PAI-1 in Human Hypertension: Relation to Hypertensive Groups

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Background: Although the renin-angiotensin system and insulin resistance (IR) have been identified as major regulators of plasminogen activator inhibitor type-1 (PAI-1), their roles in hypertensive subjects is not clearly defined.

Methods: We examined the effect of dietary salt restriction on PAI-1 levels in 239 hypertensive subjects from three centers. Subjects were placed on a 200 and 10 mmol/day sodium diets for 1-week periods. Plasma renin activity (PRA) and PAI-1 levels were measured on the last day of both diets and fasting insulin, glucose, and aldosterone (ALDO) levels, only on the low salt diet.

Results: Sodium restriction increased PAI-1 levels from 32.1 ± 2.5 ng/mL to 39.8 ± 3.2 ng/mL ($P = .009$). There was a strong positive correlation between PAI-1 levels and PRA ($r = 0.228$, $P = .0004$), IR ($r = 0.222$, $P = .001$), triglycerides ($r = 0.275$, $P < .001$), and ALDO ($P = .018$ for linear trend). The patients were divided into low renin (low IR and ALDO levels), nonmodulators (normal PRA, high IR, and low ALDO levels), and modulators (normal PRA, intermediate IR, and normal ALDO levels) groups to assess the relative contribution of each factor to PAI-1 levels. Modulators had significantly ($P = .019$) higher PAI-1 levels compared to the low renin and nonmodulators who had similar PAI-1 levels.

Conclusions: Plasma renin activity, IR, and ALDO all correlate with PAI-1 levels in the hypertensive subjects. However, the data suggest that ALDO may be an important factor contributing to the variability of PAI-1 levels in individual hypertensive subjects. Am J Hypertens 2002; 15:683–690 © 2002 American Journal of Hypertension, Ltd.

Key Words: Hypertension, renin-angiotensin system, fibrinolysis.

Recently the fibrinolytic system has attracted intense interest as a potential pathophysiologic explanation of increased risk of atherothrombotic disease. Most of this interest has focused on plasminogen activator inhibitor type-1 (PAI-1). The PAI-1 is the principal inhibitor in plasma of plasminogen activators (tPA and uPA). An imbalance in the fibrinolytic system either by a decrease in the activity of plasminogen activators or by an increase in the activity of plasminogen activator inhibitors could lead to thrombotic events such as myocardial infarction or stroke. Most clinical data suggested that this imbalance is likely due to increased levels of PAI-1.[1–4]

Among the factors that regulate PAI-1 production or release in humans, two may be particularly relevant in modifying cardiovascular risk: the activity of the renin-angiotensin-aldosterone system (RAAS)[5–7] and insulin or insulin resistance (IR). For example, both insulin and angiotensin II (Ang II) in vitro increase PAI-1 production in different cell types.[8–10] We have shown that PAI-1 levels are increased in normotensive subjects with chronic activation of the RAAS by either dietary sodium restriction[10] or diuretic use[11] and decreased by the interruption of the RAAS system with an angiotensin converting enzyme (ACE) inhibitor.[12–14] We also have documented that aldosterone stimulates PAI-1 expression in vitro,[15] and PAI-1 levels correlate closely with aldosterone levels in normal human subjects.[10] Finally, PAI-1 antigen and activity are increased in patients with IR.[16]

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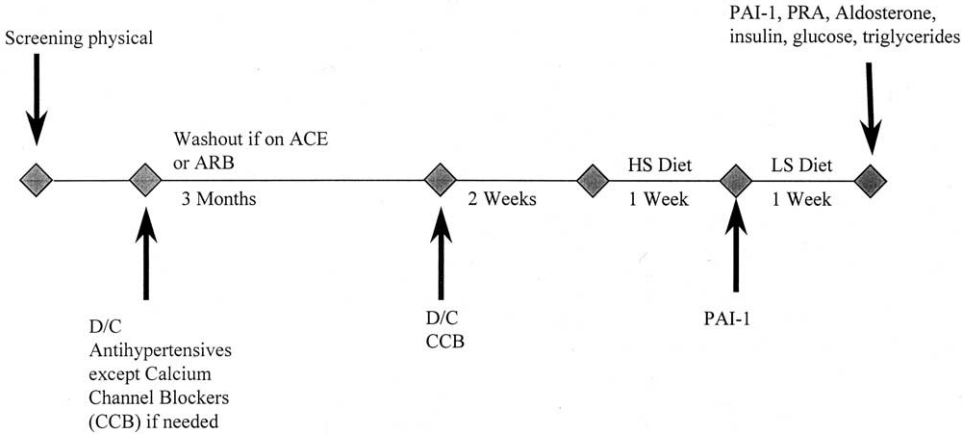

