



## ORIGINAL CONTRIBUTIONS

### Clustering of Procoagulation, Inflammation, and Fibrinolysis Variables with Metabolic Factors in Insulin Resistance Syndrome

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The known metabolic cardiovascular disease risk factors associated with insulin resistance syndrome (IRS) do not adequately explain the excess cardiovascular disease risk attributed to this syndrome, and abnormalities in hemostatic variables may contribute to this excess risk. Using data from 322 nondiabetic elderly men and women (aged 65–100 years) participating in the Cardiovascular Health Study during 1989–1990, the authors performed factor analysis on 10 metabolic risk factors associated with IRS and 11 procoagulation, inflammation, and fibrinolysis variables to examine the clustering of the metabolic and hemostatic risk markers. Factor analysis of the metabolic variables confirmed four uncorrelated factors: body mass, insulin/glucose, lipids, and blood pressure. Adding the hemostatic variables yielded three new factors interpreted as inflammation, vitamin K-dependent proteins, and procoagulant activity. Plasminogen activator inhibitor-1 clustered with the body mass factor, supporting the hypothesis that obesity is related to impaired fibrinolysis. Fibrinogen clustered with the inflammation summary factor rather than procoagulant activity, supporting the position that fibrinogen principally reflects underlying inflammation rather than procoagulant potential. The authors conclude that should hemostatic variables be shown to contribute to IRS-related cardiovascular disease, apart from plasminogen activator inhibitor-1, they may do so independently of the established metabolic abnormalities. *Am J Epidemiol* 2000;152:897–907.

aged; aged, 80 and over; factor analysis, statistical; hemostasis; inflammation; insulin resistance; metabolism; multivariate analysis

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The known metabolic cardiovascular disease risk factors associated with insulin resistance syndrome do not ade-

quately explain the excess cardiovascular disease morbidity and mortality attributed to this syndrome. In general, insulin resistance syndrome consists of hypertension, glucose intolerance, obesity, and dyslipidemia (1, 2). Hemostatic variables have increasingly been shown to predict cardiovascular disease events (3–5), and some may mediate part of the excess cardiovascular disease risk associated with insulin resistance syndrome. There is also growing evidence that occlusive arterial events result from an interrelation between hemostatic and metabolic factors (6–8). Hemostatic variables may be independently causal or, since they are highly intercorrelated, may predict disease because they represent a class of variables that reflect a larger underlying disease mechanism.

Knowledge of how hemostatic variables cluster and how these clusters relate to metabolic variables could help clarify the role of hemostatic variables in cardiovascular disease pathophysiology and help researchers interpret and develop epidemiologic multivariate models. Although statistical analysis of clustering (factor analysis) is an uncommon

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Abbreviations: CV, coefficient of variation; PAI-1, plasminogen activator inhibitor-1; VKDP, vitamin K-dependent protein.

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approach in cardiovascular disease epidemiology, several studies (9–20) have applied factor analysis to metabolic variables associated with insulin resistance syndrome, providing insights into the underlying disease process. To our knowledge, factor analysis has not been used to describe the relations among metabolic and hemostatic variables.

The purpose of this study was to describe the clustering of hemostatic variables with metabolic variables in a well characterized subset of an elderly cohort (the Cardiovascular Health Study cohort). These data were used to confirm and expand on the factor analysis work of others by adding procoagulant, inflammation, and fibrinolysis variables, including those reported to be closely associated with insulin resistance syndrome: plasminogen activator inhibitor 1 (PAI-1), fibrinogen, and factor VIIc (7, 21–25).

## MATERIALS AND METHODS

### Population

The initial Cardiovascular Health Study cohort of 5,201 men and women aged 65–100 years was recruited from random samples of Medicare eligibility lists at four field centers, as reported previously (26, 27). In 1989 and 1990, baseline medication and lifestyle histories and phlebotomy samples were obtained and physical examinations were conducted (26).

From Cardiovascular Health Study participants who were free of clinical cardiovascular disease at the baseline examination ( $n = 3,352$ ), a subset of 400 persons was chosen for specialized coagulation assays. They were evenly distributed by sex and age strata (65–69, 70–74, 75–79, 80–84, and  $\geq 85$  years). Of the 400 participants in the subset, four subjects on warfarin therapy and 74 with diabetes mellitus were excluded, resulting in a total of 322 participants. A validation group ( $n = 2,681$ ) was selected from the entire Cardiovascular Health Study cohort; this group, comprising 1,690 women and 991 men, 96.3 percent of whom were White, was free of prevalent clinical cardiovascular disease, diabetes, and warfarin use and had values for the metabolic variables but not the hemostatic and inflammation variables.

