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The Fibrinolytic Enzyme System: New Markers of Potential Interest in Cardiovascular Disease

by

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Abstract

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Impaired fibrinolytic function, mainly due to elevated plasma levels of PAI-1, is a common finding in patients with coronary heart disease. Determination or evaluation of the plasma PAI-1 concentrations are complicated, due to a natural variation in blood plasma or due to other reasons, such as a poor stability under physiological conditions.

A polymorphism is located 675 bp upstream of the transcriptional start site in the promoter region of the PAI-1 gene, consisting of a single guanosine (G) insertion/deletion variation, and resulting in two alleles containing either 4 or 5 G in a row. Individuals who are homozygous for the 4 G allele have increased PAI-1 concentrations in plasma. An increased risk for cardiovascular disease has also been hypothesized. We therefore elaborated and characterized a rapid and reliable allele specific PCR method to determine this polymorphism.

The content of PAI-1 in platelets is quite high. Because of the rapid conversion of active PAI-1 to the latent form under physiological conditions and due to that the normal turnover of platelets in the circulation is 5-10 days, it would be expected that less than 1 % of PAI-1 in platelets would be active. We found that platelet PAI-1in healthy individuals was about 10 % active, which is much higher than expected. The reason for this is not understood, but it may indeed explain why platelet rich thrombi are difficult to lyse by tPA.

The tPA activity constitutes a very small portion of tPA antigen in plasma. The major portion is inactive and constitutes complexes with different serpins (antiplasmin, C1-inhibitor or PAI-1). A method to specifically measure the complex between tPA and PAI-1 in plasma samples was developed. It was found that the concentration of this complex correlated well with PAI-1 activity or with tPA antigen in plasma. However, no correlation was found between tPA activity and tPA antigen in the plasma samples. It was considered important to further evaluate measurement of this complex, especially in connection with myocardial infarction (MI).

In several prospective studies it has been found that elevated plasma PAI-1 or tPA antigen concentrations in patients with manifest coronary heart disease are linked to future cardiovascular events such as MI. Distribution, correlation and interaction of plasma concentrations of tPA/PAI-1 complex were analyzed in 886 MI patients and 1198 matched control individuals in the Stockholm Heart Epidemiological Program (SHEEP). Analysis of coagulation, other fibrinolytic, lipoprotein and inflammatory compounds were also performed. The data clearly demonstrated that the plasma concentration of tPA/PAI-1 complex was significantly associated with the risk of MI, in both genders. Synergistic interactions were observed in men for the co-exposure to high plasma concentrations of tPA/PAI-1 complex in combination with smoking (OR=4.6) or with diabetes mellitus (OR=7.9).

Using a cohort within the SHEEP material, constituting the control individuals in this study, the tPA/PAI-1 complex was prospectively evaluated as a predictor of MI. During a follow-up period of 6-8 years, 58 out of the 1611 controls had suffered a first myocardial infarction. High plasma concentrations of tPA/PAI-1 complex or of PAI-1 significantly predicted a forthcoming MI.

In conclusion, an impaired fibrinolytic function seems to be important in cardiovascular disease.

Key words: Fibrinolysis, myocardial infarction, tissue plasminogen activator, PAI-1

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LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Falk G, Almqvist Å, Nordenhem A, Svensson H and Wiman B.
Allele specific PCR for detection of a sequence polymorphism in the promoter region of plasminogen activator inhibitor-1 (PAI-1) gene.
Fibrinolysis, 1995;9:170-174.

II Nordenhem A and Wiman B.
Plasminogen activator inhibitor-1 (PAI-1) content in platelets from healthy individuals, genotyped for the 4G/5G polymorphism in the PAI-1 gene.
Scand J Clin Lab Invest, 1997;57: 453-462

III Nordenhem A and Wiman B.
Tissue plasminogen activator (tPA) in plasma: correlation with different tPA/inhibitor complexes. Scand J Clin Lab Invest, 1998;58: 475-483

IV Nordenhem A, Leander K, Hallqvist J, de Faire U, Sten-Linder M and Wiman B.
The complex between tPA and PAI-1: Risk factor for myocardial infarction as studied in the SHEEP project. Thromb Res, 2005; 116: 232-233

V Nordenhem A, Leander K, Hallqvist J, de Faire U, Sten-Linder M and Wiman B.
tPA/PAI-1 complex is a major risk marker for development of a first myocardial infarction among healthy controls in the SHEEP study. Manuscript

LIST OF ABBREVIATIONS

APTT	Activated partial thromboplastin time
BMI	Body mass index
CABG	Coronary artery by pass grafting
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
factor VIIIa	Activated factor FVIII
DVT	Deep vein thrombosis
ECG	Electrocardiogram
FDP	Fibrin degradation products
GP	Glycoprotein
HR	Hazard ratio
HDL	High density lipoprotein
HRP	Horseradish peroxidase
IU	International Unit
LDL	Low density lipoprotein
MI	Myocardial infarction
NIDDM	Non-insulin-dependent diabetes mellitus
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor 1
PTCA	Percutaneous transluminal coronary angioplasty
RR	Relative risk
scuPA	Single-chain urokinase plasminogen activator
S	Synergy index
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
tPA	Tissue plasminogen activator
uPA	Urokinase plasminogen activator

INTRODUCTION

Haemostasis is a complex interaction between the vessel wall and components of blood, and has the function of preventing excessive blood loss after vascular damage while maintaining a viable circulation by preventing thromboembolic conditions. The haemostatic processes can be divided into primary and secondary events. Primary events involve blood vessel constriction after vessel injury, followed by the adhesion and activation of platelets. The formation of an aggregated platelet plug stops blood flow temporarily, and is sufficient for the control of bleeding in the smallest vessels. However, these primary reactions are not enough alone to maintain haemostasis in larger vessels and additional activation of secondary processes is needed. These processes involve the activation of the coagulation system, causing generation of thrombin and ultimately leading to the formation of a fibrin matrix stabilising the platelet plug. In addition, extensive regulation of the coagulation system is necessary to avoid inappropriate thrombus formation, as well as a fibrinolytic system for the degradation and removal of fibrin clots, or deposits.

The vessel wall produces and secretes substances with both procoagulant and anticoagulant effects. The procoagulant factors include von Willebrand factor, tissue factor, and platelet activating factor. The anticoagulant factors are coagulation inhibitors such as tissue factor pathway inhibitor, endothelium derived relaxing factor or nitric oxide, tPA and prostacyclin (1). Exposure of subendothelial tissue is highly thrombogenic and results in activation of the platelets and the coagulation system. Upon activation, the platelet receptors glycoprotein Ib and glycoprotein IIb/IIIa become exposed at the cell surface and interact with von Willebrand factor and fibrinogen, leading to adhesion to the subendothelium and subsequently to aggregation.

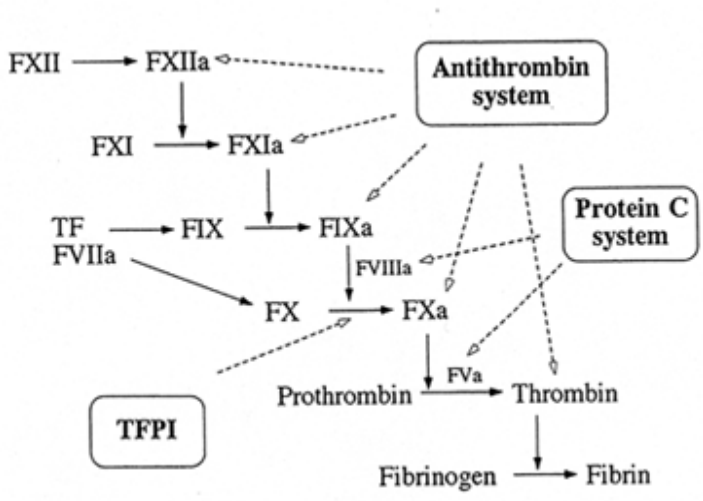

Figure 1. Schematic overview of the coagulation system. The extrinsic pathway (FVII and tissue factor) is what we understand today the most important pathway for the in vivo initiation of blood coagulation. Activation of FXII in the intrinsic pathway is initiated by exposure of high molecular mass kininogen and prekallikrein to negatively charged surfaces such as glass or kaolin in vitro. In addition to the coagulation factors shown here, the presence of phospholipids (from activated platelets) and Ca^{2+} is essential for activation of the cascade. Arrows with broken lines indicate inhibition. Negatively charged phospholipids become exposed on the surface of the activitated platelets where vitamin K-dependent coagulation factors may bind in the presence of Ca^{2+} ions. Activated platelets can release substances from three types of granulae; alfa-granulae, dense granulae and lysosomes (acid hydrolases). Contraction of platelets releases adenosine diphosphate, serotonin and thromboxane A_2. These inducers of platelet aggregation are capable of recruiting additional circulating platelets, which in turn adhere and form a platelet plug.

The fibrinolytic enzyme system is involved in many physiological and pathophysiological processes. Removal of fibrin deposits from blood vessels and prevention of formation of fibrin clots in the circulatory system are perhaps the best-known functions. These processes have given the name fibrinolytic to this proteolytic cascade system that operates through plasminogen activation and plasmin proteolysis. This system in a way counteracts the coagulation system and has important regulatory functions in haemostasis. Today, however, it is known that degradation of fibrin is only one of several important functions for this system. Other important functions of this system include activation of metalloproteinases, which in turn have the capacity to degrade extracellular matrices. This mechanism is important in remodeling of tissues as needed in e.g., wound healing and for invasive growth. The fibrinolytic system also seems to have an important role in the mechanisms involved in cell migration.

Activity in the fibrinolytic system may become decreased due to several different disturbances, such as plasminogen deficiency, deficient activator production or storage, or increased fibrinolysis inhibition, usually resulting from elevated plasma levels of PAI-1. There seems to be a connection between an impaired fibrinolytic function and MI, as has been described in a number of prospective studies. This association is, however, complicated by the strong correlation that seems to exist between raised plasma PAI-1 levels or tPA-antigen and insulin resistance, which is also correlated to MI. Measurement and interpretation of PAI-1 or tPA concentrations is not easy, because of a natural variation in blood plasma and due to other reasons, such as a poor stability of PAI-1 under physiological conditions. In this work, reasons for variation of the PAI-1 concentrations have been studied. In addition, a method for determination of tPA/PAI-1 complex have been elaborated and evaluated. Finally, some of the methods have been used in a major clinical material, the SHEEP study, to evaluate their clinical importance.

THE COAGULATION SYSTEM

Activation of the blood coagulation cascade initiates a chain of reactions leading to the formation of the enzyme thrombin, a potent enzyme that converts soluble fibrinogen into insoluble fibrin. Thrombin has also other effects of importance for regulation of the coagulation process (see below).

The coagulation system consists of a cascade of enzymatic and non-enzymatic reactions (2-5). Ca^{2+} is necessary for membrane-binding of the vitamin K-dependent coagulation factors (protrombin, factors VII, IX and X) and anticoagulant factors (Protein C and Protein S). Furthermore, Ca^{2+} is needed for a correct organisation of the multimolecular complexes formed during the cascade. The coagulation cascade may be activated in two different ways, “extrinsic” and “intrinsic”. The “extrinsic pathway” (tissue factor dependent) as we understand it today is the most important for the in vivo initiation of blood coagulation. The “intrinsic”pathway is still important when the coagulation test APT time is used. Both pathways converge in the common pathway with the activation of factor IX.

The "extrinsic pathway" is initiated by vascular injury. The integral membrane protein tissue factor localized in the adventitia becomes exposed and factor VII binds to tissue factor, resulting in activation of factor VII to factor VIIa in the presence of Ca^{2+}. For the initial activation of factor VII, factor Xa seems to be of importance (6). Tissue factor in complex with factor VIIa then activates factor IX to factor IXa and factor X to factor Xa. Factor IXa with membrane bound factor VIIIa activates factor X to factor Xa. Factor Xa together with factor Va as a cofactor in the presence of Ca^{2+} and a phospholipid surface, converts prothrombin to thrombin by cleavage and releasing fragment 1+2 from protrombin.

The "intrinsic pathway" only uses factors contained within the blood. The "intrinsic pathway" is initiated upon exposure of factor XII, factor XI, prekallikrein and high molecular mass kininogen in the blood to a negatively charged surface, e. g. connective tissue or col-

lagen. The activation of factor XII causes a sequential activation of factors XI, IX and X. Patients with deficiencies of factors XII, prekallikrein or high molecular mass kininogen, never have any bleeding symptoms, in spite of very prolonged APT times (7).

Thrombin is a key enzyme with many different functions. It splits off the fibrinopeptides A and B from fibrinogen, thereby converting soluble fibrinogen to fibrin monomers, which spontaneously polymerizes. Thrombin also activates factor XIII, which crosslinks fibrin into a stable covalently stabilyzed fibrin clot. In addition, thrombin activates platelets, which provide the surface needed for an efficient activation of the coagulation process. Thrombin also activates factors V, VIII and XI to their active forms, creating regulatory very important positive feedbacks.

The complicated function of thrombin is illustrated by its role in activation of the protein C system, which shuts off the coagulation system by inactivation of factor Va and factor VIII (8). This occurs after thrombin binding to thrombomodulin, which dramatically changes the specificity of this enzyme. Thrombin/thrombomodulin efficienly activates protein C, in the presence of protein S.

In addition to thrombin, additional components serve as regulators of the coagulation system. Tissue factor pathway inhibitor (TFPI) inhibits the tissue factor - factor VIIa - factor Xa complex (for a review see Broze and Tollefsen) (9). Tissue factor pathway inhibitor-2 (TFPI-2) is another recently discovered inhibitor of the tissue factor - factor VIIa complex (10). Antithrombin is an important serine proteinase inhibitor (serpin) that inhibits mainly thrombin but also factors Xa, IXa, XIa, XIIa and kallikrein. Serpins are a superfamily of proteins in human plasma, which are involved in the regulation of serine proteinases in coagulation, fibrinolysis and inflammation (11). They are suicidal inhibitors which all react with their target proteinases in a 1:1 molar ratio, forming tight complexes which are quite rapidly cleared from the circulation. Heparin cofactor II is another heparin-dependent thrombin inhibitor in plasma but the physiological importance is not known, for a review see Broze and Tollefsen (9).

Fibrinogen is the final protein in the coagulation cascade, which after polymerization forms the blood clot or thrombus. It is also important from the cardiovascular point of view, since it has been reported as an independent risk indicator for MI. The fibrinogen molecule is a glycoprotein containing two copies of three polypeptide chains (α, β, and γ) (12). Fibrinogen is encoded by three distinct genes located on the long arm of chromosome 4 (q23-32). It is synthesized by hepatocytes, and synthesis of the ß-chain seems to be the rate limiting step. The half-life of circulating fibrinogen is 3 to 4 days in healthy individuals. Besides being a vital part of a clot, fibrinogen also participates in platelet activation by binding to platelet GP IIb/IIIa receptors, resulting in platelet aggregation and release of the platelet granulae contents. The plasma level of fibrinogen is determined genetically and by several lifestyle and metabolic factors. Smoking, age, diabetes mellitus and hypercholesteremia are associated with increased plasma concentrations (13, 14). Fibrinogen is an acute phase reactant and its plasma levels are elevated in many conditions, such as infection, surgery and trauma. Several studies have addressed the risk of cardiovascular disease for high in comparison with low fibrinogen levels in plasma (15-19). A meta-analysis of 22 of these studies (13 prospective, five cross-sectional and four case-control) showed that elevated plasma fibrinogen levels (within the highest tertile) are associated with an overall twofold increased risk of cardiovascular disease in both healthy subjects and high-risk subjects (20).

