Association of Plasminogen Activator Inhibitor-1 With Insulin Resistance in Japan Where Obesity Is Rare

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The association between plasminogen activator inhibitor-1 (PAI-1) and insulin resistance is established in western countries. The major component of this association is obesity. Accordingly, we examined this association in Japan where the prevalence of obesity is low. Data for fasting PAI-1 levels of 404 subjects were obtained from a general population in a farming area. We measured body mass index (BMI), systolic and diastolic blood pressure, high-density lipoprotein (HDL)-cholesterol, triglycerides, fasting plasma glucose (FPG), insulin, creatinine, and uric acid. The use of alcohol was ascertained by a questionnaire. The formula for the homeostasis model assessment (HOMA) score was used as an index of insulin resistance. Uni- and multivariate analyses were applied for the determinants of plasma PAI-1. Age and sex did not affect plasma PAI-1. The average BMI was 23.0 ± 3.2 kg/m^2. Thus, most of the subjects were not obese. Because, even in this population, BMI ($P < .001$) was the strongest determinant for PAI-1 after univariate analysis, we performed multiple linear regression analyses after adjustment for BMI. The significance of triglycerides, FPG, insulin, and the HOMA score still remained. PAI-1 levels were linearly related to the HOMA score. From the subanalysis of the non-obese subjects (BMI < 25; n = 298), waist-hip ratio, triglycerides, FPG, and HOMA scores were significant determinants of PAI-1. This is the first demonstration that increased PAI-1 levels were significantly related to insulin resistance in a Japanese general population. PAI-1 levels are associated with insulin resistance, irrespective of obesity.

PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) is a potent inhibitor of fibrinolysis by binding to and inactivating tissue plasminogen activator.[1] Increased plasma levels of PAI-1 and decreased fibrinolytic activity have been demonstrated in patients with coronary artery disease and is a prognostic factor for myocardial infarction.[2-4] Moreover, insulin resistance includes a cluster of risk factors for cardiovascular diseases, such as obesity, increased triglycerides, and decreased high-density lipoprotein (HDL).[5]

Several studies have shown elevated PAI-1 levels in conditions associated with insulin resistance.[6,7] It is suggested that PAI-1 contributes to the increased cardiovascular risk encountered with insulin resistance.[2,6,8-11] However, these previous studies[6,9-11] have been conducted in western countries where the prevalence of obesity is high. Because the key component of the relationship between increased PAI-1 and insulin resistance is obesity,[12-16] it may be interesting to examine this relationship in a non-obese population. Accordingly, we examined this relationship in Japan where the prevalence of obesity is low, the incidence of coronary artery disease is low,[17] and the dietary habit is different from western countries.[18]

SUBJECTS AND METHODS

Subjects

In 1999, in a farming community in southwestern Japan (Tanushimaru town), a total of 450 people received health examinations. Of these, 26 were not on an overnight fast, and we selected 424 subjects (183 men and 241 women). Because 19 subjects were taking hypoglycemic agents and 1 subject was missing data for PAI-1, 20 subjects were eliminated from this analysis. Finally, complete data sets for 404 subjects were available in this study.

Data Collection

The medical history, use of smoking, and alcohol were ascertained by a questionnaire. Smoking and alcohol were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI, kilograms per meter squared) was calculated as an index of obesity. Waist circumference was measured at the level of the umbilicus in the standing position, and hip circumference was measured at the level of greater trochanters. The waist-to-hip ratio (WHR) was used as a measure of upper body adiposity. Blood pressure was measured twice with the subjects in the supine position. The second blood pressure after 5 deep breaths with fifth phase diastolic pressure was used for analysis. Hypertensives were defined as blood pressure ≥ 140 and/or 90mm Hg or receiving antihypertensive medication. Systolic blood pressure was measured in the arms and in the right dorsalis pedis artery. The resting ankle brachial pressure index (ABI) was determined for the right leg by dividing the systolic blood pressure at the ankle by brachial pressure.

