Plasma sE-Selectin Level Is Positively Correlated with Neutrophil Count and Diastolic Blood Pressure in Japanese Men

Kazuki MOCHIZUKI^{1,2}, Seiya INOUE¹, Rie MIYAUCHI¹, Yasumi MISAKI¹, Masaya SHIMADA¹, Nobuhiko KASEZAWA³, Kazushige TOHYAMA³ and Toshinao GODA^{1,*}

¹Laboratory of Nutritional Physiology and Global COE Program, University of Shizuoka, Graduate School of Nutritional and Environmental Sciences, Shizuoka 422–8526, Japan

²Research Branch of Food and Nutritional Sciences, Department of Local Produce and Food Sciences, Faculty of Life and Environmental Sciences, University of Yamanashi, Yamanashi, Japan

³SBS Shizuoka Health Promotion Center, Shizuoka 422–8670, Japan

(Received April 16, 2013)

Summary Increased levels of circulating soluble type of E-selectin (sE-selectin), neutrophil counts and blood pressure are associated with the development of cardiovascular diseases (CVD). In this study, we conducted a cross-sectional study of men who participated in health check-ups, and selected those who were not diagnosed with or being treated for metabolic diseases such as diabetes, hypertension and lipid abnormality according to the health check-ups. We measured their basic clinical parameters including blood pressure and neutrophil count, plasma sE-selectin concentration and lifestyle factors, and assessed their interrelations by multivariate linear regression (MLR) analysis. A total of 351 subjects aged 47.5 ± 8.41 (range, 30–64) y were recruited. Significantly correlated with sE-selectin concentration were neutrophil count, diastolic blood pressure (DBP), and systolic blood pressure (SBP) (Pearson's correlation coefficient, 0.194, 0.220 and 0.175, respectively). MLR analysis showed that sE-selectin concentration was independently positively related with DBP and neutrophil count, whereas neutrophil count was positively associated with sE-selectin concentration but not DBP. DBP, but not SBP, was independently positively correlated with sE-selectin concentration but not neutrophil count. These results indicate that circulating sE-selectin concentration may be a biomarker for indicating subsequent development of metabolic diseases, in particular CVD, from a healthy state.

Key Words sE-selectin, neutrophil, blood pressure, Japanese

It is known that metabolic abnormalities, which are frequently related to lifestyle factors such as dietary habits, exercise and smoking, induce cardiovascular diseases (CVD) and related diseases such as heart failure. Many studies have demonstrated that hypertension, lipid abnormalities and hyperglycemia are associated with increased subsequent incidence of CVD (1). It is necessary to develop useful predictors for indicating subsequent development and progression of CVD, in particular from healthy subjects.

Many epidemiological studies have demonstrated that the circulating concentration of the soluble type of E-selectin (sE-selectin) is associated with CVD risk factors such as hypertension and lower high-density lipoprotein

cholesterol (HDL-C)/high low-density lipoprotein cholesterol (LDL-C) in healthy subjects and people with metabolic diseases in Western and Asian populations (2–5). E-selectin plays roles on the initial step of attachment of leukocytes (rolling adhesion) such as neutrophils and monocytes to vascular cells, and activates these leukocytes (6). Neutrophil attachment to vascular cells and leukocyte activation induces production of reactive oxygen species and release of destructive proteases, leading to vascular injury (7). In addition, monocyte attachment to vascular cells induces atherosclerosis by differentiating the macrophages and forming foam cells between the vascular cells (8). Therefore, increasing sE-selectin concentration would induce CVD by injuring vascular cells through activation of neutrophils and monocytes.

Several studies have demonstrated that a higher neutrophil count is associated with increased mortality in Asian and Western patients with chronic and acute heart failure (9–11). In addition, neutrophil count is positively associated with CVD risk factors in healthy Asian and Western people (12, 13). Because E-selectin is related to neutrophil function as mentioned above, circulating sE-selectin concentration may be closely associated with neutrophil count in healthy subjects

*To whom correspondence should be addressed.

E-mail: gouda@u-shizuoka-ken.ac.jp

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular diseases; DBP, diastolic blood pressure; γ -GTP, γ -glutamyl-transpeptidase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ln, natural logarithm; R^2 , coefficient of determination; SBP, systolic blood pressure; SD, standard deviation; SE, standard error; TC, total cholesterol; TG, triacylglycerol.

as well as in patients with heart failure. Indeed, animal studies have demonstrated that deficiency of E-selectin by gene targeting leads to reduced neutrophil counts as well as to CVD in mice (6, 14). These results suggest that E-selectin has a direct role in increasing neutrophil count. However, it is still unknown whether sE-selectin concentration is positively associated with neutrophil counts in healthy subjects as well as in patients with CVD.