### Definitions

Hypertension was defined as systolic blood pressure  $\geq 160$  mmHg or diastolic pressure  $\geq 95$  mmHg, or self-reported high blood pressure and use of antihypertensive medication. Obesity was defined as a body weight more than 20 percent over ideal body mass using the body mass index (weight (kg)/height (m)<sup>2</sup>). Waist:hip ratio was defined as waist circumference divided by hip circumference. Impaired glucose tolerance was defined as a fasting glucose level greater than 7.8 mmol/liter and a 2-hour post-glucose challenge level between 7.8 mmol/liter and 11.0 mmol/liter. Diabetes mellitus was defined as a fasting glucose level of  $>7.8$  mmol/liter or a glucose level of  $>11.1$  mmol/liter 2 hours after a glucose load, or use of insulin or oral hypoglycemic agents.

### Laboratory assays

Information on general quality assurance procedures and laboratory methods has been published elsewhere (28). The metabolic variables we utilized were weight, waist circumference, postload and fasting insulin, postload and fasting glucose, high density lipoprotein cholesterol, fasting triglycerides, and supine systolic and diastolic blood pressure (9). Lipids were measured by enzymatic methods under Centers for Disease Control and Prevention guidelines (28). Insulin was assayed by radioimmunoassay (Diagnostic Products Corporation, Malvern, Pennsylvania) (coefficient of variation (CV) = 19.0 percent at 73.9 pmol/liter); high density lipoprotein cholesterol by dextran sulfate/magnesium sulfate precipitation (Dow Diagnostics, Indianapolis, Indiana) and enzymatic methods (Kodak Ektachem 700; Kodak, Rochester, New York) (CV = 3.6 percent); and glucose and triglycerides by enzymatic methods (Kodak Ektachem 700) (CVs = 1.9 percent and 1.1 percent, respectively).

The hemostatic variables were grouped under the following headings for analytical purposes: inflammation (C-reactive protein, factor VIIIc, and fibrinogen); procoagulation (factors VIIc, IXc, and Xc, prothrombin fragment F1-2, and fibrinopeptide A); and fibrinolysis (plasmin- $\alpha_2$ -antiplasmin, fibrin fragment D-dimer, and PAI-1). Methods used were as follows. Fibrinogen was analyzed by means of the clot-rate assay (29, 30) (CV = 3 percent). Factors VIIc, VIIIc, IXc, and Xc were analyzed by one-stage clotting assays (CVs = 5 percent, 10 percent, 6 percent, and 5 percent, respectively). Fibrinopeptide A was measured on bentonite-extracted, fibrinogen-free plasma by radioimmunoassay (31, 32) (Byk-Sangtec Diagnostica, Dietzenbach, Germany) (CVs = 12.4 percent at 1.5 ng/ml and 8.4 percent at 7.7 ng/ml). C-reactive protein (33), prothrombin fragment F1-2 (32, 34), plasmin- $\alpha_2$ -antiplasmin (35), fibrin fragment D-dimer (36, 37), and PAI-1 (38) were analyzed by enzyme-linked immunosorbent assay (CVs = 8.9 percent, 8.5 percent, 3.6 percent, 7.0 percent, and 8.4 percent, respectively).

### Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences (39). Variables were age-adjusted by linear regression, and non-normally distributed variables were natural log-transformed. Bivariate Pearson's correlations between the variables were examined. Since a large number of analyses were carried out, a  $p$  value of 0.01 or less was used to indicate statistical significance.

Factor analysis (40–42), a linear method of data reduction, was performed using principal component analysis, a technique for reducing a number of original variables into fewer summary factors or principal components. The variables “cluster” on the basis of the linear correlations that exist among them. The resultant principal components are calculated to retain as much of the variance that exists in the original variables as possible, and to represent weighted summations of the original variables. In factor analysis, only the smallest number of factors that 1) yields a clear, interpretable pattern accurately reflecting the underlying physi-

ology and 2) explains a majority of the variance in the original variables should be retained.

The principal components are transformed (rotated) to enhance interpretation. We used varimax rotation, an orthogonal rotation method that retains the property of independence among the newly created principal components. Rotated principal components are referred to as summary factors. Eigenvalues are the sum of squared correlations between the original independent variables and the principal components, and they represent the amount of variance attributable to the components. Standardized variables each have a variance equal to 1.0. Thus, any summary factor with an eigenvalue of 1 or more indicates a factor accounting for more total variance than any original standardized variable. Only summary factors with an eigenvalue greater than or equal to 1 were selected for this analysis. In exploratory analyses, we constrained the principal component analyses to fewer factors than were retained based on the above-described criterion to determine whether more parsimonious models could accurately reflect the underlying physiology.

