Inflammation-Sensitive Plasma Proteins, Diabetes, and Mortality and Incidence of Myocardial Infarction and Stroke

A Population-Based Study

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This study explores the relationship of inflammation-sensitive plasma proteins (ISPs) with the prevalence of diabetes and the interrelationships between ISPs and diabetes in the prediction of death and incidence of myocardial infarction and stroke. Plasma levels of fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, and orosomucoid were assessed in 6,050 men, aged 28–61 years. All-cause and cardiovascular mortality and incidence of myocardial infarction and stroke were monitored over 18.7 ± 3.7 years. Prevalence of diabetes (n = 321) was significantly associated with ISP levels among overweight and obese men but not among men with BMI <25 kg/m^2. The association was similar for insulin resistance according to homeostasis model assessment. High ISP levels (two or more ISPs in the top quartile) increased the cardiovascular risk among diabetic men. The risk factor–adjusted relative risks for cardiovascular mortality, myocardial infarction, and stroke were 2.8 (CI 1.8–4.5), 2.2 (1.5–3.2), and 2.5 (1.4–4.6), respectively, for diabetic men with high ISP levels (reference: nondiabetic men with low ISP levels). The corresponding risks for diabetic men with low ISP levels were 1.8 (1.1–3.0), 1.3 (0.8–2.1), and 1.2 (0.6–2.5), respectively. In conclusion, in this population-based cohort, diabetes was associated with increased ISP levels among overweight and obese men but not among men with normal weight. High ISP levels increased the cardiovascular risk similarly in diabetic as compared with nondiabetic men. *Diabetes* 52:442–447, 2003

Diabetes and impaired glucose tolerance have been associated with increased plasma concentrations of various inflammation-sensitive plasma proteins (ISPs), including fibrinogen, haptoglobin, α1-antitrypsin, serum amyloid A, C-reactive protein, and orosomucoid (1–6). Prospective studies have reported associations among various markers of inflammation and incidence of diabetes (7–10), and it has been proposed that inflammation has a causal role for the development of diabetes (4). In particular, inflammatory cytokines formed in the adipose tissue are associated with glucose dysregulation (4,11–13). However, the joint effect of overweight and inflammation on the prevalence of diabetes has not been studied extensively in the general population. Whether prevalence of diabetes shows similar relationships with ISPs in lean and obese subjects is largely unknown.

Many prospective studies have reported relationships between moderately increased plasma concentrations of ISPs and increased incidences of myocardial infarction and stroke (14–17). It is now widely accepted that inflammation has a role in the development of atherosclerosis. Diabetes is another risk factor for myocardial infarction and stroke (18–20). The relationships between diabetes and other traditional cardiovascular risk factors, e.g., an adverse lipid profile, obesity, hypertension, and physical inactivity, cannot completely explain the increased risk in diabetic individuals (18). Even though it has been suggested that inflammation contributes to the increased incidence of cardiovascular diseases among diabetic subjects, few prospective studies have addressed this question.

This study explored *1*) whether plasma levels of five ISPs (fibrinogen, orosomucoid, α1-antitrypsin, ceruloplasmin, and haptoglobin) are associated with the prevalence of diabetes and insulin resistance in a large population-based cohort of men and *2*) whether these proteins are similarly associated with the risk of mortality and incidence of myocardial infarction and stroke in nondiabetic and diabetic men.

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HOMA-IR, homeostasis model assessment for insulin resistance; ISP, inflammation-sensitive plasma protein; OGTT, oral glucose tolerance test; RR, relative risk.

RESEARCH DESIGN AND METHODS

Between 1974 and 1983, 22,444 men participated in a screening program for detection of individuals with high risk for cardiovascular diseases (21). The participation rate was 71%. Determination of the five plasma proteins was performed for 6,193 men (30% of cohort), aged 28–61 years, who were selected at random. After exclusion of men with a history of myocardial infarction, stroke, or cancer (according to questionnaire), as well as men with missing data on blood glucose, 6,050 men remained. The health service authority of the city of Malmö approved the screening program. All participants gave informed consent.