In the present study, we investigated relations among circulating sE-selectin concentration, neutrophil count, and blood pressure in middle-aged Japanese men who were not diagnosed with or being treated for metabolic diseases such as diabetes, hypertension and lipid abnormality according to their health check-ups.

MATERIALS AND METHODS

Subjects. We conducted a cross-sectional study of 740 men aged 30–64 y who participated in health check-ups at Shizuoka Medical Center (Shizuoka Prefecture, Japan) between July 2005 and March 2007. Excluded were subjects being treated for stroke, hypertension, cardiac disease, diabetes, hyperlipidemia, liver diseases, kidney diseases, prostate gland diseases, gout, allergic diseases, psychiatric diseases, cancer and ulcer prior to the check-ups. Finally, we enrolled 351 subjects. Anthropometric data were recorded and blood samples collected from each participant by trained medical staff. The participants were asked about their smoking status and physical activity. Number of cigarettes consumed per day and duration of smoking were recorded and smoking status was indicated by the Brinkman index, which multiplies the number of cigarettes smoked per day by the duration of smoking (years). Physical activity was recorded as exercise times per week. Alcohol and energy intake during the preceding month were assessed by the Brief Self-administered Diet History Questionnaire (15). All subjects gave informed consent for the use of their personal information for this study. The study protocol was approved by the Ethics Committee of the University of Shizuoka (Shizuoka, Japan), based on the Declaration of Helsinki.

Measurements. After an overnight fast, height, weight, and blood pressure (diastolic blood pressure (DBP) and systolic blood pressure (SBP)) were measured and blood samples collected from each subject. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Plasma samples were kept at -80°C for subsequent assays. Total cholesterol (TC), HDL-C, LDL-C, triacylglycerol (TG), fasting serum glucose, hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl-transpeptidase (GTP), creatine, leukocyte count, and high-sensitive C-reactive protein (CRP) (latex agglutination method) were assessed. HbA1c in plasma was determined by the method based on the Japan Diabetes Society (JDS); values were converted to units of National Glycohemoglobin Standardization Program as “JDS value plus 0.4%.”

Plasma sE-selectin concentration was measured by

Table 1. Physical characteristics, anthropometric and body composition measures and lifestyle habits in 351 middle-aged men.

Characteristics	
sE-selectin (ng/mL)	32.9 ± 13.8
Age (y)	47.5 ± 8.41
BMI (kg/m^2)	24.2 ± 2.85
SBP (mmHg)	116.7 ± 14.2
DBP (mmHg)	74.9 ± 11.0
TC (mg/dL)	214.1 ± 30.9
HDL-C (mg/dL)	56.0 ± 14.8
LDL-C (mg/dL)	133.1 ± 27.5
TG (mg/dL)	$124 (83.5, 178)$
Fasting blood glucose (mg/dL)	97.6 ± 15.5
HbA1c (%)	5.57 ± 0.65
γ -GTP (U/L)	$38 (26, 61)$
AST (U/L)	24.5 ± 8.64
ALT (U/L)	$25 (18, 35)$
Creatinine (mg/dL)	0.86 ± 0.11
White blood cell count ($/\mu\text{L}, \times 10^3$)	6.27 ± 1.54
Neutrophil count ($/\mu\text{L}, \times 10^3$)	3.47 ± 1.22
Lymphocyte count ($/\mu\text{L}, \times 10^3$)	2.03 ± 0.52
Eosinophil count ($/\mu\text{L}, \times 10^3$)	0.23 ± 0.15
Basophil count ($/\mu\text{L}, \times 10^3$)	0.06 ± 0.03
Monocyte count ($/\mu\text{L}, \times 10^3$)	0.34 ± 0.11
Large unstained cell count ($/\mu\text{L}, \times 10^3$)	0.14 ± 0.05
CRP (mg/dL)	0.18 ± 0.37
Smoking status (Brinkman index)	$42 (0, 467.5)$
Physical activity (times/wk)	2.06 ± 2.50
Alcohol intake (g/d)	$21.1 (4.01, 55.9)$
Energy intake (kcal/d)	$2,229 \pm 579$

Data are expressed as mean $\pm$ SD or median (lower quartile, upper quartile).

Milliplex MAP Human Cardiovascular Immunoassay (Cat# HCVD1–67AK, Millipore, Tokyo, Japan) according to manufacturer's instructions. Plasma samples and standard proteins were incubated with sE-selectin antibody-immobilized beads at 4°C for 16 h. After washing, the beads were incubated with detection antibody conjugated with biotin at room temperature for 1 h and then the solutions were incubated with antibody conjugated with streptavidin-phycoerythrin at room temperature for 30 minutes. After washing, signals were detected by a Luminex instrument (Luminex Corporation, Austin, TX).

