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Markers of Inflammation and Cellular Adhesion Molecules in Relation to Insulin Resistance in Nondiabetic Elderly: The Rotterdam Study

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Insulin resistance, which is highly prevalent in the elderly, is suggested to be accompanied by an increased acute phase response. Until now, it is unclear whether cellular adhesion molecules are involved in the clustering of insulin resistance.

In the present study, we examined the relationship of insulin resistance (measured by postload insulin) with levels of markers of inflammation and cellular adhesion molecules in a random sample of 574 nondiabetic elderly men and women participating in the Rotterdam Study. Associations were assessed by regression analysis, with ln-insulin as the dependent variable [regression coefficient (95% confidence interval)].

In our population, insulin was strongly and significantly ($P < 0.001$) associated with the markers of inflammation C-reactive protein [1.52 (0.96–2.08)], α-1-antichymotrypsin [1.25 (0.82–1.69)], and IL-6 [2.60 (1.69–3.52)], adjusted for age and gender. Associations weakened, to some extent, after additional adjustment for measures of obesity, smoking, and cardiovascular disease. Insulin was associated with the soluble intercellular adhesion molecule 1 [2.22 (1.29–3.16; $P < 0.001$)], whereas no association with the soluble vascular cell adhesion molecule 1 was found. The strength of the associations of insulin with C-reactive protein, α-1-antichymotrypsin, IL-6, and soluble intercellular adhesion molecule 1, as assessed by standardized regression coefficients, was comparable with the strength of the associations of insulin with high-density lipoprotein cholesterol, body mass index, and waist-to-hip ratio.

The results of this population-based study indicate that low-grade inflammation and the cellular adhesion molecule soluble intercellular adhesion molecule 1 are an integral part of insulin resistance in nondiabetic elderly. These factors may contribute to the well-known relationship between insulin resistance and cardiovascular disease risk and might potentially become therapeutic targets in insulin resistant subjects. (*J Clin Endocrinol Metab* 86: 4398–4405, 2001)

THE INSULIN RESISTANCE syndrome involves clustering of several metabolic cardiovascular disease risk factors: raised insulin, dyslipidemia, obesity, increased abdominal fat, and hypertension (1–3). Insulin resistance is highly prevalent in the elderly (4) and is associated with cardiovascular disease risk. Recent data suggest that inflammation plays a crucial role in atherogenesis (5) and that also insulin resistance may be accompanied by an increased acute-phase response, both in subjects with (6) and without diabetes mellitus (7, 8). A link between insulin resistance and the inflammatory state is further suggested by increased levels of the acute-phase proteins plasminogen activator inhibitor-1 and fibrinogen in the insulin resistance syndrome (9, 10), and by the finding that dyslipidemia in the insulin resistance syndrome and during the acute phase response show strong similarities (11, 12).

Increased levels of circulating cellular adhesion molecules have been shown among diabetic subjects, compared with nondiabetic controls (13, 14). Cellular adhesion molecules mediate the attachment and transmigration of leukocytes across the endothelial surface in response to several inflammatory cytokines (15), and are hypothesized to play an important role in the initiation of atherosclerosis (16). Until now, it is unclear whether cellular adhesion molecules are involved in the clustering of insulin resistance.

To further clarify whether inflammation and endothelial activation are an integral part of the insulin resistance syndrome, we cross-sectionally examined associations of levels of markers of inflammation and cellular adhesion molecules with insulin resistance (measured by postload insulin) in a population of nondiabetic elderly men and women participating in the Rotterdam Study.

Abbreviations: ACT, α-1-Antichymotrypsin; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; ECG, electrocardiogram; HDL, high-density lipoprotein; PAD, peripheral arterial disease; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; WHO, World Health Organization; WHR, waist-to-hip ratio.

Subjects and Methods

Study population

The Rotterdam Study is a population-based cohort study aiming to assess the occurrence of chronic diseases in an aging population and to clarify their determinants (17). The cohort includes 3105 men and 4878 women, 55 yr old and older (78% of the eligible population), living in a defined district in Rotterdam, The Netherlands. Baseline data were

collected from August 1990 until July 1993. Information on current and past health, medication, lifestyle, and risk factors for chronic diseases was gathered during a home interview by a trained research assistant. The participants were subsequently invited to a research center for clinical examination. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

Levels of inflammatory markers and cellular adhesion molecules were determined in plasma in a gender-stratified random sample of 720 subjects. As part of the Rotterdam Study, glucose metabolism was studied using a nonfasting oral glucose tolerance test. Within the sample of 720 subjects, postload insulin levels were available for 602 participants not prescribed glucose-lowering medication. Because postload insulin is not considered to be a valid measure of insulin resistance in diabetic subjects, subjects with newly diagnosed diabetes mellitus on basis of postload serum glucose levels (≥11.1 mM, n = 28) were excluded, leaving a population of 574 nondiabetic subjects. Given gender distribution, the prevalence of cardiovascular disease risk factors in the 574 subjects was comparable with the prevalence of these risk factors in the total nondiabetic population of the Rotterdam Study.

