Serum vaspin levels are positively associated with carotid atherosclerosis in a general population

Eishi Esaki[1], Hisashi Adachi[1, 2], Yuji Hirai[1], Sho-ichi Yamagishi[3], Tatsuyuki Kakuma[4], Mika Enomoto[1], Ako Fukami[1], Eita Kumagai[1], Kyoko Ohbu[1], Aya Obuchi[1], Ayako Yoshimura[1], Sachiko Nakamura[1], Yume Nohara[1], Tomoki Fujiyama[1], Yoshihiro Fukumoto[1], Tsutomu Imaizumi[5]

[1] Department of Internal Medicine, Division of Cardio-Vascular Medicine, Kurume University School of Medicine
[2] Department of Community Medicine, Kurume University School of Medicine
[3] Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine
[4] Biostatistics Center, School of Medicine, Kurume University
[5] Fukuoka Sanno Hospital and International University of Health and Welfare, Fukuoka

Abstract=249
Total word count=3,037
References=33
Four tables and one figure

Running head: Serum vaspin and carotid atherosclerosis

Address for correspondence;
Hisashi Adachi, M.D., Ph.D.
Department of Community Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830-0011, Japan
TEL:+81-942-35-3311(ext 3746)
FAX:+81-942-33-6509
E-mail: hadac@med.kurume-u.ac.jp

ABSTRACT

Objective: Vaspin is a novel adipocytokine with potential insulin-sensitizing properties. Insulin resistance (IR) plays a role in the development and progression of atherosclerosis. However, the relationship between serum vaspin levels and atherosclerosis remains unknown. Therefore, we investigated whether vaspin was correlated with carotid intima-media thickness (c-IMT).

Methods: Data for fasting vaspin levels of 201 subjects (78 men and 123 women aged over 40 years) were obtained from a general population in Japan. We obtained anthropometric parameters and blood chemistries, and calculated homeostasis model assessment-IR (HOMA-IR) index. C-IMT was measured by B-mode ultrasonography. The mean values of each parameter by tertiles of vaspin were compared with analysis of variance, and the associations of vaspin with IR and c-IMT were evaluated by multiple stepwise regression analysis.

Results: Univariate analysis revealed that vaspin levels were positively correlated with BMI, insulin, HOMA-IR index, estimated glomerular filtration rate (eGFR), c-IMT and hypertensive medication. Multiple stepwise regression analysis revealed that HOMA-IR index, c-IMT and eGFR were significantly and independently associated with vaspin. We performed multivariate analyses with c-IMT as the dependent variable. Age,

hypertensive medication and vaspin were significant for c-IMT. Moreover, a mediation analysis demonstrated that vaspin was significantly related to c-IMT, independently of IR.

Conclusions: The present study not only confirmed the previous finding of the positive association of vaspin with IR but also demonstrated that serum vaspin level was positively associated with c-IMT, independently of IR in a general population. Our results may suggest a role of vaspin in atherosclerosis in humans.

Keywords: Vaspin, Insulin resistance, Carotid atherosclerosis, Epidemiology

1. Introduction

Vaspin was first identified from visceral adipose tissues of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes mellitus with obesity and insulin resistance (IR) [1]. It is one of the visceral adipose tissue-derived adipocytokines, which belongs to the family of serine protease inhibitor (serpin) [2]. Adipose tissue expression of vaspin was significantly increased when obesity peaked in OLETF rats and administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity [1]. Thereafter, many in vitro and in vivo animal studies demonstrated insulin-sensitizing capacity of vaspin [3-5].

In humans, vaspin appears to have an insulin sensitizing properties [6] as well. Some clinical studies regarding to regulation of human vaspin have shown that serum levels are positively associated with IR [7,8] and the values are decreased following weight reduction and short-term intensive lifestyle modification [9], and restrictive bariatric surgery and laparoscopic Roux-en-Y gastric bypass (RYGB) surgery [10,11]. Thus, observations of animal and human studies suggest that vaspin could be a novel adipocytokine with an insulin-sensitizing property.

