Plasma Fibrinogen, Tissue Plasminogen Activator, Plasminogen Activator Inhibitor 1, and Their Related Factors in Three Japanese Population Samples

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Abstract

To examine population mean variations in plasma fibrinogen and fibrinolytic variables, and their relations with cardiovascular risk characteristics among Japanese middle-aged men, a cross-sectional study was conducted for a total of 245 men aged 50-59 years in three population-based samples: residents in rural communities of northeast and central Japan and urban white-collar workers. Age-adjusted mean value of plasma fibrinogen, tissue plasminogen activator antigen (t-PA antigen), plasminogen activator inhibitor 1 antigen (PAI-1 antigen) did not differ significantly among the populations. Mean value of tissue plasminogen activator activity (t-PA activity) was lower in central rural residents than in northeast rural men. According to multiple linear regression analyses, there were positive associations of t-PA and PAI-1 antigens with serum triglyceride levels, serum insulin and waist-hip ratio within each population and the total samples. A positive association between these fibrinolytic variables and usual ethanol intake was also observed. Smoking was significantly associated with plasma fibrinogen and PAI-1 antigen but not with t-PA antigen or activity. Activity of t-PA was inversely associated with body mass index, and a mean difference in t-PA activity was in part explained by a mean difference in body mass index. In conclusion, population mean values of plasma fibrinogen and fibrinolytic variables did not differ among three Japanese populations except for mean t-PA activity. Reduced fibrinolysis expressed as increased PAI-1 antigen was associated with smoking and the status of insulin resistance, such as high levels of serum insulin, serum triglycerides and waist-hip ratio.

Key words: plasama fibrinogen, tissue plasminogen activator, plasminogen activator inhibitor 1, insulin resistance, Japanese

Introduction

Hemostatic variables such as fibrinogen, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) were interrelated closely under various physiological and pathological conditions.¹⁾ Fibrinogen is converted into fibrin, allowing stabilization of aggregated platelets by the formation of thrombin. Tissue plasminogen activator (t-PA) triggers fibrinolysis by

converting plasminogen into plasmin to dissolve the fibrin. This fibrinolysis process is controlled by plasminogen activator inhibitor (PAI-1), neutralizing t-PA by the formation of t-PA and PAI-1 complex. Contributions of these hemostatic factors to development of cardiovascular disease have been reported from prospective studies in the United State and Europe.²⁻⁹⁾ Our recent prospective data also showed that plasma fibrinogen increased risk of coronary heart disease in middle-aged Japanese men.¹⁰⁾ For prevention of cardiovascular disease, it is important to examine cardiovascular risk characteristics, i.e. lifestyle, constitutional and biochemical factors, which affect these hemostatic variables.

We conducted a cross-cultural study in 1990 on hemostatic

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variables between Japanese and Caucasian Americans, which showed higher mean values of plasma fibrinogen, t-PA antigen and PAI-1 antigen in Caucasian Americans than in Japanese.^{11,12} These results corresponded to the higher mortality rate from coronary heart disease in the United States than in Japan. In that cross-cultural study, we used Japanese samples only from a rural community of northeast Japan. A study of hemostatic variables in other Japanese population samples is warranted to confirm the racial difference between Caucasians and Japanese.

We conducted a cross-sectional study in population-based samples of men aged 50-59 years in three Japanese populations including the northeast rural Japanese to examine population mean variations in plasma fibrinogen and fibrinolytic variables, and their relations with cardiovascular risk characteristics. Activity of tissue plasminogen activator (t-PA activity) was also examined as an indicator of fibrinolytic function.

Methods

Three population-based samples of men aged 50 to 59 were used: residents in northeast rural community of Akita, central rural community of Ibaraki, urban white-collar workers in Osaka. Most men in this age range were involved in rice farming in Akita, green-house farming or light industry in Kyowa, and clerical work in Osaka. Systematic samples, a total of 245 men without histories of coronary heart disease or stroke, were used from the participants in ongoing cardiovascular risk surveys.¹² All subjects were examined between March and November in 1992.