TABLE 1
Prevalence of cardiovascular risk factors in relation to diabetes and zero to one versus two to five elevated ISPs

	No diabetes		Diabetes	
	Zero to one ISP in top quartile	Two to five ISPs in top quartile	Zero to one ISP in top quartile	Two to five ISPs in top quartile
n	3,808	1,921	186	135
Age (years)	46.7 ± 3.6	47.1 ± 3.8	46.4 ± 4.3	47.5 ± 4.3
BMI (kg/m^2)	24.8 ± 3.1	24.9 ± 3.4	26.2 ± 4.0	27.9 ± 4.8
Cholesterol (mmol/l)	5.6 ± 1.0	5.8 ± 1.1	5.8 ± 1.4	5.8 ± 1.1
Triglycerides (mmol/l)	1.5 ± 0.8	1.7 ± 1.0	2.3 ± 3.1	2.5 ± 1.7
Creatinine (μmol/l)	94 ± 19	94 ± 22	93 ± 13	93 ± 14
Systolic BP (mmHg)	128 ± 15	129 ± 16	136 ± 17	139 ± 19
Diastolic BP (mmHg)	87 ± 10	87 ± 10	89 ± 11	92 ± 12
Smokers (%)	38	69	36	61
Physical inactivity (%)	19	24	21	21
Self-reported diabetes [*n* (%)]			35 (19)	30 (22)

Data are means ± SD unless noted otherwise. BP, blood pressure.

Baseline examinations. Subjects were categorized as smokers and nonsmokers. Smokers were categorized into <10, 10–19, and ≥20 cigarettes/day. Blood pressure (mmHg) was measured twice in the right arm after a 10-min rest. The average of two measurements was used. A sphygmomanometer and a rubber cuff of appropriate size were used. Use of antihypertensive medication was assessed in a questionnaire. Blood samples were taken after an overnight fast and analyzed at the Department of Clinical Chemistry at Malmö University Hospital. Plasma cholesterol concentrations were analyzed with enzymatic methods. Physical activity was assessed by questionnaire. Two categories of physical activity were used, sedentary or not sedentary.

A 2-h oral glucose tolerance test (OGTT; 30 g glucose/m^2 body surface area) was performed among 5,312 (88%) men (22). Blood glucose was analyzed with a hexokinase method. Men with fasting whole blood glucose ≥6.1 mmol/l and/or 2-h glucose ≥10.0 mmol/l, as well as men who reported that they had diabetes, were considered diabetic (23). To exclude type 1 diabetes and men with treatment for diabetes, some analyses were performed without those who had previously known diabetes (n = 65).

Insulin (mIU/l) was measured with a nonspecific radioimmunoassay (24). The limit for detection of insulin was 3 mIU/l. Intra- and interassay coefficients of variation were 5 and 8%, respectively. We used the homeostasis model formula of Matthews et al. (25) (fasting insulin × fasting glucose/22.5) to calculate a score for insulin resistance (homeostasis model assessment for insulin resistance [HOMA-IR]). Because treatment of diabetes could affect the insulin levels, all men with previously known diabetes were excluded from the analysis of HOMA-IR. In an additional analysis, all diabetic men were excluded. There was no significant difference between men with and without information on HOMA-IR with respect to BMI and number of elevated ISPs. BMI was calculated as weight divided by height squared (kg/m^2).

ISPs. Electroimmunoassay was used to analyze the plasma levels of five ISPs (26). We have previously shown that the correlation coefficients between the individual proteins range from 0.31 to 0.56 and that the cardiovascular risk increases with the number of ISPs in the top quartile (14). In accordance with the previous studies, the sample was categorized into those who had two to five ISPs in the top quartile (high ISP levels) and those with zero to one ISP in the top quartile (low ISP levels) (15).

Follow-up. All cases were followed from the baseline examination until death or 31 December 1997. Information on cause of deaths was retrieved from the Swedish Causes of Deaths register. Autopsy rate was ~40%. A cardiac event was defined as fatal or nonfatal myocardial infarction (code 410 according to ICD-9) or death due to chronic ischemic heart disease (i.e., ICD codes 412–414). First events of nonfatal myocardial infarction were retrieved from the Malmö Myocardial Infarction Register (27). Stroke was defined as cases coded 430 (subarachnoid hemorrhage), 431 (intracerebral hemorrhage), 434 (ischemic stroke), or 436 (unspecified stroke) according to the ICD. The Malmö Stroke Register (28), which has continuously searched for and validated patients with stroke since 1989, was used for case retrieval. Cases of stroke that occurred before 1989 were retrieved from the administrative register of the University Hospital and validated by review of medical records using the same procedure as the Malmö Stroke Register. Computed tomography scans were available for 172 (of 204) of the strokes that occurred in the city of Malmö. The National Hospital Discharge Register was used for retrieval of cases (n = 34) who moved out of Malmö. These diagnoses were settled by the physician at the time of hospital discharge.