Recently, it has been considered that vaspin may possess anti-atherosclerotic properties. However, there have been a few data available for the association of vaspin

with atherosclerosis [12,13]. Accordingly, we investigated whether serum vaspin levels were correlated with intima-media thickness of the carotid artery, a marker of subclinical atherosclerosis in a general population.

2. Methods

2.1. Study population

A total of 226 subjects (86 men and 140 women) aged over 40 years received a population-based health examination in a fishing community in southwestern Japan, Uku town, in 2008. This town is an isolated island in Sasebo city, located in Nagasaki prefecture, and the total population is about 3,700. Of these, we excluded 25 subjects whose vaspin data were missing or who rejected the blood tests. Consequently, 201 subjects (78 men and 123 women) were enrolled in this study.

2.2. Data collection

Height and weight were measured, and body mass index (BMI) was calculated as weight (kilograms) divided by the square of height (square meters) as an index of the presence or absence of obesity. Waist circumference was measured at the level of the umbilicus in a standing position. Blood pressure (BP) was measured twice with the

subjects in the sitting (first) and supine (second) position. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurements. The second BP with the fifth phase diastolic pressure was used for analysis. Blood was drawn from the antecubital vein for determinations of lipids profiles (high-density lipoprotein cholesterol [HDL-cholesterol], low-density lipoprotein cholesterol [LDL-cholesterol] and triglycerides), creatinine, uric acid (UA), fasting plasma glucose (FPG), insulin, glycated hemoglobin A1c [HbA1c (NGSP)] and serum vaspin levels in a morning after 12-hour fasting. Fasting blood samples were centrifuged within 1 hour after the collection. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation modified with a Japanese coefficient [14]. Serum vaspin levels were measured with an enzyme-linked immunosorbent assay (ELISA) system [1]. Intra- and inter-assay coefficients of variation of vaspin in a commercially available laboratory (AdipoGen Inc. Incheon, Korea) were 2.8% and 6.1%, respectively.

HOMA-IR index was calculated from FPG and insulin levels [FPG (mg/dl) × insulin (μU/ml)/405] as a marker of IR. Subjects with type 2 diabetes mellitus were defined as those with FPG≥126 mg/dl or HbA1c≥6.5%, or those taking oral hypoglycemic agents and receiving insulin injection. C-IMT of the common carotid

artery was determined by using duplex ultrasonography (Sonosite“TITAN”, ALOKA) with a 10-MHz transducer in the supine position. Longitudinal B-mode images at the diastolic phase of the cardiac cycle were recorded by a single trained technician who was blinded to the subjects’ background. We measured the only far wall of c-IMT. The images were magnified and measured on the screen, and printed with a high-resolution line recorder (LSR-100A, Toshiba). We measured c-IMT according to the originally described method published in Circulation. Briefly, the c-IMT defined by Pignoli et al. [15,16] was measured as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. The first line represented the lumen-intimal interface; the collagen-containing upper layer of the tunica adventitia formed the second line. At each longitudinal projection, the site of the greatest thickness, including plaque, was sought along the arterial walls nearest the skin and farthest from the skin from the common carotid artery to the internal carotid artery. Three determinations of c-IMT of one artery were conducted at the site of the greatest thickness and at 2 other points, 1 cm upstream and 1 cm downstream from this site. The averaged value among the 6 IMTs (3 from the left and 3 from the right) was used as the representative value for each individual.

The mayor and the welfare section of Uku town approved this study. The

ethical committee of Kurume University also approved this study. All participants gave informed consent.

