Review Article

Plasminogen activator inhibitor-type 1: its plasma determinants and relation with cardiovascular risk

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Summary

The habitual level of PAI-1 is influenced by many factors, of which obesity and insulin resistance are the most important. It is possible to reduce plasma PAI-1 by changes in life style, e.g. weight reduction and physical activity. Data on potential interactions between environmental and metabolic variables on one hand, and the 4G/5G-polymorphism on the other hand, are still scarce. It becomes more and more clear that PAI-1 may possibly not be a major (causal) factor in cardiovascular disease, but its role in inflammation deserves further attention. In the presence of the 4G-allele not only the PAI-1 response was more pronounced, but also the response of other acute-phase reactants, which implies that the increases of these reactants are secondary to the increase in PAI-1. A myocardial infarction also provokes an acute phase response. It can thus be hypothesized that the 4G-allele might exacerbate tissue injury during the acute phase after a myocardial infarction, and thereby negatively affect the prognosis.

Keywords

PAI-1, determinants, review

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Introduction

Plasminogen activator inhibitor-type 1 (PAI-1) is the main, fast-acting inhibitor of fibrinolysis activation, which plays an important role in vascular disease prevention by removing thrombi from the vascular system. Plasmin, the active component of fibrinolysis, breaks down fibrin into its degradation products. Plasminogen and tissue-type plasminogen activator (t-PA) bind to the surface of fibrin where t-PA cleaves inactive plasminogen into active plasmin. T-PA is inhibited by PAI-1, which inactivates t-PA by forming an irreversible 1:1 complex.

Elevated PAI-1 is considered a potential risk factor for cardiovascular disease because of its role in fibrinolysis. Apart from the well-documented function in fibrinolysis, PAI-1 is also involved in other processes, like cell migration and tissue remodeling. Importantly, PAI-1 is a strong acute-phase reactant, reflected by the possibility of the fast and large increase in plasma levels (up to > 100 fold) during the acute-phase response (1).

In this review, we will first give a broader introduction on PAI-1 and the determinants of its concentration in plasma (e.g. genetic, metabolic and lifestyle factors), followed by an overview of the epidemiological evidence for the association between PAI-1 and cardiovascular disease.

PAI-1: characteristics and regulation

PAI-1 is present in three forms in plasma: an active, an inactive and a latent form. The active form spontaneously converts into the latent form with a half-life of about one hour (2). The latent form is more stable, but can also be reconverted in the active form. Active PAI-1 in plasma can form a complex with vitronectin, resulting in a two- to fourfold increased half-life of

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PAI-1 in the circulation (3). PAI-1 in plasma can be measured either as PAI-1 activity or as PAI-1 antigen. PAI-1 antigen comprises all PAI-1 (active and inactive or latent, free or in complex with vitronectin and/or t-PA), while PAI-1 activity is a measure of the amount of t-PA that can be inhibited. Methods to measure PAI-1 antigen can vary largely and PAI-1 antigen should, therefore, be interpreted with caution. The functional measurement of PAI-1 activity should, therefore, be preferred. However, in most situations the two measures are highly intercorrelated and both are frequently used. From now on, we will refer to plasma PAI-1 levels to indicate either activity or antigen, unless specified otherwise. Plasma PAI-1 undergoes a circadian pattern with peak levels observed in the early morning (4, 5).

A second pool of PAI-1 is found in the α-granules of platelets, where PAI-1 is stored in mainly (~90%) the latent, free form (6, 7). Platelet PAI-1 is released after vessel trauma, and substantial amounts of active PAI-1 can accumulate from activated platelets at sites of arterial platelet-rich thrombi, protecting a thrombus clot from premature lysis (6, 8).

Regulation of plasma PAI-1 is a complex process, mainly occurring at the level of transcription of the PAI-1 gene. PAI-1 is produced by different cell types, including endothelial cells, hepatocytes, and adipocytes (9), and multiple regulatory factors have been identified that play a role in PAI-1 transcription, e.g. growth factors (TGF-β), inflammatory cytokines (IL-1, TNF-α), hormones, glucose, insulin, and glucocorticoids (10). PAI-1 is removed from the circulation mainly by the liver (11). Activity of PAI-1 is additionally regulated by the spontaneous inactivation of PAI-1 with a half-life of 1-2 hours (12).

