

The -675 4G/5G polymorphism at the Plasminogen Activator Inhibitor 1 (PAI-1) gene modulates plasma Plasminogen Activator Inhibitor 1 concentrations in response to dietary fat consumption

P. Pérez-Martínez^{1*}, M. D. Adarraga-Cansino¹, R. A. Fernández de la Puebla¹, A. Blanco-Molina¹, J. Delgado-Lista¹, C. Marín¹, J. M. Ordovás², J. López-Miranda¹ and F. Pérez-Jiménez¹

¹Lipids and Atherosclerosis Research Unit, Hospital Universitario Reina Sofía, CIBER, Fisiopatología, Obesidad y Nutrición, Avda. Menéndez Pidal, s/n. 14004 Córdoba, Spain

²Nutrition and Genomics Laboratory, US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA

(Received 29 March 2007 – Revised 10 July 2007 – Accepted 30 July 2007)

The objective of the study was to determine whether Plasminogen Activator Inhibitor Type 1 (PAI-1) -675 4G/5G polymorphism is associated with the response of functional plasma PAI-1 concentrations to changes in the amount and quality of dietary fat in healthy subjects. PAI-1 is the major inhibitor of fibrinolysis, and a lower level of fibrinolytic activity could be implicated in an increased risk of IHD. Fifty-nine healthy Spanish volunteers (ten 4G/4G homozygotes, twenty-eight heterozygotes 4G/5G and twenty-one 5G/5G homozygotes) consumed three diets for periods of 4 weeks each: a SFA-rich diet (38 % fat, 20 % SFA), followed by a carbohydrate-rich diet (30 % fat, 55 % carbohydrate) and a MUFA-rich diet (38 % fat, 22 % MUFA) according to a randomized crossover design. At the end of each dietary period plasma lipid and functional plasma PAI-1 concentrations were determined. Subjects carrying the 4G allele (4G/4G and 4G/5G) showed a significant decrease in PAI-1 concentrations after the MUFA diet, compared with the SFA-rich and carbohydrate-rich diets (genotype × diet interaction: $P=0.028$). 5G/5G homozygotes had the lowest plasma PAI-1 concentrations compared with 4G/4G and 4G/5G subjects (genotype: $P=0.002$), without any changes as a result of the amount and the quality of the dietary fat. In summary, no differences in plasma PAI-1 concentration response were found after changes in dietary fat intake in 5G/5G homozygotes, although these subjects displayed the lowest concentrations of PAI-1. On the other hand, carriers of the 4G allele are more likely to hyper-respond to the presence of MUFA in the diet because of a greater decrease in PAI-1 concentrations.

PAI-1 -675 4G/5G polymorphism: Nutrigenetics: Dietary fat: Functional PAI-1: MUFA-rich diet

Plasminogen Activator Inhibitor Type 1 (PAI-1) is a protein that reduces plasma fibrinolytic capacity. Its production is encoded by a gene with several polymorphisms. The presence of the 4G allele at a common insertion–deletion polymorphism in the promoter of the *PAI-1* gene has been associated with elevated plasma PAI-1 concentration and activity¹. Carriers of the 4G allele (4G/4G and 4G/5G) may be at increased risk of IHD^{2,3}, but this could depend on interactions with other environmental factors, which may explain why results obtained from Western populations have been ambiguous. On the other hand, previous studies have shown that the presence of the 5G allele in the -675 4G/5G polymorphism decreases gene expression, which predisposes to lower plasma PAI-1 concentrations⁵. A recent meta-analysis suggests the importance of this polymorphism because of a per-allele relative risk of about 1.06 of coronary disease in individuals with the -675 4G variant of the *PAI-1* gene⁶.

However, it is known that plasma PAI-1 concentration is influenced by many other factors, including diet, smoking, exercise, obesity, fasting plasma TAG concentrations and the insulin resistance syndrome^{7,8}. In this context, Sanders *et al.* have demonstrated in a group of middle-age men that postprandial variations in fibrinolytic activity are modulated by the PAI-1 -675 4G/5G genotype but not by the fat content of a meal⁹. Some data show that the substitution of dietary SFA by MUFA causes a decrease in plasma PAI-1 concentrations^{10,11}. In this respect, nutrigenetics is emerging as a multidisciplinary field that focuses on studying the interactions between nutritional and genetic factors, and health outcomes¹¹. However, it has not been determined whether the presence of the -675 4G/5G polymorphism modifies the response to different diets. In view of the previous evidence, the aim was to determine whether this polymorphism is related to the response of functional plasma PAI-1 concentrations to changes in the amount and quality of dietary fat in healthy subjects.