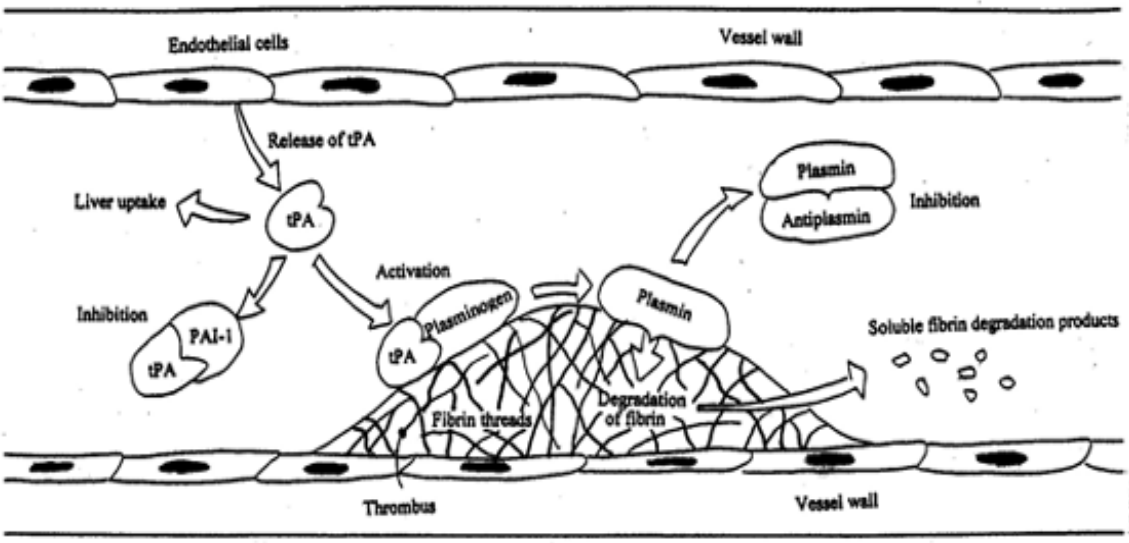

Figure 2. A simplified diagram of the fibrinolytic system (adapted from Wiman, MFR informerar, 1987)

THE FIBRINOLYTIC SYSTEM

The fibrinolytic system is responsible for the degradation and removal of fibrin clots, thereby restoring eventually obstructed circulation. This system is also involved in tissue repair, macrophage function, ovulation and malignant transformation. Plasminogen plays a central role in fibrinolysis. It is an inactive proenzyme, which upon activation to plasmin may degrade fibrin. Activation of plasminogen is catalysed by tPA or urokinase-type plasminogen activator (uPA). uPA may also be involved in pericellular proteolysis. Two inhibitors of the fibrinolytic system are PAI-1 (21-23), which inhibits tPA and uPA, and antiplasmin, which is the main inhibitor of plasmin (24-26). Plasmin bound to the fibrin surface is relatively protected from inactivation by antiplasmin. Both tPA and uPA are serine proteases, which exist as single- or two-chain molecules. The role of uPA in thrombolysis is less well-defined, but it is rather believed to be involved in other physiological processes such as cell migration, cell invasion and metastasis (27).

Components

Plasminogen and plasmin

Plasminogen is the precursor molecule of plasmin and is produced by the liver. The plasma concentration of plasminogen is about 100-150 mg/L and the half-life in the circulation about 2.8 days (28). Plasminogen is a single chain glycoprotein of M_r about 90,000, consisting of 790 amino acid, and about 2% carbohydrate. The plasminogen gene, located on chromosome 6, spans 53.5 kb and consists of 19 exons (29). Plasminogen is activated to the two-chain proteolytically active plasmin by tPA or urokinase by cleavage of a single peptide bond, Arg_{560}-Val_{561} (30). The two chains are held together by two disulphide bonds. There is a heavy (A) chain (M_r 60,000) and a light (B) chain (M_r 25,000). The light chain contains the serine protease catalytic triad (31). The heavy chain contains five kringles and mediates interactions of plasmin(ogen) with fibrin(ogen), inhibitors and cell-surface receptors.

Tissue plasminogen activator (tPA)

The tPA molecule is synthesized by endothelial cells (32, 33) and released into the blood stream by various stimuli such as stress, physical exercise and nicotinic acid (34). The release of tPA is also stimulated by vasoactive

agents such as bradykinin (35), antiduretic hormone and catecholamines (36, 37). However, the regulation of tPA synthesis and secretion is far from clear.

The tPA molecule contains 530 amino acids and has a molecular weight of about 67,000. tPA is released in a single chain form, but can be converted into a two-chain form by plasmin (38). The A-chain (M_r 38,000), is derived from the NH_2-terminal portion, and the B-chain (M_r 30,000) containing the active site from the COOH-terminal portion (39)). The A-chain contains four domains; a finger domain, a growth factor-like domain and two kringle domains. The finger and kringle 2 domains seem to be involved in binding to fibrin (40). The tPA gene is located on chromosome 8 (41), spans 33 kb and has 14 exons (42).

Plasma concentrations of tPA measured as tPA antigen are low, about 5-10 µg/L and varies greatly under different physiological and pathological conditions. The half-life for unbound tPA in plasma is short, about 5 minutes. The tPA is mainly expressed in the vascular endothelial cells. Most of the tPA in plasma is present in a complex with its primary inhibitor PAI-1 (22), or to some other protease inhibitors in plasma (43, 44).

The regulatory processes for the occurrence of active tPA in blood are: (*a*) secretion of tPA from the endothelial cells within the blood vessels; (*b*) inhibition of tPA by PAI-1 in blood plasma; and (*c*) elimination by the liver (44-47). The difference in molar ratio between tPA antigen concentration and tPA activity is typically explained by the presence of PAI-1, forming an inactive complex with tPA (48). To some extent also antiplasmin and C1-inhibitor contributes to this effect. It is possible that the complexes of tPA with these inhibitors have a longer half-life in the circulation, as compared to free tPA, allowing the complexes to accumulate (47).

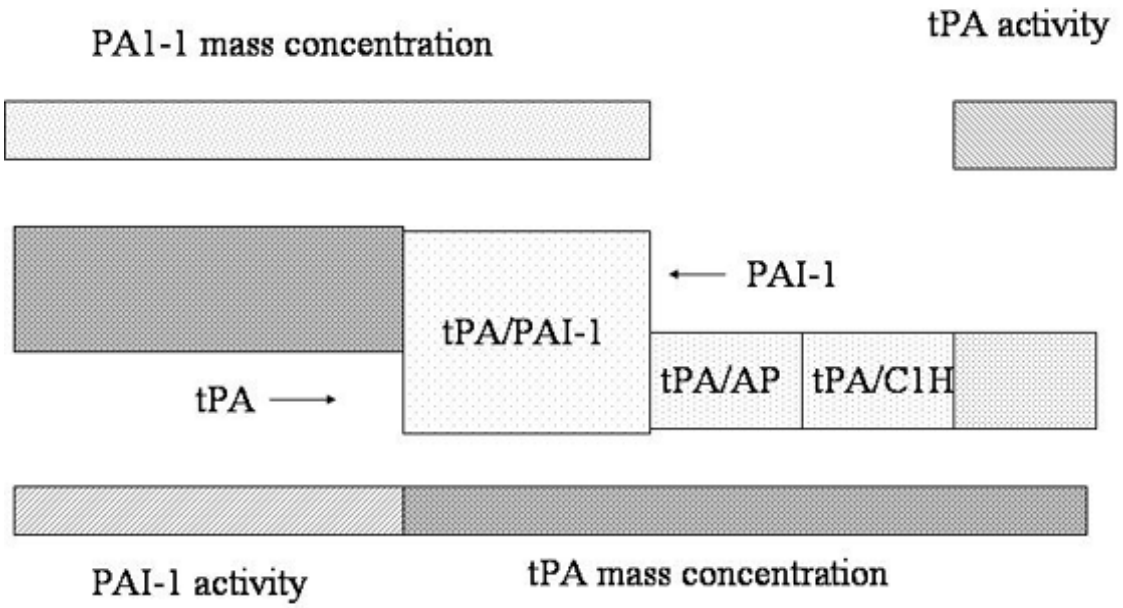

Figure 3. A presentation of the different components constituting PAI-1 and tPA mass concentrations. AP denotes antiplasmin and C1H C1-inhibitor.

Many studies have been devoted to the identification and characterization of potential tPA

receptors in the liver, as reviewed by Otter *et al.* (49). The liver parenchymal cells express a high affinity receptor for the uptake and degradation of tPA/PAI-1 complexes, which have been identified as low density lipoprotein receptor-related protein or α_2-makroglobulin receptor (50).

When single-chain tPA is bound to fibrin and plasminogen, the enzyme seems to be fully active prior to the formation of the two-chain molecule (39). This is the only known serine protease with almost full activity in its single-chain form. Only a small portion of the tPA in the circulating blood is active. Most of it is inactive and bound to the protease inhibitors PAI-1, antiplasmin, or C1-inhibitor.

In an in vivo study no correlation was found between circulating plasma levels and the local tPA release from the endothelium. Baseline measurements from venous plasma concentrations of tPA and PAI-1 cannot be used for assessment of the fibrinolytic activity locally. Activity of tPA at the organ level is dependent on the tPA release from the endothelium rather than on the circulating tPA or inhibitor of tPA in blood (51).

Urokinase (uPA)

Urokinase was originally described in human urine (52) at a concentration of 200-300 µg/L. It was later found in plasma at a concentration of 3.5 µg/L (53, 54). Urokinase is probably important in keeping the urinary tract free from fibrin deposits, but also in pericellular proteolysis, such as tissue remodelling and cell migration. The role of uPA in hemostasis and fibrinolysis is still unclear. Single-chain urokinase plasminogen activator (scuPA) has a molecular weight of about 54,000. scuPA is converted to the enzymatically active two-chain uPA by plasmin and kallikrein (55). Urokinase contains one growth factor-like domain and one kringle domain but no finger domain like tPA. The uPA gene located on chromosome 10, spans 6.5 kb and has 11 exons. Urokinase does not display any affinity for fibrin.

Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is the most important physiological inhibitor of tPA and u-PA in human plasma and forms a 1:1 complex with the respective activator. PAI-1 was discovered in conditioned media from cultured cells (33, 56) and was later found in plasma (22, 23). PAI-1 has been demonstrated in a variety of cultured cells including hepatocytes, platelets, megacaryocytes and smooth muscle cells (57). These cell-types are therefore candidates for the biosynthesis or storage of PAI-1 (33, 56, 58, 59). Synthesis or release of PAI-1 by endothelial cells is stimulated by factors such as thrombin, endotoxin, dexamethasone, interleukin-1, tumour necrosis factor and transforming growth factor β, for a review see Kruithof (60). Adipose tissue has also been shown to be a potential source of PAI-1 (61, 62).

PAI-1 is a single chain glycoprotein with a molecular weight of 52 000 consisting of 379 amino acid residues. It lacks cysteine residues and has therefore no disulfide bridges (63, 64). The PAI-1 gene, located on chromosome 7 (64) spans 12.2 kb and consists of 9 exons (65).

PAI-1 is secreted as an active molecule (66), but it is spontaneously transformed into a latent form under physiological conditions (67). Active PAI-1 is stabilized by its binding to vitronectin in plasma as well in tissues (68-70). The vitronectin-PAI-1 complex may work as an inhibitor of thrombin (71). PAI-1 bound to vitronectin in plasma has a half-life of about 4 hours, compared to about 2 hours when PAI-1 is not bound to vitronectin. The concentration of PAI-1 in plasma under normal physiological conditions is low, about 10 µg/L (72). In plasma about 60% of the PAI-1 antigen is functionally active. It is also present in platelets where the major pool of PAI-1 (100-200 µg/L) is inactive and found in the α-granulae (73-75). The platelet and plasma PAI-1 pools has been suggested to be independent of each other (73). It is known that platelets are the main source of PAI-1 in blood, but there are conflicting data in literature regarding the actual specific activity of platelet PAI-1. Published figures range from almost completely inactive to very active. The reaction between PAI-1 and plasminogen acti-

vators is fast with second order rate constant above 10^7 $M^{-1}s^{-1}$ (76).

When plasma PAI-1 concentration is increased, the half-life of functional tPA in the circulation is considerably shortened. This causes a decreased fibrinolytic potential (77). A negative correlation has been found between the plasma concentration of PAI-1 and levels of plasmin-antiplasmin complex in plasma (78, 79).

Several polymorphisms have been identified in the PAI-1 locus. An eight-allele (CA)n repeat polymorphism (80) in intron 3, and a two-allele *Hind*III restriction fragment length polymorphism of the 3´flanking region of the gene have been demonstrated to be associated with the plasma PAI-1 levels (81). In young postinfarction patients and matched controls the 1/1 genotype of *Hind*III correlated to higher levels of PAI-1 activity in plasma than the 2/2 genotype. Individuals with *Hind*III genotype 1/1 showed a two-three fold increase in the plasma PAI-1 with an increase in VLDL triglycerides as compared to individuals with genotype 2/2.

An additional polymorphism has been located 675 basepairs upstream of the transcriptional start site. It consists of a single guanosine insertion/deletion variation (4G or 5G) (82). Individuals, who are homozygous for the 4G allele typically have higher PAI-1 activity in plasma than individuals with other genotypes (82-87). Several studies have been performed regarding a possible association between the 4G/5G polymorphism in the PAI-1 gene and the development of MI with varying results (83, 85-94). However, the largest study published so far did not display any significant difference in the 4G/5G polymorphism in patients with MI as compared to the controls (95). In patients with sepsis or multitrauma, individuals homozygous for the 4G allele have been reported to have a worse outcome than individuals with the other genotypes (96, 97).

Plasminogen activator inhibitor-2 (PAI-2)

PAI-2 was first demonstrated in extracts of human placenta (98). PAI-2 has also been found in monocytes, leukocytes, macrophages and in monocyte-like cell-lines (99). PAI-2 has been purified from placenta and from plasma of pregnant women (100). The plasma concentration of PAI-2 increases during pregnancy, above 35 µg/L, and falls sharply after delivery (101). This suggests that PAI-2 may have a haemostatic role during pregnancy and delivery. It has also in a few cases been demonstrated in plasma from men and non-pregnant women (100). PAI-2 exists in two forms: a secreted glycosylated form with M_r 70 000 and an intracellular non-glycosylated form with M_r 46 000. Pregnant women with placental insufficiency have low concentration of PAI-2 (102). Preeclampsia patients have lower concentrations of PAI-2 and higher concentrations of PAI-1 than women with a normal pregnancy (103).

Thrombin-activatable fibrinolysis inhibitor (TAFI)

TAFI is a glycoprotein with M_r 60 000, consisting of 417 amino acids. It is synthesised in the liver and it is also known as procarboxypeptidase U or plasma procarboxypeptidase B. TAFI can be activated by thrombin, trypsin, kallikrein or plasmin into the active enzyme TAFIa. The most efficient activator seems to be the complex of thrombin and thrombomodulin (104, 105). TAFIa can potentially decrease the fibrinolytic activity by removing carboxyterminal lysine residues from partially degraded fibrin, thereby decreasing plasminogen binding to the surface of fibrin (106). TAFI is therefore not an inhibitor, but an enzyme that may modulate fibrinolytic activity.

