Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 59–64

Journal of Nutritional Biochemistry

Sesamol regulates plasminogen activator gene expression in cultured endothelial cells: a potential effect on the fibrinolytic system

Pey-Rong Chen[a,b], Chun-Chung Lee[c], Hang Chang[c], Chingmin E. Tsai[a,d,*]

[a]*Department of Nutrition and Food Sciences, Fu Jen University, Taipei 242, Taiwan*
[b]*Department of Dietetics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei 100, Taiwan*
[c]*Department of Medical Education and Research, Shin Kong Wu Ho-Su Memorial Hospital, Taipei 111, Taiwan*
[d]*Department of Bioscience Technology, Chung Yuan University, Chungli 320, Taiwan*

Received 15 November 2003; received in revised form 10 June 2004; accepted 3 July 2004

Abstract

Sesamol is a component in the nutritional makeup of sesame that was identified as an antioxidant. In recent years, the importance of the plasminogen activator (PA) and its adjustment factor, plasminogen activator inhibitor-1 (PAI-1), in the prevention of atherosclerosis has gradually received recognition. The objective of this in vitro study was to demonstrate the effects of sesamol on PA and PAI-1. We also compared the effects of sesamol with two well-known antioxidants, vitamins C and E, by using human umbilical vein endothelial cells as an experimental model and by treating them with the above-mentioned three nutrients with doses up to 100 μmol/L. After 24 h, cells and cultural medium were collected for analysis. The concentrations of tissue PA (tPA), urokinase PA (uPA) and PAI-1 were measured by an enzymatic immunity method. Northern blot method was used to analyze the expression of mRNA of these three types of proteins. The results showed that sesamol increased the production of uPA and tPA significantly and also up-regulated the mRNA expressions of these proteins. On the other hand, vitamins C and E could induce tPA but not uPA. As for PAI-1, none of the nutrients induced any evident response. These findings suggest that the overall vascular fibrinolytic capacity may be enhanced by using sesamol to regulate PA gene expression.

Keywords: Sesamol; Plasminogen activator; Plasminogen activator inhibitor-1; Gene expression; Endothelial cells

1. Introduction

Sesame seeds, from *Sesamum indicum*, are one of the most important edible oil seeds. Sesame seeds and sesame oil have been known as traditional health foods and have been used in ancient Chinese medicine for a long time. However, the scientific evidence of their miraculous functions, especially in the prevention of aging—as the food has often been prescribed for in traditional medicine, has not been well established. Sesamol is a potent phenolic antioxidant found mainly in roasted sesame or in processed sesame oil [1]. The biological effects of sesamol on health have been determined as follows: sesamol exhibited powerful inhibitory effects on lipid peroxidation [2], carried out the synergistic suppression of carcinogenesis and combined with other antioxidants [3]. An in vitro study indicated that sesamol inhibited the mutagenicity of mutagens in various tester stains of *Salmonella typhimurium* [4]. Sesamol could also attenuate the production of nitric oxide and hydrogen peroxide and reduce monoamine oxidase (MAO) activity in glial astrocyte cells [5]. Since a distinct relationship exists between MAO activity and the development of neurodegenerative diseases associated with aging such as Alzheimer's disease and stroke, sesamol might play a role in the prevention of these types of diseases. Fibrinolysis defects play a pivotal role in cardiovascular diseases such as atherosclerosis and atherothrombosis. Furthermore, it is considered as a risk factor for severe cardiovascular diseases such as myocardium infarction and stroke [6–8]. The blood fibrinolytic system is composed of an active enzyme, plasmin, which degrades fibrin into soluble fibrin degradation products. The plasmin is converted from its inactive proenzyme, plasminogen, by a

* Corresponding author. Department of Bioscience Technology, Chung Yuan University, Chungli 320, Taiwan. Tel.: +886 3 265 3500; fax: +886 3 265 3599.
E-mail address: jtsai@cycu.edu.tw (C.E. Tsai).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.07.008

plasminogen activator (PA) [9,10]. Two major forms of PAs, tissue type [tissue PA (tPA)] and urokinase type [urokinase PA (uPA)], have been identified. Tissue PA-mediated plasminogen activation occurs upon the dissolution of fibrin in the circulation whereas the main role of uPA appears to be in the induction of pericellular proteolysis. The fibrinolytic system is regulatively inhibited by PAI-1, a high level of which is associated with myocardial function and coronary artery disease [11,12]. Therefore, it is crucial to understand the interaction between these fibrinolytic regulated proteins and to study the correlation of their activity with occurrences of cardiovascular diseases. The aim of this in vitro study was to demonstrate the effects of sesamol on the gene and protein expression of PAs and PAI-1 in human umbilical vein endothelial cells (HUVECs). While sesamol and vitamins C and E antioxidantal properties have been well documented, their effects on PA and PAI have not been verified by recent studies. As such, the objective of this report is twofold: to demonstrate the effects that these three antioxidants have on PA and PAI and to further explore sesamol's medicinal values.