FIG. 1. Protocol outline. ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; HS = high sodium diet; LS = low salt diet; D/C = discontinue; PAI-1 = plasminogen activator inhibitor type-1; PRA = plasma renin activity.

In hypertensive subjects, the risk of myocardial damage or stroke is increased compared to normotensive individuals. Yet, for an individual patient, this increased risk is quite variable. This variability cannot be entirely explained by differences in level or duration of hypertension. Given the variability in the levels of activity of the RAAS and IR in hypertensive subjects and linkage of these factors to levels of PAI-1, one possible factor contributing to the heterogeneity of cardiovascular risk in hypertensives is PAI-1. To address this possibility, the present report takes advantage of the extensive physiologic data available on hypertensive subjects studied by the HyperPath (Hypertension Pathotypes) group. These patients had undergone extensive evaluation under controlled dietary conditions and can be divided into three major pathophysiologic groups: low renin, nonmodulating normal renin, and modulating normal renin hypertensive patients. Each of these types has varying levels of activity of the three principal mediators of PAI-1 production. Low renin patients have low renin and relatively low aldosterone levels and are relatively insulin sensitive. Nonmodulators have normal to high renin levels, relatively low aldosterone levels, and are insulin resistant. Modulating hypertensive patients have normal or elevated renin levels, normal aldosterone levels, and are intermediate in their insulin sensitivity.[17,18] Using data from these patients, PAI-1 levels was determined to assess the relative importance of each regulator in hypertensive subjects.

Methods

The data reported herein were collected from the HyperPath group. This group was formed in 1992 to evaluate the genetics of human hypertension by using intermediate phenotypes. The characteristics of this population have been previously reported.[19,20] Subjects at each site underwent an identical protocol, which consisted of several hormonal and vascular assessments when these individuals were in balance on 200- and 10-mmol sodium intakes.

Study

In the current study data from 239 hypertensive subjects from three HyperPath centers (Boston, MA [$n = 80$]; Salt Lake City, UT [$n = 57$], and Paris, France [$n = 102$]) are reported. Subjects from Paris had PAI-1 levels measured on low salt diet only and, therefore, were not included in the analysis of the effect of sodium loading. Hypertension was defined as a history of hypertension with a diastolic blood pressure (BP) more than or equal to 100 mm Hg off all medications, diastolic BP more than or equal to 90 mm Hg on one antihypertensive agent, or the use of two or more hypertensive medications at the time of the screening visit. The Institutional Review Boards of the respective hospitals approved the protocols, and written informed consent was obtained from all subjects. Although some of the information on some subjects has been reported previously, the PAI-1 data and all of the analyses are original.

All subjects had screening physical and laboratory examinations. Those with secondary forms of hypertension, diabetes mellitus, renal insufficiency, or any significant medical illnesses were excluded. Patients taking an ACE inhibitor had it changed to a different class of medication 3 months before study and all antihypertensive medications were discontinued 2 weeks before the study.

Beginning on day 1 each subject was provided a low sodium diet (10 mmol/day) for 7 days or a high sodium diet (200 mmol/day) for 3 to 7 days and then studied on the reverse diet (Fig. 1). In general, subjects were studied on high salt first and low salt second. Both diets contained 100 mmol of potassium and 800 mg of calcium. When studies on one diet (high or low salt) were completed, the subjects were placed on the alternate diet and restudied. On the next to the last day on each diet subjects were admitted to the General Clinical Research Center. Sodium balance was ensured by measurement of urinary sodium and creatinine in a 24-h urine collection and fasting, supine PAI-1 levels was obtained. Subjects in the low salt phase had fasting, supine insulin and glucose samples

obtained and a posture study done, where plasma renin activity (PRA) was measured in the morning after 90 min of standing or quiet walking. Data from this was used to stratify patients into low (<2.4 ng of angiotensin I/mL/h) and normal/high renin groups.[21] On the low salt diet Ang II was infused at 3 ng/kg/min for 55 min and serum aldosterone levels determined before and at the end of the infusion. The results were used to classify normal or high renin hypertensive patients into modulators and nonmodulators (increments in aldosterone, ≤15 ng/dL) as previously reported.[22]