The factor loading of a variable on a summary factor equals the Pearson correlation coefficient between that variable and the summary factor. Only variables that shared  $\geq 15$  percent variance with a summary factor were used for interpretation (corresponding to a loading factor of  $\geq 0.40$ ).

Principal component analysis was performed first on metabolic variables and second with the addition of hemostatic variables. To examine whether the summary variables were equivalent to an underlying variable (43), we repeated the final analysis using principal axis factoring, which assumes that the observed original variables are causally influenced by a small number of latent variables. The summary factors represent these latent variables. Principal component analysis does not assume that the summary factors are causally related.

## RESULTS

### Descriptive statistics

The mean age of the 322 participants in our subset was 77 years (table 1). One third were hypertensive, and although persons known to have diabetes mellitus were excluded from the study, 33 percent of participants exhibited impaired glucose tolerance. Forty percent were obese. Sixteen percent of the women were on hormone replacement therapy.

### Bivariate associations

Among the metabolic variables, weight and waist circumference were positively associated with fasting insulin and glucose levels (table 2). High density lipoprotein cholesterol was negatively correlated with body mass (weight and waist circumference), fasting and postload insulin and glucose, and triglycerides, whereas triglycerides were correlated only with high density lipoprotein cholesterol and fasting and postload insulin. Systolic and diastolic blood pressure were related only to each other, and not to insulin, glucose, weight, or waist circumference.

Correlations among the hemostatic and metabolic variables are reported in table 3. Fibrinopeptide A and prothrombin fragment F1-2 did not correlate significantly with markers of inflammation, unlike the vitamin K-dependent proteins (VKDPs) (factors VIIc, IXc, and Xc). Among the VKDPs, factor IXc correlated the most strongly with inflammatory markers. Fibrin fragment D-dimer correlated strongly with both procoagulant variables and inflammatory markers.

As table 4 shows, PAI-1 levels were positively associated with body mass, glucose, and insulin. Neither fibrinogen nor markers of thrombin activity (prothrombin fragment F1-2 and fibrinopeptide A) were strongly related to metabolic factors. The VKDPs were strongly associated with triglycerides. Factor IXc, plasmin- $\alpha_2$ -antiplasmin, and C-reactive protein exhibited weaker but significant associations with several other metabolic factors.

### Factor analysis

Principal component analysis of the metabolic variables resulted in four summary factors: body mass, insulin/glucose, lipids, and blood pressure (table 5). This is consistent with previous findings in several nondiabetic populations (9–11, 13, 15, 19, 44). These four factors together explained 70 percent of the total variance (table 5). Substitution of body mass index and waist:hip ratio for weight and waist circumference, respectively, resulted in similar summary factors in the subset. Confirmatory analysis in the validation group ( $n = 2,681$ ) gave rise to four factors that we interpreted identically, though minor variations in the factor loading pattern were noted (table 6). In particular, fasting insulin and glucose loaded on the insulin/glucose factor in the confirmatory analysis but not in the original analysis, and fasting glucose loaded on the body mass factor in the original analysis but not in the confirmatory analysis. While relaxation of the factor loading threshold to 0.30 would have left only one point of difference between the analyses—the failure of fasting glucose to load on the insulin/glucose factor in the original analysis (factor loading = 0.28, vs. 0.48 in the confirmatory analysis)—we elected to use a stringent factor loading threshold of 0.40 to minimize the risk of identifying a spurious factor pattern in the small subset analysis.

Inclusion of the hemostatic variables in the analysis resulted in minimal change in the previously obtained summary factors, and it produced three additional summary factors (table 7, figure 1) when a factor loading threshold of 0.4 was employed. PAI-1 grouped with the body mass factor rather than with other fibrinolytic variables such as plasmin- $\alpha_2$ -antiplasmin and fibrin fragment D-dimer, or with the inflammation variables. Fibrinogen aggregated with inflammation-sensitive proteins rather than markers of procoagulant activity. Triglycerides clustered with both the lipid factor and the VKDP factor. Exclusion of participants using either diuretics/beta-blockers or hormone replacement therapy ( $n$  for analysis = 233) resulted in similar findings, with the exception that PAI-1 loaded less on body mass (factor loading = 0.17) and more on the insulin factor (0.45) and the lipid factor (0.35).