Statistical analysis. Clinical and biochemical data are presented as mean $\pm$ standard deviation (SD), percent, or median (lower quartile, upper quartile), calculated by Excel 2007 (Microsoft, Tokyo, Japan). Pearson's correlation coefficients were used to calculate correlations for all subjects. Tukey's multiple range tests based on one way analyses of variance (ANOVAs) for sE-selectin concentration, neutrophil count, SBP, and DBP were performed among tertiles (T1, T2 and T3) for age, BMI, SBP, DBP, TG, fasting blood glucose, ALT, neutrophil count, CRP, Brinkman index, physical activity, alcohol intake and energy intake. Multivariate linear regression (MLR) analyses for the dependent variables (sE-selectin,

Table 2. Correlation between sE-selectin, neutrophil count, DBP, SBP and other characteristics.

	sE-selectin	Neutrophil	SBP	DBP
sE-Selectin (ng/mL)	—	0.194***	0.175***	0.220***
Age (y)	−0.049	−0.128*	0.274***	0.204***
BMI (kg/m ²)	0.154**	0.148**	0.240***	0.239***
SBP (mmHg)	0.175***	0.043	—	0.791***
DBP (mmHg)	0.220***	0.041	0.791***	—
TC (mg/dL)	0.140**	−0.027	0.104	0.110*
HDL-C (mg/dL)	−0.025	−0.207***	−0.022	−0.009
LDL-C (mg/dL)	0.071	0.028	0.053	0.038
TG (mg/dL)	0.122*	0.064	0.172**	0.191***
Fasting blood glucose (mg/dL)	0.147**	0.083	0.173**	0.193***
HbA1c (%)	0.056	0.099	0.063	0.077
γ-GTP (U/L)	0.208***	0.029	0.122*	0.116*
AST (U/L)	0.379***	0.051	0.152**	0.180***
ALT (U/L)	0.400***	0.075	0.134*	0.171**
Creatinine (mg/dL)	−0.095	−0.032	0.048	0.064
White blood cell count (/μL, ×10 ³)	0.193***	0.912***	0.068	0.043
Neutrophil count (/μL, ×10 ³)	0.194***	—	0.043	0.041
Lymphocyte count (/μL, ×10 ³)	0.060	0.157**	0.097	0.050
Eosinophil count (/μL, ×10 ³)	0.016	0.062	−0.035	−0.083
Basophil count (/μL, ×10 ³)	0.180***	0.230***	0.070	0.077
Monocyte count (/μL, ×10 ³)	0.135*	0.675***	0.045	0.027
Large unstained cell count (/μL, ×10 ³)	0.171**	0.311***	0.007	−0.045
CRP (mg/dL)	0.063	0.263***	0.062	0.030
Smoking status (Brinkman index)	0.030	0.200***	0.014	−0.012
Physical activity (times/wk)	−0.089	−0.091	0.098	0.063
Alcohol intake (g/d)	0.111*	−0.022	0.125*	0.154***
Energy intake (kcal/d)	0.026	0.070	0.058	0.013

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

neutrophil count, SBP, and DBP) were performed using independent variables that were associated with the dependent variables by Pearson's correlation coefficient: DBP, HDL-C, TG, fasting blood glucose, ALT, neutrophil count, CRP, age, BMI, and lifestyle factors (Brinkman index, physical activity, alcohol intake and energy intake). We excluded SBP, LDL-C, γ-GTP activity, AST activity and counts of leukocytes except for neutrophils in the analysis because these factors were collinear with the selected independent variables. All statistical analyses were performed using Excel Statistics 2008 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

Physical characteristics, anthropometric and body composition measures and lifestyle habits in the subjects are shown in Table 1. Five, 124, 26 and 28 subjects had fasting blood glucose >125 mg/dL, TG >149 mg/dL, SBP >139 mmHg, and DBP >89 mmHg, respectively.

Table 2 shows Pearson's correlation coefficients of sE-selectin, neutrophil count, SBP or DBP, with other characteristics. Circulating sE-selectin concentration was positively associated with BMI, SBP, DBP, TC, TG, fasting blood glucose, γ-GTP, AST, ALT, white blood cell count, neutrophil count, basophil count, monocyte count, large unstained cell count and alcohol intake. The neutrophil count was positively associated with sE-selectin, BMI, white blood cell count, lymphocyte count, basophil

count, monocyte count, large unstained cell count, CRP, Brinkman index, and negatively associated with age and HDL-C. SBP was positively associated with sE-selectin, age, BMI, DBP, TG, fasting blood glucose, γ-GTP, AST, ALT and alcohol intake. DBP was positively associated with sE-selectin, age, BMI, SBP, TC, TG, fasting blood glucose, γ-GTP, AST, ALT and alcohol intake.