Abbreviations: CHO, carbohydrate; PAI-1, Plasminogen Activator Inhibitor Type 1.

* **Corresponding author:** Dr Pablo Pérez-Martínez, fax +34 957 218250, email pablopermar@yahoo.es

Experimental methods

Subjects and diet

Fifty-nine healthy Spaniard normolipaemic volunteers (thirty men and twenty-nine women) participated in the study. All subjects were less than 30 years of age, with no evidence of any chronic illness or unusually high levels of physical activity. Mean initial BMI was 21 and remained constant throughout the experimental period. They were encouraged to maintain their regular physical activity and lifestyle, and were asked to record in a diary any event that could affect the outcome of the study. They consumed <30 g/d alcohol during the study.

The study design included an initial 28 d period during which all subjects consumed a SFA-enriched diet containing 15% protein, 47% CHO and 38% fat (20% SFA, 12% MUFA, 6% PUFA). After this period, thirty subjects received a MUFA-enriched diet for 28 d in a randomized, crossover design. This diet contained 15% protein, 47% CHO and 38% fat (<10% SFA, 6% PUFA, 22% MUFA). The MUFA-enriched diet was followed for 28 d by a high-CHO diet containing 15% protein, 55% CHO and <30% fat (<10% SFA, 6% PUFA, 12% MUFA). The other twenty-nine subjects received the CHO diet before the MUFA diet. Cholesterol content remained constant (under 300 mg/d) during the three periods. Virgin olive oil, used for cooking, salad dressing and as a spread, provided 80% of the MUFA diet. The CHO component of the high-CHO diet was based on the consumption of biscuits, jam and bread. Butter and palm oil were used during the SFA dietary period.

The Human Investigation Review Committee at Reina Sofia University Hospital approved the study. The compositions of the experimental diets have been described previously¹². Dietary compliance was verified by analysing the fatty acids in LDL-cholesterol esters at the end of each dietary period¹³.

Blood sampling and biochemical determinations

Venous blood for analysis of insulin, glucose, lipid and lipoprotein was collected from the subjects in tubes containing EDTA after a 12 h overnight fast at the end of each dietary period. Each analysis was performed in triplicate. Total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG were assayed by procedures described previously^{14–17}.

Plasminogen Activator Inhibitor Type 1 activity

For analysis, blood samples anticoagulated with sodium citrate were collected and PAI-1 activity (control range 3–15 IU/ml) was determined according to Chemielewska *et al.* using a commercial kit (Spectrolyse (fibrin); Bio-Pool, Umea, Sweden)¹⁸.

Genetic analysis

DNA extraction was performed using standard procedures. The PAI-1 genotypes of the -675 4G/5G polymorphism were determined by means of an allele-specific PCR, which was performed for each allele determination according to conditions previously described¹⁹.

Statistical analyses

Statistical analyses were carried out using the SPSS statistical package version 13 (SPSS Inc., Chicago, IL, USA). ANOVA for repeated measures was used to analyse the differences in plasma lipid and PAI-1 activity between dietary phases. When statistically significant effects were demonstrated, Tukey's *post hoc* test was used to identify between-group differences. Correlation analysis was performed with Pearson's coefficient of correlation. A value of $P < 0.05$ was considered significant. All data are presented as means and standard deviations.

Results

The study sample comprises a group of fifty-nine volunteers. Of these, ten were 4G/4G homozygotes, twenty-eight were 4G/5G heterozygotes and twenty-one were 5G/5G homozygotes. Fatty acid composition during each dietary period was analysed on the cholesterol ester fraction of plasma LDL, as we have previously published²⁰. An enrichment in palmitic acid was observed following the SFA diet, and in oleic acid after the MUFA diet, suggesting that there had been good adherence to the dietary protocol. No differences were recorded between genotypes with respect to age, BMI, smoking status, TAG, HDL-cholesterol and apo A-1 plasma concentration at baseline. However, 4G/4G homozygotes showed higher concentrations of total cholesterol ($P = 0.03$), LDL-cholesterol ($P = 0.01$) and apo B ($P = 0.01$) than did 4G/5G and 5G/5G subjects.