2.3. Statistical analysis

Because of skewed distributions, the natural logarithmic (ln) transformations were performed for triglycerides, insulin, HOMA-IR and vaspin. Log-transformed values were reconverted to antilogarithm forms in the tables. The medications for hypertension, dyslipidemia, and type 2 diabetes mellitus were coded as dummy variables. First, we performed multivariate analyses with vaspin as a dependent variable, and then with the c-IMT as a dependent variable. Finally, we performed a mediation analysis by creating a structural equation model to see whether vaspin was directly related to c-IMT. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using the SAS system (Release 9.3, SAS Institute, Cary, NC).

3. Results

Characteristics of the subjects stratified by tertiles of vaspin are shown in Table 1. BMI ($p<0.05$), insulin ($p<0.05$), HOMA-IR ($p<0.01$), c-IMT ($p<0.05$) and hypertensive medication ($p<0.05$) were positively associated with serum vaspin level,

whereas eGFR ($p<0.05$) was inversely associated. Characteristics of the subjects stratified by tertiles of c-IMT are shown in Table 2. Age ($p<0.001$), vaspin ($p<0.05$) and hypertensive medication ($p<0.001$) were positively associated with c-IMT, whereas eGFR ($p<0.01$) was inversely associated.

Multiple stepwise regression analysis revealed that serum vaspin level was significantly and independently associated with eGFR ($p<0.01$; inversely), HOMA-IR ($p<0.05$) and c-IMT ($p<0.05$) (Table 3a). Multiple stepwise regression analysis also revealed that c-IMT was significantly and independently associated with age ($p<0.001$), hypertensive medication ($p<0.01$) and vaspin ($p<0.05$) (Table 3b).

The result of the mediation analysis is presented in the Table 4 with path diagram in the Figure 1. We found that HOMA-IR was not related to c-IMT, and vaspin was directly related to c-IMT although vaspin was related to both c-IMT and HOMA-IR.

4. **Discussion**

In this cross-sectional cohort study in a general population, multiple stepwise regression analysis revealed that serum vaspin levels were significantly and independently associated with HOMA-IR index and c-IMT, markers of IR and carotid atherosclerosis, respectively. Moreover, a mediation analysis demonstrated that vaspin was directly related to c-IMT, independently of IR.

Vaspin and Insulin Resistance

Our results are consistent with previous studies showing a close association of serum vaspin level with IR [6,17,18] in humans. Previously, it was demonstrated that serum vaspin levels were associated with not only IR but also BMI in adults [6,17,18], children [19,20] and obese women with polycystic ovary syndrome [21,22]. However, there was no association of vaspin with BMI in our study. Because obesity and IR is closely related, no association of BMI with vaspin levels in our study may be puzzling. As apparent from Table 1, most enrolled subjects had normal BMI. Thus, absence of obesity in our population may explain this discrepant result from previous ones. Previous studies [22-24] demonstrated the influence on the regulation of vaspin by therapeutic agents such as statin, metformin and oral contraceptives. Thus, we should

not overlook effects of drugs on serum vaspin level. In our study, twenty subjects were prescribed statin, but the results were same after excluding subjects on statin. Moreover, the subjects on metformin or oral contraceptives were not included.

Because the clinical nature of our study, we are not able to state whether the high vaspin levels in insulin resistant subjects are primary or secondary. Basic in vitro and animal data suggest the compensatory elevation of vaspin in insulin resistant subjects [5,7,25].

Vaspin and Atherosclerosis

In this study, we for the first time demonstrated a close association of serum vaspin with c-IMT. Careful literature search revealed information for serum vaspin with atherosclerosis is very scanty. In a clinical study of 37 women with type 2 diabetes [26], the presence of microvascular complications was associated with low vaspin levels. However, the report was for microvascular diseases but not for atherosclerosis. The association of serum vaspin with carotid stenosis was reported in patients with acute ischemic stroke [27] and patients undergoing carotid endarterectomy [28]. No association was found in either study [27,28]. One clinical study reported a positive

association of serum vaspin with coronary atherosclerosis in asymptomatic women with metabolic syndrome [29]. However, no studies have been performed for the investigation of c-IMT, a surrogate marker of subclinical atherosclerosis, in a general population. We, for the first time, demonstrated a positive association of serum vaspin level with atherosclerosis in a general population. Although in order to elucidate the role of vaspin in atherosclerosis, it may be desirable to investigate the relationship before atherosclerosis has fully developed, it is necessary to investigate the relationship in established atherosclerotic patients as well.