Table 3 shows ANOVA among tertiles (T1, T2 and T3) for age, BMI, SBP, DBP, TG, fasting blood glucose, ALT activity, neutrophil count, CRP, Brinkman index, physical activity, alcohol intake and energy intake. For SBP, DBP, fasting blood glucose, ALT, neutrophil count and CRP, sE-selectin concentration was higher in T3 than in T1. sE-selectin concentration in T1 of physical activity and in T3 of alcohol intake were higher than in T2. Neutrophil count was higher in T3 of CRP and Brinkman index than in T1. Neutrophil count in T1 of physical activity was higher than in T2. SBP was higher in T3 of age, BMI, DBP, fasting blood glucose and CRP than in T1. SBP in T3 of TG and energy intake was higher than in T2. DBP was higher in T3 of age, BMI, SBP, TG, fasting blood glucose and CRP than in T1. SBP in T1 and T3 of the Brinkman index and SBP in T3 of alcohol intake were higher than in T2.

Table 4 shows MLR analyses for the dependent variables (sE-selectin, neutrophil count, SBP, and DBP) using independent variables for sE-selectin, age, BMI, DBP, HDL-C, TG, fasting blood glucose, ALT, neutrophil

Table 3. Correlation of sE-selectin, neutrophil count or blood pressure with other characteristics.

Factor	Category	n	sE-selectin			Neutrophils			SBP			DBP		
			Means	SD	p	Means	SD	p	Means	SD	p	Means	SD	p
Age (y)	T1 (30–40)	109	32.8	16.9		3.67	1.52		112.6	10.3	a	72.2	10.4	a
	T2 (41–50)	114	34.2	13.3		3.40	0.89		115.7	15.3	a	74.6	11.0	ab
	T3 (51–64)	128	31.7	11.1		3.35	1.18		121.2	14.9	b	77.5	11.1	b
BMI (kg/m ²)	T1 (17.4–22.5)	107	30.7	13.9		3.30	1.17		113.0	13.9	a	72.7	11.3	a
	T2 (22.6–25.0)	122	32.8	12.5		3.45	1.40		115.3	13.2	a	73.1	10.6	a
	T3 (25.1–34.4)	122	34.8	14.7		3.63	1.05		121.4	14.2	b	78.7	10.3	b
SBP (mmHg)	T1 (86–110)	118	29.8	12.5	a	3.28	0.93		—			65.6	7.5	a
	T2 (111–120)	116	33.1	13.6	ab	3.64	1.59					74.5	6.9	b
	T3 (121–180)	117	35.7	14.7	b	3.48	1.02					84.8	8.8	c
DBP (mmHg)	T1 (39–69)	115	28.8	12.5	a	3.29	0.98		105.4	9.0	a	—		
	T2 (70–79)	121	32.6	13.1	a	3.66	1.27		115.6	8.6	b			
	T3 (80–108)	115	37.2	14.6	b	3.43	1.36		129.3	13.0	c			
TG (mg/dL)	T1 (30–99)	132	30.7	14.9		3.24	1.17		115.8	14.7	ab	73.0	11.9	a
	T2 (100–149)	95	33.2	12.3		3.61	1.12		114.3	13.7	a	74.3	10.1	ab
	T3 (150–1,101)	124	34.9	13.4		3.60	1.32		119.5	13.6	b	77.4	10.4	b
Fasting blood glucose (mg/dL)	T1 (77–90)	92	30.2	13.1	a	3.48	1.28		112.6	11.7	a	71.6	9.9	a
	T2 (91–99)	146	31.8	12.6	a	3.36	1.01		115.4	14.1	a	74.5	11.0	a
	T3 (100–287)	113	36.4	15.1	b	3.59	1.41		121.8	14.8	b	78.1	11.2	b
ALT (U/L)	T1 (9–20)	124	29.9	12.9	a	3.36	1.39		115.0	14.9		73.5	11.1	
	T2 (21–30)	109	30.7	10.3	a	3.37	1.05		116.7	14.1		75.2	11.7	
	T3 (31–218)	118	38.0	16.0	b	3.67	1.16		118.6	13.4		76.2	10.2	
Neutrophil count (/μL, ×10 ³)	T1 (1.21–2.89)	116	29.7	11.8	a	—			116.8	13.5		75.3	10.8	
	T2 (2.90–3.79)	119	31.7	13.2	a				115.7	14.4		73.9	11.3	
	T3 (3.80–13.64)	116	37.2	15.2	b				117.7	14.7		75.6	11.1	
CRP (mg/dL)	T1 (0.01–0.09)	106	29.9	12.4	a	3.08	0.84	a	115.3	12.9	a	74.2	11.1	a
	T2 (0.10–0.12)	116	31.8	12.8	a	3.30	1.03	a	114.0	12.6	a	72.5	10.3	a
	T3 (0.13–5.26)	129	36.3	15.0	b	3.94	1.48	b	120.3	15.8	b	77.8	11.1	b
Smoking status (Brinkman index)	T1 (0)	172	32.0	14.5		3.26	0.96	a	117.9	14.1		76.3	11.3	b
	T2 (1–450)	90	34.7	15.0		3.41	1.15	a	113.7	13.1		71.6	10.7	a
	T3 (460–1,600)	89	32.8	10.8		3.93	1.58	b	117.6	15.2		75.7	10.2	b
Physical activity (times/wk)	T1 (0)	169	34.9	15.0	b	3.64	1.28	b	115.4	13.7		74.9	10.9	
	T2 (1–4)	97	30.0	12.6	a	3.25	1.13	a	117.8	16.1		74.1	11.7	
	T3 (5–7)	85	32.1	12.1	ab	3.37	1.16	ab	118.2	12.7		75.9	10.6	
Alcohol intake (g/d)	T1 (0)	43	34.8	13.7	ab	3.41	1.00		116.5	13.8		74.6	11.1	ab
	T2 (0.01–29.99)	164	30.8	14.0	a	3.53	1.34		115.2	13.2		73.4	11.0	a
	T3 (30.00–187.91)	144	34.7	13.3	b	3.41	1.14		118.5	15.2		76.8	10.9	b
Energy intake (kcal/d)	T1 (935–1,999)	139	31.7	13.4		3.37	1.13		116.5	14.6	ab	74.9	11.3	
	T2 (2,000–2,499)	108	34.5	13.2		3.47	1.08		114.5	11.6	a	74.1	10.1	
	T3 (2,500–4,255)	104	32.7	14.8		3.58	1.46		119.3	15.8	b	75.8	11.7	