**TABLE 1. Characteristics of subjects in a subset of Cardiovascular Health Study participants (n = 322), 1989–1990**

Characteristic	Mean	Standard deviation	Range	Reference range*
Age (years)	77	7.4	65–100	
Female gender (%)	51.2			
Obesity† (%)	40.7			
Hypertension† (%)	31.3			
Impaired glucose tolerance‡ (%)	33.2			
Fasting glucose (mmol/liter)	5.5	0.6	4.4–7.7	4.2–6.9
Postload glucose (mmol/liter)	7.2	1.8	3.3–11.0	<7.8
Fasting insulin (pmol/liter)	92.8	53.4	35.9–430.5	50–244
Postload insulin (pmol/liter)	523	383	36–2,870	222–538
Triglycerides (mmol/liter)	1.4	0.7	0.6–6.8	0.7–2.8
High density lipoprotein cholesterol (mg/dl)	1.4	0.4	0.6–3.3	0.9–2.5
Body mass index‡	25.5	4.3	16.3–44.0	
Weight (kg)	69.3	14.4	37.2–114.3	
Waist circumference (cm)	92.4	12.6	64.5–135.4	
Waist:hip circumference ratio	0.93	0.08	0.61–1.18	
Systolic blood pressure (mmHg)	139	20	96–242	
Diastolic blood pressure (mmHg)	70	11	38–110	
C-reactive protein (mg/liter)	2.32	4.25	0.08–47.30	0.21–5.06
Fibrin fragment D-dimer (ng/ml)	281	441	12–5,184	6.9–190
Prothrombin fragment F1-2 (nmol/liter)	0.41	0.43	0.12–6.88	1.07–12.74
Factor IXc (%)	117	21.2	5–186	63–134
Factor VIIc (%)	121	26.5	69–237	64–149
Factor VIIIc (%)	122	35.3	42–234	72–168
Factor Xc (%)	115	19.4	30–182	72–192
Fibrinogen (g/liter)	3.22	0.67	1.09–6.56	2.16–5.04
Fibrinopeptide A (ng/ml)	9.13	38.9	0.9–496	1.59–16.41
Plasminogen activator inhibitor-1 (ng/ml)	35.5	29.6	3–293	4.8–67.0
Plasmin- $\alpha_2$ -antiplasmin (nmol/liter)	6.58	3.22	2.57–39.31	2.07–5.77

\* Reference ranges for glucose, insulin, triglycerides, high density lipoprotein cholesterol, and fibrinogen were established for the Cardiovascular Health Study as a whole (84). Reference ranges for other variables were obtained from a biovariability study of hemostatic factors conducted at the University of Vermont (85).

† See text for definition.

‡ Weight (kg)/height (m)<sup>2</sup>.

As table 7 shows, adoption of a more lenient factor loading threshold of 0.3 would yield additional overlap of variables across factors, rendering interpretation of the loading patterns more difficult in the context of our present understanding of the underlying biologic mechanisms. Fasting glucose would

load on both the body mass factor (0.34) and the insulin/glucose factor (0.36), as would PAI-1 (0.44 and 0.34, respectively). Plasmin- $\alpha_2$ -antiplasmin would load on the body mass factor (-0.34) in addition to the inflammation factor (0.70). Constraining the 21-variable factor analysis to six factors did

**TABLE 2. Age-adjusted Pearson correlations between metabolic variables associated with insulin resistance syndrome in a subset of Cardiovascular Health Study participants (n = 322), 1989–1990**

Variable	Waist circumference	Fasting insulin	Postload insulin	Fasting glucose	Postload glucose	High density lipoprotein cholesterol	Tri-glycerides	Systolic blood pressure	Diastolic blood pressure
Body weight	0.82**	0.43**	0.18*	0.35**	0.14	-0.33**	0.10	0.08	0.12
Waist circumference		0.46**	0.27**	0.29**	0.22**	-0.30**	0.11	0.06	0.08
Fasting insulin			0.45**	0.35**	0.20**	-0.25**	0.20**	0.03	0.00
Postload insulin				0.16*	0.53**	-0.15*	0.15*	0.03	0.00
Fasting glucose					0.30**	-0.24**	0.08	-0.01	0.09
Postload glucose						-0.16*	0.15	0.07	0.06
High density lipoprotein cholesterol							-0.42**	0.03	-0.09
Triglycerides								0.12	0.01
Systolic blood pressure									0.52**

\*  $p \leq 0.01$ ; \*\*  $p \leq 0.001$ .

**TABLE 3. Age-adjusted Pearson correlations between hemostatic and inflammatory variables in a subset of Cardiovascular Health Study participants (n = 322), 1989–1990**

