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

Associations with insulin resistance and cardiometabolic risk profile

SUSANNE R. DE ROOIJ, PHD1
GIEL NJIPPELS, MD, PHD1
PETER M. NILSSON, MD, PHD2
JOHN J. NOLAN, MD1
RAFAEL GABRIEL, MD, PHD4
ELISABETTA BOBBIONI-HARSCH, PHD5
GERTRUDE MINGRONE, MD, PHD6
JACQUeline M. DEKKER, PHD1

FOR THE RELATIONSHIP BETWEEN INSULIN SENSITIVITY AND CARDIOVASCULAR DISEASE (RISC) INVESTIGATORS*

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

From the 1Institute for Research in Extramural Medicine, VU University Medical Center, Amsterdam, the Netherlands; the 2Department of Clinical Sciences, Lund University, University Hospital, Malmo, Sweden; the 3Metabolic Research Unit, St. James’ Hospital, Trinity College, Dublin, Ireland; the 4Unidad de Investigacion, Hospital Universitario La Paz, Madrid, Spain; the 5Division of Therapeutical Teaching for Chronic Diseases, University Hospital, Geneva, Switzerland; and the 6Department of Internal Medicine, Catholic University, Rome, Italy.

Corresponding author: Susanne R. de Rooij, s.r.derooij@amc.uva.nl.

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* A complete list of the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) investigators and recruiting centers is available in an online appendix.

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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. The mean all-center variance was 0.2 ± 0.5 mm Hg. ESR was measured by the modified Westergren method. The mean all-center variance of the ESR was 52.5 mm Hg, whereas the between-center variances were 0.2 × 10^12/l and 52.5 mm Hg. 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) × 10^12/l in men and 5.8 (2.0) × 10^12/l in women (P = 0.73 for difference). Median values and interquartile ranges for ESR were 5.0 (7.0) mm Hg in men and 8.0 (8.0) mm Hg 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 × 10^12/l in men and from 4.9 to 6.4 × 10^12/l in women. Medians for ESR ranged from 2.0 to 9.0 mm Hg in men and from 5.0 to 15.0 mm Hg in women. WBC and ESR were significantly correlated with each other and were stronger in men (ρ = 0.14, P < 0.01) than in women (ρ = 0.08, P = 0.04).

WBC, ESR, and M/I WBC was negatively correlated with M/I in both men (ρ = −0.19, P < 0.01) and women (ρ = −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,
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, proinsulin, 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 fasting proinsulin in men and women remained significant after adjustment for both $M/I$ and waist circumference.

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

|                  | WBC | ESR |
|------------------|-----|-----|
|                  | 3 lowest quartiles | Highest quartile | 3 lowest quartiles | Highest quartile |
|                  | 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·kgFFM⁻¹·1⁻¹)‡ | 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).
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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 WBC in M/I was 0.7% (0.1–1.4) in men and 0.6% (0.2–1.0) in women.

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 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
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-

|                     | 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).
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 induction 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 number 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 MI 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 hyperinsulinememic-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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References

1. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286–1292.
2. Festa A, D’Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42–47.
3. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999;353:1649–1652.
4. Natali A, L’Abbate A, Ferrannini E. Erythrocyte sedimentation rate, coronary atherosclerosis, and cardiac mortality. Eur Heart J 2003;24:639–648.
5. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998;279:1477–1482.
6. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972–978.
7. Shim WS, Kim HJ, Kang ES, Ahn CW, Lim SK, Lee HC, Cha BS. The association of total and differential white blood cell count with metabolic syndrome in type 2 diabetic patients. Diabetes Res Clin Pract 2006;73:284–291.
8. Vozrova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tatarami PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002;51:455–461.
9. Ferrannini E, Balkau B, Coppack SW, Dekker JM, Mari A, Nolan J, Walker M, Natali A, Beck-Nielsen H. Insulin resistance, insulin response, and obesity as indicators of metabolic risk. J Clin Endocrinol Metab 2007;92:2885–2892.
10. Hills SA, Balkau B, Coppack SW, Dekker JM, Mari A, Natali A, Walker M, Ferrannini E. The EGIR-RISC STUDY (The European group for the study of insulin.
resistance: relationship between insulin sensitivity and cardiovascular disease risk). I. Methodology and objectives. Diabetologia 2004;47:566–570

11. Festa A, D’Agostino R Jr, Mykkanen L, Tracy RP, Zaccaro DJ, Hales CN, Haffner SM. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance. The Insulin Resistance Atherosclerosis Study (IRAS). Arterioscler Thromb Vasc Biol 1999;19:562–568

12. Uysal KT, Wiesbrock SM, Hotamisligil GS. Functional analysis of tumor necrosis factor (TNF) receptors in TNF-α-mediated insulin resistance in genetic obesity. Endocrinology 1998;139:4832–4838

13. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-α, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 2003;278:45777–45784

14. Park S, Hong SM, Sung SR, Jung HK. Long-term effects of central leptin and resistin on body weight, insulin resistance, and β-cell function and mass by the modulation of hypothalamic leptin and insulin signaling. Endocrinology 2008;149:445–454

15. Meyer C, Pimenta W, Woerle HJ, Van HT, Szoke E, Mittrakou A, Gerich J. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. Diabetes Care 2006;29:1909–1914

16. Senn JJ, Kloper PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 2002;51:3391–3399

17. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S. Insulin inhibits intranuclear nuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 2001;86:3257–3265

18. Kreier F, Yilmaz A, Kalsbeek A, Romijn JA, Sauerwein HP, Fliers E, Buijs RM. Hypothesis: shifting the equilibrium from activity to food leads to autonomic unbalance and the metabolic syndrome. Diabetes 2003;52:2652–2656

19. Rogge MM. The case for an immunologic cause of obesity. Biol Res Nurs 2002;4:43–53

20. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45:1169–1196

21. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Abedini S, Hansen JF. Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent heart disease. Eur Heart J 2004;25:363–370

22. Marx N, Walcher D, Raichle C, Aleksic M, Bach H, Grub M, Hombach V, Libby P, Zieske A, Homma S, Strong J. C-peptide colocalizes with macrophages in early atherosclerotic lesions of diabetic subjects and induces monocyte chemotaxis in vitro. Arterioscler Thromb Vasc Biol 2004;24:540–545

23. van Oostrom AJ, Rabelink TJ, Verseyden C, Sijmonsma TP, Plokker HW, De Jager P, Cabezas MC. Activation of leukocytes by postprandial lipemia in healthy volunteers. Atherosclerosis 2004;177:175–182

24. Brown DW, Giles WH, Croft JB. White blood cell count: an independent predictor of coronary heart disease mortality among a national cohort. J Clin Epidemiol 2001;54:316–322

25. Erikssen G, Liestol K, Bjornholt JV, Stormorken H, Thaulow E, Erikssen J. Erythrocyte sedimentation rate: a possible marker of atherosclerosis and a strong predictor of coronary heart disease mortality. Eur Heart J 2000;21:1614–1620