Chronic Subclinical Inflammation as Part of the Insulin Resistance Syndrome

The Insulin Resistance Atherosclerosis Study (IRAS)

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Background—Inflammation has been suggested as a risk factor for the development of atherosclerosis. Recently, some components of the insulin resistance syndrome (IRS) have been related to inflammatory markers. We hypothesized that insulin insensitivity, as directly measured, may be associated with inflammation in nondiabetic subjects.

Methods and Results—We studied the relation of C-reactive protein (CRP), fibrinogen, and white cell count to components of IRS in the nondiabetic population of the Insulin Resistance Atherosclerosis Study (IRAS) (n=1008; age, 40 to 69 years; 33% with impaired glucose tolerance), a multicenter, population-based study. None of the subjects had clinical coronary artery disease. Insulin sensitivity (SI) was measured by a frequently sampled intravenous glucose tolerance test, and CRP was measured by a highly sensitive competitive immunoassay. All 3 inflammatory markers were correlated with several components of the IRS. Strong associations were found between CRP and measures of body fat (body mass index, waist circumference), SI, and fasting insulin and proinsulin (all correlation coefficients .0.3, P<0.0001). The associations were consistent among the 3 ethnic groups of the IRAS. There was a linear increase in CRP levels with an increase in the number of metabolic disorders. Body mass index, systolic blood pressure, and SI were related to CRP levels in a multivariate linear regression model.

Conclusions—We suggest that chronic subclinical inflammation is part of IRS. CRP, a predictor of cardiovascular events in previous reports, was independently related to SI. These findings suggest potential benefits of anti-inflammatory or insulin-sensitizing treatment strategies in healthy individuals with features of IRS. (Circulation. 2000;102:42-47.)

Key Words: inflammation • proteins • insulin resistance syndrome • insulin • atherosclerosis

A relationship between C-reactive protein (CRP), a sensitive marker of inflammation, and the development of atherosclerotic disease has been observed in experimental and epidemiological studies. It is still unknown, however, whether elevated CRP levels merely reflect an epiphenomenon accompanying established atherosclerotic disease or whether the protein itself is involved in the initiation and/or progression of atherosclerosis.

Previous reports suggest a positive association between components of the insulin resistance syndrome (IRS) and markers of the acute-phase response, including CRP and fibrinogen. CRP levels were associated with body mass index (BMI), serum lipids, and fasting glucose. Elevated levels of inflammatory markers (including CRP) were also found in type 2 diabetic patients with features of IRS.

The nature of the association of CRP with IRS, however, is poorly understood. We hypothesized that insulin insensitivity and/or hyperinsulinemia may be associated with circulating CRP levels. Such an association would potentially provide insights into the role of CRP in atherosclerotic disease and further clarify the association of hyperinsulinemia with cardiovascular disease.

We studied the relation of inflammatory markers (CRP, fibrinogen, white cell count) and components of IRS, including insulin sensitivity, as directly measured by a frequently sampled intravenous glucose tolerance test. Furthermore, we sought to investigate whether CRP levels were independently related to insulin (or its precursors), insulin sensitivity, or both. The analyses were restricted to nondiabetic subjects without clinical coronary artery disease to avoid possible confounding by preexisting cardiovascular disease. It has been shown previously that patients with type 2 diabetes present with higher levels of inflammatory markers and a high prevalence of atherosclerosis, including clinically undetected disease.
Methods

Study Subjects
The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, population-based epidemiological study exploring relationships between insulin resistance, cardiovascular risk factors, and cardiovascular disease across different ethnic groups and various states of glucose tolerance. A full description of the design and methods of the IRAS has been published. The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

A total of 1088 nondiabetic individuals participated in the IRAS. Subjects with a current acute illness (including clinically significant infectious disease) were excluded from IRAS examination. Subjects with clinically overt coronary artery disease, defined as past myocardial infarction, PTCA or CABG, or ECG evidence of ischemic heart disease, were excluded from the present analyses. This report includes data on 1088 nondiabetic subjects in whom CRP and fibrinogen levels were measured. Cigarette smoking was dichotomized into “never” and “ever” (including past and current) by use of a standard questionnaire. BMI (weight/height^2 [kg/m^2]) was used as an estimate of overall adiposity. Waist circumference (estimate of visceral fat) was measured at the natural indentation between the 10th rib and the iliac crest (minimum waist).

