Relationship of Hepatic and Peripheral Insulin Resistance With Plasminogen Activator Inhibitor-1 in Pima Indians

D.K. Nagi, R. Tracy, and R. Pratley

Plasminogen activator inhibitor-1 (PAI-1) is related to insulin resistance and several components of the insulin resistance syndrome, and PAI-1 levels are elevated in subjects with non–insulin-dependent diabetes mellitus. Many Pima Indians are obese, insulin-resistant, and hyperinsulinemic, and they have high rates of diabetes but a low risk of ischemic heart disease. In contrast to whites and Asians, PAI-1 activity is similar between nondiabetic and diabetic Pima Indians. We therefore examined the association of PAI-1 with hepatic and peripheral insulin action measured using the hyperinsulinemic-euglycemic clamp. To investigate if insulin per se has any effect on PAI-1 in vivo, we also assessed the effects of endogenous (during a 75-g oral glucose load) and exogenous (during hyperinsulinemic clamp) insulin on PAI-1 antigen. Twenty-one (14 men and seven women; mean age, 26.3 ± 4.8 years) Pima Indians underwent a 75-g oral glucose tolerance test (OGTT) and a sequential hyperinsulinemic-euglycemic clamp. Peripheral insulin action was measured as absolute glucose uptake (M value) and normalized to estimated metabolic body size (EMBS). Hepatic insulin action was measured as percent suppression of basal hepatic glucose output during hyperinsulinemia. PAI-1 antigen was determined using a two-site enzyme-linked immunosorbent assay that detects only free PAI-1. PAI-1 antigen concentrations were significantly related to body mass index ([BMI] $r_s = .54$, $P = .012$), waist ($r_s = .52$, $P = .016$) and thigh ($r_s = .63$, $P = .002$) circumference, and fasting plasma insulin concentration ($r_s = .59$, $P = .004$). PAI-1 antigen concentrations were not significantly associated with peripheral glucose uptake (M value) during either low-dose ($r_s = -.01$, P = NS) or high-dose ($r_s = -.11$, P = NS) insulin infusion. PAI-1 antigen was negatively correlated with basal hepatic glucose output ($r_s = -.57$, $P = .013$) and percent suppression of hepatic glucose output during hyperinsulinemia ($r_s = -.69$, $P = .005$). However, this relationship was largely due to the confounding effects of BMI, waist and thigh girth, fasting insulin, and 2-hour postload glucose concentrations, and was not significant when controlled for these variables (partial $r_s = -.30$, P = NS). There was no significant relationship of PAI-1 antigen concentration with glucose storage or glucose oxidation. Despite a threefold increase in plasma insulin concentrations during the OGTT, there were no significant changes in PAI-1 antigen concentrations (median, 57, 61, 55, and 44 ng/mL at 0, 60, 120, and 180 minutes, respectively; P = NS by ANOVA). During the hyperinsulinemic clamp, mean plasma insulin concentrations at the end of low-dose (240 pmol/m²/min) and high-dose (2,400 pmol/m²/min) infusions were 1,005 and 14,230 pmol/L, respectively. However, PAI-1 antigen concentrations at the end of low-dose and high-dose insulin infusions were similar to those at baseline (median, 63, 43, and 58 ng/mL, respectively; P = NS by ANOVA). PAI-1 antigen in Pima Indians is related to several components of the insulin resistance syndrome. However, direct measurement of insulin resistance indicates that hepatic but not peripheral insulin resistance is related to PAI-1 antigen. Neither endogenous nor exogenous hyperinsulinemia for short periods had any significant effect on PAI-1 antigen concentrations. Short-term hyperinsulinemia is unlikely to be an important regulator of PAI-1 in Pima Indians. The relationship of PAI-1 antigen to hepatic insulin resistance is largely dependent on the relationship of PAI-1 to indices of obesity and fasting insulin concentrations.

