European Review for Medical and Pharmacological Sciences 2016; 20: 1544-1558

Age-, sex- and glucose-dependent correlation of plasma soluble vascular adhesion protein-1 concentration with cardiovascular risk factors and subclinical atherosclerosis

D.-W. CHEN[1,2], Y. JIN[1], R.-M. ZHAO[3], L.-J. LONG[4], J. ZHANG[1], C.-L. HAN[5], A. ROIVAINEN[5], J. KNUUTI[5], S. JALKANEN[6], J.-C. WANG[1]

[1]Geriatric Institute, the General Hospital of the Air Force, PLA (the Chinese People's Liberation Army), Haidian District, Beijing, China
[2]Neurology Department, the General Hospital of the Air Force, PLA (the Chinese People's Liberation Army), Haidian District, Beijing, China
[3]Clinical Laboratory Center, the General Hospital of the Air Force, PLA (the Chinese People's Liberation Army), Haidian District, Beijing, China
[4]Health Examination Center, the General Hospital of the Air Force, PLA (the Chinese People's Liberation Army), Haidian District, Beijing, China
[5]Turku PET Centre, Turku University Hospital and University of Turku, Turku, Finland
[6]The Medicity Research Laboratory, and Department Medical Microbiology and Immunology, University of Turku, Turku, Finland

Abstract. – OBJECTIVE: Soluble vascular adhesion protein-1 (sVAP-1) may act as a biomarker for atherosclerosis and cardiovascular diseases. The associations of sVAP-1 concentration with cardiovascular risk factors and subclinical atherosclerosis at the population level have not been reported.

PATIENTS AND METHODS: This cross-sectional study included 834 asymptomatic subjects (49.1 ± 9.3 years). sVAP-1 was measured by enzyme-linked immunosorbent assay. Subclinical atherosclerosis was assessed by brachial-ankle pulse wave velocity (baPWV) and carotid intima-media thickness (CIMT).

RESULTS: sVAP-1 increased with age. Women had a higher concentration than men in age > 40 years. In women, sVAP-1 was negatively associated with estradiol ($p < 0.01$) and body mass index (BMI) ($p < 0.05$). In men, sVAP-1 was negatively associated with apolipoprotein A (ApoA) ($p < 0.01$), alcohol intake ($p < 0.01$) and uric acid ($p < 0.05$), but positively associated with ApoB/ApoA ($p < 0.05$). In hyperglycemia subjects, sVAP-1 positively correlated with fasting plasma glucose ($p < 0.05$) and hemoglobin A1c ($p < 0.05$), but in normoglycemic subjects, sVAP-1 negatively correlated with BMI ($p < 0.01$), triglyceride ($p < 0.05$), alcohol intake ($p < 0.05$). sVAP-1 independently influenced CIMT ($\beta = 0.001$, $p = 0.040$) and carotid plaques [odds ratio 1.380 (95% confidence interval 1.051-1.813, $p = 0.021$)] in hyperglycemia, and baPWV ($\beta = 31.605$, $p = 0.014$) in age > 55 years.

CONCLUSIONS: sVAP-1 concentration correlates with cardiovascular risk factors and subclinical atherosclerosis in an age-, sex- and glucose-dependent manner.

Key Words:
Vascular adhesion protein-1, Semicarbazide-sensitive amine oxidase, Risk factors, Atherosclerosis, Arterial stiffness.

Introduction

Vascular adhesion protein-1 (VAP-1) is an adhesion molecule and involved in leukocyte rolling, adhesion, and transmigration from the blood into sites of inflammation[1], which is important for the pathogenesis of atherosclerosis. VAP-1 also has semicarbazide-sensitive amine oxidase (SSAO) activity, which catalyzes the breakdown of primary amines to aldehyde, hydrogen peroxide, and ammonia[2]. Aldehyde and hydrogen peroxide can mediate endothelial injury, protein modification and oxidative stress that might lead to initiation or progression of atherosclerosis[3]. In addition, hydrogen peroxide also induces the expression of other endothelial adhesion molecules (e.g. E- and P-selectin),

Corresponding Author: Jian-Chang Wang, MD; e-mail: kzdw66@sohu.com

chemokines (e.g. CXCL8) and transcription factors (e.g. NF-kB), thereby increasing inflammatory damage[2]. VAP-1 is a transmembrane molecule but also presents as a soluble form (sVAP-1) in plasma[4]. Therefore, plasma sVAP-1 might be a novel biomarker for atherosclerosis and cardiovascular diseases.

Plasma sVAP-1 can be determined as sVAP-1 concentration or SSAO activity[5], and the former may be a better biomarker for atherosclerosis and cardiovascular diseases than the later. First, sVAP-1 concentration can be measured easily in extensive clinical series in routine laboratories[6,7]. Second, protein concentration seems to be more stable than enzyme activity during the storage[4,7,8]. Thirdly, compared with SSAO activity, sVAP-1 is also a biomarker for endothelial inflammation and injury that is important for atherosclerosis[9,10]. Fourthly, sVAP-1 concentration is more sensitive than SSAO activity to be associated with early cardiovascular diseases and atherosclerosis[6].

At present, some studies reported the association of sVAP-1 concentration with cardiovascular disorders and their related risk factors in special clinical patients (e.g. diabetes mellitus[11], psoriasis[12], or coronary heart disease[6]). The cardiovascular risk factors associated with sVAP-1 included glucose, blood lipids and smoking[6,11-15]. In the present study, we measured sVAP-1 concentration and observed its association with traditional and new cardiovascular risk factors, such as apolipoprotein A (ApoA), apolipoprotein B (ApoB), lipoprotein(a), homocysteine, uric acid, estradiol and C-reactive protein (CRP) in a large population of Han Chinese. We also studied correlations of sVAP-1 concentration with the Chinese cardiovascular risk score[16], and in a subpopulation with carotid intima-media thickness (CIMT) and brachial-ankle pulse wave velocity (baPWV). Plasma sVAP-1 concentration was independently associated with some cardiovascular risk factors, with CIMT and carotid plaques in hyperglycemic subjects, and with baPWV in subjects of age > 55 years.

Patients and Methods

Study Sample

In this cross-sectional study, the asymptomatic subjects who underwent a health examination at the General Hospital of Air Force PLA (the Chinese People's Liberation Army) in Beijing city during 2013-2014, were invited to participate in this study. Eligibility criteria included the following: (1) age ≥ 30 years; (2) Han Chinese ethnicity; (3) living in Beijing over 10 years. We excluded the subjects with following diseases affecting plasma sVAP-1 concentration[4]: (1) inflammatory liver diseases; (2) end-stage renal disease; (3) inflammatory skin diseases (e.g. psoriasis and atopic eczema); (4) inflammatory nervous system diseases (e.g. multiple sclerosis and Alzheimer's disease); (5) inflammation-related ocular diseases (e.g. uveitis, age-related macular degeneration); (6) congestive heart failure. In a preliminary study with a sample size of 40 subjects, the mean and standard deviation (SD) of sVAP-1 were 340.5 ng/mL and 88.5 ng/mL respectively. Based on these observations, assuming a permissible error of 10 ng/mL and a 5% significance level, a sample size of 310 subjects would be needed. A total of 900 subjects was planned in this survey to allow for subgroups analysis and subject exclusions. Participants provided written informed consent to participate in the study. The study was approved by the Institutional Review Committee of Air Force General Hospital.