A standard 75-g oral glucose tolerance test was performed. Glucose tolerance status was based on World Health Organization criteria. Laboratory Measurements
Plasma glucose was measured with the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Equipment Co). Insulin was measured with the dextran-charcoal radioimmunoassay. This insulin assay cross-reacts with proinsulin. Fasting serum intact proinsulin and 32–33 split proinsulin were determined at the Department of Clinical Biochemistry at Addenbrooke’s Hospital, Cambridge, UK (Professor C.N. Hales), by means of highly specific 2-site monoclonal antibody–based immunoradiometric assays.

Insulin sensitivity was assessed by a frequently sampled intravenous glucose tolerance test with minimal model analysis. Two modifications of the original protocol were used: (1) an injection of regular insulin, rather than tolbutamide, to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance and (2) the reduced sampling protocol because of the large number of subjects. Insulin sensitivity, expressed as the insulin sensitivity index (SI), was calculated by mathematical modeling methods (MINMOD, version 3.0, 1994).

Plasma lipoprotein measurements were obtained from fasting single fresh plasma samples through Lipid Research Clinic methods at the central IRAS laboratory at Medlantic Research Institute, Washington, DC (Professor B.V. Howard).

CRP was measured by in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem) with an interassay coefficient of variation of 8.9%. Fibrinogen was measured in citrated plasma with a modified clot-rate assay by use of the Diagnostica STAGO ST4 instrument, as described previously.

Complete blood cell counts were performed with standard techniques.

Statistical Analysis
Statistical analyses were performed with the SAS statistical software system. Descriptive statistics (mean±SE) and number/percent are shown on Table 1. CRP levels differed by sex and ethnicity in the present population, and age and smoking were determinants of CRP levels in previous reports; therefore, multivariate models (partial Spearman correlations, multiple linear regression analysis) were tailored to account for these possible confounders. Partial Spearman correlations (adjusting for age, sex, ethnicity, clinic, and smoking status) for inflammatory markers with components of the IRS were estimated for the overall population (Table 2) and stratified by ethnicity and glucose tolerance status (normal [NGT] versus impaired [IGT] glucose tolerance). In these models, we also tested for interactions between the independent variables of interest (BMI, fasting glucose, insulin, proinsulin, split proinsulin, and SI) and ethnicity and glucose tolerance status, respectively. The distribution of CRP levels was highly skewed. Logarithmically transformed values of CRP (log CRP) were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. Thus, mean values of log CRP (adjusted for age, sex, ethnicity, clinic, and smoking status) in relation to the number of metabolic disorders were calculated by ANCOVA (Figure 1). Fur-

| TABLE 1. Descriptive Data in Nondiabetic Subjects Without Clinical Coronary Artery Disease: The IRAS |
|---------------------------------|----------|----------|
| n                               | 1008     |          |
| Male sex, %                     | 43       |          |
| Impaired glucose tolerance, %   | 33       |          |
| Age, y                          | 54.7±0.3 |          |
| BMI, kg/m²                      | 28.4±0.2 |          |
| Waist circumference, cm         | 90.4±0.4 |          |
| Systolic BP, mm Hg              | 119.6±0.5|          |
| Diastolic BP, mm Hg             | 77.1±0.3 |          |
| Triglyceride, mmol/L            | 1.51±0.03|          |
| Total cholesterol, mmol/L       | 5.48±0.03|          |
| LDL cholesterol, mmol/L         | 3.66±0.03|          |
| HDL cholesterol, mmol/L         | 1.23±0.01|          |
| Fasting glucose, mmol/L         | 5.47±0.02|          |
| Fasting insulin, pmol/L         | 94.1±2.7 |          |
| Proinsulin, pmol/L              | 6.0±0.2  |          |
| Split proinsulin, pmol/L        | 8.3±0.3  |          |
| S×10^-4, min^-1, μU^-1, mL^-1   | 2.19±0.07|          |
| CRP, mg/L                       | 3.53±0.18|          |
| CRP >10 mg/L, n (%)             | 70 (6.9) |          |
| White cell count, ×10^3/mm3     | 5.81±0.07|          |
| Fibrinogen, mg/dL               | 276.4±1.8|          |