Determinants of plasma PAI-1

Many cardiovascular risk factors are known to influence plasma PAI-1, and may thus confound the association between plasma PAI-1 and cardiovascular risk in epidemiological studies. Therefore, a good understanding of the factors that influence plasma PAI-1 is needed when studying the role of PAI-1 as a causal risk factor of cardiovascular risk. We will now provide an epidemiological overview of the most important determinants of plasma PAI-1 levels, e.g. genetic, metabolic and lifestyle factors. The main effects are summarized in Table 1.

Genetic determinants of plasma PAI-1

Estimates on heritability of plasma PAI-1 levels range from 26% to 71% (13-17). So far, nine different polymorphisms have been detected in the PAI-1 gene, which have been reviewed by Nordt and colleagues (18). Three of them are located in the promoter region, one in intron 4, one in exon 8 and four in the 3'-untranslated region of the PAI-1 gene. The most extensively studied polymorphism in relation to PAI-1 levels is the 4G/5G-polymorphism in the promoter region of the gene.

4G/5G-polymorphism

The 4G/5G polymorphism is a common single base pair insertion/deletion polymorphism in the promoter region of the PAI-1 gene that affects gene transcription (19). The 4G-allele has a sequence of four guanosines. The 5G-allele has a fifth guanosine inserted, which creates an additional binding site for an inhibitor, resulting in an attenuated response to transcription factors (19, 20). The 4G/4G-genotype has been associated with higher PAI-1 levels compared to the 5G/5G-genotype, with the heterozygous genotype having intermediate levels (21-23). The genotype-related differences in PAI-1 concentrations, however, were not present in all studies (14, 24) and in studies in which it was, only a small part of the variance in PAI-1 was explained by the 4G/5G-polymorphism (16, 21). More recently, evidence is accumulating that, although this polymorphism may not strongly affect basal PAI-1 levels, it may affect PAI-1 responses. *In vitro* studies suggest, for example, strongest PAI-1 increases in response to interleukin-1 (19, 25), and VLDL (26) for the 4G-allele. Further evidence supporting this hypothesis comes from the observation of a much stronger increase in PAI-1 in the acute-phase after acute-trauma for patients with the 4G-allele than for those with the 5G-allele (27). The nature of the polymorphism can thus be described as response polymorphism, which implies that the difference in PAI-1 levels between 4G and 5G becomes more obvious in the presence of environmental and/or disease factors, which stimulate PAI-1 expression. Therefore, for the other determinants of plasma PAI-1, we will also describe the evidence for interactions with the 4G/5G-polymorphism.

Other polymorphisms in the PAI-1 gene

In vitro studies in human endothelial cells showed that the HindIII-polymorphism in the 3'-untranslated region of the PAI-1 gene affects PAI-1 transcription in response to insulin and lipoproteins (28, 29). The 1/1-genotype showed the strongest response in PAI-1 after stimulation with insulin, and the 2/2 genotype after triggering with both VLDL or Lp(a) (29, 30). Linkage disequilibrium, with small variations in the promoter region of the gene, could possibly explain these results. No strong linkage disequilibrium exists between the HindIII-polymorphism and the 4G/5G-polymorphism (18).

The inability to account for all observed heritability of plasma PAI-1 by polymorphisms in the PAI-1 gene may be due to an undiscovered genetic variation in promoter or distant enhancers. Alternatively, it can be hypothesized that the heritability may originate in heritability of determinants of PAI-1 levels such as lipids, insulin resistance and chronic inflammation.

Metabolic determinants

The metabolic syndrome comprises a cluster of cardiovascular risk factors, including (abdominal) obesity, glucose intoler-

Table 1: Overview of the main determinants of plasma PAI-1 levels.