Procedure of Health Examination

Subjects were asked to have an overnight fast and to avoid intensive exercise overeating and consuming caffeine and alcohol 12 hours before the health examination. First, height and weight were measured, and blood pressure (BP) measured two times by an electric sphygmomanometer after 5 minutes at a supine position. Body mass index (BMI). was calculated as weight (kg)/height (m^2), and underweight defined as BMI < 18.5 and overweight as BMI ≥ 25. Second, venous blood samples were drawn for analysis of glucose, hemoglobin AIc (HbA1c), triglycerides, low and high density lipoproteins, ApoA, ApoB, ApoB/ ApoA, lipoprotein(a), uric acid, homocysteine, estradiol, progesterone, and C-reactive protein (CRP) at the clinical chemical laboratory center of the hospital. Plasma samples were stored at -70°C for the measurement of sVAP-1 concentration. Third, questionnaires were used to collect information on taking medications for hypertension or diabetes mellitus, smoking and alcohol drinking. Smokers or alcohol users was defined as regular smoking or alcohol consumption daily or every week for more than 6 months. Hypertension was defined as taking anti-hypertension medications or exhibiting an average brachial artery systolic/diastolic BP

equal to or greater than 140/90 mmHg. Diabetes mellitus was defined by treatment with insulin, oral anti-diabetic agents, or by a fasting glucose level ≥ 7.0 mmol/L. Impaired fasting glucose (IFG) was defined as fasting glucose level ≥ 6.1 mmol/L and < 7.0 mmol/L. Hyperglycemia included IFG and Diabetes mellitus. Cardiovascular risk scores was calculated by the Chinese absolute risk for 10-year risk of ischemic cardiovascular disease (ICVD)[16]. At last, CIMT and baPWV were measured in noninvasive vascular evaluation laboratory according to the subjects' willingness and selection.

Measurement of Plasma sVAP-1 Concentration

sVAP-1 concentration was assessed by enzyme-linked immunosorbent assay (ELISA) kit (MedSystem Diagnostic GmbH, Vienna, Austria) according to the manufacturer's instructions. All the samples was performed by a single technician blinded for subject identification. All analyzed serum samples were diluted 1:100 with a buffer supplied with the kit and then transferred to microtiter plates coated with anti-VAP-1 antibody. After addition of mouse antibody conjugated with biotin, the plate was incubated at room temperature for 2 h on a shaker (300 rounds/min). After incubation, streptavidin conjugated with horseradish peroxidase was added to each vial and after another 1 h of incubation on a shaker (300 rounds/min) the reaction was visualized using tetramethylbenzidine (TMB) reagent and phosphatic acid. Finally, the absorbance for each sample was read at 450 nm, using the Model 450 microplate reader (BIO-RAD Laboratories Inc., USA), and applying a reference wavelength of 620 nm. The linearity (R^2) of the standard curves was 0.999-1.000. The coefficient of variation (CV) of the standard curves was 0.3-3.6%. The intra-assay CV was 0-5.4% and inter-assay CV was 0.3-9.5%. The calibration range was from 31.5 ng/ml to 2000 ng/ml. The intraclass correlation coefficients (ICC) for the reliability of sVAP-1 was 0.96 (0.92-0.98).

Measurement of CIMT and Carotid Plaques

CIMT and baPWV were measured noninvasively in a subsample of the participants. Carotid ultrasonography was performed by a single technician blinded for subject identification. Images of the extracranial carotid artery walls were obtained in three projections by an ultrasound machine (IU22, Philips Medical System, Andover, MA, USA) equipped with an 5- to 12-MHz transducer with the patient supine and neck in sight hyperextension. Two measurements of carotid IMT from the media-adventitia interface to lumen-intima interface were determined at the far wall of both common carotid arteries 10 mm caudal to the bulb. A semi-automated edge-detection software package was used to derive the average CIMT of a 10 mm length region in the artery. The larger value of both sides was taken for further analyses. The presence of plaques in the carotid artery was assessed by evaluating the ultrasonographic images of the common and bifurcation sites of the carotid artery. The plaque was defined as a focal structure of at least 0.5 mm or 50% above the surrounding IMT value, which encroaches into the arterial lumen. High risk CIMT was defined as CIMT thickness ≥ 0.1 cm and/or carotid plaque. The ICC for the reliability of CIMT was 0.97 (0.88-0.99).

Measurement of baPWV

BaPWV was measured by a single technician blinded for subject identification using an automatic device (Colin VP 1000, Omron co., Kyoto, Japan). The distance between the brachium and the ankle for baPWV was calculated by the height of the patient. PWV was calculated as the distance between the brachial and the ankle divided by the time delay between the two arterial points, expressed as centimeters per second (cm/s). After examinations were performed on both the left and right sides, the larger value was used for further analyses. The ICC for the reliability of baPWV was 0.97 (0.89-0.99).

Statistical Analysis

Categorical variables were reported as the percentage of patients in the subgroups. The distributions of continuous variables were examined by the Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean and SD. Continuous variables with skewed distribution were analyzed after square root or Blom's Formula transformation into normal distribution and were presented as medians (interquartile ranges). In univariate analysis, two class comparisons were by the Student t test and multiple-class comparisons were by one-way ANOVA for continuous variables with normal distribution. Nonparametric tests were used for comparisons of continuous variables without normal distribution.

χ^2 test was used for the comparisons of categorical variables. Pearson correlation coefficient *t*-test was used for the correlation analysis of normally distributed continuous variables. For multivariable models, linear regression models were used for continuous variables, and logistic regression models for categorical variables. The statistical significance was defined as $p < 0.05$. The requirement of adjustment for *p* value due to the multiple testing, which was carried out by means of Benjamini and Hochberg False Discovery Rate, was considered for data with significant results. All statistical analyses were performed using the SPSS software version 16.0.

Results

A total of 834 participants (303 females and 531 males) with a mean age of 49.1 ± 9.3 years (30-86 years) were included in this study (Supplemental Figure 1). As shown in Table I, data of sVAP-1 concentration, cardiovascular risk scores and the traditional cardiovascular risk factors were available from almost all the subjects. Most of subjects had data of the new cardiovascular risk factors. In addition, 190 females had measurements of progesterone and estradiol, 568 subjects had measurements of baPWV, and 354 had measurements of CIMT and carotid plaques.

Correlation of sVAP-1 Concentration with Age and Sex

sVAP-1 concentration was positively correlated to age in all subjects and both sexes (Table II). Women had slightly higher sVAP-1 concentration than men in all subjects (Table II). As shown in the Figure 1, when the subjects were divided into 4 groups according to age, this gender difference existed in 41-50 y and 51-60 y groups, but not in 30-40 y group. Compared with men, women had a tendency toward higher