From our study, we are not able to say why serum vaspin levels are increased in subjects with carotid atherosclerosis. Previous animal and human studies suggest vaspin may have anti-atherosclerotic properties [12,13,30]. Thus, it is likely that vaspin levels may be compensatory elevated. Although it is the beyond the scope of our study, we like to consider the anti-atherosclerotic properties of vaspin as follows. In cultured human aortic endothelial cells, vaspin prevented free fatty acid-induced apoptosis of endothelial cells through a stimulatory effect on the insulin-signaling pathway [30]. Thus, it is possible that vaspin will exert its anti-atherosclerotic properties through the insulin-sensitizing capacity [2,5-7]. However, our results suggest rather the direct effect of vaspin on atherosclerosis because the association of vaspin with c-IMT was

independent of HOMA-IR. We carried out an additional analysis to investigate direct and indirect relationship of vaspin with c-IMT by using a structural equation model with observed variables. The mediation analysis demonstrated that vaspin was directly related to c-IMT, independently of IR (Table 4 with path diagram in the Figure 1). The change in c-IMT for 10% increase in vaspin was 0.022.

The following basic studies may support our hypothesis [12,13,30]. A recent report demonstrated that vaspin increased nitric oxide bioavailability through the reduction of ADMA, an endogenous inhibitor of nitric oxide synthesis, in vascular endothelial cells [12]. It was shown that vaspin inhibited apoptosis as a ligand for the cell-surface GRP78 (78-kDa glucose-regulated protein)/voltage-dependent anion channel complex) in endothelial cells and inhibited apoptosis of endothelial cells [13]. Moreover, Phalitakul S, et al. [31] demonstrated that vaspin inhibited platelet-derived growth factor-BB-induced migration of vascular smooth muscle cells (SMCs) and protected the development of atherosclerosis in rats.

Multiple stepwise regression analysis revealed that serum vaspin levels were significantly and inversely associated with eGFR (Table 3a) suggesting renal function may affect serum vaspin levels. In fact, the molecular size of vaspin is too big (approximately 50-kDa) to be secreted from the kidney [2,32]. Although we have no

good explanation, the small number of enrolled subjects may be responsible. Our finding may be similar to that of Seeger et al. [33] who also demonstrated that circulating vaspin was inversely associated with eGFR in univariate analyses in control subjects. However, this association was lost after adjusting for age and gender. Thus, further studies are needed to elucidate the association of serum vaspin and renal function.

5. Limitations

The present study has several limitations. First, this study was cross-sectional and comparatively a small number of cases. Thus, nothing conclusive for the association of vaspin with atherosclerosis is stated. Prospective studies with a large number are needed to investigate the role of vaspin in the development of atherosclerosis. Second, our population was relatively healthy, and most of them had BMI, c-IMT and HOMA-IR index within normal limits. Thus, it is necessary to investigate serum vaspin levels in heterogeneous populations with wide ranges of atherosclerosis.

6. Conclusions

In conclusion, our data indicated that human circulating vaspin was positively and independently associated with HOMA-IR index and c-IMT in a general population. Vaspin may have a role in atherosclerosis.

Conflicts of interest

None declared.

Acknowledgements

This study was supported in part by the Kimura Memorial Heart Foundation, Fukuoka, by Grant-in-Aid for Scientific Research (C), from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We are grateful to members of the elected officials and residents of Uku town, and the team of physicians in Department of Internal Medicine, Division of Cardio-Vascular Medicine, Kurume University School of Medicine for their help in performing the health examinations.