Clinical examination and laboratory methods

Height, weight, and waist and hip circumferences were measured while the study participants wore indoor clothes without shoes. Body mass index (BMI, weight divided by height squared) and waist-to-hip ratio (WHR) were computed. Sitting systolic and diastolic blood pressures were measured with a random-zero sphygmomanometer by a trained research assistant, after a 5-min rest, and a standard 12-lead electrocardiogram (ECG) was obtained (ACTA ECG recorder, Esoate, Florence, Italy). The presence of peripheral arterial disease (PAD) was evaluated by measuring the systolic blood pressure of the posterior tibial artery at both the right and the left leg using an 8-MHz continuous-wave Doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random-zero sphygmomanometer. For each leg, a single blood pressure reading was taken with the subject in the supine position. The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm was calculated for each leg.

A venipuncture was performed, and nonfasting blood samples were obtained and were directly put on ice. Serum samples were processed within 30 min, after which they were kept frozen at −20 C. We used an automated enzymatic procedure to determine serum total cholesterol level (18). High-density lipoprotein (HDL) cholesterol was measured similarly, after precipitation of the non-HDL fraction. All participants not prescribed glucose-lowering medication received a glucose drink of 75 g in 200 mL water after a first venipuncture. Two hours later, a second venous blood sample was obtained. Glucose levels were determined in both blood samples by the glucose hexokinase method, whereas insulin was measured by RIA (Medgenix Diagnostics, Brussels, Belgium). This assay has a cross-reactivity with proinsulin of 40%. Because subjects using glucose-lowering medication did not undergo the glucose tolerance test, insulin was not measured in this group. The coefficients of variation of glucose and insulin measurements were less than 2.5% and 6.0%, respectively.

Levels of inflammatory markers and cellular adhesion molecules were measured in plasma. For the collection of plasma, blood was collected in tubes containing 0.129 M sodium citrate. All tubes were stored on ice before and after blood sampling. Plasma was obtained by centrifugation of 30 min, at 10,000 rotations/min, at 10 C, and was immediately frozen in liquid nitrogen and stored at −80 C. Plasma concentrations of C-reactive protein (CRP) and α-1-antichymotrypsin (ACT) were measured by kinetic nephelometry (Behring Nephelometer BN200, Marburg, Germany) after a 5× dilution using Behring's N-dilutent. Levels of IL-6, soluble intercellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule 1 (sVCAM-1) were determined by means of enzyme-linked immunosorbent assay (IL-6: Quantikine; sICAM-1 and sVCAM-1: Parameter, R&D Systems Europe, Oxon, United Kingdom). Interassay coefficients of variation were 4.4%, 2.8%, 8.7%, 6.9%, and 5.0% for CRP, ACT, IL-6, sICAM-1, and s-VCAM, respectively. Corresponding intraassay coefficients of variation were 2.6%, 3.7%, 5.7%, 5.0%, and 3.1%, respectively. For 16, 16, 6, 3, and 3 subjects, respectively, we could not determine levels of CRP, ACT, IL-6, sICAM-1, and sVCAM-1 because of insufficient plasma for analysis. Levels of CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were therefore available for analysis in the following number of subjects: 562 (265 men, 297 women), 562 (264 men, 298 women), 570 (271 men, 299 women), 571 (271 men, 300 women), and 572 (271 men, 301 women), respectively.

Metabolic disorders

Diabetes mellitus was defined as the use of glucose-lowering medication or a random or postload serum glucose level ≥ 11.1 mM according to the World Health Organization (WHO) criteria (19). Impaired glucose tolerance was considered present when the postload serum glucose level was between 7.8 and 11.1 mM in subjects without diabetes mellitus (4). Postload insulin was used as a measure of insulin resistance. Dyslipidemia was defined as a total cholesterol level ≥ 8.0 mM, and/or an HDL cholesterol level < 0.9 mM (20), and/or use of lipid lowering medication. We defined obesity as BMI ≥ 30.0 kg/m^2 in both genders, and/or waist circumference ≥ 102 cm in men, and/or waist circumference ≥ 88 cm in women according to WHO criteria (21). Hypertension was defined as systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg and/or use of antihypertensive medication, encompassing grade 2 and grade 3 hypertension according to WHO criteria (22).

Prevalent cardiovascular disease

The presence of myocardial infarction was assessed by self-report and by analysis of the standard 12-lead ECGs, which were stored digitally and analyzed by the modular ECG analysis system (23, 24). From subjects with self-reported myocardial infarction without ECG evidence, we collected additional information from the general practitioner or cardiologist, and myocardial infarction was confirmed if the information in the medical records met standard diagnostic criteria. From subjects without self-reported myocardial infarction but with ECG evidence, in whom the absence of symptoms was confirmed by reviewing the medical records, ECGs were reviewed by an experienced cardiologist, and (silent) myocardial infarction was included when the ECG met standard diagnostic criteria for myocardial infarction. We combined both types of myocardial infarctions to one variable for the analyses (25). Information on a history of coronary artery bypass graft or percutaneous transluminal coronary angioplasty was obtained during the interview. A history of stroke was determined on the basis of interview data and medical information from the general practitioner or from hospital discharge records (26). PAD was considered to be present if the ankle-arm systolic blood pressure index was less than 0.90 in either leg (27).