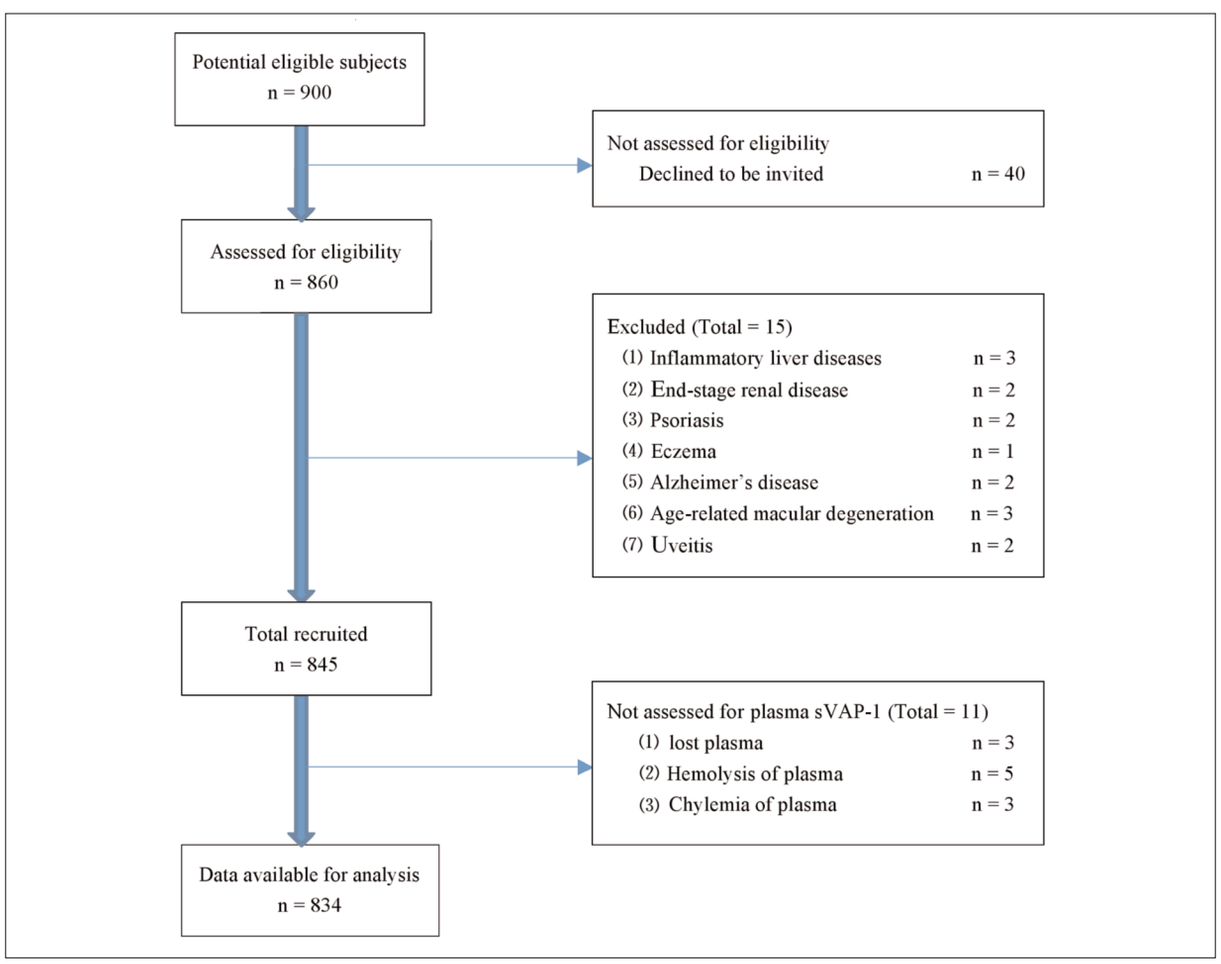

Figure 1 ***(Supplement).*** Subjects Flow Diagram.

Table I. Baseline clinical characteristics of the study subjects according to sVAP-1 tertiles.

Serum sVAP-1 tertiles (ng/mL)	All		≤ 280		280-360		≥ 360		p
	n	Value	n	Value	n	Value	n	Value	
Background									
Age (years)	834	49.1 ± 9.3	269	45.9 ± 8.5	306	48.4 ± 8.9	259	53.14 ± 9.1	□0.001
Female	834	303 (36.3)	269	68 (25.3)	306	105 (34.3)	259	130 (50.2)	□0.001
Smoking	834	224 (26.9)	269	78 (29.0)	306	85 (27.8)	259	61 (23.6)	0.333
Alcohol use	834	284 (34.1)	269	121 (45.0)	306	108 (34.3)	259	55 (21.2)	□0.001
Hypertension	834	199 (23.9)	269	61 (22.7)	306	69 (22.5)	259	69 (26.6)	0.449
Diabetes mellitus	834	89 (10.7)	269	17 (6.3)	306	24 (7.8)	259	48 (18.5)	□0.001
Physical Measures									
Systolic BP (mmHg)	825	124 ± 13	264	123 ± 12	303	124 ± 14	258	125 ± 14	0.090
Diastolic BP (mmHg)	825	77 ± 10	264	77 ± 9	303	78 ± 10	258	76 ± 11	0.184
BMI (kg/m^2)	825	25.4 ± 3.2	264	25.9 ± 3.3	303	25.3 ± 3.0	258	25.2 ± 3.4	0.031
Blood Laboratory Tests									
FPG (mmol/L)	829	5.62 ± 1.23	268	5.47 ± 0.87	305	5.46 ± 0.94	256	5.98 ± 1.69	□0.001
HbA1c (%)	668	5.65 ± 0.79	210	5.56 ± 0.62	247	5.55 ± 0.64	211	5.87 ± 1.04	□0.001
Triglycerides (mmol/L)	829	1.95 ± 1.36	268	2.16 ± 1.58	305	1.89 ± 1.21	256	1.81 ± 1.24	0.008
LDL (mmol/L)	829	3.13 ± 0.74	268	3.01 ± 0.68	305	3.13 ± 0.74	256	3.26 ± 0.78	□0.001
HDL (mmol/L)	829	1.34 ± 0.31	268	1.30 ± 0.30	305	1.34 ± 0.30	256	1.37 ± 0.33	0.024
ApoB (g/L)	667	0.80 ± 0.18	216	0.79 ± 0.17	242	0.80 ± 0.18	209	0.82 ± 0.20	0.245
ApoA (g/L)	667	1.43 ± 0.25	216	1.44 ± 0.24	242	1.43 ± 0.25	209	1.42 ± 0.26	0.880
ApoB/ApoA	667	0.58 ± 0.16	216	0.56 ± 0.14	242	0.58 ± 0.16	209	0.60 ± 0.19	0.122
Lipoprotein (a) (mg/L)	667	100.0 (47.0-193.0)	216	95.5 (40.5-163.0)	242	88.5 (49.0-194.0)	209	112.0 (54.5-211.0)	0.051
Uric acid (μmol/L)	829	303.1 ± 87.8	268	320.2 ± 86.4	305	302.1 ± 89.9	256	286.7 ± 83.5	□0.001
Homocysteine (μmol/L)	625	11.20 (9.21-13.39)	197	11.39 (9.68-13.67)	228	11.10 (9.14-13.33)	200	11.03 (8.84-13.27)	0.229
Progesterone (nmol/L)	190	0.62 (0.49-1.91)	40	0.57 (0.47-2.10)	65	0.96 (0.50-4.39)	85	0.56 (0.481.48)	0.080
Estradiol (< 100 pmol/L)	190	99 (52.1)90	40	16 (40.0)	65	27 (41.5)	85	56 (65.9)	0.003
CRP (≥ 3 mg/L)	478	164 (34.3)	142	52 (36.6)	170	50 (29.4)	166	62 (37.3)	0.243
sVAP-1 (ng/mL)	834	314.0 (226.3-377.1)							
Others									
Chinese absolute risk	820	1.9 (1.0-3.6)	263	1.9 (0.7-3.3)	302	1.9 (0.7-3.6)	255	2.6 (1.0-5.0)	0.001
Maximal baPWV (cm/s)	568	1546 ± 328	159	1500 ± 228	203	1526 ± 345	206	1603 ± 647	0.006
Maximal CIMT (cm)	354	0.090 ± 0.017	102	0.091 ± 0.016	136	0.089 ± 0.017	116	0.091 ± 0.018	0.593
Carotid plaques	354	57 (16.1)	102	10 (9.8)	136	19 (14.0)	116	28 (24.1)	0.011
High risk CIMT	354	122 (34.5)	102	30 (29.4)	136	45 (33.1)	116	47 (40.5)	0.207

Continuous variables were presented as mean ± SD (*standard deviation*) or median (*interquartile range*). Categorical variables were presented as number (*percentage*). High risk CIMT included CIMT thickness ≥ 0.1 cm and/or carotid plaque. One-way ANOVA or nonparametric tests were used for comparisons of continuous variables between sVAP-1 tertiles, and χ^2 test was used for the comparisons of categorical variables between sVAP-1 tertiles.