Variable	Factor IXc	Factor Xc	Pro-thrombin fragment F1-2	Fibrinopeptide A	Fibrin fragment D-dimer	Plasmin- $\alpha_2$ -antiplasmin	Plasminogen activator inhibitor-1	Factor VIIIc	Fibrinogen	C-reactive protein
Factor VIIIc	0.40**	0.44**	0.14	0.09	0.09	0.22**	0.13	0.14	0.15*	0.13
Factor IXc		0.48**	0.04	0.07	0.25**	0.24**	0.19**	0.35**	0.37**	0.45**
Factor Xc			0.06	-0.04	-0.01	0.16*	0.18**	0.16*	0.22**	0.27**
Prothrombin fragment F1-2				0.55**	0.34**	0.22**	0.00	0.04	0.04	0.09
Fibrinopeptide A					0.24**	0.12	0.05	0.01	0.05	0.11
Fibrin fragment D-dimer						0.39**	0.01	0.22**	0.19**	0.28**
Plasmin- $\alpha_2$ -antiplasmin							-0.20**	0.35**	0.43**	0.23**
Plasminogen activator inhibitor-1								-0.06	0.14	0.25**
Factor VIIIc									0.27**	0.20**
Fibrinogen										0.47**

\*  $p \leq 0.01$ ; \*\*  $p \leq 0.001$ .

not alter the pattern of variables loading on the inflammation, VKDP, and procoagulation factors, and the blood pressure factor remained the most “isolated” of the factors, with no variable other than systolic and diastolic blood pressure having a factor loading greater than 0.10 (data not shown). Principal axis factoring, whether applied to all 21 variables or only to the 10 metabolic variables (in either the subset or the validation group), produced results similar to those of principal component analysis (data not shown).

**DISCUSSION**

The major findings of this study were: 1) the confirmation of previously reported metabolic summary factors (10, 13)

in the nondiabetic healthy elderly: lipids, body mass, insulin/glucose, and blood pressure; 2) the identification of three new summary factors upon the addition of hemostatic variables: inflammation-sensitive proteins, markers of procoagulant activity, and VKDPs; 3) the clustering of PAI-1 with the body mass summary factor, rather than either other fibrinolytic variables or inflammation variables; and 4) the clustering of fibrinogen with inflammation variables rather than markers of procoagulation such as fibrinopeptide A and prothrombin fragment F1-2.

The four metabolic factors we identified are similar to those identified in several previous reports on nondiabetic populations (9–11, 13, 15, 19, 44), including two that studied metabolic variables in older populations (10, 13). A

**TABLE 4. Age-adjusted Pearson correlations between metabolic variables and hemostatic and inflammatory variables in a subset of Cardiovascular Health Study participants (n = 322), 1989–1990**

Variable	Weight	Waist circumference	Fasting insulin	Postload insulin	Fasting glucose	Postload glucose	High density lipoprotein cholesterol	Tri-glycerides	Systolic blood pressure	Diastolic blood pressure
<b>Procoagulation</b>										
Factor VIIIc	-0.12	-0.07	0.03	0.12	-0.07	0.10	0.17*	0.40**	0.12	-0.06
Factor IXc	0.21**	0.31**	0.14	0.26**	0.12	0.27**	-0.09	0.31**	0.04	-0.03
Factor Xc	0.03	0.11	0.11	0.30**	0.06	0.15*	0.01	0.32**	0.03	-0.05
Prothrombin fragment F1-2	-0.15*	-0.10	-0.05	-0.03	-0.15*	-0.04	0.07	0.10	0.00	-0.09
Fibrinopeptide A	-0.03	0.03	0.01	0.00	-0.11	0.05	0.03	0.05	0.04	0.04
<b>Fibrinolysis</b>										
Fibrin fragment D-dimer	0.01	0.07	-0.11	-0.11	-0.06	-0.05	-0.04	0.07	0.00	-0.01
Plasmin- $\alpha_2$ -antiplasmin	-0.24**	-0.22**	-0.27**	-0.14*	-0.16*	-0.09	0.13	0.00	0.02	0.02
Plasminogen activator inhibitor-1	0.26**	0.29**	0.30**	0.32**	0.20**	0.19**	-0.20**	0.11	0.08	0.03
<b>Inflammation</b>										
Factor VIIIc	0.00	0.02	0.04	0.14	-0.01	0.08	0.00	0.06	0.00	-0.02
Fibrinogen	0.05	0.11	0.12	0.11	0.16*	0.05	-0.15*	0.10	0.02	-0.02
C-reactive protein	0.20**	0.24**	0.18**	0.18*	0.14	0.17*	-0.21**	0.13	0.09	-0.01

\*  $p \leq 0.01$ ; \*\*  $p \leq 0.001$ .