Laboratory Analyses

Blood samples were collected on ice and centrifuged for 20 min. All samples (plasma, serum, and urine) were frozen without preservatives and stored at −20°C until assayed. The PRA, aldosterone, PAI-1, sodium, potassium, and creatinine levels were measured as previously reported.[5,19,20] The PAI-1 antigen levels were determined using a two-site immunosorbent assay (Biopool AB, Umea, Sweden), as previously described.[22] All of the laboratory analyses were done centrally in one laboratory. The PAI-1 was done at Vanderbilt University, Nashville, TN, and the rest were done at the Brigham and Women's Hospital, Boston, MA, core laboratory and specimens were transported overnight in dry ice to the respective laboratories.

Statistics

Data are presented as mean ± SEM unless otherwise noted. Patient groups were compared using independent sample *t* test. Analysis was performed on raw data and log-transformed data where appropriate to normalize data distribution. Because all PAI-1 measurements were obtained in the supine position after an overnight fast, correlations with PRA, aldosterone, insulin, and glucose were made on their levels obtained from the supine samples. However, characterization of the hypertensive groups took advantage of data obtained in the upright position and after Ang II infusion. Statistical analyses including correlation analysis were performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 9. All tests were conducted using an α level of 0.05.

Results

Relation Between PAI-1 and Activity of the RAAS

Sodium loading and restriction was used to modify the activity of the RAAS. Each subject included in the analysis had a urine sodium excretion of more than 160 mEq/day on the high salt diet and less than 30 mEq/day on the low salt diet. Activation of the RAAS was documented by the fourfold increase in the supine PRA level from 0.5 to 2.2 ng/mL/h with sodium restriction. As we have previously reported in healthy subjects, sodium restriction also significantly (P = .009) increased PAI-1 levels in hypertensive subjects from 32.1 ± 2.5 ng/mL to 39.8 ± 3.2 ng/mL. To further evaluate this relationship, we determined the correlation between PAI-1 and PRA levels when subjects were in balance on the low salt diet. Because the raw data were not normally distributed, the log-transformed data were used. A significant correlation was documented (r = 0.23, P = .0004; Fig. 2). Because previous studies have documented an effect of body mass index (BMI) and age on PAI-1 levels, a general linear model analysis was performed with ln of PAI-1 as the dependent variable. Age (P = .006), BMI (P < .001), and ethnicity (P = .001) were significant covariates, gender was not (Table 1). The BMI was positively and age negatively correlated with PAI-1 levels. African Americans (24.7 ng/mL) had lower PAI-1 levels than whites (37.7 ng/mL). Finally, the correlation between PAI-1 and supine PRA remained significant using a general linear model with BMI, ethnicity, age, and gender as covariates.

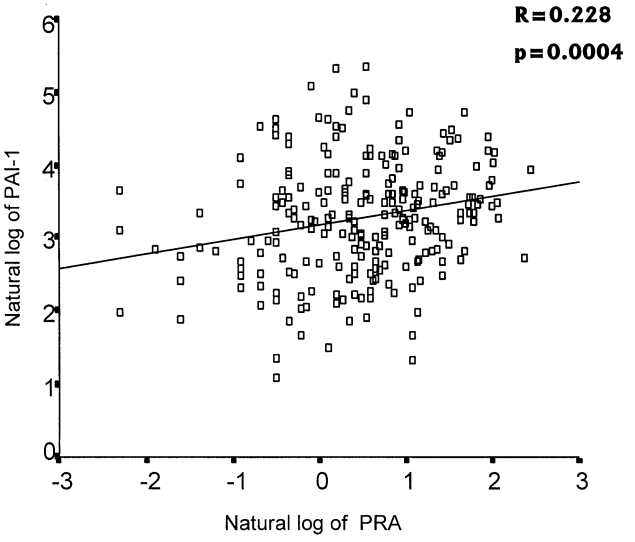

FIG. 2. Relationship of PAI-1 and PRA in subjects on a low salt diet. Data presented as natural logs. Abbreviations as in Fig. 1.

