity

비만 여성에서 혈청 혈관내피성장인자와 복부지방 분포의 연관성

연구배경: 지방세포에서 분비되는 혈관내피성장인자(vascular endothelial growth factor;VEGF)는 강력한 혈관생성인자로 지방조직 증식과 연관된 비만에 관여하는 것으로 알려져 있다. 본 연구는 비만 여성에서 비만도 및 지방 분포와 혈청 혈관내피성장인자의 연관성에 대해 알아보고자 하였다.

방법: 서울아산병원 가정의학과를 내원한 체질량지수(body mass index;BMI) 30 kg/m^2 이상인 비만 여성 37명을 대상으로 신체계측과 혈압, 혈청 지질, 혈당, 인슐린, 혈관내피성장인자를 측정하였으며, fat measured computed tomography로 피하지방과 내장지방 면적을 산출하였다.

결과: 혈청 혈관내피성장인자는 연령 보정 후 BMI와(r = 0.478, *P* = 0.024) 내장지방 면적과(r = 0.449, *P* = 0.036) 유의한 양의 상관관계를 보였지만, 피하지방 면적과는 유의한 상관관계를 보이지 않았다. 다변량 회귀분석 결과 내장지방 면적이 혈청 혈관내피성장인자의 농도에 가장 중요한 결정인자로 나타났다.

결론: 내장지방 축적은 혈청 혈관내피성장인자의 농도와 유의한 양의 상관성이 있는 것으로 나타났으며, 비만에서 항 혈관신생(anti-angiogenic)은 내장지방 분포에 영향을 미칠 수 있을 것으로 향후 이에 대한 연구가 필요하다.

중심단어: 혈관내피성장인자, 체질량지수, 내장지방, 비만

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Corresponding author: Kyung Eun Yun, Health Screening Center, Kangbuk Samsung Hospital, Sungkyunkwan University, School of Medicine, Samsung Main Bilg B1.,250,Taepyeongno 2-ga, Jung-gu, Seoul, Korea
Tel: 02-2001-2626, Fax: 02-757-0436, E-mail: eun3579@hanmail.net, Mobile: 011-9936-3579

Introduction

Adipose tissues express and release a variety of bioactive molecules that play important roles in inflammation, cell growth, energy metabolism, and the action of insulin.[1)] Expanded fat mass leads to dysregulation of adipocyte function, increased secretion of deleterious adipocytokines, and decreased secretion of beneficial adipocytokines.[1,2)] Recent observations suggest that regulation of adipose tissue vasculature is an important component of obesity, and angiogenesis appears to reciprocally modulate adipogenesis and obesity.[3)] Indeed, in an animal model of obesity, the expansion of adipose tissue was associated with active angiogenesis.[4)]

Vascular endothelial growth factor (VEGF) is a very potent angiogenic factor expressed in various cell types, including endothelial, epithelial, and mesenchymal cells.[5)] VEGF is abundantly expressed and secreted from adipocytes[6,7)], and VEGF-mediated angiogenesis is necessary for the adipose tissue expansion associated with human obesity.[8)] A recent study reported that administration of anti-VEGF antibodies inhibited not only angiogenesis but also adipose tissue development, indicating that angiogenesis and adipogenesis are functionally coupled and that VEGF is a key mediator of this process.[4)] The cardiometabolic risk factors are associated with impaired angiogenesis.[9)] A previous study reported that expressions of cardiac VEGF are down-regulated and capillary density is decreased in the myocardium in insulin-resistant states.[10)] However, circulating levels of VEGF are increased in obese subjects.[11)]

A recent study demonstrated that both subcutaneous and visceral adipose tissue expressed angiogenic factors without significant difference for VEGF expression.[8)] In one study, serum VEGF concentrations were associated with visceral fat accumulation in overweight or obese human subjects[12)], while another study found that serum VEGF was positively correlated with subcutaneous fat in normal weight and overweight subjects.[13)] Thus, the relationship between abdominal fat distribution and serum VEGF concentrations are still unclear.