THE INSULIN RESISTANCE syndrome (syndrome X) is an aggregation of hyperinsulinemia, glucose intolerance, high triglyceride, low high-density lipoprotein cholesterol, and hypertension, the underlying common factor being insulin resistance.[1] Subsequently, abdominal obesity and lack of physical activity were also suggested to be an integral part of this syndrome.[2] Subjects with features of this syndrome are excessively prone to cardiovascular disease.[3]

Plasminogen activator inhibitor-1 (PAI-1) is a fast-acting inhibitor of fibrinolysis,[4] and its activity is elevated in a number of disease states, including non–insulin-dependent diabetes mellitus.[5-8] PAI-1 is related to several components of the insulin resistance syndrome such as obesity and body fat distribution,[9,10] serum triglyceride concentration,[11,12] plasma insulin concentration,[7,8] and blood pressure.[13] This may be an indication of the relationship of PAI-1 with insulin resistance.[14] Indeed, studies in white subjects have shown that PAI-1 is related to insulin resistance as determined by the hyperinsulinemic-euglycemic clamp.[15,16]

Many Pima Indians are obese and hyperinsulinemic, and insulin resistance is a predominant metabolic abnormality in this ethnic group.[17,18] However, PAI-1 activity in nondiabetic Pima Indians is similar to that seen in whites and is unrelated to diabetes in this ethnic group.[19] These observations therefore prompted us to analyze the relationship of PAI-1 with insulin resistance measured directly by the hyperinsulinemic-euglycemic clamp in this group.

SUBJECTS AND METHODS

We studied 21 Pima Indians with no previously known abnormality of glucose tolerance. These subjects were admitted to the Clinical Research Center and fed a weight-maintaining diet for 3 days. Each subject underwent a 3-hour 75-g oral glucose tolerance test (OGTT), followed by a two-step hyperinsulinemic-euglycemic clamp 3 to 5 days after the OGTT to measure insulin action as described previously.[20] Blood pressure was measured twice using a wide cuff. Waist and thigh girth measurements were made to assess

From the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, AZ; and the Thrombosis Research Center, Burlington, VT.

Submitted December 15, 1995; accepted April 26, 1996.

Supported in part by a British Diabetic Association-Imperial Chemical Industries (BDA-IC) Transatlantic Fellowship (D.K.N.).

Address reprint requests to D.K. Nagi, MBBS, MRCP, PhD, Edna Coates Center, Pinderfields Hospital Trust, Aberford Road, Wakefield, WF1 4DG, England.

0026-0495/96/4510-0009$03.00/0

distribution of fat. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Percent body fat and fat-free mass were determined by underwater weighing as described by Bogardus et al.[21]

Measurement of Peripheral and Hepatic Insulin Action

A low-dose primed-continuous insulin infusion (240 pmol/m^2/min) was started at 8 AM and continued for 100 minutes. A variable 20% glucose infusion was administered to maintain plasma glucose at the basal level, and plasma glucose was determined at 5-minute intervals throughout the test. Immediately after the low-dose infusion, a high-dose insulin infusion (2,400 pmol/m^2/min) was administered for another 100 minutes. Steady-state plasma insulin concentrations were determined from blood samples obtained during the last 30 minutes of each clamp period. To account for differences in metabolic body size among subjects, glucose uptake rates (M values) were normalized for estimated metabolic body size ([EMBS] = fat-free mass + 17.7). This estimate of metabolic body size was derived from resting metabolic rate measurements with indirect calorimetry as previously described by Lillioja and Bogardus.[22]

The rate of glucose oxidation and glucose storage (calculated as M value − glucose oxidation) was measured at the end of the low- and high-dose insulin infusion. The hepatic glucose production rate was measured in the fasting state and during hyperinsulinemia. Hepatic insulin sensitivity was expressed as percent suppression of basal hepatic glucose output during hyperinsulinemia.

To assess the effect of endogenous insulin production on PAI-1, these subjects also underwent a prolonged OGTT with measurement of PAI-1 antigen and insulin concentrations during the test. PAI-1 antigen concentrations were determined using an enzyme-linked immunosorbent assay method[23] that detects only free PAI-1 (ie, uncomplexed with tissue plasminogen activator [tPA]), and plasma insulin was determined by double-antibody radioimmunoassay.[24] Plasma glucose was determined by the glucose oxidase method with a Beckman glucose analyzer (Fullerton, CA). Diabetes was diagnosed by World Health Organization criteria.[25]

Statistics

The data are shown as the mean ± SD or as the median and range. ANOVA was used to compare group means at different time points during the OGTT. Correlation analyses were performed using Spearman correlation coefficients.