Statistics. ANOVA, Pearson's correlation, and logistic regression were used to study the relationships among diabetes, ISP levels, and other cardiovascular risk factors. A general linear model was used to adjust the mean HOMA-IR values (log transformed) for potential confounders. Cox proportional hazards model was used for the analysis of the event rates in categories of diabetes and ISP with adjustment for potential confounders. A backward stepwise model was used to reduce the number of covariates when diabetic men were analyzed separately (P for remove = 0.10). Survival plots of the different risk factor categories confirmed the fit of the proportional hazards model. Interaction terms were entered into the multivariate models to assess interactions among diabetes, HOMA-IR, ISP, and BMI.

RESULTS

Study cohort. A total of 321 (5.3%) men had diabetes. Fasting glucose was ≥6.1 mmol/l for 270 (4.5%) men. Among the 5,312 (88%) men who performed an OGTT, 84 (1.6%) had 2-h glucose values ≥10.0 mmol/l. Forty-three male subjects had both a fasting glucose value ≥6.1 and 2-h OGTT value ≥10.0 mmol/l. Sixty-five men had previously known diabetes according to questionnaire, 55 (85%) of whom had fasting glucose values ≥6.1 mmol/l.

Median and interquartile ranges for the five ISPs were 3.47 g/l (3.0–4.0) for fibrinogen, 1.30 g/l (0.90–1.76) for haptoglobin, 0.30 g/l (0.26–0.35) for ceruloplasmin, 0.80 g/l (0.67–0.93) for orosomucoid (α1-glucoprotein), and 1.27 g/l (1.10–1.42) for α1-antitrypsin. The correlations between age and the individual ISPs were small and ranged from r = 0.000004 (haptoglobin) to r = 0.09 (α1-antitrypsin).

Prevalence of cardiovascular risk factors in relation to diabetes and ISPs. The levels of cardiovascular risk factors in diabetic and nondiabetic men with high and low ISP levels are shown in Table 1. As expected, smoking was more prevalent in groups with high ISP levels. Among the diabetic men, the number with self-reported diabetes was similar in those with high and low ISP levels.

Prevalence of diabetes in relation to ISPs. Prevalence of diabetes increased with the number of elevated ISPs (Table 2). However, the increased prevalence of diabetes among men with two to five ISPs in the top quartile was confined to men with BMI ≥25 kg/m^2, and no clear relationship was observed among men with BMI <25 kg/m^2 (Fig. 1 and Table 2). The relationships were similar after the exclusion of men with self-reported diabetes.

Adding an interaction term to the logistic regression model tested the interaction between BMI and ISP levels for the prevalence of diabetes. The interaction term was statistically significant (P = 0.04).

TABLE 2
Prevalence of diabetes in relation to ISP levels and BMI

	BMI (kg/m^2)			
	<25	25–29.9	≥30	All men
n	3,297	2,318	435	6,050
Men with diabetes (*n*)	118	130	73	321
Fibrinogen Q1–3 vs. Q4 (% with diabetes)	3.4 vs. 4.2	4.8 vs. 7.8	14.2 vs. 21.6	4.6 vs. 7.3
Haptoglobin Q1–3 vs. Q4 (% with diabetes)	3.7 vs. 3.2	4.7 vs. 8.4	14.8 vs. 21.6	4.8 vs. 6.7
Ceruloplasmin Q1–3 vs. Q4 (% with diabetes)	3.4 vs. 4.2	5.3 vs. 6.7	16.3 vs. 18.4	5.0 vs. 6.3
Orosomucoid Q1–3 vs. Q4 (% with diabetes)	3.9 vs. 2.7	5.1 vs. 6.9	14.6 vs. 20.9	5.0 vs. 6.1
α1-Antitrypsin Q1–3 vs. Q4 (% with diabetes)	3.4 vs. 3.9	5.4 vs. 6.2	15.9 vs. 19.2	5.1 vs. 5.8
No ISP in top Q (% with diabetes, *n*/*n*)	3.3 (45/1,373)	3.8 (35/930)	13.9 (19/137)	4.1 (99/2,440)
One ISP in top Q (% with diabetes, *n*/*n*)	4.0 (33/816)	6.3 (39/621)	12.8 (15/117)	5.6 (87/1,554)
Two ISPs in top Q (% with diabetes, *n*/*n*)	3.3 (16/490)	6.7 (23/341)	20.8 (15/72)	6.0 (54/903)
Three ISPs in top Q (% with diabetes, *n*/*n*)	5.4 (16/299)	6.9 (16/231)	16.9 (10/59)	7.1 (42/589)
Four or five ISPs in top Q (% with diabetes, *n*/*n*)	2.5 (8/319)	8.7 (17/195)	28.0 (14/50)	6.9 (39/564)
Zero to one ISP in top Q (% with diabetes)	3.6	4.8	13.4	4.7
Two to five ISPs in top Q (% with diabetes)	3.6	7.3	21.5	6.6
Adjusted OR for diabetes in men with two to five ISPs*	1.09 (0.72–1.6)	1.64 (1.13–2.4)	1.74 (1.03–3.0)	1.39 (1.09–1.77)

