Diabetologia (1996) 39: 1512–1518

Diabetologia

Promoter (4G/5G) plasminogen activator inhibitor-1 genotype in Pima Indians: relationship to plasminogen activator inhibitor-1 levels and features of the insulin resistance syndrome

L. J. McCormack[1], D. K. Nagi[2], M. H. Stickland[1], M. W. Mansfield[1], V. Mohamed-Ali[3], J. S. Yudkin[3], W. C. Knowler[4], P. J. Grant[1]

[1] Unit of Molecular Vascular Medicine, Research School of Medicine, University of Leeds, UK
[2] Edna Coates Diabetes Centre, Pinderfields, Wakefield, UK
[3] Department of Medicine, University College London Medical School, Whittington Hospital, London, UK
[4] National Institutes of Health, NIDDK, Phoenix, Arizona, USA

Summary Elevated plasminogen activator inhibitor-1 may contribute to vascular disease in diabetes mellitus. Pima Indians have a low incidence of cardiovascular disease despite having a high prevalence of non-insulin-dependent diabetes mellitus (NIDDM) which in this population is not associated with elevated plasminogen activator inhibitor-1 activity. In Caucasians an insertion/deletion (4G/5G) polymorphism in the promoter region of the plasminogen activator inhibitor-1 gene that has been related to activity levels of its protein in plasma differentially binds repressor and enhancer elements. In 265 Pima Indians (133 diabetic, 132 non-diabetic, 129 male, 136 female, mean age 46.6, range 34–68 years) the promoter genotype frequencies were 23.0 % for 4G/4G, 49.8 % for 4G/5G and 27.2 % for 5G/5G compared to 35.4 %, 50.8 % and 13.8 % respectively ($\chi^2 = 15.3$, 2 *df*, $p < 0.0005$) previously reported in Caucasians with NIDDM. The mean plasma activity levels in the three genotypes in the Pima Indians were 18.2, 19.1 and 18.1 U/ml, respectively. Plasminogen activator inhibitor-1 activities correlated with plasma insulin ($r = 0.38$, $p < 0.0001$), body mass index ($r = 0.24$, $p < 0.0001$), and with triglyceride level ($r = 0.12$, $p = 0.054$) but there was no relationship between promoter genotype and activity. A steeper regression slope between plasminogen activator inhibitor-1 activity and triglycerides has been observed in Caucasians with the 4G/4G genotype as compared to Caucasians with the other genotypes. This was not found in the Pima population which may indicate a functional difference in this gene associated with reduced cardiovascular risk and may be involved in the lack of association of plasminogen activator inhibitor-1 levels with NIDDM in Pima Indians. [Diabetologia (1996) 39: 1512–1518]

Keywords Plasminogen activator inhibitor-1, fibrinolysis, Pima Indians, polymorphism, genetic, diabetes mellitus, non-insulin-dependent.

In Caucasians, the two to threefold increase in cardiovascular disease seen in subjects with non-insulin-dependent diabetes mellitus (NIDDM) accounts for a large part of the excess total mortality of this condition [1, 2]. The increased cardiovascular risk in NIDDM is greater than can be explained by established risk factors such as dyslipidaemia and obesity [3]. Interest has therefore focused on other features of NIDDM, that may contribute to the elevated vascular risk in subjects with NIDDM, including abnormalities of the coagulation and fibrinolytic cascades. The elevated circulating levels of the fibrinolytic inhibitor plasminogen activator inhibitor-1 (PAI-1) seen in NIDDM [4–8] offer two possible pathways by which vascular damage could occur and lead to the high prevalence of macrovascular disease. First, through impaired fibrinolysis contributing to the formation of atherosclerotic plaques, of which fibrin is an important constituent [9]; second, impaired fibrinolysis may result in a pro-thrombotic state predisposing to formation of thrombi and arterial occlusion.

Received: 7 May 1996 and in revised form: 21 August 1996

Corresponding author: L. McCormack, Unit of Molecular Vascular Medicine, G- Floor, Martin Wing, The General Infirmary Leeds, Leeds, LS1 3EX, UK

Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; PAI-1, plasminogen activator inhibitor-1; CHD, coronary heart disease; PCR, polymerase chain reaction.

There is evidence that genetic factors may be important in determining PAI-1 levels [10–13]. A common insertion/deletion polymorphism has been reported in the promoter region of the PAI-1 gene that has been shown to be related to PAI-1 activity in some populations [11–14]. Environmentally influenced factors, such as serum triglyceride concentrations, have been reported to interact with the PAI-1 genotype to regulate plasma PAI-1 levels in some populations [10, 12, 15].

