Low-Grade Chronic Inflammation in the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Population

Associations with insulin resistance and cardiometabolic risk profile

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OBJECTIVE — Low-grade chronic inflammation has been hypothesized to underlie the constellation of cardiometabolic risk factors, possibly by inducing insulin resistance. In the present study, we investigated associations between inflammation markers, insulin sensitivity (expressed as the ratio of the *M* value to the mean plasma insulin concentrations measured during the final 40 min of the clamp [*M/I*]), and a range of cardiometabolic risk factors in a large, healthy population.

RESEARCH DESIGN AND METHODS — The Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) cohort includes 1,326 nondiabetic European men and women, aged between 30 and 60 years. We measured cardiometabolic risk factors and performed a hyperinsulinemic-euglycemic clamp. We determined total white blood cell count (WBC) and erythrocyte sedimentation rate (ESR) as markers of chronic inflammation.

RESULTS — WBC and ESR were both strongly associated with *M/I*. WBC and ESR were further associated with a range of cardiometabolic risk factors. Associations between WBC and HDL cholesterol, triglycerides, heart rate, fasting C-peptide, and insulin and 2-h insulin in men and women and between WBC and 2-h glucose in women remained significant after adjustment for both *M/I* and waist circumference. Associations between ESR and HDL cholesterol, heart rate, fasting, and 2-h insulin in men and women and between ESR and fat mass in women remained significant after adjustment for *M/I* and waist circumference.

CONCLUSIONS — This study showed that low-grade chronic inflammation is associated with the cardiometabolic risk profile of a healthy population. Insulin resistance, although strongly associated with inflammation, does not seem to play a large intermediary role.

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Several inflammation markers have been shown to be associated with cardiometabolic risk profile. A study in a diabetic population showed that abnormalities of the immune system including elevated levels of acute-phase reactants, interleukin-6 (IL-6), C-reactive protein (CRP), and cortisol were all associated with the metabolic syndrome (1). The Insulin Resistance Atherosclerosis Study (IRAS) showed in a nondiabetic population that white blood cell count (WBC), CRP, and fibrinogen were all related to elements of the metabolic syndrome (2). In a range of prospective studies, inflammation markers have also been shown to relate to the development of type 2 diabetes and coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) study reported associations between raised WBC, fibrinogen, and low serum albumin and diagnosis of diabetes 7 years later in a large middle-aged population (3). In a large cohort study in patients undergoing angiography, the erythrocyte sedimentation rate (ESR) was related to coronary atherosclerosis and was a predictor of cardiac death in patients with probable ischemic heart disease (4). A meta-analysis of prospective studies investigating the relationship between inflammatory factors and subsequent coronary heart disease showed associations between fibrinogen, CRP, albumin, and WBC and the development of coronary heart disease (5).

Low-grade inflammation may lead to cardiometabolic disease by inducing insulin resistance, a major contributor to the development of cardiovascular and metabolic disease. Insulin resistance has been shown to be associated with several inflammatory factors (2,6,7). A prospective study in Pima Indians showed that a high WBC predicted development of diabetes, independent of body fat (8). The effect seemed to be mediated by a worsening of insulin sensitivity during the 5 years of follow-up.

Limitations of the existing literature on the association between low-grade inflammation, insulin resistance, and cardiometabolic disease include the lack of direct measurement of insulin sensitivity by the standard technique, the hyperinsulinemic-euglycemic clamp, in a large healthy population. Often, fasting hyperinsulinemia has been used as a surrogate measure of insulin sensitivity (6,7). However, along with others, our group has shown that hyperinsulinemia contributes

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to cardiovascular risk independently of insulin resistance as measured by the hyperinsulinemic-euglycemic clamp (9). The question is whether low-grade inflammation is associated with insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp and whether clamp-derived insulin resistance mediates the association between inflammation and cardiometabolic disease. In the present study we tried to answer these questions by using data from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study cohort.

RESEARCH DESIGN AND METHODS

The RISC cohort consists of clinically healthy men and women aged between 30 and 60 years. Cohort members were recruited by 19 research centers in 14 European countries. Detailed information on inclusion can be found elsewhere (10). The local medical ethics committee of each participating research center approved the study. All participants gave written informed consent.

Basal measurements

As indicators for cardiometabolic disease risk, we included waist circumference, fat mass, fasting glucose, proinsulin, insulin and C-peptide, 2-h glucose and insulin, insulin resistance, HDL cholesterol, triglycerides, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate.