RESULTS

Clinical details of the subjects are shown in Table 1. Three subjects were diagnosed as having diabetes and three had impaired glucose tolerance using World Health Organization criteria. Steady-state plasma glucose concentrations and coefficients of variation for glucose during low- and high-dose clamps are shown in Table 2. Percent suppression of basal hepatic glucose output during hyperinsulinemia was 90% (10% to 100%).

Table 1. Clinical Characteristics of the Subjects (N = 21)

Characteristic	Mean ± SD
Age (yr)	26.3 ± 4.8
Sex (M/F)	14/7
BMI (kg/m^2)	32.9 ± 5.4
Body fat (%)	32 ± 7
Waist (cm)	107.4 ± 13.7
Thigh (cm)	66.4 ± 6.5
Systolic blood pressure (mm Hg)	132 ± 26
Diastolic blood pressure (mm Hg)	82 ± 17

Table 2. Data for the Sequential Hyperinsulinemic-Eyglycemic Clamp

Variable	Basal (0 min)	End of Low-Dose Insulin Infusion (240 pmol/m^2/min)	End of High-Dose Insulin Infusion (2,400 pmol/m^2/min)
Steady-state plasma insulin (pmol/L)	—	888 (522-2,742)	14,393 (7,049-24,442)
Mean steady-state plasma glucose (mmol/L)	—	5.83 ± 0.42	5.59 ± 0.20
Coefficient of variation for glucose	—	1.97 ± 0.46	3.70 ± 0.72
Glucose disposal (mg/min/kg EMBS)	—	3.33 ± 1.31	9.21 ± 1.85
Hepatic glucose output (mg/min/kg EMBS)	1.98 ± 0.23	—	—
PAI-1 antigen (ng/mL)	58 (18-169)	63 (9-199)	43 (15-183)

NOTE. Data are shown as the mean ± SD or as the median (range); n = 20.

Relationships of PAI-1 Antigen With Peripheral and Hepatic Insulin Action

PAI-1 antigen concentrations were significantly related to BMI, waist circumference, thigh circumference, and plasma insulin concentration, but not to percent body fat (Table 3). PAI-1 antigen concentrations measured at baseline were not significantly related to peripheral glucose uptake (M value) during either low-dose ($r_s = -.10$; Fig 1a) or high-dose ($r_s = -.12$) insulin infusion (Fig 1b). There was no relationship between basal hepatic glucose output and percent suppression of hepatic glucose output during hyperinsulinemia. Suppression of hepatic glucose output was significantly and negatively correlated with BMI ($r_s = -.62$, $P = .006$), waist girth ($r_s = -.69$, $P = .001$), thigh girth ($r_s = -.052$, $P = .025$), fasting insulin concentration ($r_s = -.69$, $P = .001$), and 2-hour postload glucose concentration ($r_s = -.57$, $P = .01$). PAI-1 antigen concentrations were negatively correlated with basal hepatic glucose output ($r_s = -.61$, $P = .027$; Fig 2a), but not with hepatic insulin resistance expressed as percent suppression of basal hepatic glucose during hyperinsulinemia ($r_s = -.29$, P = NS). However, we reanalyzed this relationship after omitting two subjects in whom suppression of hepatic glucose

Table 3. Spearman Correlation Coefficients for PAI-1 Antigen With Baseline Clinical and Biochemical Variables

Variable	r_s	P
Weight	.46	.034
BMI	.54	.012
Waist circumference	.52	.016
Thigh circumference	.63	.002
Waist to hip ratio	.14	NS
Percent fat	.14	NS
Fasting plasma insulin	.59	.011
Fasting plasma glucose	.41	.070

NOTE. N = 21 except for percent fat (n = 19).