Total cholesterol, LDL-cholesterol and HDL-cholesterol were significantly reduced ($P < 0.001$) following the consumption of the CHO and MUFA diets as compared with the SFA-enriched diet (Table 1). An effect of the interaction of genotype and diet was observed, because the consumption of a MUFA diet decreases plasma PAI-1 concentrations in subjects carrying the 4G allele (4G/4G, 4G/5G) more than the SFA and CHO diets ($P = 0.028$; Fig. 1). An effect associated with genotype was also noted, the 5G/5G homozygote subjects showed the lowest plasma PAI-1 concentrations, without any changes as a result of the amount and quality of the dietary fat ($P = 0.002$; Fig. 1).

Discussion

The present results show that the consumption of a MUFA-rich diet for 4 weeks decreases plasma PAI-1 concentrations in subjects carrying the 4G allele (4G/4G, 4G/5G) in the *PAI-1* gene promoter, compared with the SFA- and CHO-rich diets, while the 5G/5G homozygotes showed the lowest plasma PAI-1 concentrations, without any changes resulting from the amount and quality of the dietary fat.

In agreement with previous studies which used different experimental designs^{10–12,21}, the present findings confirm that the isoenergetic substitution of dietary SFA by MUFA is associated with lower plasma levels of the fibrinolysis inhibitor. To the best of our knowledge, the current study is the first to examine the association between the -675 4G/5G polymorphism at the *PAI-1* gene and functional plasma PAI-1 concentrations in response to dietary intervention. Our most relevant finding, which has not been described before, is the observation that there exists an interaction between

Table 1. Plasma lipid, lipoprotein and Plasminogen Activator Inhibitor Type 1 (PAI-1) activity values after each dietary period
(Mean values and standard deviations)

	SFA diet		CHO diet		MUFA diet		P value
	Mean	SD	Mean	SD	Mean	SD	
Total cholesterol (mmol/l)	4.27	0.3	3.6*	0.2	3.74*	0.3	0.001
LDL-cholesterol (mmol/l)	2.80	0.6	2.32*	0.5	2.34*	0.6	0.001
HDL-cholesterol (mmol/l)	1.12	0.3	0.99*	0.2	1.03*	0.1	0.001
TAG (mmol/l)	0.77	0.2	0.78	0.2	0.79	0.3	0.437
apo A-1 (mmol/l)	3.67	0.01	2.78*	0.1	2.86*	0.01	0.001
apo B (mmol/l)	1.88	0.3	1.70*	0.2	1.70*	0.2	0.001
PAI-1 activity (UA/ml)	7	2	7	2	5*†	2	0.05

CHO, carbohydrate.

Mean values were significantly different from those of the SFA diet (ANOVA test for repeated measures): * $P < 0.05$.

Mean value was significantly different from that of the CHO diet (ANOVA test for repeated measures): † $P < 0.05$.

the intake of dietary fat and the presence of the 4G allele. Thus, the expected reduction in plasma PAI-1 concentrations was observed only in subjects carrying the 4G allele but not in the 5G/5G homozygotes, although these subjects showed the lowest plasma levels. The lack of a differential response to different dietary fats which we observed in 5G/5G subjects is in agreement with a previous study that tested the response after a test meal that was very high in butter fat²². In that study, subjects carrying the 4G allele also showed an increase in PAI-1 activity. These data confirm the findings of previous reports that PAI-1 activity in 5G/5G homozygotes is lower than in subjects who carry one or more 4G alleles^{23,24}. In a previous study, Sanders *et al.* have demonstrated a lower plasma TAG concentration in the subjects homozygous for the 5G allele²⁵. All these data suggest that the *PAI-1* promoter -675 4G variant is associated with a higher risk of myocardial infarction. However, subjects are more likely to hyper-respond to the presence of MUFA in the diet because of a greater decrease in PAI-1 concentrations.

A potential mechanism to explain the observed changes in PAI-1 concentrations resulting from different fat intakes lies in the identification by Eriksson *et al.*²⁶ of a VLDL-inducible

factor that binds a putative VLDL response element located to residues -672 to -657. The VLDL-induced factor was found to bind to the region adjacent to and partly overlapping the binding site of the 5G allele. Competition between the 5G allele-specific transcriptional repressor protein⁵ and the VLDL-induced factor could explain the 4G/5G allele-specific relations between VLDL TAG and PAI-1 activity levels in plasma. In addition, competitive binding between the 5G allele-specific repressor and the common transcriptional activator could explain the differences in basal transcriptional activity²⁶. This competition between the activator and the repressor will not be present or decreased in 4G/4G and 4G/5G subjects, supporting the lower overall levels of circulating PAI-1 observed in the present study for 5G/5G homozygotes. Alternatively, Chen *et al.*²⁷ have identified a fatty acid response element, distinct from the VLDL response element²⁶, and shown a NEFA-induced increase in the expression of PAI-1 independent of previously reported effects mediated by TAG or lipoproteins. This could mediate the differential responses observed for the various dietary fatty acids in the current study. However, PAI-1 regulation appears to be highly complex and probably tissue dependent and this hypothesis needs to be further tested *in vitro* and *in vivo*.