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Figure Legend

Figure 1: Path diagram by the result of mediation analysis is presented.

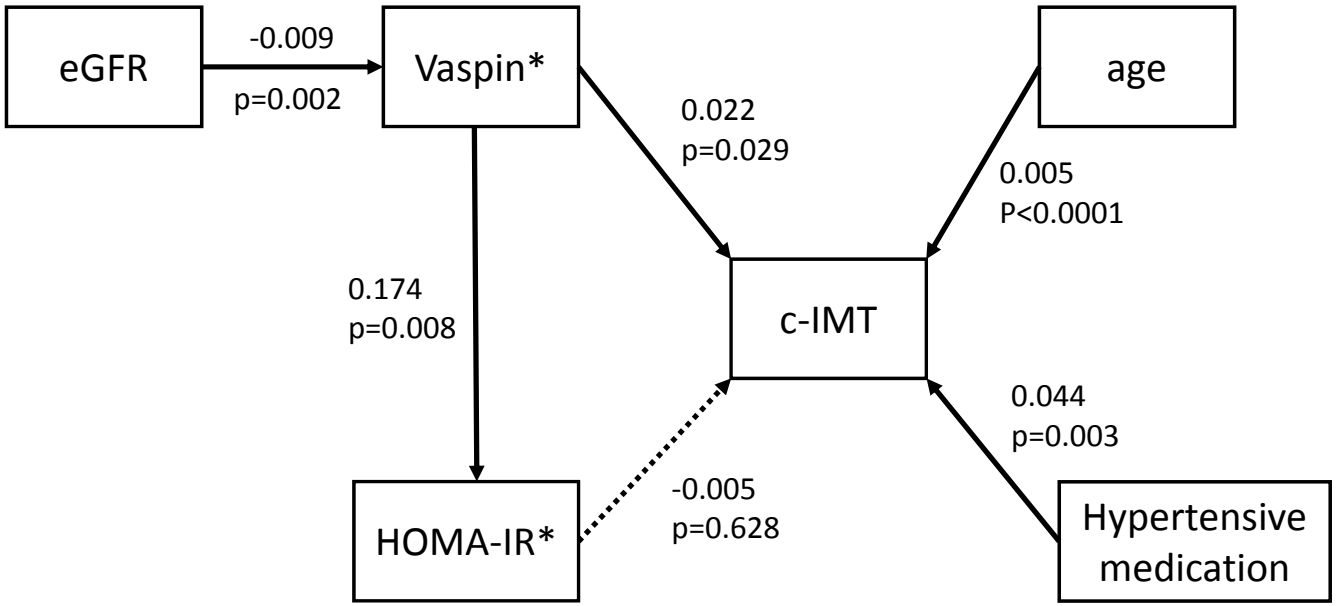

Table 4. Results of the mediation analysis

Endogenous variable	Exogenous variable	Estimate	SE	z	p-value	95%CI
c-IMT	**intercept**	**0.359**	**0.048**	**7.55**	**0.000**	**(0.266, 0.452)**
	Vaspin*	**0.022**	**0.010**	**2.19**	**0.029**	**(0.002, 0.042)**
	HOMA-IR*	**−0.005**	**0.011**	**−0.48**	**0.628**	**(−0.027, 0.016)**
	Age	**0.005**	**0.001**	**7.11**	**0.000**	**(0.004, 0.007)**
	Hypertensive medication	**0.044**	**0.015**	**2.99**	**0.003**	**(0.010, 0.073)**
Vaspin*	**intercept**	**0.413**	**0.226**	**1.83**	**0.068**	**(−0.015, −0.003)**
	eGFR	**−0.009**	**0.003**	**−3.04**	**0.002**	**(−0.030, 0.855)**
HOMA-IR*	**intercept**	**0.043**	**0.048**	**0.89**	**0.374**	**(−0.052, 0.137)**
	Vaspin*	**0.174**	**0.065**	**2.67**	**0.008**	**(0.046, 0.303)**

*:These variables are shown in the original scale after analysis using log (natural)-transformed values.