We measured height on a clinic stadiometer, weight and fat-free mass with the Tanita bioimpedance balance (Tanita, Middlesex, U.K.), and waist circumference with a tape measure. Blood pressure and heart rate were measured three times by an automatic blood pressure measuring device (OMRON, Hoofddorp, the Netherlands). We used the median value of the three measurements. Fasting blood samples were taken to assess WBC, ESR, and lipids. We performed a 75-g oral glucose tolerance test (OGTT). A lifestyle and medical history questionnaire was used to collect information on socioeconomic status, family history of diabetes, smoking and alcohol drinking habits, and physical activity.

Insulin sensitivity

On a separate day within 1 month of the OGTT, a hyperinsulinemic-euglycemic clamp was performed in all participants included in the study. Fasting samples were taken to assess proinsulin and C-peptide. Exogenous insulin was infused at a rate of 240 pmol · min^{-1} · m^{-2} simultaneously with a 20% dextrose infusion. Plasma glucose was measured at 5- to 10-min intervals to ensure that it remained within 0.8 mmol/l (±15%) of the target glucose concentration (4.5–5.0 mmol/l). The steady-state period for the calculation of the *M* value was between 80 and 120 min and normalized by the fat-free mass. Insulin sensitivity was expressed as the ratio of the *M* value to the mean plasma insulin concentrations measured during the final 40 min of the clamp (*M/I*) (9). All clamps were performed between June 2002 and October 2004.

Laboratory measurements

Both WBC and ESR were determined in local laboratories cooperating with each participating research center. Total WBC was measured by automated cell counters. ESR was measured by the modified Westergren method. The mean all-center variance of the WBC was 2.8×10^{12}/l and of ESR was 52.5 mm/h, whereas the between-center variances were 0.2×10^{12}/l and 52.5 mm/h. All other variables were measured by a central laboratory. Detailed information on further laboratory assessments can be found elsewhere (9).

Statistical methods

Fat mass was calculated by subtracting fat-free mass from total body weight. Variables with skewed distributions are represented by medians and interquartile ranges and were log-transformed when used in a multivariate regression analysis. Because of large sex differences in ESR values, we performed all analyses and show all data separately for men and women. We split the cohort according to sex-specific quartiles of WBC concentrations and ESR values.

We used logistic and linear regression analysis to analyze possible differences between the quartiles on general, lifestyle, and clinical characteristics. We used Spearman's ρ to analyze correlations between WBC and ESR, *M/I*, and the cardiometabolic risk factors. To detect possible effects of infections, we analyzed correlations again after exclusion of clinically abnormal WBC values (>10×10^{12}/l) and ESR (>15 mm/h). Finally, we used linear regression analysis to study the associations between WBC, ESR, insulin sensitivity, and cardiometabolic risk factors. We ran four models: in the first model we adjusted for the basic variables age, smoking, and study center; in the second model we adjusted for the basic variables and waist circumference; in the third model we adjusted for the basic variables and *M/I*; and in the fourth model we adjusted for the basic variables, waist circumference, and *M/I*. In all other statistical models, we adjusted for age, smoking, and study center. To make the resulting regression coefficients comparable, we report standardized β values.

RESULTS

Study group

A total of 1,538 men and women participated in the RISC cohort. After the basal measurements, 180 cohort members not satisfying the inclusion criteria were excluded and 32 participants dropped out. We excluded another 13 participants, because both WBC and ESR values were missing. Of the resulting group of 1,313 participants, 591 (45%) were men. The mean ± SD age of the study group was 43.8 ± 8.3 years (Table 1).

WBC, ESR, and general and lifestyle characteristics

Median values and interquartile ranges for WBCs were 5.5 (2.2) $\times 10^{12}$/l in men and 5.8 (2.0) $\times 10^{12}$/l in women ($P = 0.73$ for difference). Median values and interquartile ranges for ESR were 5.0 (7.0) mm/h in men and 8.0 (8.0) mm/h in women ($P < 0.01$ for difference).