**TABLE 5. Results of factor analysis with metabolic variables, factors, and factor loadings, Cardiovascular Health Study subset (*n* = 322), 1989–1990**

Variable	Factor			
	Body mass	Insulin/glucose	Blood pressure	Lipids
Body weight	0.92*	-0.03	0.09	0.09
Waist circumference	0.89*	0.08	0.05	0.07
Fasting insulin	0.59*	0.38	-0.07	0.17
Fasting glucose	0.50*	0.28	0.02	0.08
Postload insulin	0.18	0.84*	-0.03	0.06
Postload glucose	0.10	0.83*	0.08	0.07
Systolic blood pressure	-0.01	0.06	0.87*	0.04
Diastolic blood pressure	0.09	-0.01	0.87*	<0.01
Triglycerides	-0.03	0.13	0.06	0.87*
High density lipoprotein cholesterol	-0.33	-0.02	0.01	-0.76*
% total variance	24.0	16.6	15.4	14.1
% cumulative variance	24.0	40.5	55.9	70.0

\* Factor loading  $\geq 0.40$ .**TABLE 6. Results of factor analysis with metabolic variables, factors, and factor loadings, Cardiovascular Health Study validation group (*n* = 2,681), 1989–1990**

Variable	Factor			
	Body mass	Insulin/glucose	Lipids	Blood pressure
Body weight	0.92*	0.06	0.14	0.05
Waist circumference	0.89*	0.13	0.15	<0.01
Postload glucose	<0.01	0.82*	<0.01	0.08
Postload insulin	0.07	0.82*	0.22	-0.03
Fasting insulin	0.44*	0.49*	0.33	-0.02
Fasting glucose	0.39	0.48*	<0.01	0.13
Triglycerides	0.02	0.23	0.85*	0.03
High density lipoprotein cholesterol	-0.32	0.01	-0.77*	-0.02
Diastolic blood pressure	0.07	-0.04	0.03	0.85*
Systolic blood pressure	<0.01	0.13	0.01	0.84*
% total variance	21.2	19.0	15.2	14.5
% cumulative variance	21.2	40.2	55.4	69.9

\* Factor loading  $\geq 0.40$ .

study of nondiabetic men and women aged 50–89 years (*n* = 606 men, 765 women) identified four factors interpreted as body size, glucose tolerance, lipids, and blood pressure (10). The pattern of variables loading on each factor was nearly identical to that observed in the validation group (table 6), differing only by 1) the failure of fasting insulin to load on the glucose tolerance factor; 2) the loading of fasting glucose on glucose tolerance only in women; and 3) the loading of low density lipoprotein cholesterol on the lipids factor (low density lipoprotein cholesterol was not studied in the present analysis) (10).

A study of older, nondiabetic Japanese-American men (mean age = 77.6 years (standard deviation 4.6); *n* = 2,760)

yielded four factors interpreted as weight/waist, blood pressure, lipids, and insulin/glucose (13). Both the pattern of variables loading on each factor and the factor loadings were similar to those in the present study, though postload glucose and insulin were excluded from the analysis. Body weight, waist circumference, and fasting insulin loaded on a weight/waist factor, with factor loadings of 0.93, 0.92, and 0.46, respectively (13), compared with loadings of 0.92, 0.89, and 0.44 on the body mass factor in this study's validation group (table 6). Similarly, systolic and diastolic blood pressure both had loadings of 0.88 on a blood pressure factor (13), compared with 0.84 and 0.85, respectively, in this study (table 6). Taken together, these data from multiple studies of populations that were similar in terms of age support the existence of distinct pathophysiologic mechanisms underlying the observed clustering of metabolic variables related to insulin resistance syndrome in older adults.

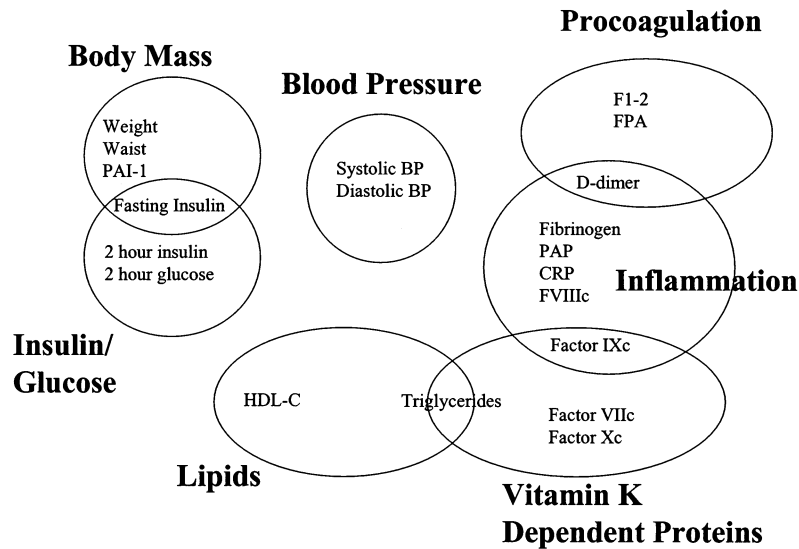
Three additional factors—inflammation, VKDPs, and procoagulation—were identified in a factor analysis involving 11 hemostatic and inflammatory variables in addition to the 10 metabolic variables. Though these factors, like the four metabolic factors, are uncorrelated, three variables loaded on more than one factor, including triglycerides, which correlated not only with the lipids factor but also with the VKDPs. This “splitting” of triglycerides into a VKDP factor and a lipid factor is of uncertain significance, and it may reflect multiple roles of triglyceride-bearing lipoproteins. Any explanation for the association between triglycerides and factor VIIc is currently speculative; it may be due to a role of triglyceride-bearing lipoproteins as procoagulant surfaces (45). Additionally, two hemostatic variables, factor IXc and fibrin fragment D-dimer, loaded not only on the VKDP and procoagulation factors, respectively, but also on the inflammation factor. Variables such as these, which are significantly related to more than one summary factor, suggest possible points of interrelation between the representative biologic processes (for example, in the case of fibrin fragment D-dimer, inflammation and thrombin activity). The statistical clustering of hemostatic factors may also provide useful information in terms of developing multivariate models. The clusters suggest potential collinear variables in multivariate models, which may lead to unreliable estimates of risk, and highly correlated variables that may represent aspects of a common pathophysiology such as inflammation (46).

