Subclinical Inflammation Is Strongly Related to Insulin Resistance But Not to Impaired Insulin Secretion in a High Risk Population for Diabetes

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Subclinical inflammation was shown to be a strong predictor of cardiovascular events and was suggested to be a part of the metabolic syndrome (MS). The aim of the present study was to investigate the relationship of the inflammatory parameters—leukocyte count, C-reactive protein (CRP), and fibrinogen level—to insulin resistance and insulin secretion, as well as to other components of the MS in a population at risk for diabetes. A total of 396 subjects (142 men and 254 women) were analyzed from the follow-up of the Risk Factors in Impaired Glucose tolerance (IGT) for Atherosclerosis and Diabetes (RIAD) study, who were at risk for type 2 diabetes, such as family history of diabetes, obesity, and/or hyper/dyslipoproteinemia. Subjects under lipid-lowering treatment or with acute infections were not eligible. A variety of risk factors within the MS were examined: lipids, glycemic parameters, coagulation, insulin fractions. and microalbuminuria. CRP was determined by a highly sensitive method, using an immunological agglutination test, and fibrinogen was measured by the method of Clauss. Insulin resistance was evaluated by the homeostasis model assessment (HOMA) and insulin secretion by HOMA and by insulin areas under curve in an oral glucose tolerance test (OGTT), insulin increment at 30 mnutes of OGTT, and insulin increment/glucose increment at 30 minutes of OGTT. By univariate analysis, fibrinogen level ($r = 0.180$, $P < .001$), leukocyte count ($r = 0.162$, $P = .001$), and CRP ($r = 0.251$, $P < .001$) were all highly significantly correlated to insulin resistance, but not to insulin secretion. A significant rise was found for the majority of the components of the MS in quartiles of the examined inflammatory parameters. In multivariate analysis of all analyzed metabolic parameters, including age, sex, physical activity, and smoking, body mass index (BMI) was found a strong independent determinant of all inflammatory markers examined. Thus, in a population at risk for type 2 diabetes we demonstrate that subclinical inflammation underlies the metabolic syndrome, through association to one of its primary anomalies—insulin resistance, whereas no association was found to impaired insulin secretion.

THE METABOLIC syndrome (MS) is characterized by clustering of glucose intolerance (or type 2 diabetes), android obesity, hypertension, hyper/dyslipidemia, impaired fibrinolysis and coagulation, and accelerated atherosclerosis, which develop on the basis of a genetic predisposition in interaction with environmental factors.[1-3] The MS is one of the most common diseases worldwide, especially among elderly subjects in the industrial countries, with a further increasing prevalence also in the developing countries and among younger individuals.[4,5]

During the last years it was found that markers of inflammation, such as C-reactive protein (CRP), leukocyte count, fibrinogen, and lipoprotein-associated phospholipase A2, are strong independent predictors of cardiovascular and cerebrovascular events.[6-11] The data available suggest that low-grade chronic inflammation not only reflects the underlying macrovascular disease, but is also actively involved in the initial and advanced stages of atherogenesis.[12-17] Although inflammation has been convincingly shown to be associated to atherosclerosis, the nature of the link is not completely understood. A possible mechanism is through the association to other risk factors within the metabolic syndrome.[18] Thus, inflammatory parameters were reported to be strongly related to various features of the MS, such as obesity, hyper/dyslipidemia, hypertension, hyperglycemia etc.[18-21]

Recent data indicate that the chronic activation of the innate immune system may underlie the MS, characterizing the common soil for the causality of type 2 diabetes and cardiovascular disease.[22] Prospective findings of the Atherosclerosis Risk in Communities (ARIC) study indicate that markers of inflammation and endothelial dysfunction predict the development of diabetes mellitus and weight gain in adults.[22,23] Since the primary defects in the pathogenesis of type 2 diabetes are insulin resistance and impaired insulin secretion, linked to each other in a vicious cycle,[1,2,24,25] it is interesting whether and to what extent chronic inflammation affects any or both of them. In a nondiabetic population from the Insulin Resistance Atherosclerosis Study (IRAS), CRP was shown to be independently related to insulin sensitivity, measured by a frequently sampled intravenous glucose tolerance test.[18] So far there are no data on the effect of chronic subclinical inflammation on insulin secretion. To address this question, a population disposed to develop diabetes would be most appropriate. Therefore, the aim of the present study was to investigate the relationship of the inflammatory parameters—leukocytes count, CRP, and fibrinogen level—to insulin resistance and insulin secretion, as well as to other components of the MS in a population at risk for diabetes.

