C-Reactive Protein, the Metabolic Syndrome, and Prediction of Cardiovascular Events in the Framingham Offspring Study

Martin K. Rutter, MD; James B. Meigs, MD, MPH; Lisa M. Sullivan, PhD; Ralph B. D’Agostino, Sr, PhD; Peter W.F. Wilson, MD

Background—Inflammation (assessed by C-reactive protein [CRP]) and the metabolic syndrome (MetS) are associated with cardiovascular disease (CVD), but population-based data are limited.

Methods and Results—We assessed the cross-sectional relations of CRP to the MetS (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III definition) in 3037 subjects (1681 women; mean age, 54 years) and the utility of CRP and the MetS to predict new CVD events (n=189) over 7 years. MetS (≥3 of 5 traits) was present in 24% of subjects; mean age-adjusted CRP levels for those with 0, 1, 2, 3, 4, or 5 MetS traits were 2.2, 3.5, 4.2, 6.0, or 6.6 mg/L, respectively (P trend <0.0001). In persons with MetS, age-adjusted CRP levels were higher in women than men (7.8 versus 4.6 mg/L; P<0.0001). MetS and baseline CRP were individually related to CVD events (for MetS: age-sex-adjusted hazard ratio [HR], 2.1; 95% CI, 1.5 to 2.8; for highest versus lowest CRP quartile: HR, 2.2; 95% CI, 1.4 to 3.5). Greater risk of CVD persisted for MetS and CRP even after adjustment in a model including age, sex, MetS (HR, 1.8; 95% CI, 1.4 to 2.5), and CRP (HR, 1.9; 95% CI, 1.2 to 2.9). The c-statistic associated with the age- and sex-adjusted model including CRP was 0.72; including MetS, 0.74; and including CRP and MetS, 0.74.

Conclusions—Elevated CRP levels are related to insulin resistance and the presence of the MetS, especially in women. Although discrimination of subjects at risk of CVD events using both MetS and CRP is not better than using either phenotype alone, both CRP and MetS are independent predictors of new CVD events. (Circulation. 2004;110:380-385.)

Key Words: C-reactive protein ■ cardiovascular diseases ■ insulin ■ prognosis ■ risk factors

Inflammation, as assessed by C-reactive protein (CRP), is emerging as a predictor of cardiovascular disease (CVD), and it may be an important precursor of the metabolic syndrome (MetS) and type 2 diabetes.1,2 Subjects with the MetS are at increased risk of CVD,2–5 and the National Cholesterol Education Program (NCEP) has proposed a practical definition of the syndrome to aid detection of these high-risk individuals.6 Several studies have demonstrated relationships between CRP and individual components of the MetS.1–3,7–16 Data on the cross-sectional relations between CRP and components of the NCEP MetS are relatively scarce in the population setting,15 and only 1 population-based study has described prospective CVD risk associated with these variables.3 We therefore examined these relationships in the Framingham Offspring cohort prospectively followed up for 7 years. We sought to determine the relationships between CRP and other risk factors associated with the MetS, CVD, and type 2 diabetes and to examine how effectively CRP and MetS predict CVD events in the Framingham population.

Methods

Study Subjects

Study subjects were participants in the Framingham Offspring Study, a community-based observational study of risk factors for CVD. From January 1991 through June 1995 (examination cycle 5), participants fasted overnight, provided written informed consent, and underwent a standardized clinical examination. Prevalent diabetes was defined as fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) at any previous examination or self-reported use of hypoglycemic drugs. Participants without diagnosed diabetes had a 75-g OGTT, and newly diagnosed diabetes was defined in accordance with 1998 World Health Organization guidelines. Of 3799 participants, we excluded those with prevalent or newly diagnosed diabetes (n=346), prevalent CVD (n=288), or missing information on covariates (n=128), which left 3037 subjects (1681 women) in this analysis.

