

# Multimarker Approach to Evaluate the Incidence of the Metabolic Syndrome and Longitudinal Changes in Metabolic Risk Factors The Framingham Offspring Study

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**Background**—The metabolic syndrome (MetS) is associated with increased cardiovascular risk. We evaluated the relative contributions of circulating biomarkers representing distinct biological pathways to the incidence of MetS and to longitudinal changes of its constituent risk factors.

**Methods and Results**—We measured 8 circulating biomarkers reflecting inflammation (C-reactive protein), hemostasis (plasminogen activator inhibitor-1, fibrinogen), neurohormonal activity (aldosterone, renin, B-type natriuretic peptide, N-terminal proatrial natriuretic peptide), and endothelial dysfunction (homocysteine) in 2292 Framingham Offspring Study participants (mean age, 57 years; 56% women). We related the biomarker panel to incidence of MetS on follow-up initially and then related biomarkers associated with incident MetS to longitudinal change in its components. On follow-up (mean, 2.9 years), 282 participants (of 1473 participants without prevalent MetS at baseline) developed MetS. After adjustment for clinical risk factors, the biomarker panel was associated with incident MetS ( $P=0.008$ ). On backward elimination, plasminogen activator inhibitor-1 and aldosterone remained associated with incident MetS (multivariable-adjusted odds ratio per 1-SD increment log marker, 1.31 [ $P=0.004$ ] and 1.21 [ $P=0.015$ ], respectively). In multivariable analyses evaluating longitudinal change in MetS components (analyzed as continuous variables), plasminogen activator inhibitor-1 was significantly and positively associated with changes in fasting glucose, systolic blood pressure, and triglycerides (all  $P<0.05$ ). Serum aldosterone was associated positively with change in systolic blood pressure ( $P=0.023$ ) and inversely with change in high-density lipoprotein cholesterol ( $P=0.001$ ).

**Conclusions**—Higher circulating plasminogen activator inhibitor-1 and aldosterone levels are associated with the development of MetS and with longitudinal change of its components, suggesting that these biomarkers and related pathways play a key role in mediating metabolic risk. (*Circulation*. 2007;116:984-992.)

**Key Words:** aldosterone ■ epidemiology ■ metabolic syndrome X ■ plasminogen activator inhibitor 1 ■ risk factors

Metabolic risk factors, including visceral obesity, glucose intolerance, and dyslipidemia, often cluster in individuals,<sup>1,2</sup> a construct called the metabolic syndrome (MetS). Although the clinical use of MetS has been questioned,<sup>3,4</sup> there is agreement that clustering of risk factors portends an increased risk of cardiovascular disease. It is

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widely accepted that insulin resistance is a key feature of MetS, although overlap between the 2 conditions is only partial.<sup>5</sup> Indeed, a variety of metabolic, neurohormonal, and inflammatory abnormalities may accompany MetS. In cross-

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sectional studies, the presence of MetS has been correlated with higher circulating concentrations of inflammatory markers,<sup>6,7</sup> hemostatic markers,<sup>8</sup> and neurohormonal activation.<sup>9,10</sup> Elevation of several of these biomarkers may antedate development of risk factors such as type 2 diabetes<sup>11–13</sup> and hypertension.<sup>14–16</sup> However, it is less clear if alterations of biomarkers are pathogenetically related to the incidence of MetS itself. Previous studies have not examined conjoint and relative contributions of multiple biomarkers representing key pathogenetic pathways for predicting the development of MetS and its components.

We hypothesized that select circulating biomarkers are associated with the incidence of MetS and with longitudinal tracking of its components. We tested this hypothesis by evaluating a comprehensive panel of biomarkers representing the following pathways: inflammation (high-sensitivity C-reactive protein), hemostasis (plasminogen activator inhibitor-1 [PAI-1], fibrinogen), neurohormonal activation (aldosterone, renin, B-type natriuretic peptide [BNP], N-terminal proatrial natriuretic peptide [N-ANP]), and endothelial dysfunction (homocysteine).

## Methods

### Study Sample

The design and selection criteria of the Framingham Offspring Study have been described previously.<sup>17</sup> Participants were eligible for the present investigation if they attended both the sixth (1995 to 1998; baseline) and seventh (1998 to 2001; follow-up visit) examination cycles. Of 3264 eligible attendees, we excluded 972 individuals for the following reasons: prevalent cardiovascular disease (coronary heart disease, cerebrovascular disease, intermittent claudication, or congestive heart failure;  $n=359$ ), prevalent diabetes (fasting plasma glucose  $\geq 126$  mg/dL or use of insulin or oral hypoglycemic agents<sup>18</sup>;  $n=228$ ), serum creatinine  $>2.0$  mg/dL ( $n=7$ ), missing covariates ( $n=226$ ), or missing biomarker data ( $n=152$ ). After exclusions, 2292 individuals (mean age, 57 years; 56% women) remained eligible for our study. This larger sample was the sampling frame for analyses of longitudinal change in individual components of MetS (see the Statistical Analyses section for rationale). A smaller sample of 1473 participants was eligible for analyses of the incidence of MetS after exclusion of 819 participants with prevalent MetS at baseline. The Institutional Review Board at Boston University Medical Center approved the study, and all participants gave written informed consent.

