PAI-1 and the Metabolic Syndrome

Links, Causes, and Consequences

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Abstract—The link between plasminogen activator inhibitor (PAI)-1 and the metabolic syndrome with obesity was established many years ago. Increased PAI-1 level can be now considered a true component of the syndrome. The metabolic syndrome is associated with an increased risk of developing cardiovascular disease, and PAI-1 overexpression may participate in this process. The mechanisms of PAI-1 overexpression during obesity are complex, and it is conceivable that several inducers are involved at the same time at several sites of synthesis. Interestingly, recent in vitro and in vivo studies showed that besides its role in atherothrombosis, PAI-1 is also implicated in adipose tissue development and in the control of insulin signaling in adipocytes. These findings suggest PAI-1 inhibitors serve in the control of atherothrombosis and insulin resistance. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

Key Words: adipose tissue ■ atherothrombosis ■ metabolic syndrome ■ obesity ■ PAI-1

In blood, fibrinolysis breaks down fibrin and maintains vessel patency, and in tissues it breaks down the extracellular matrix and controls cell adhesion and migration and thus participates in tissue remodeling. Fibrinolysis is primarily regulated by plasminogen activator inhibitor type-1 (PAI-1), which prevents the escape of this potentially destructive protease system.1-3

Increased PAI-1 levels may predispose patients to the formation of atherosclerotic plaques prone to rupture with a high lipid-to-vascular smooth muscle cells ratio as a result of decreased cell migration.4 In humans, there is clinical evidence that increased PAI-1 levels are associated with atherothrombosis.5,6 In large epidemiological studies, elevated plasma PAI-1 levels have been identified as a predictor of myocardial infarction.7-11 Remarkably, the predictive ability of PAI-1 disappears after adjustment for markers of the metabolic syndrome (MetS) such as body mass index (BMI), triglycerides, and high-density lipoprotein cholesterol,8,12-16 suggesting that the MetS is a prerequisite to high plasma PAI-1 levels in patients prone to atherothrombosis.

The purpose of this review is to update our understanding of the connections between PAI-1 and the MetS. We discuss the possible mechanisms linking increased circulating PAI-1 levels to the MetS and the recent findings implicating PAI-1 in adipose tissue development and insulin signaling, making PAI-1 more an actor rather than a simple marker of the MetS.

Contribution of MetS to PAI-1 Synthesis

Description of MetS

The MetS consists of a cluster of metabolic abnormalities that cooccur in an individual more often than by chance. These abnormalities include obesity with a distribution of the fat in the central part of the body (visceral or android obesity), impaired glucose tolerance, hyperinsulinemia, dyslipidemia with elevated triglyceride level, low high-density lipoprotein cholesterol concentration, increased proportion of small dense lipoparticles, and hypertension, all well-documented risk factors for cardiovascular disease.17 Individuals with the MetS are at increased risk for diabetes mellitus and cardiovascular disease.18,19 Some investigators called into question the existence of this syndrome in the sense of its having a single underlying cause20 and argued that the MetS does not add to global cardiovascular risk as assessed by current algorithms, which already include some of the MetS features. It is clear that the MetS has more than one cause.21 The extent to which the MetS adds to the global risk of cardiovascular disease has to be defined, given its growing prevalence worldwide. Ectopic fat depots such as the visceral one may play a central part in linking the MetS to cardiovascular disease. Ectopic fat could be secondary to a defect of peripheral fat cell proliferation, as observed in severe insulin resistance associated with hereditary lipodystrophy, or to the failure of fat cells to increase their size and therefore to accommodate an increased energy influx, leading to a reorientation of fat storage.22 The resulting ectopic fat depots may be a source of toxicity toward the surrounding tissues by releasing active substances such as free fatty acid23 and deleterious adipokines such as inflammatory cytokines and PAI-1.24
ing PAI-1 is increased in obese subjects with the MetS, as well as in patients with type II diabetes. The more severe the MetS, the higher the plasma level of PAI-1. The MetS explains a major part of plasma PAI-1 level variability, with this relationship being stronger in men than in women (45% versus 26%). Interventional studies reported that if insulin resistance is improved, plasma PAI-1 levels decrease. Decreased plasma PAI-1 concentrations were observed after weight reduction by a hypocaloric diet and were associated with decreased body fat. In addition, treatment with insulin-sensitizing drugs like metformin or troglitazone decrease plasma PAI-1 levels in subjects with type II diabetes and to some extent in normal obese subjects. It has been proposed to consider increased PAI-1 levels as a true component of the MetS.