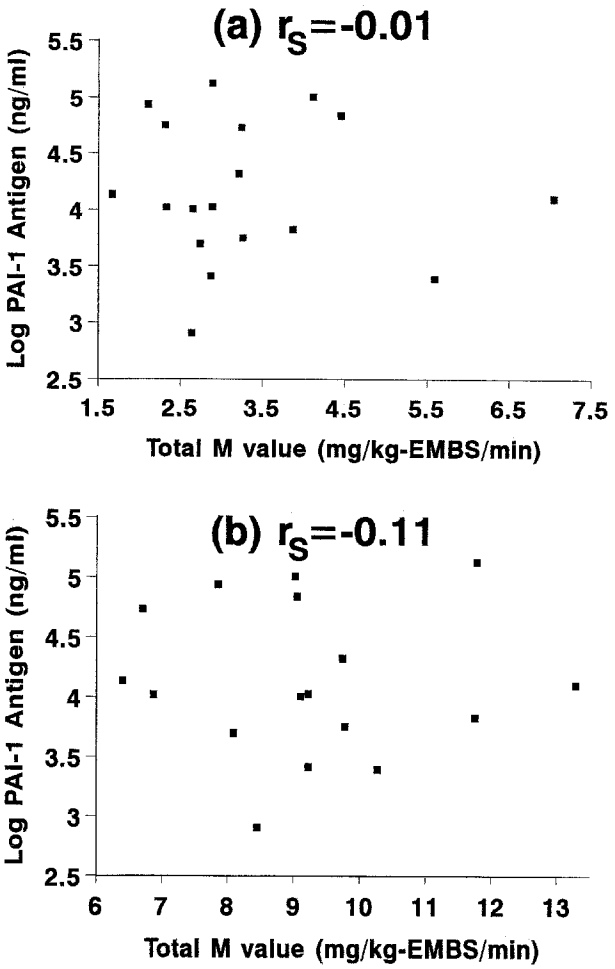

Fig 1. Relationship of peripheral glucose uptake (M value) with PAI-1 antigen concentration during (a) low-dose (n = 18) and (b) high-dose (n = 17) insulin infusion.

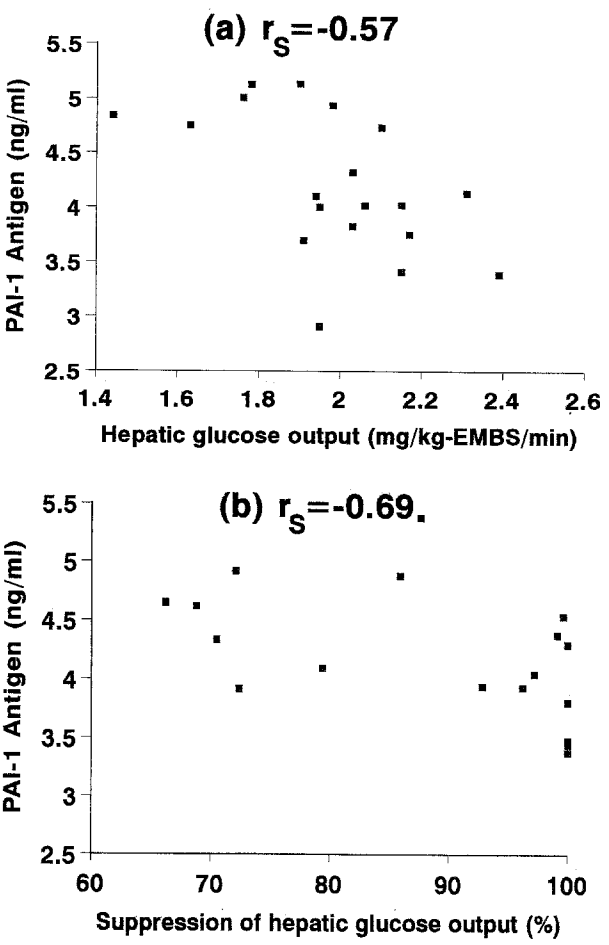

Fig 2. Relationship of (a) basal hepatic glucose output (n = 19) and (b) percent suppression of hepatic glucose output (n = 18) with PAI-1 antigen concentration during hyperinsulinemia.

output was minimal (<15%) and found a highly significant relationship of PAI-1 with percent suppression of hepatic glucose output ($r_s = -.69$, $P = .005$; Fig 2b). However, this relationship was no longer significant when controlled for the effects of BMI, waist and thigh girth, fasting insulin, and 2-hour postload glucose concentrations (partial $r_s = -.30$, P = NS).