### Assessment of Clinical Covariates

At each Framingham Heart Study examination, participants undergo routine medical history, physical examination using a standardized protocol (including blood pressure [BP] measurement and anthropometry), and laboratory assessment of risk factors. A physician measured BP using a mercury column sphygmomanometer after the participant had rested in a seated position for 5 minutes; the average of 2 readings obtained on the participant's left arm constituted the examination BP. Fasting levels of plasma glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured with standardized assays. Cigarette smoking was defined by self-reported use within the year preceding the baseline examination.

At examination cycle 5 ( $\approx 4$  years before the study baseline), fasting plasma glucose and insulin levels were measured. We calculated homeostasis model assessment insulin resistance index (HOMA-IR) using this formula:

$$(1) \quad \frac{\text{fasting insulin} \times \text{fasting glucose}}{22.5}^{19}$$

### Biomarker Measurements at Baseline Examination

At the baseline examination, phlebotomy was performed on fasting participants who had rested for 5 to 10 minutes in a supine position, typically between 8 and 9 AM. Specimens were stored at  $-80^{\circ}\text{C}$  without freeze-thaw cycles until assay.

High-sensitivity C-reactive protein was measured with a Dade Behring BN100 nephelometer. ELISA methods were used to measure plasma levels of PAI-1 antigen (TintElize PAI-1, Biopool, Ventura, Calif). Plasma fibrinogen was determined by the Clauss method. Serum aldosterone was measured by radioimmunoassay (Quest Diagnostics, Cambridge, Mass), and plasma renin was measured by immunochemiluminometric assay (Nichols assay, Quest Diagnostics). Plasma BNP and N-terminal proatrial natriuretic peptide were measured with high-sensitivity immunoradiometric assays (Shionogi, Osaka, Japan). Total plasma homocysteine was measured with high-performance liquid chromatography with fluorometric detection. The average interassay coefficients of variation for the biomarkers were as follows: high-sensitivity C-reactive protein, 2.2%; PAI-1, 7.7%; fibrinogen, 2.6%; aldosterone, 4.0% for high concentrations and 9.8% for low concentrations; renin, 2.0% for high concentrations and 10.0% for low concentrations; BNP, 12.2%; N-terminal proatrial natriuretic peptide, 12.7%; and homocysteine, 9%.

### Incidence of MetS

The incidence of MetS was ascertained at the seventh examination (mean, 2.9 years from baseline; range, 1.0 to 6.2 years) among 1473 individuals (878 women) free of the condition at baseline. MetS was defined according to the modified definition of the National Cholesterol Education Program Adult Treatment Panel III guidelines<sup>20</sup> as the presence of  $\geq 3$  of the following: increased waist circumference ( $\geq 102$  cm for men,  $\geq 88$  cm for women), elevated BP ( $\geq 130$  mm Hg systolic,  $\geq 85$  mm Hg diastolic) or treatment for high BP, hyperglycemia (fasting glucose  $\geq 100$  mg/dL) or treatment with oral hypoglycemic agents or insulin, hypertriglyceridemia ( $\geq 150$  mg/dL) or treatment with lipid-lowering treatment, and low HDL cholesterol ( $<40$  mg/dL in men,  $<50$  mg/dL in women).

### Longitudinal Changes in Component Risk Factors on Follow-Up

For biomarkers related to MetS incidence, we also evaluated the relations to longitudinal changes in levels of metabolic risk factors that define MetS (see below for conservative analytical strategy). Thus, we evaluated changes in levels of the following risk factors between the baseline and follow-up examinations: waist circumference, systolic and diastolic BPs, plasma glucose, triglycerides, and HDL cholesterol. The larger sample of 2292 participants was eligible for these analyses to maximize our statistical power; exclusion of people with prevalent MetS was not necessary for these analyses; rather, we excluded individuals on treatment at baseline for a given risk factor.

### Statistical Analyses

All biomarkers, except fibrinogen and BNP, displayed right-skewed distributions and were logarithmically transformed. Because more than one quarter of BNP values were below the assay detection limit and were assigned the minimum detectable value, we used sex-specific quartiles of BNP in our models. Additionally, all other markers were sex standardized to facilitate easier comparisons and to account for sex differences in their distributions. This sex standardization was accomplished by subtracting from each value the sex-specific mean and dividing by sex-specific SD. Pairwise Pearson correlation coefficients were calculated to assess the relations among the biomarkers.