The Pima Indians of the Gila River Indian Community in Arizona have the world's highest reported prevalence of NIDDM [16–19]. Despite this, coronary heart disease (CHD) is less common [17, 19–21] with a rate of fatal CHD of half that in diabetic subjects from the Framingham study when controlled for age and sex [19]. A study of the effect of diabetes on PAI-1 levels in three different racial groups found PAI-1 activity levels in diet-treated diabetic Pima Indians were similar to non-diabetic Pima Indians and that levels in these non-diabetic Pima Indians did not differ significantly from non-diabetic Caucasian and Asian subjects [22]. This finding prompted us to investigate the relationship between features of the insulin resistance syndrome and PAI-1 activity levels and the genetic regulation of PAI-1 in Pima Indians. Genotype at a single base-pair insertion/deletion polymorphism was identified and the relationship between levels of PAI-1 and those of other metabolic variables by genotype was investigated in a multiple regression model. Genotype data was obtained on all subjects in the earlier study but insulin-treated subjects were excluded from this study to enable comparison with studies of this polymorphism in other ethnic groups. The low prevalence of CHD and the lack of association between PAI-1 levels and NIDDM in the Pima Indians have yet to be explained and could be due to environmental or genetic factors.

Subjects and methods

Subject recruitment. Subjects were randomly recruited as part of a longitudinal epidemiological study [17] of NIDDM among the Pima Indian residents of the Gila River Indian Community between November 1991 an October 1992. Every 2 years residents of the community over the age of 5 years are asked to undergo a research examination which includes the determination of venous glucose concentration 2 h after the ingestion of 75 g of glucose. The diagnosis of diabetes was made by World Health Organisation criteria [23] (plasma glucose ≥ 11.1 mmol/l 120 min after 75 g carbohydrate load). All subjects aged between 35 and 70 years, attending between November 1991 and October 1992, were included unless excluded by the following criteria; pregnancy, a clinical history of ischaemic heart disease, use of thiazide diuretics, oral contraceptives or insulin treatment. Height and weight were recorded in subjects wearing light clothing without shoes. body mass index was calculated from the subject's weight in kilograms divided by the square of the height measured in meters.

Plasma glucose was measured by a hexokinase method (Ciba-Corning EXPRESS 550 analyser, Ciba-Corning, Norwood Massachusetts). Total serum cholesterol and serum triglycerides were measured by enzymatic methods using a Hitachi 717 analyser (Boehringer Mannheim Indianapolis, Indiana).

Plasma insulin was measured using previously characterised antibodies [24] in a modified two-site microplate immunoenzymometric assay [25]. The assay was sensitive to 2.0 pmol/l with an intra-assay coefficient of variation of 8.7 % for concentrations above 15 pmol/l and inter-assay coefficient of variation of 12 %.

Blood was collected into EDTA before lysing of the erythrocytes and pelleting of the leukocytes for later DNA extraction. After digestion with proteinase K, DNA was extracted by a standard phenol-chloroform method.

Determination of PAI-1 genotype. PAI-1 4G/5G promoter genotype was established for each subject by polymerase chain reaction (PCR) amplification of genomic DNA using the allele specific primers [26]: insertion 5G allele; 5′-GTC TGG ACA CGT GGG GG-3′, deletion 4G allele; 5′-GTC TGG ACA CGT GGG GA-3′ each in a separate PCR reaction together with the common downstream primer 5′-TGC AGC CAG CCA CGT GAT TGT CTA G-3′ and a control upstream primer 5′-AAG CTT TTA CCA TGG TAA CCC CTG GT-3′ to verify the occurrence of DNA amplification in the absence of the allele on the genomic DNA. The PCR was carried out in a final volume of 25 μl containing 100 ng DNA, 50 pmol of specific and downstream primers, 3 pmol of upstream primers, 0.2 μmol/l dNTPs, and 1 U Taq polymerase (Gibco, Paisley, UK). The PCR cycle conditions were 94 °C for 60 s, 65 °C for 45 s then 72 °C for 75 s for 30 cycles. The amplified DNA fragments were separated by agarose gel electrophoresis and, after staining with ethidium bromide, viewed under ultraviolet light. Each subject was classified by two observers into one of the three possible genotype groups: 4G/4G, 4G/5G or 5G/5G. To validate this method control subjects of each genotype, as shown by direct sequencing, were run with each batch of samples.