(a, b) Different letters denote significant differences among tertiles by Tukey's test based on one way ANOVA in each group. Each factor was divided into tertiles (T1, T2 and T3) and parenthetic value indicates range of the factor in each group.

count, CRP, Brinkman index, physical activity, alcohol intake and energy intake. MLR analysis for sE-selectin concentration, neutrophil count, and SBP or DBP as dependent variables disclosed that plasma sE-selectin concentration was positively independently associated

with DBP, ALT, and neutrophil count. Neutrophil count was positively independently associated with sE-selectin, CRP, and Brinkman index and negatively independently associated with age, and HDL-C. SBP was independently positively associated with age, BMI, TG, and alcohol

Table 4. MLR analysis of parameters as independent variables for sE-selectin, neutrophil count, SBP and DBP.

	sE-selectin ($R^2=0.231^{***}$)				Neutrophil count ($R^2=0.220^{***}$)				SBP ($R^2=0.215^{***}$)				DBP ($R^2=0.217^{***}$)			
	β	SE	p		β	SE	p		β	SE	p		β	SE	p	
sE-selectin (ng/mL)	0.020	—	0.820		0.016	0.005	<0.001***		0.099	0.056	0.080		0.099	0.044	0.025*	
Age (y)	—	0.087	0.605		—0.025	0.008	0.001**		0.507	0.088	<0.001***		0.306	0.068	<0.001***	
BMI	—0.141	0.273	0.025*		0.032	0.024	0.185		1.004	0.278	<0.001***		0.780	0.216	<0.001***	
DBP (mmHg)	0.151	0.067	0.314		0.006	0.006	0.318		—	—	—		—	—	—	
HDL-C (mg/dL)	0.054	0.053	0.711		—0.014	0.005	0.003**		0.055	0.055	0.325		0.056	0.043	0.191	
TG (mg/dL)	0.003	0.008	0.208		—0.001	0.001	0.153		0.019	0.009	0.023*		0.017	0.007	0.009**	
Fasting blood glucose (mg/dL)	0.056	0.044	0.001***		0.004	0.004	0.287		0.064	0.046	0.162		0.066	0.036	0.064	
ALT (U/L)	0.255	0.037	<0.001***		—0.006	0.003	0.077		0.040	0.041	0.324		0.039	0.032	0.219	
Neutrophil count ($/\mu\text{L} \times 10^3$)	2.013	0.601	<0.001***		—	—	—		0.627	0.634	0.324		0.493	0.493	0.318	
CRP (mg/dL)	0.499	1.882	0.791		0.749	0.163	<0.001***		1.428	1.956	0.466		0.319	1.521	0.834	
Smoking status (Brinkman index)	—0.001	0.002	0.683		0.001	0.000	<0.001***		—0.005	0.002	0.026*		—0.005	0.002	0.007**	
Physical activity (times/wk)	—0.121	0.277	0.663		—0.044	0.025	0.077		0.521	0.287	0.071		0.340	0.223	0.129	
Alcohol intake (g/d)	0.039	0.020	0.056		—0.003	0.002	0.103		0.049	0.021	0.021*		0.053	0.016	0.001**	
Energy intake (kcal/d)	—0.002	0.001	0.206		0.000	0.000	0.165		—0.001	0.001	0.519		—0.002	0.001	0.044*	
Intercept	3.063	9.393	0.745		3.074	0.821	<0.001***		49.727	9.589	<0.001***		26.464	7.455	<0.001***	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

intake and independently negatively associated with the Brinkman index. DBP was independently positively associated with sE-selectin, age, BMI, TG, and alcohol intake and independently negatively associated with the Brinkman index and energy intake (Table 4).