### Multimarker Panel and Incidence of MetS

To minimize multiple statistical testing, we used a conservative 2-step strategy to relate the biomarkers to the incidence of MetS. First, we used multivariable logistic regression to relate the entire panel of biomarkers to the incidence of MetS. We adjusted for the following covariates (all defined at the baseline examination): age,

sex, systolic and diastolic BPs, fasting glucose, HDL cholesterol, logarithm of triglycerides, and sex-standardized waist circumference. We estimated a global probability value for the biomarker panel as a whole using a likelihood ratio test in which  $-2 \log$  likelihood for the model with clinical covariates and biomarkers was subtracted from  $-2 \log$  likelihood for the model with clinical covariates only. The purpose of this global probability value was to determine whether the biomarker panel as a whole was associated with MetS incidence. We performed further analyses of individual biomarkers only if the global probability value was  $<0.05$ . Second, we selected a final set of informative biomarkers with backward elimination using an individual threshold of  $P<0.05$  for retention of biomarkers in the model. In secondary analyses, we added HOMA-IR as a covariate to the multivariable model to explore whether the association of biomarkers with incident MetS was confounded by insulin resistance.

Additional analyses focused on the biomarkers that were individually retained on backward elimination (referred to as informative biomarkers for simplicity). We assessed the effect modification of the biomarker-MetS relations by age and sex by incorporating appropriate interaction terms in multivariable models. We also assessed their conjoint influence on the incidence of MetS by cross-classifying participants according to sex-standardized median values and determining whether individuals with values above the median (high) on  $\geq 1$  biomarkers alone and in combination had a greater risk of MetS compared with those with concentrations of informative biomarkers below the median (low).

In addition, we constructed separate receiver-operating characteristics curves for the informative biomarkers to estimate sex-specific cutoff points that maximized the sums of sensitivity and specificity for predicting new-onset MetS.<sup>21</sup> These cutoff points were used to examine the sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) for incident MetS. We estimated the  $c$  statistic (area under the receiver-operating characteristics curve) for multivariable models incorporating the informative biomarkers and compared it with that for models without the biomarkers.

#### **Informative Biomarkers and Longitudinal Change in Metabolic Risk Factors**

For the informative biomarkers in the panel, we examined the relations of the baseline biomarker levels (modeled together) to longitudinal changes in the metabolic risk factors constituting MetS, with separate analyses for each risk factor. We excluded participants using BP-lowering or lipid-lowering treatment at baseline for analyses of changes in BP and triglycerides (individuals with diabetes at baseline were excluded from the study sample as noted earlier). We used sex-pooled multivariable linear regression models with the change in levels of risk factors as the dependent continuous variables; change in waist circumference was standardized within sex. For systolic BP, diastolic BP, plasma glucose, and logarithm of triglycerides, we used censored normal regression to account for treatment for high BP, diabetes, or dyslipidemia at the follow-up examination.<sup>22</sup> Models were adjusted for age, sex, baseline body mass index (except for models with waist circumference as the dependent variable), and baseline level of the individual risk factor analyzed. Two-sided values of  $P<0.05$  were considered statistically significant. All analyses were performed with SAS 9.1 (SAS Institute, Cary, NC) and STATA 8.2 (Stata Corp, College Station, Tex).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## **Results**

### **Baseline Biomarkers and Clinical Characteristics**

The baseline clinical characteristics of the study sample are shown in Table 1. Most of the biomarkers demonstrated a modest pairwise correlation (correlation coefficients ranging from  $-0.23$  to  $0.31$ ; Table 2). Stronger correlations were

evident for CRP and fibrinogen ( $r=0.52$ ) and for the 2 natriuretic peptides ( $r=0.57$ ).

### **Relations of the Multimarker Panel and Incidence of MetS**

On follow-up, 282 participants (142 women) developed new-onset MetS. The panel of 8 biomarkers was associated with incidence of MetS (global test,  $P=0.008$ ; Table 3). In stepwise logistic regression with backward elimination of biomarkers (adjusting for age, sex, systolic and diastolic BPs, glucose, high-density lipoprotein (HDL) cholesterol, logarithm of triglycerides, and sex-standardized waist circumference, all forced into the model), PAI-1 and aldosterone were significantly associated with MetS ( $P=0.004$  and  $P=0.015$ , respectively; Table 3). There was no effect modification by age or sex for either of these markers (interaction terms were not significant).

When HOMA-IR measured 4 years before the baseline was added as a covariate to the multivariable model, the relations of PAI-1 (multivariable-adjusted odds ratio [OR] per SD of log marker, 1.30; 95% CI, 1.07 to 1.58;  $P=0.007$ ) and aldosterone (multivariable-adjusted OR per SD of log marker, 1.22; 95% CI, 1.04 to 1.43;  $P=0.014$ ) to incident MetS remained robust; SD of log PAI-1 corresponds to 18.9 ng/mL, and SD of log aldosterone corresponds to 7.2 ng/dL increase above the median in original units. Additionally, HOMA-IR was significantly associated with incidence of MetS (multivariable-adjusted OR, 1.13; 95% CI, 1.04 to 1.23 per SD [1.8];  $P=0.004$ ). These results remained essentially identical when insulin resistance, defined as the highest quartile of HOMA-IR, was incorporated into the model as a binary variable instead of as a continuous variable.

