C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction
A Potential Role for Cytokines Originating From Adipose Tissue?

John S. Yudkin, C.D.A. Stehouwer, J.J. Emeis, S.W. Coppack

Abstract—C-reactive protein, a hepatic acute phase protein largely regulated by circulating levels of interleukin-6, predicts coronary heart disease incidence in healthy subjects. We have shown that subcutaneous adipose tissue secretes interleukin-6 in vivo. In this study we have sought associations of levels of C-reactive protein and interleukin-6 with measures of obesity and of chronic infection as their putative determinants. We have also related levels of C-reactive protein and interleukin-6 to markers of the insulin resistance syndrome and of endothelial dysfunction. We performed a cross-sectional study in 107 nondiabetic subjects: (1) Levels of C-reactive protein, and concentrations of the proinflammatory cytokines interleukin-6 and tumor necrosis factor-α, were related to all measures of obesity, but titers of antibodies to Helicobacter pylori were only weakly and those of Chlamydia pneumoniae and cytomegalovirus were not significantly correlated with levels of these molecules. Levels of C-reactive protein were significantly related to those of interleukin-6 ($r=0.37$, $P<0.0005$) and tumor necrosis factor-α ($r=0.46$, $P<0.0001$). (2) Concentrations of C-reactive protein were related to insulin resistance as calculated from the homeostasis model assessment model, blood pressure, HDL, and triglyceride, and to markers of endothelial dysfunction (plasma levels of von Willebrand factor, tissue plasminogen activator, and cellular fibronectin). A mean standard deviation score of levels of acute phase markers correlated closely with a similar score of insulin resistance syndrome variables ($r=0.59$, $P<0.00005$), this relationship being weakened only marginally by removing measures of obesity from the insulin resistance score ($r=0.53$, $P<0.00005$). These data suggest that adipose tissue is an important determinant of a low level, chronic inflammatory state as reflected by levels of interleukin-6, tumor necrosis factor-α, and C-reactive protein, and that infection with H pylori, C pneumoniae, and cytomegalovirus is not. Moreover, our data support the concept that such a low-level, chronic inflammatory state may induce insulin resistance and endothelial dysfunction and thus link the latter phenomena with obesity and cardiovascular disease. (Arterioscler Thromb Vasc Biol. 1999;19:972-978.)

Key Words: C-reactive protein ■ insulin resistance ■ obesity ■ endothelial dysfunction ■ interleukin-6

Inflammatory processes have important roles in the etiology of coronary heart disease (CHD), but the mechanisms underlying this relationship are poorly understood. Several studies have shown that elevated plasma levels of fibrinogen, C-reactive protein (CRP), and interleukin-6 (IL-6) are associated with the risk of CHD and the severity of atherosclerosis. Whether these molecules play a causative role, or simply act as markers of the acute phase reaction, is debatable. Elevated IL-6 levels have been reported in patients with unstable angina where inflammatory processes may facilitate the transition from the clinically stable to unstable atherosclerotic plaques. However, it has also been shown that CRP levels are associated with CHD in healthy subjects, both in a cross-sectional study in general practice, and longitudinally in the US Physicians Health Study, the MONICA-Augsburg Cohort Study, and the MRFIT Study, where CRP levels predicted cardiovascular events or CHD mortality during a follow-up of between 2 and 17 years. These observations imply that atheroma progression, as well as plaque rupture, may be predicted by raised CRP levels. It has nevertheless remained an issue of debate as to whether the relationship between CRP and cardiovascular disease reflects inflammation in the vascular wall, perhaps because of chronic infections such as Chlamydia pneumonia, or inflammation originating in a more remote site, with secondary effects on the vascular wall through cytokines and other mediators.