MATERIALS AND METHODS

Subjects were analyzed from the follow-up of the Risk Ractors in Impaired Glucose tolerance (IGT) for Atherosclerosis and Diabetes (RIAD) study. In brief, the baseline examination consisted of 1,139 subjects, aged 40 to 70 years, who were at risk for the development of type 2 diabetes, such as family history of diabetes, obesity, and/or

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hyper/dyslipoproteinemia.[26,27] Known diabetes, medication affecting glucose tolerance, liver and kidney diseases, thyroid gland functional disorders, and acute infections were exclusion criteria. Follow-up evaluations were performed 2 to 3 years after baseline. Of the original subjects examined at baseline, 597 appeared for the follow-up examination. Of these, 201 were receiving lipid-lowering drugs and were therefore excluded from the analysis, because statins and fibrates are known to reduce inflammatory parameters.[28-30] Basic characteristics of the examined subjects (n = 396; 142 men and 254 women) are listed in Table 1. Two hundred seventy-one had a normal glucose tolerance, 82 had IGT, and 43 had newly detected asymptomatic type 2 diabetes.

Laboratory Examination

Venous blood was drawn after an overnight fast of at least 10 hours for the measurement of inflammation parameters and various components of the MS.[26,27]

CRP was determined by a highly sensitive method, using an immunological agglutination test (Boehringer Mannheim test kits, Mannheim Germany). Fibrinogen was measured by the method of Clauss (Fibrinogen; Boehringer Mannheim; coefficient of variation [CV], 2.9% to 5.5%). Complete blood cell counts were performed with standard techniques.

A standard oral glucose tolerance test (OGTT) was performed with 75-g glucose (Glucodex; Rougier Inc, Chambly, Canada). Plasma glucose was measured by the hexokinase method (interassay CV, 1.5%). HbA_{1c} was examined by high-performance liquid chromatography on a Diamat analyzer (Bio-Rad Laboratories, Munich, Germany). Triglycerides, total cholesterol, and free fatty acids were measured enzymatically on a Ciba Corning Express Plus analyzer (Boehringer Mannheim). High-density lipoprotein (HDL)-cholesterol was determined after precipitation with dextran sulfate on a Ciba Corning Express Plus analyzer (Boehringer Mannheim).

Proinsulin was analyzed by highly specific enzyme immunoassay (DGR Instruments, Marburg, Germany). Specific insulin and C-peptide were also measured by enzyme immunoassays (Medgenix Diagnostics, Fleurus, Belgium). Specific insulin (interassay CV, 7.6%) showed no cross-reactivity to human proinsulin. Insulin resistance was evaluated by the homeostasis model assessment (HOMA)[31] and insulin secretion by HOMA and by insulin areas under the curve in OGTT, insulin increment at 30 minutes of OGTT, and insulin increment/glucose increment at 30 minutes of OGTT.

The concentration of active plasminogen activator inhibitor-1 (PAI-1) antigen was determined using commercially available enzyme immunassay (Immuno AG, Heidelberg, Germany). Tissue plasminogen activator (tPA) was measured by enzyme immunoassay (TintElize; Biopool, Umea, Sweden) and von Willebrandt factor antigen by electroimmunoassay (Immuno AG). Urine was collected as fresh morning urine samples. Albuminuria was measured by nephelometry (Nephelometer BNII; Behring, Marburg, Germany).

Statistics

Data evaluation was conducted using the SPSS/PC + program. The distribution of values was assessed by the Kolmogorov-Smirnov test for homogeneity of variances. The distribution of CRP level was highly skewed. Therefore logarithmically transformed values of CRP were used to analyze the correlation to insulin resistance and insulin secretion. Metabolic parameters were evaluated in quartiles of fibrinogen, CRP, and leukocytes count using analysis of variance (ANOVA). For nonparametric variables, values were transformed logarithmically. Data are presented as the mean ± SD. HOMA insulin resistance was assessed in tertiles of leukocytes count and body mass index (BMI), as well as in tertiles of CRP and BMI. Multivariate analysis was conducted by multiple linear regression to demonstrate the independent determinants of the inflammatory markers. To decide which variables should be included into the model, at first age, sex, smoking status, physical activity, BMI, waist-to-hip ratio (WHR), blood pressure, HbA_{1c}, plasma glucose (fasting and postprandial), insulin (fasting and postprandial), C-peptide (fasting and postprandial), free fatty acids, plasma triglycerides, HDL-cholesterol, PAI, tPA, microalbuminuria, and von Willebrandt factor were included into a univariate analysis to evaluate the correlation of any of these to the examined inflammatory parameters, after which the ones that were significantly correlated were included into the multivariate analysis.