Statistical analysis

To obtain normal distributions, insulin, CRP, and IL-6 were natural-log transformed (ln-transformation).

The associations between levels of postload insulin and markers of inflammation and cellular adhesion molecules were assessed by separate linear regression models with ln-insulin as the dependent variable and with levels of markers of inflammation and cellular adhesion molecules as independent variables. Associations were examined for the total population and for men and women separately. Models were initially adjusted for age and, if appropriate, gender. In subsequent models, we additionally adjusted for BMI, WHR, smoking (never, former, current), and presence of cardiovascular disease.

Furthermore, multivariate-adjusted levels of markers of inflammation and cellular adhesion molecules were assessed in tertiles of levels of postload insulin. For these analyses, we constructed variables with the values 1–3 for subsequent tertiles of levels of postload insulin for the total population, and for men and women separately. These variables were entered in general linear models as continuous independent variables with levels of markers of inflammation and cellular adhesion molecules as dependent variables. Tests of significance for the coefficients of the ordered variables of insulin were considered to be tests for trend.

Subsequently, we compared the strength of the associations of postload insulin with markers of inflammation and cellular adhesion molecules to the strength of the associations of insulin with variables classically considered to be clustered within the insulin resistance syndrome. For this endeavor, we performed separate linear regression

analyses with postload ln-insulin as the dependent variable and levels of markers of inflammation, cellular adhesion molecules, and HDL cholesterol, BMI, WHR, and systolic blood pressure as independent variables, and presented standardized regression coefficients of multivariate-adjusted analyses.

In addition, we computed levels of markers of inflammation and cellular adhesion molecules in subjects according to the presence of the number of metabolic disorders known to be clustered within the insulin resistance syndrome: impaired glucose tolerance, dyslipidemia, obesity, and hypertension. For these analyses we constructed a variable with value 0–4 according to the number of metabolic disorders present. This variable was entered in general linear models as a continuous independent variable, with levels of markers of inflammation and cellular adhesion molecules as dependent variables. Tests of significance for the coefficients of the ordered variable of the number of metabolic disorders present were considered to be tests for trend.

We considered P-values < 0.05 to be statistically significant. SPSS 9.0 for Windows (SPSS Inc., Chicago, IL) was used for all analyses.

Results

Characteristics of the population are described in Table 1. Levels of CRP ranged from 0.01–48.7 mg/liter; 35 subjects had values greater than 10 mg/liter, the cut-point generally used to identify clinically relevant inflammation.

Correlations between the levels of the three markers of inflammation were moderate; CRP–IL-6 r = 0.53, CRP–ACT r = 0.40, ACT–IL-6 r = 0.26, $P < 0.001$, adjusted for age and gender. The levels of CRP, ACT, and IL-6 were strongly associated with levels of postload insulin (Table 2). Associations with CRP tended to be somewhat stronger in women than in men. Multivariate adjustment decreased the strength of the associations to some extent (Table 2). Adjusting the association between CRP and postload insulin for the other two inflammatory markers removed the association (β = 0.42, $P = 0.23$), whereas the effect of controlling for the other two inflammatory markers on the strength of the association between ACT and insulin, and IL-6 and insulin was less pronounced ($\beta = 0.95$, and $\beta = 1.87$, $P \leq 0.001$, respectively, adjusted for age and gender). Levels of the cellular adhesion molecule sICAM-1 were associated with levels of CRP, ACT, and IL-6 (correlation coefficients r = 0.28, r = 0.20, and r = 0.24, respectively, all $P < 0.001$), whereas no associations between levels of sVCAM-1 and levels of markers of inflammation were found (r = 0.05, r = −0.02, r = 0.08, respectively, not statistically significant), all adjusted for age and gender. Levels of sICAM-1 showed a strong association with postload insulin as well, whereas levels of sVCAM-1 did not (Table 2). Adjustment of the associations between levels of sICAM-1 and postload insulin for levels of CRP, ACT, or IL-6 did not materially affect the results (data not shown). Levels of sICAM-1 were associated with other parameters of the insulin resistance syndrome, namely WHR (r = 0.09, P = 0.045) and HDL cholesterol (r = −0.15, $P = 0.001$), adjusted for age and gender, whereas levels of sVCAM-1 were not (r = 0.06 and −0.05, respectively, not statistically significant). Exclusion of subjects with impaired glucose tolerance slightly weakened the strength of the described associations (data not shown). Exclusion of subjects with levels of CRP more than 10 mg/liter did not affect the results (data not shown). As-