Relation Between PAI-1 and Insulin Resistance

The PAI-1 production is stimulated not only by Ang II but also by insulin, and also IR states are associated with

Table 1. General linear model analysis of PAI-1 levels on low salt diet as dependent variable (N = 234)

Covariates	F	P
Age	5.60	.006
Sex	0.75	.401
BMI	13.68	<.001
Race	9.59	.001

PAI-1 = plasminogen activator inhibitor type 1; BMI = body mass index.

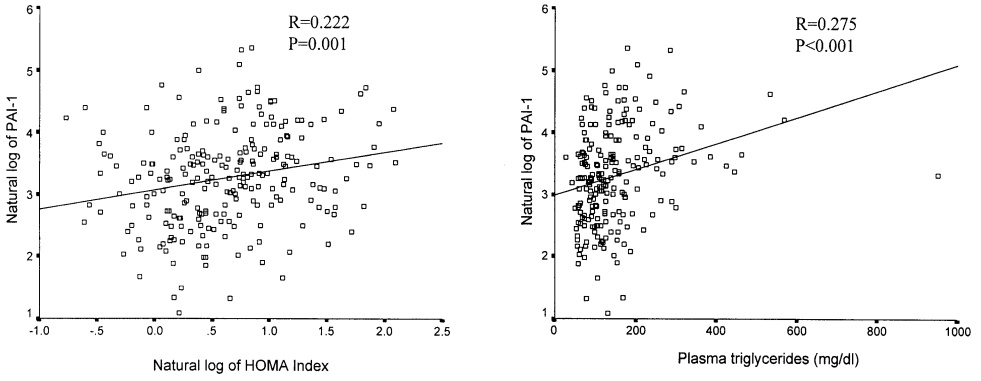

FIG. 3. Relationship between insulin resistance syndrome (Homeostasis Model Assessment Index [HOMA Index], triglycerides, and PAI-1]. Data presented as natural logs. Other abbreviation as in Figs. 1 and 2.

higher PAI-1 levels.[23,24] To determine whether this occurred in our hypertensive population, IR was determined in each subject by calculating the Homeostasis Model Assessment Index (HOMA Index = glucose (in millimoles per liter) × insulin (in micro units per milliliter)/22.5) using values from the fasting, supine sample. The HOMA index has been shown to correlate well with IR using clamp techniques.[25,26] There was a significant correlation between the HOMA index and PAI-1 levels (r = 0.22, $P = .001$; Fig. 3). Furthermore, triglyceride levels, which highly correlate with the degree of IR, also significantly correlated with PAI-1 levels (r = 0.28, $P < .001$; Fig. 3).

Relation Between PAI-1 and Aldosterone

Supine, fasting low salt serum aldosterone levels were not normally distributed nor were the log-transformed values. Therefore, to analyze the relationship between PAI-1 and aldosterone levels we assessed the levels of PAI-1 in the subjects divided into quintiles by their aldosterone levels. There was a gradual increase in PAI-1 levels from the lowest to the highest quintile (Fig. 4), which was significant for linear trend ($P = .018$).

PAI-1 Levels in Hypertensive Subject Groups

Of the 239 hypertensive subjects studied, 50 subjects were in the low renin group and 189 were in the normal/high renin group. Subjects in the normal/high renin group had a higher PAI-1 level (36.9 ± 2.3 ng/mL) than the low renin subjects (30.9 ± 4.1 ng/mL), although this did not reach statistical significance ($P = .112$). Because of the effect of changes in salt intake on modifying PAI-1 and renin levels, this was an unexpected finding. The differences in the PRA levels between the low renin and normal/high renin subjects on the low salt diet was as great, if not greater, than the difference in the PRA levels when dietary sodium intake was changed from low to high. Because the normal/high renin group was heterogeneous, consisting of both modulating hypertensive subjects and nonmodulators, we then compared PAI-1 levels in the three hypertensive groups (Table 2). The modulators were younger than the other groups ($P = .005$) and had a lower systolic