Trained technicians drew and processed blood samples according to methods adapted from the Atherosclerosis Risk in Community (ARIC) Study.¹³ Blood was drawn between 8:30 and 10:30 a.m. from seated participants after at least an 8-hour fast because of circadian fluctuations of t-PA and PAI-1 antigens and t-PA activity.^{14,15} A 21-gauge needle was used with minimal stasis. The blood was collected into a 4.5 ml siliconized glass tubes containing 0.5 ml of 3.8 % of sodium citrate. Platelet stabilizing agents were not used because the tubes were immediately centrifuged at 1,500 x g for 20 minutes at 4 °C.

Plasma fibrinogen was measured by the clotting assay of Clauss,¹⁶ using reagents obtained from General Diagnostics (Organon-Technika Co., Morris Plains, N.J.). Antigens of tissue plasminogen activator (t-PA antigen) and plasminogen activator inhibitor (PAI-1 antigen) and activity of t-PA were measured by enzyme linked immunosorbent assay using kits of TintElizeTMtPA (Biopool, Sweden) and TintElizeR PAI-1 (Biopool, Sweden), and Spectrolyse/fibrin (Biopool, Sweden), respectively. Serum insulin was measured by enzyme-immunoassay using a kit of ELA test insulin II (Berlinger Mannheim Yamanouchi, Japan). For analysis of t-PA activity, 250 μ L of 1.0 mol/L sodium citrate (pH 3.9) was added in the 500 μ L plasma. The plasma was separated and stored at -70 °C until measurement within 6 months in University of Tsukuba.

For measurement of serum lipids, glucose and plasma fibrinogen, the serum and plasma samples were transported on

dry ice to the Osaka Medical Center for Cancer and Cardiovascular Diseases, and stored again at -70 °C until the measurement. Total cholesterol and triglyceride were measured by an enzymatic method (SMAC, Technicon Instrument Corp., Terrytown, New York).¹⁷ HDL-cholesterol was measured by a heparin-manganese method following the Liebermann-Burchard method (Autoanalyzer II, Technicon Instrument Corp.). LDL-cholesterol was estimated from these three lipids by the equation: LDL-cholesterol = total cholesterol - HDL-cholesterol - 0.2 x triglycerides.¹⁸ The measurements in the laboratory were standardized by the Lipid Standardization Program, Center for Disease Control, Atlanta.¹⁷ Serum glucose was measured by enzyme assay (Autoanalyzer II, Technicon Instrument Corp.).

Height in stocking feet and weight in light clothing were measured, and a body mass index (BMI) was calculated as weight(kg)/height(m)². Systolic and diastolic blood pressures were measured using a standard mercury sphygmomanometer on the right arm of seated participants after a five-minute rest. Blood pressure technicians were trained according to American Heart Association methods.¹⁹ An interview was conducted to ascertain usual intake of alcohol and smoking status. Trained technicians measured to the nearest centimeter waist circumference at the umbilical level and hip circumference at the symphysis pubic at the maximum protrusion of the hips. Waist-hip ratio (WHR) was calculated as waist-hip circumference ratio.

Analysis of variance was used to compare means of continuous variables among the three populations. The chi-square test was used to compare prevalence rates. The significance of Spearman correlation coefficients between cardiovascular risk characteristics and hemostatic variables in each population was tested using the t-test. Multiple linear regression was used to examine the relation of selected cardiovascular risk characteristics and hemostatic variables in each population and in the total samples. All p-values for statistical tests on means were two-tailed, and p < 0.05 was regarded as significance level.

Results

Table 1 summarizes age-adjusted means and prevalence of cardiovascular risk characteristics for three Japanese population samples. Mean age was between 54 and 55. Mean values of body mass index (BMI), waist-hip ratio (WHR) were high in central rural men and intermediate in urban white-collar workers and low in northeast rural men. Blood pressure levels and the prevalence of hypertension were higher in central and northeast rural men than in urban white-collar workers. Mean ethanol intake and mean glucose were lower in central rural men than in the other populations.

Mean values of fibrinogen, t-PA, or PAI-1 antigens did not differ significantly among the three populations although mean PAI-1 antigen tended to be lower in the central rural residents than in the other two populations (Table 2). There was a significant population difference in mean t-PA activity; mean t-PA activity was significantly lower in central rural men than in northeast rural men.

Table 1 . Age-adjusted mean values (standard errors) of cardiovascular risk characteristics in men aged 50-59 years of three Japanese populations.