TABLE 1. Clinical and biochemical characteristics of the study population

Variable	All subjects, n = 574	Men, n = 272	Women, n = 302
Age, yr	70.2 ± 8.9	69.7 ± 8.3	70.7 ± 9.3
BMI, kg/m^2	26.4 ± 3.4	25.9 ± 2.8	26.8 ± 3.8
Waist circumference, cm	91 ± 11	94 ± 9	87 ± 11
WHR, cm/cm	0.91 ± 0.09	0.96 ± 0.07	0.87 ± 0.08
Obesity, % (n)[a]	32 (181)	18 (50)	43 (131)
Smoking status, % (n)			
Never	34 (198)	9 (25)	57 (173)
Past	44 (250)	64 (174)	25 (76)
Current	22 (126)	27 (73)	18 (53)
Systolic blood pressure, mm Hg	139 ± 21	138 ± 20	139 ± 22
Diastolic blood pressure, mm Hg	73 ± 11	74 ± 11	73 ± 11
Hypertension, % (n)[b]	26 (147)	24 (65)	27 (82)
Total cholesterol, mM	6.6 ± 1.2	6.3 ± 1.2	6.9 ± 1.2
HDL cholesterol, mM	1.3 ± 0.4	1.2 ± 0.4	1.5 ± 0.3
Dyslipidemia, % (n)[c]	24 (135)	26 (70)	21 (65)
Glucose, mmol/liter	6.4 ± 1.6	6.2 ± 1.6	6.6 ± 1.6
Postload insulin, mU/liter[d]	52.7 (31.0–76.0)	51.0 (29.0–73.0)	53.0 (35.0–81.0)
Impaired glucose tolerance, % (n)[e]	19 (107)	14 (37)	23 (70)
Cardiovascular disease, % (n)[f]	22 (127)	23 (63)	21 (64)
CRP, mg/liter[d,g]	1.6 (0.8–3.5)	1.4 (0.7–3.6)	1.8 (0.8–3.4)
ACT, mg/dl[g]	47.1 ± 14.0	46.1 ± 14.1	48.0 ± 13.8
IL-6, pg/ml[d,g]	1.8 (1.2–3.0)	1.9 (1.4–3.1)	1.7 (1.2–2.8)
sICAM-1, ng/ml[g]	220.9 ± 64.6	224.4 ± 72.5	217.7 ± 56.5
sVCAM-1, ng/ml[g]	541.8 ± 180.8	547.6 ± 169.5	536.5 ± 190.5

Data are mean ± SD, median (interquartile range) for variables with skewed distributions, or percentages (number of subjects).

[a] BMI ≥ 30.0 kg/m^2 in both genders, and/or waist circumference ≥ 102 cm in men, and/or waist circumference ≥ 88 cm in women.

[b] Systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg, and/or use of antihypertensive medication.

[c] Total cholesterol level ≥ 8.0 mM, and/or HDL cholesterol level < 0.9 mM, and/or use of lipid lowering medication.

[d] Skewed data.

[e] Postload serum glucose between 7.8 and 11.1 mM in subjects without diabetes mellitus.

[f] Presence of PAD and/or history of myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, or stroke.

[g] Levels of CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were available in the following numbers of subjects: 562 (265 men, 297 women), 562 (264 men, 298 women), 570 (271 men, 299 women), 571 (271 men, 300 women), and 572 (271 men, 301 women), respectively.

TABLE 2. Regression coefficients for ln-insulin[a] as the dependent variable and markers of inflammation and cellular adhesion molecules as independent variables

	All subjects		Men		Women	
	β^b	95% CI	β^b	95% CI	β^b	95% CI
CRP, mg/liter[c]						
Model 1[d]	1.52	(0.96; 2.08)	1.10	(0.26; 1.94)	1.97	(1.22; 2.72)
Model 2[e]	0.88	(0.24; 1.51)	0.43	(−0.49; 1.35)	1.40	(0.49; 2.30)
ACT, 100 mg/dl						
Model 1[d]	1.25	(0.82; 1.69)	1.26	(0.60; 1.92)	1.28	(0.70; 1.85)
Model 2[e]	1.09	(0.62; 1.60)	1.18	(0.48; 1.87)	1.09	(0.42; 1.75)
IL-6, pg/ml[c]						
Model 1[d]	2.60	(1.69; 3.52)	2.60	(1.26; 3.94)	2.62	(1.37; 3.87)
Model 2[e]	1.91	(0.92; 2.90)	2.02	(0.62; 3.42)	1.88	(0.46; 3.31)
sICAM-1, 1 μg/ml						
Model 1[d]	2.22	(1.29; 3.16)	2.10	(0.85; 3.36)	2.44	(1.01; 3.87)
Model 2[e]	1.94	(0.96; 2.92)	1.57	(0.24; 2.90)	2.26	(0.77; 3.76)
sVCAM-1, 10 μg/ml						
Model 1[d]	1.78	(−1.72; 5.27)	2.45	(−3.23; 8.12)	1.30	(−3.11; 5.71)
Model 2[e]	0.75	(−3.07; 4.56)	0.64	(−5.26; 6.55)	0.91	(−4.13; 5.95)