The synthesis of CRP by the liver is largely regulated by IL-6. Although the activated leukocyte is widely assumed to be the major source of circulating IL-6, with additional contributions from fibroblasts and endothelial cells, novel
observations from our laboratory have proposed a previously unsuspected source for this cytokine. Using the technique of arteriovenous difference measures across a subcutaneous adipose tissue bed and radio-xenon measures of adipose tissue blood flow, we have demonstrated IL-6 production by human subcutaneous adipose tissue in vivo.\textsuperscript{13} The production of IL-6, as well as systemic concentrations, increase with adiposity, and we have estimated that \( \approx 30\% \) of total circulating concentrations of IL-6 originate from adipose tissue in healthy subjects.\textsuperscript{13} Both IL-6 and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) are expressed in adipose tissue\textsuperscript{14,15} and in vitro release of TNF-\( \alpha \) by adipocytes has been reported.\textsuperscript{16} Among the known effects of these cytokines are inhibition of insulin signaling\textsuperscript{17} and induction of both hypertriglyceridemia\textsuperscript{18} and endothelial activation.\textsuperscript{19}

These observations have led us to explore the links of levels of acute phase markers and concentrations of proinflammatory cytokines with two of their proposed determinants, ie, obesity and chronic infection with 3 organisms proposed to be related to risk of CHD.\textsuperscript{11,20–22} We have also explored relationships of acute phase markers with features of the insulin resistance syndrome\textsuperscript{23} and of markers of endothelial dysfunction, ie, with the proposed consequences of a chronic low-level inflammatory state.\textsuperscript{17,19} We hypothesized that: (1) If adipose tissue were responsible for production of proinflammatory cytokines, then circulating concentrations of C-reactive protein and of proinflammatory cytokines would be related to measures of obesity; (2) If IL-6 were responsible for the metabolic and vascular consequences of obesity, then measures of IL-6 and of CRP would relate to insulin resistance syndrome and endothelial markers, independently of measures of adiposity.

We have explored these relationships in a population of 107 healthy subjects in whom a large number of measures had been assessed, recognizing that this size of study, and its cross-sectional design, must, by its nature be hypothesis generating. We have explored associations both between individual measures of obesity, insulin resistance syndrome, endothelial and acute phase activation, as well as between predefined groups of these variables.

**Methods**

**Subjects**

We studied 107 white nondiabetic subjects as a follow-up investigation of cardiovascular risk factors.\textsuperscript{24–28} In summary, we originally investigated subjects aged 40 to 75 randomly selected from the age-sex register of a north London general practice, and 36 (SD5) months later restudied 125 of those with normal glucose tolerance. In 107 of the recall subjects, sufficient serum and plasma was available 3 months later restudied 125 of those with normal glucose tolerance. In 107 of the recall subjects, sufficient serum and plasma was available to study a range of other variables, these being similar to the total population in age, gender, ratio, and levels of the risk factors under investigation. The details of the study methods for anthropometry, blood pressure, daytime and overnight albumin excretion rate, cardiovascular disease history, and Minnesota code classification of electrocardiograms have been described previously.\textsuperscript{24–27}

**Methods**

Fasting blood from these 107 subjects was collected, spun at 2000 g for 15 minutes, and used for assay of total and HDL cholesterol, triglycerides, insulin, proinsulin, des 32,32 proinsulin, plasminogen activator inhibitor-1 (PAI-1) activity, and fibrinogen as previously described.\textsuperscript{24–27} and for additional measures of endothelial and acute phase markers and other related variables. Tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and interleukin-6 (IL-6) were measured by ELISA (R&D Systems). Thrombomodulin, von Willebrand factor, cellular fibronectin,\textsuperscript{29} tissue plasminogen activator (tPA) antigen, PAI-1 antigen, and C-reactive protein (CRP) were measured at the Gaubius Laboratory, TNO-PG, Leiden, Netherlands. Thrombomodulin was assayed using an ELISA kit (Stago);\textsuperscript{30} von Willebrand factor antigen was measured by an ELISA essentially as described\textsuperscript{31} using polyclonal antibodies from DAKO; and cellular fibronectin was measured with a sandwich ELISA using a monoclonal antibody IST-9 (Harlan Sera Labs) against the ED-A domain for capture, and a peroxin-conjugated polyclonal antibody (DAKO).\textsuperscript{32} Cellular fibronectin was measured by a microtiter plate ELISA. Tissue plasminogen activator and PAI-1 antigens were measured by ELISA (Organon Teknika), which recognizes both free forms of the factors and complexes of tPA with PAI-1. C-reactive protein was measured using a highly sensitive ELISA procedure,\textsuperscript{32} with a range of 0.25 to 10.25 \( \mu \)g/mL and an interassay coefficient of variation (CV) of 8\%. Antibody titters to Helicobacter pylori were measured using an enzyme immunoassay (Helico-G, Porton Cambridge). C pneumoniae IgG antibody titters were determined by ELISA according to a published method.\textsuperscript{33} Cytomegalovirus (CMV) IgG titters were determined using a standard microtiter complement fixation assay, using in-house CMV antigen prepared from the AD169 strain of CMV. These assays were performed in the Department of Microbiology and Virology, UCL Hospitals, London. Insulin sensitivity was calculated using the homeostasis model assessment (HOMA) model,\textsuperscript{34} a mathematical estimate of insulin sensitivity based on fasting glucose and insulin concentrations.