Measurement of PAI-1 activity. All samples were taken after an overnight fast, between 08.00 and 10.00 hours to minimise the effect of diurnal variation. Blood for PAI-1 activity was collected without venous stasis with a wide-bore needle into prechilled tubes containing 3.8 % sodium citrate solution. Samples were cold centrifuged immediately and the plasma stored at –70 °C. PAI-1 activity was measured by a kit chromogenic substrate method (Kabi Vitrum, Uxbridge, Middlesex, UK), modified to a microplate method. Results are expressed as arbitrary units (AU/ml), one unit of inhibitor being defined as the amount that inhibits one international unit of tissue plasminogen activator. The intra-assay coefficient of variation is 4.5 % and inter-assay coefficient of variation is 8.5 % and detection limit of the assay is 5 AU/ml.

Statistical analysis

Variables with a $\log_e$-normal distribution, insulin, triglycerides, cholesterol and blood glucose, were $\log_e$ transformed and are shown as the geometric mean with its 95 % confidence interval. Other data are presented as arithmetic mean and 95 % confidence interval. Continuous variables were compared using the Student's *t*-test for independent samples. Categorical variables were compared using the chi-square test. Pearson

correlation coefficients between the metabolic parameters and PAI-1 levels were calculated.

One-way ANOVA was used to compare values between more than two groups of subjects. The relationship of various independent variables to PAI-1 activity levels was assessed by stepwise multiple linear regression analysis with entry criterion as $p < 0.05$ and removal criterion as $p > 0.1$ for each independent variable.

Two techniques were used to detect the influence of genotype on PAI-1 activity. First, genotype, expressed as two indicator variables was entered in a regression analysis model. Second, the PAI-1 activity data was adjusted for the influence of factors that showed significant correlation with PAI-1 activity, i. e. *ln* insulin and age, and then adjusted levels were compared in the three genotype groups.

The regression slope for the relationship between each metabolic factor (insulin, body mass index, triglyceride, glucose) and PAI-1 was calculated for each genotype and the slopes compared using the method described by Armitage and Berry [27].

Results

Clinical data. The clinical characteristics of the 265 Pima Indians are shown in Table 1. Subjects with diabetes had higher fasting glucose, HbA_{1c} and were older. There was no significant difference in the body mass index of the two groups.

Fibrinolytic data. PAI-1 activity levels and Pearson correlation coefficients between the metabolic parameters and PAI-1 levels have been reported previously for 213 of these subjects [22]. As there was no difference between the PAI-1 activity levels in the diabetic and non-diabetic subjects (Table 1) these groups were combined to look at the effects of metabolic and genetic variables on PAI-1 activity. In the 265 Pima Indians studied PAI-1 activity correlated with plasma insulin ($r = 0.38$, $p < 0.0001$), body mass index ($r = 0.24$, $p < 0.0001$), and weakly with triglycerides ($r = 0.12$, $p = 0.054$). There was also a negative correlation between age and PAI-1 activity ($r = –0.16$, $p < 0.010$) and no significant correlation with glucose, cholesterol or HbA_{1c}.

To determine the independent predictors of PAI-1, linear multiple regression analysis was performed with PAI-1 activity as the dependent variable and body mass index, age, sex, insulin, triglycerides, glucose, cholesterol and genotype as the initial independent variables in a stepwise model. Age and insulin remained in the model as the only independent and significant predictors of PAI-1 activity according to the mathematical expression:

PAI-1 activity = 4.95 *ln* insulin – 0.16 age + 2.96 ($F = 25.8$, $p < 0.00001$)

This model accounts for 16.5 % of the variance in PAI-1 levels between individuals.

Genotype data. There were 61 subjects with the 4G/4G genotype, 134 with 4G/5G and 72 with 5G/5G.

Table 1. Subjects characteristics

	Diabetic	Non-diabetic	*p* value
Number	133	132	NS
Male : Female	65 : 68	64 : 68	NS
Age (years)	48.7 (47.2–50.2)	44.5 (43.2–45.9)	0.03
Body mass index (kg/m^2)	33.2 (32.0–34.3)	35.7 (34.3–37.1)	0.07
PAl-1 activity (AU/ml)	18.7 (17.1–20.3)	18.6 (17.1–20.1)	NS
HbA_{1c} (%)	8.7 (8.3–9.0)	5.5 (5.4–5.6)	< 0.0001
Fasting glucose[a] (mmol/l)	9.8 (9.1–10.5)	5.4 (5.4–5.5)	< 0.0001
Cholesterol[a] (mmol/l)	4.42 (4.27–4.58)	4.36 (4.22–4.49)	NS
Triglycerides[a] (mmol/l)	1.49 (1.35–1.65)	1.29 (1.18–1.42)	NS
Insulin[a] (pmol/l)	105.7 (93.6–119.3)	104.7 (93.4–117.4)	NS
Treatment (Diet: Sulphonylurea)	74 : 59		