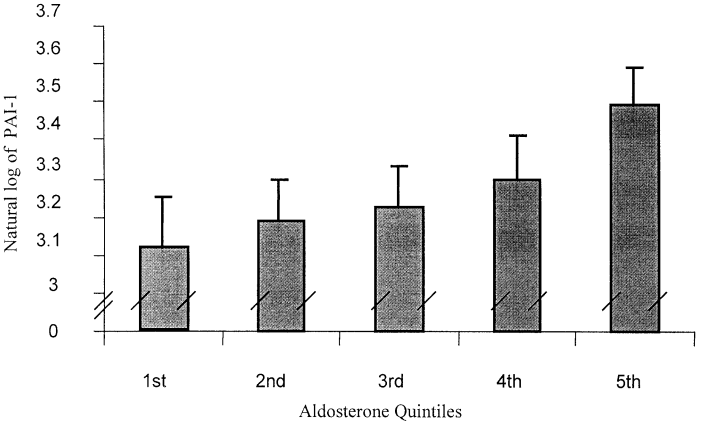

FIG. 4. PAI-1 levels by aldosterone quintiles (mean ± SEM). *P* for linear trend = .018. Abbreviation as in Figs. 1–3.

Table 2. Demographic data in the three hypertensive subgroups (mean ± SEM)

	Low Renin (*N* = 50)	Nonmodulators (*N* = 59)	Modulators (*N* = 130)	*P*
Age (y)	50.2 ± 0.9	49.3 ± 1.1	46.7 ± 0.6	.005
Body mass index	26.6 ± 0.4	28.0 ± 0.5	27.7 ± 0.3	.1
% Males	40	78	52	<.001
% Whites	84	73	91	.002
Systolic blood pressure (mm Hg) low salt, supine	141.2 ± 2.2	136.6 ± 2.2	133.5 ± 1.6	.037
Diastolic blood pressure (mm Hg) low salt, supine	82.6 ± 1.3	82.0 ± 1.3	81.5 ± 0.9	.81

BP ($P = .037$) (Table 2). Body mass index ($P = .1$) and diastolic BP ($P = .81$), however, were similar in all three groups. Nonmodulators, as previously described, had a preponderance of male subjects ($P < .001$). Striking associations are documented in Fig. 5. Low renin and nonmodulators had similar PAI-1 levels, whereas modulators had a third higher value ($P = .019$). Of the three factors evaluated in this study that can modify PAI-1 levels, only aldosterone could account for these findings in PAI-1 levels. Renin activity was five times higher in the nonmodulators and modulators than in the low renin subjects, yet, nonmodulators and low renin patients had similar PAI-1 levels. Insulin resistance, as defined by the HOMA index, was highest in the nonmodulators and lowest in the low renin subjects—a dissociation between insulin (or IR) and PAI-1 levels. In contrast, plasma aldosterone levels were significantly higher in the modulators ($P < .01$), consistent with the increased PAI-1 levels and not significantly different between the low renin and nonmodulators (Fig. 5).

Discussion

The present study confirms a previous report in normotensive subjects and a smaller group of hypertensive patients that the level of sodium intake significantly modifies PAI-1 antigen levels in patients with essential hypertension. It expands on these observations to document a strong correlation between the PAI-1 levels and PRA, the degree of IR and aldosterone levels in patients with essential hypertension. Perhaps more important, the present report may provide clues as to why there is variability in cardiovascular risk in hypertensive groups and the potential role PAI-1 may play in mediating this variability. Finally, it reinforces a substantial role for aldosterone as a likely mediator of variability in PAI-1 levels.