**Statistical Methods**

Linear correlation was used to look at relationships between variables, with logarithmic transformation of skewed variables. Comparison of groups was performed using unpaired Student’s \( \text{t} \) test. Multiple regression analysis was used to explore the independence of observed relationships between clusters of variables, with forced entry of age, gender, smoking, and prevalent CHD, followed by the standard deviation scores (see below) for the putative independent variables. Data are presented as mean \( \pm \) SD or as median (interquartile range) for skewed variables. Significance levels are shown for all comparisons and relationships where \( P < 0.05 \) although, because of the number of tests being performed, a more rigorous criterion of significance should be applied. Nevertheless, because the purpose is to explore relationships of acute phase markers with obesity, insulin resistance syndrome, and endothelial activation, another approach has also been used.

To explore the association between predefined clusters of variables, we created mean standard deviation scores for insulin resistance variables, endothelial markers, and acute phase markers for each subject. This approach was taken to reduce the influences of biological variability of each measure,\textsuperscript{35} which would make the usual multivariate approach less suitable, as well as to reduce the number of associations explored. We also preferred this approach to a formal factor analysis, as we were interested in possible etiological relationships between three predefined, and ostensibly distinct, groups of variables. For each subject, each variable was expressed as standard deviations of difference from the population mean, if necessary after logarithmic transformation, a value that ranged from \( -2.5 \) to \( 2.5 \). The mean scores were calculated as the mean of these standard deviation scores as follows: (1) Insulin resistance score = (systolic blood pressure + diastolic blood pressure + triglyceride + [HDL cholesterol \( \times (–1) \)] + [insulin sensitivity \( \times (–1) \)] + body mass index + waist-to-hip ratio + subscapular-to-triceps ratio)/6. (2) Endothelial marker score = (thrombomodulin + cellular fibronectin + von Willebrand factor + mean albumin excretion rate)/4. (3) Acute phase marker score = (fibrinogen + C-reactive protein + IL-6 + TNF-\( \alpha \))/4.

For some of the analyses, including those shown in Table 2, the obesity variables were omitted from the insulin resistance score as follows: (systolic blood pressure + diastolic blood pressure + triglyceride + [HDL cholesterol \( \times (–1) \)] + [insulin sensitivity \( \times (–1) \)])/5.

For some analyses we also derived an obesity score as a mean standard deviation score: (body mass index + waist-to-hip ratio + subscapular-to-triceps ratio)/3.

Where results were missing, for insulin (\( n = 2 \)), albumin excretion rate (\( n = 1 \)), thrombomodulin (\( n = 10 \)), or fibrinogen (\( n = 4 \)), the mean standard deviation scores were calculated for the smaller denomina-
RESULTS

The characteristics of these middle-aged white subjects with normal glucose tolerance are shown in Table 1. The low levels of CRP are similar to those found in other healthy populations.\(^7,8\)

To explore the possible determinants of the acute phase markers and of the levels of proinflammatory cytokines, we explored their relationships with titers of IgG antibodies to 3 organisms which have been proposed as playing a potential role in atherogenesis.\(^11,20–22\) Concentrations of C-reactive protein correlated weakly with titers of \textit{H pylori}, \textit{C pneumoniae}, and cytomegalovirus antibodies (Table 2). However, the only significant correlation seen between titers of such antibodies and concentrations of cytokines was that of IL-6 with \textit{H pylori}.