The incidence of MetS increased across quartiles of PAI-1 and aldosterone (Table 4). There was a statistically significant 19% to 23% increase in risk of MetS per quartile increment in PAI-1 and aldosterone (trend tests, Table 4). Table 4 also presents results for the risk of MetS for different combinations of high versus low PAI-1 and aldosterone (defined on the basis of sex-standardized median levels), with the group with both PAI-1 and aldosterone under the median serving as the referent. Individuals with levels of both PAI-1 and aldosterone above the median experienced a doubling in the risk for incident MetS (Table 4).

### **Evaluation of Performance of PAI-1 and Aldosterone for Predicting MetS**

To evaluate the potential clinical usefulness of these markers for predicting new-onset MetS, we calculated the sensitivities, specificities, PPVs, and NPVs for sex-specific cut points derived from receiver-operating characteristics curve analyses (based on maximizing the sum of sensitivity and specificity). The cut points chosen for PAI-1 were  $\geq 21.9$  ng/mL for men (sensitivity, 0.75; specificity, 0.57; PPV, 0.61; NPV, 0.72) and  $\geq 27.7$  ng/mL for women (sensitivity, 0.53; specificity, 0.86; PPV, 0.69; NPV, 0.75). The threshold chosen for aldosterone was  $\geq 11.0$  ng/dL for both men and women (sensitivity, 0.46; specificity, 0.63; PPV, 0.53; NPV, 0.57 for men; and sensitivity, 0.54; specificity, 0.55; PPV, 0.42; NPV, 0.67 for women). The  $c$  statistic for predicting incident MetS

**TABLE 1. Baseline Characteristics of the Study Sample\***

	Total Cohort (n=2292)		Sample Excluding Participants With MetS at Baseline (n=1473)	
	Men (n=1000)	Women (n=1292)	Men (n=595)	Women (n=878)
<b>Clinical features</b>				
Mean age, y	57±10	57±9	56±10	56±9
Systolic BP, mm Hg	129±16	125±19	124±15	120±17
Diastolic BP, mm Hg	78±9	74±9	76±8	72±8
BP ≥130/85 mm Hg or treatment, %	56.0	43.8	38.7	27.8
Hypertension, %	38.2	31.5	23.5	18.5
Treatment for hypertension, %	23.3	19.6	15.1	10.5
BMI, kg/m <sup>2</sup>	28.4±4.3	26.8±5.2	27.0±3.6	25.2±4.2
BMI ≥30 kg/m <sup>2</sup> , %	29.1	21.8	16.2	12.4
Weight, kg	87.6±14.4	70.0±14.2	83.4±12.6	66.2±12.0
Waist circumference, cm	101±11	93±14	97±9	88±12
Increased waist circumference, %†	42.2	59.1	24.4	42.7
Total cholesterol, mg/dL	201±34	211±38	200±34	207±37
Triglycerides, mg/dL	138±88	124±67	108±54	101±48
High triglycerides, %‡	36.6	31.1	15.8	12.8
HDL-C, mg/dL	44±12	59±16	49±12	64±15
Low HDL-C, %§	37.5	28.9	17.8	13.7
Change in weight, kg	0.5±4.9	0.6±4.4	0.7±4.2	0.7±4.5
Current smoker, %	13.3	14.4	14.3	13.9
Fasting plasma glucose, mg/dL	100±9	94±10	97±8	91±7
Impaired fasting glucose, %¶	47.1	25.6	29.4	9.7
HOMA-IR	7.4±3.2	6.3±2.5	6.3±1.9	5.6±1.6
<b>Biomarkers, median (quartiles 1, 3)</b>				
CRP, mg/L	1.7 (0.8, 3.3)	2.1 (0.9, 4.9)	1.2 (0.6, 2.7)	1.5 (0.7, 3.6)
PAI-1, ng/mL	24.5 (16.2, 34.3)	18.5 (11.4, 29.3)	20.6 (13.7, 28.6)	15.2 (9.9, 23.8)
Fibrinogen, mg/dL	314 (280, 358)	332 (288, 379)	305 (272, 349)	325 (287, 359)
Aldosterone, ng/dL	9 (7, 13)	10 (7, 14)	9 (6, 13)	10 (7, 14)
Renin, mU/L	14 (8, 24)	11 (6, 18)	14 (9, 24)	11 (6, 18)
BNP, pg/mL	5.3 (4.0, 12.0)	9.4 (4.0, 18.8)	5.2 (4.0, 11.5)	9.6 (4.3, 19.6)
N-ANP, pmol/L	271 (185, 381)	347 (252, 481)	273 (185, 373)	353 (261, 489)
Homocysteine, nm/mL	9.6 (8.2, 11.6)	8.3 (6.9, 10.0)	9.6 (8.2, 11.5)	8.1 (6.8, 9.8)

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol.