Antiplasmin

Antiplasmin was first described in 1976 (24-26), as the principal plasmin inhibitor in plasma. Antiplasmin is a single-chain glycoprotein with M_r 70 000. It is a member of the serine protease inhibitor super family of proteins (serpins). The plasma concentration is about 70 mg/L and its half-life in the circulation is about 2.6 days (107).

The reaction between plasmin and antiplasmin in circulation is rapid, with a rate constant above 10^7 $M^{-1}s^{-1}$ (108). In contrast, antiplasmin reacts much slower with fibrin-bound

plasmin (109). Small amounts of antiplasmin become cross-linked to fibrin during clotting by the aid of factor XIIIa (110). This protects the clot from premature lysis and problems within this process may result in a bleeding tendency (111). Deficiency or dysfunction of the antiplasmin molecule may also cause a bleeding tendency due to increased fibrinolytic activity (112).

The fibrinolytic system in disease

Impaired fibrinolysis in venous thrombosis

An impaired fibrinolytic function is a common finding in patients with different kinds of thrombotic disease, for a review see Wiman (113). In deep vein thrombosis (DVT), an impaired fibrinolytic function has been found in 30-40% of the patients (114). The value of many of these investigations is limited due to that the data are retrospective and nonspecific assays have frequently been used. Today we know that the decreased fibrinolytic activity mainly is caused by increased plasma levels of PAI-1. Occasionally, this is observed together with a poor release of tPA on venous occlusion (115). An increased plasma concentration of PAI-1 as seen in individuals with thrombotic disease, results in a fast formation of the inactive complex and shortens the half-life time of tPA to <60 s (77). DVT patients often have a long-lasting inflammatory response, which contributes to the increased PAI-1 levels in plasma. Retrospective studies therefore give limited information and are difficult to evaluate regarding the question of cause or consequence. In a prospective study of recurrent DVT a weak but statistically significant correlation between PAI-1 or tPA antigen levels in plasma and recurrent DVT has been reported (116). Some families with recurrent DVT seem to have a combination of increased levels of PAI- 1 and increased levels of histidine-rich glycoprotein in plasma (117).

Impaired fibrinolysis in coronary heart disease

The history of atherosclerosis often starts early in life and involves several components such as lipid deposition in the subendothelial layer, inflammation and endothelial dysfunction, eventually followed by thrombosis. The atheroma precedes fatty streaks that may cause symptoms by it self or develops into vulnerable plaques at risk to rupture (118). If that happens thrombotic stimulating factors are released and as a consequence thrombosis may occur. Atherothrombosis is a progressive disease, causing clinical problems, such as, acute myocardial infarction, stroke and peripherial arterial disease. Atherothrombosis of the coronary arteries is the major cause of death and disability in the western world (119).

In European community as a whole, age standardized mortality from coronary heart disease increased from 146.2/100 000 in the second half of the 1960s to 158.9/100 000 at the end of 1970, but declined thereafter to reach 99.6/100 000 in 1995-97, corresponding to a decline of 37 % since the late 1970s (120).

As mentioned above, a decreased fibrinolytic activity in blood is mainly due to increased plasma PAI-1 levels (115). Also elevated plasma concentrations of tPA antigen, mostly tPA/PAI-1 complex, correlates with a decreased fibrinolytic activity. Thus, there is a strong correlation between plasma PAI-1 levels and tPA antigen or tPA/PAI-1 complex, but there is no correlation between tPA activity and tPA antigen (44, 121, 122).

The first report demonstrating that elevated plasma PAI-1 concentrations was connected with MI was a case control study of of men who had survived an MI occurring before the age of 45 years (123). In a follow-up study it was demonstrated that plasma concentration of PAI-1 activity independently correlated with reinfarction occurring within 3 years of the primary event (124). In addition, PAl-I remained an independent predictor of cardiac death within 6 to 9 years of follow-up (125). Since then many prospective studies have been published indicating that a decreased fibrinolytic activity, due to increased PAI-1 concentrations (85, 89, 95, 121, 126-132) or increased tPA antigen in plasma (127, 130, 133-144) predicts MI**.** Later on it was demonstrated that tPA antigen to a large extent constitutes the complex between tPA and PAI-1

(44). In this thesis a method to specifically measure this complex in plasma was developed and later evaluated in clinical materials (121, 145). Impaired fibrinolysis activity measured as increased PAI-1 concentrations or tPA/PAI-1 concentrations correlates with insulin concentrations, triglycerides and body mass index.

Impaired fibrinolysis and insulin resistance syndrome

There is an association of impaired fibrinolytic function caused by increased plasma PAI-1 concentrations and insulin resistance syndrome (146, 147), which has been identified in several studies (148-153). The insulin resistance syndrome comprises central obesity, hyperinsulinaemia, impaired glucose tolerance, dyslipidaemia and hypertension.

It has been demonstrated that PAI-1 in plasma is correlated with serum triglycerides, blood glucose, insulin levels, body mass index (BMI), waist-to-hip circumference ratio and hypertension. Increased plasma PAI-1 levels have also been found to correlate with the amount of visceral fat in obese humans (154), and PAI-1 is commonly and predictably elevated in individuals with insulin resistance or type II diabetes (155). It has prospectively been found that PAI-1 also predicts the development of type II diabetes mellitus (156)**.**

The biological mechanisms underlying the association between insulin resistance and haemostatic variables are not yet completely clear. The effect of diabetes on PAI-1 levels has been studied on blood samples and small tissue specimens from the mammary artery, obtained from diabetic and non-diabetic individuals in connection with coronary artery bypass graft surgery (157). PAI-1 related immunofluorescence was elevated in the arterial wall and reduced fibrinolytic activity was found in plasma in diabetic patients.

Increased fibrinolytic activity and bleeding tendency

A bleeding problem has been observed in patients with absent or reduced plasma antiplasmin activity (158-160). In patients with absent or reduced plasma PAI-1 concentrations, a weak bleeding tendency has been observed (161-163). An excessive fibrinolysis due to increased tPA activity levels may also be associated with a bleeding tendency, which has been described in a few patients (164, 165). In a study of patients referred to a hospital coagulation unit due to bleeding symptoms, a low PAI-1 activity were more frequently found among these patients as compared to matched controls (166).

The fibrinolytic system in cancer

The role of the fibrinolytic system in cancer has attracted considerable interest during many years. In this respect, focus at present, is at the urokinase pathway of this system. The main components of the urokinase plasminogen activator (uPA) system include; uPA, PAI-1 and uPAR (167). Data has shown that the uPA system plays an important role in cancer invasion and metastasis. It has been suggested that the uPA system plays a broad role in cancer and is involved at multiple stages in the formation and progression of the disease (168). The importance of the fibrinolytic system in breast cancer is well documented and it has been shown that high levels of uPA antigen and PAI-1 in the primary tumour are associated with poor prognosis of the patient (169, 170).

THE PRESENT INVESTIGATION

AIMS OF THE STUDY

1. To elaborate a simple and reliable allele-specific PCR method to determine the 4G/5G polymorphism in the PAI-1 gene promoter region.

2. To perform a systematic study measuring PAI-1 activity and antigen content in platelets in order to investigate the degree of PAI-1 activity in platelets.

3. To develope specific ELISA methods for determination of the protease/inhibitor complexes constituting tPA antigen in plasma. To investigate if tPA/PAI-1 complex in plasma is a better discriminator for arterial thrombotic disease as compared to tPA antigen.

4. To explore the distribution, correlation and interaction of plasma concentrations of tPA/PAI-1 complex in MI patients and matched controls in the Stockholm Heart Epidemiology Program (SHEEP).

5. To evaluate the role of plasma concentrations of tPA/PAI-1 complex as predictor of MI in healthy individuals (SHEEP controls) in the SHEEP study.

MATERIALS AND METHODS

A PCR method for the 4G/5G polymorphism in the promoter region of the PAI-1 gene

In paper I, we present a simple and reliable PCR method, utilizing allele specific primers, to determine the 4G/5G polymorphism in the PAI-1 gene promoter region. Our allele specific PCR method uses two 17-mer allele specific primers, 5′-GTCTGGAC AGTGGGGG-3′(PAI-5G) and 5′-GTCTGGACAGTGGG GA-3′ (PAI-4G) in combination with a downstream primer 5′-TGCAGCCAGCCACGTG ATTGTCTAG-3′ (PAI-2d), resulting in DNA fragments of 140 and 139 bp, respectively. A fourth primer located upstream of the polymorphic region, 5′-AAGCTTTTACCATGG TAACCCCTGGT-3′ (PAI-u), was used as a positive control in the PCR reaction and results in a DNA fragment of 257 bp in combination with the downstream primer (PAI-2d). The PCR amplification was performed in a total volume of 50 µl using 1 µg of genomic DNA. The reaction mixture contained 10 mmol/L Tris-HCL, pH 8.8, 1.5 mmol/L $MgCl_2$, 50 mmol/L of each nucleoside triphosphate, 0.2 µmol/L of each primer, except for the upstream control primer which where a concentration of 0.05 mmol/L was used and 2 units of Dynazyme (a thermostable DNA polymerase purchased from Finnzymes Oy, Espoo, Finland). The mixture was subjected to 30 cycles of 94 °C (45s), 65 °C (45s) and 72 °C (75s). Amplified DNA fragments were analysed by 4% agarose gel electrophoresis. Using the described method, 308 healthy blood donors were genotyped regarding this polymorphism. In order to establish the reliability of the assay, 120 of these samples were reanalysed in a random and blind fashion, with identical results.

PAI-1 activity and PAI-1 antigen measurements

For determination of PAI-1 activity in plasma Spectrolyse®/pL PAI (Biopool) was used. The method is a two-stage, indirect enzymatic assay. In stage one, a fixed amount of tPA is added to the plasma sample and allowed to react with the PAI-1 present. Then the sample is acidified to destroy alpha-2-antiplasmin and other potential plasmin inhibitors that would otherwise interfere with the tPA assay. Subsequently, the sample is diluted. In stage two, the residual tPA activity is measured by adding the sample to a mixture of Glu-plasminogen, poly-D-lysine and chromogenic substrate at neutral pH. The residual tPA activity in the sample will catalyse the conversion of plasminogen to plasmin, which in turn will hydrolyse the chromogenic substrate. The amount of colour developed is proportional to the amount of tPA activity in the sample. Poly-D-lysine is present as a stimulator of the

tPA catalysed conversion of plasminogen to plasmin. The PAI-1 content of the sample is then identified as the difference between the amount of tPA added and the amount of tPA recovered. One unit of PAI-1 activity is defined as the amount of PAI-1 that inhibits one international unit of human single chain tPA. Highest PAI-1 activity value accurately measured is 28 lU/ml. Samples with PAI-1 levels above 28 lU/ml had to be diluted and reassayed. According to the manufacturer, at the level 9 IU/ml, the within assay CV and between assay CV are 11.4% and 9.4%, respectively.

Determination of PAI-1 antigen in plasma was performed by an ELISA method (TintElize®, Biopool). For capturing purpose a monoclonal antibody against PAI-1 were used, while horseradish peroxidase (HRP)-conjugated polyclonal anti-PAI-1 antibodies were used for measuring purpose. The minimal detectable concentration was 0.5 ng/ml of PAI-1 antigen. The within assay CV and between assay CV are typically less than 5 % at 40 ng/ml.

Determination of tPA antigen utilizes the double antibody principle (TintElize, Biopool). Plasma sample or standard containing tPA is added to a microtiter plate well which is coated with goat anti-tPA IgG. After an incubation to bind tPA, HRP-labelled Fab fragments from anti-tPA IgG are used for measuring purpose. According to the manufacturer the within assay CV and between assay CV are 5.5 % and 3.5%, respectively at a tPA antigen concentration of 6 ng/ml.

To measure tPA/PAI-1 complex we combined the two kits for measuring PAI-1 and tPA antigen (kits from Biopool). Thus microtiter plates coated with polyclonal antibodies against human tPA was used in combination with a HRP-conjugated monoclonal antibody (MAI-12) to PAI-1. The colour developed was found to be directly proportional to the concentration of tPA/PAI-1 complex in the sample. The detectionlimit of was about 0.25 µg/L. Samples with tPA/PAI-1 complex levels above 20 µg/L had to be diluted in tPA/PAI-1 free plasma or PET buffer. Later a commercially available kit (TintElize tPA/PAI-1®, Biopool) was used. Using this kit at a level of 9.1 µg/L a within-assay CV of 4.0 % and between-assay CV of 3.5% was achieved (according to the manufacturer). This kit was used in papers IV and V. Standardization of the commercial kit was somewhat different as compared to the method described in paper III, which is most likely the reason for that discrepant levels was measured in plasma from healthy individuals with the two methods.

Quantification of the specific complexes between tPA and the different inhibitors (PAI-1, antiplasmin and C1-inhibitor) was performed by classical two-site ELISA methods. Goat anti-tPA IgG was used as capture antibodies in all systems (identical microtiter plates as used in Tintelize tPA a kind gift from Biopool). For detection, HRP-conjugated polyclonal antibodies towards the different inhibitors (PAI-1, antiplasmin and C1-inhibitor) were used. Immunization was performed with purified proteins as described (171-173). The antibodies was conjugated with HRP according to Tijssen (174).

The rate constants for the reactions between tPA and antiplasmin or C1 inhibitor have not been reported previously and were therefore determined using pseudo first-order conditions. The experiments were performed as follows: tPA at a final concentration of 0.38 mmol/L (16000 IU/ml) was mixed with antiplasmin (final concentration 14 mmol/L) or C1 inhibitor (final concentration 14 mmol/L). After various incubation times (0 - 6 h), samples were taken for measurement of residual tPA activity, utilizing the chromogenic substrate Flavigen tPA (final concentration 0.5 mmol/l). The buffer used in these experiments was 0.04 mol/l sodium phosphate buffer pH 7.3, containing 0.1 mol/l NaCl, 0.1 g/l Tween 80 and 0.01 mmol/L NaN_3. In the experiments with C1 inhibitor, heparin (final concentrations 0 or 20 U/ml) was occasionally added to the buffer. The decline in tPA activity was plotted in a semilogarithmic graph and used to calculate the half-lives, which subsequently were used to calculate the reaction rate constants.
In order to clarify the role of vitronectin in stabilizing PAI-1 activity within the platelets, an ELISA method was developed to specifically

determine the concentration of PAI-1/vitronectin complex in platelet lysates. This was performed by a classical two-site ELISA method, utilizing polyclonal monospecific vitronectin IgG for coating microtitre plates and an HRP-labelled monoclonal antibody towards PAI-1 (MAI-12, Biopool) for detection (175).

Extraction of PAI-1 from fresh platelets from 5 mL citrated or acidified (Stabilyte tubes) blood was performed as described below. To obtain platelet rich plasma, blood was first centrifuged for 15 minutes at 140 × g (4 °C), followed by a second centrifugation at 2750 × g for 15 min (4 °C) to collect the platelets. These were then resuspended and washed once with either 5 mL of 0.01 mol/L sodium phosphate buffer, pH 7.0, containing 0.15 mol/L NaCl (citrated tubes) or 5 mL of 0.02 mol/L sodium acetate buffer, pH 5.5, containing 0.15 mol/L NaCl (Stabilyte tubes). After centrifugation, the platelets in the pellet were completely lysed by treatment with 1 mL of 50 mL/L Triton X 100 for 15 minutes at room temperature. The lysates were centrifuged for 15 minutes at 2750 × g (+ 4 °C) and the supernatants stored at -70 °C in aliquots. By this procedure we have avoided activation of the platelets and release of PAI-1, since this might lead to a decrease in PAI-1 activity concentrations due to thrombin formation and subsequent inactivation of PAI-1 (176).