Blood was drawn from the antecubital vein for determination of lipids (total cholesterol, HDL-cholesterol, and triglycerides), free fatty acid (FFA), fasting plasma glucose (FPG), insulin, creatinine, uric acid, and PAI-1 levels in the morning after a 12-hour fast. PAI-1 was measured in citrated plasma using enzyme-linked immunosorbent assay[19] that is sensitive to free PAI-1, but not to PAI-1 complexed with t-PA. The citrated sample was centrifuged for a minimum of 10 minutes to make sure that there was no contamination from platelet PAI-1. The estimate of insulin resistance by homeostasis model assessment (HOMA) score was calculated with the formula: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 as described by Matthews et al.[20] Other chemistries, such as serum total cholesterol, HDL-cholesterol (enzymatic assay method), and creatinine (enzymatic assay method) were measured at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan).

The Ukiha branch of the Japan Medical Association, by the mayor, and the welfare section of Tanushimaru district approved this study. The Ethics Committee of Kurume University School of Medicine also approved this study. All participants gave informed consent.

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Table 1. Background of Subjects

	Men (183)	Women (241)	Total (424)
Age (yr)	62.8 ± 11.9	62.4 ± 12.7	62.6 ± 12.4
Systolic BP (mm Hg)	135.6 ± 21.5	132.4 ± 20.3	133.8 ± 20.8
Diastolic BP (mm Hg)	80.8 ± 13.3	76.2 ± 11.4	78.2 ± 12.2
ABI	1.05 ± 0.11	1.03 ± 0.09	1.04 ± 0.10
BMI (kg/m^2)	23.1 ± 3.1	23.0 ± 3.2	23.0 ± 3.2
Waist-hip ratio	0.88 ± 0.06	0.81 ± 0.06	0.84 ± 0.07
Total cholesterol (mmol/L)	4.88 ± 0.90	5.33 ± 0.80	5.13 ± 0.88
HDL-cholesterol (mmol/L)	1.33 ± 0.38	1.47 ± 0.35	1.41 ± 0.36
Triglycerides (mmol/L)	1.21 ± 0.02	1.07 ± 0.02	1.13 ± 0.02
FFA (mEq/L)	0.55 ± 0.33	0.59 ± 0.35	0.57 ± 0.34
FPG (mmol/L)*	5.33 ± 0.07	5.15 ± 0.06	5.23 ± 0.07
Insulin (pmol/L)*	26.4 ± 11.4	27.6 ± 10.2	27.0 ± 10.8
HOMA score*	1.04 ± 0.68	1.05 ± 0.61	1.05 ± 0.64
Creatinine (μmol/L)	86.6 ± 22.9	68.1 ± 10.6	76.0 ± 19.4
Uric acid (μmol/L)	350.9 ± 83.3	267.7 ± 71.4	303.3 ± 89.2
PAI-1 (ng/mL)*	23.1 ± 1.8	23.4 ± 1.8	23.3 ± 1.8
Current smoking (% yes)	78 (42.6)	7 (2.9)	84 (19.8)
Alcohol intake (% yes)	96 (52.4)	15 (6.2)	111 (26.2)
Medication (% yes)	44 (24.0)	47 (19.5)	91 (21.5)
Diabetes mellitus	11 (6.0)	8 (3.3)	19 (4.5)
Hyperlipidemia	4 (2.9)	14 (5.8)	18 (4.2)

Abbreviations: BP, blood pressure; ABI, ankle-brachial index; BMI, body mass index; FFA, free fatty acid; FPG, fasting plasma glucose.
*Log-transformed values were used.

Table 2. Univariate Analyses for Determination of Plasma PAI-1 Levels

Variables	β^+	SE	*t* Value	*P*
Age	−0.016	0.002	−0.320	.749
Sex	−0.034	0.060	0.679	.497
Systolic BP	0.068	0.001	1.378	.169
Diastolic BP	0.142	0.002	2.884	.004
ABI	0.043	0.305	0.868	.386
BMI	0.235	0.009	4.862	.0001
Waist-hip ratio	0.184	0.416	3.763	.001
Total cholesterol	0.058	0.001	1.166	.244
HDL-cholesterol	−0.118	0.002	−2.395	.017
Triglycerides†	0.222	0.067	3.627	.0001
FFA	−0.056	0.087	−1.127	.261
FPG†	0.174	0.272	2.813	.005
Insulin†	0.290	0.059	4.819	.0001
HOMA score†	0.308	0.055	5.157	.0001
Creatinine	0.026	0.135	0.530	.597
Uric acid	0.123	0.020	2.479	.014
Current smoking	−0.010	0.074	−0.198	.843
Alcohol intake	0.059	0.068	1.190	.235

NOTE. β^+, standardized regression coefficients.
Abbreviations: SE, standard error, BP, blood pressure; ABI, ankle-brachial index; BMI, body mass index; FFA, free fatty acid; FPG, fasting plasma glucose.
*Log-transformed values were used.