Possible Mechanisms Linking PAI-1 Overexpression to MetS

Obviously, induction of PAI-1 overexpression is a complex process and several inducers may be involved at the same time at several sites of synthesis, including vessel walls. Factorial analysis showed that elevated circulating PAI-1 levels during obesity are not associated with the interleukin (IL)-6 driven inflammation (C-reactive protein [CRP], fibrinogen), as one would expect because PAI-1 is considered an acute phase protein whose synthesis is induced by IL6. PAI-1 levels are also not associated with dyslipidemia but rather with the fat redistribution phenotype assessed by the measure of waist circumference and the insulin resistance state. In this context, the role of ectopic fat depots as sites of PAI-1 synthesis may be relevant.

Adipose Tissue and Ectopic Fat Depots as Sites of PAI-1 Overexpression

Several groups have described the ability of adipocyte cell lines and murine adipose tissue to synthesize PAI-1. Subsequent reverse-transcription polymerase chain reaction (RT-PCR) and in situ hybridization studies suggested that the increased plasma PAI-1 originates primarily from the adipocyte in response to chronically elevated levels of tumor necrosis factor (TNF), insulin, and transforming growth factor (TGF)-beta. PAI-1 is also produced by human adipose tissue explants, but it is mainly localized in the stromal compartment of the adipose tissue. PAI-1 antigen was detected in purely stromal area and in small cells in direct contact with adipocytes of macrophage origin. During human preadipocyte differentiation, PAI-1 secretion appears to be produced by contaminant macrophages. These results suggest that macrophages infiltrating adipose tissues may be one of the main source of PAI-1 in patients with a MetS.

Several groups have stressed the exclusive association between high plasma PAI-1 levels and visceral obesity. For example, changes in plasma PAI-1 levels during a weight-reducing program correlated with changes in visceral fat depot but not in subcutaneous fat depot. In obese rats PAI-1 mRNA is found in both types of fat tissue but its level increased only in visceral fat during the development of obesity. In obese patients, abdominal visceral fat expressed 5-fold more PAI-1 than subcutaneous tissue.

Ectopic fat accumulation in human liver was also associated with a strong expression of PAI-1 close to fat cells. This could be related to the strong relationship between circulating PAI-1 antigen and hepatic but not peripheral insulin resistance in Pima Indians. All these findings suggest that circulating PAI-1 levels are not closely dependent on fat mass but rather that they reflect fat redistribution and may be considered as a biomarker of ectopic fat storage. However, several questions remain unanswered. Is there a direct ectopic fat mass effect, an indirect connection between ectopic fat and PAI-1 through a mediator, or a common ground with a parallel evolution of PAI-1 and ectopic fat without a real connection?

Arguments for the Contribution of TNF and TGF-beta to PAI-1 Overexpression

TNF is involved in insulin resistance. The group of Loskutoff was the first to emphasize the contribution of TNF in PAI-1 regulation during obesity. In ob/ob mice, deletion of both TNF receptors (TNF R1 and RII) significantly reduced the plasma PAI-1 levels as well as the adipose tissue PAI-1 mRNA levels. TNF-neutralizing antibodies decreased plasma PAI-1 levels, proving a direct link between TNF and PAI-1 during obesity. Moreover, the invalidation of both TNF receptors decreased TGF-beta expression in the adipose tissue and in humans, TNF receptors, TGF-beta, and PAI-1 levels were strongly correlated within adipose tissue. These results suggest that the TNF and TGF-beta pathways are connected within adipose tissue and may both control PAI-1 expression. The possible connection between insulin resistance, TGF-beta, and PAI-1 is further supported by the lowered expression of PAI-1 in FOXC2+/- mice in response to TGF-beta1 treatment because FOXC2 has been implicated in insulin resistance. Thiazolidinediones decrease plasma PAI-1 levels and inhibit PAI-1 synthesis through anti-TNF properties in the absence of inducible PPARgamma activation, indicating that the TNF pathway is a potential PAI-1 inducer during the MetS and underscores the ability of thiazolidinedione to exert anti-inflammatory properties independent of PPARgamma activation in several cell types.

In summary, the parallelism between circulating PAI-1 levels and the features of the MetS may be the reflection of an active TGF-beta pathway that modulates both insulin resistance and PAI-1 synthesis.