Data shown as the geometric mean (95 % confidence interval) or where log transformed[a] as geometric mean and anti-logged (95 % confidence interval). Parameters were compared using the *t* -test for independent samples or chi-square test where appropriate

The genotype frequencies were not different from those predicted by Hardy-Weinberg equilibrium. The allele frequencies were 0.48 and 0.52 for 4G and 5G, respectively (Table 2). There was no difference in the genotype distribution between diabetic and non-diabetic Pima Indians ($\chi^2 = 1.94$, 2 *df*, $p = 0.38$). The genotype frequencies in the Pima Indians were however significantly different from those observed in a previously reported study of 189 Caucasian subjects with NIDDM in Leeds, UK [15] ($\chi^2 = 15.2$, 2 *df*, $p < 0.0005$) (Fig. 1). There was no relationship between PAI-1 genotype and PAI-1 concentrations alone or when adjusted for features of the insulin resistance syndrome (Table 2). Power calculations estimate this study had an 80 % likelihood of detecting a difference in PAI-1 activity of 4.5 mU/l between the 4G/4G and 5G/5G groups at the $p < 0.05$ level. This would represent a 25 % difference between the highest level of 19 mU/l and the lowest levels, a difference less than was detected between 4G/4G subjects and 5G/5G subjects in NIDDM subjects in the UK [12].

Gene-environment interactions. Table 3 shows the results of the regression analyses. There was no difference in the regression slopes of PAI-1 on triglycerides, insulin or glucose between genotype groups. For PAI-1 on body mass index the regression slope in the 4G/4G group was significantly steeper than in the 4G/5G group ($p = 0.022$), although no adjustment has been made for multiple comparisons.

Table 2. Relationship between genotype and PAl-1 activity levels

	PAl-1 genotype			
	4G/4G	4G/5G	5G/5G	All
Number of subjects	61	132	72	265
Diabetic: non-diabetic	26 : 35	68 : 64	39 : 33	133 : 132
PAl-1 activity (AU/ml)	18.2 (15.9–20.5)	19.1 (17.5–20.7)	18.1 (16.0–20.2)	18.6 (17.5–19.7)
Adjusted PAl-1 activity[a]	18.1 (15.9–20.2)	19.0 (17.6–20.5)	18.3 (16.5–20.2)	18.6 (17.6–19.6)
PAl-1 activity (AU/ml) in diabetic subjects	18.0 (13.9–22.0)	19.1 (16.8–21.4)	18.4 (15.3–21.4)	18.7 (17.1–20.3)
PAl-1 activity (AU/ml) in non-diabetic subjects	18.4 (15.6–21.2)	19.1 (16.8–21.4)	17.7 (14.7–20.6)	18.6 (17.1–20.1)

[a] PAl-1 activity (AU/ml) adjusted for insulin and age in a multiple regression model, shown as mean and (95 % confidence interval)

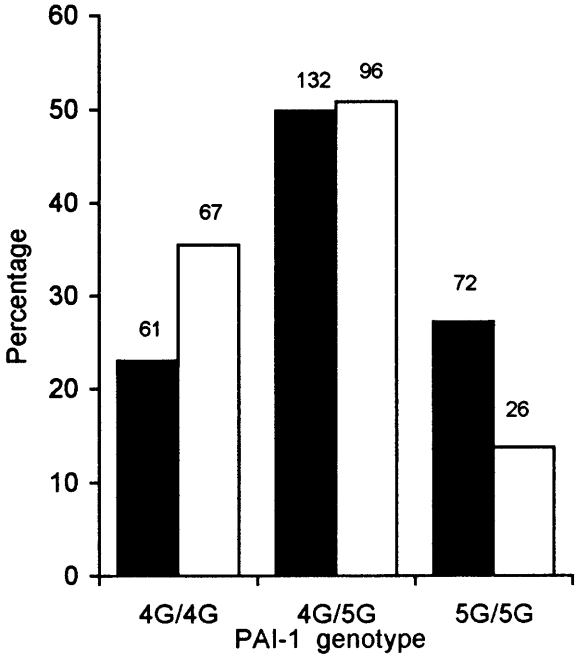

Fig. 1. The frequency of each genotype in Pima Indians (■) compared to Caucasian subjects (□) with NIDDM in Leeds. ($\chi^2 = 15.2$, 2 *df*, $p < 0.0005$). The number of individuals is shown above each bar