Both IL-6 and TNF-\(\alpha\) are expressed in adipose tissue,\(^14,15\) and we have recently described the release of the former, but not the latter, from a subcutaneous adipose tissue bed in vivo.\(^13\) Concentrations of IL-6, TNF-\(\alpha\), and C-reactive protein were strongly related to measures of total, and particularly central, obesity (Table 2).

\begin{table}
\centering
\caption{Characteristics of Study Subjects}
\begin{tabular}{lrr}
\hline
Variable & Mean & SD \\
\hline
Number (M/F) & 107 (59/48) \\
Age (y) & 59.0 ± 10.9 \\
Body mass index (kg/m\(^2\)) & 25.9 ± 4.5 \\
Waist-to-hip ratio & 0.86 ± 0.08 \\
Subscapular-to-triceps ratio & 1.31 ± 0.59 \\
Systolic blood pressure (mm Hg) & 124.8 ± 18.4 \\
Diastolic blood pressure (mm Hg) & 80.4 ± 11.0 \\
Triglyceride (mmol/L) & 1.3 (1.0, 1.7) \\
HDL cholesterol (mmol/L) & 1.38 ± 0.37 \\
LDL cholesterol (mmol/L) & 3.62 ± 1.05 \\
Fasting plasma glucose (mmol/L) & 4.8 ± 0.5 \\
2-h plasma glucose (mmol/L) & 4.9 ± 1.1 \\
PAI-1 activity (AU/mL) & 8.1 (4.2, 15.9) \\
PAI-1 antigen (ng/mL) & 95.6 ± 58.9 \\
tPA (ng/mL) & 21.0 ± 9.4 \\
von Willebrand factor (%) & 109.7 ± 40.9 \\
Thrombomodulin (ng/mL) & 33.7 (10.9, 121.3) \\
Cellular fibronectin (%) & 108 (71, 159) \\
Mean albumin excretion rate (\(\mu\)g/min) & 10.2 (7.0, 20.6) \\
Fibrinogen (mg/mL) & 289.2 ± 75.9 \\
CRP (\(\mu\)g/mL) & 1.35 (0.57, 2.18) \\
TNF-\(\alpha\) (pg/mL) & 3.65 (2.98, 4.53) \\
IL-6 (pg/mL) & 2.19 (1.18, 4.40) \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Relationships of Concentrations of Proinflammatory Cytokines and C-reactive Protein With Antibody Titers and Obesity}
\begin{tabular}{lrrrr}
\hline
Antibody & TNF-\(\alpha\) & IL-6 & CRP \\
\hline
\textit{H pylori} titer (n=80) & 0.18 & 0.28* & 0.24* \\
\textit{C pneumoniae} titer (n=70) & 0.21 & 0.15 & 0.25* \\
CMV titer (n=80) & 0.21 & 0.17 & 0.23* \\
Body mass index & 0.33† & 0.19* & 0.41† \\
Waist-to-hip ratio & 0.51† & 0.41‡ & 0.32† \\
Subscapular-to-triceps ratio & 0.37† & 0.26† & 0.21† \\
CRP & 0.46† & 0.37‡ & – \\
\hline
\end{tabular}
\end{table}

Concentrations of CRP correlated both with those of IL-6 (\(r=0.37, P<0.0005\)) and of TNF-\(\alpha\) (\(r=0.46, P<0.0001\)). In Table 3 the relationships of concentrations of IL-6, TNF-\(\alpha\), and C-reactive protein with the components of the insulin resistance syndrome and with endothelial markers are shown. Univariate correlations are given as these were little affected by adjustment for age and gender. Concentrations of TNF-\(\alpha\) were related to all insulin resistance variables, including proinsulin-like molecules, tPA, and PAI-1. Concentrations of IL-6 were also related to several of the insulin resistance syndrome and endothelial markers, including albumin excretion rate, although the relationships for C-reactive protein were generally stronger. Although there is a weak relationship between concentrations of low-density LDL cholesterol and those of CRP, no such relationships are seen with TNF-\(\alpha\) or IL-6.