Clinical Examination and Laboratory Methods

Serum concentrations of CRP were measured with an enzyme immunoassay (Hemagen Diagnostics, Inc) as previously described.17,18 MetS was defined according to 2001 NCEP Adult Treatment Panel III (ATP) guidelines.6 Plasma glucose was measured in fresh specimens with a hexokinase reagent kit (A-Gent...
Glucose Test, Abbott). Glucose assays were run in duplicate; the intra-assay coefficient of variation was <3%. Fasting insulin levels were measured in plasma as total immunoreactive insulin and were standardized to serum levels for reporting purposes. The lower limit of sensitivity was 8.0 pmol/L (1.1 μU/mL), and the intra- and interassay coefficients of variation ranged from 5.0% to 10.0%. Insulin resistance was assessed from fasting insulin and glucose levels and the previously validated homeostasis model assessment (HOMA-IR): HOMA-IR=[fasting glucose (mmol/L)×fasting insulin (μU/mL)]/22.5. Total cholesterol and triglyceride levels were measured enzymatically, and the HDL cholesterol fraction was measured after precipitation of LDL and VLDL particles with dextran sulfate magnesium. The Framingham laboratory participates in the lipoprotein cholesterol laboratory standardization program administered by the Centers for Disease Control and Prevention (Atlanta, Ga). Blood pressure was assessed as the average of 2 measurements taken after subjects had been seated for ≥5 minutes. Waist circumference was measured in subjects in a standing position at the level of the umbilicus. Subjects who reported smoking at least 1 cigarette per day during the year before the examination were classified as current smokers.

CVD Assessment and Follow-Up

Incident CVD was assessed with standard Framingham Heart Study criteria and included the following: new-onset angina, fatal and nonfatal myocardial infarction or stroke, transient ischemic attack, heart failure, or intermittent claudication. Subjects free of CVD at the seventh examination if free of a CVD event.

Statistical Analysis

Descriptive statistics were generated for all study variables, including mean and SD for continuous variables and relative frequencies for categorical variables. The distributions of CRP, fasting insulin, and HOMA-IR were examined graphically, and because they were skewed, values were log transformed to promote normality in all regression models. Summary statistics were calculated on raw data for those measures. Multiple linear regression analysis was used to test for differences in mean age-adjusted CRP levels between men and women at each level of the respective risk factors and then to test for interactions between levels of the risk factors and sex on mean CRP. Male and female subjects were classified as having 0, 1, 2, 3, 4, or 5 components of MetS, and age-adjusted mean CRP levels were estimated for each group. Trends were assessed with χ² tests. Similar analyses were performed in younger (<55 years) and older subjects and compared men with women not taking hormone replacement therapy (HRT). Cox proportional-hazards regression analysis was used to assess the association of CRP levels and MetS with incident CVD. Models were adjusted for age and sex, and CRP was modeled both as an indicator and as a continuous variable. Hazards ratios (HRs) and 95% CIs are presented to assess effect. Model performance was assessed with the c-statistic, the area under the receiver-operating characteristic curve. All analyses were performed with SAS 8.2; 2-sided values of P<0.05 were considered statistically significant.

Results

The mean age of the study population was 54 years (range, 26 to 82 years), and the MetS was present in ≈1 in 4 of the individuals studied, similar to what has been reported by NHANES (Table 1).10 MetS prevalence was higher in men than in women (P=0.0001), and a higher proportion of men than women exceeded thresholds defined by the NCEP ATP III for blood pressure, HDL cholesterol, triglycerides, and fasting glucose (all P<0.02). Although waist circumference measurements were greater in men than women (P=0.0001), a similar proportion of women and men exceeded NCEP ATP III gender-specific thresholds (P=0.2). Sixty-three percent of the women were postmenopausal at baseline, and 15% of these were using HRT. One in 5 of the study population was a current smoker. Mean±SD body mass index (BMI) and the proportion of subjects with BMI >30 kg/m² were higher in men than in women (28±4 versus 26±5 kg/m², P=0.0001; and 26% versus 19%, P=0.001, respectively). Measures of insulin resistance, assessed by fasting insulin and HOMA-IR, were higher in men than in women (31±12 versus 28±10 μU/mL, P<0.0001; and 8±3 versus 7±3 U, P=0.002, respectively). CRP levels were higher in women than in men (P=0.002), and this relationship persisted (P=0.04) when women using HRT were excluded from analysis. Quartiles (Q) for the population distribution for CRP were as follows: Q1, 0.01 to 0.25 mg/dL; Q2, 0.26 to 1.46 mg/dL; Q3, 1.47 to 4.49 mg/dL; and Q4, 4.50 to 256 mg/dL.