In a multiple regression analysis, age, sex, BMI, thigh girth, fasting insulin, and insulin resistance (peripheral and hepatic) explained 60% of the variability in PAI-1 antigen concentrations. However, the contribution of peripheral and hepatic insulin resistance to the variability of PAI-1 antigen concentrations was 12% (data not shown).

Effect of Exogenous and Endogenous Hyperinsulinemia on PAI-1

Mean insulin concentrations at the end of low-dose and high-dose insulin infusions were 1,005 and 14,230 pmol/L, respectively. However, PAI-1 antigen concentrations at these times were not significantly different from those at baseline (median, 58, 63, and 43 ng/mL at 0, 100, and 200 minutes, respectively, P = NS by ANOVA; Table 2).

During the OGTT, plasma insulin concentrations increased significantly ($P < .001$), but there was no change in PAI-1 antigen concentrations (median, 57, 61, 55, and 44 ng/mL at 0, 60, 120, and 180 minutes, respectively, P = NS by ANOVA; Table 4). There was no relationship between changes in PAI-1 antigen concentrations and changes in insulin ($r_s = -.16$, P = NS), and plasma glucose ($r_s = -.17$, P = NS) concentrations during the OGTT.

DISCUSSION

The results of this study show no significant relationship of peripheral insulin action with PAI-1 antigen concentrations in a group of young Pima Indians. However, hepatic insulin action was significantly related to PAI-1 antigen, although this relationship was confounded by the relationship of PAI-1 antigen with BMI and fasting insulin concen-

Table 4. Biochemical Data During 3-Hour OGTT (N = 21)

Time (min)	Plasma Glucose (mmol/L)	Plasma Insulin (mU/L)	PAI-1 Antigen (ng/mL)
0	4.5 ± 0.4	239 (125-586)	57 (28-216)
30	8.1 ± 1.7	1,282 (488-3,785)	—
60	8.4 ± 1.9	1,197 (322-3,964)	61 (13-190)
120	7.3 ± 2.3	790 (169-5,106)	55 (17-201)
180	4.9 ± 1.6	387 (125-3,533)	44 (20-193)

NOTE. Data shown as mean ± SD or as median (range).

tration. Whether the relationship of PAI-1 with hepatic insulin resistance is of major significance in determining circulating PAI-1 is unclear, since a previous study showed that PAI-1 activity was not significantly different between nondiabetic and diabetic Pima Indians.[19] The lack of an association of peripheral insulin resistance with PAI-1 antigen concentration in Pima Indians contrasts with those seen in whites, in whom PAI-1 is related to insulin resistance,[15] and subjects with type II diabetes mellitus have higher levels of PAI-1 than nondiabetic subjects.[7,8] In previously published studies, one investigation showed a weak relationship of PAI-1 activity with peripheral glucose uptake,[16] whereas in another the relationship of PAI-1 with insulin resistance was much stronger.[15] In the study by Potter van Loon et al,[15] PAI-1 antigen was strongly associated with both hepatic and peripheral insulin action, with an r value of .67 and .87, respectively, when expressed as the ED_{50}, ie, the insulin concentration at which hepatic suppression of glucose or peripheral glucose uptake was half-maximal. However, the relationship was weaker ($r = .57$, $P < .05$) with a maximal rate of peripheral glucose uptake. In the latter study, insulin resistance and intact proinsulin concentration together explained the majority of variation in PAI-1 antigen. However, it is worth noting that insulin action was expressed as the peripheral insulin concentration at which peripheral glucose utilization was half-maximal (ED_{50}), and not as the absolute M value of glucose, as we used in this study. Although these methodological differences may partly explain differences between the results of our study and those reported by Potter van Loon et al, they are unlikely to be the sole explanation. Nevertheless, these data strongly suggest that there may be ethnic differences in the relationship of PAI-1 with peripheral insulin resistance. These findings also indicate that the previously reported relationship of peripheral insulin resistance with PAI-1 is not interlinked with the effect of insulin on glucose metabolism at the sites of peripheral glucose uptake, ie, skeletal muscle.