Other biochemical analyses

Total cholesterol, HDL cholesterol and triglycerides were analysed in serum from fresh serum samples with a colometric method (Kodak Echtachem). LDL cholesterol was calculated according to the Fridewald formula (177, 178). Serum insulin was measured using commercial RIA kits, RIA 100, Pharmacia (Uppsala, Sweden). C-reactive protein (CRP) levels were measured in plasma using a highly sensitive immunonephelometric system (Dade-Behring, Marburg, Germany) and an automated BN II nephelometer. Apolipoprotein A1 and apolipoprotein B were determined by immunochemical techniques. Plasma fibrinogen was determined in samples that had been kept frozen at -70 ºC and analysed by polymerisation test according to the method described by Vermylen et al. (179).

Patients and controls in the SHEEP study

In papers IV and V, the Stockholm Heart Epidemiology Program (SHEEP), a large population based case-control study, is used. Cases were all first-time acute MI occurrences and diagnosed according to standardized criteria by the Swedish Association of Cardiologists in 1991. All acute MI were diagnosed according to (1) certain symptoms according to case history information; (2) specified changes in blood levels of the enzymes serum creatine kinase (CK) and serum lactate dehydrogenase (LD); and (3) specified electrocardiogram changes. All hospitalised cases were diagnosed according to these criteria. To certify that only cases with first-time MI events were selected in the study, each case was checked for previous MI's from 1975 and forward in the hospital discharge registry. Male cases were identified during a 2 years period (1992-1993), while female cases were collected during a 3-year period (1992-1994). During the period January 1 to October 31, 1992, the upper age limit for subjects was 65 years; from November 1, 1992, and onwards it was 70 years. Cases were recruited from three sources; (i) all coronary and intensive care units at the internal medicine departments of the 10 emergency hospitals within the county of Stockholm (89% of male cases and 80% of female cases, or 97% of all non-fatal cases), (ii) the computerized Hospital Discharge Register for the County, and (iii) death certificates form the National Register for Causes of Death maintained by Statistics Sweden (SCB). Cases were included at time of disease onset. The cases identified in SHEEP were classified as non-fatal if they survived at least 28 days after the day of their diagnosis (an internationally accepted definition), and as fatal if they did not. One control per case was randomly sampled from the computerized register of the Stockholm County population within 2 days of the case occurrence. The controls (individuals with a history of coronary heart disease were excluded) were matched to cases by sex, age (within a 5-year interval) and residential area within the Stockholm County. Five control candidates were identified at the same time, so that another one could simply replace potentially non-responding controls. Occa-

sionally both the initial and a substitute control were included, due to a late response from the initial one. Therefore, somewhat more controls than cases were finally included.

Postal questionnaires covering a large set of life style, behaviour or occupation questions were distributed to non-fatal cases and to their controls. The non-fatal cases and their controls were subjected to a physical examination (in the morning), which also included blood sampling (fasting samples), blood pressure measurements and anthropometrical tests. Examination took place about 3 months after the disease onset (and inclusion in SHEEP). This time interval was chosen in order to allow for cases to regain a metabolically stable state (123, 180). The examination date for the controls was set as close as possible to that of the corresponding case in order to avoid bias due to seasonal variation in the blood parameters.

Collection of blood samples and analyses performed

After 10 minutes of rest in the supine position, the patients had blood drawn from an antecubital vein into evacuated tubes (0.129 mol/L sodium citrate, EDTA, or nothing for serum samples) using minimal stasis. Many of the routine biochemical analyses were performed immediately at the local laboratories within the different participating hospitals. For storage, the blood samples were typically centrifuged within 30 minutes, and plasma or serum were immediately frozen in aliquots and stored at -70 ºC until used. The samples were then analysed at the Department of Clinical Chemistry, Karolinska Hospital. Due to an unfortunate break down of one of the freezers about 15% of the samples of the SHEEP material were destroyed.

Definition of standard risk factor and exposures

One definition of the word, risk factor is: "Risk factor: an environmental, behavioral, or biologic factor confirmed by temporal sequence, usually in longitudinal studies, which if present directly increases the probability of a disease occurring, and if absent or removed reduces the probability. Risk factors are part of the causal chain, or expose the host to the causal chain. Once disease occurs, removal of a risk factor may not result in a cure" (181). Risk factors can be divided into two groups; modifiable and fixed. In the modifiable group lifestyle and behavioural factors are categorized for and contains cholesterol, blood pressure, diabetes, smoking and physical activity. The fixed group represents sex and age. In Sweden, cardiovascular disease accounts for about 21% of death in women and 26% in men (182).

Smoking

Subjects who smoked when included in the study, or who had stopped smoking within the last two years, were classified as current smokers. Subjects who had stopped smoking more than two years before inclusion in the study were classified as ex-smokers. Subjects who had never smoked regularly for at least one year were considered never-smokers. In the analyses of potential interaction between smoking and the concentration of tPA/PAI-1 complex, never- and ex-smokers were compared with current smokers.

Overweight

The individual Body Mass Index (BMI) value was calculated from height and weight measurements from the health examination. Subjects with BMI values >28kg/m^2 were classified as suffering from overweight.

Waist-Hip ratio

The waist-hip ratio (W/H) was calculated at the health examination to estimate distribution of adipose tissue. W/H was calculated as the circumference midway between the lower rib margin and the iliac crest (waist) divided by the circumference at the widest point between hip and buttock (hip). Women with a W/H ratio >0.82 were categorized as exposed. For men the cut-off ratio used was >1.0.

Diabetes mellitus

Subjects were classified as diabetics if they were controlling the diabetes with insulin treatment, drug treatment, or diet at the time of inclusion in the study.

Hypertension
The blood pressure was recorded during the health examination and taken as the mean of two readings in supine position after five minutes of rest. Subjects fulfilling one or more of the following criteria were classified as hypertensives: (1) on antihypertensive drug therapy (for reason of hypertension); (2) a history of regular antihypertensive drug therapy during the last five years (or a part of that time); (3) a systolic blood pressure ≥160mm Hg or a diastolic blood pressure ≥90mm Hg without history of anti-hypertensive drug therapy.

Physical inactivity
Questions about physical activity included conditions at work, household, homework and leisure time activities. In this work, subjects who reported inactive leisure time, including occasional walks, during the last 5-10 years were categorized as exposed to physical inactivity.

Hypertriglyceridemia
Subjects with fasting serum triglyceride levels ≥2.3mmol/L were classified as exposed.

Hypercholesterolemia
Subjects with fasting levels of total cholesterol ≥6.5mmol/L or receiving lipid lowering medication were classified as exposed.

Blood parameters
All variables were dichotomized. The 75th percentile was used as cut-off for male and female control group, respectively, when determinig exposure to high apolipoprotein B, high LDL cholesterol, high CRP, high von Willebrand factor, high PAI-1 activity, high homocystein, high tPA/PAI-1 complex and high insulin. The 25th percentile was used as cut-off for the male and female control groups, respectively, when determinig exposure to low apolipoprotein A1 and low HDL cholesterol.

Statistical methods
Statistical analysis in paper I was performed using non parametric methods (Statistica for Windows, StatSoft Inc, Tulsa, OK USA). In paper II, comparison within or between groups were performed with paired or unpaired Student's t-tests (two tailed), respectively. Since PAI-1 concentrations (activity or antigen) in plasma are not normally distributed, logarithmic transformations were performed for these analytes prior to statistical calculations. Regressions were calculated by the least square method using a computer program (Sigmaplot, Jandel Scientific). Statistical analysis in paper IV and V were performed using SAS (versions 6.11; 6.12; 8e). The relationship between continuous variables was quantified by Spearman's rank-order correlation coefficient. Because the distributions of plasma concentrations of tPA/PAI-1 complex and PAI-1 were skewed, the non- parametric Kruskal-Wallis test was applied when comparing these values between different groups. In paper IV the relative risk of MI for the exposure to high plasma tPA/PAI-1 complex levels was calculated as odds ratios (OR). The influence of confounders was adjusted for using unconditional logistic regression when calculating OR with 95% confidence intervals (CI). In paper V the hazard ratios (HR) for developing MI due to the exposure to different risk factors were calculated. The influence of confounders was adjusted for using the Cox proportional hazards model when calculating HR with 95% confidence intervals (CI). Age (in five-year age groups), residential area, and smoking were controlled for by means of dummy variables. SAS 8e under Windows NT was used for the epidemiological and statistical analyses (183). Interaction between two risk factors was defined in accordance with Rothman and Greenland (1998) (184). Thus, the empirical criterion of interaction is deviation from additive effects of the compared risk factors. To evaluate possible interactions, we calculated Synergy index scores (S), which give the ratio of the combined effects to the sum of the effects of two risk factors. A Synergy index score exceeding 1.0 indicates a synergistic interaction and an index below 1.0 indicates an antagonistic interaction. Synergy index scores with 95% CI were calculated using a SAS program, which also allowed for confounding control (185). Missing values, however, were deleted in the analyses.

RESULTS AND DISCUSSION

Paper I

The occurence of the 4G/5G polymorphism in the PAI-1 promoter region, 675 basepair (bp) upstream of the transcriptional start site, consisting of a single guanosine insertion/deletion variation, resulting in two alleles containing either 4 or 5 guanosines in a row, correlates to the plasma PAI-I concentration. In paper I, a simple and reliable PCR method, utilizing allele specific primers, to determine the 4G/5G polymorphism in the PAI-1 gene promoter region is presented.

Using this method, more than 300 blood donors of varying age and sex (about 70% were men) were genotyped. The following allele distribution was found: 52.3% 4G and 47.7% 5G. The genotype distribution was: 4G/4G, 28.2 %, 4G/5G, 48.1% and 5G/5G, 23.7%. This is in agreement with what would be expected if the data are in Hardy-Weinberg equilibrium. A closer examination of the data by dividing the material into two age groups, one 19-51 and the other 52-71 years of age gave interesting results. The frequency of individuals homozygous for the 4G allele was much higher in the younger group (34.7%) as compared to the older (22.1%, p=0.014). The reason for this difference between the age groups is not known. The result from this analysis was still based on relatively few individuals. Therefore it is not possible to draw far-reaching conclusions. Also, a study of 4G/5G polymorphism in 124 healthy centenarians compared to an equally large group of young individuals did not reveal any major differences in allele distribution (186). In another study this polymorphism was investigated in different ethnic groups, such as Blacks, Hispanics, and Non-Hispanic Whites. The allele frequencies were significantly different among the 3 ethnic groups: the frequencies for the 4G and the 5G alleles were 0.52 and 0.48 in non-Hispanic whites, 0.38 and 0.62 in Hispanics, and 0.28 and 0.72 in blacks, respectively (187).

A meta analysis regarding the 4G allele in the PAI-1 gene and its relation to acute myocardial infarction has been performed (188). This study included 7 case control studies with a total of 2813 patients. The results indicated a weak, but significant association between the 4G allele and increased risk of myocardial infarction with OR 1.20, 95% CI 1.04 to 1.39, (P =0.04) (188). Thereafter, two large case controls studies have been published (95, 189). One study of 2819 Japanese myocardial infarction patients (2003 men and 816 women) and 2242 controls (1306 men and 936 women) demonstrated a significant correlation of the occurrence of the 4G allele and myocardial infarction only in women, but not in men (189). The other large study with 1212 (851 men and 361 women) with first-time MI and 1556 controls (1051 men and 505 women) demonstrated similar results (95).

Paper II

Platelets are the main source of PAI-1 in blood, but there are conflicting data in literature regarding the specific activity of platelet PAI-1. Published data range from almost completely inactive to very active (57, 58, 73, 74, 190-196). Some studies have indicated that platelet PAI-1 could be of pathophysiological importance, especially regarding the sensitivity of a thrombus to thrombolytic treatment with tPA (197-199). Our data suggested that PAI-1 in platelets in most individuals is about 10 % active, but occasionally it is almost as active as in plasma. We avoided activation of platelets during preparation and extraction of PAI-1, since this may cause inactivation of PAI-1 by the action of thrombin, if formed. Nevertheless, even if small standardization problems are taken into account, the PAI-1 activity found in the platelets was higher than expected, if conversion into the latent form had occurred normally. Vitronectin could be detected in the platelet lysates in low concentrations and a negative correlation was found between vitronectin and PAI-1 in the platelet lysates. In order to clarify the role of vitronectin in stabilizing PAI-1 activity within the platelets, attempts were performed to specifically determine the concentration of PAI-1/vitronectin complex in the platelet lysates. However, we were not able to find detectable concentrations of this complex in the platelet lysates. The reason for that PAI-1 within the platelets is more active than ex-

pected is therefore so far not well understood. A possible mechanism could be a continuous de novo synthesis of PAI-1 (200). It is known that platelets lack nuclear DNA. However, they still contain mRNA from the megakaryocytes and it has been demonstrated that protein synthesis still may occur for some proteins (201). It has also been proposed that the increased activity of PAI-1 in the platelets could be due to that active PAI-1 molecules are packed with other large α-granule proteins in a calcium-dependent process (202).

We also demonstrated that the 4G/5G polymorphism influences the PAI-1 activity content in platelets in the same way as previously has been shown for PAI-1 in plasma (82). The content of PAI-1 antigen in platelets was not significantly different among the different genotypes, although a tendency in the same direction was observed. However, as was expected, the PAI-1 levels in platelets were not subjected to any diurnal variation, in contrast to what has been found in plasma (203). Also, our data suggested that a diurnal variation of PAI-1 in plasma may exist independently of genotype regarding the 4G/5G polymorphism. In a much larger study it was subsequently demonstrated that the diurnal variation with very high levels of plasma PAI-1 in the early morning was much more pronounced in individuals with the 4G/4G genotype (204). In the same study, tPA antigen showed a weak diurnal variation, which did not differ among the genetic variants regarding the 4G/5G-polymorphism. These results have been confirmed in another and independent study (205).

The plasma PAI-1 diurnal variation seems to be dependent on a transcription factor CLIF (cyclelike factor), which has been recognized in regulating the circadian variation of PAI-1 gene expression (206). In the PAI-1 promoter, 2 E-box elements (CACGTG*)* are responsible for the activation of the PAI-1 gene by CLIF. One of the E-boxes is located at -677 to -672, overlapping the sequence of the 4G/5G polymorphism in the PAI-1 promoter, explaining why the different PAI-1 genotypes behave differently regarding the diurnal variation.
A strong correlation was found between the content of PAI-1 and β-thromboglobulin in platelets. The reason for this is unclear. One suggestion could be a co-regulation of the genes for the two proteins. Another possibility is that β-thromboglobulin or PAI-1 content merely reflects the number of α-granulaes within the platelets. In addition, no correlation was found between plasma PAI-1 activity, plasma PAI-1 antigen or PAI-1 in platelet lysates with platelet counts or mean platelet volumes. This is most likely due to that the studies were performed on healthy individuals with a very limited variation in platelet counts ($252 \pm 42 \times 10^9$/L) or in mean platelet volumes (8.6 ± 1.4 fL).

Paper III

The concentration of tPA antigen in plasma has attracted considerable interest due to its correlation with development of cardiovascular disease (128, 131, 135-137, 139, 140, 143). The plasma concentration of tPA antigen typically correlates well with PAI-1 activity rather than with tPA-activity. Thus, an increased plasma tPA antigen concentration reflects a decreased fibrinolytic activity rather than an increased fibrinolytic activity. Plasma tPA antigen is a heterogeneous mixture consisting of low concentration of active tPA (only a few percent), but mainly of tPA in complex with various inhibitors, such as PAI-1 (43, 45, 207, 208), antiplasmin (209) or C1-inhibitor (43, 45, 207, 208).