In summary, the presence of the 4G allele at the *PAI-1* gene promoter is associated with higher levels of PAI-1. This group, which makes up 40% of the general population, is sensitive to reductions in SFA or CHO in the diet intake, and responds with a reduction in plasma PAI-1 concentrations when the recommendation to consume a MUFA-enriched diet is followed. In conclusion, the present study suggests that the cardioprotective effect of a chronic intake of a MUFA diet enriched in olive oil could be due, at least in part, to its protective effect on fibrinolytic activity.

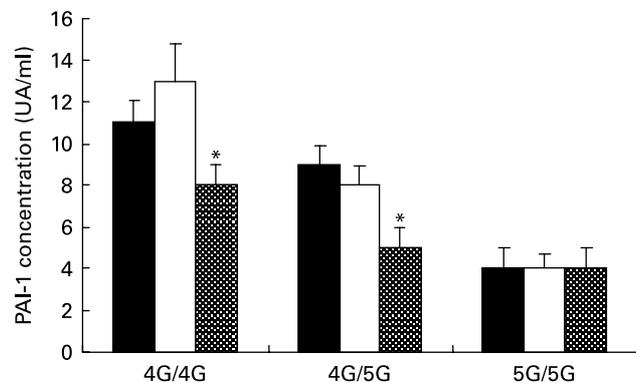


Fig. 1. Functional Plasminogen Activator Inhibitor Type 1 (PAI-1) levels (UA/ml) during the three dietary periods (■, SFA; □, carbohydrate (CHO); ▨, MUFA) according to genotype (4G/4G, n 10; 4G/5G, n 28; 5G/5G, n 21). Values are means with their standard deviations depicted by vertical bars. Mean values were significantly different from those of the SFA and CHO diet: * $P < 0.05$. P genotype = 0.002; P diet = 0.003; P genotype \times diet interaction = 0.028.

Acknowledgements

This work was supported by research grants from CIBER (CBO/6/03), Instituto de Salud Carlos III; CICYT (SAF 01/2466-C05 04 to F. P.-J., SAF 01/0366 to J. L.-M.), the Spanish Ministry of Health (FIS 01/0449); Fundación Cultural "Hospital Reina Sofía-Cajasar" (to C. M. and P. G.); Consejería de Salud, Servicio Andaluz de Salud (00/212, 00/39, 01/239, 01/243, 02/64,

02/65, 02/78); Consejería de Educación, Plan Andaluz de Investigación, Universidad de Córdoba and by NIH/NHLBI grant no. HL54776 and contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research Service.