Age-adjusted CRP levels were greater when components of the MetS or the MetS itself were present (Table 2). CRP levels were also related to HRT use, smoking status, BMI, and measures of insulin resistance. For individual components of the MetS, there was a significant gender interaction, and CRP levels were higher in women than men with MetS risk factor abnormalities (all P<0.01). For example, in subjects with higher blood pressure, CRP levels in women exceeded those in men. Similar relationships were observed for BMI, insulin, and HOMA-IR (all P≤0.0002). In current smokers, CRP levels were similar in men and women. Confirming the results of previous research, CRP levels were less strongly related to total and LDL cholesterol levels than to the individual components of the MetS (not shown).

A highly significant relationship was observed between the number of components of the MetS present and mean age-adjusted CRP levels (the Figure). As shown, there was a significant gender interaction; age-adjusted CRP levels were significantly higher in women than in men when ≥2 components of the MetS were present (all P<0.02). Almost identi-

### TABLE 1. Clinical and Biochemical Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Women (n=1681)</th>
<th>Men (n=1356)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>122 ± 19</td>
<td>128 ± 16</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>72±10</td>
<td>77±10</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>57±15</td>
<td>44±11</td>
</tr>
<tr>
<td>Waist circumference, in</td>
<td>34±5</td>
<td>39±4</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>127±76</td>
<td>151±103</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL, %</td>
<td>93±10</td>
<td>97±9</td>
</tr>
<tr>
<td>BP ≥130/85 mm Hg, %</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>HDL &lt;50 (women) or &lt;40 (men) mg/dL, %</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Waist &gt;35 (women) or &gt;40 (men) in, %</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>Triglycerides ≥150 mg/dL, %</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>Fasting glucose ≥110 md/dL, %</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>ATP III MetS, %</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.6 (0.2–5.1)</td>
<td>1.3 (0.3–4.0)</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. Data are mean±SD or median (interquartile range) unless stated.
During 7 years of follow-up, there were 189 CVD events (new-onset angina, 54; fatal and nonfatal myocardial infarction or stroke, 41; transient ischemic attack, 14; heart failure, 19; intermittent claudication, 17; other cerebrovascular event, 28; coronary heart disease death, 6; coronary insufficiency, 7). In age- and sex-adjusted models, both CRP and MetS at baseline were individually related to subsequent CVD events (Table 3). The c-statistic, reflecting the overall predictive power of the model, was similar for CRP or MetS. In an age- and sex-adjusted model that included both CRP and MetS, each of these variables was independently related to CVD events, increasing risk ∼2-fold. However, inclusion of both variables produced only a minimal increase in the c-statistic compared with that obtained with either variable on its own.

**Discussion**

**Cross-Sectional Study**

Genetic and environmental factors contribute to the pathogenesis of the MetS, but of the modifiable risk factors, obesity and physical inactivity are the most important. The cross-sectional relations of CRP and features of the MetS in this and other studies suggest that inflammation is strongly associated with insulin resistance and the MetS, and it supports the hypothesis that inflammation plays an impor-
CRP production. The potential molecular mechanisms linking inflammation and insulin resistance and glucose uptake, as originally suggested by Pickup and Crook. An important link between these conditions and obesity could be the proinflammatory cytokines produced by adipose tissue such as tumor necrosis factor-α and interleukin-6. These cytokines can influence insulin resistance and glucose uptake, promote hepatic fatty acid synthesis, and increase hepatic CRP production. The potential molecular mechanisms linking inflammation and insulin resistance have been reviewed recently by Schmidt and Duncan. One such mechanism involves tumor necrosis factor-α, which can inhibit insulin-mediated phosphorylation of the insulin receptor and of insulin receptor substrate-1 and thereby reduce cell surface translocation of GLUT4 transporters. A central role for obesity in the pathogenesis of insulin resistance could explain why some studies have shown that the relationship between inflammatory markers and the risk of developing diabetes is attenuated after correction for obesity. Alternative explanations for our cross-sectional results seem less likely but are possible and are not mutually exclusive. For instance, individual features of the metabolic syndrome such as hypertension and dyslipidemia could directly cause endothelial dysfunction and subclinical atherosclerosis, leading to inflammation and raised CRP. Alternatively, insulin resistance could increase hepatic CRP production by blocking insulin-mediated inhibition of acute-phase protein gene expression.