In plasma samples from 30 healthy individuals we found no correlation between tPA antigen on the one hand and tPA/C1-inhibitor complex or tPA/antiplasmin complex on the other hand. The tPA/C1-inhibitor complex seems to be present in plasma in quite high concentrations, which is somewhat surprising since the reaction rate between tPA and C1-inhibitor is low (3 to 5 $mol^{-1}s^{-1}$). This has been reported earlier (208) and it is likely that the reaction rate in circulating blood is increased e.g. at the endothelial surface, due to the presence of heparane-sulphate. The tPA/C1-inhibitor concentrations might also have been overestimated, to some extent, because of standardization problems. It is possible that some of the tPA/inhibitor complexes partly dissociates after binding to the tPA antibodies in the mi-

crotiter plates, thereby causing measuring problems. Such problems might be worse for the complex with C1-inhibitor, as compared to the complexes with the other inhibitors. However, as could be expected a strong positive correlation was found between tPA antigen and tPA/PAI-1 complex in plasma samples. For this reason it is possible that the concentration of tPA/PAI-1 complex in plasma would have the edge over tPA antigen regarding the power to predict thrombotic events.

In an unpublished study together with Dr. Elisabeth Moor (Dept of Cardiology, Karolinska University Hospital in Solna) we have measured plasma tPA/inhibitor complexes from 100 men who underwent elective coronary artery bypass grafting and compared the data with data from matched controls (Table 1). These patients had significantly higher concentrations of tPA/PAI-1 complex ($p < 0.005$) and also of tPA/C1-inhibitor complex ($p < 0.05$) in plasma as compared to matched controls. The results regarding tPA/antiplasmin complex and tPA antigen were not different between the patients and the matched controls. There were no differences in any of the tPA/inhibitor complexes between patients without occlusions and patients with occlusions of one or more vein grafts within three months of surgery.

Table 1. Plasma tPA antigen (μg/L) and tPA/inhibitor complexes (μg/L) in 100 men, who subsequently were subjected to elective coronary artery bypass grafting. The data for 107 matched controls are also shown. The values are given as mean ± SD. Student's t test has been used for calculation of p-values.

	Patients (n = 100)	**Controls (n = 107)**	**p-value**
tPA antigen	9.6 ± 2.8	10.2 ± 3.5	0.119
tPA/PAI-1	2.9 ± 1.7	1.6 ± 1.0	0.003
tPA/antiplasmin	0.8 ± 1.1	0.6 ± 0.5	0.08
tPA/C1-inhibitor	6.8 ± 11.6	4.6 ± 4.6	0.038

Paper IV

Today many different types of risk factors for MI has been described including several laboratory parameters, such as serum cholesterol fractions, apolipoproteins and fibrinogen. Inflammatory markers have also become increasingly interesting. In addition to this, an impaired fibrinolytic function due to increased plasma levels of PAI-1, the most important inhibitor of the physiological plasminogen activator tPA, has been demonstrated to corre late to the development of MI in several prospective studies(123, 127, 134, 210-212). In other studies it was found that tPA antigen (about 40% of which constitutes tPA/PAI-1 complex) or specifically analysed tPA/PAI-1 complex was an even better predictor of MI than PAI-1 (122, 134, 139, 143).

In paper IV we explored the distribution, correlation and interaction of plasma tPA/PAI-1 complex in patients and matched controls in the SHEEP study. About 3 months after the primary event, 1267 (893 men and 374 women) survivors of the initial MI had been subjected to blood sampling. At this time most of the patients were considered to be in a metabolically stable condition. In other studies the blood sampling have been further delayed (213, 214)**,** but then patients with an early

reinfarction would have been excluded from the study. An equal number of matched individuals served as controls. In the present study 886 patients (591 men and 295 women) and 1198 matched controls (753 men and 445 women)was included, and all had been analysed for plasma tPA/PAI-1 complex concentration. The result showed that the plasma concentration of tPA/PAI-1 complex was significantly associated with the risk of MI, for both genders. Synergistic interactions were observed in men for the co-exposure to high plasma tPA/PAI-1 complex concentrations in combination with smoking (OR=4.6) or diabetes mellitus (OR=7.9). Synergisms were also found in combination with exposure to serum hypercholesterolemia or increased levels of apolipoprotein B. Among men an antagonistic effect of the co-exposure to high tPA/PAI-1 complex and hypertension was found. A similar tendency was found among women. An antagonistic effect of increased waist/hip ratio and increased tPA/PAI-1 complex was only observed among the women.

In successive blood samples, intraindividual tPA antigen levels are relatively constant when not taken in the acute phase (215), while intraindividual PAI-1 levels vary considerably over time (215). This may at least partly explain why tPA antigen levels were significantly better associated with cardiovascular events than PAI-1 levels.

Both tPA and PAI-1 are subject to diurnal variation with highest levels in the early morning for PAI-1 activity and highest concentrations in the afternoon for tPA activity. This may offer an explanation to the higher incidence of myocardial infarction in the early morning (216, 217).

The definition of diabetes mellitus used in the study is a conservative one, as it does not consider blood glucose values. It has been revealed that "unknown" diabetes may occur commonly in patients with MI (218). It could be expected that with measuring the blood glucose values in the MI patients to find more diabetes mellitus patients. Impaired fibrinolysis as elevated plasma levels of PAI-1 and tPA are documented as an integral feature of the insulin resistance syndrome (146, 219, 220). An increased abdominal adipose tissue is hypothesised to be responsible for a mild chronic inflammatory state, which may induce insulin resistance and endothelial dysfunction, markers of which include t-PA antigen levels (220, 221).

In some instances a decreased fibrinogen concentratin in plasma could be due to that patients stopped smoking after MI, or because of treatment with statins. Statins were a relatively new drug when the SHEEP study started and only few patients were treated with this type of drug.

ACE-inhibitors, which have been suggested to lower plasma PAI-1 concentrations, were used only by about 200 MI patients and a about 50 controls.

Blood pressure usually falls after a MI and may still be affected 3 months afterwards due to the damage of the heart muscle. Thus, the fraction of patients with hypertension in the material is probably underestimated.

Life style changes in patients with MI and the prescribed medication may affect blood pressure levels. Prior to the MI in the cases, medications that lower blood pressure were around 30% in the MI cases and 20 in the controls. Medications for lowering blood lipids were scares, 5% in the MI cases and 4% in controls.

Paper V

It has previously been described that the tPA/PAI-1 complex is an important biochemical marker for myocardial reinfarction in the SHEEP study (122). In the present work we have prospectively followed a cohort constituting the control group in the SHEEP study for about 6-8 years. During that time 58 of those individuals (48 men and 10 women), who at the time of inclusion were considered to be healthy regarding cardiovascular disease, developed a first MI. The results clearly demonstrated that the individuals with increased plasma concentrations of tPA/PAI-1 complex (or PAI-1) had a significantly increased risk of developing MI during the follow-up period. In addition, CRP and HDL cholesterol, as well as

smoking, the occurrence of diabetes mellitus or hypertension also predicted MI. However, regarding the parameters hypercholesterolemia, high LDL cholesterol, high apolipoprotein B, low apolipoprotein A1, high fibrin D-dimer, high von Willebrand factor, hypertriglyceridemia, or high fibrinogen did not predict MI in this study. The material was too small to be divided according to gender or to analyse synergisms.

Prospective studies of initially healthy individuals regarding an impaired fibrinolytic function and MI are very limited. Ridker et al followed 231 healthy men from the Physicians' Health Study cohort who developed MI within 5 years, demonstrating that the baseline concentrations of tPA antigen were higher in MI than controls (139). Thögersen *et al*. (129) demonstrated significantly higher concentrations of PAI-1 and tPA antigen in predicting the occurrence of a first MI, but in a population with a high prevalence of CHD in both genders. Another study (Caerphilly) (133), in wich nearly 2000 middle aged men were followed for a period of 5 years, it was demonstrated that tPA antigen, but not PAI-1 activity, was associated with the development of major CHD. Recently, from the same study, with a longer follow-up period of 13 years, it was demonstrated that both tPA antigen and PAI-1 activity were risk factors for major CHD (222). Our study constituting the control subjects within the SHEEP study is however the first prospective investigation of previously “healthy” individuals with respect to specifically measured tPA/PAI-1 complex as risk indicator for MI. The results suggest that measurements of this complex, especially in individuals suffering from diabetes mellitus or in those who smoke, could be of practical value to assess the future risk of MI.

GENERAL DISCUSSION

The fibrinolytic enzyme system is involved in many physiological and pathophysiological processes. Removal of fibrin deposits from blood vessels and prevention of formation of fibrin clots in the circulatory system are perists (220). It has prospectively been demonhaps the best-known functions. These processes have given the name fibrinolytic system to this proteolytic cascade system that operates through plasminogen activation and plasmin proteolysis. A number of prospective studies of fibrinolytic parameters associated with MI have appeared. Most of them demonstrate a clear association between an impaired fibrinolytic function and development of MI.

The 4G/5G polymorphism in the PAI-1 promoter region correlates to the individual plasma PAI-I concentration. However, the association of this polymorphism to MI is weak and only significant in women (95, 188, 189).

Studies have shown that increased plasma PAI-1 concentrations diminish the efficiency of thrombolytic therapy in connection with MI in the acute stage, by preventing or delaying clot dissolution (223, 224).

Overweight and obesity assessed as BMI or WH-ratio are associated with increased cardiovascular morbidity and mortality (225, 226). Even a moderate weight loss results in regression of coronary arterial lesions and significantly reduces cardiac events and total mortality (227). In most studies (228-231), an association between the change in weight, BMI or body fat and the changes in PAI-1 levels has been found. The reduction in plasma PAI-1 concentrations in weight reduction appears to be more closely related to changes in adipose tissue than to changes in metabolic variables. In most studies a relation between alterations in plasma PAI-1 concentrations and changes in triglycerides or insulin has been found (229, 231, 232). It has been demonstrated that the occurrence of the 4G/4G genotype of PAI-1 is associated with an increased risk of obesity (233).

The insulin resistance syndrome is associated with increased cardiovascular morbidity and mortality. PAI-1 is an acute-phase protein regulated by the cytokines interleukin-6, TNF-α and TGF-β. An association between plasma PAI-1 and hormones, such as insulin also ex

strated that PAI-1 predicts the development of

type II diabetes mellitus (156). The disruption of the PAI-1 gene in genetically obese ob/ob mice was associated with reduced adiposity and improvement of the metabolic profile (234). These data indeed suggest a direct role of PAI-1 in obesity and insulin resistance. Accelerated atherothrombotic processes in the insulin resistance syndrome are attributed to metabolic abnormalities, inflammation and to impaired fibrinolysis due to increased plasma PAI-1 levels.

Decreasing PAI-1 activity may reduce the incidence of cardiovascular events and may increase the effectiveness of thrombolytic therapy. Research has resulted in a number of different compounds that inhibit PAI-1 activity i.e. monoclonal antibodies (235), peptides (236) and low molecular weight compounds (237). Even though extensive research has been carried out with respect to PAI-1 inhibition no positive clinical results have been reported so far. In addition some pharmaceutical drugs indirectly may lower plasma PAI-1 concentrations. These are ACE inhibitors, pravastatin and oestrogens.

Ramipril is one ACE inhibitor that decreases the concentration of plasma PAI-1. In a study with post-MI patients treated with this drug, a decrease of 44% regarding PAI-1 antigen and 22% of plasma PAI-1 activity was found (238).. In another large study (n = 9297), the outcome demonstrated that this ACE inhibitor significantly decreased the incidence of MI and cardiovascular death (239). The results were most likely independent of the blood pressure–lowering effects of ramipril, since most patients were not hypertensive and only limitedblood pressure reductions were observed. Thus, it is possible that one of the mechanisms for decreasing MI and cardiovascular death is indeed decreased plasma PAI-1 concentrations. As mentioned above, a weight loss is also accompanied by both decreased plasma PAI-1 concentrations and a diminished risk of MI.

In summary, an impaired fibrinolytic function cannot yet be considered as a fully established risk factor for MI in an epidemiological meaning, since prospective studies of initially healthy individuals including measurements of fibrinolytic parameters are very few. The data produced in this study emphasize the importance of further evaluation of the parameters of the fibrinolytic system (PAI-1, tPA antigen, or tPA/PAI-1 complex) as risk factors in future prospective studies. However, data are accumulating that an impaired fibrinolytic function may very well become a true risk factor for MI. In addition, our data suggest that measurement of the tPA/PAI-1 complex could be of practical value in assessing the risk for developing MI in smokers or in individuals with type II diabetes mellitus.

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REFERENCES

1. Pearson J. Endothelial cell biology. Haemostasis and Thrombosis (ed. by In:Bloom AL, Forbes CD, Thomas DP, Tuddenhammar EG), p 219 Churchill Livingstone, Edingburgh.; 1994.
2. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry. 1991 Oct 29;30(43):10363-70.
3. Davie EW. Biochemical and molecular aspects of the coagulation cascade. Thromb Haemost. 1995 Jul;74(1):1-6.
4. Furie B, Furie BC. Molecular and cellular biology of blood coagulation. N Engl J Med. 1992 Mar 19;326(12):800-6.
5. Furie B, Furie BC. The molecular basis of blood coagulation. Cell. 1988 May 20;53(4):505-18.
6. Broze G. The tissue factor pathway of coagulation: factor VII, tissue factor, and tissue factor pathway inhibitor. Haemostasis and Thrombosis (ed. by: Bloom AL, Thomas DP, Tuddenhammar EG), Churchill Livingstone, Edingburgh. 1994:pp. 349-67.
7. Rizza C. Haemophilia and related inherited coagulation defects. Haemostasis and Thrombosis (ed. by: Bloom AL, Forbes, CD, Thomas DP, Tuddenhammar EG), Churchill Livingstone, Edingburgh. 1994:p. 819.
8. Dahlback B, Stenflo J. A natural anticoagulant pathway: protein C, protein S, C4b-binding protein and thrombomodulin. Haemostasis and Thrombosis (ed. by: Bloom AL, Forbes, CD, Thomas DP, Teg D), Churchill Livingstone, Edingburgh. 1994;Third ed. vol. 1.
9. Broze G, Tollefsen D. Regulation of blood coagulation by protease inhibitors. The molecular basis of blood diseases (ed. by: Stamatoyannopoulos G, Nienhuis AW, Majerus PW and Varmus H) W.B. Saunders, Philadelphia. 1994:p. 629.
10. Sprecher CA, Kisiel W, Mathewes S, Foster DC. Molecular cloning, expression, and partial characterization of a second human tissue-factor-pathway inhibitor. Proc Natl Acad Sci U S A. 1994 Apr 12;91(8):3353-7.
11. Travis J, Guzdek A, Potempa J, Watorek W. Serpins: structure and mechanism of action. Biol Chem Hoppe Seyler. 1990 May;371 Suppl:3-11.
12. Blomback B. Fibrinogen and fibrin--proteins with complex roles in hemostasis and thrombosis. Thromb Res. 1996 Jul 1;83(1):1-75.
13. Lee AJ, Smith WC, Lowe GD, Tunstall-Pedoe H. Plasma fibrinogen and coronary risk factors: the Scottish Heart Health Study. J Clin Epidemiol. 1990;43(9):913-9.
14. Folsom AR, Wu KK, Davis CE, Conlan MG, Sorlie PD, Szklo M. Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. Atherosclerosis. 1991 Dec;91(3):191-205.
15. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med. 1984 Aug 23;311(8):501-5.
16. Yarnell JW, Sweetnam PM, Elwood PC, Eastham R, Gilmour RA, O'Brien JR, et al. Haemostatic factors and ischaemic heart disease. The Caerphilly study. Br Heart J. 1985 May;53(5):483-7.
17. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. Jama. 1987 Sep 4;258(9):1183-6.
18. Sweetnam PM, Thomas HF, Yarnell JW, Beswick AD, Baker IA, Elwood PC. Fibrinogen, viscosity and the 10-year incidence of ischaemic heart disease. Eur Heart J. 1996 Dec;17(12):1814-20.
19. Bolibar I, Kienast J, Thompson SG, Matthias R, Niessner H, Fechtrup C. Relation of fibrinogen to presence and severity of coronary artery disease is independent of other coexisting heart disease. The ECAT Angina Pectoris Study Group. Am Heart J. 1993 Jun;125(6):1601-5.
20. Maresca G, Di Blasio A, Marchioli