Table 1. Characteristics of study subjects stratified by tertiles of serum vaspin levels

Variable	Tertiles of serum vaspin levels			p-value
	T1 (n=67)	T2 (n=67)	T3 (n=67)	
Vaspin* (ng/ml)	0.36 (0.17-0.58)	0.76 (0.59-1.00)	1.68 (1.01-1.74)	**<0.001**
Age (years)	64.5 (10.1)	66.6 (9.8)	66.1 (9.8)	0.454
Male gender: n (%males)	24 (35.8)	30 (44.8)	24 (35.8)	0.306
BMI (kg/m^2)	23.4 (2.8)	23.6 (3.2)	24.6 (2.8)	**0.038**
Waist (cm)	84.8 (9.1)	84.6 (9.4)	86.7 (9.1)	0.384
Waist-Hip ratio	0.91 (0.06)	0.91 (0.05)	0.92 (0.05)	0.476
Systolic BP (mmHg)	138.5 (17.7)	136.4 (19.9)	138.5 (17.0)	0.753
Diastolic BP (mmHg)	79.3 (9.6)	80.8 (11.5)	81.3 (10.2)	0.533
LDL-cholesterol (mg/dl)	119.4 (31.7)	117.1 (29.0)	122.1 (27.5)	0.617
HDL-cholesterol (mg/dl)	61.4 (15.3)	58.5 (12.1)	57.0 (14.4)	0.184
Triglycerides* (mg/dl)	83.0 (37-286)	82.0 (32-218)	93.3 (34-393)	0.252
FPG (mg/dl)	93.3 (17.6)	99.0 (27.6)	97.4 (12.6)	0.254
Insulin* (μU/ml)	3.7 (0.5-16.4)	4.1 (0.9-11.6)	5.0 (0.9-18.2)	**0.011**
HOMA-IR*	0.85 (0.12-3.93)	0.98 (0.23-5.54)	1.19 (0.20-4.58)	**0.009**
HbA_{1c} (%, NGSP)	5.0 (0.7)	5.0 (0.6)	5.0 (0.4)	0.894
eGFR ($ml/min/1.73m^2$)	75.4 (15.8)	72.0 (16.2)	67.9 (14.4)	**0.023**
Uric Acid (mg/dl)	5.1 (1.4)	5.3 (1.4)	5.5 (1.4)	0.228
c-IMT (mm)	0.69 (0.12)	0.72 (0.11)	0.74 (0.10)	**0.043**
Alcohol intake: n (%yes)	17 (32.7)	16 (30.8)	19 (36.5)	0.836
Current smoking: n (%yes)	8 (38.1)	5 (23.8)	8 (38.1)	0.623
Medications: n (%yes)				
Hypertension	22 (32.8)	28 (41.8)	37 (55.2)	**0.035**
Type 2 diabetes mellitus	1 (1.5)	4 (6.0)	3 (4.5)	0.227
Dyslipidemia	9 (13.4)	10 (14.9)	5 (7.5)	0.391

Data are means (SD), geometric mean, range, or percent.

*:These variables are shown in the original scale after analysis using log (natural)-transformed values.

eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) study equation [$eGFR=194\times Creatinin^{-1.094}\times Age^{-0.287}$ ($\times 0.739$ if female)].