In men, there was a positive association between age and WBC that showed a trend toward significance ($P = 0.07$), whereas in women the association was statistically significant ($P < 0.01$). Smoking status ($P < 0.01$) was significantly and positively associated with WBC in men and women. Study center was associated with WBC in men only ($P < 0.01$). ESR was significantly associated with study center ($P < 0.01$) and negatively associated with alcohol intake ($P < 0.01$) in both men and women. Medians for WBC for the different study centers ranged from 4.5 to 7.4 $\times 10^{12}$/l in men and from 4.9 to 6.4 $\times 10^{12}$/l in women. Medians for ESR ranged from 2.0 to 9.0 mm/h in men and from 5.0 to 15.0 mm/h in women. WBC and ESR were significantly correlated with each other and were stronger in men ($\rho = 0.14$, $P < 0.01$) than in women ($\rho = 0.08$, $P = 0.04$).

WBC, ESR, and *M/I*

WBC was negatively correlated with *M/I* in both men ($\rho = -0.19$, $P < 0.01$) and women ($\rho = -0.10$, $P = 0.01$) (Table 2). Per unit increase in WBC, *M/I* decreased by 6.7% (95% CI 4.2–9.3, adjusted for age,

Table 1—*WBC and ESR values for general, lifestyle, and clinical characteristics according to the lowest versus highest quartiles of WBC and ESR*

	Men				Women				
	WBC		ESR		WBC		ESR		
	3 lowest quartiles	Highest quartile	3 lowest quartiles	Highest quartile	3 lowest quartiles	Highest quartile	3 lowest quartiles	Highest quartile	All
n	441	146	440	140	540	176	535	176	1,313
General characteristics									
Age (years)	43.6	41.8	42.8	44.2*	44.7	43.0*	43.7	46.1*	43.8 ± 8.3
Family history of diabetes (%)	25	33	25	33	28	29	25	37	27
Lifestyle characteristics									
Frequency of smoking (%)	20	51†	27	30	19	46†	27	21	27
Alcohol intake (g/week)‡	80	70	81	62	30	39	35	26§	49 (90)
Clinical characteristics									
Waist circumference (cm)	92.9	95.3§	92.4	97.2§	80.9	81.9§	79.8	84.9§	86.6 ± 12.8
Fat mass (kg)	18.4	20.6§	18.1	21.7§	22.3	23.4§	21.6	25.8§	20.9 ± 8.9
HDL cholesterol (mmol/l)	1.3	1.1§	1.3	1.1§	1.6	1.5§	1.6	1.5§	1.4 ± 0.4
Triglycerides (mmol/l)‡	1.0	1.3§	1.0	1.3§	0.8	0.9§	0.8	0.9§	0.9 (0.6)
SBP (mmHg)	123	120	122	122	113	113	113	114	117 ± 12
DBP (mmHg)	77	76	76	77	73	73§	72	74	74 ± 8
Heart rate (bpm)	65	71§	65	70§	70	72	69	72§	68 ± 10
Fasting glucose OGTT (mmol/l)†	5.2	5.3	5.2	5.2	4.9	5.0	4.9	5.0	5.1 (0.7)
2-h glucose OGTT (mmol/l)‡	5.5	5.6	5.5	5.7	5.5	5.8§	5.6	5.7	5.6 (1.9)
Fasting C-peptide (pmol/l)	542	669§	555	634§	497	561§	493	569§	540 ± 232
Fasting proinsulin (pmol/l)‡	6.0	7.0§	6.0	7.0§	5.0	5.0	5.0	6.0§	6.0 (4.0)
Fasting insulin OGTT (pmol/l)‡	31	42§	30	41§	27	35§	27	37§	31 (23)
2-h insulin (pmol/l)‡	126	160§	125	160§	151	181§	148	210§	148 (155)
M/I (μmol · min^{-1} kg$_{FFM}$$^{-1}$ · nmol^{-1} · l^{-1})‡	114	96§	117	91§	146	144	151	129§	130 (88)

Data are means ± SD, medians (interquartile range), or frequencies. *Statistically significant difference ($P < 0.05$) from mean in 3 lowest quartiles (linear regression analysis, adjusted for smoking and study center) †Statistically significant difference ($P < 0.05$) from proportion in three lowest quartiles (logistic regression analysis, adjusted for sex, age, and study center). ‡Median. §Statistically significant difference ($P < 0.05$) from mean/median in 3 lowest quartiles (linear regression analysis, adjusted for sex, age, smoking, and study center).

study center, and smoking) in men and by 4.3% (2.1–6.5) in women. Additional adjustment for waist circumference led to a *M/I* decrease of 4.5% (2.1–6.8) per unit WBC in men and 3.2% (1.0–5.3) in women.