Discussion

Elevated circulating levels of PAI-1 seen in Caucasian subjects with NIDDM [4–8] may contribute to vascular damage and hence to the high prevalence of macrovascular disease in this disorder. Evidence to support this view comes from studies in which elevated levels of PAI-1 predict recurrent infarction in a group of young survivors of myocardial infarction [28, 29] and may predict poor outcome from myocardial infarction [30].

The causes of high levels of PAI-1 seen in Caucasians in NIDDM remain to be established. Insulin resistance and its associated features have all been implicated in this process. Insulin resistance has a strong correlation with PAI-1 levels in glucose-clamp studies in hypertensive [31], obese [32], and subjects with NIDDM [33]. This relationship has also been found in population studies where insulin resistance is identified indirectly by the presence of its associated features [8, 28, 29, 31, 33–38].

It is difficult to distinguish the effects of insulin resistance from those of hyperinsulinaemia, hypertriglyceridaemia, male pattern obesity, high BMI, systolic hypertension, low HDL-cholesterol and glucose intolerance. In vitro cell stimulation experiments provide evidence to implicate insulin [39–41] very low density lipoproteins from hypertriglyceridaemic subjects [42] and high glucose levels [43–44] in the production of elevated PAI-1 levels. However, in vivo studies have failed to demonstrate an effect of hyperinsulinaemia [45–48] or hypertriglyceridaemia [45] on PAI-1 levels. While some studies find a significant correlation between PAI-1 and glycaemia [12], others have shown only a weak correlation [15, 49, 50].

Evidence that genetic factors are important in determining PAI-1 levels comes from clinical studies. Initially, a 3′ Hind III RFLP and an intronic dinucleotide (CA) repeat were shown to be related to elevated PAI-1 levels [10]. More recently, an insertion/deletion (4G/5G) polymorphism has been related to circulating levels of PAI-1 in several large studies of NIDDM and non-diabetic subjects. Levels are highest in subjects with the 4G/4G genotype and generally about one-third higher than subjects homozygous for the 5G allele [11–13]. Recent studies have shown an association between genotype at this site and coronary artery disease itself [13, 51]. Evidence that the informative nature of the 4G/5G polymorphism lies in it being a functional site comes from in vitro studies demonstrating differential enhancer/repressor binding at the 4G and 5G sites [11, 13]. Studies of gene-environment interactions in patients have provided further information on the functional nature of the 4G/5G mutation, with three studies having

Table 3. Regression slopes for PAl-1 activity on the metabolic variables for the different PAl-1 genotypes

	Genotype[a]	*R*	slope	SE slope	*p*-value
ln Insulin	4G/4G	0.44	5.77	1.51	0.0003
	4G/5G	0.31	4.22	1.13	0.0003
	5G/5G	0.44	5.69	1.4	0.0001
Age	4G/4G	0.09	0.09	0.14	0.05
	4G/5G	0.22	–0.23	0.09	0.01
	5G/5G	0.26	–0.27	0.12	0.03
Body mass index[b]	4G/4G	0.42	0.61	0.17	0.001
	4G/5G	0.12	0.14	0.10	NS
	5G/5G	0.35	0.39	0.13	0.003
ln Triglyceride	4G/4G	0.06	1.25	2.78	NS
	4G/5G	0.09	1.44	1.34	NS
	5G/5G	0.19	2.93	1.85	NS
ln Glucose	4G/4G	0.02	0.42	3.28	NS
	4G/5G	0.07	–1.44	1.84	NS
	5G/5G	0.21	4.74	2.67	NS

[a] Number of subjects 4G/4G = 61, 4G/5G = 132, 5G/5G = 72; [b] 4G/4G genotype v 4G/5G $p = 0.022$

demonstrated an interaction between triglycerides and genotype in relation to PAI-1 levels [10, 12, 15].

In contrast to the studies in Caucasians that have not shown a significant difference between PAI-1 levels in the different genotypes [15], in the Pima Indians not even a trend towards higher levels was seen with possession of one or more 4G alleles. Pre-existing vascular disease may be necessary for expression of the genotype effect on PAI-1 levels, and this could explain the absence of an effect of genotype on PAI-1 levels in Pima Indians.