Possible Contribution of Local Cortisol Production

Clinical observations have highlighted the link between glucocorticoids and visceral obesity. Dexamethasone and cortisol are potent inducers of PAI-1 synthesis by cultured adipocytes and human adipose tissue, making cortisol a possible inducer of PAI-1 during visceral obesity. Excess of circulating cortisol has failed to be demonstrated during obesity but a local cortisol production may occur within adipose tissue. 11-beta-hydroxysteroid dehydrogenase (11beta-HSD1) is expressed in most tissues. It potentiates the action of endogenous glucocorticoids by converting inactive cortisone into cortisol. Several recent experiments suggested that
MetS may result from elevated 11β-HSD1 activity.\textsuperscript{69,70} Interestingly, in adipose tissue, 11β-HSD1 levels paralleled those of PAI-1. Using human adipose tissue explants we observed that inactive cortisone stimulated PAI-1 secretion in an 11β-HSD1–dependent manner.\textsuperscript{71} 11-beta-HSD1 may be a central player in linking PAI-1, the MetS, and atherosclerosis because beside its improvement of MetS 11-beta-HSD1 inhibition prevents progression of atherosclerosis in mice.\textsuperscript{72}

### The Culprit May Be Glucidolipidic Disturbances Associated With MetS

Several groups as well as ours have suggested hyperinsulinemia and hypertriglyceridemia contribute to PAI-1 synthesis. Most cell culture experiments confirm that excesses of insulin or proinsulin,\textsuperscript{73–75} free fatty acid, or very-low-density lipoprotein (VLDL),\textsuperscript{76–78} directly increase PAI-1 synthesis. The apparent contradiction between the inability of insulin to induce glucose uptake and its capacity to stimulate PAI-1 synthesis led some investigators to demonstrate that some genes became insulin-resistant, whereas others, including PAI-1, continued to respond normally to insulin although insulin resistance was established.\textsuperscript{79} Moreover the signaling pathway of PAI-1 synthesis differs in normal and insulin-resistant adipocytes.\textsuperscript{80} This difference supports the hypothesis that the signaling pathways that remain insulin-sensitive may contribute to vascular disease associated with obesity and type II diabetes.

Such a direct effect of insulin or lipoprotein is not always supported by clinical observations. Postprandial hyperinsulinemia and hypertriglyceridemia as well as hyperinsulinemia induced during a hyperinsulinemic euglycemic clamp are not associated with increased circulating level of PAI-1 in healthy or obese subjects.\textsuperscript{55,81–83} Even more, low-dose insulin infusion was shown to decrease Egr-1, a pro-inflammatory transcription factor, in mononuclear cells as well as some prothrombotic factors such as tissue factor and PAI-1 plasma levels in obese individuals.\textsuperscript{84}

### Possible Contribution of the Renin Angiotensin System

The renin-angiotensin system mainly controls blood pressure. Angiotensin-converting enzyme inhibition significantly reduces plasma PAI-1 in obese subjects.\textsuperscript{85} The renin angiotensin system is completely expressed in human adipose tissue and several reports suggested it plays a role on PAI-1 synthesis. Angiotensin II promotes PAI-1 production and release in cultured human adipocytes via the angiotensin II type 1 receptor (AT1 receptor), and AT1 receptor blockade reduces basal PAI-1 release and abolishes angiotensin II-stimulated PAI-1 release from adipocytes.\textsuperscript{86} It is not known how angiotensin II acts on PAI-1 synthesis. Angiotensin II may promote PAI-1 secretion in many ways because it has been involved in local uptake, synthesis, and oxidation of lipids, inflammation, as well as cellular migration and proliferation mechanisms, but a more direct pathway cannot be ruled out.\textsuperscript{87,88}

### Oxidative Stress as a Central Player

In nondiabetic humans, fat accumulation as well as PAI-1 level closely positively correlates with the markers of systemic oxidative stress.\textsuperscript{89} Production of reactive oxygen species (ROS) increased selectively in adipose tissue of obese mice, partly because of augmented expression of NADPH oxidase and lowered expression of antioxidative enzymes induced by fat accumulation. This locally increased oxidative stress dysregulates the production of adipokines by adipose tissue such as TNF, MCP-1, and PAI-1.\textsuperscript{80} Macrophages infiltrated in adipose tissue\textsuperscript{84} may be involved in this process by elevating ROS production in the obese adipose tissue. Remarkably, obese mice treated with an NADPH oxidase...
inhibitor, adipocynin, showed reduced ROS production, improved diabetes and hyperlipidemia, and attenuated dysregulation of adipokines. Thus, oxidative stress in adipose tissue and probably in other tissues may play a central role in linking most of the features that characterize the MetS and plasma PAI-1 levels.