This apparent discrepancy might reflect differences in the degree of obesity or other characteristics of the study subjects. In the present study, we investigated the associations between serum VEGF and abdominal fat distribution in obese Korean women.

Methods

1. Study Subjects

We recruited 37 obese women (body mass index $\geq$ 30 kg/m^2) who visited the obesity clinic at the Department of Family Medicine (Asan Medical Center, Seoul, Korea) from April to December 2007. The number of study subjects was estimated by calculating sample size based on effect size (0.45), two-sided α-value (0.05), and β-value (0.20). We excluded individuals with secondary causes of obesity, pregnant or lactating women, subjects with evidence of malignancy and severe hepatic diseases (serum albumin levels < 3.3 g/dL) or renal diseases (serum creatinine levels > 1.3 mg/dL), and subjects who were using medication that could affect weight or metabolism (appetite suppressants, lipase-inhibitors, and thyroid hormone). A part of study subjects were taking anti-hypertensive agents (n = 20), oral hypoglycemic agents (n = 8), and lipid lowering agents (n = 8). Twenty-one women (56.8%) had entered menopause. All participants gave written informed consent, and the study was approved by the Institutional Review Board of Asan Medical Center.

2. Anthropometric Measurements and Estimation of Abdominal Fat Distribution

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. Waist circumference was measured at the mid-point between the lower border of the rib cage and the iliac crest. All anthropometric measurements were taken by a single person throughout the study, in order to minimize interpersonal variations. The distribution of abdominal fat was assessed by computed tomography (CT) on a Siemens Somatom Scanner (Erlangen, Germany). Subjects were placed in a supine position, and a cross-sectional scan of 10-mm thickness centered at the L4-L5 vertebral disc space was obtained using a skeletal radiograph as a reference to establish the position of the scans to the nearest millimeter. Area of total abdominal fat tissue was measured by delineation with a graph pen, followed by computation of the adipose tissue area, using an

attenuation range of -190 to -30 Hounsfield units. The area of visceral fat tissue was measured by drawing a line within the muscle wall surrounding the abdominal cavity, whereas the area of subcutaneous abdominal fat tissue was calculated by subtracting the visceral fat tissue area from the total abdominal fat tissue area.

3. Measurement of Cardiometabolic Risk Factors

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 taken using a standard mercury sphygmomanometer on the patient's right arm. Two successive blood pressure readings were obtained at 5-min intervals, and the results were averaged. Blood samples were obtained after a 12-hr overnight fast, and serum was separated by centrifugation. Serum glucose concentration was measured by the glucose oxidase method, and insulin concentration was measured using a human insulin radioimmunoassay (RIA) kit (TFB, Japan). The homeostasis model assessment for insulin resistance (HOMA) index was calculated according to the following formula: fasting serum insulin (μU/mL) × fasting serum glucose (mg/dL) / 405, as previously described.[14] Total cholesterol and triglyceride levels were determined by enzymatic procedures using an autoanalyzer (Hitachi-747; Hitachi, Japan). The HDL-cholesterol fraction was measured enzymatically after MnCl2 precipitation of apo-B-containing lipoproteins. Serum VEGF concentrations were measured with an enzyme-linked immunosorbent assay (ELISA) system (R&D Systems, USA).

4. Statistical Analyses

The observed values are presented as mean ± SD. The data were tested for normal distribution; prior to analysis, variables such as triglycerides, insulin, and HOMA-IR levels were logarithmically transformed to approach a normal distribution. Pearson's correlation coefficients were used to assess the correlations between serum VEGF concentrations and anthropometries, abdominal fat distributions, and cardiometabolic risk factors. Partial correlation coefficients were estimated after adjustment for age. Stepwise multiple regression analysis was performed using the serum VEGF concentration as a dependent variable and the other factors as independent variables. All analyses were carried out using SPSS (version 14.0 for Windows; SPSS, USA). For all tests, *P*-values < 0.05 were considered statistically significant.