2. Materials and methods

2.1. Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord vein by collagenase treatment as described previously [13]. Human umbilical cords from normal deliveries or cesarean sections were collected. In a tissue culture hood, both ends of each cord (1 cm long) were excised with a sterile scalpel to expose a sterile surface. The umbilical vein was perfused with 1× PBS and was then perfused with 0.2% collagenase (Boehringer Mannheim) in PBS solution until the vein was distended. The two ends of the cord were clamped with sterile clamps and the cords were incubated for 30 min at 37°C. Then, the cords were gently massaged to facilitate endothelial cell detachment from the vessel wall. The solution was flushed out and the lumen of the vein was washed with M199 medium (containing 1% penicillin–streptomycin). The cell suspension was collected and centrifuged at 1000 rpm for 5 min at 4°C. The pellet was resuspended in medium M199 (containing 10% serum and 1% penicillin–streptomycin). The cells were plated on a 10-cm culture plate and were incubated in 37°C and 5% CO_2 overnight. After attaching overnight, the cells were washed with 1× PBS and grown in endothelial cell growth medium (BioWhittaker, #CC-3121) supplemented with 12% fetal bovine serum (Gibco). After they have fully grown, HUVECs were divided or stored in liquid nitrogen (medium containing 20% DMSO when stored). Before treatment, the cells (at a density of 5×10^5 cells/plate, 6-cm dish) were seeded in the medium containing 2% fetal bovine serum (Gibco, #10270-106) overnight. HUVECs between passages 3 and 7 were used for experimentation.

2.2. Cell treatments

Sesamol (Sigma, #S-8518) was predissolved in ethanol with concentrations of 1, 10 and 100 mmol/L and then diluted with culture medium to obtain three final concentrations of 1, 10 and 100 μmol/L. α-Tocopherol (Sigma, #T-3251) was predissolved in ethanol with 100 mmol/L and then diluted into the final concentration of 100 μmol/L with medium, whereas L(+)-ascorbic acid (Merck, #100127) was diluted directly in the culture medium with 100 μmol/L. The cultured medium and cells with various concentrations, solvent control (ethanol) and control treatments were isolated after 24 h.

2.3. Enzyme-linked immunosorbent assay

Imubind enzyme-linked immunosorbent assay kits, PAI-1 (American diagnostica no. 821), uPA (no. 894) and tPA (no. 860) were used for measuring the concentrations of the three proteins in cell extract and culture medium. These concentrations were adjusted by total protein of cell extracts. Total protein of cell extract was measured by Bradford protein assay (Bio-Rad, #69466A) [14].

2.4. Probes

Probes for Northern blot analysis were synthesized by reverse transcriptase polymerase chain reaction (PCR). The oligonucleotides PAI-1-5′ (gtctttggtgaagggtctgct) and PAI-1-3′ (ctcgtgaagtcagcctgaaa) were used to amplify the PAI-1 complementary DNA (cDNA) with 943 bp. The oligonucleotides uPA-F (gccaccatgagagccctgct) and uPA-R (cctgtagatggccgcaaacc) were used to amplify the uPA cDNA with 600 bp. The oligonucleotides tPA-5′ (ttccgccccaccacctgacg) and tPA-3′ (gaggagtcgggtgttcctgg) were used to amplify the tPA cDNA with 622 bp. The oligonucleotides glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-5′ (tggtatcgtggaaggactca) and GAPDH-3′ (agtgggtgtcgctgttgaag) were used to amplify the GAPDH cDNA with 370 bp. The PCR conditions for those probes were the same. The initial denaturation phase lasted 5 min at 94°C and was followed by a 35-cycle amplification phase consisting of 1 min at 94°C, 1 min at 60°C and 3 min at 72°C. Amplification was terminated after 7 min at 72°C. After PCR, the DNA fragments were purified with a QIA quick gel extraction kit (Qiagen). Twenty nanograms of DNA fragments was labeled with α-^{32}P-dCTP (Amersham) by a random prime labeling system (Rediprime, Amersham) for Northern blot assay.