BP indicates blood pressure. Data are mean±SE.

| TABLE 2. Partial Spearman Correlation Analysis of Inflammation Markers With Variables of IRS Adjusted for Age, Sex, Clinic, Ethnicity, and Smoking Status |
|---------------------------|----------|----------|
|                          | CRP      | WBC      | Fibrinogen |
| BMI                      | 0.40†    | 0.17†    | 0.22†      |
| Waist                    | 0.43‡    | 0.18‡    | 0.27‡      |
| Diastolic BP             | 0.17†    | 0.01     | 0.11†      |
| Systolic BP              | 0.20‡    | 0.08*    | 0.11†      |
| Triglyceride             | 0.23†    | 0.15‡    | 0.03       |
| Cholesterol (total)      | 0.10†    | −0.01    | 0.01       |
| HDL cholesterol          | −0.11†   | −0.12†   | −0.15‡     |
| LDL cholesterol          | 0.09*    | −0.01    | 0.05       |
| Fasting glucose          | 0.18‡    | 0.13‡    | 0.07*      |
| Fasting insulin          | 0.33‡    | 0.24‡    | 0.18‡      |
| Proinsulin (intact)      | 0.30†    | 0.17‡    | 0.20‡      |
| Proinsulin (split)       | 0.32†    | 0.21‡    | 0.20‡      |
| S_i                      | −0.37†   | −0.24‡   | −0.18‡     |

BP indicates white cell count; BP, blood pressure. *P<0.05, †P<0.005, ‡P<0.0001.
thermore, we calculated (unadjusted) mean values of log CRP by tertile for SÍ, BMI, and triglycerides and for hypertension (Figure 2).

Stepwise linear regression models were fit for log CRP as a dependent variable, including all variables of interest at the same time as independent variables to demonstrate the relative contribution of each of these variables to the outcome variable. After age, sex, ethnicity, clinic, and smoking status were forced into the model, the following independent variables were considered for the model: BMI, diastolic and systolic blood pressures (SBP), fasting glucose, fasting insulin, SÍ, and proinsulin (intact). Only variables that had a \( P \leq 0.05 \) were considered in the final fitted model. \( R^2 \) for the model=26.0%.

Results

Table 1 shows descriptive data. All 3 inflammatory markers were correlated with several components of IRS (Table 2). The associations were generally stronger for CRP than for white cell count and fibrinogen. Strong associations (correlation coefficients >0.3) were found between CRP and measures of body fat (BMI, waist circumference), SÍ, fasting insulin, and proinsulin. There was a linear increase in CRP levels with an increase in the number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, hypertension; Figure 1). The respective mean log of CRP levels...
The associations as shown in the overall population (Table 2) were also consistent among the 3 ethnic groups. Correlation coefficients for CRP in non-Hispanic whites (n=399), blacks (n=267), and Hispanics (n=342) were 0.43, 0.43, and 0.38 (BMI); 0.43, 0.46, and 0.39 (waist); 0.35, 0.29, and 0.36 (fasting insulin); 0.30, 0.31, and 0.29 (intact proinsulin); 0.38, 0.28, and 0.29 (split proinsulin); and −0.41, −0.33, and −0.38 (Sₜ), respectively (all P<0.0001). The correlation coefficient of CRP with fasting glucose was 0.16 (P<0.005) in non-Hispanic whites, 0.12 (P=NS) in blacks, and 0.25 (P<0.0001) in Hispanics. The association of fasting insulin with CRP and fibrinogen was less pronounced in blacks compared with non-Hispanic whites and Hispanics (P<0.005 and P<0.05 for interaction terms), respectively, although the associations were clearly in the same direction and, for CRP, highly significant in all 3 ethnic groups. All other interaction terms were not statistically significant.