Results

1. Characteristics of Subjects

The anthropometric and metabolic characteristics of the study participants are presented in Table 1. The mean age and BMI were 50.3 ± 11.8 years and 32.8 ± 3.0 kg/m^2. The mean visceral and subcutaneous fat areas were 144.2 ± 47.4 cm^2 and 351.8 ± 89.7 cm^2, respectively, while the mean serum VEGF concentration was 408.8 ± 223.9 pg/mL.

Table 1. Basic characteristics of the study participants

Variable	Mean ± SD
Age (years)	50.3 ± 11.8
Body mass index (kg/m^2)	32.8 ± 3.0
Waist circumference (cm)	98.6 ± 6.9
Visceral fat area (cm^2)	144.2 ± 47.4
Subcutaneous fat area (cm^2)	351.8 ± 89.7
Systolic blood pressure (mmHg)	131.9 ± 16.2
Diastolic blood pressure (mmHg)	80.6 ± 10.5
Fasting serum glucose (mg/dL)	108.0 ± 15.3
Fasting serum insulin (μU/mL)	14.1 ± 8.4
HOMA index	4.1 ± 3.6
Total cholesterol (mg/dL)	185.9 ± 38.4
Triglycerides (mg/dLl)	153.6 ± 95.6
HDL cholesterol (mg/dL)	57.0 ± 13.8
Serum VEGF concentration (pg/mL)	408.8 ± 223.9

HOMA, homeostasis model assessment for insulin resistance index; VEGF, vascular endothelial growth factor.

2. Correlations between Serum VEGF Concentration with Anthropometric Measurements and Cardiometabolic Variables

Table 2 shows the correlations between serum VEGF concentration with anthropometric measurements and cardiometabolic variables. Our results revealed that BMI (r = 0.529, *P* = 0.001) and visceral fat area (r = 0.457, *P* = 0.021) significantly correlated with serum VEGF concentration. Even after adjustment for age, the correlations between serum VEGF concentration and BMI (r = 0.478, *P* = 0.024) and visceral fat area (r = 0.449, *P* = 0.036) remained significant. However, no significant correlations between serum VEGF concentrations and cardiometabolic risk factors could be found.

3. Multiple Regression Analysis to Study the Relationship between Serum VEGF Concentration with Anthropometric Measurements and Cardiometabolic Risk Factors

Stepwise multiple regression analysis was performed to investigate the relationship between serum VEGF concentration (as the dependent variable) with anthropometric measurements and cardiometabolic risk factors (as independent variables) (Table 3). The results revealed that visceral fat area was independently related to serum VEGF concentration. No other variable entered the equation at a significant level.

Discussion

The present study demonstrates that among obese Korean women, BMI and visceral fat accumulation are significantly associated with serum VEGF concentration, and visceral fat accumulation is an independent factor associated with serum VEGF concentration. This close relationship between the circulating VEGF concentration and visceral fat could result from the functional coupling

Table 2. Correlations between serum VEGF concentration, anthropometric measirements, and cardiometabolic risk factors in the study participants

Variable	r	*P*-value	r*	*P*-value
Body mass index (kg/m^2)	0.529	0.001	0.478	0.024
Waist circumference (cm)	0.321	0.056	0.196	0.382
Visceral fat area (cm^2)	0.457	0.021	0.449	0.036
Subcutaneous fat area (cm^2)	0.315	0.125	0.332	0.131
Systolic blood pressure (mmHg)	0.136	0.431	0.279	0.208
Diastolic blood pressure (mmHg)	0.024	0.889	0.171	0.448
Fasting serum glucose (mg/dL)	0.246	0.143	0.087	0.701
Fasting serum insulin (μU/mL)	-0.093	0.584	-0.271	0.222
HOMA index	-0.035	0.835	-0.259	0.244
Total cholesterol (mg/dL)	-0.116	0.508	-0.254	0.225
Triglycerides (mg/dL)	-0.159	0.362	-0.109	0.629
HDL cholesterol (mg/dL)	0.008	0.966	-0.017	0.941

HOMA, homeostasis model assessment for insulin resistance index; VEGF, vascular endothelial growth factor.
* adjusted for age.