Determinant	Effect	Interaction with 4G/5G	Mechanism of action
4G/5G-polymorphism	In general slightly higher levels for the 4G-allele, the effects are however small[16,21]	N.A.	Attenuated response for the 5G-allele to transcription factors due to an extra guanosine[19,20]
Obesity	(Central) obesity increases PAI-1[35]	No consistent evidence	- PAI-1 production and secretion by adipocytes[36-38] - increased hepatic PAI-1 production by adipocyte-derived cytokines[39]
Blood lipids	Less favourable lipid profile is associated with increased PAI-1 levels[51]	Interaction with triglyceride levels[48,60]	VLDL: effect on PAI-1 transcription[26] and possibly on stability of PAI-1 mRNA[53] LDL: mechanism not clear; effect seems independent of LDL-receptor[54,55]
Insulin resistance	Positive association[61]	No evidence	(Pro)-insulin stimulates PAI-1 transcription[64,65] and possibly an effect of glucose[69,70]
Renin-Angiotensin system	Association with ACE-levels and with a polymorphism in the ACE-gene[76,77]	Only limited data[79]	Angiotensin II stimulates PAI-1 production[73-75]
Sex hormones	Estrogen decreases PAI-1 levels, no clear effect of progestogen[98]	Inconsistent results, only limited data[91-93,99]	Estrogen may directly decrease PAI-1 biosynthesis or may increase the clearance rate[90]. Alternatively, the effects may be through effects on body composition and insulin resistance[82]
N3-fatty acids	Small positive effect on PAI-1, dependent on type of fatty acids[104]	No data	Not fully understood
Alcohol	Increases PAI-1[108-113]	No data	Unknown
Physical activity	Physical activity results in lower PAI-1 levels (both short-term[129-131] and long-term effects[133-135])	No data for acute effects and only limited data for long-term effects which does not provide strong evidence for an interaction[142]	Short-term: probably due to increased clearance Long-term: probably mediated by effects on body weight and blood lipids
Circadian pattern	Strong effect, peak levels in the early morning[4,5,143-145]	More pronounced effect for the 4G/4G-genotype[146,147]	Mediated by CLIF, the binding site of this transcription factor overlaps with the 4G/5G-polymorphism[148]
Acute-phase response	Strong positive effect on plasma PAI-1[10]	Stronger response for the 4G-allele[19,27]	Proinflammatory cytokines (e.g. IL-1, TNF-α and TGF-β) stimulate PAI-1 transcription[10]

ance/type II diabetes mellitus, dyslipidaemia and hypertension. No uniform definition of the metabolic syndrome is used, and it has been argued that PAI-1 should be considered part of it (31). PAI-1 is associated with many of the components of the metabolic syndrome, as will be discussed below in greater detail.

Obesity

Obesity, especially central fat, is associated with increased PAI-1 levels (32), and weight reduction has been shown to be effective in lowering PAI-1 (33-34). The relationship between PAI-1 and obesity has recently been reviewed by Mutch et al. (35). PAI-1 is synthesized and secreted directly by adipose tissue (36-38), but adipose tissue might further increase plasma PAI-1 by increased hepatic PAI-1 production in response to adipocyte-derived cytokines (TNF-α en TGF-β) (39).

In vitro studies showed higher PAI-1 production for human visceral fat than for subcutaneous fat (38, 40). Recent data suggest that stromal cells, and thus not the adipocytes itself, are the most important source of PAI-1 within adipose tissue (40). Visceral fat contains a higher amount of stromal cells than subcutaneous fat, which might explain the regional differences in PAI-1 production (40). However, not all studies show a higher

PAI-1 expression in visceral than in subcutaneous fat (41), or even show opposite results (42). The authors of the latter study (42) suggested that their unexpected finding might be explained by the adipose tissue in their study being derived from more obese subjects than in the other studies. They hypothesize that obesity *per se* may influence regional differences in PAI-1 secretion.

In addition, adipocytes from obese subjects produced more PAI-1 than adipocytes from lean subjects (43, 44), even after adjusting for adipocyte size (44). In contrast with these findings is the observation that PAI-1 expression in human subcutaneous adipose tissue increased after weight reduction (45). The adipose secretion rates of PAI-1 in human abdominal subcutaneous adipose tissue did not differ across the 4G/5G-genotypes (46).