DISCUSSION

The present study focused on relationships among circulating sE-selectin concentration, neutrophil count, and blood pressure in middle-aged Japanese men who were not diagnosed with or being not treated for metabolic diseases according to their health check-ups. Previous studies have demonstrated that circulating sE-selectin concentration is positively associated with BMI, blood pressure and TG in male and female white and South Asian subjects who are free from coronary heart disease, stroke and other cardiovascular disease and diabetes (4, 16). In this study, we have demonstrated that circulating sE-selectin concentration is positively associated with BMI, blood pressure and TG as well as TC and fasting blood glucose levels in the middle-aged Japanese apparently healthy subjects. These findings supplement previous reports in Japanese type 2 diabetic patients in whom circulating sE-selectin concentration was positively associated with TG and fasting blood glucose levels (17). Interestingly, we found a strong positive association of circulating sE-selectin concentration with neutrophil count in our subjects. Moreover, no association between neutrophil count and blood pressure was observed. From the results in the current study as well as those in previous reports, we hypothesized in this study that circulating sE-selectin concentration would be independently associated with neutrophil count and blood pressure and might be a useful biomarker for metabolic disorders related to increased levels of neutrophil count and blood pressure such as CVD.

To explore the hypothesis, we performed MLR analyses using variables associated with sE-selectin concentration, neutrophil count, SBP or DBP and lifestyle factors. Interestingly, circulating sE-selectin concentration was independently positively associated with neutrophil count as well as DBP, but not SBP, whereas no independent association between neutrophil count and DBP was observed. Previous studies have demonstrated that high blood pressure is one of the major risk factors of CVD (1). In addition, neutrophil count is associated with mortality in Asian and Western patients with chronic and acute heart failure (9–11). One of the causes of chronic and acute heart failure is induction of vascular injury by increasing numbers of neutrophils, because attachment and activation of neutrophils induces production of reactive oxygen species and release of destructive proteases (7). The hypothesis is supported by many studies including this study showing that the inflammation and oxidative stress marker CRP is positively associated with neutrophil count, but not DBP, in men without treatment for metabolic diseases (13) and patients with chronic stable angina (18). This combination of hypertension with increasing neutrophil count would induce chronic and acute heart failure from the

pre-stage of CVD. Our results suggest that E-selectin may have roles in elevating blood pressure and increasing neutrophil count. Indeed, deficiency of E-selectin in mice by gene targeting leads to reduced neutrophil count and CVD (6, 14), although the function of the sE-selectin is still unknown. sE-selectin is considered to be a deviating protein; however, the average plasma concentration in apparently healthy Japanese men in the current study was not low (32.9 ± 13.8 ng/mL). Thus, sE-selectin may have functions of elevating blood pressure and increasing neutrophil count. It should be examined in animal studies whether injection of sE-selectin into animals induces elevation of blood pressure, neutrophil count and incidents of CVD. Furthermore, these results indicate that damage of vascular injury by E-selectin may have occurred from a healthy state, and sE-selectin concentration, neutrophil counts and blood pressure would be useful biomarkers for preventing development and progression of CVD from a healthy state.

The reason why the association of sE-selectin concentration with DBP was stronger than that with SBP in this population is still unclear. It has been reported that subjects with systolic hypertension had a higher risk of stroke than those with diastolic hypertension (19, 20). Our results are in contrast with these previous studies. It is already known that older people with hypertension tend to have higher SBP than DBP (21). It has also been reported that DBP is a strong risk factor for developing CVD in the population under 50 y, whereas SBP is a strong risk factor in the population over 60 y (22). Subjects in the current study are almost all middle aged, from 40 to 64 y (47.5 ± 8.41), and their values for SBP and DBP are mostly within a normal range (6 and 28 subjects among 351 had high SBP and DBP, respectively). Thus, the stronger association of sE-selectin concentration with DBP than with SBP in this study may be caused by the younger study group and earlier developmental stage for CVD.

It should be mentioned that association between circulating sE-selectin concentration and monocyte count was lower than that between sE-selectin concentration and neutrophil count in this population, although many animal studies have demonstrated that macrophages differentiated from monocytes induce atherosclerosis (8). Previous studies have demonstrated that monocyte count is also positively associated with CVD in Western and Asian people (23, 24). However, many studies have demonstrated that neutrophil count rather than monocyte count is positively associated with development of CVD in Western and Asian countries (10, 13, 25, 26). These results indicate that neutrophils may contribute to and predict the development and progression of CVD. Our results indicate that neutrophil count is positively associated with circulating sE-selectin concentration more than monocyte count, suggesting that E-selectin may be related to the development of CVD by increasing neutrophil count.