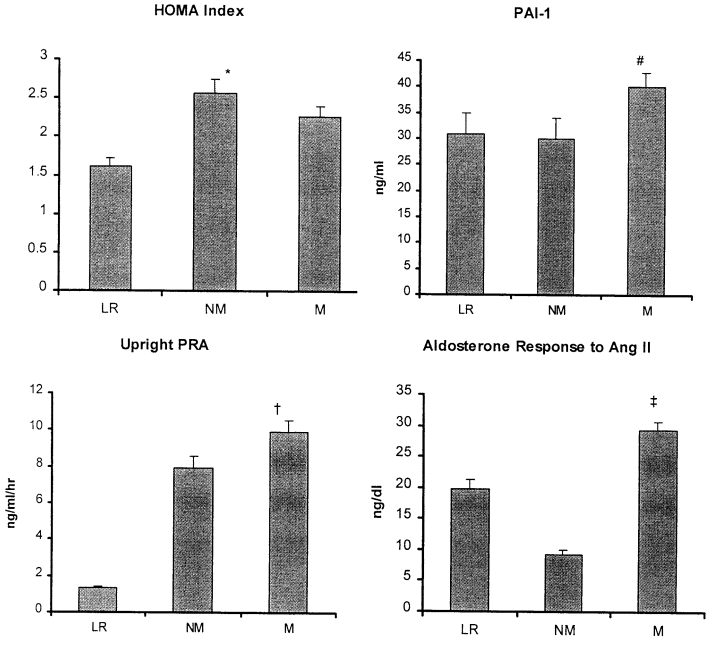

FIG. 5. Relationship between hypertensive groups and PAI-1, insulin resistance (HOMA Index), aldosterone, and PRA. LR = low renin; NM = nonmodulators; M = modulators. ANOVA: M significantly different from NM and LR, #$P = .019$, and ‡$P < .001$; M significantly different than LR, †$P < .001$; NM significantly different than LR, *$P < .001$. Other abbreviations as in Figs. 1–4.

An increasing body of literature has documented that PAI-1 antigen or activity is likely a major factor in determining overall thrombolytic activity[27] and in mediating the risk of myocardial infarction and strokes, particularly in hypertensive subjects. These data include increased PAI-1 levels or activity in humans with thrombotic events,[28] transgenic mice who overexpress human PAI-1 gene developed thrombosis,[29] humans with little or no detectable PAI-1 have lifelong bleeding problems,[30,31] young survivors of myocardial infarction have increased PAI-1 levels compared to controls,[32] and PAI-1 levels are increased in those individuals who are susceptible of a recurrent myocardial infarction.[33] The association of PAI-1 to systolic and diastolic BP[34] and the increased cardiovascular risk seen with increased levels of PAI-1,[2,3,35,36] seem to be independent of other traditional cardiovascular risk factors. The three major regulators of PAI-1 production have also been implicated in cardiovascular risk. For nearly three decades it has been suggested that individuals with low renin hypertension have a decreased risk of cardiovascular morbidity and mortality compared to those with an activated renin-angiotensin system (RAS).[37] Whether this applies to African American hypertensive subjects is unclear. Several studies suggest that this risk appears to be independent of BP[37,38] and that the administration of a converting enzyme inhibitor reduces this risk in individuals with hypertension with or without left ventricular dysfunction and in normotensive individuals who have an increased cardiovascular risk.[14] Insulin resistance also has been associated with increased cardiovascular risk in hypertensive subjects, patients with diabetes, and in normotensive individuals who have abnormal lipid levels. Finally, aldosterone may also be associated with increased cardiovascular risk. Patients with primary aldosteronism and glucocorticoid remediable aldosteronism have an increased risk of stroke compared to individuals who have essential hypertension. Furthermore, administration of an aldosterone antagonist, spironolactone, reduces mortality in individuals with class 3 and 4 heart failure treated with ACE inhibitors and diuretics.[39] In a recent publication Sawathiparnich et al[40] showed a significant correlation between PAI-1 levels and serum aldosterone ($r^2 = 0.57$, $P = .003$) in hypertensive subjects and spironolactone therapy abolished this correlation. Subjects on spironolactone showed no increase in PAI-1 levels, although their Ang II levels were significantly elevated. A common mediator of all three of these risk factors (aldosterone, RAS, and IR) could be mediated by increased levels of PAI-1.