The population was dichotomized into those with high and those with low concentrations of CRP, based on the median

\begin{table}
\centering
\caption{Relationship of Concentrations of Proinflammatory Cytokines and of C-reactive Protein With Components of Insulin Resistance Cluster and Endothelial Markers}
\begin{tabular}{lrrrr}
\hline
Variable & TNF-\(\alpha\) & IL-6 & CRP & Mean albumin excretion rate \\
\hline
Insulin sensitivity§ & −0.35‡ & −0.09 & −0.22* & – \\
Triglyceride§ & 0.37‡ & 0.03 & 0.27† & – \\
HDL cholesterol & 0.27† & −0.26† & −0.21* & – \\
LDL cholesterol & 0.08 & 0.04 & 0.25* & – \\
Systolic blood pressure & 0.33‡ & 0.31† & 0.34† & – \\
Intact proinsulin§ & 0.33‡ & 0.16 & 0.16 & – \\
Des 31,32 proinsulin§ & 0.28† & 0.11 & 0.08 & – \\
PAI-1 antigen & 0.35‡ & 0.18 & 0.19* & – \\
tPA antigen & 0.40† & 0.32‡ & 0.40‡ & – \\
von Willebrand factor & 0.38‡ & 0.11 & 0.31‡ & – \\
Thrombomodulin§ & 0.32† & −0.05 & 0.13 & – \\
Cellular fibronectin§ & 0.36‡ & 0.13 & 0.28† & – \\
Mean albumin excretion rate§ & 0.25* & 0.20* & 0.07 & – \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Comparison of CRP and Tumor Necrosis Factor-\(\alpha\) Levels Between Groups of Subjects}
\begin{tabular}{lrrrr}
\hline
Group & CRP & TNF-\(\alpha\) & IL-6 & Mean albumin excretion rate \\
\hline
Obese & 0.37† & 0.26† & 0.21† & – \\
Normal & 0.46† & 0.37‡ & – & – \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Comparison of CRP and Tumor Necrosis Factor-\(\alpha\) Levels Between Groups of Subjects}
\begin{tabular}{lrrrr}
\hline
Group & CRP & TNF-\(\alpha\) & IL-6 & Mean albumin excretion rate \\
\hline
Obese & 0.37† & 0.26† & 0.21† & – \\
Normal & 0.46† & 0.37‡ & – & – \\
\hline
\end{tabular}
\end{table}

\textsuperscript{§}Data logarithmically transformed.
value of 1.35 mg/mL (Table 4). Subjects with high concentrations of CRP were more obese than those with lower levels, and had higher levels of blood pressure, triglyceride, von Willebrand factor, cellular fibronectin, PAI-1, tPA, and of the proinflammatory cytokines TNF-α and IL-6, but did not differ in titers of antibodies to Helicobacter, Chlamydia, or cytomegalovirus.

To overcome the problems of biological variability of the different measures, and to explore these inter-relationships further while controlling for potential confounds, we used summary scores for the insulin resistance syndrome variables, for endothelial dysfunction, and for acute phase markers by calculating a mean of a standard deviation score for each group of variables (see Methods). The relationships of these are shown in Figure 1. Whereas the insulin resistance syndrome and endothelial scores correlate with a coefficient of 0.32 (P=0.0008), there is a strong relationship between the insulin resistance syndrome and acute phase scores (r=0.59, P<0.00005). The third of these correlations, between endothelial and acute phase scores, is also significant (r=0.43, P<0.00005). A sum obesity score correlated with measures of both endothelial (r=0.33, P=0.001) and acute phase (r=0.54, P<0.00005) scores. Nevertheless, if the 3 measures of obesity are removed from the insulin resistance syndrome score, the relationship with the acute phase score was only slightly weakened (r=0.53, P<0.00005). Moreover, the strength of the relationship was not substantially affected by omitting any particular variable from either score. In multiple regression models, controlling for age, gender, smoking, and prevalent CHD, if the acute phase and endothelial scores were included in the same model, the former remained significantly associated with insulin resistance syndrome score (partial r=0.61, P<0.00005), but not the latter (partial r=−0.02, P=0.82). We have also approached the analysis of clustering of the variables using factor analysis, with generally similar results. The insulin resistance variables associate as two clusters, one comprising altered lipid concentrations with central obesity, and the other blood pressure with body mass index. Although both clusters correlate with acute phase markers, it is the second that relates more closely to endothelial dysfunction (data not shown).