Statistical Methods

Because of skewed distributions, the natural logarithmic (ln) transformations were performed for triglycerides, FPG, insulin, HOMA score, and PAI-1. Mean values with upper and lower 95% confidence intervals (CI) were exponentiated and presented as geometric mean ± standard deviation (SD), in which SD was approximated as the difference of the exponentiated CI divided by 3.92, which is the number of SD in a 95% CI where data are normally distributed. Results are presented as mean ± SD. The medications for hypertension, hyperlipidemia, and diabetes were coded as dummy variables. Multiple linear regression analysis was performed for determinants of plasma PAI-1 levels adjusted for BMI. Mean plasma PAI-1 levels by tertiles of the increasing HOMA score were compared using analysis of covariance, adjusted for age, sex, and BMI as covariates. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of the SPSS system (SPSS, Chicago, IL).

RESULTS

Backgrounds of the subjects are presented in Table 1. Most of them were normotensive, non-obese, nondiabetic, and normolipidemic, but the prevalence of smoking was high in men. PAI-1 levels did not differ between men and women. Determinants of plasma PAI-1 levels are shown in Table 2. Parameters statistically significantly related to PAI-1 levels were diastolic blood pressure ($P < .05$), BMI ($P < .001$), WHR ($P < .01$), HDL-cholesterol (inversely, $P < .05$), triglycerides ($P < .001$), FPG ($P < .01$), insulin ($P < .001$), HOMA score ($P < .001$), and uric acid ($P < .05$). Because BMI was the strongest determinant of PAI-1, multiple linear regression analysis was performed after adjustment for BMI. The significance of triglycerides ($P < .05$), FPG ($P < .05$), insulin ($P < .01$), and HOMA score ($P < .001$) still remained (Table 3). HOMA score was the strongest determinant for plasma PAI-1 levels. Statistical significance and dose-response relationship are demonstrated for HOMA score in Fig 1.

To further investigate the impact of obesity on the relationship between PAI-1 and insulin resistance, univariate analysis was performed in the subgroup of non-obese subjects (BMI < 25, n = 298), again BMI ($P < .05$), WHR ($P < .01$), triglycerides ($P < .05$), FPG ($P < .05$), insulin ($P < .05$), and HOMA score ($P < .05$) were significant determinants of PAI-1 (Table 4).

DISCUSSION

PAI-1 and Insulin Resistance

A positive association between fasting plasma insulin levels and PAI-1 has been demonstrated by several investigators in

Table 3. Multiple Linear Regression Analysis for Determination of Plasma PAI-1 Levels Adjusted for BMI

Variables	β^+	SE	*t* Value	*P*
Diastolic BP	0.095	0.002	1.918	.056
Waist-hip ratio	0.089	0.474	1.586	.113
HDL-cholesterol	−0.064	0.002	−1.282	.201
Triglycerides*	0.160	0.068	2.565	.011
FPG*	0.141	0.260	2.322	.021
Insulin*	0.207	0.067	3.040	.003
HOMA score*	0.231	0.062	3.417	.001
Uric acid	0.075	0.020	1.520	.129

NOTE. β^+, standardized regression coefficients.
Abbreviations: SE, standard error, BP, blood pressure; FPG, fasting plasma glucose.
*Log-transformed values were used.