\*Values for clinical features are mean±SD or percentages.

†Waist circumference ≥40.2 in (102 cm) in men or ≥34.6 in (88 cm) in women.

‡Triglycerides ≥150 mg/dL or treatment.

§HDL-C &lt;40 mg/dL in men or &lt;50 mg/dL in women.

||Change in weight from examination 6 to 7.

¶Plasma glucose ≥100 mg/dL.

was 0.81 for a model that incorporated age, sex, and the aforementioned clinical covariates. If PAI-1 and aldosterone were incorporated simultaneously into the model, the *c* statistic increased to 0.82 ( $P=0.11$  versus model without the 2 biomarkers).

### Relations of PAI-1 and Aldosterone and Longitudinal Tracking of Metabolic Risk Factors

Given that PAI-1 and aldosterone were the biomarkers retained in analyses relating the panel to incident MetS, analyses of individual components of MetS focused on these

2 biomarkers (modeled together). In multivariable-adjusted models, PAI-1 was significantly and positively associated with longitudinal changes in fasting glucose, systolic BP, and triglycerides (Table 5). Aldosterone was significantly associated with an increase in mean systolic BP and a decrease in mean HDL cholesterol on follow-up (Table 5).

### Discussion

#### Principal Findings

In a community-based sample, we jointly examined the association of MetS and its components with 8 biomarkers

**TABLE 2. Correlations Among Biomarkers\***

	hsCRP	PAI-1	Fibrinogen	Aldosterone	Renin	BNP	N-ANP	Homocysteine
hsCRP	1.00	0.31	0.52	0.09	0.01	0.01	-0.01	0.04
PAI-1	...	1.00	0.19	0.08	0.05	-0.08	-0.14	0.09
Fibrinogen	...	...	1.00	0.03	0.08	0.06	0.02	0.08
Aldosterone	...	...	...	1.00	0.24	-0.17	-0.18	0.02
Renin	...	...	...	...	1.00	-0.22	-0.23	0.07
BNP	...	...	...	...	...	1.00	0.57	0.05
N-ANP	...	...	...	...	...	...	1.00	0.11
Homocysteine	...	...	...	...	...	...	...	1.00

hsCRP indicates high-sensitivity C-reactive protein.

\*Values are Pearson correlation coefficients (n=2292) for sex-standardized, log-transformed CRP, PAI-1, aldosterone, renin, N-ANP, homocysteine, sex-standardized fibrinogen, and sex-specific BNP quartiles.

representing distinct biological pathways a priori hypothesized to be related to incidence of MetS. In multivariable analyses relating the biomarkers conjointly to MetS, both PAI-1 and aldosterone were significantly associated with the incidence of MetS, relations that remained robust on adjustment for HOMA-IR. PAI-1 and aldosterone also were associated with longitudinal change in several metabolic risk factors. Specifically, higher PAI-1 was associated with longitudinal increases in fasting plasma glucose, systolic BP, and triglycerides. Higher aldosterone was directly related to change in systolic BP and inversely related to change in HDL cholesterol. These findings notwithstanding, receiver-operating characteristics curve analyses evaluating the potential utility of these biomarkers for predicting MetS demonstrated a suboptimal performance overall, suggesting that these biomarkers are unlikely to prove useful as screening tools. Nonetheless, the association of both PAI-1 and aldosterone with tracking of risk factors is of great pathophysiological interest.

### Previous Studies of the Biomarkers and MetS

Many cross-sectional studies have demonstrated that individual biomarkers are associated with MetS.<sup>6–10,23</sup> Other studies have demonstrated that elevation of select biomarkers antedates the development of weight gain,<sup>24,25</sup> diabetes,<sup>11–13</sup> and hypertension.<sup>14–16</sup> For instance, higher CRP levels predict the incidence of diabetes<sup>13</sup> and hypertension,<sup>15</sup> suggesting a potential role for inflammation in the pathogenesis of these conditions. Furthermore, biomarkers of endothelial dysfunc-

tion have been reported to predict incident diabetes.<sup>12</sup> Data from some randomized trials indicate that therapy with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers is associated with a reduced risk of diabetes,<sup>9</sup> providing indirect evidence that neurohormonal activation may precede glucose intolerance. Prior studies have not examined the joint and comparative value of a panel of biomarkers representing distinct biological pathways for predicting the development of MetS.