**Discussion**

There has been much interest in the prognostic significance of raised levels of C-reactive protein in patients with angina,36 with the proposal that it points to release of IL-6 by activated macrophages in an unstable plaque.37 More recently, however, the observations that raised concentrations of CRP in

---

**TABLE 4. Characteristics of Subjects With Low (<1.35 μg/mL) and High (≥1.35 μg/mL) of C-Reactive Protein**

<table>
<thead>
<tr>
<th></th>
<th>Low CRP</th>
<th>High CRP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (M/F)</td>
<td>53 (27/26)</td>
<td>54 (32/26)</td>
<td>0.38</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55.9±11.0</td>
<td>62.1±9.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Smokers (non/ex/current)</td>
<td>37/3/13</td>
<td>26/6/22</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.3±4.0</td>
<td>27.5±4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.84±0.08</td>
<td>0.89±0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Subscapular-to-triceps ratio</td>
<td>1.15±0.51</td>
<td>1.47±0.62</td>
<td>0.004</td>
</tr>
<tr>
<td>H pylori titer</td>
<td>1/&lt;10 (1/&lt;10, 1/48)</td>
<td>1/24 (1/&lt;10, 1/70)</td>
<td>0.57</td>
</tr>
<tr>
<td>C pneumoniae titre</td>
<td>1/100 (1/&lt;100, 1/400)</td>
<td>1/200 (1/&lt;100, 1/200)</td>
<td>0.55</td>
</tr>
<tr>
<td>Cytomegalovirus titre</td>
<td>1/20 (1/&lt;5, 1/80)</td>
<td>1/40 (1/10, 1/80)</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>21.4 (14.4, 42.9)</td>
<td>32.8 (19.1, 46.0)</td>
<td>0.032</td>
</tr>
<tr>
<td>Insulin sensitivity (HOMA) (%)</td>
<td>107.6 (55.9, 165.6)</td>
<td>73.8 (51.0, 127.6)</td>
<td>0.027</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>118.7±15.7</td>
<td>130.8±18.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>77.7±11.0</td>
<td>83.0±10.3</td>
<td>0.011</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.2 (0.9, 1.6)</td>
<td>1.35 (1.18, 1.93)</td>
<td>0.036</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.44±0.39</td>
<td>1.33±0.35</td>
<td>0.14</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>4.8±0.4</td>
<td>4.8±0.5</td>
<td>0.40</td>
</tr>
<tr>
<td>PAI-1 activity (AU/mL)</td>
<td>6.5 (3.6, 12.2)</td>
<td>10.7 (4.8, 17.2)</td>
<td>0.051</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/mL)</td>
<td>83.4±55.1</td>
<td>107.6±60.5</td>
<td>0.033</td>
</tr>
<tr>
<td>tPA (ng/mL)</td>
<td>17.1±8.6</td>
<td>24.9±8.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>von Willebrand factor (%)</td>
<td>101.5±37.8</td>
<td>117.8±42.6</td>
<td>0.038</td>
</tr>
<tr>
<td>Thrombomodulin (ng/mL)</td>
<td>33.2 (27.7, 39.6)</td>
<td>34.8 (28.1, 41.1)</td>
<td>0.35</td>
</tr>
<tr>
<td>Cellular fibronectin (%)</td>
<td>84.0 (62.0, 138.5)</td>
<td>130.0 (83.3, 178.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean albumin excretion rate (µg/min)</td>
<td>10.8 (7.3, 19.4)</td>
<td>9.6 (6.8, 21.6)</td>
<td>0.34</td>
</tr>
<tr>
<td>Fibrinogen (mg/mL)</td>
<td>275.2±68.7</td>
<td>301.8±80.4</td>
<td>0.22</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>3.18 (2.63, 3.69)</td>
<td>4.12 (3.58, 5.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.35 (0.89, 3.11)</td>
<td>3.22 (1.79, 5.39)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Variables are presented as mean±SD, or as median (interquartile range) for skewed variables.
healthy subjects predicted the incidence of CHD over a period of years\textsuperscript{8–10} have suggested a role for inflammation in the initiation of atherosclerosis as well as in the precipitation of an acute event. The synthesis of CRP is predominantly under the control of IL-6,\textsuperscript{12} which in turn has been assumed to originate largely from activated leukocytes, either in the vessel wall itself or at a remote site of infection.\textsuperscript{2,11} We found that concentrations of C-reactive protein were related to titers of IgG antibodies, evidence of previous infection, for each of the 3 organisms we investigated. However, the correlations between these antibody titers and concentrations of fibrinogen, TNF-\(\alpha\), and IL-6 were weaker and generally insignificantly. In this study, Chlamydia antibodies were measured using an ELISA method, and it is possible that antibody titers by an immunofluorescence assay would have been more closely related to markers of inflammation.\textsuperscript{11,38}