RESULTS

In univariate analysis, fibrinogen level ($r = 0.180$, $P < .001$), leukocyte count ($r = 0.162$, $P = .001$), and CRP ($r = 0.251$, $P < .001$) were all highly significantly correlated to HOMA insulin resistance, which was confirmed after adjustment for age and sex. In multivariate analysis, considering all examined variables of the MS, after including BMI, none of the inflammatory parameters remained significantly related with insulin resistance. By univariate analysis, the inflammatory parameters were not significantly correlated with HOMA insulin secretion.

Table 2 lists the levels of various metabolic parameters in quartiles of fibrinogen. A significant rise was found for BMI, HbA_{1c}, fasting and 2-hour postprandial plasma glucose in OGTT, fasting insulin, C-peptide (fasting and postprandial), HOMA insulin resistance (but not HOMA insulin secretion, insulin areas under the curve in OGTT, insulin increment at 30 minutes of OGTT, or insulin increment/glucose increment at 30 minutes of OGTT), total cholesterol, and microalbuminuria, as well as age.

The risk factors of the metabolic syndrome in quartiles of CRP are listed in Table 3. A significant elevation in the top quartiles was observed for BMI, blood pressure (systolic and diastolic), HbA_{1c}, plasma glucose (fasting and postprandial), insulin (fasting and postprandial), C-peptide (fasting and postprandial), free fatty acids (fasting), insulin resistance (but not HOMA insulin secretion, insulin areas under the curve in OGTT, insulin increment at 30 minutes of OGTT, or insulin increment/glucose increment at 30 minutes of OGTT), plasma triglycerides, and PAI active (no significant trend), as well as a significant reduction of HDL-cholesterol.

Table 4 lists the metabolic parameters in quartiles of leukocytes count. A significant rise was observed for BMI, WHR (no significant trend), HbA_{1c}, plasma glucose (fasting and postprandial), C-peptide (fasting and postprandial), free fatty acids (fasting), HOMA insulin resistance (but not insulin secretion, insulin areas under the curve in OGTT, insulin increment at 30 minutes of OGTT, or insulin increment/glucose increment at 30 minutes of OGTT), plasma triglycerides, and microalbuminuria, and a significant decrease of HDL-cholesterol.

HOMA insulin resistance was significantly correlated with BMI ($r = 0.545$, $P < .001$), CRP ($r = 0.243$, $P < .001$), and leukocyte count ($r = 0.103$, P .04). No correlation was found between the inflammatory parameters and HOMA insulin secretion, insulin areas under curve in OGTT, insulin increment at 30 minutes of OGTT, and insulin increment/glucose increment at 30 minutes of OGTT in the total group, as well as in the different glucose tolerance stages.

Figure 1 shows HOMA insulin resistance in tertiles of BMI

Table 1. Basic Characteristics of the Examined Subjects in Total and In Glucose Tolerance Stages (mean ± SD)

Parameter	Total	NGT	IGT	DM
No. (men/women)	396 (142/254)	271 (98/173)	82 (25/57)	43 (19/24)
Age (yr)	56.8 ± 8.7	55.9 ± 8.5	58.8 ± 9.0	59.2 ± 8.4
BMI (kg/m^2)	27.1 ± 4.8	26.4 ± 4.7	28.3 ± 4.7	29.1 ± 4.7
WHR	0.87 ± 0.09	0.86 ± 0.1	0.87 ± 0.08	0.91 ± 0.07
HbA_{1C} (%)	5.5 ± 0.6	5.4 ± 0.5	5.5 ± 0.4	6.2 ± 0.7
Plasma glucose, fasting (mmol/L)	5.6 ± 0.9	5.4 ± 0.5	5.8 ± 0.5	7.2 ± 1.3
Plasma glucose, 2 hour in OGTT (mmol/L)	7.0 ± 2.8	5.5 ± 1.2	9.0 ± 0.9	12.5 ± 3.6
Blood pressure, systolic (mm Hg)	128.0 ± 20.5	125.0 ± 19.9	133.1 ± 19.1	137.7 ± 22.3
Blood pressure, diastolic (mm Hg)	80.7 ± 10.8	79.7 ± 10.5	82.5 ± 10.2	83.7 ± 13.3

Abbreviations: NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus.

and CRP. A significant rise in trend was found parallel to the increase of BMI in all CRP tertiles. Also, in the second tertile for BMI, a significant increase in trend was observed parallel to the elevation of CRP.

HOMA insulin resistance in dependence on BMI and leukocyte count is presented in Fig 2. In all tertiles for leukocyte count, a significant rise is seen parallel to the increase of BMI. In addition, in the top tertile for BMI, a significant elevation of insulin resistance is observed with the increase of leukocyte count.

In multivariate analysis, including all analyzed metabolic parameters (age, sex, physical activity, and smoking status), BMI ($P = .001$), smoking ($P = .006$), and PAI ($P = .01$) were found as independent determinants of leukocytes count; BMI ($P = .002$), age ($P = .015$), and low physical activity ($P = .045$) were independently related to fibrinogen; and BMI ($P < .001$) and postprandial C-peptide ($P = .002$) were independent determinants of CRP.