Table II. Associations between plasma sVAP-1 concentration and cardiovascular risk factors adjusting for age and sex.

Characteristic	All*			Women**			Men**		
	n	r/mean ± SD	p	n	r/mean ± SD	p	n	r/mean ± SD	p
Continuous variables									
Age	834	0.286	< **0.001**$^{\#\#}$	303	0.257	< **0.001**$^{\#\#}$	531	0.266	< **0.001**$^{\#\#}$
Systolic BP (mmHg)	825	0.020	0.561	301	-0.039	0.499	524	0.063	0.151
Diastolic BP (mmHg)	825	0.036	0.301	301	0	0.990	524	0.061	0.161
BMI (kg/m^2)	825	-0.118	< **0.001**$^{\#\#}$	301	-0.223	< **0.001**$^{\#\#}$	524	-0.046	0.296
FPG (mmol/L)	829	0.128	< **0.001**$^{\#\#\#}$	300	0.124	0.032$^{\#}$	529	0.215	< **0.001**$^{\#\#}$
HbA1c (%)	668	0.163	< **0.001**$^{\#\#\#}$	251	0.136	0.032$^{\#}$	417	0.182	< **0.001**$^{\#\#\#}$
Triglycerides (mmol/L)	829	-0.053	0.128	300	-0.099	0.087	529	-0.042	0.334
LDL (mmol/L□	829	0.068	0.052	300	0.085	0.142	529	0.057	0.190
HDL (mmol/L)	829	0.001	0.966	300	0.045	0.434	529	-0.029	0.502
ApoB (g/L)	667	0.033	0.393	249	0.049	0.442	418	0.025	0.614
ApoA (g/L)	667	-0.065	0.096	249	0.102	0.109	418	-0.162	< **0.001**$^{\#\#\#}$
ApoB/ApoA	667	0.082	0.035#	249	-0.011	0.862	418	0.132	0.007$^{\#\#}$
Uric acid (μmol/L)	829	-0.079	0.023$^{\#}$	300	-0.025	0.663	529	-0.108	0.013$^{\#\#}$
Normal score of lipoprotein(a)	667	0.031	0.481	249	-0.051	0.427	418	0.080	0.102
Normal score of homocysteine	625	-0.013	0.743	241	-0.035	0.593	384	0.006	0.900
Normal score of progesterone	–	–	–	189	-0.018	0.812	–	–	–
Normal score of Chinese absolute risk for ICVD***	820	–	–	298	0.167	0.004$^{\#\#}$	522	0.257	< **0.001**$^{\#\#\#}$

Table continued

Table II *(Continued)*. Associations between plasma sVAP-1 concentration and cardiovascular risk factors adjusting for age and sex.

Characteristic		All*			Women**			Men**		
		n	r/mean ± SD	*p*	n	r/mean ± SD	*p*	n	r/mean ± SD	*p*
Categorical variables										
Sex	Women	303	18.73 ± 2.83	< **0.001**$^{###}$						
	Men	531	17.47 ± 2.53							
Smoking	yes	224	17.65 ± 2.36	0.300	2	16.42 ± 3.61	0.343	222	17.66 ± 2.36	0.221
	no	610	18.03 ± 2.82		301	18.75 ± 2.82		309	17.34 ± 2.65	
Alcohol use	yes	284	17.04 ± 2.64	< **0.001**$^{###}$	5	18.64 ± 2.64	0.864	279	17.02 ± 2.64	< **0.001**$^{###}$
	no	550	18.39 ± 2.63		298	18.73 ± 2.83		252	17.98 ± 2.31	
Hypertension	yes	199	18.02 ± 2.61	0.689	50	18.98 ± 2.54	0.371	149	17.70 ± 2.56	0.91
	no	635	17.9 0 ± 2.74		253	16.68 ± 2.88		382	17.38 ± 2.52	
Diabetes mellitus	yes	89	19.16 ± 3.02	< **0.001**$^{###}$	18	20.75 ± 3.77	0.003$^{##}$	71	18.76 ± 2.68	< 0.001$^{###}$
	no	745	17.78 ± 2.64		285	18.60 ± 2.71		460	17.27 ± 2.45	
BMI (kg/m^2)	< 18.5	35	19.02 ± 3.10	**0.007**$^{##}$	28	19.10 ± 3.36	0.007$^{##}$	7	18.70 ± 1.87	0.358
	18.5-25	348	18.21 ± 2.92		165	18.87 ± 2.88		183	17.61 ± 2.84	
	≥ 25	442	17.64 ± 2.46		108	18.39 ± 2.57		334	17.39 ± 2.38	
Estradiol (pmol/L)	< 100	99	19.54 ± 2.51	**0.001**$^{###}$						
	≥ 100	91	17.99 ± 2.42							
CRP (mg/L)	< 3	314	18.11 ± 2.44	0.859	139	18.82 ± 2.56	0.337	175	17.54 ± 2.18	0.334
	≥ 3	164	18.15 ± 2.77		49	18.77 ± 2.54		115	17.89 ± 2.83	
Chinese absolute risk for ICVD* **										
Low risk (< 5%)		646			258	18.59 ± 2.84	0.020$^{#}$	388	17.13 ± 2.48	< **0.001**$^{###}$
Middle risk (5-10%)		154			36	19.20 ± 2.56		118	18.36 ± 2.36	
High risk (≥ 10%)		20			4	22.23 ± 1.98		16	19.70 ± 2.77	

sVAP-1 was square root-transformed (SQRT). Lipoprotein(a), homocysteine, progesterone and Chinese absolute risk for ICVD were transformed into normal distribution by Blom's Formula. Correlations of continuous variables with SQRT (sVAP-1) were reported as r. SQRT (sVAP-1) was reported as mean ± SD according to categorical variables. Correlations were analyzed by partial correlations. *Adjusting for age and sex, **Adjusting for age and ***No adjustments. Corrections for p-value of < 0.05 due to multiple testing were carried out by means of Benjamini and Hochberg False Discovery Rate. $^{#}$Corrected $p \geq 0.05$, $^{##}$Corrected $p < 0.05$ and $^{###}$Corrected $p < 0.01$. sVAP-1 = soluble vascular adhesion protein 1; BP = blood pressure; BMI = body mass index; FPG = Fasting plasma glucose HbA1c = hemoglobin AIc; LDL = low density lipoprotein; HDL = high density lipoprotein; ICVD = ischemic cardiovascular disease; CRP = C-reactive protein.