PAI-1 loaded significantly only on the body mass summary factor, and not on the inflammation or procoagulation factors. The fact that PAI-1 clustered with body weight, waist circumference, and fasting insulin fits well with previous observations of associations between PAI-1 and both insulin (47, 48) and body mass (49, 50), though the relative strengths of these associations, as well as the association between PAI-1 and lipids (51, 52), have been debated. PAI-1 is also considered an acute-phase protein, responsive to changes in inflammatory status (53). Our results are most consistent with an association of PAI-1 with obesity, possibly mediated through the production of PAI-1 by adipose tissue (49, 50, 54), though adipocyte synthesis of PAI-1 *in vivo* remains controversial (55). Alternatively, obesity may

**TABLE 7. Results of factor analysis with metabolic, hemostatic, and inflammatory variables, factors, and factor loadings, Cardiovascular Health Study subset (n = 322), 1989–1990**

Variable	Factor						
	Body mass	Inflam-mation	VKDPs*	Insulin/glucose	Pro-coagula-tion	Blood pressure	Lipids
Waist circumference	0.90†	0.08	0.01	0.07	<0.01	0.04	0.05
Body weight	0.90†	0.03	-0.07	<0.01	-0.08	0.09	0.10
Fasting insulin	0.57†	-0.05	0.07	0.40†	-0.03	-0.06	0.16
Plasminogen activator inhibitor-1	0.44†	-0.03	0.19	0.34	0.10	0.04	0.04
Fibrinogen	0.06	0.74†	0.06	0.09	-0.03	-0.01	0.14
Plasmin- $\alpha_2$ -antiplasmin	-0.34	0.70†	0.11	-0.21	0.16	0.06	-0.08
Factor VIIIc	-0.06	0.62†	0.10	0.10	-0.05	-0.03	-0.09
C-reactive protein	0.28	0.62†	0.12	0.14	0.12	0.03	0.12
Factor IXc	0.28	0.54†	0.53†	0.14	0.02	-0.02	0.03
Fibrin fragment D-dimer	0.08	0.50†	-0.04	-0.25	0.48†	<0.01	0.06
Factor VIIc	-0.10	0.10	0.83†	0.02	0.11	0.05	<0.01
Factor Xc	0.08	0.22	0.73†	0.17	-0.08	-0.04	<0.01
Postload glucose	0.05	0.09	0.04	0.81†	0.02	0.08	0.09
Postload insulin	0.20	0.04	0.20	0.80†	<0.01	-0.04	-0.02
Fasting glucose	0.34	0.13	-0.18	0.36	-0.22	0.03	0.25
Prothrombin fragment F1-2	-0.11	0.05	0.09	-0.02	0.85†	-0.07	0.01
Fibrinopeptide A	0.03	0.02	-0.02	0.07	0.84†	0.06	-0.03
Diastolic blood pressure	0.04	0.01	-0.12	0.03	-0.03	0.87†	0.05
Systolic blood pressure	0.04	-0.01	0.14	0.02	0.03	0.87†	-0.02
High density lipoprotein cholesterol	-0.25	-0.09	0.17	-0.11	0.04	<0.01	-0.84†
Triglycerides	0.03	-0.02	0.55†	0.03	0.09	0.05	0.73†
% total variance	12.7	11.7	9.7	9.1	8.6	7.4	6.7
% cumulative variance	12.7	24.4	34.1	43.2	51.8	59.1	65.9

\* VKDPs, vitamin K-dependent proteins.  
 † Factor loading  $\geq 0.40$ .