	Northeast rural	Central rural	Urban white-collar	P for difference
Number	82	83	80	
Age, years	55.4 (0.3)	54.2 (0.3)	54.1 (0.3)	0.009
Body mass index, kg/m ²	22.8 (0.3)	24.0 (0.3)	23.2 (0.3)	0.01
Waist-hip ratio	0.89(0.01)	0.93(0.01)	0.92(0.01)	<0.001
Ethanol intake, g/wk	11.4 (0.9)	8.3 (0.9)	10.2 (0.9)	0.05
Current smoker, %	66	49	49	0.04
Systolic blood pressure, mmHg	126.5 (2.0)	129.5 (1.9)	120.2 (2.0)	0.003
Diastolic blood pressure, mmHg	81.0 (1.2)	82.8 (1.2)	77.4 (1.2)	0.007
Hypertensives, %	18	25	15	0.24
Serum total cholesterol, mmol/L	4.99 (0.09)	5.20 (0.09)	5.18 (0.09)	0.19
LDL-cholesterol, mmol/L	2.81 (0.09)	3.24 (0.09)	2.90 (0.10)	0.003
HDL-cholesterol, mmol/L	1.58 (0.04)	1.29 (0.04)	1.45 (0.04)	<0.001
Triglycerides, mmol/L	1.18 (0.10)	1.47 (0.10)	1.68 (0.10)	0.003
glucose, mmol/L	5.82 (0.07)	5.50 (0.06)	5.66 (0.07)	0.002
Insulin, pmol/L	55 (4)	66 (4)	61 (4)	0.06
Insulin, pmol/L	55 (4)	66 (4)	61 (4)	0.06

Conversion factor for serum lipids and glucose from units of mmol/L to mg/dL is 38.67 for cholesterol, 88.57 for triglycerides, and 18.01 for glucose.

Table 2 . Age-adjusted mean values (standard errors) of hemostatic variables in men aged 50-59 years of three Japanese populations.

	Northeast rural	Central rural	Urban white-collar	P for difference
Number	82	83	80	
Fibrinogen, mg/dl	2.85 (0.07)	2.83 (0.07)	2.74 (0.07)	0.47
t-PA antigen, ng/ml	7.5 (0.3)	8.3 (0.3)	7.6 (0.3)	0.23
t-PA activity, IU/ml	6.4 (0.3)	2.8 (0.3)	4.2 (0.3)	<0.001
PAI-1 antigen, ng/ml	15.9 (0.8)	13.3 (0.8)	15.4 (0.8)	0.05

t-PA: tissue plasminogen activator ; PAI-1 : plasminogen activator inhibitor-1

Table 3 indicates Spearman correlation coefficients of these hemostatic variables with known cardiovascular risk factors in each population. Plasma fibrinogen was not correlated consistently with these fibrinolytic variables; correlation coefficient (r) = -0.01 to 0.16 with t-PA antigen, r = -0.36 to 0.08 with t-PA activity and r = -0.03 to 0.20 with PAI-1 antigen. T-PA antigen and PAI-1 antigen were strongly intercorrelated; r = 0.64 for northeast rural men, r = 0.68 for central rural men, and r = 0.64 for urban white-collar workers. T-PA activity was significantly and inversely correlated with PAI-1 antigen in each population; r = -0.39, r = -0.31 and r = -0.43, respectively. T-PA activity was inversely correlated with t-PA antigen in northeast rural men but no significant correlation was found in the other populations; r = -0.33, r = -0.11 and r = -0.18, respectively. The correlations between age and these hemostatic variables were weak and inconsistent.

Plasma fibrinogen was positively correlated with WHR, current smoking, blood pressure levels, and inversely correlated with HDL-cholesterol in each population. Moderate and significant correlation between smoking and plasma fibrinogen was observed in northeast rural men and urban white-collar workers but the correlation was not significant in central rural men. Fibrinogen was not correlated consistently with other risk characteristics.

T-PA antigen was positively correlated with BMI, WHR, ethanol intake, current smoking, blood pressure levels, triglycerides, glucose and insulin, and inversely correlated with HDL-cholesterol in each population. There were moderate and significant correlations of t-PA antigen with WHR, triglycerides and insulin in each population.