### PAI-1 and MetS

Several cross-sectional studies have demonstrated that higher PAI-1 levels are associated with prevalent MetS<sup>26</sup> and its components (such as obesity,<sup>27</sup> increased blood glucose levels,<sup>28</sup> or dyslipidemia<sup>29</sup>). Increased PAI-1 levels have even been proposed to be a true component of MetS.<sup>26,30</sup> Furthermore, a higher PAI-1 level also has been demonstrated to predict the incidence of diabetes in community-based samples.<sup>31</sup> However, it is unclear whether PAI-1 levels predict incident MetS prospectively.

Several potential mechanisms may explain the association of PAI-1 with new-onset MetS and with tracking of several metabolic risk factors. It has been suggested that PAI-1 participates in the development and regulation of adipose tissue. Studies indicate that PAI-1 synthesis is increased by insulin, glucocorticoids, angiotensin II, fatty acids, and cytokines.<sup>32</sup> Furthermore, experimental studies indicate that PAI-1 may have a causal role in the development of obesity

**TABLE 3. Biomarker Concentrations and Incident MetS Excluding Participants With Prevalent MetS (n=1473)**

	Adjusted OR* (95% CI)	$\chi^2$ Statistic	P
Global test of all biomarkers	...	20.61	0.008
Final model†			
Log PAI-1, per 1-SD increment	1.31 (1.09 to 1.57)	8.15	0.004
Log aldosterone, per 1-SD increment	1.21 (1.04 to 1.41)	5.90	0.015

\*Odds of incident MetS associated with a 1-SD increment in the natural logarithm of the biomarker. Odds ratios were adjusted for age, sex, sex-standardized waist circumference, systolic and diastolic BPs, high-density lipoprotein cholesterol, fasting glucose, and log triglycerides.

†Results are shown only for variables retained by stepwise selection with  $P<0.05$  as the threshold for retention.

**TABLE 4. Logistic Regression Analysis Examining Biomarkers in Quartiles\* and in Combinations to Incidence of MetS Excluding Participants With Prevalent MetS at Baseline**

	Cases/People at Risk	Unadjusted Incidence Rates, % (95% CI)	Multivariable Adjusted† OR (95% CI)	P
Individual markers				
PAI-1				
Q1	48/506	9.5 (7.2 to 12.4)	Referent	...
Q2	76/426	17.8 (14.5 to 21.8)	1.23 (0.80 to 1.88)	0.34
Q3	80/342	23.4 (19.2 to 28.2)	1.27 (0.82 to 1.96)	0.28
Q4	78/199	39.2 (32.7 to 46.1)	1.81 (1.12 to 2.92)	0.02
Trend	...	...	1.19 (1.02 to 1.38)	0.02
Aldosterone				
Q1	66/406	16.3 (13.0 to 20.2)	Referent	...
Q2	60/381	15.7 (12.4 to 19.8)	0.76 (0.50 to 1.17)	0.22
Q3	77/353	21.8 (17.8 to 26.4)	1.40 (0.93 to 2.12)	0.11
Q4	79/333	23.7 (19.5 to 28.6)	1.64 (1.08 to 2.48)	0.02
Trend	...	...	1.23 (1.08 to 1.41)	0.002
Marker combinations				
Both $\leq$ median	55/509	10.8 (8.4 to 13.8)	Referent	...
PAI-1 $>$ median, aldosterone $\leq$ median	71/278	25.5 (20.8 to 31.0)	1.31 (0.85 to 2.03)	0.23
Aldosterone $>$ median, PAI-1 $\leq$ median	69/423	16.3 (13.1 to 20.1)	1.76 (1.16 to 2.69)	0.008
Both $>$ median	87/263	33.1 (27.7 to 39.0)	2.19 (1.43 to 3.37)	$<0.001$

n=1473.

\*The quartiles (Q) were based on the total sample (n=2292), which explains why the number at risk differs for each quartile.

†Multivariable models are adjusted for age, sex, sex-standardized waist circumference, systolic and diastolic BPs, HDL cholesterol, glucose, and log triglycerides.

and insulin resistance.<sup>33-35</sup> PAI-1-deficient mice develop lower body weight, glucose levels, and triglyceride levels, as well as lesser insulin resistance, compared with wild-type mice when fed a high-energy diet.<sup>34,35</sup> These PAI-1-deficient animals also have normal levels of peroxisome proliferator-activated receptor- $\gamma$  and adiponectin mRNA, key regulators of glucose and lipid metabolism, when fed a high-energy diet, in contrast to downregulation of these pathways in their

wild-type counterparts with normal PAI-1.<sup>34,35</sup> These findings are consistent with the notion that the absence of PAI-1 in adipocytes protects against insulin resistance by promoting glucose uptake and adipocyte differentiation via increased peroxisome proliferator-activated receptor- $\gamma$  expression.<sup>33</sup> Furthermore, it was demonstrated recently that a pharmacological inhibitor of PAI-1 can diminish glucose-stimulated PAI-1 increases and reduce differentiation in human adipo-