ESR was also negatively correlated with *M/I* in men ($\rho = -0.27$, $P < 0.01$) and women ($\rho = -0.21$, $P < 0.01$) (Table 3). *M/I* decreased by 2.1% (95% CI 1.3–2.9) per unit increase in ESR in men and by 1.2% (0.8–1.6) in women. With adjustment for waist circumference, the decrease was 1.3% (0.6–2.0) in men and 0.9% (0.5–1.3) in women.

WBC, cardiometabolic risk factors, and *M/I*

Correlations between WBC and all cardiometabolic risk factors except SBP, DBP, fasting glucose in both men and women, and 2-h glucose in men were significant (Table 2). Overall, correlations were stronger in men than in women. In a multivariable regression model, WBC was a statistically significant predictor variable for waist circumference, fat mass, HDL cholesterol, triglycerides, heart rate, 2-h glucose, fasting C-peptide, proinsulin and insulin, 2-h insulin, and *M/I* in men and in women and also for dBP in women (adjusted for age, study center, and smoking). After addition of *M/I* to the regression model, associations between WBC and 2-h glucose and fasting proinsulin in men and between WBC and DBP in women became statistically nonsignificant. Associations between WBC and HDL cholesterol, triglycerides, heart rate, fasting C-peptide, and insulin and 2-h insulin in men and women and between WBC and 2-h glucose in women remained significant after adjustment for both *M/I* and waist circumference.

ESR, cardiometabolic risk factors, and *M/I*

Correlations between ESR and all cardiometabolic risk factors but fasting glucose in men and women and sBP, dBP, and fasting proinsulin in men were significant (Table 3). In a multivariable regression model, ESR was a statistically significant predictor variable for waist circumference, fat mass, HDL cholesterol, triglycerides, heart rate, fasting C-peptide, proinsulin and insulin, 2-h insulin, and *M/I* in men and women and for 2-h glucose in men. When *M/I* was added to the regression model, associations between ESR and fasting proinsulin in men and women and HDL cholesterol, 2-h glucose, and fasting C-peptide in men became statistically nonsignificant. Associations between ESR and HDL cholesterol, heart rate, fasting, and 2-h insulin in men and women and between ESR and fat mass in women remained significant after adjustment for *M/I* and waist circumference.

WBC, ESR, and fasting insulin compared with *M/I*

Correlations between WBC and fasting insulin were stronger than those between WBC and *M/I* for both men ($\rho = 0.28$,

Table 2—*Spearman rank correlations and standardized regression coefficients for the associations between WBC and cardiometabolic risk factors*

	Correlation	Model 1	Model 2	Model 3	Model 4
Men					
Waist circumference (cm)	0.15*	0.157†	—	0.096†	—
Fat mass (kg)	0.19*	0.198†	0.062†	0.131†	0.047
HDL cholesterol (mmol/l)	−0.32*	−0.294†	−0.243†	−0.245†	−0.219†
Triglycerides (mmol/l)	0.23*	0.221†	0.158†	0.163†	0.126†
sBP (mmHg)	−0.08	−0.045	−0.090†	−0.051	−0.083
dBP (mmHg)	0.01	−0.020	−0.059	−0.041	−0.064
Heart rate (bpm)	0.24*	0.231†	0.205†	0.177†	0.166†
Fasting glucose OGTT (mmol/l)	−0.03	0.009	−0.020	0.019	−0.003
2-h glucose OGTT (mmol/l)	0.06	0.102†	0.065	0.072	0.063
Fasting C-peptide (pmol/l)	0.25*	0.243†	0.173†	0.170†	0.132†
Fasting proinsulin (pmol/l)	0.21*	0.173†	0.109†	0.096	0.063
Fasting insulin OGTT (pmol/l)	0.28*	0.259†	0.182†	0.171†	0.128†
2-h insulin (pmol/l)	0.18*	0.217†	0.158†	0.136†	0.119†
M/I	−0.19*	−0.227†	−0.150†	—	—
Women					
Waist circumference (cm)	0.12*	0.162†	—	0.109†	—
Fat mass (kg)	0.11*	0.175†	0.047†	0.125†	0.044
HDL cholesterol (mmol/l)	−0.19*	−0.186†	−0.137†	−0.150†	−0.123†
Triglycerides (mmol/l)	0.14*	0.157†	0.119†	0.127†	0.108†
sBP (mmHg)	−0.02	0.050	0.008	0.031	0.004
dBP (mmHg)	0.04	0.095†	0.048	0.076	0.047
Heart rate (bpm)	0.14*	0.122†	0.110†	0.121†	0.118†
Fasting glucose OGTT (mmol/l)	0.04	0.081	0.033	0.071	0.034
2-h glucose OGTT (mmol/l)	0.08*	0.133†	0.109†	0.109†	0.100†
Fasting C-peptide (pmol/l)	0.20*	0.241†	0.172†	0.200†	0.163†
Fasting proinsulin (pmol/l)	0.11*	0.148†	0.097†	0.098†	0.072
Fasting insulin OGTT (pmol/l)	0.27*	0.301†	0.225†	0.256†	0.215†
2-h insulin (pmol/l)	0.16*	0.204†	0.171†	0.163†	0.151†
M/I	−0.10*	−0.154†	−0.115†	—	—