*Adjusted for age, physical activity, smoking, and BMI (all men only). OR, odds ratio; Q, quartile.

Insulin resistance in relation to ISPs. Insulin resistance was compared between categories of BMI and ISP. After exclusion of men with self-reported diabetes, information on HOMA-IR was available in 2,985 men. Among men with BMI ≥30 kg/m^2, HOMA-IR (log transformed) was significantly higher among men with two to five ISPs in the top quartile (Fig. 2). After adjustments for age, smoking, and physical inactivity, mean HOMA-IR (±SE) among men with BMI ≥30 kg/m^2 was 1.26 ± 0.07 and 1.48 ± 0.08 for men with zero to one and two to five ISPs in the top quartile, respectively ($P = 0.03$). The corresponding values were 0.65 ± 0.03 and 0.75 ± 0.04, respectively, for men with BMI 25–29.9 kg/m^2 ($P = 0.05$) and 0.43 ± 0.02 and 0.38 ± 0.03, respectively, for men with normal weight ($P = 0.26$). An interaction term was added to the model in order to test the interaction between BMI and high ISP levels. The interaction term was statistically significant ($P < 0.01$). Results were virtually identical when all diabetic men were excluded.

Incidence of cardiovascular diseases. A total of 910 men (15.0%) died during the follow-up, 372 (41%) of them from cardiovascular diseases (ICD-9 codes 390–440). A total of 608 (10.0%) men experienced a fatal or nonfatal cardiac event. Two hundred thirty-seven (3.9%) men suffered a stroke, 9 a subarachnoid hemorrhage, 29 an intracerebral hemorrhage, 169 an ischemic stroke, and 30 cases were unspecified.

Event rates in relation to diabetes and ISP levels. The men were categorized according to diabetes and ISP levels (zero to one versus two to five ISPs in the top quartile). The highest cardiovascular mortality and incidence of cardiac events and stroke was found among those who had diabetes and high ISP levels (Table 3 and Fig. 3). The combined effects of diabetes and high ISP levels on

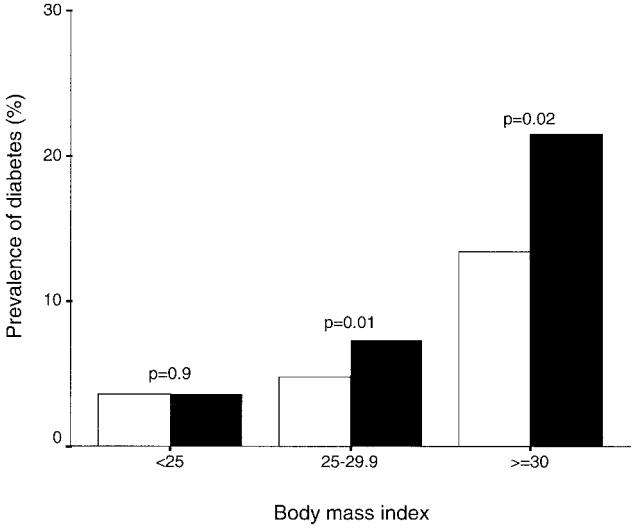

FIG. 1. Prevalence of diabetes in relation to BMI in men with zero to one (□) or two to five (■) ISPs in the top quartile.