In Pima Indians CHD is infrequent despite the high prevalence of diabetes and obesity. This population, in which the prevalence of CHD is low even in subjects with NIDDM, could give important clues to the factors that form the link between NIDDM and CHD in other populations. In these Pima Indians there was no significant difference between PAI-1 activity levels in diabetic and non-diabetic subjects despite a similar relationship between PAI-1 activity and the features of insulin resistance to that observed in studies of Caucasians.

The prevalence of the different alleles at the 4G/5G locus has mainly been studied previously in Caucasian populations and demonstrate broadly similar prevalences whether patients are derived from Swedish or British populations. In the present study the Pima Indian population was found to have a significantly lower prevalence of the potentially deleterious 4G/4G genotype than in Caucasian subjects. Additionally, unlike some of the previous studies, there was no relationship between possession of a particular genotype at this locus and circulating levels of PAI-1. This variation in genotype prevalence may merely reflect random ethnic differences of no biological significance, alternatively this may be one of several genes predisposing an individual to CHD which may account for the lower prevalence of heart disease seen in this ethnic group.

In this group of Pima Indians we found a similar though weaker correlation between PAI-1 and triglyceride levels than previously found in Caucasian subjects with NIDDM. No relationship was found between PAI-1 genotype at the 4G/5G polymorphic site and the regression slope for PAI-1 activity on triglyceride or glucose concentration. The regression of PAI-1 on body mass index showed a steeper slope in the 4G/4G group compared to the 4G/5G group, although this may be a chance finding.

The reason for the absence in this ethnic group of the genotype specific relationship between PAI-1 activity and triglyceride that has been observed in Caucasians is unclear. However, one possibility is that binding of transcription factors differs in Pima Indians leading to alterations in gene regulation compared to Caucasians. Differences in triglyceride levels could also be involved. Although the allele frequencies in the Pima Indians were significantly different from those observed in Caucasian populations, it is unlikely that the absence of a relationship between 4G/5G genotype and PAI-1 levels in Pima Indians would be explained solely by differences in genotype frequency suggesting changes in other regulatory functions.

In summary, this study demonstrates that in Pima Indians there is no relationship between the PAI-1 4G/5G polymorphism, PAI-1 levels and most features of the insulin resistance syndrome. However, a genotype specific relationship between body mass index and PAI-1, with a steeper slope in the 4G/4G group was observed. In contrast to most studies in Caucasian subjects, there was no direct relationship between PAI-1 genotype and levels of PAI-1. These data suggest that this polymorphic site is not involved in the regulation of PAI-1 synthesis in Pima Indians. This may indicate a functional difference in the PAI-1 gene in this population. Further studies are required to elucidate the relationship between the PAI-1 gene, circulating PAI-1 levels and the presence of vascular disease in the Pima population.

Acknowledgements. The authors wish to thank the members of the Gila River Indian Community for participating in these studies. D. K. Nagi was supported by the British Diabetic Association-Imperial Chemical Industries Transatlantic fellowship.

References

1. Kannel WB, McGee DL (1979) Diabetes and cardiovascular disease, the Framingham Study. JAMA 241: 2035–2038
2. Multiple Risk Factor Intervention Trial (MRFIT) Research Group (1982) Multiple risk factor intervention trial: risk factor changes and mortality results. JAMA 248: 1465–1477