T-PA activity was inversely correlated with BMI, WHR, ethanol intake, current smoking in all populations but was not

correlated consistently with other risk characteristics. The correlation between t-PA activity and BMI was significant in each population.

PAI-1 antigen was positively correlated with BMI, WHR, ethanol intake, current smoking, blood pressure levels, triglycerides, glucose and insulin, and inversely correlated with HDL-cholesterol in each population. There were moderate and significant correlations of PAI-1 antigen with BMI, WHR, ethanol intake, triglycerides and insulin in each population.

Table 4 shows predicted changes in hemostatic variables in relation to predictor variables according to multiple linear regression in each population and total samples. Predictor variables were risk characteristics which showed consistent correlations of these hemostatic variables as well as age. For the analyses of total samples, population was adjusted using indicator variables. Estimates of mean (standard error) changes in hemostatic variables associated with change of one standard deviation of continuous predictor variables were presented. For plasma fibrinogen, significant association was found with current smoking and diastolic blood pressure in the total samples although the association did not always reach statistical significance in each population. For t-PA activity, significant inverse associations with BMI and ethanol intake were found in total samples although the significant relations were not consistently observed in each population.

Triglycerides were significantly associated with t-PA antigen in each population. A 0.95 mg/dl greater triglycerides was associated with a 0.94 ng/ml higher t-PA antigen in total samples. WHR, ethanol intake and serum insulin were also significantly associated with t-PA antigen in the total samples although the association did not always reach statistical significance in each population.

Table 3 . Spearman correlation coefficients between hemostatic variables and cardiovascular risk characteristics in men aged 50-59 years of three Japanese populations.

	Age	BMI	WHR	Ethanol	Smoker	SBP	DBP	LDL	HDL	TG	Glucose	Insulin
Fibrinogen												
Northeast rural men	-0.01	-0.12	0.02	0.25 [†]	0.32 [†]	0.09	0.10	-0.02	-0.10	-0.05	-0.05	-0.03
Central rural men	0.03	-0.03	0.09	-0.08	0.13	0.17	0.24 [†]	0.20*	-0.05	-0.04	0.01	-0.03
Urban white-collar	0.21*	0.18	0.28 [†]	0.11	0.31 [‡]	0.21*	0.14	0.08	-0.25 [†]	0.24 [†]	0.12	0.10
t-PA antigen												
Northeast rural men	-0.02	0.06	0.19*	0.41 [†]	0.22 [†]	0.12	0.12	-0.09	-0.08	0.35 [†]	0.19*	0.32 [†]
Central rural men	-0.02	0.31 [†]	0.42 [†]	0.16	0.14	0.10	0.28 [†]	-0.03	-0.20*	0.61 [†]	0.06	0.25 [†]
Urban white-collar	0.02	0.19*	0.34 [†]	0.35 [†]	0.32 [†]	0.22 [†]	0.08	0.11	-0.21*	0.48 [†]	0.10	0.26 [†]
t-PA activity												
Northeast rural men	0.01	-0.24 [†]	-0.13	-0.25 [†]	-0.03	0.10	0.05	0.004	-0.05	-0.19	0.04	-0.19
Central rural men	0.08	-0.29 [†]	-0.11	-0.10	-0.27 [†]	-0.28 [†]	-0.06	-0.16	0.04	-0.09	-0.01	0.06
Urban white-collar	0.03	-0.27 [†]	-0.33 [†]	-0.16	-0.27 [†]	-0.12	-0.07	0.13	0.15	-0.18	-0.26 [†]	-0.20*
PAI-1 antigen												
Northeast rural men	-0.12	0.19*	0.19*	0.36 [†]	0.26 [†]	0.08	0.16	-0.10	-0.14	0.41 [†]	0.11	0.28 [†]
Central rural men	-0.30 [†]	0.42 [†]	0.47 [†]	0.34 [†]	0.23 [†]	0.13	0.26 [†]	0.09	-0.28 [†]	0.57 [†]	0.19	0.33 [†]
Urban white-collar	0.02	0.32 [†]	0.50 [†]	0.41 [†]	0.36 [†]	0.25 [†]	0.23 [†]	0.12	-0.38 [†]	0.57 [†]	0.26 [†]	0.30 [†]

* P < 0.10, [†] P < 0.05, [‡] P < 0.01

t-PA:tissue plasminogen activator ; PAI-1:Plasminogen activator inhibitor-1, SBP:Systolic blood pressure ; DBP:Diastolic blood pressure; LDL: Low-density lipoprotein cholesterol; HDL:High-density lipoprotein cholesterol ; TG: Triglycerides

Table 4. Predicted changes in hemostatic variables associated with selected cardiovascular risk characteristics.