The associations were also consistently seen in subjects with NGT and IGT. Correlation coefficients for CRP in subjects with NGT and IGT were 0.34 and 0.41 (BMI), 0.37 and 0.40 (waist), 0.32 and 0.23 (fasting insulin), 0.23 and 0.29 (intact proinsulin), 0.25 and 0.31 (split proinsulin), and −0.35 and −0.26 (Sₜ), respectively (all P<0.0001). The correlation of CRP with fasting glucose was weak in NGT (r=0.11, P<0.05) and not significant in IGT (r=0.09). The association of fasting insulin with CRP was stronger in subjects with NGT than with IGT (P<0.0001 for interaction term). All other interaction terms were not statistically significant.

Multivariate linear regression analyses showed that BMI, systolic blood pressure, and Sₜ (inversely) were independently associated with log CRP levels in the overall population (Table 3).

Discussion
In the present study, we have shown that in healthy, nondiabetic subjects, CRP, a sensitive marker of inflammation that has previously been associated with cardiovascular disease, was independently related to insulin sensitivity. Chronic subclinical inflammation emerged as part of IRS. The findings were consistent across the 3 ethnic groups of the IRAS (non-Hispanic whites, blacks, and Hispanics) and in subjects with NGT and IGT.

Previously, in various populations, CRP levels were associated with BMI, triglyceride level, HDL cholesterol level (inversely), total cholesterol level, and blood pressure. Mendall et al found an association between CRP levels and fasting glucose, confirmed by Tracy et al in the elderly but only in a nonsmoking subset. However, information about the association of serum levels of inflammatory markers with insulinemia and insulin sensitivity, hallmarks of the IRS, is scarce. Recently, we have reported that the IRAS associations of fibrinogen and plasminogen activator-1 with several components of IRS, including insulin, proinsulin, and Sₜ. In 3 other studies, fibrinogen levels were independently associated with fasting insulin levels in nondiabetic subjects. In 107 nondiabetic subjects, CRP levels were related to insulin resistance, as calculated with the homeostasis model assessment model. The present study clearly corroborates and extends these results, indicating that chronic, subclinical inflammation is part of IRS. We have shown that various components of IRS were correlated to inflammatory markers (Table 2 and Figure 2) and that an increasing number of components of IRS (dyslipidemia, abdominal obesity, low Sₜ, and hypertension) paralleled increasing levels of CRP (Figure 1). The results were consistent across a variety of ethnic groups that differ in insulin sensitivity, indicating that the relations found in our study apply to populations with high and low Sₜ.

There are several possible explanations for these findings, which are not necessarily exclusive. First, it is possible that chronic inflammation may represent a triggering factor in the origin of IRS, and eventually type 2 diabetes, as previously discussed by Pickup and Crook. According to this hypothesis, stimuli such as overnutrition would result in cytokine hypersecretion and eventually lead to insulin resistance and diabetes in genetically or metabolically predisposed individuals. Cytokines, mainly interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, exert major stimulatory effects on hepatic synthesis of acute-phase proteins.

Second, clustering of cardiovascular risk factors as typically encountered in subjects with the IRS may yield cardiovascular disease (yet undetected), and elevated CRP levels thus would be the result of preexisting atherosclerosis. Previous cross-sectional analyses show an association between CRP levels and atherosclerotic disease. CRP was also elevated in elderly women with subclinical atherosclerosis in the Cardiovascular Health Study; however, in a larger cohort from the same population, no significant association of CRP with carotid intimal-medial thickness was found. Atherosclerosis starts very early in life, and insulin resistance potentially accelerates this process. Therefore, it is highly likely that in our “healthy” middle-aged population, atherosclerosis prevails, particularly in those with features of IRS. However, the degree and extent of atherosclerosis operative in increasing CRP levels is unknown; therefore, even relatively sensitive methods of assessing preclinical atherosclerosis (such as carotid ultrasound) may lack accuracy in this respect.