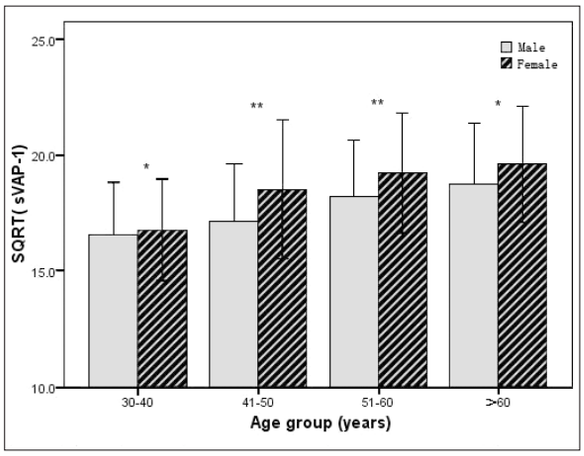

Figure 1. Effects of age and sex on plasma sVAP-1 concentration. sVAP-1 concentration was transformed into normal distribution by SORT (square root). SORT (sVAP-1) was significantly higher in women than in man in 41-50 years (18.5 vs. 17.2) and 51-60 years (19.2 vs. 18.2), but not in 30-40 years (16.8 vs. 16.6). In addition, women had a tendency toward higher sVAP-1 in subjects ≥ 60 years (19.6 vs. 18.8). *$p \geq 0.05$, **$p < 0.001$. sVAP-1 = soluble vascular adhesion protein 1.

sVAP-1 concentration in subjects of older than 60 y. The women with higher estradiol (≥ 100 pmol/L) had a lower sVAP-1 concentration than those with lower estradiol (< 100 pmol/L), but progesterone did not correlate with sVAP-1 concentration in women (Table II).

Correlation of sVAP-1 Concentration with Other Cardiovascular Risk Factors

The correlations between plasma sVAP-1 concentration and other cardiovascular risk factors were analyzed adjusting for age and sex, and separately for women and men because of the effects of age and sex on sVAP-1 concentration. The correlations between plasma sVAP-1 concentration and metabolic factors were analyzed separately for hyperglycemic and normoglycemic subjects, since sVAP-1 has insulin-like actions and is involved in glucose metabolism[3].

Blood pressure (BP) and hypertension did not correlate with sVAP-1 concentration (Table II). The sVAP-1 concentration in the subjects with diabetes mellitus was significantly higher than in those without diabetes mellitus in both women and men (Table II). Fasting plasma glucose level and hemoglobin A1c (HbA1c) also correlated positively with sVAP-1 concentration in all subjects (Table II) and in hyperglycemic subjects (Table III). Body mass index (BMI) correlated negatively with sVAP-1 concentration in women and normoglycemic subjects after adjusting for age and sex, but not in men and hyperglycemic subjects (Tables II and III). When compared to overweight women, lean women had higher sVAP-1 concentrations (Table II). In men, sVAP-1 concentration had an inverse correlation with ApoA and a positive correlation with ApoB/ApoA, but not with other type of lipids after adjusting for age (Table II). In women, sVAP-1 concentration had no correlation with any type of lipids after adjusting for age (Table II). In normoglycemic subjects, an inverse association between triglycerides and sVAP-1 concentration was observed after adjusting for age and sex, but not in hyperglycemic subjects (Table III). sVAP-1 concentration of current smokers was not different from non-smokers in either sex (Table II) or in either glucose level (Table III). However, subjects with current alcohol use had lower sVAP-1 concentration than those without alcohol use in men and normoglycemic subjects, but not in women and hyperglycemic subjects after adjusting for age and sex. Uric acid also had an inverse correlation to sVAP-1 concentration in men, but not in women after adjusting for age (Table II). Homocysteine and CRP had no correlation to sVAP-1 concentration in either sex or in either glucose level (Tables II and III).

Correlation of sVAP-1 Concentration with Cardiovascular Risk Score

As a total cardiovascular risk assessment (both coronary heart disease and stroke), Chinese cardiovascular risk score is calculated by age and separately for women and men. Therefore, the association between plasma sVAP-1 concentration and cardiovascular risk score was analyzed not by adjusting for age and sex, but in women and men separately. In both women and men, sVAP-1 concentration correlated positively with cardiovascular risk score (Table II). In men, compared to the subjects with middle risk (5-10%), those with low risk (< 5%) had lower sVAP-1 level and those with high risk (≥ 10%) had higher sVAP-1 level (Table II).

Correlation of sVAP-1 Concentration with Subclinical Atherosclerosis

Since age, sex, BP and glucose levels are all associated with the CIMT and baPWV, we analyzed the effect of sVAP-1 concentration on CIMT and baPWV in subgroups according to

Table III. Associations between plasma sVAP-1 concentration and metabolic factors in subgroups according to glucose level.

Characteristic		Hyperglycemic subjects			Normoglycemic subjects		
		n	r/mean ± SD	p	n	r/mean ± SD	p
Continuous variables							
BMI (kg/m^2)		154	-0.164	**0.043**$^{\#}$	671	-0.129	**0.001**$^{\#\#\#}$
FPG (mmol/L)		155	0.272	**0.001**$^{\#\#}$	673	0	0.989
HbA1c (%)		127	0.239	**0.007**$^{\#\#}$	541	-0.022	0.607
Triglycerides (mmol/L)		155	-0.004	0.956	673	-0.103	**0.007**$^{\#\#}$
LDL (mmol/L)		155	0.145	0.073	673	0.040	0.296
HDL (mmol/L)		155	0.088	0.277	673	0.003	0.946
ApoB (g/L)		129	0.107	0.229	538	-0.011	0.808
ApoA (g/L)		129	-0.121	0.177	538	-0.033	0.449
ApoB/ ApoA		129	0.200	**0.024**$^{\#}$	538	0.017	0.702
Uric acid (μmol/L)		155	-0.109	0.182	673	-0.080	**0.037**$^{\#}$
Normal score of lipoprotein(a)		129	0.135	0.131	538	0	1.000
Normal score of homocysteine		124	0.125	0.171	501	-0.035	0.434
Categorical variables							
Smoking	Yes	51	18.24 ± 2.70	0.791	173	17.48 ± 2.23	0.167
	No	104	18.95 ± 3.05		506	17.84 ± 2.74	
Alcohol use	Yes	55	17.80 ± 2.69	0.170	229	16.86 ± 2.60	□0.001$^{\#\#}$
	No	100	19.21 ± 2.98		450	18.20 ± 2.52	

Hyperglycemia included diabetes mellitus and impaired fasting glucose. sVAP-1 was square root-transformed (SQRT). Lipoprotein(a), homocysteine were transformed into normal distribution by Blom's Formula. Correlations of continuous variables with SQRT (sVAP-1) were reported as r. SQRT (sVAP-1) was reported as mean ± SD according to categorical variables. Correlations were analyzed by partial correlations. *Adjusting for age and sex, **Adjusting for age and ***No adjustments. Corrections for p-value of < 0.05 due to multiple testing were carried out by means of Benjamini and Hochberg False Discovery Rate. $^{\#}$Corrected $p \geq 0.05$, $^{\#\#}$Corrected $p < 0.05$ and $^{\#\#\#}$Corrected $p < 0.01$. sVAP-1 = soluble vascular adhesion protein 1; BMI = body mass index; FPG = Fasting plasma glucose HbA1c = hemoglobin AIc; LDL = low density lipoprotein; HDL = high density lipoprotein.

age, sex, hypertension and hyperglycemia. Although old age is defined as > 60 years in the developing countries such as China, we divided our subjects into younger and older groups by 55 years because of the small number of > 60 years persons in our study.

There was no association between CIMT and sVAP-1 concentration in all subjects after adjusting for age, sex, smoking, alcohol use, hypertension, diabetes mellitus, BMI, low-density lipoprotein (LDL) and triglycerides (Table IV). However, sVAP-1 concentration was positively associated with CIMT in subjects with hyperglycemia, but not in normoglycemic subjects (Table IV). Because there were only 57 subjects with carotid plaques (Table I), we selected high risk CIMT (CIMT thickness ≥ 0.1 cm and/or carotid plaque) as the dependent variable to determine the effect of sVAP-1 concentration on carotid plaques. Similar to CIMT, sVAP-1 concentration was an independent risk factor for carotid plaques only in hyperglycemic subjects (Table V). Although there was also no association between baPWV and sVAP-1 concentration in all subjects after adjusting for above confounders, sVAP-1 concentration was positively associated with baPWV in subgroups of age > 55 years (Table IV).