[a] Postload insulin.
[b] β indicates regression coefficient; an increase of the independent variable by 1 U is associated with an increase of insulin by a factor e^{β}.
[c] CRP and IL-6 were ln-transformed to obtain a better model fit as assessed by residual analysis; an increase of CRP and IL-6 by 1% yield an increase of postload insulin by 0.1 β%.
[d] Model 1. Adjusted for age, and, if appropriate, gender. Number of subjects in models with CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were 562 (265 men, 297 women), 562 (264 men, 298 women), 570 (271 men, 299 women), 571 (271 men, 300 women), and 572 (271 men, 301 women), respectively.
[e] Model 2. Adjusted for age, BMI, WHR, smoking (never, former, current), presence of cardiovascular disease, and, if appropriate, gender. Numbers of subjects in models with CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were 486 (230 men, 256 women), 486 (229 men, 257 women), 494 (235 men, 259 women), 495 (235 men, 260 women), and 495 (235 men, 260 women), respectively.

sociations between postload insulin and levels of markers of inflammation and sICAM-1 were still present after exclusion of subjects with prevalent cardiovascular disease (data not shown).

Multivariate-adjusted levels of CRP, ACT, IL-6, and sICAM-1 increased in subsequent tertiles of levels of postload insulin (Fig. 1). All tests for trend were statistically significant, except for the trend analyses regarding the association between tertiles of levels of postload insulin and CRP and sICAM-1 in men. Additional adjustment of the levels of sICAM-1 in tertiles of levels of postload insulin for markers of inflammation did not materially affect the results (data not shown). Again, we found no association between levels of sVCAM-1 and levels of postload insulin.

The strengths of the multivariate-adjusted associations between levels of postload insulin and CRP, ACT, IL-6, and sICAM-1, as expressed by standardized regression coefficients, were comparable with the strengths of the associations between levels of insulin and HDL cholesterol, BMI, and WHR (Table 3). Adjustment of the association between levels of postload insulin and sICAM-1 for levels of markers of inflammation again did not materially affect the results (data not shown). In our population, no association between systolic blood pressure and postload insulin was found.

The number of subjects categorized in categories 0–4 indicating the number of metabolic disorders present was 192, 232, 111, 31, and 5, respectively. Because of missing data, 3 subjects could not be categorized accordingly. Because of the low number of subjects with 3 or 4 metabolic disorders, we combined these categories into 1 category for analyses. Multivariate-adjusted levels of CRP, ACT, IL-6, and levels of sICAM-1 increased with the increasing number of metabolic disorders present (Fig. 2). All tests for trend reached statistical significance. Additional adjustment of levels of sICAM-1 according to the number of metabolic disorders for markers of inflammation did not materially affect the results (data not shown). No association was found between levels of sVCAM-1 and the number of metabolic disorders present. After stratification by gender, results were comparable with those presented for the total population, apart from CRP in men ($p_{trend} = 0.34$), and sICAM-1 in women ($p_{trend} = 0.17$) (data not shown).

Discussion

Our results indicate that, in an elderly population, markers of inflammation are strongly and independently associated with insulin resistance, as measured by postload insulin. In addition, the cellular adhesion molecule sICAM-1 is associated with insulin, whereas sVCAM-1 is not.

Some methodological issues should be taken into account when interpreting our results. Nonfasting postload insulin was used as a measure of insulin resistance. Previous results from the Rotterdam Study indicate that these levels are similar to fasting postload levels (28), and it is shown that postload insulin provides a good measure of insulin resistance in subjects without diabetes mellitus (29). If anything, the validity of our results does not depend on the precision of the measurement of insulin resistance used. The immunoassay used to measure insulin is known to cross-react with proinsulin. Although proinsulin is increased in impaired glucose tolerance, it constitutes only a minor part of the total insulin measured (30) and is therefore probably not responsible for the observed association with levels of markers of inflammation and cellular adhesion molecules. Levels of markers of inflammation and cellular adhesion molecules