DISCUSSION

In this analysis we confirm the notion that subclinical inflammation is an integral part of the MS and is strongly related

Table 2. Metabolic Parameters (mean ± SD) in Quartiles of Fibrinogen

Parameter	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P‡
No.	103	98	97	98	
Age (yr)	55.5 ± 9.2	54.9 ± 8.3	58.6 ± 7.8	58.6 ± 8.5	3, 4 to 1, 2
BMI (kg/m^2)	26.1 ± 3.7	26.4 ± 3.8	27.4 ± 5.0	28.7 ± 6.0	4 to 1, 2
WHR	0.87 ± 0.11	0.86 ± 0.09	0.88 ± 0.09	0.88 ± 0.1	NS
Blood pressure, systolic (mm Hg)	126 ± 18	127 ± 19	130 ± 21	130 ± 24	NS
Blood pressure, diastolic (mm Hg)	81 ± 10	81 ± 9	80 ± 12	81 ± 12	NS
HbA_{1C} (%)	5.3 ± 0.6	5.4 ± 0.5	5.6 ± 0.6	5.6 ± 0.5	3, 4 to 1, 2
Plasma glucose, fasting (mmol/l)	5.5 ± 0.6	5.6 ± 0.9	5.7 ± 1.1	5.8 ± 0.8	3, 4 to 1
Plasma glucose, 2-hour in OGTT (mmol/L)	6.8 ± 2.6	6.4 ± 2.4	7.0 ± 3.1	7.6 ± 3.1	4 to 1, 2
Insulin, fasting* (pmol/L)	69.2 ± 75.8	67.8 ± 40.0	72.0 ± 56.8	74.4 ± 38.8	4 to 1
Insulin, 2-hour in OGTT* (pmol/L)	326 ± 333	285 ± 270	327 ± 342	338 ± 276	NS
Proinsulin, fasting (pmol/L)	4.4 ± 4.1	9.8 ± 45.4	4.8 ± 4.3	3.8 ± 2.3	NS
Proinsulin, 2-hour in OGTT (pmol/L)	20.8 ± 18.7	27.0 ± 44.9	19.4 ± 11.6	19.9 ± 11.2	NS
C-peptide, fasting (pmol/L)	1,159 ± 624	1,188 ± 533	1,204 ± 549	1,322 ± 443	4 to 1
C-peptide, 2-hour in OGTT (pmol/L)	3,814 ± 1,498	3,647 ± 1,380	3,741 ± 1,409	4,234 ± 1,400	4 to 1, 2, 3
Free fatty acids, fasting (mmol/L)	0.57 ± 0.25	0.55 ± 0.22	0.61 ± 0.28	0.59 ± 0.23	NS
Insulin resistance (HOMA)*	17.3 ± 20.1	17.6 ± 12.5	19.1 ± 16.7	19.4 ± 11.4	4 to 1
Insulin secretion (HOMA)*	710 ± 640	575 ± 781	707 ± 730	682 ± 327	NS
Insulin areas under the curve during 2-hour OGTT (pmol/L)	42.987 ± 28.976	42.247 ± 28.551	43.952 ± 32.710	46.353 ± 29.204	NS
Insulin increment, 30 min in OGTT (pmol/L)	322 ± 253	287 ± 209	286 ± 189	303 ± 243	NS
Insulin increment/glucose increment, 30 min in OGTT · 10^{-7} *†	0.73 ± 0.37	1.39 ± 0.71	0.71 ± 0.11	0.37 ± 0.44	NS
Triglycerides* (mmol/L)	1.4 ± 0.8	1.6 ± 1.8	1.8 ± 2.8	1.4 ± 0.8	NS
Total cholesterol (mmol/L)	5.4 ± 0.8	5.8 ± 1.2	6.0 ± 1.2	5.8 ± 1.1	1 to 2, 3, 4
HDL-cholesterol (mmol/L)	1.6 ± 0.4	1.6 ± 0.5	1.6 ± 0.4	1.5 ± 0.4	NS
PAI active* (ng/mL)	15.2 ± 37.5	12.6 ± 18.7	16.7 ± 30.6	14.3 ± 13.2	NS
Microalbuminuria* (mg/L)	18.2 ± 20.3	15.5 ± 27.8	16.5 ± 18.7	23.0 ± 27.9	4 to 2

NOTE. Quartile 1, ≤ 2.7 g/L; Quartile 2, > 2.7 and ≤ 3.1 g/L; Quartile 3, > 3.1 and ≤ 3.6 g/L; Quartile 4, > 3.6 and ≤ 9.9 g/L.