Discussion

Our study showed that plasma sVAP-1 concentration was higher in older participants, women, and diabetes mellitus. sVAP-1 concentration correlated with the cardiovascular risk factors and subclinical atherosclerosis in an age-, sex- and glucose-dependent manner.

Our results were in line with previous reports in which sVAP-1 concentration positively correlated with age in a Finnish population[7,8] and in a Taiwanese population[11,15]. There are two possible mechanisms for the observed correlation between sVAP-1 concentration and age. Firstly, abundant experimental and clinical data show that aging is associated with chronic low-grade inflammation[17]. Even in normal healthy aging, there is an up-regulation of inflammatory cytokines, chemokines and

Table IV. Effect of plasma sVAP-1 concentration on baPWV and CIMT in multivariable linear regression.

Sub-groups	n	Model 1			Mode 2			Model 3			Model 4			Model 5		
		β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*	β	SE	*p*
								Maximal baPWV								
All	568	-0.256	4.282	0.952												
Age ≤ 55 years	424	-5.886	3.919	0.134												
Age ≥ 55 years	144	31.605	12.752	**0.014**												
Women	210				3.129	6.522	0.632									
Men	358							-1.805	5.744	0.748						
Hypertension (yes)	162										7.578	11.059	0.494			
Hypertension (no)	406										-3.920	4.205	0.352			
Hyperglycemia (yes)	117													9.680	11.119	0.386
Hyperglycemia (no)	451													-2.599	4.497	0.564
								Maximal CIMT								
All	354	0	0	0.675												
Age ≤ 55 years	226	0	0	0.970												
Age > 55 years	88	0	0.001	0.508												
Women	128				0	0.001	0.307									
Men	266							0	0	0.773						
Hypertension (yes)	87										0.001	0.001	0.144			
Hypertension (no□	267										0	0	0.117			
Hyperglycemia (yes)	63													0.001	0.001	0.040
Hyperglycemia (no)	291													0	0	0.225

sVAP-1 was transformed into normal distribution by square root. sVAP-1 = soluble vascular adhesion protein 1; baPWV = brachial-ankle pulse wave velocity; CIMT = carotid intima-media thickness. Model 1 was adjusted for all the variables (age, sex, smoking, alcohol use, hypertension, diabetes mellitus, body mass index, triglyceride, low density lipoproteins); Model 2 were adjusted for all the variables except sex, smoking and alcohol use because of few smoking and alcohol users in women; Model 3 was adjusted for all the variables except sex; Model 4 was adjusted for all the variables except hypertension; Model 5 was adjusted for all the variables except diabetes mellitus.

Table V. Effect of plasma sVAP-1 concentration on high risk CIMT in logistic regression.

Sub-groups	Number of high risk CIMT (yes/no)	Model 1		Model 2		Model 3		Model 4		Model 5	
		Adjusted Odds Ratio (95% CI)	p	Adjusted Odds Ratio (95% CI)	p	Adjusted Odds Ratio (95% CI)	p	Adjusted Odds Ratio (95% CI)	p	Adjusted Odds Ratio (95% CI)	p
All	122/232	0.999 (0.903-1.105)	0.983								
Age ≤ 55 years	67/199	0.979 (0.869-1.104)	0.731								
Age > 55 years	55/33	1.090 (0.875-1.358)	0.440								
Women	43/85			0.930 (0.793-1.090)	0.368						
Men	79/147					1.053 (0.910-1.219)	0.488				
Hypertension (yes)	43/44							1.253 (0.952-1.647)	0.107		
Hypertension (no)	79/188							0.941 (0.384-1.061)	0.321		
Hyperglycemia (yes)	25/38									1.308 (1.051-1.813)	0.021
Hyperglycemia (no)	97/194									0.946 (0.804-1.065)	0.359

sVAP-1 was transformed into normal distribution by square root. sVAP-1 = soluble vascular adhesion protein 1; baPWV = brachial-ankle pulse wave velocity; CIMT = carotid intima-media thickness. Model 1 was adjusted for all the variables (age, sex, smoking, alcohol use, hypertension, diabetes mellitus, body mass index, triglyceride, low density lipoproteins); Model 2 were adjusted for all the variables except sex, smoking and alcohol use because of few smoking and alcohol users in women; Model 3 was adjusted for all the variables except sex; Model 4 was adjusted for all the variables except hypertension; Model 5 was adjusted for all the variables except diabetes mellitus.

adhesion molecules both in laboratory rodents and in primates[18]. In humans, plasma concentrations of several inflammatory markers (e.g. soluble vascular cell adhesion protein 1, soluble intercellular adhesion molecule 1, E-selectin) are positively correlated with age, independent of other cardiovascular risk factors[18]. Since sVAP-1 is also an adhesion molecule and involved in inflammation[1], it is logical to assume that sVAP-1 concentration increases with age similar to other adhesion molecules. Secondly, previous studies have reported that fasting plasma glucose levels increase with age[19], which may be associated with impaired insulin secretion and insulin resistance[20]. Many studies demonstrate thatβ-cell function (or insulin secretion) and insulin sensitivity decrease with age in human[20]. Stolen et al[21] demonstrate that hyperglycemia alone can up-regulate sVAP-1 concentration in mice, and Abella et al[22] have shown that insulin can down-regulate the release of VAP-1 into the culture media of 3T3-L1 adipocytes. In humans, the insulin is a negative regulator[6], and acute and chronic hyperglycemia are positive regulators for plasma sVAP-1 concentration[13,14]. The relationship between sVAP-1 concentration and glucose/insulin level may be likely due to the insulin-like effects of sVAP-1[3]. This relationship can explain that sVAP-1 concentration will increase with age to compensate for decrease of insulin secretion and insulin sensitivity.

We found that women had higher sVAP-1 concentration than men in the subjects older than 40 years that is close to mean natural menopause age[23]. Because the estradiol level after menopause is < 100 pmol/L[24], we compared the sVAP-1 concentration of women with estradiol ≥ 100 pmol/L (premenopausal) with those with estradiol < 100 pmol/L (postmenopausal). The result showed that a low estrogen level corresponded to a higher sVAP-1 concentration, which suggested that women might have a higher sVAP-1 concentration than men after 40 years old due to decrease of estrogen level. Both menopause and ovariectomy may cause low-grade systemic inflammation which may be prevented by treatment with low doses of estrogen, suggesting that estrogen might have anti-inflammatory effects[25]. Thus, the inverse association between estrogen and sVAP-1 observed in the present study may be explained by their contrasting roles in the inflammatory state.

Previous studies[6,8,26] have produced conflicting results on the relation of sVAP-1 activity with

BMI and blood lipids. We think that glucose level and sex might be two confounders for these associations. First, we unexpectedly found that sVAP-1 concentration was inversely correlated with BMI and triglycerides only in normoglycemic subjects, but not in hyperglycemic subjects. Human adipocytes express a membrane-bound SSAO/sVAP-1 that interplays with glucose and lipid metabolism by exerting insulin-like effects[27]. Hydrogen peroxide, one of the products of SSAO activity, has been considered as an insulin mimicker[28]. These insulin-like actions include stimulation of glucose uptake by recruitment of the glucose transporter type 4 (GLUT4) to the cell membranes, improvement of peripheral glucose utilization, inhibition of fat cell lipolysis, and stimulation of lipogenesis[3,27]. Therefore, in hyperglycemic subjects, hyperglycemia and lower insulin induce the elevated plasma SSAO/sVAP-1[26], which masks the real relationship between plasma SSAO/sVAP-1 and BMI or lipids. Second, we observed inverse correlations of sVAP-1 concentration with BMI in women, which is in line with the earlier findings[8]. In men, we found that sVAP-1 had an inverse correlation to ApoA and positive correlation to ApoB/ApoA. Since ApoB and ApoA are equivalent or superior to cholesterol-based indices with respect to risk of coronary artery disease, sVAP-1 may have an association with different atherogenic forms of lipids in different sexes. Many studies have reported differences of cardiovascular risk factors including BMI and lipids between both sexes[29], which may be the reason for sex-dependent correlation of sVAP-1 to BMI and lipids.