There has been a long-lasting debate about the role of ROS in oxygen sensing. Interestingly, hypoxia and ROS increase PAI-1 expression in adipocytes via distinct signaling pathways, suggesting that both may participate in PAI-1 overexpression during obesity.

**Pl of the Circadian Rhythm of PAI-1**

Finally, one could wonder whether the dysregulation of PAI-1 synthesis observed during the MetS may not be driven by dysregulation of the circadian clock, an endogenous self-sustained machinery of rhythmically acting transcriptional loops. Both clock:bmal1 and clock:bmal2 heterodimers activate the PAI-1 promoter. Deletion of the Clock and Bmal1 genes results not only in circadian disturbances but also in metabolic abnormalities of lipid and glucose homeostasis, a phenotype resembling the MetS. It could thus be suspected that PAI-1 synthesis dysregulation in obese patients is secondary to alteration of the self-regulated circadian clock, but this dysregulation needs to be elucidated.

In conclusion, the causes of PAI-1 overexpression in the MetS are complex, with much interference between biological systems. Establishment of inflammation or oxidative stress at the macrophage level as fundamental precursors is tempting and may reveal interesting avenues for a better understanding of the link between atherosclerosis and the MetS.

**Contribution of PAI-1 to the Development of Adipose Tissue and Insulin Resistance**

**Clinical Evidence that PAI-1 Is Diabetogenic**

High PAI-1 levels may help to identify a high-risk population with the potential of developing atherosclerotic disease and type II diabetes. Indeed, Festa et al showed that high plasma PAI-1 levels predict the development of diabetes. In their study the association of CRP and fibrinogen with incident diabetes was significantly attenuated after adjustment for body fat, waist circumference, or insulin sensitivity. In a logistic regression model that included age, sex, ethnicity, clinical center, smoking, BMI, insulin sensitivity, physical activity, and family history of diabetes, PAI-1 still remained significantly related to incident type II diabetes. Furthermore, the same group has recently shown that progression of PAI-1 levels over time, in addition to high baseline PAI-1 levels, is associated with incident diabetes. Similar findings were obtained in 2 other populations. Based on these results it has been hypothesized that PAI-1 participates in the development of key features of the MetS. This hypothesis is also sustained by the relationship between PAI-1 gene polymorphisms, obesity, and insulin resistance in population studies. The PAI-1 gene polymorphism 4G/5G in the promoter region in position −675 has been especially studied; the 4G allele is associated with increased PAI-1 transcription compared with the 5G allele in in vitro studies and with increased plasma PAI-1 levels in vivo. In some studies, 4G allele carriers were more prone to obesity and MetS but not in others. We have recently shown there is an interaction between insulin and pro-insulin levels and the −675 4G/5G PAI-1 gene polymorphism for the risk of myocardial infarction. Patients with the highest pro-insulin levels were at risk for myocardial infarction only if they were homozygous for the 4G allele, suggesting that PAI-1 genotype may influence the vascular risk associated with hyperinsulinemia. All together, these results are in favor of a role of PAI-1 gene variability in the modulation of obesity-associated phenotypes.

**In Vitro Support of the Role of PAI-1 in Insulin Signaling and Adipocyte Differentiation**

Studies on cultured fibroblasts showed that PAI-1 interferes with insulin signaling by preventing binding of vitronectin to αvβ3 and αvβ5, and can inhibit insulin-induced protein kinase B phosphorylation. PAI-1 also binds IGF-5 binding protein and thus impairs insulin action. Studies with adipocytes revealed interesting results. Overexpression of PAI-1 by adenovirus-mediated gene transfer inhibited differentiation. Conversely, preadipocytes from PAI-1−/− mice showed greater differentiation than those issued from wild type mice and exhibited enhanced basal as well as insulin-stimulated glucose uptake. Inhibition of PAI-1 with a neutralizing antibody promoted 3T3 adipocyte differentiation. Remarkably, PAI-1 deficiency was able to blunt the deleterious effect induced by TNFα on glucose uptake and on adipocyte differentiation marker expression levels. Intriguingly, using a synthetic PAI-1 inhibitor we recently observed not an increase but a decrease in human adipocyte differentiation.