2.5. Northern blot analysis

In order to detect the expression level of uPA, tPA and PAI-1 genes, Northern blot assay was used for detecting gene expression. Total RNA was isolated by the method that was described in the Tri-Reagent's protocol (Molecular Research Center). Ten to 20 μg of total RNA was separated on 1% agarose/formaldehyde gels as described previously [15] and was then transferred onto nylon membrane (Hybond-N+, Amersham). Hybridization was performed at

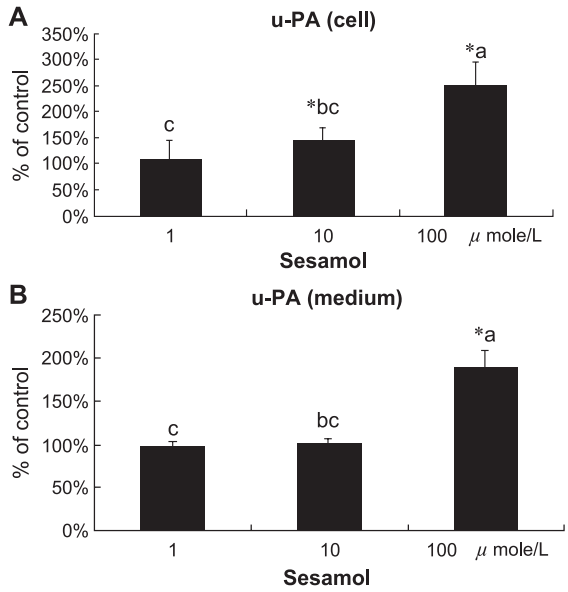

Fig. 1. The % changes compared with their control of uPA in the cells (A) and medium (B) treated with three dosages of sesamol after 24 h. Values are means±S.D. of three independent experiments. Asterisk indicates that the treatment group is significantly different from its control. Columns not sharing a common superscript are significantly different, $P<.05$.

65°C for 16 h in a hybridization buffer (Rapid-hyb buffer, Amersham). The membrane was then washed once with solution A (5× SSC, 0.1% SDS) at 65°C for 20 min and twice with solution B (0.1× SSC, 0.1% SDS) at 65°C for 20 min. Finally, the membrane was put into autoradiography.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 8.1 statistical software system (SPSS). The significance of differences among groups was tested by one-way ANOVA,

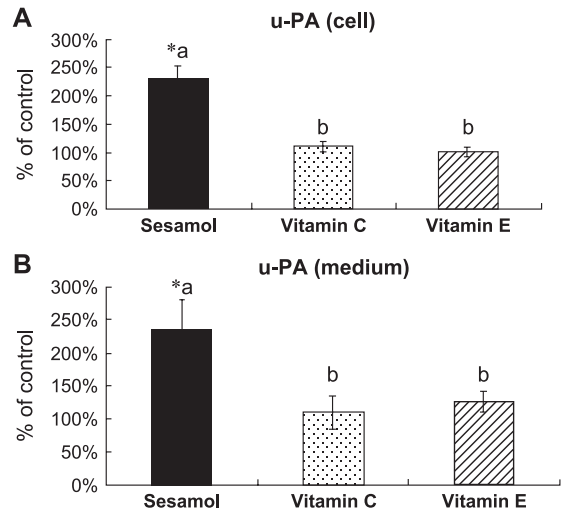

Fig. 2. The % changes compared with their control of uPA in the cells (A) and medium (B) treated with sesamol, vitamin C or vitamin E after 24 h. Values are means±S.D. of three independent experiments. Asterisk indicates that the treatment group is significantly different from its control. Columns not sharing a common superscript are significantly different, $P<.05$.