**Gender Influence**

Most cross-sectional studies of CRP and the MetS have undertaken sex-adjusted analysis or have been performed in single-sex cohorts. Of those studies reporting sex-specific analysis, none have compared CRP levels in men and women in relation to the number of components of the MetS as defined by NCEP ATP III. Here, we have shown in a population-based cohort that age-adjusted CRP levels were strongly related to the individual components of the NCEP ATP III MetS and that this relationship was stronger in women than in men. Our results are in keeping with those from the Mexico City Diabetes Study in which CRP levels were more strongly related to insulin resistance and features of the MetS in women. That study also showed that CRP levels predicted the development of the MetS and diabetes in women but not in men. Two more recent reports have shown that in a cross-sectional analysis, markers of inflammation, including CRP, were more strongly related to insulin resistance and/or the NCEP MetS in women than in men.

Our cross-sectional data suggest that the gender differences observed are not explained by HRT use, but it is possible that endogenous estrogen is responsible. An alternative explanation is that in subjects with the metabolic syndrome, women might have greater quantities of total body adipose tissue compared with men, and this could be the source of proinflammatory cytokines. Finally, Han and coworkers have suggested that inflammation might have a greater effect on insulin resistance in women than in men. This important hypothesis is supported by data in Table 2 showing stronger relationships in women than in men between CRP levels and measures of insulin resistance and all the features of the MetS. Laboratory studies have shown that the proinflammatory cytokine interleukin-6 can influence estradiol production by granulosa cells and therefore that chronic inflammation could theoretically mitigate the protective effect of estrogen on insulin resistance and body fat distribution. We observed higher levels of CRP in women than in men but higher levels of insulin resistance in men than in women, suggesting that inflammation as assessed by CRP is not the main pathophysiological process leading to insulin resistance. However, a gender-specific effect of chronic inflammation on insulin resistance might help to explain the results from the San Antonio Heart Study that showed that women who developed diabetes experienced a greater change in MetS-associated risk factors compared with the corresponding group of men. The same pathobiology might help to explain why the development of diabetes is associated with a greater increase in dyslipidemia and relative risk for CVD in women than in men.

**Prospective Study**

Our observation that CRP and NCEP ATP III MetS are independent risk factors for CVD is concordant with results from the Women’s Health Study, the West of Scotland Coronary Prevention Study, and a study of Turkish men and

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**TABLE 3. Age- and Sex-Adjusted HR for CVD Events Associated With Upper Quartile CRP Levels and/or the MetS**

<table>
<thead>
<tr>
<th>CRP upper quartile</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>2.2</td>
<td>...</td>
<td>1.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.4–3.5</td>
<td>...</td>
<td>1.2–2.9</td>
</tr>
<tr>
<td>P</td>
<td>0.0004</td>
<td>...</td>
<td>0.008</td>
</tr>
<tr>
<td>MetS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>...</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>...</td>
<td>1.5–2.8</td>
<td>1.4–2.5</td>
</tr>
<tr>
<td>P</td>
<td>...</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>c-Statistic</td>
<td>0.72</td>
<td>0.74</td>
<td>0.74</td>
</tr>
</tbody>
</table>

HR comparisons are with subjects with lowest quartile CRP levels and those without the MetS.
women with low cholesterol levels. Previous studies have been limited for several important reasons such as use of selected, single-sex cohorts derived from clinical trials and incomplete or absent data for waist circumference, fasting glucose, or glucose tolerance. Here, we extend the findings of previous studies to a population-based cohort of well-characterized men and women from the United States. We also show that combining CRP and MetS data does not add clinically relevant prognostic information regarding population CVD risk as assessed by the c-statistic. Further research is required to determine whether combining CRP and MetS data could have utility in the risk assessment of individual patients.