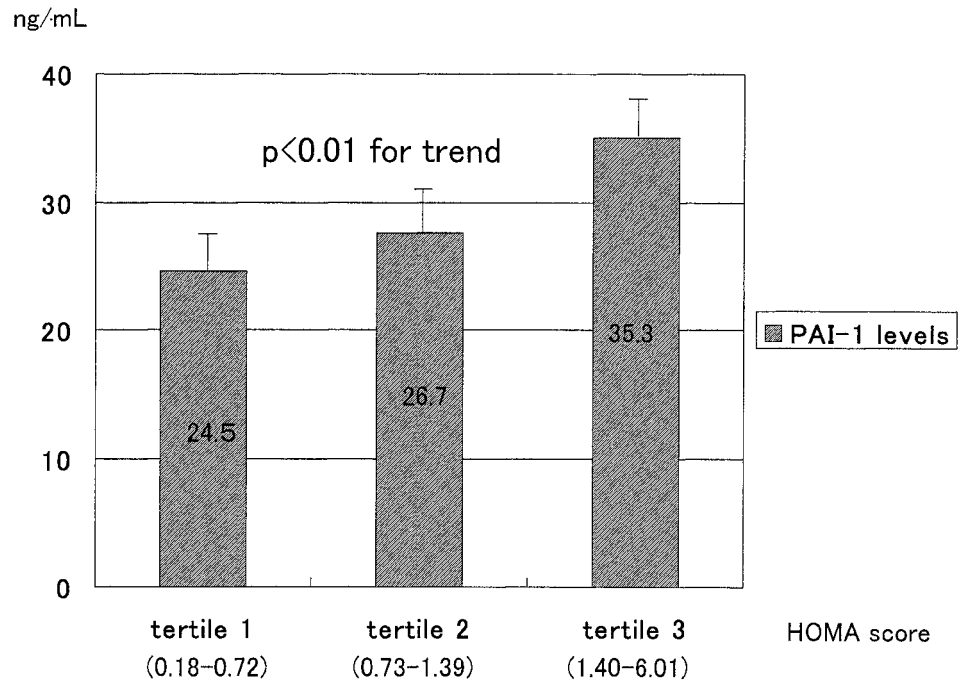

Fig 1. Age, sex, and BMI-adjusted mean plasma PAI-1 levels stratified by HOMA index tertiles.

western countries[6,7,10,12,15] where the incidence of coronary artery disease is high, the prevalence of obesity is high, and the dietary and social habits are different from those of the Japanese. However, there are no epidemiologic studies regarding this relationship in Japan. As shown in Table 1, most of the subjects enrolled in this study were non-obese, normolipidemic, and nondiabetic, but the prevalence of smokers was high. It may be interesting to note that the mean cholesterol levels in both men and women were compatible with those in western countries.[15,21] On the above-described backgrounds, the mean level of PAI-1 was not much different from those reported in western countries.[21,22] As reported previously,[11,14] age and gender did not affect PAI-1 levels in this study, as well.

We found that typical parameters of insulin resistance were associated with plasma PAI-1 levels. We measured FPG, insulin levels, and calculated HOMA score, which is used for an index of insulin resistance in epidemiologic studies.[23-25] Although diabetics were rare in our population (Table 1), we excluded subjects who were taking hypoglycemic agents in the analysis. The results of analyses then indicated that the association between increased PAI-1 with insulin resistance does exist.

Table 4. Univariate Analysis for Determination of Plasma PAI-1 Levels

Variables	β^+	SE	*P*
BMI	0.120	0.015	.040
Waist-hip ratio	0.166	0.498	.004
Total cholesterol	−0.015	0.035	.794
HDL-cholesterol	−0.122	0.002	.055
Triglycerides*	0.181	0.078	.012
FPG*	0.151	0.296	.035
Insulin*	0.152	0.074	.034
HOMA score*	0.178	0.069	.013
Uric acid	0.072	0.024	.217

NOTE. Non-obese subjects; BMI < 25; n = 298. β^+, standardized regression coefficients.

Abbreviations: SE, standard error, BMI, body mass index; FPG, fasting plasma glucose.

*Log-transformed values were used.

Impact of Obesity on the Association Between PAI-1 and Insulin Resistance

It has been demonstrated that obesity is a key component of the association between increased PAI-1 and insulin resistance.[10,12-16] In this study, most subjects were not obese as shown in Table 1. Even in this population, BMI was a strong ($P < .001$) determinant of PAI-1 (Table 2), indicating the importance of obesity for the association between increased PAI-1 and insulin resistance. To investigate the impact of obesity, multiple linear regression analysis was performed after adjustment for BMI (Table 3), which demonstrated the positive association between increased PAI-1 and triglycerides, FPG, plasma insulin levels, and HOMA score. This analysis indicated that the association between increased PAI-1 and insulin resistance does exists, irrespective of obesity. To further elucidate the impact of obesity, we subanalyzed the non-obese population with BMI < 25 kg/m^2 (Table 4), which showed the association with PAI-1, BMI, WHR, triglycerides, FPG, plasma insulin level, and HOMA score. Thus, these careful analyses suggested the positive association between increased plasma PAI-1 levels and insulin resistance, irrespective of obesity.