Studies of obesity and PAI-1 in strata of the 4G/5G-polymorphism do not provide consistent evidence for genotype-specific associations. One study showed a clear association between PAI-1 levels and the 4G/5G-polymorphism in obese, but not in lean subjects (32). In Pima Indians, body mass index (BMI) and PAI-1 were associated in both the 4G/4G and 5G/5G-genotypes, but not in the 4G/5G-genotype (47). In patients with angiographically determined coronary disease the association between PAI-1 and BMI was strongest for the 5G/5G genotype (48).

Based on a study in PAI-1 knockout mice, the hypothesis was raised that PAI-1 might also promote the evolution of obesity (49). A study in 505 humans showed that the prevalence of obesity was twofold higher in carriers of the 4G-allele than of the 5G-allele (50), which is in agreement with this hypothesis. The mechanism by which PAI-1 might promote the development of obesity is not yet clear, but might involve effects of PAI-1 on cell migration and angiogenesis (50).

In summary, it is evident that (central) obesity is an important determinant of plasma PAI-1 levels. The association differs across the genotypes of the 4G/5G-polymorphism, but this should be further explored. The hypothesis, that PAI-1 might influence the evolution of obesity has only been marginally investigated, and deserves further research.

Blood lipids

PAI-1 is associated with cholesterol, LDL-cholesterol, VLDL and triglyceride levels, and negatively with HDL-cholesterol (51). *In vitro*, VLDL has consistently been shown to induce a concentration-dependent increase in PAI-1 expression in endothelial (52), and hepatic cells (53). It has been shown that VLDL stimulation of PAI-1 expression in endothelial cells is mediated through transcriptional activation of the PAI-1 gene, and a VLDL-response element has been identified in the promoter region of the PAI-1 gene (26). Besides effects of VLDL on PAI-1 gene transcription, VLDL might also affect the stability of the PAI-1 mRNA transcripts (53). The effects of LDL-cholesterol are less consistent. Generally, native-LDL does not induce PAI-1 synthesis *in vitro*, unless high concentrations are used (52, 54) or when LDL is oxidized (55), or glycated (56). LDL effects do not appear to be dependent on interactions with the LDL-receptor (54, 55).

Chronic and acute hypertriglyceridemia have been associated with changes in plasma PAI-1 (57). In a recent study in 10 healthy males a rise in plasma PAI-1 was observed after a triglyceride infusion (58). However, intravenous administration of a fat emulsion did not affect PAI-1 in another study in healthy males (59). The association between triglyceride levels and PAI-1 was genotype specific, with a steeper slope in subjects with the 4G/4G-genotype in patients with coronary artery disease (48), and in type 2 diabetics (60).

In summary, blood lipids are consistently associated with plasma PAI-1, but the exact mechanisms should be further unraveled, and also interactions with the 4G/5G-polymorphism should be further explored.

Insulin resistance and diabetes

Bastard and colleagues reviewed the role of plasma PAI-1 in insulin resistance and concluded that high plasma PAI-1 levels are undoubtedly related to insulin resistance, and that the mechanisms appear to be multi-factorial and remain to be elucidated (61). Cross-sectional studies show positive associations between fasting insulin levels and PAI-1 both in subjects with normal (62, 63), and impaired glucose tolerance (62, 63) and also in type 2 diabetics (63). Additional evidence for an association between PAI-1 and insulin is provided by *in vitro* studies, demonstrating that both insulin and proinsulin stimulate transcription of the PAI-1 gene in several tissues (64, 65). However, acute intravenous administration of insulin in humans did not increase PAI-1 levels (66-68). An alternative explanation for increased levels of PAI-1 in the presence of diabetes could be that PAI-1 is elevated because of high serum glucose levels. Evidence for this hypothesis is provided by *in vitro* studies in which glucose stimulates PAI-1 expression in endothelial and vascular smooth muscle cells (69, 70). In a prospective study of 1,047 non-diabetic subjects, PAI-1 was an independent risk factor for the development of type 2 diabetes (71). However, in healthy type I diabetics, PAI-1 is lower than in normal individuals (72), arguing against glucose as a mediator.