We have found close relationships between circulating CRP and cytokine concentrations and each of the anthropometric measures of obesity, compatible with an adipose tissue origin for TNF-\(\alpha\) and IL-6. Mendall et al have previously shown associations of circulating concentrations of CRP,\textsuperscript{7} and of TNF-\(\alpha\),\textsuperscript{39} but not of IL-6,\textsuperscript{39} with BMI, and the relationship of CRP levels with obesity were also noted in the MRFIT cohort,\textsuperscript{10} and, among nonsmokers, in the Cardiovascular Health Study.\textsuperscript{46} By contrast, we found no significant influence of smoking status on the relationships between acute phase markers and obesity. Both cytokines are expressed in, and released by, adipose tissue.\textsuperscript{13–16} We have recently reported significant in vivo release of IL-6, but not TNF-\(\alpha\), by a subcutaneous adipose tissue depot.\textsuperscript{13} However, the relationships of circulating concentrations of TNF-\(\alpha\) with obesity suggests that adipose tissue, perhaps in other sites, may contribute to circulating levels. Alternatively, the expression of one of the TNF-\(\alpha\) soluble receptors by adipose tissue\textsuperscript{41} raises the possibility that the circulating cytokine is in the form of a complex, the relationships with measures of obesity representing secretion of the soluble receptor. Even if free, it is likely that the presence of this cytokine in the circulation represents spillover from the interstitial compartment in adipose tissue, and perhaps from adipocytes within muscle.

We report a relationship between circulating concentrations both of CRP and of two proinflammatory cytokines with a number of features of the insulin resistance syndrome,\textsuperscript{23} reflecting our previous report of a relationship between fibrinogen concentrations and measures of insulin resistance.\textsuperscript{25} Although relationships of CRP levels with triglycerides, HDL, glucose, and diabetes have been noted previously,\textsuperscript{7,40} no such relationship appears to have been reported with insulin concentrations or measures of insulin resistance. It is clearly not possible, in a cross-sectional study, to attribute causality to one of a set of correlated variables, but we have explored some hypotheses in this setting. The relationship between elevated concentrations of CRP and of the proinflammatory cytokines with the insulin resistance syndrome could represent associations produced by a confounding variable, such as adiposity. However, the relationships between a derived insulin resistance syndrome standard deviation score and one for the acute phase variables was only slightly weakened by removing all obesity measures from the former score. We also excluded PAI-1 from the calculation of an insulin resistance score, both because the measure of antigen may represent inactive PAI-1 (complexed to tPA or released from platelets), and also because PAI-1 is recognized to respond to acute phase stimuli.

Our observations could suggest that the cytokines, arising in part from adipose tissue, might themselves be partly responsible for the metabolic, hemodynamic, and hemostructural abnormalities that cluster with insulin resistance. Although not itself an inducer of acute phase proteins, TNF-\(\alpha\) induces production of IL-6,\textsuperscript{42} which is itself the major determinant of the acute phase response.\textsuperscript{12} Among the known metabolic effects of TNF-\(\alpha\) are inhibition of the action of lipoprotein lipase\textsuperscript{43} and stimulation of lipolysis,\textsuperscript{18} these actions being shared with IL-6.\textsuperscript{44,45} Furthermore, TNF-\(\alpha\) impairs the function of the insulin signaling pathway by effects on phosphorylation of both the insulin receptor and its substrate, IRS-1.\textsuperscript{1,17,46}