Model 1 adjusted for age, center, and smoking status; model 2 adjusted for age, center, smoking status, and waist circumference; model 3 adjusted for age, center, smoking status, and *M/I*; and model 4 adjusted for age, center, smoking status, waist circumference, and *M/I*. *Statistically significant correlation ($P < 0.05$). †Statistically significant standardized regression coefficient ($P < 0.05$).

$P < 0.01$) and women ($\rho = 0.27$, $P < 0.01$). Per unit increase in WBC, fasting insulin increased by 8.1% (95% CI 5.4–10.8) in men and by 9.8% (7.3–12.3) in women. When we adjusted for waist circumference and *M/I*, the increase was 4.1% (1.7–6.4) in men and 7.0% (4.8–9.1) in women. With adjustment for waist circumference and fasting insulin, the decrease per unit of WBC in *M/I* became statistically nonsignificant in both men (–1.8% [95% CI −0.5 to 4.0]) and women (–0.7% [−2.8 to 1.4]).

In men, the correlation between ESR and *M/I* was somewhat stronger than the correlation between ESR and fasting insulin ($\rho = 0.23$, $P < 0.01$). In women, it was the other way around ($\rho = 0.26$, $P < 0.01$). Per unit increase in ESR, fasting insulin increased by 2.2% (95% CI 1.4–3.0) in men and 1.5% (1.0–2.0) in women; adjusted for waist circumference and *M/I,* the increase was 0.9% (0.3–1.6) in men and 0.6% (0.2–1.0) in women. With adjustment for waist circumference and fasting insulin, the decrease per unit WBC in *M/I* was 0.7% (0.1–1.4) in men and 0.6% (0.2–1.0) in women.

Additional analyses

To examine whether the associations between WBC, ESR, and fasting C-peptide and proinsulin could be explained by fasting insulin, we added fasting insulin to regression model 1. Results showed that associations between WBC, ESR, and proinsulin and between ESR and C-peptide disappeared in both men and women. However, the association between WBC and fasting C-peptide remained (standardized β in men 0.094 and in women 0.075).

Twenty-three participants had a WBC $>10 \times 10^{12}$/l, and 190 participants had an ESR value >15 mm/h. Exclusion of high WBC and ESR values had some effect on the size of the correlations with the cardiometabolic variables. However, except for the correlation between WBC and fasting C-peptide in men ($P = 0.07$) and correlations between ESR and SBP ($P = 0.07$) and DBP ($P = 0.07$) in women, all of the reported correlations remained statistically significant.

CONCLUSIONS — In a large, healthy European cohort, we found that two general markers of inflammation, WBC and ESR, were associated with insulin sensitivity, as measured by the hyperinsulinemic-euglycemic clamp, and with a wide range of other cardiometabolic risk factors. Insulin resistance was related to several inflammatory factors previously (2,6,7). However, in these studies, fasting insulin was often used as a surrogate measure of insulin resistance (6,7). We have now shown that insulin sensitivity as measured by the standard technique, the

Table 3—*Spearman rank correlations and standardized regression coefficients for the associations between ESR and cardiometabolic risk factors*