Regulation of PAI-1 by the renin-angiotensin system

The renin-angiotensin system (RAS) plays a key role in the regulation of blood pressure. Renin converts angiotensinogen to angiotensin 1, which in turn is converted to the vasoconstrictor angiotensin 2 by angiotensin 1 converting enzyme (ACE). Inhibition of the RAS by ACE-inhibitors lowers blood pressure, a widely used therapy for hypertension. The fibrinolytic and the renin-angiotensin systems are linked by angiotensin II, that stimulates production of PAI-1 both *in vivo* (73) and *in vitro* (74,

75). Also, its metabolite angiotensin IV stimulated PAI-1 production in human adipocytes in an *in vitro* study (74). Vaughan has recently reviewed the link between the RAS and the fibrinolytic system (76).

ACE levels have been correlated to PAI-1 concentrations and a polymorphism in the ACE-gene has been shown to be predictive for PAI-1 levels (77). Furthermore, intervention studies with ACE-inhibitors have shown decreases in PAI-1 levels (78), but the effects appear to largely depend on the type of ACE-inhibitor. Further evidence for a link between RAS and fibrinolysis is provided by the observation that during a period of activation of RAS by salt depletion, the concentration of PAI-1 significantly increased, both in normotensive subjects (78) and in subjects with essential hypertension (79). The latter study furthermore showed that this effect was only present within subjects with the 4G/4G-genotype (79).

Hormonal influences

Menopausal status and hormone replacement therapy

In observational epidemiological studies it has consistently been observed that PAI-1 levels increase after menopause (80, 81). In the Framingham Offspring Study, 32% higher PAI-1 concentrations were observed in postmenopausal compared to premenopausal women (80).

Observational studies show 15-50% lower PAI-1 levels in postmenopausal women using hormone replacement therapy (HRT) compared to non-users (82, 83). In the Cardiovascular Health Study (82), HRT-users were thinner and had less abdominal fat than non-users, which largely accounted for the observed difference in PAI-1 between users and non-users. However, estrogen dose was inversely associated with PAI-1 antigen, but not with BMI in this study, which is favors a true effect of HRT on PAI-1.

Several randomized controlled trials with HRT have been conducted, which consistently show a decline in PAI-1 (84-86). In the large randomized HOPE-trial (87) a dose response association was observed for conjugated equine estrogen (CEE), which was weakened by progestogen. In general, smaller effects on PAI-1 are observed for transdermal HRT, possibly because this does not first pass the liver (88, 89).

Estrogen may directly decrease PAI biosynthesis and secretion, or may increase the clearance rate (90). The effect of sex hormones could furthermore be through effects on body composition and insulin resistance. Given the strong effects of sex hormones on PAI-1, these latter mechanisms may only explain a small part of the observed effects.

Three studies (91-93) investigated the effects of HRT stratified by the 4G/5G-polymorphism. A trial with transdermal HRT in 38 postmenopausal women with coronary artery disease showed the strongest decrease in PAI-1 for the 4G/4G-genotype, while PAI-1 in the 5G/5G-genotype remained unchanged (91). However, two other intervention studies (92, 93) did not observe different effects of estrogen administration across the variants of the 4G/5G-polymorphism.

In summary, postmenopausal status and HRT are consistent determinants of plasma PAI-1, but data on interactions with the 4G/5G-polymorphism is limited and inconsistent.

Oral contraceptives

The use of oral contraceptives (OC) has consistently been associated with lower PAI-1 levels in cross-sectional studies (94, 95). Experimental data show that OC use, both second and third generation, leads to a substantial decrease in PAI-1 (96, 97). Estrogen is generally considered the compound responsible for this decrease, but also administration of progesteron-only pills lowered PAI-1, although not significantly (98). The decrease in PAI-1 is already achieved after a short period of OC use, and after cessation PAI-1 returns to baseline levels within 8 days (97). No different effect of OC use on plasma PAI-1 levels was present for the different genotypes of the 4G/5G-polymorphism in an intervention study among 95 women (99). As far as we know, no other studies have been reported on the effect of OC use separately for the 4G/5G-polymorphism.