This is the first study to find a negative association of sVAP-1 with current alcohol use and uric acid in human, and these associations were gender- and glucose-dependent. Indeed, uric acid is the most abundant aqueous antioxidant, accounting for up to 60% of plasma anti-oxidative capacity[30]. At the same time, wine consumption elevated the plasma antioxidant capacity mediated by two separate factors, wine phenols and plasma uric acid[31]. In addition, alcohol intake also has an anti-inflammatory action independent of the type of beverage consumed in the population[32]. Moderate alcohol consumption appears to reduce expression of some inflammatory molecules, such as ICAM-1 (intercellular adhesion molecule 1), IL-6 and CRP[33]. Some prospective studies also show an inverse relationship between moderate alcohol consumption and these inflammatory markers[34]. Since sVAP-1 has both inflammatory and oxidative function[1,2], the inverse correlation of sVAP-1 to alcohol use and uric acid is not surprising. In our study (data not shown) and other studies, men have higher serum uric acid concentration than women by approximately 1.4 mg/dL[35], and men are heavier alcohol drinkers than women[36]. Therefore, these gender differences may determine the inverse association of sVAP-1 with uric acid and alcohol use only in men. Moreover, the reason that this negative association of sVAP-1 with current alcohol use was only in normoglycemic subjects, may be that the hyperglycemia influences the sVAP-1 concentration[8,14] and counteract the effect of alcohol use on sVAP-1.

Both we and Aalto et al[8] showed that sVAP-1 was positively associated with global risk assessment of ischemic cardiovascular diseases in a population level. In addition, Li et al[11] showed that sVAP-1 could predict 10-year cardiovascular mortality in type 2 diabetic mellitus, independent of traditional risk factors. Recently, a study7] showed that inclusion of sVAP-1 in the Framingham model could improve the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) of incident major adverse cardiovascular events. Therefore, sVAP-1 might be a favorable biomarker associated with cardiovascular diseases.

Similar to some early studies[8,13,14], our work also showed that high sVAP-1 concentration, positively correlated with fasting plasma glucose and HbA1c, was an independent risk factor for CIMT increase and carotid plaques in hyperglycemic subjects, but not in normoglycemic subjects. Therefore, glucose level may not influence sVAP-1 concentration in normoglycemia subjects, but stimulates sVAP-1 increase in hyperglycemic subjects to reach sufficient high concentration leading to vascular complications.

For the first time, our report has shown that sVAP-1 concentration was positively associated with baPWV in subjects aged > 55 years after adjusting for other confounding factors. This suggests that sVAP-1 concentration is an independent risk factor for arterial stiffness in the older participants. The association between sVAP-1 and arterial stiffness has been demonstrated in the previous animal studies. Two investigations[37,38] have shown that the overexpression of SSAO/VAP-1 can affect the elastic fibers of arterial wall and lead to an increased arterial stiffness in the transgenic mice.

An increase in SSAO activity can regulate vascular smooth muscle cell (VSMC) tone by the production of hydrogen peroxide in the rats[39]. Two reasons may explain that this association exists only in the older participants in our study. First, PWV may be a more sensitive marker for arterial stiffness in older persons since it shows a concave curvilinear relationship with age[40,41]. Second, because sVAP-1 increases with age, it is reasonable to assume that sVAP-1 has an effect on arterial stiffness only in older persons with higher sVAP-1 concentration.

The strength of the study is that we analyzed the association of sVAP-1 concentration with cardiovascular risk factors and subclinical atherosclerosis in subgroups stratified by age, sex and glucose level. However, the inconsistent results of some previous studies[8] may be due to not considering the effects of age, sex and glucose level on sVAP-1. In addition, we analyzed for the first time the association of sVAP-1 concentration with sex hormone, homocysteine, uric acid and baPWV.

The present study has a number of limitations: (1) Investigation of testosterone is not included. In our paper, an inverse association of sVAP-1 with estradiol is found, therefore, probably testosterone is also related to sVAP-1. Testosterone will be included in the future study. (2) Detailed information of daily and accumulated alcohol consumption and cigarette is absent, therefore the dose-dependent relationship between sVAP-1 and alcohol and cigarette is not available. The detailed information will be included in the future study. (3) All subjects in this study are only Han Chinese, thus it should be careful to extrapolate this finding to other population.

Conclusions

sVAP-1 concentration is an independent determinant of CIMT and carotid plaques in hyperglycemic subjects and of baPWV in the older participants. Plasma sVAP-1 concentration is also associated with both traditional and new cardiovascular risk factors, and these associations are dependent on age, sex and glucose level. In addition, sVAP-1 concentration correlates with the global risk score for Chinese ICVD. These results suggest that sVAP-1 concentration correlates to cardiovascular risk factors and subclinical atherosclerosis in an age-, sex- and glucose-dependent manner.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Merinen M, Irjala H, Salmi M, Jaakkola I, Hänninen A, Jalkanen S. Vascular adhesion protein-1 is involved in both acute and chronic inflammation in the mouse. Am J Pathol 2005; 166: 793-800.
2) Jalkanen S, Salmi M. VAP-1 and CD73, endothelial cell surface enzymes in leukocyte extravasation. Arterioscler Thromb Vasc Biol 2008; 28: 18-26.
3) Obata T. Diabetes and semicarbazide-sensitive amine oxidase (SSAO) activity: a review. Life Sci 2006; 79: 417-422.
4) Boomsma F, Hut H, Bagghoe U, van der Houwen A, van den Meiracker A. Semicarbazide-sensitive amine oxidase (SSAO): from cell to circulation. Med Sci Monit 2005; 11: RA122-126.
5) Salmi M, Jalkanen S. VAP-1: an adhesin and an enzyme. Trends Immunol 2001; 22:211-216.
6) Salmi M, Stolen C, Jousilahti P, Yegutkin GG, Tapanainen P, Janatuinen T, Knip M, Jalkanen S, Salomaa V. Insulin-regulated increase of soluble vascular adhesion protein-1 in diabetes. Am J Pathol 2002; 161: 2255-2262.
7) Aalto K, Havulinna AS, Jalkanen S, Salomaa V, Salmi M. Soluble vascular adhesion protein-1 predicts incident major adverse cardiovascular events and improves reclassification in a Finnish prospective cohort study. Circ Cardiovasc Genet 2014; 7: 529-535.
8) Aalto K, Maksimow M, Juonala M, Viikari J, Jula A, Kähönen M, Jalkanen S, Raitakari OT, Salmi M. Soluble vascular adhesion protein-1 correlates with cardiovascular risk factors and early atherosclerotic manifestations. Arterioscler Thromb Vasc Biol 2012; 32: 523-532.
9) Koc-Zorawska E, Malyszko J, Malyszko JS, Mysliwiec M. VAP-1, a novel molecule linked to endothelial damage and kidney function in kidney allograft recipients. Kidney Blood Press Res 2012; 36: 242-247.
10) Eirin A, Zhu XY, Woollard JR, Herrmann SM, Gloviczki ML, Saad A, Juncos LA, Calhoun DA, Rule AD, Lerman A, Textor SC, Lerman LO. Increased circulating inflammatory endothelial cells in blacks with essential hypertension. Hypertension 2013; 62: 585-591.
11) Li HY, Jiang YD, Chang TJ, Wei JN, Lin MS, Lin CH, Chiang FT, Shih SR, Hung CS, Hua CH, Smith DJ, Vanio J, Chuang LM. Serum vascular adhesion protein-1 predicts 10-year cardiovascular and cancer mortality in individuals with type 2 diabetes. Diabetes 2011; 60: 993-999.
12) Nemati H, Khodarahmi R, Rahmani A, Ebrahimi A, Amani M, Eftekhari K. Serum lipid profile in psori-

atic patients: correlation between vascular adhesion protein 1 and lipoprotein (a). Cell Biochem Funct 2013; 31: 36-40.