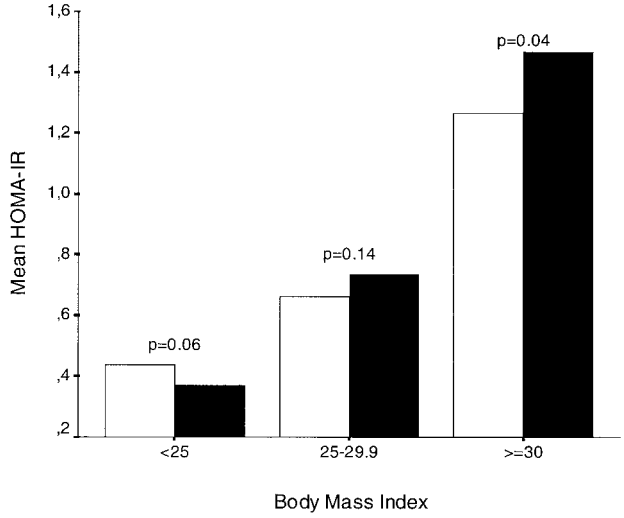

FIG. 2. Mean HOMA-IR (log transformed) in relation to BMI in men with zero to one (□) or two to five (■) ISPs in the top quartile.

TABLE 3
All-cause and cardiovascular death and incidence of myocardial infarction and stroke in relation to diabetes and ISPs

	No diabetes		Diabetes	
	Zero to one ISP in top quartile	Two to five ISPs in top quartile	Zero to one ISP in top quartile	Two to five ISPs in top quartile
n	3,808	1,921	186	135
All-cause mortality (*n*)	416	410	38	46
Age-adjusted RR	reference	2.1 (1.8–2.4)	2.1 (1.5–2.9)	3.8 (2.8–5.1)
Risk factor adjusted*	reference	1.6 (1.4–1.8)	1.8 (1.3–2.6)	2.5 (1.8–3.5)
Age-adjusted RR			reference	1.8 (1.2–2.8)
Risk factor adjusted*			reference	1.5 (0.93–2.4)
CVD mortality (*n*)	159	174	16	23
Age-adjusted RR	reference	2.3 (1.9–2.9)	2.3 (1.4–3.9)	4.9 (3.2–7.6)
Risk factor adjusted*	reference	1.7 (1.4–2.2)	1.8 (1.1–3.0)	2.8 (1.8–4.5)
Age-adjusted RR			reference	2.2 (1.1–4.1)
Risk factor adjusted†			reference	2.2 (1.2–4.2)
Cardiac events (*n*)	277	280	21	30
Age-adjusted RR	reference	2.2 (1.8–2.6)	1.7 (1.1–2.7)	3.7 (2.6–5.4)
Risk factor adjusted*	reference	1.6 (1.3–1.9)	1.3 (0.83–2.1)	2.2 (1.5–3.2)
Age-adjusted RR			reference	2.2 (1.2–3.8)
Risk factor adjusted†			reference	2.2 (1.2–3.8)
Stroke (*n*)	122	94	8	13
Age-adjusted RR	reference	1.7 (1.3–2.2)	1.5 (0.8–3.2)	3.9 (2.2–6.9)
Risk factor adjusted*	reference	1.4 (1.1–1.9)	1.2 (0.6–2.5)	2.5 (1.4–4.6)
Age-adjusted RR†			reference	2.4 (0.98–5.8)
Ischemic stroke (*n*)	100	80	8	11
Age-adjusted RR	reference	1.7 (1.3–2.3)	1.9 (0.91–3.8)	4.0 (2.1–7.4)
Risk factor adjusted*	reference	1.4 (1.0–1.9)	1.5 (0.7–3.1)	2.5 (1.3–4.8)
Age-adjusted RR			reference	2.0 (0.80–5.0)

*RR adjusted for age, BMI, smoking, tobacco consumption, cholesterol, triglycerides, physical inactivity, systolic blood pressure, and medication for hypertension. †Number of covariables were reduced in a backward stepwise Cox regression due to the small number of events. Age, cholesterol, and ISP levels remained in the final models for cardiovascular death and cardiac events. Age and ISP levels remained in the final model for stroke.

cardiovascular mortality, incidence of cardiac events, and stroke were similar or somewhat higher than what could be expected from addition of relative risks (RRs). The interaction term between ISP levels and diabetes was nonsignificant, however, in all Cox models. The results were essentially the same when men with self-reported diabetes were excluded.