	Correlation	Model 1	Model 2	Model 3	Model 4
Men					
Waist circumference (cm)	0.24*	0.186†	—	0.095†	—
Fat mass (kg)	0.22*	0.194†	0.048	0.102†	0.034
HDL cholesterol (mmol/l)	−0.17*	−0.121†	−0.076	−0.032	−0.023
Triglycerides (mmol/l)	0.19*	0.171†	0.121†	0.102†	0.082
sBP (mmHg)	−0.03	−0.013	−0.063	−0.038	−0.067
dBP (mmHg)	−0.04	0.002	−0.040	−0.041	−0.060
Heart rate (bpm)	0.18*	0.180†	0.151†	0.139†	0.130†
Fasting glucose OGTT (mmol/l)	−0.02	−0.029	−0.048	−0.039	−0.045
2-h glucose OGTT (mmol/l)	0.13*	0.110†	0.069	0.020	0.032
Fasting C-peptide (pmol/l)	0.12*	0.130†	0.053	0.038	0.012
Fasting proinsulin (pmol/l)	0.08	0.122†	0.033	−0.002	−0.026
Fasting insulin OGTT (pmol/l)	0.23*	0.231†	0.144†	0.134†	0.100†
2-h insulin (pmol/l)	0.19*	0.199†	0.145†	0.080†	0.075†
M/I	−0.27*	−0.231†	−0.145†	—	—
Women					
Waist circumference (cm)	0.24*	0.198†	—	0.142†	—
Fat mass (kg)	0.24*	0.227†	0.070†	0.173†	0.065†
HDL cholesterol (mmol/l)	−0.18*	−0.181†	−0.113†	−0.127†	−0.083†
Triglycerides (mmol/l)	0.18*	0.112†	0.061	0.068	0.039
SBP (mmHg)	−0.09*	0.005	−0.054	−0.009	−0.052
DBP (mmHg)	−0.08*	0.008	−0.059	−0.013	−0.059
Heart rate (bpm)	0.17*	0.145†	0.128†	0.124†	0.116†
Fasting glucose OGTT (mmol/l)	−0.05	−0.002	−0.056	−0.003	−0.048
2-h glucose OGTT (mmol/l)	0.14*	0.062	0.027	0.006	0.001
Fasting C-peptide (pmol/l)	0.19*	0.153†	0.055	0.082†	0.022
Fasting proinsulin (pmol/l)	0.17*	0.124†	0.036	0.044	−0.009
Fasting insulin OGTT (pmol/l)	0.26*	0.234†	0.136†	0.148†	0.090†
2-h insulin (pmol/l)	0.26*	0.217†	0.179†	0.133†	0.120†
M/I	−0.21*	−0.218†	−0.161†	—	—

Model 1 adjusted for age, center, and smoking status; model 2 adjusted for age, center, smoking status, and waist circumference; model 3 adjusted for age, center, smoking status, and *M/I*; and model 4 adjusted for age, center, smoking status, waist circumference, and *M/I*. *Statistically significant correlation ($P < 0.05$). †Statistically significant standardized regression coefficient ($P < 0.05$).

hyperinsulinemic-euglycemic clamp, is associated with inflammation. In addition, we have shown that fasting hyperinsulinemia is independently and at least as strongly associated with inflammation as insulin resistance. The RISC study previously showed that insulin resistance and hyperinsulinemia are independent contributors to cardiovascular risk (9). The present results are supported by Festa et al. (11), who showed that fasting proinsulin and insulin were related to fibrinogen and plasminogen activator inhibitor 1 independently of insulin resistance as estimated by an intravenous glucose tolerance test.

When we adjusted for waist circumference, the associations between WBC, ESR, and insulin resistance were reduced but remained strong. A number of proinflammatory cytokines are known to directly affect insulin sensitivity: tumor necrosis factor-α and leptin have been shown to affect insulin sensitivity in animal models, whereas IL-6 has been shown to induce insulin resistance in humans (12–14). Hypothetically, the inflammatory effect on fasting insulin concentrations could occur in the liver. Fasting hyperinsulinemia has been suggested to reflect insulin resistance in the liver (15). IL-6 has been shown to inhibit insulin signaling in hepatocytes (16). Because of the cross-sectional nature of our data, a reversed pathway is also possible: the state of insulin resistance and/or hyperinsulinemia itself promotes inflammation. Insulin has an anti-inflammatory effect in that it can lessen the acute-phase response (17). Insulin resistance may prevent the anti-inflammatory effect of insulin. Another possibility is that low-grade inflammation, insulin resistance, and hyperinsulinemia are all manifestations of another underlying pathological condition, for example, dysfunction of the autonomic nervous system as a consequence of a disturbed food and activity behavior pattern. This dysfunction has been hypothesized to underlie the metabolic syndrome and its precursors (18).