Our observation that CRP levels and MetS have similar discriminatory power regarding subsequent CVD risk has several possible interpretations. First, the data are consistent with the hypothesis that inflammation causes CVD largely through its influence on MetS variables. This is supported by the strong cross-sectional correlation between CRP levels and individual features of the MetS and the minimal increase in c-statistic when CRP is added to the MetS CVD prediction model. Alternatively, CRP could simply be acting as a marker for subclinical atherosclerosis “caused by” MetS variables.

Our observation that CRP and MetS are independent predictors of CVD events supports a direct role for CRP in atherosclerosis, thrombosis, and plaque rupture, as evidenced by much recent laboratory work. Additionally, CRP could be acting as a marker of risk factors related to the MetS such as hyperinsulinemia, small dense LDL, and adiponectin; or again, it may be acting as a marker of silent atherosclerosis.

Study Limitations

Limitations of this study should be noted. First, CRP was assessed from 1 blood sample only, and data on subjects with CRP levels >10 mg/dL were included in the analysis. Second, we excluded subjects with diabetes or CVD at baseline, and our analysis did not control for diabetes developing during follow-up. Bias introduced here might have caused some reduction of the HR and c-statistic associated with CRP in CVD risk prediction models. Third, power calculations showed that we were underpowered to perform either a sex-specific survival analysis or an analysis relating to individual CVD end points. Accepting these limitations, a sex-specific analysis showed that MetS and baseline CRP were individually related to CVD events in both men and women (women: age-adjusted HR, 2.4; 95% CI, 1.5 to 3.9 for MetS, and HR, 3.1; 95% CI, 1.4 to 6.7 for CRP, highest versus lowest quartile; men: HR, 1.9; 95% CI, 1.3 to 2.8 for MetS, and HR, 1.8; 95% CI, 1.0 to 3.1 for CRP). In women, greater risk of CVD persisted for MetS and CRP after adjustment in a model that included age, MetS (HR, 1.9; 95% CI, 1.1 to 3.1; P=0.01), and CRP (HR, 2.4; 95% CI, 1.1 to 5.4; P=0.03). However, in men, CRP was not independently related to CVD events (HR, 1.6; 95% CI, 0.9 to 2.7; P=0.12) in an age-adjusted model that included MetS (HR, 1.8; 95% CI, 1.2 to 2.6; P=0.003). A further exploratory analysis suggested that CRP may be a stronger predictor of CVD risk in subjects without than in subjects with the MetS (age- and sex-adjusted HRs [highest versus lowest CRP quartiles] were 2.0; 95% CI, 1.2 to 3.5; P=0.01, and 1.6; 95% CI, 0.7 to 3.8; P=0.29, respectively). Although it is not possible to draw firm conclusions from these data, further investigation seems indicated. Fourth, the complex interactions between the MetS and inflammation in the pathogenesis of CVD are difficult to disentangle by this analysis of population-based data. For example, the true impact of inflammation in the pathogenesis of CVD might be best reflected by the results of model 1 in which the influence of CRP was unadjusted for the presence of the MetS. Finally, our study sample was largely white, limiting the generalizability to other ethnic groups.

Conclusions

This population-based study of men and women has shown that inflammation as assessed by CRP is strongly related to all components of the NCEP ATP III MetS and that this relationship is stronger in women than in men. Both CRP and MetS are independent risk factors for CVD and have similar discriminatory ability with respect to subsequent CVD risk, but combining these variables adds little to overall risk prediction. Further research should aim at establishing whether combining CRP and MetS data could have utility in the risk assessment of individual patients.

Disclosure

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References


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