The impact of high ISP levels on the cardiovascular risk in diabetic men was similar in the three categories of BMI (data not shown). The interaction terms between BMI and diabetes, as well as between BMI and ISP levels, were nonsignificant for all end points.

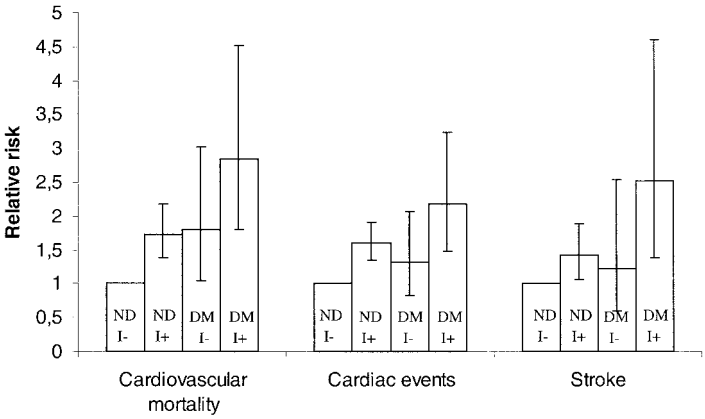

FIG. 3. RRs of cardiovascular mortality, cardiac events, and stroke in nondiabetic (ND) and diabetic (DM) men with low (I−) and high (I+) ISP levels. Adjusted for age, BMI, smoking, tobacco consumption, cholesterol, triglycerides, physical inactivity, systolic blood pressure, and medication for hypertension.

The age-adjusted RR for cardiovascular mortality was 2.35 (CI 1.7–3.3) for men with diabetes. Adjustments for number of ISPs in the top quartile (categorical variable) reduced the RR to 2.18 (1.6–3.0), corresponding to ~13% of the excess risk. The risk factor–adjusted RR (adjusted for age, BMI, smoking, cigarettes, cholesterol, triglycerides, physical inactivity, systolic blood pressure, and antihypertensive medication) for cardiovascular mortality was reduced from 1.69 (1.2–2.4) to 1.63 (1.1–2.3) by further adjustments for ISP. The increased RRs for cardiac events and stroke among diabetic men were similarly marginally reduced by adjustments for ISP.

DISCUSSION

In recent years, much attention has been given to the relationships among adiposity, inflammation, and diabetes. However, the combined effect of overweight and ISP levels on the prevalence of diabetes has not been studied extensively in population-based studies. Prevalence of diabetes and HOMA-IR were significantly associated with number of elevated ISPs among overweight and obese men. No association was observed among men with normal weight. Another important finding was that the cardiovascular risk differs markedly between diabetic men with and without elevated ISP levels. High ISP levels increased the cardiovascular risk similarly or slightly more in diabetic as compared with nondiabetic men.

Studies of apparently healthy subjects have reported increased incidences of cardiovascular diseases in men with elevated ISP levels (14–17). Studies of diabetic subjects have reported increased incidences of cardiovascular diseases or increased diabetes complications among subjects with high fibrinogen (29) and other markers of inflammation (6,30–32). According to the present results, joint effects of diabetes and high ISP levels on the incidence of cardiovascular diseases are similar to or tend to be slightly higher than what could be expected from risk addition on a risk ratio scale. Measurement of ISP may be useful for assessment of the cardiovascular risk in diabetic patients. Whether high ISP levels in diabetic patients should be targets for specific treatments, e.g., with thiazolidinediones, statins, or other drugs with anti-inflammatory effects, awaits trial. It is noteworthy, however, that treatment with statins has been associated with reduced coronary event rates in nondiabetic subjects with high ISP levels, even though their cholesterol levels were low (33).