*Logarithmically transformed values in ANOVA.

†Data given as mean ± SEM.

‡Significant difference, $P < .05$. NS, not significant.

Table 3. Metabolic Parameters (mean ± SD) in Quartiles of CRP

Parameter	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* ‡
No.	183	16	101	96	
Age (yr)	56.6 ± 8.5	54.3 ± 11.6	57.3 ± 8.7	57.2 ± 8.4	NS
BMI (kg/m^2)	25.6 ± 3.5	26.0 ± 3.8	27.7 ± 5.0	29.4 ± 5.7	4 to 1, 2, 3; 3 to 1
WHR	0.87 ± 0.10	0.86 ± 0.10	0.87 ± 0.08	0.88 ± 0.09	NS
Blood pressure, systolic (mm Hg)	126 ± 20	122 ± 18	128 ± 21	133 ± 20	4 to 1, 2
Blood pressure, diastolic (mm Hg)	79 ± 10	80 ± 11	81 ± 11	83 ± 12	4 to 1
HbA_{1C} (%)	5.4 ± 0.6	5.5 ± 0.4	5.5 ± 0.5	5.5 ± 0.7	3, 4 to 1
Plasma glucose, fasting (mmol/L)	5.5 ± 0.7	5.7 ± 1.0	5.6 ± 0.8	5.9 ± 1.1	4 to 1, 3
Plasma glucose, 2-hour in OGTT (mmol/L)	6.4 ± 2.6	6.6 ± 2.2	7.2 ± 2.8	7.8 ± 3.1	3, 4 to 1
Insulin, fasting* (pmol/L)	66.1 ± 67.6	66.7 ± 40.4	69.1 ± 35.7	81.1 ± 44.8	3, 4 to 1
Insulin, 2-hour in OGTT* (pmol/L)	271 ± 274	335 ± 430	341 ± 315	389 ± 314	3, 4 to 1
Proinsulin, fasting (pmol/l)	4.0 ± 3.7	5.8 ± 7.9	9.0 ± 43.7	4.4 ± 4.4	NS
Proinsulin, 2-hour in OGTT (pmol/L)	18.8 ± 15.0	32.2 ± 48.8	25.0 ± 39.1	23.3 ± 14.5	NS
C-peptide, fasting (pmol/L)	1,102 ± 544	1,161 ± 522	1,249 ± 461	1,390 ± 572	3, 4 to 1
C-peptide, 2-hour in OGTT (pmol/L)	3,534 ± 1,384	3,745 ± 1,552	4,040 ± 1,442	4,333 ± 1,365	3, 4 to 1
Free fatty acids, fasting (mmol/L)	0.56 ± 0.24	0.55 ± 0.26	0.60 ± 0.24	0.62 ± 0.26	4 to 1
Insulin resistance (HOMA)*	16.5 ± 17.6	17.9 ± 13.9	17.5 ± 10.4	22.3 ± 15.6	3, 4 to 1; 4 to 3
Insulin secretion (HOMA)*	686 ± 698	633 ± 330	609 ± 763	704 ± 351	NS
Insulin areas under the curve in 2-hour OGTT (pmol/L)	41.060 ± 30.235	43.915 ± 29.530	43.752 ± 26.801	49.540 ± 31.967	NS
Insulin increment, 30 min in OGTT (pmol/L)	301 ± 223	297 ± 179	298 ± 223	305 ± 240	NS
Insulin increment/glucose increment 30 min in OGTT · 10^{-7}*†	0.65 ± 0.27	0.85 ± 0.52	1.34 ± 0.61	0.49 ± 0.38	NS
Triglycerides* (mmol/L)	1.4 ± 0.8	1.7 ± 2.3	1.7 ± 2.7	1.7 ± 1.6	3, 4 to 1
Total cholesterol (mmol/L)	5.7 ± 1.0	5.5 ± 2.1	5.7 ± 1.1	5.8 ± 1.1	NS
HDL-cholesterol (mmol/L)	1.6 ± 0.4	1.4 ± 0.4	1.6 ± 0.5	1.5 ± 0.4	4, 2 to 1
PAI active* (ng/mL)	13.7 ± 32.1	17.6 ± 18.1	12.7 ± 11.0	17.9 ± 28.9	4 to 1
Microalbuminuria* (mg/L)	18.8 ± 26.4	10.4 ± 6.3	17.1 ± 18.4	19.3 ± 24.9	NS

NOTE. Quartile 1, ≤ 1.0 mg/L; Quartile 2, > 1.0 and ≤ 1.6 mg/L; Quartile 3, > 1.6 and ≤ 4.2 mg/L; Quartile 4, > 4.2 and ≤ 76.4 mg/L.
*Logarithmically transformed values in ANOVA.
†Data given as mean ± SEM.
‡Significant difference, $P < .05$. NS, not significant.

to one of its primary anomalies, insulin resistance. We also demonstrate for the first time that low-grade inflammation is not associated with an impaired insulin secretion in a population at risk for type 2 diabetes mellitus.