Table 3. Multiple regression analysis for the relationship between serum VEGF concentration with anthropometric measurements and cardiometabolic risk factors

Independent variable	Parameter estimate	*P*-value
Age		0.436
Body mass index		0.108
Waist circumference		0.886
Visceral fat area	1.958	0.034
Subcutaneous fat area		0.239
Systolic blood pressure		0.253
HOMA index		0.100
Total cholesterol		0.090
R^2	0.205	

HOMA, homeostasis model assessment for insulin resistance index; VEGF, vascular endothelial growth factor.

between VEGF-mediated angiogenesis and adipogenesis.[4,8] Consistent with this notion, a previous study showed that adipokines and VEGF were released at greater levels by visceral adipose tissue than by subcutaneous adipose tissue in obese humans.[15] Cell fractionation studies of visceral fat have shown that adipocytes are the primary sources of VEGF.[7] Additionally, adipose stromal cells and infiltrated inflammatory cells can also significantly contribute to VEGF production.[16]

Adipose tissue can stimulate angiogenesis in physiological[17,18] and pathological[19,20] models; conversely, angiogenic factors such as VEGF can modulate adipocyte differentiation.[21] Adipose tissue mass is sensitive to angiogenesis inhibitors, potentially suggesting that these agents could have applications in the regulation of overweight and obesity.[22] Since adipocytes are surrounded by extensive capillary networks, it is likely that adipose tissue growth would be profoundly affected by treatment with anti-angiogenic agents.[22]

However, a recent study regarding the regulation of adipose tissue angiogenesis demonstrated that VEGF was expressed at similar levels in subcutaneous and visceral adipose tissues in humans.[8] Contrary to our present findings, a previous study reported that serum VEGF concentrations were positively correlated with subcutaneous fat accumulation, not with visceral fat accumulation, in overweight and normal weight men (mean BMI 25.7 ± 3.8 kg/m^2).[10] However, another study showed that serum VEGF concentrations were associated with visceral fat accumulation in obese or overweight human subjects (mean BMI 33.8 ± 5.1 kg/m^2).[9] Although the exact cause of the discrepancy is not yet known, it may arise from differences in the degree of obesity or other characteristics of the study subjects. Considering both of the above-mentioned two studies together,[9,10] the association between serum VEGF concentrations and visceral fat accumulation emerges as the more obese subjects are included in the study. In line with this, a strong correlation between serum VEGF level and visceral fat area was observed in our subjects (women with BMI ≥ 30 kg/m^2). In addition, the prior study showed that body weight reduction therapy reduced serum VEGF concentrations as visceral fat area decreased.[9] In the same token serum VEGF-A levels, along with the levels of other cytokines related to adipose tissue, have been shown to decrease after weight loss following bariatric surgery.[23]

Our results showed that there were no significant correlations between serum VEGF concentrations and cardiometabolic risk factors. In accordance with our results, a recent study demonstrated that serum VEGF concentrations were not significantly correlated with HOMA-IR even though those were significantly correlated with BMI. In that study, circulating levels of soluble VEGF receptor-2 rather than VEGF are significantly increased in subjects with metabolic syndrome associated with insulin resistance.[24] However, another study showed a significant correlation of serum VEGF concentrations with HOMA index, but not with fasting plasma glucose concentration.[25] Thus, the associations between serum VEGF concentrations and cardiometabolic risk factors remain controversial. In our study, one of the reasons of non-significant correlations between serum VEGF concentrations and cardiometabolic risk factors might be that a part of patients taking medications for control of blood pressures, plasma glucose, and lipid profiles were involved in the study subjects. We need to a further study having a large number of subjects to present a strong statistical power.

Certain limitations may be pointed out from our study. First, our findings might be hard to generalize, due to the small number of obese (BMI ≥ 30 kg/m^2) Korean women enrolled in this study. Second, the cross-sectional design makes it difficult to determine the causality of the observed relationships. However, our data contribute to a growing body of evidence suggesting that visceral fat accumulation is an independent factor associated with the elevation of serum VEGF concentrations in obese subjects.

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