In addition to their associations with insulin resistance syndrome variables, elevated levels of CRP and of cytokines were associated with a series of indicators of endothelial dysfunction. Tracy et al have previously reported associations of levels of CRP with a variety of measures of procoagulant activity and fibrinolysis,\textsuperscript{40} and have suggested that these represent consequences either of inflammation in underlying atherothrombotic disease or of inflammatory cells activated by products of ongoing coagulation processes. TNF-\(\alpha\) is known to influence endothelial cell function,\textsuperscript{19,47} and a recent study suggests that IL-6 may also induce endothelial expression of chemokines and adhesion molecules in the presence of IL-6 soluble receptor, which is released in inflammatory states.\textsuperscript{48} If endothelial dysfunction, perhaps as a consequence of elevated concentrations of cytokines, resulted in impair-
ment of vasodilatation of resistance vessels, it could be postulated that the cluster of variables that have been attributed to insulin resistance (dyslipidemia, hypertension, and impaired fibrinolysis), as well as insulin resistance itself, might all result as consequences of a common antecedent. The strong relationship between concentrations of C-reactive protein and insulin resistance variables (Table 3), compared with those seen for IL-6, may simply reflect the longer half-life of C-reactive protein providing a more stable marker of acute phase mediators. Levels of C-reactive protein are predominantly modulated by hepatic effects of IL-6, which suggests a more important role for this cytokine in the cluster than suggested by the correlations shown in Table 3. If circulating TNF-α represents spillover from adipose tissue and muscle, where the local concentrations would be more likely to approximate to those required to exert metabolic effects in vitro, this might imply autocrine or paracrine, and not endocrine, metabolic effects of TNF-α. Adipose tissue release of IL-6, also induced by TNF-α, may then be responsible for systemic effects on endothelial and lipids.

In conclusion, we have shown, in healthy subjects, relationships between levels of CRP and measures of obesity, consistent with our finding of adipose tissue release of IL-6 in vivo and implicating adipose tissue as a major source for circulating IL-6. We have also found associations between levels of acute phase proteins and of proinflammatory cytokines not only with blood pressure and dyslipidemia, but both with a measure of insulin resistance and with markers of endothelial dysfunction. Furthermore, the association of acute phase markers with insulin resistance variables is independent of anthropometric measures of obesity. We are suggesting a more general role for both IL-6 and TNF-α in atherogenesis and thrombosis, influencing as they do, the risk factors which have been termed the insulin resistance syndrome, endothelial function and expression of prothrombotic factors and adhesion molecules, and acute phase proteins, which in turn may increase cardiovascular risk. Our paradigm provides a novel explanation for the association of insulin resistance and cardiovascular risk, as well as a putative mechanism for the deleterious effects of obesity, and in particular central adiposity, in heart disease risk. Of necessity, however, this study has merely developed a hypothesis about the common antecedence of adipose tissue-generated proinflammatory cytokines in insulin resistance and endothelial dysfunction, which will require further testing in both epidemiological and clinical investigative studies.

Acknowledgments

We are grateful for the help of Mairi Gould on the work in the screening phase of the Goodinge Study, and Maryam Fernández for work on the recall phase; to Dr John Griffin in performing the assays of cytokines; and to Geoffrey Ridgway, Dr Nicola Brink, and Dr John Holton for antibody tier assays. We thank Ms Christine Andrés for albumin and PAI-1 activity assays, and Dr Vidya Mohamed-Ali for measurement of insulin and proinsulin-like molecules. Aspects of this study were supported by grants from the Wellcome Trust (12441/1.5) and British Heart Foundation (PG92133), and a Program Grant from the British Diabetic Association. Dr Stehouwer is supported by a fellowship from the Diabetes Fonds Netherlands and the Netherlands Organization for Scientific Research (NWO), and Dr Emeis by Grant 28–2623 from the Praeventiefonds.

References


C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction: A Potential Role for Cytokines Originating From Adipose Tissue?

John S. Yudkin, C. D. A. Stehouwer, J. J. Emeis and S. W. Coppack

doi: 10.1161/01.ATV.19.4.972
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/4/972

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/