In addition, sE-selectin concentration was independently positively associated with ALT activity. ALT activity is known as a marker indicating liver damage,

particularly, fatty liver. Thus, circulating sE-selectin concentration may be elevated by liver damage. Neutrophil count is independently positively associated with CRP concentration and independently inversely associated with HDL cholesterol. Many previous studies have demonstrated that CRP is a strong risk factor for CVD (27, 28) and that low HDL-C is a major risk factor for the development of CVD. Neutrophil counts are also positively associated with CVD risk factors in healthy Asian and Western people (12, 13, 29). In addition, one of the causes of chronic and acute heart failure is thought to be the induction of vascular injury through an increased neutrophil count, because neutrophil activation induces their attachment to vascular cells, the production of reactive oxygen species, and the release of destructive proteases from the neutrophils (7).

We showed strong association between circulating sE-selectin concentration and neutrophil count by MLR analyses. However, R^2 for MLR analyses was relatively low (R^2 of sE-selectin, neutrophil count, SBP, and DBP, were 0.231, 0.220, 0.215 and 0.217, respectively). Because R^2 of MLR analyses indicates the relevance of the multiple regression equation, it is difficult to predict circulating sE-selectin concentration by independent variables using the equation, and other factors including blood parameters and lifestyle factors would also be related to circulating sE-selectin concentration. However, the population in our study was apparently healthy men. Thus, our results indicate that association between sE-selectin and neutrophil count or blood pressure can be observed from the healthy state. In addition, our results indicate that associations among sE-selectin, neutrophil count, and blood pressure may be affected by lifestyle factors and related blood parameters.

In summary, circulating sE-selectin concentration is independently positively associated with neutrophil count and blood pressure in Japanese men who were not diagnosed with or being not treated for metabolic diseases according to their health check-ups. Our results suggest that circulating sE-selectin is related to increased neutrophil count and elevated DBP, and those associations may be related to the development of CVD, but this hypothesis should be examined in well-designed cohort studies.

Acknowledgments

We thank Drs. S. Sasaki and K. Murakami for providing the BDHQ and for helpful discussions. This study was financially supported by the Global COE program for the Center of Excellence for Innovation in Human Health Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a grant from Suzuken Memorial Foundation.

REFERENCES

- 1) Mottillo S, Filion KB, Genest J, Joseph L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. 2010. The metabolic syndrome and cardiovascular risk—a systematic review and meta-analysis. *J Am Coll Cardiol* **56**: 1113–1132.