Results from prospective as well as experimental studies suggest that inflammation is involved in the pathogenesis of both diabetes (4) and atherosclerosis (34). The nature of this relationship is unclear. Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycemia and insulin resistance could also promote inflammation, and inflammation may be a factor linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress (35), by the formation of advanced glycation end products (36), and by increasing the transcription factor nuclear factor κB (37). Yet another possibility is that the inflammatory response is a result of vascular complications following diabetes. The present results persisted after excluding men with known diabetes. The cohort was relatively young, probably with relatively few macrovascular complications, and the cardiac events mostly occurred after >10 years of follow-up. Other studies have shown that the inflammatory markers are often elevated before diabetes has developed (7–9). Nevertheless, the possibility that macrovascular complications increased the ISP levels cannot completely be excluded.

Studies of obesity, insulin resistance, and type 2 diabetes have shown relationships with proinflammatory cytokines and acute-phase reactants (4,11–13). Obesity has been associated with oxidative stress (38). Obesity is also associated with elevated levels of the proinflammatory cytokine tumor necrosis factor-α, and weight loss is associated with decreasing levels (39). Elevated levels of fibrinogen have also been associated with future weight increase (40). This suggests that obesity could cause inflammation and may also be an effect of inflammation. Prevalence of diabetes was significantly associated with the number of elevated ISPs. However, the relationship between ISP and prevalence of diabetes was limited to men with BMI >25 kg/m^2, and no relationship was observed among men with low BMI. It is generally agreed that type 2 diabetes is a heterogeneous disorder with several different molecular mechanisms (41). For example, obese and lean diabetic subjects differ with respect to their sensitivity to insulin (42). Our results are compatible with the view that inflammation and overweight interact in the pathogenesis of diabetes and that this relationship, at least partially, is explained by impairment of insulin sensitivity (11,13). Among men with normal weight, noninflammatory mechanisms seem to be more important and no positive relationship between inflammation and insulin resistance was observed. It is also noteworthy, however, that even among overweight and obese men, diabetes was not uncommon among those without any evidence of inflammation.

The absence of a relationship between ISP and diabetes in men with normal weight partially disagrees with recently reported results from Festa et al. (7). They found associations among plasminogen activator inhibitor 1, fibrinogen, C-reactive protein, and incidence of diabetes in lean subjects during a 5-year follow-up. However, they used a higher cutoff for lean men (BMI <27.4 kg/m^2), and the study population was older. Differences with respect to study populations, study designs, and inflammatory markers are possible explanations for the disparate results.

Several potential confounders have been taken into account in the analysis. However, a limitation of the study is that no information on complications, treatment, duration, or type of diabetes was available. The proportion with known diabetes was small and similar among those with high and low ISP levels. The results were essentially the same after excluding men with known diabetes, and it is very unlikely that differences at baseline with respect to the occurrence of type 1 diabetes or treatment of diabetes explain the results. We also do not know whether the cardiovascular risk factors and treatment of diabetes changed during the follow-up. Men with diabetes, hypertension, and hypercholesterolemia were referred for further evaluation and treatment. Smokers were recommended to stop but were not offered any help to do so. Because these factors were more common among men with high ISP levels, they should benefit most from the interventions. Another limitation is that we do not know whether the subfractions of cholesterol, e.g., LDL and lipoprotein(a), differed between the groups. However, the LDL-to-HDL ratio is correlated to levels of triglycerides, and the results were adjusted for triglyceride levels as a proxy for dyslipidemia.

BMI was used to assess degree of overweight. We had no information on the distribution of body fat. Previous studies have shown that the correlation between BMI and waist circumference is ~0.9 (43). It is hence unlikely that the results would be substantially different if the waist circumference was used instead of BMI. The number of subjects with OGTT glucose ≥10.0 mmol/l, as well as the overlap between those who fullfilled the fasting glucose and OGTT criteria, was small. This could be explained by the fact that the glucose load in this study was lower (50–60 g) than the standardized dose of 75 g used in most recent studies. The fact that men with known diabetes did not perform an OGTT also reduced the overlap between the groups. Because assessment of insulin sensitivity with the clamp technique was not feasible in this epidemiological study, HOMA was used as a surrogate method. However, it is noteworthy that the results for HOMA-IR and prevalence of diabetes were very consistent, even when diabetic men were excluded from the analysis of HOMA-IR.

In conclusion, in this population-based cohort, plasma levels of ISP were associated with diabetes and HOMA-IR

among overweight and obese men, and no relationship was found among men with normal weight. The cardiovascular risk differed markedly between diabetic men with and without elevated ISP levels. High ISP levels increased the cardiovascular risk similarly in diabetic as compared with nondiabetic men.

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