All inflammatory parameters were significantly correlated with insulin resistance, calculated by HOMA, and this remained significant after age and sex adjustment. This is compatible with results of the IRAS, in which insulin resistance was determined by the frequently sampled intravenous glucose tolerance test.[18]

Furthermore, in quartiles of fibrinogen, CRP, and leukocyte count we observed a significant rise for many important components of the MS, as well as for insulin resistance. This is consistent with previous reports.[18-21] Thus, white blood cell count was strongly related with plasma glucose, insulin, BMI, blood pressure, plasma triglycerides, fibrinogen, and HDL-cholesterol (inversely) in healthy males.[20] CRP was also shown to be associated with obesity, insulin resistance, and endothelial dysfunction in healthy subjects.[19,21] In a nondiabetic population from the IRAS without clinical coronary artery disease, CRP, white blood cell count, and fibrinogen were correlated with several components of the insulin resistance syndrome.[18] In various populations, an association was found between CRP and BMI,[8,21,32,33] triglycerides,[8,21,32,33] HDL-cholesterol (inverse),[21,33] and blood pressure.[21,32] In the previous works, patients under lipid-lowering drug treatment were not excluded from analysis, although this medication has a significant anti-inflammatory effect, which could have influenced the results. In the present work, the relationship of inflammatory parameters was studied in relation to a broad spectrum of risk factors of the MS in a population at risk for type 2 diabetes, after excluding subjects receiving statins or fibrates. In this population, the inflammatory markers were most strongly related to BMI, glycemia (HbA_{1c}, fasting and postprandial plasma glucose in OGTT), and insulin resistance. The CRP concentration was more strongly associated to the other components of the MS than fibrinogen or leukocytes count, which is consistent with data of the IRAS.[18]

We found no association between subclinical inflammation and insulin secretion, evaluated by HOMA and by insulin areas under the curve in OGTT, insulin increment at 30 minutes of OGTT, and insulin increment/glucose increment at 30 minutes of OGTT, in this population at risk for diabetes. This was also confirmed for the different glucose tolerance stages. This points to the principle difference between type 1 and type 2 diabetes, since in the second case the chronic subclinical inflammation does not affect the function of the beta cells.

Recently the question was raised whether type 2 diabetes is a disease of the innate immune system.[34] In accordance with the results of the present study the answer seems to be positive,