R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol. 1999 Jun;19(6):1368-77.
21. Wiman B, Chmielewska J, Ranby M. Inactivation of tissue plasminogen activator in plasma. Demonstration of a complex with a new rapid inhibitor. J Biol Chem. 1984 Mar 25;259(6):3644-7.
22. Chmielewska J, Ranby M, Wiman B. Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. Thromb Res. 1983;31:427-36.
23. Kruithof EK, Tran-Thang C, Ransijn A, Bachmann F. Demonstration of a fast-acting inhibitor of plasminogen activators in human plasma. Blood. 1984 Oct;64(4):907-13.
24. Collen D. Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. Eur J Biochem. 1976 Oct 1;69(1):209-16.
25. Moroi M, Aoki N. Isolation and characterization of alpha2-plasmin inhibitor from human plasma. A novel proteinase inhibitor which inhibits activator-induced clot lysis. J Biol Chem. 1976 Oct 10;251(19):5956-65.
26. Mullertz S, Clemmensen I. The primary inhibitor of plasmin in human plasma. Biochem J. 1976 Dec 1;159(3):545-53.
27. Carmeliet P, Collen D. Targeted gene maipulation and transfer of the plasminogen and coagualtion system in mice. Fibrinolysis. 1996;10:195-213.
28. Collen D, Tytgat G, Claeys H, Verstraete M, Wallen P. Metabolism of plasminogen in healthy subjects: effect of tranexamic acid. J Clin Invest. 1972 Jun;51(6):1310-8.
29. Magnaghi P, Citterio E, Malgaretti N, Acquati F, Ottolenghi S, Taramelli R. Molecular characterisation of the human apo(a)-plasminogen gene family clustered on the telomeric region of chromosome 6 (6q26-27). Hum Mol Genet. 1994 Mar;3(3):437-42.
30. Robbins KC, Summaria L, Hsieh B, Shah RJ. The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. J Biol Chem. 1967 May 25;242(10):2333-42.
31. Wiman B. Primary structure of the B-chain of human plasmin. Eur J Biochem. 1977 Jun 1;76(1):129-37.
32. Astrup T. Tissue activators of plasminogen. Fed Proc. 1966 Jan-Feb;25(1):42-51.
33. Loskutoff DJ, van Mourik JA, Erickson LA, Lawrence D. Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. Proc Natl Acad Sci U S A. 1983 May;80(10):2956-60.
34. Gram J, Jespersen J. Assessment of fibrinolysis in patients with thrombosis - from laborotory to clinical pratice. Hypercoagulable states. (ed. by Seghatchian MJ, Samama MM, Hecker SP) Boca Raton: CRC press. 1996:pp. 139-45.
35. Egberg N, Gallimore M, Gréen K, Jakobsson J, Vesterqvist O, Wiman B. Effects of plasma kallikrein and bradykinin infusion into pigs on plasma fibrinolytic variables and urinary excretion of thromboxane and prostacyclin metabolites. Fibrinolysis. 1988;2:101-6.
36. Smith D, Gilbert M, Owen WG. Tissue plasminogen activator release in vivo in response to vasoactive agents. Blood. 1985 Oct;66(4):835-9.
37. Larsson PT, Wiman B, Olsson G, Angelin B, Hjemdahl P. Influence of metoprolol treatment on sympatho-adrenal activation of fibrinolysis. Thromb Haemost. 1990 Jun 28;63(3):482-7.
38. Wallen P, Rånby M, Bergsdorf N, Kok P. Purification and characterization of tissue plasminogen activator: on the occerence of two different forms and their enzymatic properties. Progress in Fibrinolysis (ed. by Davidsson JF, Nilsson IM, Åstedt B) ,. 1981;vol. 5:pp. 16-32.
39. Wallen P, Bergsdorf N, Ranby M. Purification and identification of two structural variants of porcine tissue plasminogen activator by affinity adsorption on fibrin. Biochim Biophys Acta. 1982 Nov 24;719(2):318-28.

40. Pannekoek H, de Vries C, van Zonneveld A. Mutants of human tissue-type plasminogen activator (t-PA): structural and functional properties. Fibrinolysis. 1988;2(123-132.).
41. Rajput B, Degen SF, Reich E, Waller EK, Axelrod J, Eddy RL, et al. Chromosomal locations of human tissue plasminogen activator and urokinase genes. Science. 1985 Nov 8;230(4726):672-4.
42. Pennica D, Holmes WE, Kohr WJ, Harkins RN, Vehar GA, Ward CA, et al. Cloning and expression of human tissue-type plasminogen activator cDNA in E. coli. Nature. 1983 Jan 20;301(5897):214-21.
43. Thorsen S, Philips M. Isolation of tissue-type plasminogen activator-inhibitor complexes from human plasma. Evidence for a rapid plasminogen activator inhibitor. Biochim Biophys Acta. 1984 Nov 6;802(1):111-8.
44. Nordenhem A, Wiman B. Tissue plasminogen activator (tPA) antigen in plasma: correlation with different tPA/inhibitor complexes. Scand J Clin Lab Invest. 1998 Oct;58(6):475-83.
45. Chandler WL, Levy WC, Stratton JR. The circulatory regulation of TPA and UPA secretion, clearance, and inhibition during exercise and during the infusion of isoproterenol and phenylephrine. Circulation. 1995 Nov 15;92(10):2984-94.
46. Chandler WL. The human fibrinolytic system. Crit Rev Oncol Hematol. 1996 Sep;24(1):27-45.
47. Chandler WL, Alessi MC, Aillaud MF, Henderson P, Vague P, Juhan-Vague I. Clearance of tissue plasminogen activator (TPA) and TPA/plasminogen activator inhibitor type 1 (PAI-1) complex: relationship to elevated TPA antigen in patients with high PAI-1 activity levels. Circulation. 1997 Aug 5;96(3):761-8.
48. Kluft C. Constitutive synthesis of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1): conditions and therapeutic targets. Fibrinolysis. 1994;8:1-7.
49. Otter M, Kuiper J, van Berkel T, Rijken D. Mechanisms of tissue-type plasminogen activator (tPA) clearance by the liver. Plasminogen Activation in Fibrinolysis, in Tissue Remodeling and Development. (ed. by Brakman P, Kluft C). The New York Academy of Sciences, New York. 1992:pp 431-42.
50. Bu G, Warshawsky I, Schwartz AL. Cellular receptors for the plasminogen activators. Blood. 1994 Jun 15;83(12):3427-36.
51. Hrafnkelsdottir T, Gudnason T, Wall U, Jern C, Jern S. Regulation of local availability of active tissue-type plasminogen activator in vivo in man. J Thromb Haemost. 2004 Nov;2(11):1960-8.
52. Sobel G, Mohler S, Jones N, Dewdy A, Guest M. Urokinase: An activator of plasma profibrinolysin extracted from urine. Am J Physiol. 1952:768-9.
53. Wijngaards G, Kluft C, Groeneveld E. Demonstration of urokinase-related fibrinolytic activity in human plasma. Br J Haematol. 1982 May;51(1):165-9.
54. Tissot JD, Schneider P, Hauert J, Ruegg M, Kruithof EK, Bachmann F. Isolation from human plasma of a plasminogen activator identical to urinary high molecular weight urokinase. J Clin Invest. 1982 Dec;70(6):1320-3.
55. Ichinose A, Fujikawa K, Suyama T. The activation of pro-urokinase by plasma kallikrein and its inactivation by thrombin. J Biol Chem. 1986 Mar 15;261(8):3486-9.
56. Gelehrter TD, Barouski-Miller PA, Coleman PL, Cwikel BJ. Hormonal regulation of plasminogen activator in rat hepatoma cells. Mol Cell Biochem. 1983;53-54(1-2):11-21.
57. Simpson AJ, Booth NA, Moore NR, Bennett B. Distribution of plasminogen activator inhibitor (PAI-1) in tissues. J Clin Pathol. 1991 Feb;44(2):139-43.
58. Kruithof EK, Tran-Thang C, Bachmann F. Studies on the release of a plasminogen activator inhibitor by human platelets. Thromb Haemost. 1986 Apr 30;55(2):201-5.
59. Laug WE, Aebersold R, Jong A,

Rideout W, Bergman BL, Baker J. Isolation of multiple types of plasminogen activator inhibitors from vascular smooth muscle cells. Thromb Haemost. 1989 Jun 30;61(3):517-21.
60. Kruithof EK. Plasminogen activator inhibitors--a review. Enzyme. 1988;40(2-3):113-21.
61. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S. Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenetic link between obesity and cardiovascular disease. Circulation. 1996 Jan 1;93(1):106-10.
62. Samad F, Yamamoto K, Loskutoff DJ. Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-alpha and lipopolysaccharide. J Clin Invest. 1996 Jan 1;97(1):37-46.
63. Ny T, Sawdey M, Lawrence D, Millan JL, Loskutoff DJ. Cloning and sequence of a cDNA coding for the human beta-migrating endothelial-cell-type plasminogen activator inhibitor. Proc Natl Acad Sci U S A. 1986 Sep;83(18):6776-80.
64. Ginsburg D, Zeheb R, Yang AY, Rafferty UM, Andreasen PA, Nielsen L, et al. cDNA cloning of human plasminogen activator-inhibitor from endothelial cells. J Clin Invest. 1986 Dec;78(6):1673-80.
65. Loskutoff DJ, Linders M, Keijer J, Veerman H, van Heerikhuizen H, Pannekoek H. Structure of the human plasminogen activator inhibitor 1 gene: nonrandom distribution of introns. Biochemistry. 1987 Jun 30;26(13):3763-8.
66. Chmielewska J, Carlsson T, Urden G, Wiman B. On the relationship between diferent molecular forms of the fast inhibitor of tissue plasminogen activator. Fibrinolysis. 1987;1(1):67-73.
67. Levin EG, Santell L. Conversion of the active to latent plasminogen activator inhibitor from human endothelial cells. Blood. 1987 Oct;70(4):1090-8.
68. Wiman B, Almquist A, Sigurdardottir O, Lindahl T. Plasminogen activator inhibitor 1 (PAI) is bound to vitronectin in plasma. FEBS Lett. 1988 Dec 19;242(1):125-8.
69. Mimuro J, Schleef RR, Loskutoff DJ. Extracellular matrix of cultured bovine aortic endothelial cells contains functionally active type 1 plasminogen activator inhibitor. Blood. 1987 Sep;70(3):721-8.
70. Declerck PJ, De Mol M, Alessi MC, Baudner S, Paques EP, Preissner KT, et al. Purification and characterization of a plasminogen activator inhibitor 1 binding protein from human plasma. Identification as a multimeric form of S protein (vitronectin). J Biol Chem. 1988 Oct 25;263(30):15454-61.
71. Ehrlich HJ, Gebbink RK, Keijer J, Linders M, Preissner KT, Pannekoek H. Alteration of serpin specificity by a protein cofactor. Vitronectin endows plasminogen activator inhibitor 1 with thrombin inhibitory properties. J Biol Chem. 1990 Aug 5;265(22):13029-35.
72. Urden G, Hamsten A, Wiman B. Comparison of plasminogen activator inhibitor activity and antigen in plasma samples. Clin Chim Acta. 1987 Nov 16;169(2-3):189-96.
73. Booth NA, Simpson AJ, Croll A, Bennett B, MacGregor IR. Plasminogen activator inhibitor (PAI-1) in plasma and platelets. Br J Haematol. 1988 Nov;70(3):327-33.
74. Kruithof EK, Nicolosa G, Bachmann F. Plasminogen activator inhibitor 1: development of a radioimmunoassay and observations on its plasma concentration during venous occlusion and after platelet aggregation. Blood. 1987 Nov;70(5):1645-53.
75. Declerck P, Alessi M, Verstreken M, Kruithof E, Juhan-Vague I, Collen D. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood. 1988;71:220-5.
76. Chmielewska J, Ranby M, Wiman B. Kinetics of the inhibition of plasminogen activators by the plasminogen-activator inhibitor. Evidence for 'second-

site' interactions. Biochem J. 1988 Apr 15;251(2):327-32.
77. Wiman B. The fibrinolytic enzyme system. Basic principles and links to venous and arterial thrombosis. Hematol Oncol Clin North Am. 2000 Apr;14(2):325-38, vii.
78. Wiman B, Haegerstrand-Björkman M. Plasmin-antiplasmin complex in plasma - a global fibrinolytic assay. [Abstract no.1958]. Thromb Haemost. 1993;69:1091.
79. Pedersen OD, Gram J, Jespersen J. Plasminogen activator inhibitor type-1 determines plasmin formation in patients with ischaemic heart disease. Thromb Haemost. 1995 May;73(5):835-40.
80. Klinger KW, Winqvist R, Riccio A, Andreasen PA, Sartorio R, Nielsen LS, et al. Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis. Proc Natl Acad Sci U S A. 1987 Dec;84(23):8548-52.
81. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb. 1991 Jan-Feb;11(1):183-90.
82. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. 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. 1993 May 25;268(15):10739-45.
83. Mansfield MW, Stickland MH, Grant PJ. Environmental and genetic factors in relation to elevated circulating levels of plasminogen activator inhibitor-1 in Caucasian patients with non-insulin-dependent diabetes mellitus. Thromb Haemost. 1995 Sep;74(3):842-7.
84. Panahloo A, Andres C, Mohamed-Ali V, Gould MM, Talmud P, Humphries SE, et al. The insertion allele of the ACE gene I/D polymorphism. A candidate gene for insulin resistance? Circulation. 1995 Dec 15;92(12):3390-3.
85. Ye S, Green FR, Scarabin PY, Nicaud V, Bara L, Dawson SJ, et al. 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 I'nfarctus du Mycocarde. Thromb Haemost. 1995 Sep;74(3):837-41.
86. Eriksson P, Kallin B, van 't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A. 1995 Mar 14;92(6):1851-5.
87. Panahloo A, Mohamed-Ali V, Lane A, Green F, Humphries SE, Yudkin JS. Determinants of plasminogen activator inhibitor 1 activity in treated NIDDM and its relation to a polymorphism in the plasminogen activator inhibitor 1 gene. Diabetes. 1995 Jan;44(1):37-42.
88. Petrovic D, Globocnik-Petrovic M, Peterlin B. 4G4G genotype of PAI-1 gene promoter polymorphism is not associated with myocardial infarction in Caucasians with type-2 diabetes. Cardiology. 2003;100(3):157-8.
89. Juhan-Vague I, Morange PE, Frere C, Aillaud MF, Alessi MC, Hawe E, et al. The plasminogen activator inhibitor-1 -675 4G/5G genotype influences the risk of myocardial infarction associated with elevated plasma proinsulin and insulin concentrations in men from Europe: the HIFMECH study. J Thromb Haemost. 2003 Nov;1(11):2322-9.
90. Hindorff LA, Schwartz SM, Siscovick DS, Psaty BM, Longstreth WT, Jr., Reiner AP. The association of PAI-1 promoter 4G/5G insertion/deletion polymorphism with myocardial infarction and stroke in young women. J Cardiovasc Risk. 2002 Apr;9(2):131-7.
91. Iacoviello L, Burzotta F, Di Castelnuovo A, Zito F, Marchioli R, Donati MB. The 4G/5G polymorphism of PAI-1 pro-