However, an indirect pathway from inflammation to insulin resistance via obesity could also be possible. A body of evidence shows that obesity causes inflammation. However, some data suggest the reverse to also be true: a state of chronic inflammation may be a causative factor in obesity (19).

Besides insulin sensitivity and fasting insulin, both WBC and ESR showed consistent and strong associations with waist circumference, fat mass, HDL cholesterol, triglycerides, heart rate, and 2-h insulin in men and women. Associations between WBC, ESR, and 2-h glucose and dBP were less consistent, and we found no associations between WBC, ESR, and sBP and fasting glucose. The strong relations between WBC, ESR, and the several cardio-

metabolic risk factors became somewhat smaller but did not consistently disappear after adjustment for insulin resistance. Although the data are cross-sectional, they suggest that a state of chronic low-grade inflammation does not lead to the development of a pattern of high cardiometabolic risk via the inducement of insulin resistance. Instead, the associations seem to be of a direct nature. Most are supported by the literature. Infection and inflammation are well known to be associated with marked changes in lipid and lipoprotein metabolism (20). An association between subclinical inflammation and elevated heart rate was found in a study of middle-aged and elderly individuals without apparent heart disease (21). Interestingly, we also found an association between WBC and fasting C-peptide, which did not disappear after adjustment for fasting insulin. A direct effect of inflammation on C-peptide is not known. However, because our observations are not of a prospective nature, the direction of the associations could also be reversed. C-peptide has been found to induce monocyte chemotaxis in vitro and may play an active role in atherogenesis (22).

Another association that could be reversed is that of inflammation and lipids. Evidence for a direct effect of lipids on the induction of a proinflammatory state is increasing. Among other studies, one in which healthy men were subjected to a water test and a 6-h fat challenge showed that after the fat challenge, neutrophil counts and activation of monocytes and neutrophils were increased compared with values after the water test (23).

The relationship between smoking, alcohol, and inflammation has long been known. Our study results confirm the major effect that smoking behavior has on inflammation: the number of smokers more than doubled in the highest quartile of WBC values compared with the numbers in lower quartiles. Results from the present study also confirm that the use of alcohol reduces inflammation.

Our study has a number of limitations. Both WBC and ESR were not centrally determined by a single laboratory, possibly inducing measurement variability. Systematic differences between study centers were observed, but we adjusted for study center in all analyses. Another drawback is that we had no information on possible infections in our participants that could have caused a high WBC or ESR. However, removal of the clinically abnormal values of WBC and ESR had no major effect on the correlations between these markers and cardiometabolic variables. Also, WBC and ESR may be viewed as suboptimal markers of inflammation compared with the currently very popular marker CRP (which we did not measure). However, both WBC and ESR have frequently been shown to be associated with cardiometabolic abnormalities and with the development of cardiometabolic disease and cardiovascular mortality (2–5,24,25). A further limitation is that because of the cross-sectional nature of our data, we can draw no conclusions on causality and directionality of the associations we found. Finally, in observational studies, the interpretation of the observed associations and the changes in the model after addition of additional variables is limited by chance and by imprecision of the measurements. In the present study, efforts were made to perform precise measurements of insulin resistance. Furthermore, all centers were centrally instructed on the measurements and central laboratories performed most assays. The consistent pattern observed—that the majority of the associations between the inflammation markers and cardiometabolic risk factors remained strong after addition of *M/I* to the model and regression coefficients only slightly decreased—suggests that insulin resistance is not a large intermediary factor.

Besides the limitations, our study has some major advantages. First, we measured inflammatory markers in a large number of healthy participants. Second, we measured insulin sensitivity with the gold standard technique. Finally, we measured a wide variety of cardiometabolic risk factors, ranging from glucose concentrations to concentrations of C-peptide.

In conclusion, this study showed that low-grade chronic inflammation is strongly associated with cardiometabolic risk in a healthy population. Insulin resistance, as measured by the hyperinsulinemic-euglycemic clamp, seems to be one of these cardiometabolic risk factors rather than an intermediary factor in the relation between inflammation and other cardiometabolic risk factors.

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