Table 4. Metabolic Parameters (mean ± SD) in Quartiles of Leukocyte Counts

Parameter	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P‡
No.	109	91	103	93	
Age (yr)	57.6 ± 7.4	57.6 ± 9.1	56.6 ± 8.7	55.4 ± 9.2	NS
BMI (kg/m^2)	26.1 ± 4.6	26.9 ± 4.1	27.9 ± 4.7	27.6 ± 5.5	3, 4 to 1
WHR	0.85 ± 0.09	0.87 ± 0.09	0.89 ± 0.09	0.88 ± 0.10	3 to 1
Blood pressure, systolic (mm Hg)	126 ± 23	127 ± 19	130 ± 19	130 ± 21	NS
Blood pressure, diastolic (mm Hg)	80 ± 11	80 ± 11	81 ± 11	81 ± 11	NS
HbA_{1C} (%)	5.4 ± 0.4	5.5 ± 0.6	5.5 ± 0.5	5.5 ± 0.7	2, 3, 4 to 1
Plasma glucose, fasting (mmol/L)	5.4 ± 0.5	5.6 ± 0.9	5.7 ± 0.9	5.8 ± 1.0	2, 3, 4 to 1
Plasma glucose, 2-hour in OGTT (mmol/L)	6.3 ± 2.2	6.7 ± 2.8	7.3 ± 2.9	7.6 ± 3.1	3, 4 to 1; 4 to 2
Insulin, fasting* (pmol/L)	64.7 ± 49.6	68.1 ± 76.8	73.6 ± 42.1	76.4 ± 47.8	NS
Insulin, 2-hour in OGTT* (pmol/L)	313 ± 355	282 ± 204	355 ± 350	324 ± 266	NS
Proinsulin, fasting (pmol/L)	4.4 ± 3.5	4.3 ± 4.7	9.1 ± 43.4	4.5 ± 3.7	NS
Proinsulin, 2-hour in OGTT (pmol/L)	20.5 ± 15.1	19.9 ± 16.6	26.9 ± 43.0	20.3 ± 12.9	NS
C-peptide, fasting (pmol/L)	1,092 ± 442	1,175 ± 619	1,255 ± 533	1,344 ± 551	3, 4 to 1; 4 to 2
C-peptide, 2-hour in OGTT (pmol/L)	3,698 ± 1,472	3,587 ± 1,205	4,027 ± 1,592	4,145 ± 1,366	3, 4 to 2; 4 to 1
Free fatty acids, fasting (mmol/L)	0.53 ± 0.224	0.56 ± 0.24	0.60 ± 0.24	0.64 ± 0.28	3, 4 to 1; 4 to 2
Insulin resistance (HOMA)*	15.5 ± 11.3	17.7 ± 21.3	19.4 ± 12.7	20.8 ± 15.7	3, 4 to 1; 4 to 2
Insulin secretion (HOMA)*	657 ± 966	657 ± 630	692 ± 343	670 ± 370	NS
Insulin areas under the curve in 2-hour OGTT (pmol/L)	43.573 ± 33.529	38.855 ± 21.900	46.690 ± 31.038	46.073 ± 30.287	NS
Insulin increment, 30 min in OGTT (pmol/L)	287 ± 189	303 ± 242	312 ± 233	303 ± 238	NS
Insulin increment/glucose increment, 30 min in OGTT · 10^{-7}*†	1.26 ± 0.62	0.61 ± 0.47	0.52 ± 0.35	0.76 ± 0.12	NS
Triglycerides* (mmol/L)	1.3 ± 0.7	1.3 ± 0.8	1.7 ± 1.6	1.9 ± 2.9	3, 4 to 1, 2
Total cholesterol (mmol/L)	5.7 ± 0.9	5.7 ± 1.1	5.9 ± 1.0	5.7 ± 1.3	NS
HDL-cholesterol (mmol/L)	1.6 ± 0.4	1.6 ± 0.5	1.5 ± 0.4	1.5 ± 0.4	4, 3 to 1
PAI active* (ng/mL)	13.3 ± 22.4	13.8 ± 28.5	17.1 ± 37.3	14.5 ± 13.6	NS
Microalbuminuria* (mg/L)	15.4 ± 17.7	16.8 ± 28.1	17.3 ± 18.6	23.7 ± 29.4	4 to 2

NOTE. Quartile 1, ≤ 4.7 gigha particles per liter (GPt/L); Quartile 2, > 4.7 and ≤ 5.5 GPt/L; Quartile 3, > 5.5 and ≤ 6.5 GPt/L; Quartile 4 > 6.5 and ≤ 16.7 GPt/L.

*Logarithmically transformed values in ANOVA.

†Data given as mean ± SEM.

‡Significant difference, $P < .05$. NS, not significant.

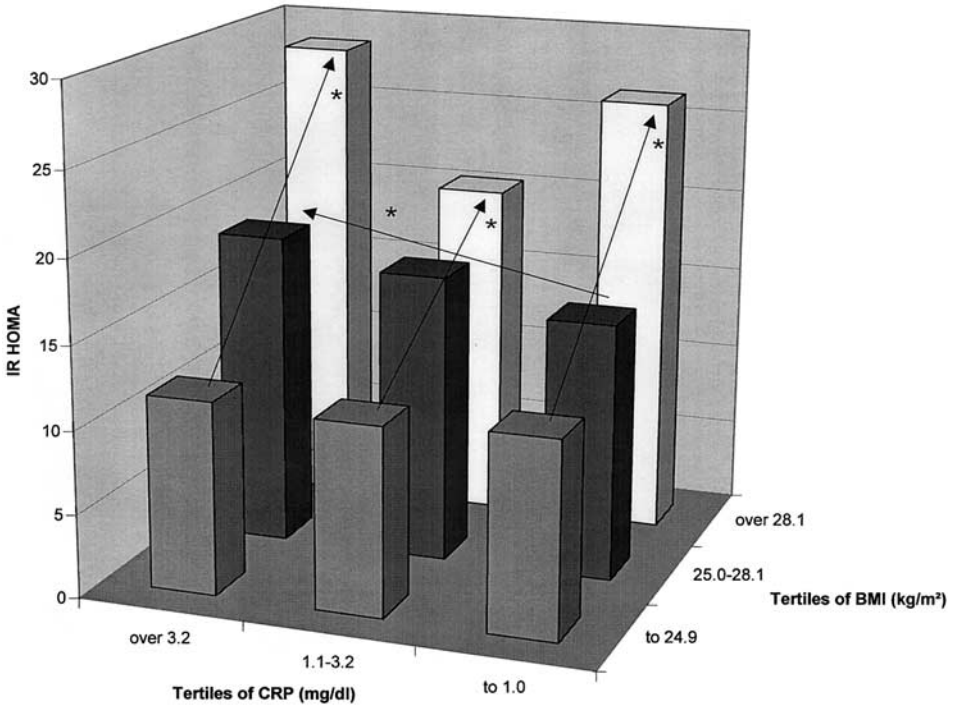

Fig 1. Insulin resistance (IR HOMA) in tertiles of BMI and CRP. *$P < .001$, ANOVA in trend.