In conclusion, this is the first demonstration that increased PAI-1 levels were significantly related to insulin resistance in a

Japanese general population in which the prevalence of obesity is low. PAI-1 levels are associated with insulin resistance, irrespective of obesity.

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REFERENCES

1. Sprengers ED, Kluft C: Plasminogen activator inhibitors. Blood 69:381-387, 1987

2. Hamsten A, Wiman B, De Faire U, et al: Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 313:1557-1563, 1985

3. Nilsson TK, Johnson O: The extrinsic fibrinolytic system in survivors of myocardial infarction. Thromb Res 48:621-630, 1987

4. Almér LO, Öhlin H: Elevated levels of the rapid inhibitor of plasminogen activator (t-PAI) in acute myocardial infarction. Thromb Res 47:335-339, 1987

5. Reaven GM.: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

6. Potter van Loon BJ, Kluft C, Radder JK, et al: The cardiovascular risk factor plasminogen activator inhibitor type I is related to insulin resistance. Metabolism 42:945-949, 1993

7. Bastard JP, Piéroni L, Hainque B: Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev 16:192-201, 2000

8. Levi M, Biemond BJ, van Zonneveld AJ, et al: Inhibition of plasminogen activator inhibitor-1 activity results in promotion of endogenous thrombolysis and inhibition of thrombus extension in models of experimental thrombosis. Circulation 85:305-312, 1992

9. Hamsten A, de Faire U, Walldius G, et al: Plasminogen activator inhibitor in plasma: Risk factor for recurrent myocardial infarction. Lancet 2:3-9, 1987

10. Vague J, Alessi MC: PAI-1, obesity, insulin resistance and risk of cardiovascular events. Thromb Haemost 78:656-660, 1997

11. Thögerson AM, Jansson JH, Boman K, et al: High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women. Circulation 98:2241-2247, 1998

12. Bastard JP, Piéroni L: Plasma plasminogen activator inhibitor 1, insulin resistance and android obesity. Biomed Pharmacother 53:455-461, 1999

13. Loskutoff DJ, Samad F: The adipocyte and hemostatic balance in obesity. Studies of PAI-1. Arterioscler Thromb Vasc Biol 18:1-6, 1998

14. Vague P, Juhan-Vague I, Chabert V, et al: Fat distribution and plasminogen activator inhibitor activity in nondiabetic obese women. Metabolism 38:913-915, 1989

15. Landin K, Stigendal L, Eriksson E, et al: Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism 39:1044-1048, 1990

16. Vague P, Juhan-Vague I, Aillaud MF, et al: Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and reactive body weight in normal and obese subjects. Metabolism 35:250-253, 1986

17. Toshima H: Coronary artery disease trends in Japan. Jpn Circ J 58:166-172, 1994

18. Hertog MG, Kromhout D, Aravanis C, et al: Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch Intern Med 155:381-386, 1995

19. Declerck PJ, Alessi MC, Verstreken M, et al: Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood 71:220-225, 1988

20. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and B-cell function from fasting plasma glucose and insulin concentration in men. Diabetologia 28:412-419, 1985

21. Haffner SM, D'Agostino R, Mykkänen L, et al: Proinsulin and insulin concentrations in relation to carotid wall thickness. Insulin Resistance Atherosclerosis Study. Stroke 29:1498-1503, 1998

22. Festa A, D'Agostino R, Mykkänen L, et al: Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance. The Insulin Resistance Atherosclerosis Study. Arterioscler Thromb Vasc Biol 19:562-568, 1999

23. Haffner SM, Gonzalez C, Miettinen H, et al: A prospective analysis of the HOMA model: The Mexico City Diabetes Study. Diabetes Care 19:1138-1141, 1996

24. Haffner SM, Miettinen H, Stern MP: The homeostasis model in the San Antonio Heart Study. Diabetes Care 20:1087-1092, 1997

25. Adachi H, Hirai Y, Tsuruta M, et al: Is insulin resistance or diabetes mellitus associated with stroke? An 18-year follow-up study. Diabetes Res Clin Pract 51:215-223, 2001