	Northeast rural		Central rural		Urban white-collar		Total	
	Predicted change(SE)	P value	Predicted change(SE)	P value	Predicted change(SE)	P value	Predicted change(SE)	P value
Fibrinogen, g/L								
Age, 3year	- 0.10 (0.07)	0.15	- 0.01 (0.07)	0.85	0.18 (0.08)	0.03	0.01 (0.04)	0.74
BMI, 3kg/m ²	- 0.16 (0.12)	0.17	- 0.05 (0.11)	0.69	0.03 (0.11)	0.82	- 0.07 (0.07)	0.25
WHR, 0.05	0.07 (0.09)	0.45	- 0.01 (0.10)	0.93	0.07 (0.10)	0.46	0.05 (0.05)	0.37
Diastolic BP, 11mmHg	0.08 (0.07)	0.25	0.12 (0.07)	0.09	0.21 (0.08)	0.01	0.13 (0.04)	0.003
Ethanol intake, 26g/day	0.10 (0.07)	0.18	-0.07 (0.06)	0.25	- 0.003 (0.09)	0.97	- 0.02 (0.04)	0.97
Current smoking, yes	0.30 (0.15)	0.05	0.14 (0.13)	0.29	0.44 (0.15)	0.004	0.29 (0.08)	< 0.001
Triglycerides, 0.95 mmol/L	- 0.06 (0.12)	0.59	- 0.05 (0.09)	0.61	- 0.05 (0.05)	0.40	- 0.05 (0.04)	0.28
Insulin, 30 mmol/L	0.03 (0.09)	0.97	- 0.05 (0.08)	0.54	- 0.03 (0.10)	0.77	- 0.02 (0.05)	0.70
t-PA antigen, ng/ml								
Age, 3year	0.31 (0.31)	0.33	0.22 (0.34)	0.52	0.14 (0.32)	0.66	0.18 (0.18)	0.32
BMI, 3kg/m ²	- 0.90 (0.53)	0.10	- 0.76 (0.54)	0.16	- 0.15 (0.44)	0.74	- 0.46 (0.29)	0.11
WHR, 0.05	0.42 (0.40)	0.30	0.85 (0.50)	0.09	0.63 (0.38)	0.11	0.64 (0.24)	0.008
Diastolic BP, 11 mmHg	- 0.01 (0.31)	0.98	0.09 (0.34)	0.79	0.12 (0.32)	0.70	0.14 (0.18)	0.45
Ethanol intake, 26g/day	0.91 (0.33)	0.008	0.17 (0.28)	0.56	0.69 (0.33)	0.04	0.54 (0.17)	0.002
Current smoking, yes	- 0.09 (0.69)	0.90	0.82 (0.63)	0.20	0.55 (0.58)	0.34	0.55 (0.36)	0.12
Triglycerides, 0.95 mmol/L	1.16 (0.54)	0.04	1.93 (0.44)	< 0.001	0.53 (0.21)	0.01	0.94 (0.18)	< 0.001
Insulin, 30 pmol/L	1.01 (0.42)	0.02	0.40 (0.38)	0.30	0.55 (0.39)	0.17	0.70 (0.22)	0.002
t-PA activity, IU/ml								
Age, 3year	- 0.03 (0.45)	0.95	0.05 (0.08)	0.58	- 0.17 (0.26)	0.51	- 0.12 (0.17)	0.49
BMI, 3kg/m ²	- 1.33 (0.76)	0.08	- 0.63 (0.13)	< 0.001	- 0.16 (0.35)	0.64	- 0.61 (0.26)	0.02
WHR, 0.05	0.72 (0.58)	0.22	0.28 (0.12)	0.03	- 0.52 (0.31)	0.10	0.12 (0.22)	0.58
Diastolic BP, 11mmHg	0.88 (0.44)	0.05	- 0.002 (0.08)	0.98	0.02 (0.26)	0.93	0.32 (0.17)	0.06
Ethanol intake, 26g/day	- 1.03 (0.47)	0.03	- 0.09 (0.07)	0.21	- 0.13 (0.27)	0.62	- 0.40 (0.16)	0.01
Current smoking, yes	0.52 (0.99)	0.60	- 0.49 (0.16)	0.002	- 0.71 (0.46)	0.13	- 0.38 (0.33)	0.26
Triglycerides, 0.95 mmol/L	- 1.43 (0.78)	0.07	0.08 (0.11)	0.46	0.03 (0.16)	0.87	- 0.14 (0.16)	0.39
Insulin, 30 pmol/L	- 0.57 (0.60)	0.34	0.19 (0.09)	0.05	- 0.21 (0.32)	0.52	- 0.20 (0.20)	0.33
PAI-1 antigen, ng/ml								
Age, 3year	- 0.44 (0.85)	0.61	- 1.88 (0.50)	< 0.001	0.53 (0.72)	0.46	- 0.56 (0.39)	0.15
BMI, 3kg/m ²	- 0.53 (1.43)	0.71	0.90 (0.80)	0.27	1.21 (0.97)	0.22	0.64 (0.61)	0.29
WHR, 0.05	0.55 (1.09)	0.62	2.49 (0.75)	0.001	1.18 (0.86)	0.17	1.15 (0.51)	0.02
Diastolic BP, 11mmHg	0.34 (0.84)	0.69	- 0.64 (0.50)	0.21	0.92 (0.71)	0.20	0.22 (0.39)	0.56
Ethanol intake, 26g/day	1.61 (0.89)	0.08	1.21 (0.41)	0.005	2.29 (0.73)	0.003	1.73 (0.37)	< 0.001
Current smoking, yes	3.78 (1.87)	0.05	2.78 (0.94)	0.004	2.71 (1.29)	0.04	2.99 (0.76)	< 0.001
Triglycerides, 0.95 mmol/L	2.75 (1.47)	0.07	1.06 (0.65)	0.11	2.24 (0.47)	< 0.001	2.14 (0.39)	< 0.001
Insulin, 30 pmol/L	1.39 (1.13)	0.22	2.28 (0.56)	< 0.001	2.09 (0.88)	0.02	1.83 (0.47)	< 0.001