- 2) Hope SA, Meredith IT. 2003. Cellular adhesion molecules and cardiovascular disease. Part II. Their association with conventional and emerging risk factors, acute coronary events and cardiovascular risk prediction. *Intern Med J* **33**: 450–462.
- 3) Sakurai S, Kitamura A, Cui R, Yamagishi K, Tanigawa T, Iso H. 2009. Relationships of soluble E-selectin and high-sensitivity C-reactive protein with carotid atherosclerosis in Japanese men. *J Atheroscler Thromb* **16**: 339–345.
- 4) Miller MA, Kerry SM, Cook DG, Cappuccio FP. 2004. Cellular adhesion molecules and blood pressure: Interaction with sex in a multi-ethnic population. *J Hypertens* **22**: 705–711.
- 5) Demerath E, Towne B, Blangero J, Siervogel RM. 2001. The relationship of soluble ICAM-1, VCAM-1, P-selectin and E-selectin to cardiovascular disease risk factors in healthy men and women. *Ann Hum Biol* **28**: 664–678.
- 6) Kakkar AK, Lefer DJ. 2004. Leukocyte and endothelial adhesion molecule studies in knockout mice. *Curr Opin Pharmacol* **4**: 154–158.
- 7) Lucchesi BR. 1990. Modulation of leukocyte-mediated myocardial reperfusion injury. *Annu Rev Physiol* **52**: 561–576.
- 8) Moore KJ, Tabas I. 2011. Macrophages in the pathogenesis of atherosclerosis. *Cell* **145**: 341–355.
- 9) Reddan DN, Klassen PS, Szczech LA, Coladonato JA, O'Shea S, Owen WF Jr, Lowrie EG. 2003. White blood cells as a novel mortality predictor in haemodialysis patients. *Nephrol Dial Transplant* **18**: 1167–1173.
- 10) Dragu R, Huri S, Zuckerman R, Suleiman M, Mutlak D, Agmon Y, Kapeliovich M, Beyar R, Markiewicz W, Hammerman H, Aronson D. 2008. Predictive value of white blood cell subtypes for long-term outcome following myocardial infarction. *Atherosclerosis* **196**: 405–412.
- 11) Straburzynska-Migaj E, Gwizdala A, Siniawski A, Ochotny R, Grajek S. 2010. Leptin and inflammation in patients with chronic heart failure. *Kardiol Pol* **68**: 1243–1247.
- 12) Nordestgaard BG, Adourian AS, Freiberg JJ, Guo Y, Muntendam P, Falk E. 2010. Risk factors for near-term myocardial infarction in apparently healthy men and women. *Clin Chem* **56**: 559–567.
- 13) Yen ML, Yang CY, Yen BL, Ho YL, Cheng WC, Bai CH. 2006. Increased high sensitivity C-reactive protein and neutrophil count are related to increased standard cardiovascular risk factors in healthy chinese men. *Int J Cardiol* **110**: 191–198.
- 14) Jones SP, Trocha SD, Strange MB, Granger DN, Kevil CG, Bullard DC, Lefer DJ. 2000. Leukocyte and endothelial cell adhesion molecules in a chronic murine model of myocardial reperfusion injury. *Am J Physiol Heart Circ Physiol* **279**: H2196–2201.
- 15) Murakami K, Mizoue T, Sasaki S, Ohta M, Sato M, Matsushita Y, Mishima N. 2008. Dietary intake of folate, other B vitamins, and ω -3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults. *Nutrition* **24**: 140–147.
- 16) Miller MA, Sagnella GA, Kerry SM, Strazzullo P, Cook DG, Cappuccio FP. 2003. Ethnic differences in circulating soluble adhesion molecules: The wandsworth heart and stroke study. *Clin Sci (Lond)* **104**: 591–598.
- 17) Matsumoto K, Sera Y, Nakamura H, Ueki Y, Miyake S. 2002. Serum concentrations of soluble adhesion molecules are related to degree of hyperglycemia and insulin resistance in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* **55**: 131–138.
- 18) Avanzas P, Arroyo-Espliguero R, Cosin-Sales J, Quiles J, Zouridakis E, Kaski JC. 2004. Multiple complex stenoses, high neutrophil count and C-reactive protein levels in patients with chronic stable angina. *Atherosclerosis* **175**: 151–157.
- 19) Qureshi AI, Suri MF, Mohammad Y, Guterman LR, Hopkins LN. 2002. Isolated and borderline isolated systolic hypertension relative to long-term risk and type of stroke: A 20-year follow-up of the national health and nutrition survey. *Stroke* **33**: 2781–2788.
- 20) Rutan GH, Kuller LH, Neaton JD, Wentworth DN, McDonald RH, Smith WM. 1988. Mortality associated with diastolic hypertension and isolated systolic hypertension among men screened for the multiple risk factor intervention trial. *Circulation* **77**: 504–514.
- 21) Okayama A, Kadowaki T, Okamura T, Hayakawa T, Ueshima H. 2006. Age-specific effects of systolic and diastolic blood pressures on mortality due to cardiovascular diseases among Japanese men (NIPPON DATA80). *J Hypertens* **24**: 459–462.
- 22) Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB, Levy D. 2001. Does the relation of blood pressure to coronary heart disease risk change with aging? The framingham heart study. *Circulation* **103**: 1245–1249.
- 23) Cortina MG, Campello AR, Conde JJ, Ois A, Voustianiouk A, Tellez MJ, Cuadrado E, Roquer J. 2008. Monocyte count is an underlying marker of lacunar subtype of hypertensive small vessel disease. *Eur J Neurol* **15**: 671–676.
- 24) Yun KH, Oh SK, Park EM, Kim HJ, Shin SH, Lee EM, Rhee SJ, Yoo NJ, Kim NH, Jeong JW, Jeong MH. 2006. An increased monocyte count predicts coronary artery spasm in patients with resting chest pain and insignificant coronary artery stenosis. *Korean J Intern Med* **21**: 97–102.
- 25) Jia EZ, Yang ZJ, Yuan B, Zang XL, Wang RH, Zhu TB, Wang LS, Chen B, Ma WZ. 2005. Relationship between leukocyte count and angiographical characteristics of coronary atherosclerosis. *Acta Pharmacol Sin* **26**: 1057–1062.
- 26) Huang G, Zhong XN, Zhong B, Chen YQ, Liu ZZ, Su L, Ling ZY, Cao H, Yin YH. 2009. Significance of white blood cell count and its subtypes in patients with acute coronary syndrome. *Eur J Clin Invest* **39**: 348–358.
- 27) Nordestgaard BG, Zacho J. 2009. Lipids, atherosclerosis and cvd risk: Is CRP an innocent bystander? *Nutr Metab Cardiovasc Dis* **19**: 521–524.
- 28) de Ferranti SD, Rifai N. 2007. C-reactive protein: A non-traditional serum marker of cardiovascular risk. *Cardiovasc Pathol* **16**: 14–21.
- 29) Oda E, Kawai R. 2010. Comparison between high-sensitivity c-reactive protein (hs-CRP) and white blood cell count (WBC) as an inflammatory component of metabolic syndrome in Japanese. *Intern Med* **49**: 117–124.