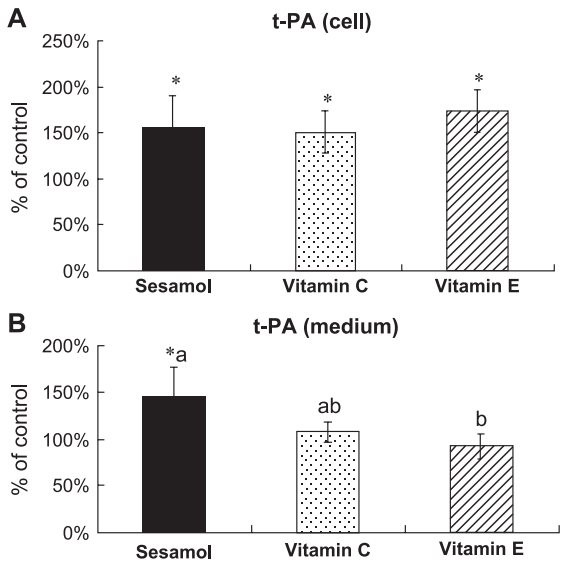

Fig. 3. The % changes compared with their control of tPA in the cells (A) and medium (B) treated with sesamol, vitamin C or vitamin E after 24 h. Values are means±S.D. of three independent experiments. Asterisk indicates that the treatment group is significantly different from its control. Columns not sharing a common superscript are significantly different, $P<.05$.

and least significant difference procedure was used for post hoc testing. Differences were considered significant when $P<.05$. Data shown in the figures were calculated to % change of control and expressed as mean±S.D. of three independent experiments.

3. Results

Since it is important to determine the correlation between the regulation of fibrinolytic proteins and the occurrences of

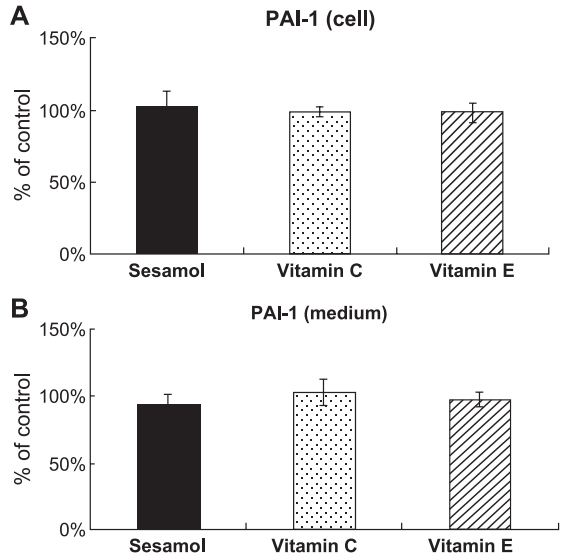

Fig. 4. The % changes compared with their control of PAI-1 in the cells (A) and medium (B) treated with sesamol, vitamin C or vitamin E after 24 h. Values are means±S.D. of three independent experiments. There are no significant differences between treatments and their controls. Also, there are no significant differences between treatment groups, $P<.05$.

cardiovascular diseases, we performed in vitro experiments to test the effect of various doses of sesamol on endothelial cells and to check the % changes of uPA, tPA and PAI-1 compared with their control in the cell lysate and medium.

Twenty-four hours after the HUVECs were treated with 100 μmol/L sesamol, the uPA protein concentration increased significantly with more than 200% in cell lysate and 150% in medium (Fig. 1A and B). We also compared sesamol-treated cells with those treated with other antioxidants, vitamins C and E, in the same concentration of 100 μmol/L. As shown in Fig. 2A and B, only sesamol significantly enhanced the production of uPA in both cell lysate and medium. Neither vitamin C nor vitamin E had any significant effect on uPA production.

As for tPA, sesamol also induced its production in both cell lysate and medium (Fig. 3A and B). We further discovered that both vitamins C (compared with blank) and E (compared with blank ethanol) significantly increased tPA concentration in cell lysate. However, in cultured medium, no significant changes in tPA concentration were detected in cells treated with either vitamin C or vitamin E.

For the PAI-1, we did not observe any of these tested nutrients having any significant effect of PAI-1 induction either in cell lysate or in cultured medium (Fig. 4A and B).

From Northern blot assays, sesamol induced the mRNA expressions of uPA and tPA dose dependently (Fig. 5A and B). In the sesamol-treated cells, the ratio of tPA mRNA induction was higher than that of uPA. Vitamin C, on the other hand, had no effect on uPA mRNA induction while vitamin E induced uPA mRNA expression only slightly. Both vitamins C and E induced tPA mRNA expression just as effectively as sesamol did. All three nutrients only slightly induced the mRNA expression of PAI-1, but the ratio of PAI-1 was less than the ratio of uPA or tPA mRNA induction.