moter gene and the risk of myocardial infarction: a meta-analysis. Thromb Haemost. 1998 Dec;80(6):1029-30.
92. Ossei-Gerning N, Mansfield MW, Stickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. Arterioscler Thromb Vasc Biol. 1997 Jan;17(1):33-7.
93. Henry M, Chomiki N, Scarabin PY, Alessi MC, Peiretti F, Arveiler D, et al. Five frequent polymorphisms of the PAI-1 gene: lack of association between genotypes, PAI activity, and triglyceride levels in a healthy population. Arterioscler Thromb Vasc Biol. 1997 May;17(5):851-8.
94. Zhan M, Zhou Y, Han Z. Plasminogen activator inhibitor-1 4G/5G gene polymorphism in patients with myocardial or cerebrovascular infarction in Tianjin, China. Chin Med J (Engl). 2003 Nov;116(11):1707-10.
95. Leander K, Wiman B, Hallqvist J, Sten-Linder M, de Faire U. PAI-1 level and the PAI-1 4G/5G polymorphism in relation to risk of non-fatal myocardial infarction: results from the Stockholm Heart Epidemiology Program (SHEEP). Thromb Haemost. 2003 Jun;89(6):1064-71.
96. Hermans PW, Hibberd ML, Booy R, Daramola O, Hazelzet JA, de Groot R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. Lancet. 1999 Aug 14;354(9178):556-60.
97. Menges T, Hermans PW, Little SG, Langefeld T, Boning O, Engel J, et al. Plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism and prognosis of severely injured patients. Lancet. 2001 Apr 7;357(9262):1096-7.
98. Kawano T, Morimoto K, Uemura Y. Urokinase inhibitor in human placenta. Nature. 1968 Jan 20;217(125):253-4.
99. Chapman HA, Jr., Stone OL. Characterization of a macrophage-derived plasminogen-activator inhibitor. Similarities with placental urokinase inhibitor. Biochem J. 1985 Aug 15;230(1):109-16.
100. Lecander I, Astedt B. Isolation of a new specific plasminogen activator inhibitor from pregnancy plasma. Br J Haematol. 1986 Feb;62(2):221-8.
101. Kruithof EK, Tran-Thang C, Gudinchet A, Hauert J, Nicoloso G, Genton C, et al. Fibrinolysis in pregnancy: a study of plasminogen activator inhibitors. Blood. 1987 Feb;69(2):460-6.
102. Reith A, Booth NA, Moore NR, Cruickshank DJ, Bennett B. Plasminogen activator inhibitors (PAI-1 and PAI-2) in normal pregnancies, pre-eclampsia and hydatidiform mole. Br J Obstet Gynaecol. 1993 Apr;100(4):370-4.
103. Bonnar J, Daly L, Sheppard BL. Changes in the fibrinolytic system during pregnancy. Semin Thromb Hemost. 1990 Jul;16(3):221-9.
104. Redlitz A, Tan AK, Eaton DL, Plow EF. Plasma carboxypeptidases as regulators of the plasminogen system. J Clin Invest. 1995 Nov;96(5):2534-8.
105. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. J Biol Chem. 1996 Jul 12;271(28):16603-8.
106. Wang W, Boffa MB, Bajzar L, Walker JB, Nesheim ME. A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activable fibrinolysis inhibitor. J Biol Chem. 1998 Oct 16;273(42):27176-81.
107. Wiman B. Human alfa2-AP. Methods Enzymol. 1981;80(32):395-408.
108. Wiman B, Collen D. On the kinetics of the reaction between human antiplasmin and plasmin. Eur J Biochem. 1978 Mar 15;84(2):573-8.
109. Wiman B, Collen D. Molecular mechanism of physiological fibrinolysis. Nature. 1978 Apr 6;272(5653):549-50.
110. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest. 1980

Feb;65(2):290-7.
111. Sakata Y, Aoki N. Significance of cross-linking of alpha 2-plasmin inhibitor to fibrin in inhibition of fibrinolysis and in hemostasis. J Clin Invest. 1982 Mar;69(3):536-42.
112. Stump DC, Taylor FB, Jr., Nesheim ME, Giles AR, Dzik WH, Bovill EG. Pathologic fibrinolysis as a cause of clinical bleeding. Semin Thromb Hemost. 1990 Jul;16(3):260-73.
113. Wiman B. Plasminogen activator inhibitor 1 in thrombotic disease. Curr Opin Hematol. 1996 Sep;3(5):372-8.
114. Isacson S, Nilsson IM. Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. Acta Chir Scand. 1972;138(4):313-9.
115. Wiman B, Hamsten A. Impaired fibrinolysis and risk of thromboembolism. Prog Cardiovasc Dis. 1991 Nov-Dec;34(3):179-92.
116. Schulman S, Wiman B. The significance of hypofibrinolysis for the risk of recurrence of venous thromboembolism. Duration of Anticoagulation (DURAC) Trial Study Group. Thromb Haemost. 1996 Apr;75(4):607-11.
117. Angles-Cano E, Gris JC, Loyau S, Schved JF. Familial association of high levels of histidine-rich glycoprotein and plasminogen activator inhibitor-1 with venous thromboembolism. J Lab Clin Med. 1993 May;121(5):646-53.
118. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005 Apr 21;352(16):1685-95.
119. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993 Apr 29;362(6423):801-9.
120. Levi F, Lucchini F, Negri E, La Vecchia C. Trends in mortality from cardiovascular and cerebrovascular diseases in Europe and other areas of the world. Heart. 2002 Aug;88(2):119-24.
121. Nordenhem A, Leander K, Hallqvist J, de Faire U, Sten-Linder M, Wiman B. The complex between tPA and PAI-1: risk factor for myocardial infarction as studied in the SHEEP project. Thromb Res. 2005;116(3):223-32.
122. Wiman B, Andersson T, Hallqvist J, Reuterwall C, Ahlbom A, deFaire U. Plasma Levels of Tissue Plasminogen Activator/Plasminogen Activator Inhibitor-1 Complex and von Willebrand Factor Are Significant Risk Markers for Recurrent Myocardial Infarction in the Stockholm Heart Epidemiology Program (SHEEP) Study. Arterioscler Thromb Vasc Biol. 2000 August 1, 2000;20(8):2019-23.
123. Hamsten A, Wiman B, de Faire U, Blomback M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med. 1985 Dec 19;313(25):1557-63.
124. Hamsten A, de Faire U, Walldius G, Dahlen G, Szamosi A, Landou C, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet. 1987 Jul 4;2(8549):3-9.
125. Malmberg K, Bavenholm P, Hamsten A. Clinical and biochemical factors associated with prognosis after myocardial infarction at a young age. J Am Coll Cardiol. 1994 Sep;24(3):592-9.
126. Scarabin PY, Aillaud MF, Amouyel P, Evans A, Luc G, Ferrieres J, et al. Associations of fibrinogen, factor VII and PAI-1 with baseline findings among 10,500 male participants in a prospective study of myocardial infarction--the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction. Thromb Haemost. 1998 Nov;80(5):749-56.
127. Juhan-Vague I, Pyke SD, Alessi MC, Jespersen J, Haverkate F, Thompson SG. Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. ECAT Study Group. European Concerted Action on Thrombosis and Disabilities. Circulation. 1996 Nov 1;94(9):2057-63.
128. Held C, Hjemdahl P, Rehnqvist N, Wallen NH, Bjorkander I, Eriksson SV, et al. Fibrinolytic variables and cardiovascular prognosis in patients with stable angina

pectoris treated with verapamil or metoprolol. Results from the Angina Prognosis study in Stockholm. Circulation. 1997 May 20;95(10):2380-6.
129. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation. 1998 Nov 24;98(21):2241-7.
130. Thogersen AM, Soderberg S, Jansson JH, Dahlen G, Boman K, Nilsson TK, et al. Interactions between fibrinolysis, lipoproteins and leptin related to a first myocardial infarction. Eur J Cardiovasc Prev Rehabil. 2004 Feb;11(1):33-40.
131. Folsom AR, Aleksic N, Park E, Salomaa V, Juneja H, Wu KK. Prospective study of fibrinolytic factors and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb Vasc Biol. 2001 Apr;21(4):611-7.
132. Cortellaro M, Cofrancesco E, Boschetti C. Fibrinolytic activity and ischaemic heart disease. PLAT Study Group. Lancet. 1993 Dec 11;342(8885):1489.
133. Lowe GD, Yarnell JW, Sweetnam PM, Rumley A, Thomas HF, Elwood PC. Fibrin D-dimer, tissue plasminogen activator, plasminogen activator inhibitor, and the risk of major ischaemic heart disease in the Caerphilly Study. Thromb Haemost. 1998 Jan;79(1):129-33.
134. Thogersen AM, Jansson J-H, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High Plasminogen Activator Inhibitor and Tissue Plasminogen Activator Levels in Plasma Precede a First Acute Myocardial Infarction in Both Men and Women : Evidence for the Fibrinolytic System as an Independent Primary Risk Factor. Circulation. 1998 November 24, 1998;98(21):2241-7.
135. Mannucci PM, Bernardinelli L, Foco L, Galli M, Ribichini F, Tubaro M, et al. Tissue plasminogen activator antigen is strongly associated with myocardial infarction in young women. J Thromb Haemost. 2005 Feb;3(2):280-6.
136. Lowe GD, Danesh J, Lewington S, Walker M, Lennon L, Thomson A, et al. Tissue plasminogen activator antigen and coronary heart disease. Prospective study and meta-analysis. Eur Heart J. 2004 Feb;25(3):252-9.
137. Gram J, Bladbjerg EM, Moller L, Sjol A, Jespersen J. Tissue-type plasminogen activator and C-reactive protein in acute coronary heart disease. A nested case-control study. J Intern Med. 2000 Feb;247(2):205-12.
138. Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. N Engl J Med. 1995 Mar 9;332(10):635-41.
139. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet. 1993 May 8;341(8854):1165-8.
140. Smith FB, Lee AJ, Fowkes FG, Price JF, Rumley A, Lowe GD. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. Arterioscler Thromb Vasc Biol. 1997 Nov;17(11):3321-5.
141. Munkvad S, Gram J, Jespersen J. A depression of active tissue plasminogen activator in plasma characterizes patients with unstable angina pectoris who develop myocardial infarction. Eur Heart J. 1990 Jun;11(6):525-8.
142. Olofsson BO, Dahlen G, Nilsson TK. Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary artery disease. Eur Heart J. 1989 Jan;10(1):77-82.
143. Jansson JH, Nilsson TK, Olofsson BO. Tissue plasminogen activator and other risk factors as predictors of cardio-

vascular events in patients with severe angina pectoris. Eur Heart J. 1991 Feb;12(2):157-61.
144. Jansson JH, Nilsson TK, Johnson O. von Willebrand factor, tissue plasminogen activator, and dehydroepiandrosterone sulphate predict cardiovascular death in a 10 year follow up of survivors of acute myocardial infarction. Heart. 1998 Oct;80(4):334-7.
145. Nordenhem A, Leander K, Hallqvist J, de Faire U, Sten-Linder M, Wiman B. The tPA/PAI-1 complex is a major risk marker for development of a first myocardial infarction among healthy controls in the SHEEP study. Manuscript.
146. Kohler HP. Insulin resistance syndrome: interaction with coagulation and fibrinolysis. Swiss Med Wkly. 2002 May 18;132(19-20):241-52.
147. Juhan-Vague I, Alessi MC. Regulation of fibrinolysis in the development of atherothrombosis: role of adipose tissue. Thromb Haemost. 1999 Aug;82(2):832-6.
148. Juhan-Vague I, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. Arterioscler Thromb. 1993 Dec;13(12):1865-73.
149. Potter van Loon BJ, Kluft C, Radder JK, Blankenstein MA, Meinders AE. The cardiovascular risk factor plasminogen activator inhibitor type 1 is related to insulin resistance. Metabolism. 1993 Aug;42(8):945-9.
150. Vague P, Juhan-Vague I, Aillaud MF, Badier C, Viard R, Alessi MC, et al. Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism. 1986 Mar;35(3):250-3.
151. Juhan-Vague I, Vague P, Alessi MC, Badier C, Valadier J, Aillaud MF, et al. Relationships between plasma insulin triglyceride, body mass index, and plasminogen activator inhibitor 1. Diabete Metab. 1987 Jul;13(3 Pt 2):331-6.
152. Nilsson TK, Boman K, Bjerle P, Hallmans G, Hellsten G. von Willebrand factor and fibrinolytic variables are differently affected in the insulin resistance syndrome. J Intern Med. 1994 May;235(5):419-23.
153. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia. 1991 Jul;34(7):457-62.
154. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med. 1996 Jul;2(7):800-3.
155. Lyon CJ, Hsueh WA. Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease. Am J Med. 2003 Dec 8;115 Suppl 8A:62S-8S.
156. Festa A, D'Agostino R, Jr., Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes. 2002 Apr;51(4):1131-7.
157. Pandolfi A, Cetrullo D, Polishuck R, Alberta MM, Calafiore A, Pellegrini G, et al. Plasminogen Activator Inhibitor Type 1 Is Increased in the Arterial Wall of Type II Diabetic Subjects. Arterioscler Thromb Vasc Biol. 2001 August 1, 2001;21(8):1378-82.
158. Kluft C, Vellenga E, Brommer EJ, Wijngaards G. A familial hemorrhagic diathesis in a Dutch family: an inherited deficiency of alpha 2-antiplasmin. Blood. 1982 Jun;59(6):1169-80.
159. Aoki N. Hemostasis associated with abnormalities of fibrinolysis. Blood Rev. 1989 Mar;3(1):11-7.
160. Kettle P, Mayne EE. A bleeding disorder due to deficiency of alpha 2-antiplasmin. J Clin Pathol. 1985 Apr;38(4):428-9.
161. Minowa H, Takahashi Y, Tanaka T, Naganuma K, Ida S, Maki I, et al. Four