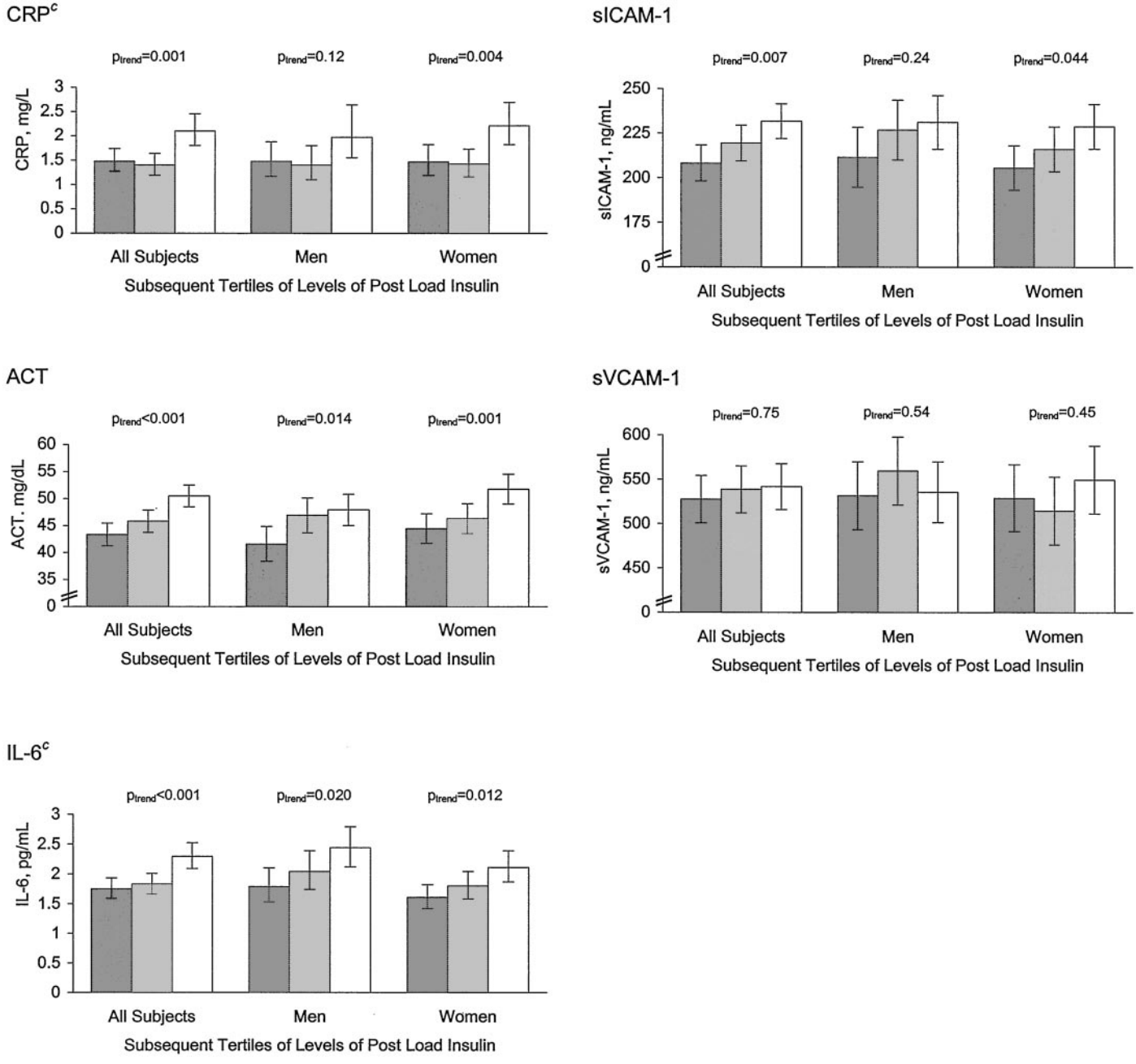

FIG. 1. Multivariate-adjusted ([a]) mean levels [95% confidence interval (CI)] of markers of inflammation and cellular adhesion molecules according to tertiles of levels of postload insulin ([b]) in the total population, and in men and women separately. [a], Adjusted for age, BMI, WHR, smoking (never, former, current), presence of cardiovascular disease, and, if appropriate, gender. Multivariate-adjusted levels of CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were available in the following numbers of subjects: 486 (230 men, 256 women), 486 (229 men, 257 women), 494 (235 men, 259 women), 495 (235 men, 260 women), and 495 (235 men, 260 women), respectively. [b], Tertiles of levels of insulin were computed for the total population, and for men and women separately. [c], Geometric mean values (95% CI) because of skewed data.

were measured in a gender-stratified random sample of subjects representative of the participants of the Rotterdam Study. We do assume that the sampling of subjects will not depend on the associations between insulin and levels of markers of inflammation and cellular adhesion molecules, making selection bias unlikely.

The results of our study are in line with recent results from the Insulin Resistance Atherosclerosis Study, in which CRP, fibrinogen, and white cell count were found to be associated with fasting insulin in nondiabetic subjects (7). In healthy middle-aged subjects, CRP was found to be related to insulin resistance as well (8). Also, in subjects with type 2 diabetes mellitus, an elevated acute-phase response was particularly marked in those with features of the metabolic syndrome (6). Factor analysis of data on healthy elderly people from the Cardiovascular Health Study, however, found inflammatory variables only weakly linked to insulin resistance (31).

The etiology of the clustering of metabolic factors in the

TABLE 3. Multivariate-adjusted[a] standardized regression coefficients for ln-insulin[b] as the dependent variable and markers of inflammation and cellular adhesion molecules and factors classically associated with insulin resistance as independent variables

	All subjects		Men		Women	
	β[c]	*P* value	β[c]	*P* value	β[c]	*P* value
CRP, mg/liter[d,e]	0.12	0.007	0.062	0.36	0.20	0.003
ACT, mg/dl[d]	0.20	<0.001	0.21	0.001	0.20	0.001
IL-6, pg/ml[d,e]	0.18	<0.001	0.19	0.005	0.17	0.010
sICAM-1, ng/ml[d]	0.17	<0.001	0.14	0.021	0.18	0.003
sVCAM-1, ng/ml[d]	0.017	0.70	0.014	0.83	0.022	0.72
HDL cholesterol, mM[d]	−0.13	0.003	−0.090	0.14	−0.17	0.004
BMI, kg/m^2[d]	0.14	0.002	0.23	0.002	0.10	0.09
WHR, cm/cm[d]	0.25	<0.001	0.16	0.019	0.24	<0.001
Systolic blood pressure, mm Hg[d]	0.031	0.49	0.060	0.35	−0.008	0.90