This effect could be attributed to the human origin of the cells or to unknown properties of the inhibitor.

**In Vivo Support of the Role of PAI-1 in Obesity and the Related Glucidolipidic Disturbances**

The effect of PAI-1 excess has been investigated in vivo. Mice overexpressing murine PAI-1 under the control of the aP2 promoter develop high PAI-1 expression within adipose tissue. These mice exhibited adipocyte hypertrophy and a higher mRNA level of a preadipocyte marker in adipose tissue, suggesting adipocyte differentiation potential is decreased. These differences were exacerbated under high-fat diet with a significant lower body weight and smaller adipocytes associated with a lower feeding efficiency in transgenic mice. These findings suggest that PAI-1 overexpression induces impaired adipose tissue growth, which is in line with the in vitro effect of PAI-1 on murine adipocyte differentiation described earlier. When looking at the metabolic parameters, it appears that old transgenic mice maintained on standard fat diet exhibit significantly higher insulinemia and a tendency to higher triglyceride levels despite lower body fat. These data indicate that PAI-1 overexpression may worsen the metabolic profile; these differences, however, were not found when younger transgenic mice were subjected to high-fat diet. One could wonder whether local and/or systemic PAI-1 contributes to this phenotype and whether
PAI-1 is directly or indirectly involved through the modulation of TNF or TGF-beta actions. PAI-1 deficiency protects against TNF effects on adipocytes and it has been recently hypothesized that PAI-1 could exert its action through inhibition of the proprotein convertase, furine, involved in TGF-beta activation and insulin receptor shedding.

Because of the improved insulin-stimulated glucose uptake and increased differentiation induced by PAI-1 inhibition in murine cultured adipocytes, one could expect PAI-1 deficiency to lead to higher subcutaneous fat accumulation in vivo under high-fat diet. Whereas 2 studies did not demonstrate any effect of PAI-1 deficiency on weight gain, 2 groups found that fat accumulation was prevented with a concomitant improvement in insulin sensitivity in mice lacking PAI-1 in 2 kinds of models, a nutritionally induced and a genetic murine model of obesity. The protection against obesity was linked to an increase in metabolic rate, total energy expenditure, and thermogenesis. These findings suggest the plasminogen activation system may be implicated in the control of fat accumulation in a more systemic way than that initially proposed. In the adult central nervous system, tissue-type plasminogen activator (tPA) is expressed at the mRNA and protein levels in many sites. tPA is considered a major relay to the hypothalamic-pituitary-adrenal axis. It could reasonably be proposed that inhibition of tPA in the central nervous system by excess systemic or local PAI-1 may affect the control of body weight by the central nervous system; this aspect needs to be investigated.

Interestingly, we recently observed that inhibition of PAI-1 with the same synthetic inhibitor previously cited may improve insulin sensitivity in mice. This synthetic, low-molecular-weight PAI-1 inhibitor was added to the normal chow of wild-type mice for 4 weeks. After insulin injection, glycemia was lower in treated animals as insulin levels after glucose injection, suggesting higher insulin sensitivity in treated mice. During high-fat diet, mice treated with the same PAI-1 inhibitor had lower body weight, glycemia, and triglyceride level than nontreated mice. Overall, these data support the concept that PAI inhibition has the potential to reduce obesity and improve insulin sensitivity and may represent a new therapeutic target. This needs to be confirmed in different experimental models and the mechanisms involved should be precisely defined.

Conclusion

Several new features have been added to the MetS over time because they were frequently found associated with the metabolic syndrome. PAI-1, the main inhibitor of the fibrinolytic system, belongs to this cluster and could be considered a true component of the MetS. The mechanisms linking PAI-1 to the MetS are complex and probably interrelated, and several inducers may act jointly and at several sites of synthesis. In vitro and in vivo studies have indicated that PAI-1 might be involved in the development of obesity. Thus PAI-1 may serve as a feedback loop to limit adipose tissue expansion. Further efforts with experimental and clinical studies are needed to better understand this complex interplay. In any case, these findings support the rationale to develop PAI-1 inhibitors as they may serve to control atherothrombosis and insulin resistance.

Disclosures

None.

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