Dietary factors and plasma PAI-1

The relations with plasma PAI-1 have been examined for several dietary factors. Nutrients that have been most intensively studied are n-3 fatty acids and alcohol. Limited data is also available on other dietary factors.

N-3 fatty acids

Several interventions with polyunsaturated n-3 fatty acids have been performed, with conflicting results. In most studies, supplementation with n-3 fatty acids was associated with an increase in PAI-1 (100, 101). However, in other studies supplementation lowered or did not change PAI-1 (102, 103). Most studies were rather small, and differences in (control) supplements and designs of the studies make comparisons difficult. Hansen and colleagues estimated, based on a total of 17 trials, that a 17.7% increase in PAI-1 could be attributed to n-3 fatty acid supplementation (104). Overall, it can be concluded that n-3 fatty acids may lead to an increase in PAI-1 levels, but that these effects are only modest and depend on the type of fat. The mechanisms are still not fully understood.

Alcohol

In cross-sectional studies, alcohol consumers consistently have higher levels of PAI-1 than alcohol-abstainers (77, 105-107). In the NHLBI Family Heart Study, a large population-based study, PAI-1 was increased only in subjects consuming more than 15 grams of alcohol per day (105). The association between alcohol consumption and PAI-1 is dose-dependent (J-shaped) (106, 107).

Several small intervention studies in male volunteers demonstrated an acute and strong increase in PAI-1 after alcohol consumption (108, 109). Intake of 40 grams of alcohol resulted in PAI-1 activity levels that were 12 times higher 5 hours after intake. This rise was independent of the type of beverage consumed (beer, wine or spirits), suggesting that ethanol is the active compound (108). In women, only one intervention study on the short-term effects of alcohol was performed (110), showing a sharp increase in PAI-1 after consumption of wine at dinner in post-menopausal, but not in premenopausal women (110).

Apart from the acute, transient effects of alcohol on PAI-1 levels, also the long-term effects have been studied. Most long-term interventions show higher PAI-1 levels after a period of alcohol intake (111-113). However, one intervention in which subjects were given about 20 grams of alcohol per day for a period of 30 days failed to increase PAI-1 (114). The specific mechanisms, by which alcohol increases PAI-1 remain uncertain, but cannot be explained by a direct effect of alcohol on PAI-1 gene transcription. On the contrary, *in vitro* studies demonstrated down-regulation of PAI-1 gene transcription in cultured human endothelial cells by ethanol (115, 116).

Other dietary factors

Antioxidants may attenuate the response to infection, and since PAI-1 is an acute phase reactant, antioxidants might decrease PAI-1 levels. The association between vitamin C and PAI-1 has only been studied in one cross-sectional study, in which an inverse association between serum ascorbate and PAI-1 was observed (117). An experiment in obese men with administration of antioxidant vitamins did not change PAI-1 levels (118). Administration of α-tocopherol led to a decrease in PAI-1 in type 2 diabetics (119).

An experimental study in a porcine model of hypercholesterolemia demonstrated that vitamin C and vitamin E reduced local and systemic PAI-1 (120). Because the association between antioxidants and PAI-1 has only rarely been studied, no firm conclusions are allowed. Present data, however, suggest that a PAI-1 lowering effect of antioxidants is possible.

Smoking

Data on the relationship between smoking and PAI-1 is scarce. Several cross-sectional studies showed higher PAI-1 levels for smokers than for former and non-smokers, who never smoked (121, 122). However, in a study of monozygotic twins discordant for smoking no significant difference was observed and associations with cigarette dose were absent (123). In the Caerphilly Study, PAI-1 levels increased gradually with the amount of tobacco, but PAI-1 levels were significantly increased only for the heaviest smokers (106). In the Northern Sweden MONICA study no association was observed between smoking status and PAI-1 activity (124). Apparently, smoking is not a major determinant of PAI-1. Triglycerides and insulin resistance may mediate the small effects observed in some of the studies. In an experimental study, transdermal nicotine administration did not affect plasma PAI-1 (125).