SE:Standard error ; BMI:Body mass index; WHR: Waist hip ratio; t-PA: tissue plasminogen activator ; PAI-1: Plasminogen activator inhibitor-1

For t-PA activity, significant inverse associations with BMI and ethanol intake were found in total samples although the significant relations were not consistently observed in each population. A 3 kg/m² higher BMI was associated with a 0.61 ng/ml lower t-PA activity, and a 26 g/day higher ethanol intake was associated with a 0.40 ng/ml lower t-PA activity in the total samples.

Current smoking was associated significantly with PAI-1 antigen in each population. Smoking was associated with a 2.99 ng/ml higher PAI-1 antigen in the total samples. WHR, ethanol intake, current smoking, triglycerides, and insulin were also significantly associated with PAI-1 antigen in the total samples although the association did not always reach statistical significance in each population.

Discussion

There are some variations in mean t-PA activity, but not in mean values of plasma fibrinogen, t-PA antigen or PAI-1 antigen among three Japanese population samples. The higher mean value of BMI in central rural men compared with that in northeast rural men may explain the population difference in mean t-PA activity because BMI was a significant correlate of t-PA activity. Our previous study indicated that physical activity estimated by a 24-hour dietary recall was lower in central rural men than in northeast rural men,²⁰ which corresponded with higher mean BMI in central rural men than in northeast rural men.

We previously reported that mean values of fibrinogen, t-PA and PAI-1 antigens were higher in Caucasians than in Japanese^{11,12} where Japanese sample was chosen from northeast rural population as the present study did. The present study indicated that mean values of these hemostatic variables in northeast rural men did not differ significantly from those in the other two populations. Therefore, the results provide extended evidence on higher mean fibrinogen and fibrinolytic variables in Caucasians than in Japanese. Because these hemostatic variables were recognized to raise risk of coronary heart disease incidence^{7,9} or recurrence⁸, the higher level of these hemostatic variables corresponded to the higher mortality rate of coronary heart disease in the United States than in Japan.