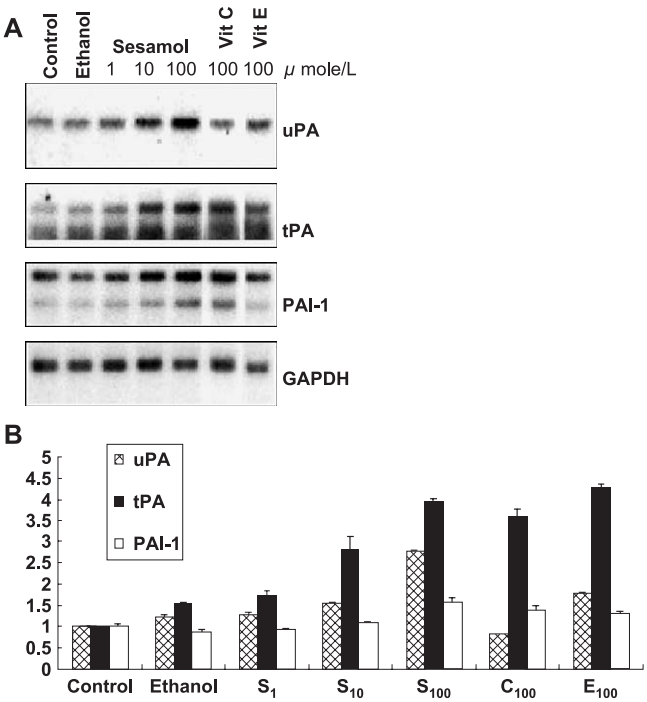

Fig. 5. The Northern blot of uPA, tPA and PAI-1 mRNA expressed in cells treated with sesamol, vitamin C or vitamin E after 24 h (A). Above signals were quantified by a densitometer and normalized with GAPDH and control (B).

4. Discussion

Many reports provide evidence showing that the concentration of PAI-1 is correlated with occurrences in diseases such as type 2 diabetes [16], diabetic nephropathy [17], myocardium infarction [18] and hyperlipidemia [19,20]. Low-density lipoprotein (LDL) particle size was inversely related to PAI-1 levels, thus providing one possible explanation for the atherogeneity of small dense LDL particles [21]. Hypofibrinolysis was present in hemodialysis patients with diabetes [22] and in patients with chronic renal failure, renal dialysis and transplantation [23]. Generally, patients with these chronic diseases have the trend to develop atherosclerotic complications.

Since an imbalance between PA and PAI-1 levels is an indicator of a fibrinolytic defect, low levels of PA or excessive levels of PAI-1 cause hypofibrinolysis. Fibrinolysis defects are related to thrombotic complications and therefore contribute to the development of atherosclerosis. Evidence shows that decreased fibrinolytic capacity (increased PAI-1) was likely to occur in fatty streak lesions and zones of severe atherosclerosis during observations of the human arterial wall [24].

Because of the correlation between hypofibrinolysis and PAI-1, several conditions such as the fact that a high glucose concentration [25] leads to an induction in PAI-1 mRNA would modify PA or PAI-1 production. Drugs such as valsartan (angiotensin type I receptor antagonist) [26] and rosiglitazone (hypoglycemic agent) [27] could decrease PAI-1 levels and even increase tPA levels [26]. Except for tPA, some studies revealed the relationship between PAI-1 and nutrition; high fiber intake was inversely associated with PAI-1 levels in epidemiology [28] and in an animal (baboon) study [29]. Vitamins C and E attenuated PAI-1 expression in a hypercholesterolemic porcine model of angioplasty [30]. A clinical study showed that the high dose of α-tocopherol supplementation (1200 IU/day) decreased PAI-1 levels in type 2 diabetic patients [31]. It can be seen from the above studies that foods or nutrients that play a potential role in fibrinolytic functions might serve as medicines in an adjunctive therapy to prevent atherosclerosis.

In the present in vitro study, we found that sesamol increased the production of uPA and tPA in endothelial cells but did not influence PAI-1 production. The regulation of these proteins was also confirmed by their mRNA expression. We also compared sesamol's effect on uPA, tPA and PAI-1 protein and gene expression with that of vitamins C and E. We found that all three nutrients induced more tPA production in endothelial cells. However, sesamol induced uPA significantly while vitamins C and E did not. None of the three

nutrients induced PAI-1 evidently. Tissue PA had been used in medical treatment as a thrombolytic agent, such as in acute myocardial infarction [32,33]. From our study, we demonstrated that sesamol increased PAs significantly but left PAI-1 largely unaffected. As a result, we predicted that sesamol might play a role in antiatherosclerosis and antithrombosis.