**FIGURE 1.** The factor loading pattern of metabolic, inflammatory, and hemostatic variables related to insulin resistance syndrome in 322 non-diabetic adults aged 65–100 years, Cardiovascular Health Study, 1989–1990. The 21 intercorrelated variables load on seven uncorrelated factors, which are represented by the large ellipses. While the seven factors are uncorrelated, there are four variables that have factor loadings  $\geq 0.40$  on more than one of the factors; these variables lie in the regions of overlap between ellipses. F1-2, prothrombin fragment 1-2; FPA, fibrinopeptide A; PAI-1, plasminogen activator inhibitor-1; BP, blood pressure; D-dimer, fibrin fragment D-dimer; PAP, plasmin- $\alpha_2$ -antiplasmin; CRP, C-reactive protein; FVIIIc, factor VIIIc; HDL-C, high density lipoprotein cholesterol.

influence PAI-1 levels through increased synthesis of tumor necrosis factor- $\alpha$ , a proinflammatory cytokine, by adipose tissue (56) and muscle (57). Both tumor necrosis factor- $\alpha$  (53) and insulin (58) have been shown to up-regulate endothelial cell production of PAI-1. However, on the basis of our findings after exclusion of participants using beta-blockers and diuretics, we cannot rule out an additional relation between insulin and triglycerides affecting PAI-1 levels, as suggested by others (59); such a linkage may also be mediated by adipocyte production of tumor necrosis factor- $\alpha$  (60).

Fibrinogen loaded on the inflammation factor but not with prothrombin fragment F1-2, fibrinopeptide A, and fibrin fragment D-dimer on the procoagulation factor, which is consistent with a hypothesis that regulation of fibrinogen concentration is more closely related to inflammation than to coagulation status in the Cardiovascular Health Study population (32). Despite associations of insulin resistance syndrome with fibrinogen (7, 25) and prothrombin fragment F1-2 (61, 62), these variables, along with other markers of inflammation and thrombin, did not cluster with a metabolic summary factor. This may suggest a weaker relation between procoagulant variables and a prediabetic syndrome, or that inflammation and procoagulation contribute to cardiovascular disease risk independently of known insulin resistance syndrome factors (63, 64). Our results are most consistent with the position that fibrinogen principally reflects the severity of underlying disease rather than confers increased procoagulant potential (65).

Given the mounting evidence that inflammation links insulin resistance syndrome and cardiovascular disease (66, 67), we might have expected to observe C-reactive protein or other acute phase proteins loading on a metabolic summary factor such as body mass or insulin/glucose in addition to the inflammation factor. Pathophysiologic bases for such an expectation include adipocyte production of proinflammatory factors, including tumor necrosis factor- $\alpha$  and leptin, that diminish insulin sensitivity (68–71) and induce synthesis of acute phase proteins (56, 72). Furthermore, markers of inflammation have recently been shown to predict the development of type II diabetes mellitus in middle-aged adults (73). However, acute phase proteins have consistently been shown to predict cardiovascular disease risk independently of other risk factors in numerous epidemiologic studies (63, 74–79); and it is possible that the advanced age of our study participants resulted in circulating levels of C-reactive protein and fibrinogen being more strongly influenced by the accumulated atherosclerotic burden than by adipose tissue mass, which is consistent with the “proximate pathophysiology” hypothesis advanced to explain the relations among coagulant activity, inflammation, and the onset of clinical cardiovascular disease (65).

The strengths of the current study include a carefully designed parent study with appropriate blood collection and storage procedures and validation of our subgroup by confirmation of the initial metabolic factor analysis in the larger cohort. We attempted to minimize the effect of the relatively small size of the subgroup by restricting our interpretation of summary factors to those variables that shared at least 15

percent variance with a summary factor (that is, variables with factor loadings of at least 0.40). We also chose to use body weight and waist circumference as anthropometric variables in the final analyses, since the use of ratio-based indices of obesity such as body mass index and waist:hip ratio has recently been called into question (80, 81) and ratio measures in general have the potential to give rise to spurious correlations (82, 83).

In conclusion, the clustering of hemostatic and metabolic variables with summary factors suggests underlying pathologic mechanisms in insulin resistance syndrome. Of the hemostatic variables examined, only PAI-1 was significantly correlated with the established metabolic summary factors of insulin resistance syndrome. This suggests that markers of inflammation and procoagulation, while often correlated with individual metabolic variables, may reflect separate underlying processes. If these hemostatic variables are proven to contribute to the cardiovascular disease complications of insulin resistance syndrome in future studies, our results suggest that they may do so apart from the established metabolic abnormalities, and that measurement of these variables may yield additional information about cardiovascular disease risk. Extension of these analyses to populations with a high prevalence of insulin resistance may be useful in further determining the role of hemostatic variables in insulin resistance syndrome.

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