13) Li HY, Lin MS, Wei JN, Hung CS, Chiang FT, Lin CH, Hsu HC, Su CY, Wu MY, Smith DJ, Vainio J, Chen MF, Chuang LM. Change of serum vascular adhesion protein-1 after glucose loading correlates to carotid intima-medial thickness in non-diabetic subjects. Clin Chim Acta 2009; 403: 97-101.

14) Li HY, Wei JN, Lin MS, Smith DJ, Vainio J, Lin CH, Chiang FT, Shih SR, Huang CH, Wu MY, Hsein YC, Chuang LM. Serum vascular adhesion protein-1 is increased in acute and chronic hyperglycemia. Clin Chim Acta 2009; 404: 149-153.

15) Wang YC, Li HY, Wei JN, Lin MS, Shih SR, Hua CH, Smith DJ, Vanio J, Chuang LM. Ann Hum Biol 2013; 40: 413-418.

16) Wu Y, Liu X, Li X, Li Y, Zhao L, Chen Z, Li Y, Rao X, Zhou B, Detrano R, Liu K, USA-PRC Collaborative Study of Cardiovascular and Cardiopulmonary Epidemiology Research Group, China Multicenter Collaborative Study of Cardiovascular Epidemiology Research Group. Estimation of 10-year risk of fatal and nonfatal ischemic cardiovascular diseases in Chinese adults. Circulation 2006; 114: 2217-2225.

17) Goto M. Inflammaging (inflammation + aging): A driving force for human aging based on an evolutionarily antagonistic pleiotropy theory? Biosci Trends 2008; 2: 218-230.

18) Ungvari Z, Kaley G, de Cabo R, Sonntag WE, Csiszar A. Mechanisms of vascular aging: new perspectives. J Gerontol A Biol Sci Med Sci 2010; 65: 1028-1041.

19) Toyoda K, Fukushima M, Mitsui R, Harada N, Suzuki H, Takeda T, Taniguchi A, Nakai Y, Kawakita T, Yamada Y, Inagaki N, Seino Y. Factors responsible for age-related elevation in fasting plasma glucose: a cross-sectional study in Japanese men. Metabolism 2008; 57: 299-303.

20) Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 1999; 104: 787-794.

21) Stolen CM, Yegutkin GG, Kurkijärvi R, Bono P, Alitalo K, Jalkanen S. Origins of serum semicarbazide-sensitive amine oxidase. Circ Res 2004; 95: 50-57.

22) Abella A, García-Vicente S, Viguerie N, Ros-Baró A, Camps M, Palacín M, Zorzano A, Marti L. Adipocytes release a soluble form of VAP-1/SSAO by a metalloprotease-dependent process and in a regulated manner. Diabetologia 2004; 47: 429-438.

23) Schoenaker DA, Jackson CA, Rowlands JV, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. Int J Epidemiol 2014; 43: 1542-1562.

24) Atkinson C, Compston JE, Day NE, Dowsett M, Bingham SA. The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2004; 79: 326-333.

25) Abu-Taha M, Rius C, Hermenegildo C, Noguera I, Cerda-Nicolas JM, Issekutz AC, Jose PJ, Cortijo J, Morcillo EJ, Sanz MJ. Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of estrogen or losartan. J Immunol 2009; 183: 1393-1402.

26) Mészáros Z, Szombathy T, Raimondi L, Karádi I, Romics L, Magyar K. Elevated serum semicarbazide-sensitive amine oxidase activity in non-insulin-dependent diabetes mellitus: correlation with body mass index and serum triglyceride. Metabolism 1999; 48: 113-117.

27) Morin N, Lizcano JM, Fontana E, Marti L, Smih F, Rouet P, Prévot D, Zorzano A, Unzeta M, Carpéné C. Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. J Pharmacol Exp Ther 2001; 297: 563-572.

28) Hayes GR, Lockwood DH. Role of insulin receptor phosphorylation in the insulinomimetic effects of hydrogen peroxide. Proc Natl Acad Sci U S A 1987; 84: 8115-8119.

29) Thomas GN, McGhee SM, Schooling M, Ho SY, Lam KS, Janus ED, Lam TH, Hong Kong Cardiovascular Risk Factor Prevalence Study Steering Committee. Impact of sex-specific body composition on cardiovascular risk factors: the Hong Kong Cardiovascular Risk Factor Study. Metabolism 2006; 55: 563-569.

30) Fabbrini E, Serafini M, Colic Baric I, Hazen SL, Klein S. Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. Diabetes 2014; 63: 976-981.

31) Boban M, Modun D. Uric acid and antioxidant effects of wine. Croat Med J 2010; 51: 16-22.

32) Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, Koenig W. Effect of alcohol consumption on systemic markers of inflammation. Lancet 2001; 357: 763-767.

33) Bau PF, Bau CH, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. Alcohol 2007; 41: 479-488.

34) Pai JK, Hankinson SE, Thadhani R, Rifai N, Pischon T, Rimm EB. Moderate alcohol consumption and lower levels of inflammatory markers in US men and women. Atherosclerosis 2006; 186: 113-120.

35) Rodrigues SL, Baldo MP, Capingana P, Magalhães P, Dantas EM, Molina Mdel C, Salaroli LB, Morelato RL, Mill JG. Gender distribution of serum uric acid and cardiovascular risk factors: population based study. Arq Bras Cardiol 2012; 98: 13-21.

36) Oliveira A, Rodríguez-Artalejo F, Lopes C. Alcohol intake and systemic markers of inflammation--shape of the association according to sex and body mass index. Alcohol 2010; 45: 119-125.

37) Göktürk C, Nilsson J, Nordquist J, Kristensson M, Svensson K, Söderberg C, Israelson M, Garpenstrand H, Sjöquist M, Oreland L, Forsberg-Nilsson K. Overexpression of semicarbazide-sensitive amine oxidase in smooth muscle cells leads to an abnormal structure of the aortic elastic laminas. Am J Pathol 2003; 163: 1921-1928.

38) Gokturk C, Sugimoto H, Blomgren B, Roomans GM, Forsberg-Nilsson K, Oreland L, Sjoquist M. Macrovascular changes in mice overexpressing human semicarbazide-sensitive amine oxidase in smooth muscle cells. Am J Hypertens 2007; 20: 743-750.

39) Vidrio H, Medina M, González-Romo P, Lorenzana-Jiménez M, Díaz-Arista P, Baeza A. Semicarbazide-sensitive amine oxidase substrates potentiate hydralazine hypotension: possible role of hydrogen peroxide. J Pharmacol Exp Ther 2003; 307: 497-504.

40) Lee HY, Oh BH. Aging and arterial stiffness. Circ J 2010; 74: 2257-2262.

41) Reece AS. Deterioration of indices of aortic augmentation and vascular age predate major cardiac events and interact with opiate dependence. Int J Cardiol 2011; 149: e8-11.