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; c-IMT, carotid intima-media thickness

Table 2. Characteristics of study subjects stratified by tertiles of c-IMT levels

Variable	Tertiles of c-IMT levels			p-value
	T1 (n=67)	T2 (n=67)	T3 (n=67)	
c-IMT (mm)	0.59 (0.07)	0.71 (0.04)	0.85 (0.06)	**<0.001**
Age (years)	59.6 (9.3)	67.4 (8.6)	70.5 (8.4)	**<0.001**
Male gender: n (%males)	25 (37.3)	22 (32.8)	31 (46.3)	0.074
BMI (kg/m^2)	23.6 (2.9)	24.1 (2.9)	23.7 (3.1)	0.585
Waist (cm)	83.6 (9.4)	86.4 (9.3)	86.1 (8.7)	0.167
Waist-Hip ratio	0.89 (0.05)	0.91 (0.05)	0.92 (0.05)	0.054
Systolic BP (mmHg)	134.4 (17.8)	138.0 (17.2)	141.3 (19.2)	0.098
Diastolic BP (mmHg)	79.9 (10.9)	80.2 (10.9)	81.2 (9.4)	0.764
LDL-cholesterol (mg/dl)	120.8 (32.9)	119.3 (29.4)	118.5 (25.5)	0.899
HDL-cholesterol (mg/dl)	61.4 (16.8)	56.9 (12.6)	58.7 (11.8)	0.173
Triglycerides* (mg/dl)	84.8 (32-371)	90.0 (37-318)	84.8 (34-393)	0.295
FPG (mg/dl)	94.0 (15.6)	98.1 (27.5)	97.6 (14.2)	0.439
Insulin* (μU/ml)	4.1 (1.0-14.7)	4.7 (0.5-18.2)	3.9 (0.9-13.3)	0.139
HOMA-IR*	0.95 (0.20-4.32)	1.11 (0.12-5.54)	0.92 (0.20-4.09)	0.187
HbA_{1c} (%, NGSP)	4.9 (0.6)	5.1 (0.7)	5.0 (0.4)	0.155
eGFR ($ml/min/1.73m^2$)	76.3 (14.8)	71.2 (15.7)	67.5 (15.7)	**0.005**
Uric Acid (mg/dl)	5.3 (1.5)	5.2 (1.3)	5.4 (1.4)	0.446
Vaspin* (ng/ml)	0.65 (0.17-4.23)	0.80 (0.17-3.17)	0.90 (0.26-5.67)	**0.024**
Alcohol intake: n (%yes)	20 (29.8)	15 (22.4)	17 (25.4)	0.508
Current smoking: n (%yes)	12 (17.9)	4 (6.0)	5 (7.5)	0.052
Medications: n (%yes)				
Hypertension	17 (25.4)	34 (50.7)	36 (53.7)	**<0.001**
Type 2 diabetes mellitus	2 (3.0)	2 (3.0)	4 (6.0)	0.495
Dyslipidemia	4 (5.9)	12 (17.9)	8 (11.9)	0.121

Data are means (SD), geometric mean, range, or percent.

*:These variables are shown in the original scale after analysis using log (natural)-transformed values.

eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) study equation [$eGFR=194\times Creatinin^{-1.094}\times Age^{-0.287}$ ($\times 0.739$ if female)].

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; c-IMT, carotid intima-media thickness

Table 3a. Multiple stepwise regression analysis for the correlates of vaspin*

Parameters	ßeta	SE	p-value
eGFR (ml/min/1.73m^2)	-0.009	0.003	**0.003**
HOMA-IR*	0.182	0.075	**0.016**
c-IMT (mm)	0.828	0.418	**0.048**
			R^2=0.091

Abbreviations: eGFR, estimated glomerular filtration rate;
HOMA-IR, homeostasis model assessment-insulin resistance;
c-IMT, carotid intima-media thickness
SE, Standard error
*:This variable is shown in the original scale after analysis using log (natural)-transformed value.

Table 3b. Multiple stepwise regression analysis for the correlates of c-IMT

Parameters	ßeta	SE	p-value
Age (years)	0.006	0.001	**<0.001**
Hypertensive medication	0.047	0.015	**0.002**
Vaspin* (ng/ml)	0.022	0.010	**0.035**
			R^2=0.295

Abbreviations: c-IMT, carotid intima-media thickness; SE, Standard error
*:This variable is shown in the original scale after analysis using log (natural)-transformed value.