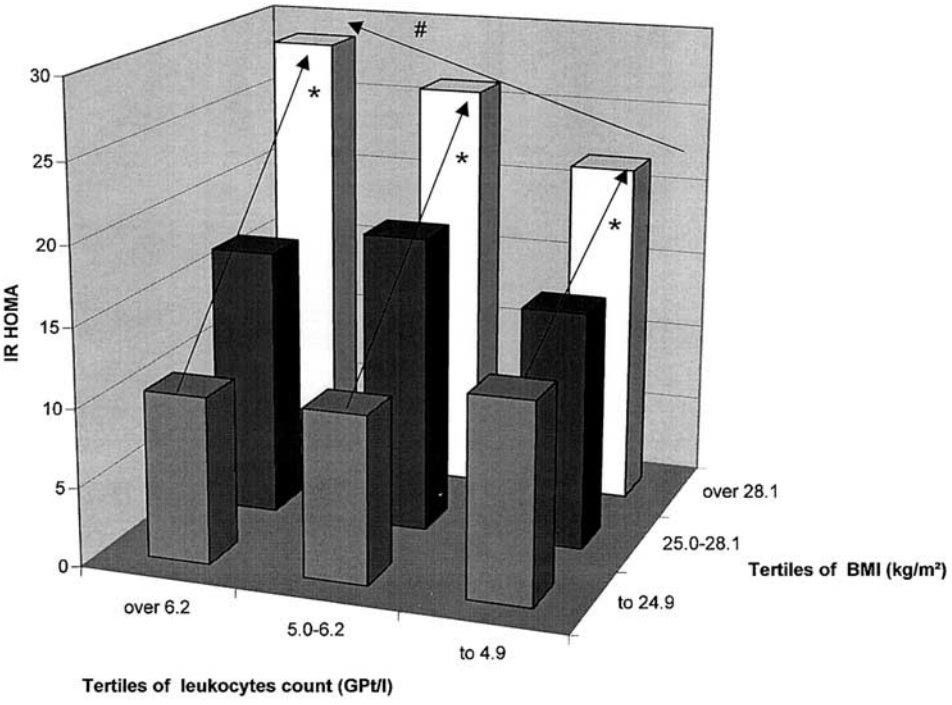

Fig 2. Insulin resistance (IR HOMA) in tertiles of BMI and leukocyte count. *P < .001, ANOVA in trend; #P < .001, Kruskal-Wallis-H test in trend.

since inflammation is associated with one of the underlying defects of type 2 diabetes: insulin resistance. In this respect, obesity plays an important link between inflammation and insulin resistance. Consistent with other investigators, we found that after including BMI into the multivariate model, the correlation between inflammatory parameters and insulin resistance disappeared.[18] Moreover, in a multivariate analysis in our study, BMI was a strong independent determinant of all examined inflammatory parameters. It is known that both interleukin-6 (IL-6) and tumor necrosis factor(TNF)-alpha are produced in adipose tissue in a considerable amount and may be able to mediate the systemic metabolic impairments of the MS.[35] The increased CRP level in subjects with the MS may reflect cytokine production by adipocytes, since IL-6 and CRP concentrations are closely related in obese subjects.[36] In addition, TNF-alpha, although acting locally, may contribute to insulin resistance of adipocytes and muscle cells.[37] In our study, for all levels of leukocyte counts and CRP, a significant rise was observed for insulin resistance parallel to BMI. On the other hand, a significant elevation of insulin resistance was found parallel to the increase of leukocyte counts in the top tertile of BMI, as well as parallel to the increase of CRP in the middle tertile of BMI.

The strength of this study lies in the selecting of a population at risk for type 2 diabetes, rather than those with the current diagnosis. Furthermore, it is the first study on the relationship between chronic inflammation and the MS that takes into consideration the effect of lipid-lowering drugs. The weak point of the study is that the homogenous population in the trial with a careful exclusion of possible biases is not representative of the general population. Prospective studies would be needed to confirm the predictive role of chronic subclinical inflammation for the development of type 2 diabetes through the association to insulin resistance, but not to insulin secretion.

In conclusion, in a population at risk for type 2 diabetes we demonstrate that subclinical inflammation underlies the MS, through association with one of its primary anomalies—insulin resistance, whereas no association was found to impaired insulin secretion.

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