**TABLE 5. Linear and Censored Normal Regression Analysis\* Examining the Relations of Change in Individual Risk Factors According to PAI-1 and Aldosterone Levels (Modeled Together)**

ΔMetS Component	No.	Mean $\pm$ SD†	Proportion Treated at Follow-Up, %	Log PAI-1		Log Aldosterone	
				Multivariable-Adjusted‡ $\beta$ (95% CI)	P	Multivariable-Adjusted‡ $\beta$ (95% CI)	P
Waist circumference§	2292	0.0 $\pm$ 0.4	NA	0.004 (-0.015 to 0.024)	0.66	0.009 (-0.008 to 0.026)	0.30
Fasting glucose	2290	1.7 $\pm$ 11.2	0.4	0.571 (0.046 to 1.095)	0.033	0.194 (-0.261 to 0.648)	0.40
Systolic BP	1804	-0.3 $\pm$ 14.0	9.6	0.847 (0.164 to 1.530)	0.015	0.757 (0.106 to 1.407)	0.023
Diastolic BP	1804	-0.9 $\pm$ 8.1	9.6	0.172 (-0.217 to 0.561)	0.39	0.344 (-0.021 to 0.710)	0.065
Triglycerides	2086	-0.003 $\pm$ 0.4	8.6	0.045¶ (0.025 to 0.064)	$<0.001$	0.013 (-0.004 to 0.029)	0.13
HDL cholesterol	2290	2.2 $\pm$ 9.2	NA	-0.299 (-0.718 to -0.120)	0.16	-0.630 (-0.993 to -0.268)	0.001

\*Standard linear regression was used for change in waist circumference and HDL cholesterol (HDL-C). Censored normal regression was used for change in fasting glucose, systolic and diastolic BPs, and triglycerides.

†Mean change ( $\Delta$ ) of component during follow-up.

‡Multivariable-adjusted  $\beta$  for change ( $\Delta$ ) in component of metabolic syndrome was adjusted for age, sex, baseline body mass index (except waist circumference model), and baseline level of the individual component, and values given are per 1-SD increment of log PAI-1 (corresponding to 18.9 ng/mL increase above the median in original units) and log aldosterone (corresponding to 7.2 ng/dL increase above the median in original units), respectively.

§Variable was sex standardized in the analysis.

||Variable was log transformed in the analysis.

¶Corresponds to 5.3 mg/dL increase from the median.

cytes.<sup>36</sup> In addition, it could decrease adiposity and levels of glucose and triglycerides in a murine model.<sup>36</sup>

Apart from the above-discussed mechanisms that might link increased PAI-1 concentrations to the obesity, hyperglycemia, and dyslipidemia components of MetS, studies also link increased PAI-1 levels to the high BP component. Higher PAI-1 levels may reflect endothelial dysfunction,<sup>37</sup> an important component of hypertension. Higher vascular PAI-1 appears to accelerate perivascular and medial fibrosis, whereas suppression of PAI-1 protects against the vascular changes observed in experimental models of hypertension.<sup>38</sup>

### Aldosterone and MetS

Two recent cross-sectional studies have demonstrated that higher aldosterone concentrations are associated with a greater prevalence of MetS and components of MetS such as increased BP, triglycerides, and waist circumference and decreased HDL cholesterol.<sup>39,40</sup> However, these findings contrast with another report<sup>41</sup> that did not demonstrate an association of aldosterone and MetS. Low HDL cholesterol also has been correlated with high aldosterone concentrations in other studies,<sup>42–44</sup> and weight loss has been shown to decrease serum concentrations of several markers in the renin-angiotensin-aldosterone system, including aldosterone.<sup>45</sup> Several recent cross-sectional studies have reported direct correlations between aldosterone and insulin resistance.<sup>40,44,46</sup> Further evidence for the involvement of the renin-angiotensin-aldosterone system in the metabolic processes underlying development of MetS is provided by clinical trials demonstrating that blockers of the renin-angiotensin-aldosterone system (angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) decrease the risk of future diabetes.<sup>47</sup> We have previously reported that higher aldosterone concentrations are associated with hypertension prospectively.<sup>48</sup> However, we are not aware of other published data relating aldosterone levels to the development of MetS and tracking of HDL cholesterol.

High aldosterone concentrations might cause increased BP through multiple mechanisms, including increased renal sodium retention, potentiation of angiotensin II action, impaired endothelial function, and reduced vascular compliance.<sup>48</sup> Less is known about the potential mechanisms linking high aldosterone concentrations to low HDL, but they might involve the metabolism of adipose tissue, which is known to have high activity of the renin-angiotensin-aldosterone system.<sup>49</sup> In vitro studies support a role for aldosterone in the adipogenesis by mediating early adipocyte differentiation,<sup>50,51</sup> but it is less clear whether this could contribute to the observed inverse relation with HDL cholesterol.