Angiotensin II can stimulate PAI-1 expression from cultured endothelial and smooth muscle cells. In humans, ACE inhibition has been shown to reduce PAI-1 levels. Our previous studies have documented that chronic endogenous activation of the RAAS by dietary salt restriction increases PAI-1 levels in both normotensive and hypertensive subjects—a finding confirmed in the present study. However, the effect of PRA on PAI-1 level is modest. Insulin levels,[41,42] visceral adiposity,[43] and triglycerides[44] have all been associated with higher PAI-1 levels. Each is also associated with IR. The present study extends these findings to include IR in hypertensive subjects. There were highly significant positive correlations between PAI-1 levels and BMI, triglyceride levels, and HOMA index. Thus, in hypertensive subjects, the increased cardiovascular risk associated with higher PAI-1 levels may be in part secondary to metabolic factors, related to IR, or vice versa. Finally, the present study also strongly supports our previous hypothesis that aldosterone is a major factor contributing to PAI-1 levels.[10] We have also documented that aldosterone interacts synergistically with Ang II to increase PAI-1 expression in vitro.[15]

Thus, all three proposed major mediators of PAI-1 production could be modulators of PAI-1 levels, and potentially cardiovascular risk, in hypertensive subjects. At least two of them, RAS and aldosterone, could mediate the increased PAI-1 levels with sodium restriction documented in this and previous reports.[10] Because of the interrelationship of these factors, it is difficult to ascribe relative weights to their individual contribution to PAI-1 levels in vivo in humans. However, several unique features of the present report allow for a reasonable speculation as to these relative weights. First, the hypertensive subjects could be divided into three specific pathophysiologic groups, each with a different constellation of activities of these three factors (Fig. 5). Second, these relationships were assessed in subjects on a controlled, low sodium intake where the RAS and aldosterone are activated, thereby enhancing the likelihood of dissecting out their relative importance in modifying PAI-1 levels. Nonmodulators and low renin subjects had similar PAI-1 levels that were lower than in modulators. Yet, these subjects had distinctly different PRA levels, thereby decreasing the likelihood that the activity of the RAS is the major factor modifying PAI-1 under these conditions. Low renin subjects and nonmodulators had contrasting degrees of IR: the later being insulin resistant and the former insulin sensitive. Thus, the similar PAI-1 levels in these two groups make IR a less likely candidate. What is shared in common with these two groups are lower aldosterone levels compared to the modulating group. Therefore, the likely strongest mediator of the variability of PAI-1 levels in hypertensive subjects under the conditions of this study is aldosterone. These results also suggest that aldosterone rather than Ang II may be the major mediator of the change in PAI-1 levels observed when dietary sodium intake is modified.

How do these results increase our understanding of the potential mediators of cardiovascular risk in patients with hypertension? From the presently available data, it is likely that the relative low cardiovascular risk in patients with low renin hypertension when compared to those hypertensives with normal/high renin levels is secondary to at least two factors that act directly on the vascular system, a lower degree of IR and lower PAI-1 levels.

Furthermore, the mechanisms that lead to decreased cardiovascular morbidity and mortality in subjects given an aldosterone receptor antagonist should include a reduction in PAI-1 levels. Can changes in PAI-1 levels explain all of the variability in cardiovascular risk in patients with hypertension? The data from this study would suggest not. We have previously documented that nonmodulators have an increased familial risk of myocardial infarction.[15] The present data suggest that this increased risk is less likely to be due to activation of PAI-1 production and more likely to be secondary to the consequences of the increase in IR that has been documented in this group of the hypertensive population.

The main limitation of our study is its cross-sectional design and the correlative data. This limits our ability to infer causal relationship between increased PAI-1 levels and the RAAS and IR. However, the data provide valuable information that allow for the development of specific hypotheses and protocols to determine whether these associations are causally related.

In conclusion, PAI-1 is the principal inhibitor of plasminogen activators in plasma and a major factor in determining the role of fibrinolytic activity. Increased PAI-1 levels have been associated with increased risk of thrombotic events and myocardial infarction. The present study documents that chronic endogenous activation of the RAS, aldosterone levels, and IR in hypertensive patients are all correlated with increased PAI-1 levels. Furthermore, the data support the hypothesis that aldosterone is likely a major mediator of PAI-1 levels in hypertensive patients. To the extent that PAI-1 levels serve as a surrogate marker for relative cardiovascular risk, these data provide further insight into the beneficial cardiovascular effects seen with ACE inhibitors and aldosterone antagonists. The present study also illustrates that regulation of PAI-1 is complex with multiple known and probable unknown determinants exerting varying effects in groups of hypertensive subjects, which may have relevance in interpreting both mechanistic and therapeutic studies.

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