cases of bleeding diathesis in children due to congenital plasminogen activator inhibitor-1 deficiency. Haemostasis. 1999;29(5):286-91.
162. Fay WP, Shapiro AD, Shih JL, Schleef RR, Ginsburg D. Brief report: complete deficiency of plasminogen-activator inhibitor type 1 due to a frame-shift mutation. N Engl J Med. 1992 Dec 10;327(24):1729-33.
163. Repine T, Osswald M. Menorrhagia due to a qualitative deficiency of plasminogen activator inhibitor-1: case report and literature review. Clin Appl Thromb Hemost. 2004 Jul;10(3):293-6.
164. Booth NA, Bennett B, Wijngaards G, Grieve JH. A new life-long hemorrhagic disorder due to excess plasminogen activator. Blood. 1983 Feb;61(2):267-75.
165. Aznar J, Estelles A, Vila V, Reganon E, Espana F, Villa P. Inherited fibrinolytic disorder due to an enhanced plasminogen activator level. Thromb Haemost. 1984 Oct 31;52(2):196-200.
166. Ågren A, Wiman B, Stiller V, Lindmarker P, Sten-Linder M, Carlsson A, et al. Evaluation of low PAI-1 activity as a risk factor for hemorrhagic diathesis. J Thromb Haemost. 2005;3:1-8.
167. Duffy MJ, Duggan C. The urokinase plasminogen activator system: a rich source of tumour markers for the individualised management of patients with cancer. Clin Biochem. 2004 Jul;37(7):541-8.
168. Duffy MJ. The urokinase plasminogen activator system: role in malignancy. Curr Pharm Des. 2004;10(1):39-49.
169. Harbeck N, Kates RE, Schmitt M, Gauger K, Kiechle M, Janicke F, et al. Urokinase-type plasminogen activator and its inhibitor type 1 predict disease outcome and therapy response in primary breast cancer. Clin Breast Cancer. 2004 Dec;5(5):348-52.
170. Look MP, van Putten WL, Duffy MJ, Harbeck N, Christensen IJ, Thomssen C, et al. Pooled analysis of prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in 8377 breast cancer patients. J Natl Cancer Inst. 2002 Jan 16;94(2):116-28.
171. Lindahl TL, Sigurdardottir O, Wiman B. Stability of plasminogen activator inhibitor 1 (PAI-1). Thromb Haemost. 1989 Sep 29;62(2):748-51.
172. Wiman B. Affinity-chromatographic purification of human alpha 2-antiplasmin. Biochem J. 1980 Oct 1;191(1):229-32.
173. Lindahl T, Wiman B. Purification of high and low molecular weight plasminogen activator inhibitor 1 from fibrosarcoma cell-line HT 1080 conditioned medium. Biochim Biophys Acta. 1989 Feb 23;994(3):253-7.
174. Tijssen P, Kurstak E. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. Anal Biochem. 1984 Feb;136(2):451-7.
175. Eklund AG, Sigurdardottir O, Ohrn M. Vitronectin and its relationship to other extracellular matrix components in bronchoalveolar lavage fluid in sarcoidosis. Am Rev Respir Dis. 1992 Mar;145(3):646-50.
176. Ehrlich HJ, Gebbink RK, Preissner KT, Keijer J, Esmon NL, Mertens K, et al. Thrombin neutralizes plasminogen activator inhibitor 1 (PAI-1) that is complexed with vitronectin in the endothelial cell matrix. J Cell Biol. 1991 Dec;115(6):1773-81.
177. Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, et al. Multilayer film elements for clinical analysis: applications to representative chemical determinations. Clin Chem. 1978 Aug;24(8):1343-50.
178. Abell L, Levy B, Brodie B, Kendell F. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem. 1952;195:357-66.
179. Vermylen C, De Vreker RA, Verstraete M. A rapid enzymatic method for assay of fibrinogen fibrin polymerization time (FPT test). Clin Chim Acta. 1963 May;8:418-24.
180. Hamsten A, Walldius G, Dahlen G,

Johansson B, De Faire U. Serum lipoproteins and apolipoproteins in young male survivors of myocardial infarction. Atherosclerosis. 1986 Feb;59(2):223-35.
181. Beck JD. Risk revisited. Community Dent Oral Epidemiol. 1998 Aug;26(4):220-5.
182. Senestrand U, Wallentin L. RIKSHIA In:. 2003.
183. SAS procedures guides [computer program]. SAS Institute Inc. 6.11. Cary, N.C.; 1990.
184. Rothman K, Greenland S. Modern epidemiology. 2 ed ed. Philadelphia: Lippincott-Raven Publishers; 1998.
185. Lundberg M, Fredlund P, Hallqvist J, Diderichsen F. A SAS program calculating three measures of interaction with confidence intervals. Epidemiology. 1996 Nov;7(6):655-6.
186. Mannucci PM, Mari D, Merati G, Peyvandi F, Tagliabue L, Sacchi E, et al. Gene polymorphisms predicting high plasma levels of coagulation and fibrinolysis proteins. A study in centenarians. Arterioscler Thromb Vasc Biol. 1997 Apr;17(4):755-9.
187. Festa A, D'Agostino R, Jr., Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Circulation. 2003 May 20;107(19):2422-7.
188. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. Circulation. 2001 Dec 18;104(25):3063-8.
189. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med. 2002 Dec 12;347(24):1916-23.
190. Booth N, Croll A, Bennet B. The activity of plasminogen inhibitor (PAI-1) of human platelets. Fibrinolysis. 1990;4:138-40.
191. Fay WP, Owen WG. Platelet plasminogen activator inhibitor: purification and characterization of interaction with plasminogen activators and activated protein C. Biochemistry. 1989 Jul 11;28(14):5773-8.
192. Erickson LA, Ginsberg MH, Loskutoff DJ. Detection and partial characterization of an inhibitor of plasminogen activator in human platelets. J Clin Invest. 1984 Oct;74(4):1465-72.
193. Sprengers ED, Akkerman JW, Jansen BG. Blood platelet plasminogen activator inhibitor: two different pools of endothelial cell type plasminogen activator inhibitor in human blood. Thromb Haemost. 1986 Jun 30;55(3):325-9.
194. Urden G, Chmielewska J, Carlsson T, Wiman B. Immunological relationship between plasminogen activator inhibitors from different sources. Thromb Haemost. 1987 Feb 3;57(1):29-34.
195. Lang IM, Marsh JJ, Moser KM, Schleef RR. Presence of active and latent type 1 plasminogen activator inhibitor associated with porcine platelets. Blood. 1992 Nov 1;80(9):2269-74.
196. Declerck PJ, Alessi MC, Verstreken M, Kruithof EK, Juhan-Vague I, Collen D. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood. 1988 Jan;71(1):220-5.
197. Braaten JV, Handt S, Jerome WG, Kirkpatrick J, Lewis JC, Hantgan RR. Regulation of fibrinolysis by platelet-released plasminogen activator inhibitor 1: light scattering and ultrastructural examination of lysis of a model platelet-fibrin thrombus. Blood. 1993 Mar 1;81(5):1290-9.
198. Torr-Brown SR, Sobel BE. Attenuation of thrombolysis by release of plasminogen activator inhibitor type-1 from platelets. Thromb Res. 1993 Dec 1;72(5):413-21.
199. Biemond BJ, Levi M, Coronel R,

Janse MJ, ten Cate JW, Pannekoek H. Thrombolysis and reocclusion in experimental jugular vein and coronary artery thrombosis. Effects of a plasminogen activator inhibitor type 1-neutralizing monoclonal antibody. Circulation. 1995 Feb 15;91(4):1175-81.
200. Brogren H, Karlsson L, Andersson M, Wang L, Erlinge D, Jern S. Platelets synthesize large amounts of active plasminogen activator inhibitor 1. Blood. 2004 Dec 15;104(13):3943-8.
201. Weyrich AS, Dixon DA, Pabla R, Elstad MR, McIntyre TM, Prescott SM, et al. Signal-dependent translation of a regulatory protein, Bcl-3, in activated human platelets. Proc Natl Acad Sci U S A. 1998 May 12;95(10):5556-61.
202. Lang IM, Schleef RR. Calcium-dependent stabilization of type I plasminogen activator inhibitor within platelet alpha-granules. J Biol Chem. 1996 Feb 2;271(5):2754-61.
203. Andreotti F, Davies GJ, Hackett DR, Khan MI, De Bart AC, Aber VR, et al. Major circadian fluctuations in fibrinolytic factors and possible relevance to time of onset of myocardial infarction, sudden cardiac death and stroke. Am J Cardiol. 1988 Sep 15;62(9):635-7.
204. Hoekstra T, Geleijnse JM, Schouten EG, Kluft C. Diurnal variation in PAI-1 activity predominantly confined to the 4G-allele of the PAI-1 gene. Thromb Haemost. 2002 Nov;88(5):794-8.
205. van der Bom JG, Bots ML, Haverkate F, Kluft C, Grobbee DE. The 4G5G polymorphism in the gene for PAI-1 and the circadian oscillation of plasma PAI-1. Blood. 2003 Mar 1;101(5):1841-4.
206. Maemura K, de la Monte SM, Chin MT, Layne MD, Hsieh CM, Yet SF, et al. CLIF, a novel cycle-like factor, regulates the circadian oscillation of plasminogen activator inhibitor-1 gene expression. J Biol Chem. 2000 Nov 24;275(47):36847-51.
207. Booth NA, Walker E, Maughan R, Bennett B. Plasminogen activator in normal subjects after exercise and venous occlusion: t-PA circulates as complexes with C1-inhibitor and PAI-1. Blood. 1987 Jun;69(6):1600-4.
208. Huisman LG, van Griensven JM, Kluft C. On the role of C1-inhibitor as inhibitor of tissue-type plasminogen activator in human plasma. Thromb Haemost. 1995 Mar;73(3):466-71.
209. Korninger C, Collen D. Neutralization of human extrinsic (tissue-type) plasminogen activator in human plasma: no evidence for a specific inhibitor. Thromb Haemost. 1981 Oct;46(3):662-5.
210. Held C, Hjemdahl P, Rehnqvist N, al e. Fibrinolytic variables and cardiovascular prognosis in patients with stable angina pectoris treated with Verapamil or Metoprolol. Circulation. 1997;95:2380-.
211. Jansson J, Olofsson B, Nilsson T. Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease. A 7-year follow-up. Circulation. 1993 November 1, 1993;88(5):2030-4.
212. Cortellaro M, Cofrancesco E, Boschetti C, Mussoni L, Donati MB, Cardillo M, et al. Increased fibrin turnover and high PAI-1 activity as predictors of ischemic events in atherosclerotic patients. A case-control study. The PLAT Group. Arterioscler Thromb. 1993 Oct;13(10):1412-7.
213. Merlini PA, Bauer KA, Oltrona L, Ardissino D, Cattaneo M, Belli C, et al. Persistent activation of coagulation mechanism in unstable angina and myocardial infarction. Circulation. 1994 Jul;90(1):61-8.
214. Reganon E, Vila V, Martinez-Sales V, Vaya A, Aznar J. Inflammation, fibrinogen and thrombin generation in patients with previous myocardial infarction. Haematologica. 2002 Jul;87(7):740-5; discussion 5.
215. Jansson JH, Norberg B, Nilsson TK. Impact of acute phase on concentrations of tissue plasminogen activator and plasminogen activator inhibitor in plasma after deep-vein thrombosis or open-heart surgery. Clin Chem. 1989 Jul;35(7):1544-

50.
216. Neerstrand H, Østergaard P, Bergqvist D, Mätzsch T, Hedner U. tPA inhibitor, tPA:Ag, plasminogen, and a2-antiplasmin after low moleular weight heparin or standard heparin. Fibrinolysis. 1987:39-43.
217. Grimaudo V, Hauert J, Bachmann F, Kruithof EK. Diurnal variation of the fibrinolytic system. Thromb Haemost. 1988 Jun 16;59(3):495-9.
218. Norhammar A, Tenerz A, Nilsson G, Hamsten A, Efendic S, Ryden L, et al. Glucose metabolism in patients with acute myocardial infarction and no previous diagnosis of diabetes mellitus: a prospective study. Lancet. 2002 Jun 22;359(9324):2140-4.
219. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. Obes Rev. 2002 May;3(2):85-101.
220. Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost. 2003 Jul;1(7):1575-9.
221. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol. 1999 Apr;19(4):972-8.
222. Smith A, Patterson C, Yarnell J, Rumley A, Ben-Shlomo Y, Lowe G. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly Study. Circulation. 2005 Nov 15;112(20):3080-7.
223. Nicholls SC, Chandler WL, Hoffer EK. Thrombolysis failure: a role for plasminogen activator inhibitor type 1 (PAI-1). Br J Haematol. 2001 May;113(2):559-60.
224. Booth NA, Robbie LA, Croll AM, Bennett B. Lysis of platelet-rich thrombi: the role of PAI-1. Ann N Y Acad Sci. 1992 Dec 4;667:70-80.
225. Rao SV, Donahue M, Pi-Sunyer FX, Fuster V. Results of Expert Meetings: Obesity and Cardiovascular Disease. Obesity as a risk factor in coronary artery disease. Am Heart J. 2001 Dec;142(6):1102-7.
226. Vega GL. Results of Expert Meetings: Obesity and Cardiovascular Disease. Obesity, the metabolic syndrome, and cardiovascular disease. Am Heart J. 2001 Dec;142(6):1108-16.
227. Van Gaal LF, Wauters MA, De Leeuw IH. The beneficial effects of modest weight loss on cardiovascular risk factors. Int J Obes Relat Metab Disord. 1997 Mar;21 Suppl 1:S5-9.
228. Lindahl B, Nilsson TK, Jansson JH, Asplund K, Hallmans G. Improved fibrinolysis by intense lifestyle intervention. A randomized trial in subjects with impaired glucose tolerance. J Intern Med. 1999 Jul;246(1):105-12.
229. Folsom AR, Qamhieh HT, Wing RR, Jeffery RW, Stinson VL, Kuller LH, et al. Impact of weight loss on plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. Arterioscler Thromb. 1993 Feb;13(2):162-9.
230. Calles-Escandon J, Ballor D, Harvey-Berino J, Ades P, Tracy R, Sobel B. Amelioration of the inhibition of fibrinolysis in elderly, obese subjects by moderate energy intake restriction. Am J Clin Nutr. 1996 Jul;64(1):7-11.
231. Mavri A, Stegnar M, Krebs M, Sentocnik JT, Geiger M, Binder BR. Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. Arterioscler Thromb Vasc Biol. 1999 Jun;19(6):1582-7.
232. Charles MA, Morange P, Eschwege E, Andre P, Vague P, Juhan-Vague I. Effect of weight change and metformin on fibrinolysis and the von Willebrand factor in obese nondiabetic subjects: the BIGPRO1 Study. Biguanides and the Prevention of the Risk of Obesity. Diabetes Care. 1998 Nov;21(11):1967-72.
233. Hoffstedt J, Andersson IL, Persson L, Isaksson B, Arner P. The common -675 4G/5G polymorphism in the plasminogen

activator inhibitor -1 gene is strongly associated with obesity. Diabetologia. 2002 Apr;45(4):584-7.
234. Schafer K, Fujisawa K, Konstantinides S, Loskutoff DJ. Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. Faseb J. 2001 Aug;15(10):1840-2.
235. Berry CN, Lunven C, Lechaire I, Girardot C, O'Connor SE. Antithrombotic activity of a monoclonal antibody inducing the substrate form of plasminogen activator inhibitor type 1 in rat models of venous and arterial thrombosis. Br J Pharmacol. 1998 Sep;125(1):29-34.
236. Xue Y, Bjorquist P, Inghardt T, Linschoten M, Musil D, Sjolin L, et al. Interfering with the inhibitory mechanism of serpins: crystal structure of a complex formed between cleaved plasminogen activator inhibitor type 1 and a reactive-centre loop peptide. Structure. 1998 May 15;6(5):627-36.
237. Gils A, Stassen JM, Nar H, Kley JT, Wienen W, Ries UJ, et al. Characterization and comparative evaluation of a novel PAI-1 inhibitor. Thromb Haemost. 2002 Jul;88(1):137-43.
238. Vaughan DE, Rouleau J-L, Ridker PM, Arnold JMO, Menapace FJ, Pfeffer MA. Effects of Ramipril on Plasma Fibrinolytic Balance in Patients With Acute Anterior Myocardial Infarction. Circulation. 1997 July 15, 1997;96(2):442-7.
239. Yusuf S, Sleight P, Pogue J, al: e. Effects of an angiotensin converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. N Engl J Med. 2000;342:145-53.