References

- Ye S, Green FR, Scarabin PY, *et al.* (1995) The 4G/5G genetic polymorphism in the promoter 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. *Etude CasTemoins de l'infarctus du Myocarde. Thromb Haemost* **74**, 837–841.
- Fu L, Jin H, Song K, Zhang C, Shen J & Huang Y (2001) Relationship between gene polymorphism of the PAI-1 promoter and myocardial infarction. *Chin Med J (Engl)* **114**, 266–269.
- Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR & Peter RJ (2001) Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. *Circulation* **104**, 3063–3068.
- Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ & Miletich JP (1997) Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. *Circulation* **95**, 59–62.
- Eriksson P, Kallin B, van't Hooft FM, Bavenholm P & Hamsten A (1995) Allele-specific increase in basal transcription of the plasminogen-activator inhibitor1 gene is associated with myocardial infarction. *Proc Natl Acad Sci U S A* **92**, 1851–1855.
- Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R & Danesh J (2006) Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. *Lancet* **367**, 651–658.
- Cesari M, Sartori MT, Patrassi GM, Vettore S & Rossi GP (1999) Determinants of plasma levels of plasminogen activator inhibitor-1. *Arterioscler Thromb Vasc Biol* **19**, 316–320.
- Kohler HP & Grant PJ (2000) Plasminogen activator inhibitor Type I and coronary artery disease. *New Engl J Med* **342**, 1792–1801.
- Sanders TA, de Grassi T, Acharya J, Miller GJ & Humphries SE (2004) Postprandial variations in fibrinolytic activity in middle-aged men are modulated by plasminogen activator inhibitor I 4G-675/5G genotype but not by the fat content of a meal. *Am J Clin Nutr* **79**, 577–581.
- Avellone G, Di Garbo V & Cordova R (1998) Effects of Mediterranean diet on lipid, coagulative and fibrinolytic parameters in two randomly selected population samples in Western Sicily. *Nutr Metab Cardiovasc Dis* **8**, 287–296.
- Junker R, Pieke B, Schulte H, Nofer R, Neufeld M, Assmann G & Wahrburg U (2001) Changes in hemostasis during treatment of hypertriglyceridemia with a diet rich in monounsaturated and n-3 polyunsaturated fatty acids in comparison with a low-fat diet. *Tromb Res* **101**, 355–366.
- López-Segura F, Velasco F, López-Miranda J, *et al.* (1996) Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arterioscler Thromb Vasc Biol* **16**, 82–88.
- Ruiz-Gutiérrez V, Prada JL & Pérez-Jiménez F (1993) Determination of fatty acid and triacylglycerol composition of human very-low-density lipoproteins. *J Chromatogr* **622**, 117–134.
- Allain CC, Poon LS, Chan CS, Richmond W & Fu PC (1974) Enzymatic determination of total serum cholesterol. *Clin Chem* **20**, 470–474.
- Fossati P & Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* **28**, 2077–2082.
- Assmann G, Schrierwer H, Schmitz G & Hagele EO (1983) Quantification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid-MgCl₂. *Clin Chem* **29**, 2026–2030.
- Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* **18**, 499–502.
- Chemielewska J, Ranby M & Wiman B (1983) Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. *Thromb Res* **31**, 427–436.
- Morange PE, Henry M, Tregouet D, Granel B, Aillaud MF, Alessi MC & Juhan-Vague I (2000) The polymorphism in the PAI-1 gene is associated with a higher risk of venous thrombosis in factor V Leiden carriers. *Arterioscler Thromb Vasc Biol* **20**, 1387–1391.
- Perez-Martinez P, Gomez P, Paz E, Marin C, Gavilan-Moral E, Lopez-Miranda J, Ordovas JM, Fernandez de la Puebla RA & Perez-Jimenez F (2001) Interaction between smoking and the SstI polymorphism of the apo C-III gene determines plasma lipid response to diet. *Nutr Metab Cardiovasc Dis* **11**, 237–243.
- Perez-Jimenez F, Castro P, Lopez-Miranda J, Paz-Rojas E, Blanco A, Lopez-Segura F, Velasco F, Marin C, Fuentes F & Ordovas JM (1999) Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* **145**, 351–358.
- Byrne CD, Wareham NJ, Martensz ND, Humphries SE, Metcalfe JC & Grainger DJ (1998) Increased PAI activity and PAI-1 antigen occurring with an oral fat load: associations with PAI-1 genotype and plasma active TGF-beta levels. *Atherosclerosis* **140**, 45–53.
- Vaisanen SB, Humphries SE, Luong LA, Penttila I, Bouchard C & Rauramaa R (1999) Regular exercise, plasminogen activator inhibitor-1 (PAI-1) activity and the 4G/5G promoter polymorphism in the PAI-1 gene. *Thromb Haemost* **82**, 1117–1120.
- van der Bom JG, Bots ML, Haverkate F, Kluft C & Grobbee DE (2003) The 4G5G polymorphism in the gene for PAI-1 and the circadian oscillation of plasma PAI-1. *Blood* **101**, 1841–1844.
- Sanders TA, de Grassi T, Acharya J, Miller GJ & Humphries SE (2004) Postprandial variations in fibrinolytic activity in middle-aged men are modulated by plasminogen activator inhibitor I 4G-675/5G genotype but not by the fat content of a meal. *Am J Clin Nutr* **79**, 577–581.
- Eriksson P, Nilsson L, Karpe F & Hamsten A (1998) Very-low-density lipoprotein response element in the promoter region of the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia. *Arterioscler Thromb Vasc Biol* **8**, 20–26.
- Chen Y, Billadello JJ & Schneider DJ (2000) Identification and localization of a fatty acid response region in the human plasminogen activator inhibitor-1 gene. *Arterioscler Thromb Vasc Biol* **20**, 2696–2701.