In this study, PAI-1 antigen was related to several components of the insulin resistance syndrome such as BMI, waist to hip ratio, thigh girth, and fasting insulin concentration, as in other populations. The reason for the noted disparity, ie, no relationship with a direct measurement of insulin action, but a significant relationship with variables associated with insulin resistance, is unclear. However, these data indicate that peripheral insulin resistance may not be an important regulator of PAI-1 antigen concentrations in Pima Indians.

Insulin has been suggested to be an important regulator of PAI-1, and in vitro studies have shown that insulin stimulates the synthesis and secretion of PAI-1 from Hep G2[26] and endothelial[27] cell lines. In the current study, endogenous hyperinsulinemia during the OGTT had no significant effect on PAI-1 antigen concentrations, confirming that short-term endogenous hyperinsulinemia is unlikely to be an important regulator of PAI-1 antigen in Pima Indians. Our results are in agreement with those of a previous study in white subjects, in whom PAI-1 concentrations did not change during an OGTT,[28,29] but are opposite to those of Medvescek et al,[30] who reported increased PAI-1 activity during a carbohydrate meal. We did not see any effect of exogenous hyperinsulinemia on PAI-1 antigen concentrations, in contrast to previous data wherein exogenous hyperinsulinemia was associated with a paradoxical suppression of PAI-1 antigen and an increase in tPA concentrations,[16] but similar to data reported by Grant et al,[31] which show no effect. The trend toward a lower PAI-1 antigen concentration during a clamp and OGTT is entirely in keeping with previously described diurnal variations in PAI-1.[32] It has been suggested that insulin may acutely enhance the clearance of circulating PAI-1:tPA complex, and any acute effect of insulin on PAI-1 may therefore be masked.[16] However, in our study, the assay only measured the free PAI-1 antigen level, and any effect of insulin on the clearance of the PAI-1:tPA complex would not have affected our results. Secondly, triglyceride-dependent stimulation of PAI-1[33,34] may also be masked by insulin due to its positive effect on triglyceride elimination, thereby further confounding the results. Thirdly, insulin also has an acute effect on the sympathetic nervous system,[35] and catecholamines have been shown to enhance fibrinolytic activity.[36] Finally, the relationship of PAI-1 with fasting plasma insulin concentration and not with an acute increase in plasma insulin concentration during the OGTT or hyperinsulinemic-euglycemic clamp suggests that it is chronic exposure to hyperinsulinemia that affects the fibrinolytic system.

Hyperglycemia stimulates PAI-1 synthesis and secretion[37] and is related to PAI-1 activity independently of its relationship with BMI, triglyceride, and fasting insulin concentration.[38] However, there was no effect of high plasma glucose levels during the OGTT on PAI-1 antigen concentrations in this study, again suggesting that high plasma glucose for only a short period has no effect or a minimal effect on PAI-1. However, our results do not discount the possibility that chronic and persistent hyperglycemia may be an important regulator of circulating PAI-1. Indeed, a recent study indicates that glucose may regulate the PAI-1 gene.[39] However, our previous observations that PAI-1 activity was similar between nondiabetic and diabetic subjects[19] would argue against a major role of glucose in PAI-1 gene regulation, at least in Pima Indians.

In summary, the results of this study show a lack of association of PAI-1 antigen concentration with peripheral insulin resistance in Pima Indians. These results are consistent with our previous observation that PAI-1 activity is similar between nondiabetic and diabetic Pima Indians. We have also shown that hyperinsulinemia over a short period is not an important regulator of PAI-1 in this population. Recently, studies have shown that the effect of triglyceride and plasma glucose concentrations, other important regulators of PAI-1, may be dependent on PAI-1 genotype,[39-41] and such studies are currently in progress in this population.