### Multiple Biomarkers and Prediction of MetS

Of the 8 biomarkers evaluated, PAI-1 and aldosterone emerged as the biomarkers most significantly associated with MetS after adjustment for clinical covariates. One possible interpretation of the findings is that PAI-1 and aldosterone are key markers of incident MetS in the community because only these markers remained significant in the multivariable models. Experimental studies support this interpretation, as discussed above. However, it must be recognized that these 2

biomarkers were chosen on the basis of backward elimination in regression models. Biomarkers with greater analytical precision or low interassay variation may be retained in models without indicating lesser biological relevance of biomarkers that are not retained in the models. These cautionary comments notwithstanding, the present investigation extends the multimarker concept to the prediction of MetS and its components, a concept that recently has been applied to cardiovascular disease<sup>52</sup> and hypertension.<sup>53</sup>

### Study Strengths and Limitations

The strengths of the present study include the large community-based sample; routine assessment of circulating biomarkers; availability of standardized clinical covariates, including insulin resistance; and use of multivariable analyses to examine the biomarkers conjointly and comparatively. Nonetheless, the present study has several limitations. First, for select biomarkers, measurement-related issues may have limited our power to detect a relation. For plasma BNP levels, a significant proportion of individuals (26% of women, 41% of men) were below the detection limit of the assay.<sup>54</sup> For plasma renin and serum aldosterone, we used single-occasion measurements obtained on a random sodium diet and without a period of longer rest in a supine position. Thus, it may be questioned whether a single reading adequately represents the renin and aldosterone profiles of participants. Second, as noted above, in a stepwise selection procedure, the biomarkers retained in the final model are not necessarily the ones that are biologically most relevant. Third, there has been considerable debate regarding the choice of different definitions of MetS<sup>23,55,56</sup> and regarding the utility of the condition itself.<sup>3,4</sup> These controversies notwithstanding, our data provide insights into which biomarkers predict the development of metabolic traits alone and in combination (exemplified by MetS). Fourth, because our sample consists predominantly of white and middle-aged to elderly participants, the generalizability of our findings to other ethnicities and younger individuals is unknown. Fifth, because measurements of insulin levels were not available at the baseline of the present investigation, we had to use measurements from the prior examination cycle ≈4 years earlier. The fact that insulin resistance measured 4 years before the baseline was a strong predictor of incident MetS after baseline suggests that these exploratory analyses were of some value. Nevertheless, the time interval between insulin measurements and the baseline examination is an important limitation, and there may be some residual confounding resulting from the lack of characterization of insulin resistance using contemporaneous measurements. In addition, the mean follow-up time in our study was only 2.9 years, so our results reflect the association of biomarkers with metabolic syndrome over a short-term period. Finally, we acknowledge that the panel of biomarkers will always be somewhat arbitrary based on practical limitations; it may not be feasible for any given study to examine all possible biomarkers.

### Conclusions

In our large community-based cohort, a conservative analytical strategy evaluating a panel of important biomarkers demonstrated that higher plasma PAI-1 and serum aldoste-

rone concentrations are associated with future development of MetS and with longitudinal changes in metabolic risk factors. Analyses of the discriminative utility of these biomarkers for predicting incident MetS demonstrated unsatisfactory performance overall, suggesting that these biomarkers are unlikely to prove useful as screening tools. Our findings are primarily of pathophysiological importance. Further studies are warranted to confirm our findings and to elucidate the pathophysiological mechanisms underlying the observed associations.

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### Disclosures

None.

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## CLINICAL PERSPECTIVE

The metabolic syndrome has been associated with increased risk of cardiovascular disease. In the present study, we evaluated the relative contributions of circulating biomarkers representing distinct biological pathways to the incidence of metabolic syndrome and to longitudinal changes of its components. We measured a panel of 8 circulating biomarkers reflecting inflammation (C-reactive protein), hemostasis (plasminogen activator inhibitor-1, fibrinogen), neurohormonal activity (aldosterone, renin, B-type natriuretic peptide, N-terminal proatrial natriuretic peptide), and endothelial dysfunction (homocysteine) in 2292 Framingham Offspring Study participants. First, we related the biomarker panel to the incidence of the metabolic syndrome. Next, we related biomarkers significantly related to the metabolic syndrome (in the first step) to longitudinal change in the individual components of the metabolic syndrome. Using a backward elimination procedure, plasminogen activator inhibitor-1 and aldosterone were associated with incident metabolic syndrome. Plasminogen activator inhibitor-1 was positively associated with longitudinal changes in fasting glucose, systolic blood pressure, and triglycerides. Aldosterone was associated positively with change in systolic blood pressure and inversely with change in high-density lipoprotein cholesterol. We conclude that higher circulating plasminogen activator inhibitor-1 and aldosterone levels are associated with development of metabolic syndrome and with longitudinal change of its components, suggesting that these biomarkers and related pathways play a key role in mediating metabolic risk.

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