Third, decreased insulin sensitivity may lead to enhanced CRP expression by counteracting the physiological effect of insulin on hepatic acute-phase protein synthesis. Clamp studies in normal subjects showed that insulin exerts selective effects on hepatic protein synthesis, with an increase in...
albumin synthesis and a decrease in fibrinogen synthesis.\textsuperscript{35} The inverse of the picture typically seen during the acute-phase response.\textsuperscript{36} Resistance to this effect would then lead to increased synthesis of acute-phase proteins, such as fibrinogen and CRP.

Finally, the effect could be indirect via body fat; we observed a strong and independent association of CRP levels with measures of body fat and triglycerides. This is in accordance with results of previous cross-sectional analyses.\textsuperscript{6,8–10,12} In a previous interventional study, the synthesis of proinflammatory cytokines by peripheral monocytes (TNF-\(\alpha\), IL-1) was suppressed by dietary fish oil supplementation,\textsuperscript{37} suggesting an effect of dietary fat on cytokine production.\textsuperscript{38} Another (speculative at this time) mechanism would be a generally enhanced adipose tissue–derived cytokine expression (TNF-\(\alpha\), IL-6). Accordingly, weight loss was associated with a decrease in CRP in the Women’s Healthy Lifestyle Project (E. Meilahn, personal communication, 1996), also supporting an association of body fat and chronic inflammation.

We found a strong and independent association of elevations in inflammatory markers, namely CRP, with decreased S\(_I\). The association of low S\(_I\) (indicating high insulin resistance) with elevated CRP levels found in the present study could potentially explain the association of hyperinsulinemia (another indicator of insulin resistance) with cardiovascular disease.\textsuperscript{14} Several experimental studies suggest a direct role of CRP in the initiation and/or progression of atherosclerotic lesions. CRP has been shown to (1) be a potent stimulator of tissue factor production by macrophages;\textsuperscript{4} (2) activate the complement system in vivo;\textsuperscript{39} (3) accumulate in early atherosclerotic lesions in human aorta\textsuperscript{2} and coronary arteries\textsuperscript{3}; (4) bind to lipoproteins, such as LDL and VLDL, thus inducing their aggregation\textsuperscript{4}; and (5) be expressed by monocytes.\textsuperscript{40} In epidemiological studies, CRP levels in the upper normal range have consistently been predictive of cardiovascular disease in various populations.\textsuperscript{5–8} Moreover, sensitive assays and the biological properties of CRP (such as its stable half-life\textsuperscript{41}) make this protein a clinically useful marker of chronic subclinical inflammation.

The results of the present study are potentially clinically important. As previously shown, treatment of several components of IRS (adiposity, dyslipidemia, hypertension) may have beneficial effects in terms of preventing type 2 diabetes\textsuperscript{42} and cardiovascular disease.\textsuperscript{43,44} Therefore, if subclinical inflammation is indeed another facet of the IRS, anti-inflammatory treatment may also be beneficial. Accordingly, it has been suggested that the effects of aspirin may, at least partly, be mediated through its anti-inflammatory rather than its antiplatelet properties.\textsuperscript{5} Furthermore, treatment aiming at improving insulin resistance, whether nonpharmacological, such as exercise and weight reduction, or pharmacological, such as metformin and thiazolidinediones, may lower CRP levels and thus provide additional therapeutic benefits beyond mere glucose lowering. Alternatively, if elevated CRP levels were merely a marker of prevalent or developing atherosclerosis, these treatment strategies would then be clinically fruitless. Prospective studies are clearly needed to address these issues.

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References
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