ACKNOWLEDGMENT

We are grateful to members of The Gila River Indian Community for their participation in this project, and the staff of the Clinical and Nutrition Diabetes Section. We are thankful to Linda Phillips for assistance in various measurments and other organizational aspects of the study. We are extremely grateful to Dr Clifton Bogardus and Dr William Knowler for organizational aspects of this study and for advice in preparation of the manuscript.

REFERENCES

1. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988
2. Zimmet PZ: Kelly West Lecture 1991. Challenges in diabetes epidemiology—From West to the rest. Diabetes Care 15:232-252, 1992
3. Stern MP: Diabetes and cardiovascular disease. The "common soil" hypothesis. Diabetes 44:369-374, 1995
4. Wiman B, Hamsten A: The fibrinolytic enzyme system and its role in the etiology of thrombo-embolic disease. Semin Thromb Haemost 26:207-216, 1990
5. Auwerx J, Bouillon R, Collen D, et al: Tissue-type plasminogen activator antigen and plasminogen activator inhibitor in diabetes mellitus. Arteriosclerosis 8:68-72, 1988
6. Garcia Frade LJ, de la Calle H, Torrado MC, et al: Hypofibrinolysis associated with vasculopathy in non–insulin dependent diabetes mellitus. Thromb Res 59:51-59, 1990
7. Nagi DK, Jain S, Garvey S, et al: Defective fibrinolysis in non–insulin-dependent diabetes mellitus relates to plasma insulin concentrations. Clin Sci 80:25p, 1991 (suppl 24, abstr)
8. Juhan-Vague I, Roul C, Alessi MC, et al: Increased plasminogen activator inhibitor activity in non-insulin dependent diabetes subjects. Relationship with plasma insulin. Thromb Haemost 61:370-373, 1989
9. Vague P, Juhan-Vague I, Chabret V, et al: Fat distribution and plasminogen activator inhibitor activity in nondiabetic obese women. Metabolism 38:913-915, 1989
10. 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
11. Mehta J, Mehta P, Lawson D, et al: Plasma tissue plasminogen activator inhibitor levels in coronary artery disease: Correlation with age and serum triglyceride concentration. J Am Coll Cardiol 9:263-268, 1987
12. Hamsten A, Wiman D, 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
13. Landin K, Tenghorn L, Smith U: Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. J Int Med 227:273-278, 1990
14. Juhan-Vague I, Alessi MC, Vague P: Increased plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia 34:457-462, 1991
15. Potter van Loon BJ, Kluft C, Radder MA, et al: The cardiovascular risk factor plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism 42:945-949, 1993
16. Landin K, Tenghorn L, Chmielewska J, et al: The acute effect of insulin on tissue plasminogen activator and plasminogen activator inhibitor in man. Thromb Haemost 65:130-133, 1991
17. Nagulesparan M, Savage PJ, Knowler WC, et al: Increased in vivo insulin resistance in non-diabetic Pima Indians compared with caucasians. Diabetes 31:952-956, 1982
18. Lillioja S, Nyomba BL, Saad MF, et al: Exaggerated early insulin release and insulin resistance in a diabetes-prone population: A metabolic comparison of Pima Indians and caucasians. J Clin Endocrinol Metab 73:866-876, 1991
19. Nagi DK, Mohamed Ali V, Yudkin JS: Ethnic differences in the association of plasminogen activator inhibitor-1 (PAI-1) with NIDDM. Diabetes 43:37A, 1994 (suppl 1, abstr)
20. Lillioja S, Mott DM, Zawadzki JK, et al: In-vivo insulin action is a familial characteristic in non-diabetic Pima Indians. Diabetes 36:1329-1335, 1987
21. Bogardus C, Lillioja S, Mott DM, et al: Relationship between degree of obesity and in-vivo insulin action in man. Am J Physiol 248:E285-E291, 1985