3. Pyörälä K (1979) Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease. Results from two population studies in Finland. Diabetes Care 2: 131–141
4. Walmsley D, Hampton KK, Grant PJ (1991) Contrasting fibrinolytic responses in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes. Diabet Med 8: 954–959
5. Auwerx J, Bouillon R, Collen D, Geboers J (1988) Tissue-type plasminogen activator antigen and plasminogen activator inhibitor in diabetes mellitus. Arteriosclerosis 8: 68–72
6. Garcìa Fraḍe LJ, de la Calle H, Torrado MC, Lara JI, Cuellar L, Garcia Avello A (1990) Hypofibrinolysis associated with vasculopathy in non insulin dependent diabetes mellitus. Thromb Res 59: 51–59
7. Gough SCL, Rice PJS, McCormack L, Chapman C, Grant PJ (1993) The relationship between plasminogen activator inhibitor-1 and insulin resistance in newly diagnosed type 2 diabetes mellitus. Diabet Med 10: 638–642
8. Juhan-Vague I, Roul C, Alessi MC, Ardissone JP, Heim M, Vague P (1989) Increased plasminogen activator inhibitor activity in non-insulin-dependent diabetic patients – relationship with plasma insulin. Thromb Haemost 61: 370–373
9. Smith EB (1986) Fibrinogen, fibrin and fibrin degradation products in relation to atherosclerosis. Clin Haematol 15: 355–370
10. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S (1991) Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 11: 183–190
11. Dawson S, Wiman B, Hamsten A, Green F, Humphries S, Henney A (1993) The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. J Biol Chem 268: 10739–10745
12. Panahloo A, Mohamed-Ali V, Lane A, Green F, Humphries S, Yudkin J (1995) Determinants of plasminogen activator inhibitor 1 activity in treated NIDDM and its relation to a polymorphism in the plasminogen activator inhibitor 1 gene. Diabetes 44: 37–42
13. Eriksson P, Kallin B, van 'T Hooft FM, Båvenholm P, Hamsten A (1995) Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 92: 1851–1855
14. Ye S, Green FR, Scarabin PY et al. (1995) The 4G/5G genetic polymorphism in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. Thromb Haemost 74: 837–841
15. Mansfield MW, Stickland MH, Grant PJ (1995) Environmental and genetic factors in relation to circulating levels of plasminogen activator inhibitor-1 in Caucasian patients with non-insulin-dependent diabetes mellitus. Thromb Haemost 74: 842–847
16. Bennett PH, Rushforth NB, Miller M, LeCompte PM (1976) Epidemiologic studies of diabetes in the Pima Indians. Recent Prog Horm Res 32: 333–376
17. Knowler WC, Bennett PH, Hamman RF, Miller M (1978) Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. Am J Epidemiol 108: 497–505
18. Knowler WC, Pettitt DJ, Savage PJ, Bennett PH (1981) Diabetes incidence in Pima Indians: contribution of obesity and parental diabetes. Am J Epidemiol 113: 144–156
19. Nelson RG, Sievers ML, Knowler WC et al. (1990) Low incidence of fatal coronary heart disease in Pima Indians despite high prevalence of non-insulin-dependent diabetes. Circulation 81: 987–995
20. Howard BV, Welty TK, Fabsitz RR et al. (1992) Risk factors for coronary heart disease in diabetic and non-diabetic native Americans. The Strong Heart Study. Diabetes 41 [Suppl 2]:4–11
21. Sievers ML, Nelson RG, Bennett PH (1990) Adverse mortality experience of a southwestern American Indian community: overall death rates and underlying causes of death in Pima Indians. J Clin Epidemiol 43: 1231–1242
22. Nagi DK, Knowler WC, Hanson RL, Mohamed Ali V, Yudkin JS (1996) Plasminogen activator inhibitor-1 (PAI-1) and non-insulin-dependent diabetes in Pima Indians, South Asians and Europeans: populations at varying risk of NIDDM and coronary artery disease. Thromb Haemost 75: 921–927
23. World Health Organisation (1985) Diabetes mellitus. Report of a WHO Study Group. Technical Report Series No. 727, WHO, Geneva
24. Sobey WJ, Beer SF, Carrington CA et al. (1989) Sensitive and specific two-site immunoradiometric assays to human insulin, proinsulin, 65–66 split and 32–33 split proinsulin. Biochem J 260: 535–541
25. Mohamed-Ali V, Yudkin JS (1992) An end-point amplified immunoenzymometric assay (IEMA) specific for human insulin. Clin Sci 82:[Suppl 27] 4P (Abstract)
26. Falk G, Almqvist A, Nordenhem A, Svensson H, Wiman B (1995) Allele specific PCR for detection of a sequence polymorphism in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene. Fibrinolysis 9: 170–174
27. Armitage P, Berry G (1987) Statistical methods in medical research. 2nd edn Blackwell, Oxford, UK, pp 273–282
28. Hamsten A, Wiman B, de Faire U, Blombäck M (1985) Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 313: 1557–1563
29. Hamsten A, de Faire U, Walldius G et al. (1987) Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 2: 3–9
30. Gray RP, Yudkin JS, Patterson DL (1993) Enzymatic evidence of impaired reperfusion in diabetic patients after thrombolytic therapy for acute myocardial infarction – A role for plasminogen activator inhibitor? Br Heart J 70: 530–536
31. Landin K, Tengborn L, Smith U (1990) Elevated fibrinogen and plasminogen activator inhibitors (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. J Int Med 227: 273–278
32. Landin K, Stigendal L, Eriksson E et al. (1990) Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism 39: 1044–1048
33. Potter van Loon BJ, Kluft K, Radder JK, Blankenstein MA, Meinders AE (1993) The cardiovascular risk factor plasminogen activator inhibitor-1 is related to insulin resistance. Metabolism 42: 945–949
34. Vague P, Juhan-Vague I, Aillaud MF et al. (1986) Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 35: 250–253
35. Juhan-Vague I, Alessi MC, Joly P et al. (1989) Plasma plasminogen activator inhibitor-1 in angina pectoris. Influence of plasma insulin and acute phase-response. Arteriosclerosis 9: 362–367