Physical activity

Cross-sectional studies suggest that individuals who regularly exercise have reduced PAI-1 levels, compared to sedentary subjects (126-128). In the Northern Sweden MONICA study a strong and dose-dependent association between PAI-1, and regular leisure time physical activity was observed both in men and women (128). Both intervention studies on the short- and long-term effects of physical activity on PAI-1 have been performed, as outlined below.

Short-term effects of exercise

An exercise test generally results in a fall in PAI-1 levels, both in healthy populations and in populations with a history of cardiovascular disease (129-131). Physical activity has been shown to increase the release of tissue-type plasminogen activator (t-PA) from the vascular endothelium (132). As a result of this increase in t-PA-antigen, PAI-1 activity is expected to decrease, because of the formation of complexes with t-PA. PAI-1 activity appears to respond faster to exercise than PAI-1 antigen (130), as expected from very rapid interaction between t-PA and PAI-1. Exercise-related changes in liver blood flow contribute to the rapid clearance of components such as t-PA-PAI-1 and possibly PAI-1, and constitute another influence on blood levels in the acute period. No studies were done on the 4G/5G-genotype specific short-term effects of exercise.

Physical training programs

Physical training programs generally result in decreases in PAI-1 (133-135). It is difficult to give an overall estimate of the strength of the effect on PAI-1, because of the large differences in populations (age, baseline condition) and exercise programs (intensity and duration), but the effects are notable. In men, a reduction of 80% was observed after a 9-month training program, and in women a reduction of 73% was observed (136). A study comparing training effects in younger and older subjects showed a stronger decline in older subjects (137). In contrast, a 6-month intensive training program in elderly only led to a moderate decrease in PAI-1 antigen (138). In the study of De Geus et al (139), the magnitude of effect clearly depended on baseline PAI-1 activity. It is expected that an effect of physical activity only lead to a fall in PAI-1 in subjects with a high body weight or insulin resistance, probably by an improvement of insulin resistance.

In contrary to other studies, the Oslo Diet and Exercise Study did not show any decrease in PAI-1 values after a one-year exercise program focused on endurance training (140). El-Sayed and colleagues (141) compared the effect of 12 weeks of

low intensity exercise with high intensity exercise and found a significant difference in PAI-1 activity, only in the high intensity group. The effects of training programs on PAI-1 are likely to be mediated by changes in body weight and blood lipids.

We are aware of only one study that investigated the effects of physical training on PAI-1 levels stratified by the 4G/5G-polymorphism (142). After three years of regular exercise, the largest decrease in PAI-1 was observed for the 4G/4G-genotype (-36% versus -5% for the other genotypes) (142). Baseline levels were (non-significantly) increased in the 4G/4G-genotype, which might partly explain the genotype-specific association.

Circadian pattern of plasma PAI-1

PAI-1 undergoes a circadian pattern with peak plasma levels observed in the early morning (4, 5, 143-145). In the Rotterdam Study, among a sub sample of 263 men and women aged 55 years and over, the morning/afternoon difference in PAI-1 antigen was more pronounced in persons with the 4G/4G-genotype than in the other genotypes (146). We also observed this in a population of 599 elderly patients (147). A biological explanation for a genotype-specific diurnal variation in PAI-1 is provided by Maemura and colleagues, who identified a transcription factor (CLIF: cycle-like factor), that is involved in the circadian pattern of PAI-1 (148). The binding site of this transcription factor overlaps with the location of the 4G/5G-polymorphism, making an interaction between the 4G/5G-polymorphism and the diurnal pattern biological plausible.