[a] Adjusted for age, BMI (apart from model with BMI as independent variable), WHR (apart from model with WHR as independent variable), smoking (never, former, current), presence of cardiovascular disease, and, if appropriate, gender.
[b] Postload insulin.
[c] β indicates standardized regression coefficient.
[d] Number of subjects in models with CRP, ACT, IL-6, sICAM-1, sVCAM-1, and HDL cholesterol to systolic blood pressure were 486 (230 men, 256 women), 486 (229 men, 257 women), 494 (235 men, 259 women), 495 (235 men, 260 women), 495 (235 men, 260 women), and 574 (272 men, 302 women), respectively.
[e] CRP and IL-6 were ln-transformed to obtain a better model fit as assessed by residual analysis.

insulin resistance syndrome remains controversial. A common view is that insulin resistance, with its compensatory hyperinsulinemia, is the underlying mechanism (1). Alternatively, abdominal obesity may be the primary defect of the clustering (2). Our data, and those of others (6–8), give support to the hypothesis that raised concentrations of proinflammatory cytokines, originating from various cells, and the resultant acute-phase response may underlie much of the metabolic clustering (32). Furthermore, a key role for the cytokine TNFα, which induces hepatic synthesis of acute-phase proteins (33), has been suggested in the pathogenesis of insulin resistance. TNFα increases serum triglycerides and very-low-density lipoprotein; stimulates insulin-independent glucose use, while inhibiting stimulated glucose uptake by fat and muscle; and causes an increase in counterregulatory hormones (34). Moreover, TNFα plays a role as a mediator of peripheral insulin resistance in obesity by inhibiting the tyrosine kinase activity of the insulin receptor and its substrate (35). The cross-sectional nature of the design of our study complicates etiological interpretation of the results. However, prospective data showed markers of inflammation to be associated with the development of diabetes mellitus, probably reflecting the pathogenesis of type 2 diabetes (36).

An alternative explanation for the association between insulin and levels of markers of inflammation might be the presence of atherosclerosis, which is associated with both insulin resistance and markers of inflammation (37). In our population, however, associations between insulin and levels of markers of inflammation were still present after adjustment for presence of cardiovascular disease (Table 2, model 2) and after exclusion of subjects with prevalent cardiovascular disease (data not shown). This suggests that atherosclerosis did not induce the association between insulin resistance and markers of inflammation. However, because we adjusted only for presence of cardiovascular disease, the assessment of degree and extent of atherosclerosis might lack accuracy in this respect. Furthermore, we have to consider the possibility that decreased insulin sensitivity leads to, rather than is the consequence of, raised concentrations of inflammatory mediators. Insulin inhibits acute-phase protein synthesis in human hepatoma cell lines (38), suggesting that insulin resistance might amplify the cytokine effect on the liver.

We are the first to describe an association between insulin and levels of the cellular adhesion molecule sICAM-1, which has been found to be associated with increased risk for future coronary events (39, 40). In our population, the cellular adhesion molecule sVCAM-1 was not associated with insulin. Previous results in healthy men participating in the Physician's Health Study describe associations of sICAM-1 with several metabolic cardiovascular risk factors encompassed in the insulin resistance syndrome, such as triglycerides, HDL cholesterol, fibrinogen, and hypertension (41). In dyslipidemic patients, increased levels of sICAM-1 and sVCAM-1 were found as well (42).

Levels of sICAM-1 were associated with levels of markers of inflammation. Adjustment of the association between insulin and sICAM-1 for CRP, ACT, or IL-6, however, did not materially affect the results. These results may indicate that inflammation is not the principal mechanism linking insulin and endothelial activation in our population (15, 43). Heterogeneity of markers of low-grade inflammation may have played a role in these findings as well. Another mechanism explaining the association between insulin and levels of sICAM-1 may be increased oxidation of LDL cholesterol (44). Moreover, a direct effect of glucose or insulin on the expression of cellular adhesion molecules has been demonstrated in rabbits (45). On the other hand, we have to consider the possibility that the association between insulin and sICAM-1 may be induced by atherosclerosis, which has been shown to be associated with higher levels of sICAM-1 (39). Associations, however, remained after adjustment for the presence of cardiovasular disease (Table 2, model 2) and were equally present in subjects without prevalent cardiovasular disease (data not shown). Further studies should determine whether our observation can be confirmed. An understanding of the role of cellular adhesion molecules in insulin resistance may lead to a potential target for prevention or treatment of atherosclerosis. Recently, for example, antibodies to ICAM-1