36. Sundell IB, Nilsson TK, Hallmans G, Hellsten G, Dahlen GH (1989) Interrelationships between plasma levels of plasminogen activator inhibitor, tissue plasminogen activator, lipoprotein(a), and established cardiovascular risk factors in a North Swedish population. Atherosclerosis 80: 9–16
37. Mussoni L, Mannucci L, Sirtori M et al. (1992) Hypertriglyceridaemia and regulation of fibrinolytic activity. Arterioscler Thromb 12: 19–27
38. Mehta J, Mehta P, Lawson D, Saldeen T (1987) Plasma tissue plasminogen activator inhibitor levels in coronary artery disease: correlations with age and triglyceride concentrations. J Am Coll Cardiol 9: 263–268
39. Alessi MC, Juhan-Vague I, Kooistra T, Declerk PJ, Collen D (1988) Insulin stimulates the synthesis of plasminogen activator-1 by hepatocellular cell line Hep G2. Thromb Haemost 60: 491–494
40. Kooistra T, Bosma PJ, Tons HAM, van den Berg AP, Meyer P, Princen HMG (1989) Plasminogen activator inhibitor 1: biosynthesis and mRNA level are increased in Hep G2 cells. Thromb Haemost 62: 723–728
41. Grant PJ, Rüegg M, Medcalf RL (1991) Basal expression and insulin-mediated induction of PAI-1 mRNA in Hep G2 cells. Fibrinolysis 5: 81–86
42. Stiko-Rahm A, Wiman B, Hamsten A, Nilsson J (1990) Secretion of plasminogen activator inhibitor-1 from cultured human umbilical vein endothelial cells is induced by very low density lipoprotein. Arteriosclerosis 10: 1067–1073
43. Maiello M, Boeri D, Podesta F et al. (1992) 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
44. Nordt TK, Klassen KJ, Schneider DJ, Sobel BE (1993) Augmentation of synthesis of plasminogen activator inhibitor type-1 in arterial endothelial cells by glucose and its implications for local fibrinolysis. Arterioscler Thromb 13: 1822–1828
45. Grant PJ, Kruithof EKO, Felley CP, Felber JP, Bachmann F (1990) Short term infusions of insulin, triacylglycerol and glucose do not cause acute increases in plasminogen activator inhibitor-1 concentrations in man. Clin Sci 79: 513–516
46. Potter van Loon BJ, deBart ACW, Radder JK, Frölich M, Kluft C, Meinders AE (1990) Acute exogenous hyperinsulinaemia does not result in elevation of plasminogen activator inhibitor-1 (PAI-1) in humans. Fibrinolysis 4 [Suppl 2]:93–94
47. Landin K, Tengborn L, Chmielewska J, von Schenck H, Smith U (1991) The acute effect of insulin on tissue plasminogen activator and plasminogen activator inhibitor in man. Thromb Haemost 65: 130–133
48. McCormack L, Stickland MH, Grant PJ (1993) Plasminogen activator inhibitor-1 antigen concentrations during insulin and oral glucose tolerance tests in obese man. Fibrinolysis 7: 225–228
49. Gunnarsson R, Nyman D, Wålinder O, Östman J (1980) Fibrinolytic activity and diabetes control – evidence for a relationship. A preliminary report. Acta Med Scand [Suppl] 639: 23–24
50. van Wersch JWJ, Donders SHJ, Westerhuis LWJJM, Venekamp WJRR (1991) Microalbuminuria in diabetic patients: relationship to lipid glyco-metabolic, coagulation and fibrinolysis parameters. Eur J Clin Chem Clin Biochem 29: 493–498
51. Mansfield MW, Stickland MH, Grant PJ (1995) Plasminogen activator inhibitor-1 (PAI-1) promoter polymorphism and coronary artery disease in non insulin dependent diabetes. Thromb Haemost 74: 1032–1034