There was a strong correlation between t-PA antigen and PAI-1 antigen in our study samples. PAI-1 antigen was also moderately and inversely correlated with t-PA activity. However, t-PA antigen was not significantly correlated with t-PA activity, which was consistent with previous findings.^{21,22} It is noteworthy that t-PA antigen levels does not necessarily reflect the activity because of the role of PAI-1, a rapid inhibitor of t-PA.²¹ For example, t-PA antigen levels are higher in the morning and lower in the afternoon whereas the activity shows an opposite diurnal fluctuation.^{14,15}

Within populations, we found significant associations of hemostatic variables with several cardiovascular risk factors. In multivariate analyses, serum insulin, serum triglycerides and WHR were inversely associated with t-PA antigen and PAI-1 antigen, but not with t-PA activity or fibrinogen. High values of serum insulin, serum triglycerides and WHR are clinical characteristics of impaired insulin sensitivity or insulin resistance. Thus, impaired insulin sensitivity was likely to be related with high antigen levels of t-PA and PAI-1.²³⁻²⁵

In vitro studies demonstrated that both insulin and very low

density lipoprotein (VLDL), rich in triglycerides, induce the synthesis and release of PAI-1 from endothelial cells²⁶ and hepatic cells.²⁷ Furthermore, an intervention study demonstrated that reduction of serum triglycerides by diet and medical treatment lead to reduction in plasma PAI-1 activity.²⁶ However, there was no report on the direct effect of insulin or VLDL on plasma t-PA antigen or activity. In the present study, t-PA activity was not significantly associated with the status of impaired insulin sensitivity unlike t-PA and PAI-1 antigen.

T-PA activity was significantly related with BMI but not with WHR in the multivariate analysis. The mechanism of the relation between BMI and t-PA activity was not known, but the similar relation was reported from a study of middle-aged Swedish men of borderline hypertension.²⁸

Current smoking was associated with fibrinogen and PAI-1 antigen in the present study. It is well known that cigarettes smoking increases plasma fibrinogen by stimulating production of fibrinogen in liver.²⁹ There is growing evidence that through increased activity of sympathetic nerve system, smoking may cause insulin resistance to glucose uptake and impaired insulin sensitivity³⁰⁻³³, which may in turn increases PAI-1 antigen or activity. However, in the present study, current smoking was not related with increased serum insulin or increased WHR although smoking was significantly associated with increased triglyceride levels in total samples; age- and population-adjusted mean values of serum insulin was 59 pmol/L for smokers and 62 pmol/L for non-smokers (*P* for difference = 0.48); that of WHR was 0.913 for smokers and 0.912 for non-smokers (*P* = 0.81); and mean triglycerides were 1.57 mmol/L for smokers and 1.28 mmol/L for non-smokers (*P* = 0.01).

Ethanol intake was positively associated with t-PA and PAI-1 antigen and inversely associated with t-PA activity among Japanese men who had a high mean ethanol intake. This result is consistent with the experimental data showing that moderate or high alcohol intake increases t-PA antigen^{34,35} and PAI-1 activity,³⁵ and decreases t-PA activity.³⁴ This result also corresponds to epidemiologic data that heavy drinking is associated with increased risk of coronary heart disease.^{36,37} Moderate ethanol intake is, however, associated with reduced coronary risk due to increased HDL-cholesterol levels,³⁷ reduced platelet aggregation,³⁸ and decreased concentrations of plasma fibrinogen and factor VII.³⁹ Therefore, it is postulated that at moderate ethanol intake, a potential adverse effect of ethanol in fibrinolytic system may be counterbalanced by these beneficial effects.

In conclusion, the present study showed that population mean values of plasma fibrinogen and fibrinolytic variables did not differ among three Japanese populations except for mean t-PA activity. Reduced fibrinolysis expressed as increased PAI-1 antigen was associated with smoking and the status of insulin resistance, such as high levels of serum insulin, serum triglycerides and waist-hip ratio.

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