We remained open-minded to opposing findings as we used the nutrition method to enhance the fibrinolytic functions. Urokinase PA is a serine protease that activates the zymogen plasminogen and is potentially responsible for initiating a cascade of fibrinolysis and extracellular proteolysis. Although Falkenberg et al. [34] proposed that a gene therapy employing local uPA overexpression might be applied as a strategy to prevent intravascular thrombosis, their follow-up study found that elevated uPA expression in atherosclerotic arteries contributed to intimal growth and constrictive remodeling leading to lumen loss. High uPA values were also observed in patients with some malignancies [35]. Overexpression might increase the risk for aneurysm formation due to activated metalloproteinase and might cause extracellular matrix degradation. Proteolysis might indeed play a role in the neovascularization and rupture of plaques or in the ulceration and rupture of aneurysms [36]. For this concern, we have performed the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) test and cell growth assay to demonstrate the effects of sesamol on the proliferation of endothelial cells. Results showed that sesamol inhibited endothelial cell growth even at the concentration of 1 μmol/L. However, sesamol did not induce cell death when the concentration level approached the 100 μmol/L (data not shown) level. Vitamin C up to 100 μmol/L had no effect on endothelial cell growth, but vitamin E could slightly promote cell growth when concentration was more than 10 μmol/L. From these results, we suggest that the main characteristic of sesamol is that it is antithrombotic.

In conclusion, we have found that sesamol may enhance overall vascular fibrinolytic capacity through regulating gene expression of PA. Sesamol performed more effectively than vitamins C and E. Confirming its antithrombotic effects, however, lies outside the scope of this study and further in vivo study is recommended.

Acknowledgments

This work was supported by a grant from the National Science Council (NSC 89-2312-B-030-001). We thank Dr. Bao Wei Wang, Dr. Shankung Lin, Ms. Ju Yun Cheng and Ms. Ya Chen Liu for their technical assistance.

References

[1] Budowski P. Recent research on sesamin, sesamolin and related compounds. J Am Oil Chem Soc 1964;41:280–5.
[2] Uchida M, Nakajin S, Toyoshima S, Shinoda M. Antioxidative effect of sesamol and related compounds on lipid peroxidation. Biol Pharm Bull 1996;19:623–6.
[3] Hasegawa R, Tiwawech D, Hirose M, Takaba K, Hoshiya T, Shirai T, et al. Suppression of diethylnitrosamine-initiated preneoplastic foci development in the rat liver by combined administration of four antioxidants at low doses. Jpn J Cancer Res 1992;83:431–7.
[4] Kaur IP, Saini A. Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity. Mutat Res 2000;470:71–6.
[5] Mazzio EA, Harris N, Soliman KFA. Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. Planta Med 1998;64:603–6.
[6] Kohler HP, Grant PJ. Plasminogen activator inhibitor type-1 and coronary artery disease. N Engl J Med 2000;342:1792–801.
[7] Koenig W. Haemostatic risk factors for cardiovascular diseases. Eur Heart J 1998;19(Suppl):C39–C43.
[8] Catto AJ, Carter AM, Stickland M, Bamford JM, Davies JA, Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. Thromb Haemost 1997;77:730–4.
[9] Hoylaerts M, Rijken DC, Lijnen HR, Collén D. Kinetics of the activation of plasminogen by human tissue plasminogen activator: role of fibrin. J Biol Chem 1982;257:2912–9.
[10] Higgins DL, Vehar GA. Interaction of one-chain tissue plasminogen activator with intact and plasmin-degraded fibrin. Biochemistry 1987;26:7786–91.
[11] Almér LO, Ohlin H. Elevated levels of the rapid inhibitor of plasminogen activator (t-PAI) in acute myocardial infarction. Thromb Res 1987;47:335–9.
[12] Aznar J, Estellés A, Tormo G, Sapena P, Tormo V, Blanch S, et al. Plasminogen activator inhibitor activity and other fibrinolytic variables in patients with coronary artery disease. Br Heart J 1988;59:535–41.
[13] Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest 1973;52:2745–56.
[14] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[15] Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1982.
[16] Reaven GM. Multiple CHD risk factors in type 2 diabetes: beyond hyperglycaemia. Diabetes Obes Metab 2002;4(Suppl):s13–18.
[17] Paueksakon P, Revelo MP, Ma LJ, Marcantoni C, Fogo AB. Microangiopathic injury and augmented PAI-1 in human diabetic nephropathy. Kidney Int 2002;61:2142–8.
[18] Hamsten M, Wiman B, de Faire U, Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 1985;313:1557–63.
[19] Wada H, Mori Y, Kaneko T, Wakita Y, Nakase T, Minamikawa K, et al. Elevated plasma levels of vascular endothelial cell markers in patients with hypercholesterolemia. Am J Hematol 1993;44:112–6.
[20] Tousoulis D, Davies G, Ambrose J, Tentolouris C, Stefanadis C, Toutouzas P. Effects of lipids on thrombotic mechanisms in atherosclerosis. Int J Cardiol 2002;86:239–47.
[21] Festa A, Agostino RD, Mykkänen JL, Tracy R, Howard BV, Haffner SM. Low-density lipoprotein particle size is inversely related to plasminogen activator inhibitor-1 levels: the insulin resistance atherosclerosis study. Arterioscler Thromb Vasc Biol 1999;19:605–10.
[22] Opatrny Jr K, Zemanova P, Mares J, Vit L, Opatrna S, Sefrna F, et al. Fibrinolysis defect in long-term hemodialysis patients with type 2 diabetes mellitus and its relation to metabolic disorders. Am J Nephrol 2002;22:429–36.
[23] Opatrny Jr K, Zemanova P, Opatrna S, Vit L. Fibrinolysis in chronic renal failure, dialysis and renal transplantation. Ann Transplant 2002;7:34–43.
[24] Schneider DJ, Ricci MA, Taatjes DJ, Baumann PQ, Reese JC, Jeffrey C, et al. Changes in arterial expression of fibrinolytic system proteins in atherogenesis. Arterioscler Thromb Vasc Biol 1997;17:3294–301.