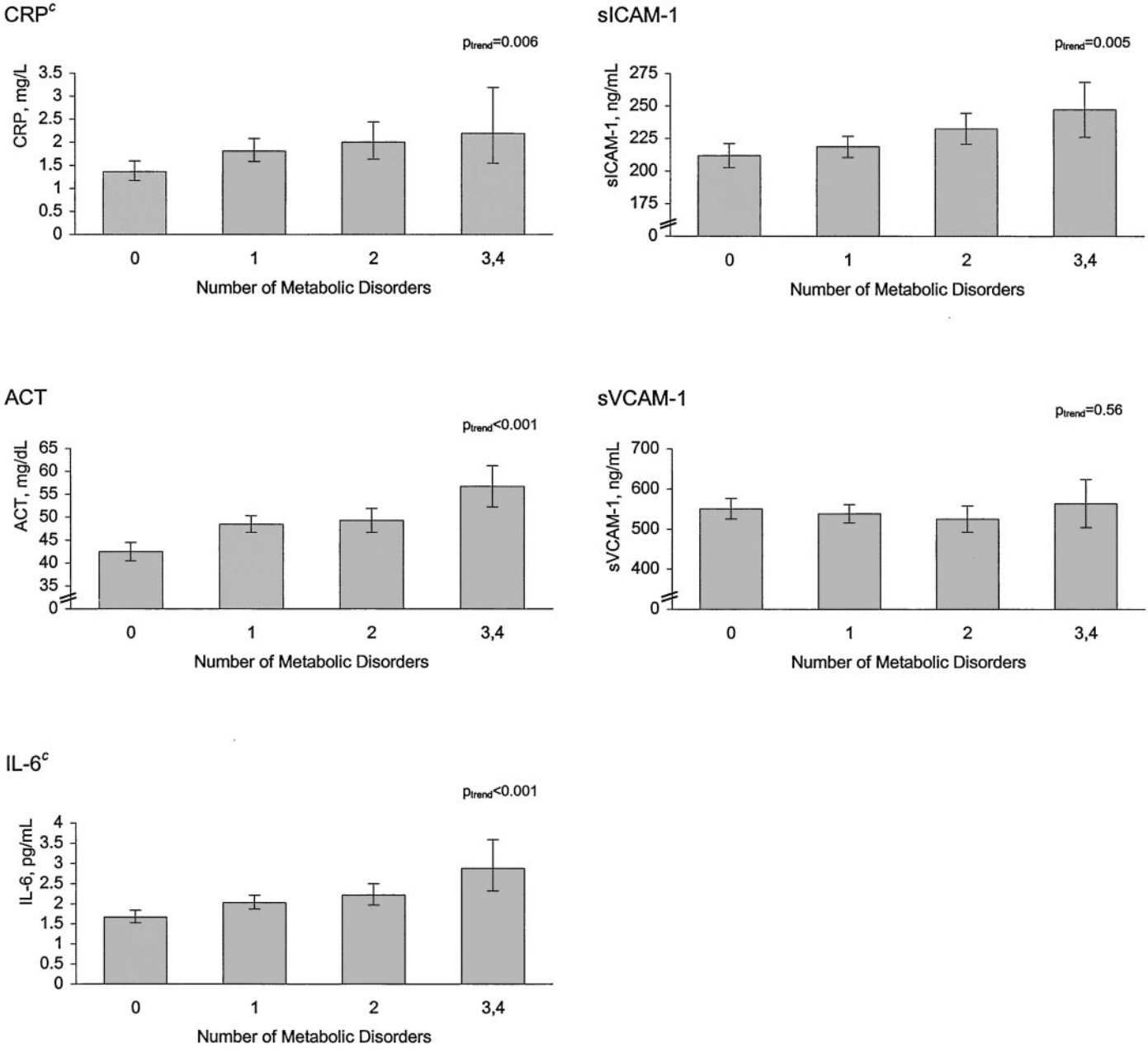

FIG. 2. Multivariate-adjusted ([a]) mean levels (95% CI) of markers of inflammation and cellular adhesion molecules according to the number of metabolic disorders present ([b]) in the total population. [a], Adjusted for age, gender, smoking (never, former, current), and presence of cardiovascular disease. Multivariate-adjusted levels of CRP, ACT, IL-6, sICAM-1, and sVCAM-1 were available in 559, 559, 559, 568, and 569 subjects, respectively. [b], Metabolic disorders encompassed impaired glucose tolerance, dyslipidemia, obesity, and hypertension. [c], Geometric mean values (95% CI) because of skewed data.

have been shown to reverse atherogenesis in hypercholesterolemic rabbits (46).

In summary, our results indicate that insulin is strongly and independently associated with markers of inflammation and the cellular adhesion molecule sICAM-1, suggesting that subclinical inflammation and endothelial activation are an integral part of the insulin resistance syndrome. These factors may contribute to the well-known relationship between insulin resistance and cardiovascular disease risk. Moreover, antiinflammatory treatment and strategies aimed at antagonizing effects of cellular adhesion molecules may possibly gain clinical importance in the treatment of insulin resistance and its complications.

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