PAI-1 and the acute-phase response

Evidence is accumulating that PAI-1 plays a crucial role in the acute-phase response. PAI-1 is an acute-phase protein, which means that PAI-1 levels strongly increase in response to inflammation or injury. Inflammatory cytokines, e.g. interleukin-1, TNF-α and TGF-β, have been shown to stimulate PAI-1 production (10). In transfected HepG2 cell lines it was demonstrated that the 4G-allele produced six times more mRNA than the 5G-allele in response to interleukin-1 (19). The acute-phase response of PAI-1 may thus be affected by the 4G/5G-polymorphism. Furthermore, in severe trauma patients the 4G/4G-genotype showed the strongest response not only in PAI-1 but also in TNF-α and interleukin-1. In particular, the difference in response in interleukin-1 was remarkable, with the largest difference across the genotypes six days after severe trauma (27). Apparently, increased PAI-1 is not only a response to proinflammatory cytokines, but PAI-1 in turn also stimulates the synthesis of cytokines itself, suggesting a central role of PAI-1 in the acute-phase response. The stronger acute-phase response for the 4G/4G-genotype may have detrimental effects on prognosis in acute conditions. Recent studies indeed provide evidence for genetic aspects of this hypothesis. The 4G-allele has been associated with the development of septic shock after meningococcal infection (149), a poor survival rate after severe trauma (27), and an increased mortality rate after aneurysm repair (150). However, the 4G/5G-polymorphism was not predictive for outcome after an acute myocardial infarction (151).

Plasma PAI-1, the 4G/5G-polymorphism and cardiovascular disease

Both PAI-1 and the 4G/5G-polymorphism have been studied extensively in relation to cardiovascular risk. First, the evidence for an association with the risk of coronary heart disease will be described followed by an overview of the studies on the risk of stroke.

PAI-1, 4G/5G-polymorphism and coronary heart disease

In most population-based prospective studies, PAI-1 was not significantly associated with the occurrence of a first event of coronary heart disease (152, 153). In the few studies in healthy populations, in which plasma PAI-1 was associated with risk of coronary events, the strength of the association was strongly reduced after adjusting for cardiovascular risk factors (154, 155). The differences reported on the prognostic value of PAI-1 for coronary risk in epidemiological studies have been suggested to be at least in part attributed to the confounding variables controlled for (51).

Case-control and cross-sectional studies showed increased plasma PAI-1 levels in patients with existing coronary heart disease (156, 157). Also, PAI-1 levels predicted future coronary events in populations with angina pectoris (158) or a history of a myocardial infarction (159, 160).

An association between the 4G/5G-polymorphism and cardiovascular disease would contribute to evidence for the hypothesis, that PAI-1 is a causal risk factor for cardiovascular disease. In a meta-analysis of 9 mainly case-control studies a modestly increased risk of myocardial infarction was observed for the 4G/4G-genotype (161) (OR = 1.20, 95% CI: 1.04-1.39). In a Japanese study, in which 112 polymorphisms were examined simultaneously, it turned out that the 4G/5G-polymorphism was one of the two polymorphisms, that were associated with myocardial infarction in women (162). However, in a recent large case-control study the 4G/5G-polymorphism was not associated with risk of an acute myocardial infarction at young age (163). Iwai and colleagues observed that the 4G/5G-polymorphism was associated with a faster progression to acute coronary syndromes after first anginal pain (164). In postmenopausal women (165) and in the elderly (166), the 4G/5G-polymorphism was not predictive for fatal myocardial infarction. In the Physicians' Health Study, the 4G/5G-polymorphism was not predictive for future myocardial infarction in men initially free of cardiovascular disease (167).

PAI-1, 4G/5G-polymorphism and stroke

Johansson and colleagues found that plasma PAI-1 was not significantly associated with incidence of (first) stroke in a population-based cohort (nested case-control design) (168). In stroke patients, increased PAI-1 levels have been observed compared to healthy controls, both in the acute-phase, and even several months after the event (156, 169, 170).

Remarkably, most studies observed a protective effect for stroke for the 4G-allele (171, 172), although in only one study was the effect strong enough to reach statistical significance (165). In a Korean case-control study, the 4G-allele was associated with an increased risk of ischemic stroke (173). The genetic background of the study population may possibly explain this discrepant finding. A protective role of the 4G-allele in stroke, as opposed to an increase in risk of myocardial infarction, may indicate a difference in pathogenesis of these diseases.

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