[25] Suzuki M, Akimoto K, Hattori Y. Glucose upregulates plasminogen activator inhibitor-1 gene expression in vascular smooth muscle cells. Life Sci 2002;72:59–66.
[26] Oubina MP, de Las Heras N, Vazquez-Perez S, Cediel E, Sanz-Rosa D, Ruilope LM, et al. Valsartan improves fibrinolytic balance in atherosclerotic rabbits. J Hypertens 2002;20:303–10.
[27] Valensi P. Rosiglitazone: a clinical development focused on cardiovascular prevention. Ann Endocrinol (Paris) 2002;63:1s35–1s40.
[28] Djoussé L, Ellison RC, Zhang Y, Arnett DK, Sholinsky P, Borecki I. Relation between dietary fiber consumption and fibrinogen and plasminogen activator inhibitor type 1: the National Heart, Lung, and Blood Institute family heart study. Am J Clin Nutr 1998;68: 68–575.
[29] Venter CS, Nel CJ, Vorster HH, Jerling JC, Oosthuizen W, Veldman FJ, et al. Soluble-fibre concentrate lowers plasminogen activator inhibitor-1 in baboons (*Papio ursinus*). Br J Nutr 1997;78:625–37.
[30] Orbe J, Rodriguez JA, Calvo A, Grau A, Belzunce MS, Martinez-Caro D, et al. Vitamins C and E attenuate plasminogen activator inhibitor-1 (PAI-1) expression in a hypercholesterolemic porcine model of angioplasty. Cardiovasc Res 2001;49:484–92.
[31] Devaraj S, Chan Jr AV, Jialal I. Alpha-tocopherol supplementation decreases plasminogen activator inhibitor-1 and P-selectin levels in type 2 diabetic patients. Diabetes Care 2002;25:524–9.
[32] Bendixen BH, Ocava L. Evaluation and management of acute ischemic stroke. Curr Cardiol Rep 2002;4:149–57.
[33] Boersma E, Mercado N, Poldermans D, Gardien M, Vos J, Simoons ML. Acute myocardial infarction. Lancet 2003;361:847–58.
[34] Falkenberg M, Tom C, DeYoung MB, Wen S, Linnemann R, Dichek DA. Increased expression of urokinase during atherosclerotic lesion development causes arterial constriction and lumen loss, and accelerates lesion growth. Proc Natl Acad Sci U S A 2002;99: 10665–70.
[35] Bell WR. The fibrinolytic system in neoplasia. Semin Thromb Hemost 1996;22:459–78.
[36] Lijnen HR. Extracellular proteolysis in the development and progression of atherosclerosis. Biochem Soc Trans 2002;30:163–7.