22. Lillioja S, Bogardus C: Obesity and insulin resistance, lessons learned from Pima Indians. Diabetes Metab Rev 4:517-540, 1988
23. DeClerk P, Alessi M, Verstreken M, et al: Measurement of plasminogen activator inhibitor 1 (PAI-1) in biological fluids with a murine antibody based enzyme-linked immunosorbent assay. Blood 71:220-225, 1988
24. Herbert V, Lau K, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965
25. World Health Organization: Diabetes mellitus: Report of a WHO study group. WHO Tech Rep Ser 727:1-113, 1985
26. Alessi MC, Juhan-Vague I, Kooistra T, et al: Insulin stimulates the synthesis of plasminogen activator inhibitor 1 by the human hepatocellular cell line HepG2. Thromb Haemost 60:491-494, 1988
27. Schneider DJ, Nordt TK, Sobel BE: Stimulation by proinsulin of expression of plasminogen activator inhibitor type-1 in endothelial cells. Diabetes 41:890-895, 1992
28. Seljeflot I, Eritsland J, Torjesen P, et al: Insulin and PAI-1 levels during oral glucose tolerance test in patients with coronary artery disease. Scand J Clin Invest 54:241-246, 1994
29. McCormack L, Stickland MH, Grant PJ: Plasminogen activator inhibitor-1 antigen concentrations during insulin and oral glucose tolerance tests in obese man. Fibrinolysis 7:225-228, 1993
30. Medvescek M, Keber D, Stegnar M, et al: Plasminogen activator inhibitor 1 response to a carbohydrate meal in obese subjects. Fibrinolysis 4:89-90, 1990
31. Grant PJ, Kruithof EKO, Felley CP, et al: Short-term infusion of triacylglycerol and glucose do not cause acute increase in plasminogen activator inhibitor-1 concentrations in man. Clin Sci 79:513-516, 1990
32. Grimaudo V, Hauert J, Bachmann F, et al: Diurnal variation of the fibrinolytic system. Thromb Haemost 59:495-499, 1988
33. Stiko-Rahm A, Wiman B, Hamsten A, et al: Secretion of plasminogen activator inhibitor-1 from cultured human umbilical vein endothelial cells is induced by very low density lipoprotein. Atherosclerosis 10:1067-1073, 1990
34. Latron Y, Alessi MC, Anfosso G, et al: Effect of low density lipoprotein on secretion of plasminogen activator inhibitor-1 (PAI-1) by human endothelial cells and hepatoma cells. Fibrinolysis 4:82-83, 1990 (suppl 2)
35. Rowe JW, Young JB, Minaker KL, et al: Effect of insulin and glucose infusions on sympathetic nervous activity in normal man. Diabetes 30:131-141, 1981
36. Gader AMA, Clarkson AR, Cash JD: The plasminogen activator and coagulation factor VIII response to adrenaline, noradrenaline, isoprenaline and salbutamol in man. Thromb Res 2:9-16, 1973
37. Maiello M, Boeri D, Podesta F, et al: Increased expression of tissue plasminogen activator and its inhibitor and reduced fibrinolytic potential of human endothelial cells cultured in elevated glucose. Diabetes 41:1009-1015, 1992
38. Nagi DK, Mohamed-Ali V, Walji S, et al: Does hyperglycaemia regulate plasminogen activator inhibitor (PAI-1) activity? Diabetic Med 11:S25, 1994 (suppl 2, abstr)
39. Mansfield MW, Stickland MH, Grant PJ: Environmental and genetic factors in relation to elevated levels of plasminogen activator inhibitor-1 in caucasian patients with NIDDM. Thromb Haemost 74:842-847, 1995
40. Dawson S, Hamsten A, Wiman B, et al: Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasminogen activator inhibitor-1 activity. Arterioscler Thromb 11:183-190, 1991
41. Panahloo A, Mohamed-Ali V, Lane A, et al: Determinants of plasminogen activator inhibitor 1 activity in treated NIDDM and its relationship to a polymorphism in the plasminogen activator inhibitor 1 gene. Diabetes 44:37-42, 1995