Abstract

Objective To investigate the association between C-reactive protein (CRP) and insulin resistance.

Materials and Methods This study included 1,624 Japanese participants (652 men and 972 women) aged 40 to 69 years who were non-diabetics or did not have medication for hypertension or dyslipidemia, a history of cardiovascular disease or CRP levels >10 mg/l. Serum CRP level, fasting glucose level, and fasting insulin level were measured, and the degree of insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR). Categories of CRP were defined by the following tertiles: <0.25 mg/l, 0.25–0.59 mg/l, and ≥0.60 mg/l.

Results Elevated CRP levels were associated with increased fasting insulin levels, fasting glucose levels, and HOMA-IR in both men and women. Although the adjustment for body mass index in addition to age, cigarette smoking, and alcohol consumption attenuated the associations between CRP and fasting insulin, fasting glucose, and HOMA-IR, elevated CRP levels were associated with increased insulin levels and HOMA-IR in both sexes. Stratified analyses by CRP level and obesity showed that obesity status was associated with increased fasting insulin levels, fasting glucose levels, and HOMA-IR in both sexes and that fasting insulin levels, fasting glucose levels, and HOMA-IR were higher among obese individuals than among non-obese individuals at the same level of CRP.

Conclusion These results suggest a possible role of subclinical inflammation in insulin resistance and glucose intolerance in Japanese, but it only partly explains the link between obesity and impaired glucose homeostasis.

Key words: C-reactive protein, fasting glucose, insulin, insulin resistance, obesity, cross-sectional study

Introduction

Insulin resistance is known to be a major risk factor in the etiology of type 2 diabetes, hypertension, and dyslipidemia and may be a risk factor for coronary heart disease (CHD) (1, 2). A hyperinflammatory trait is hypothesized to be an important factor underlying insulin resistance syndrome (3). C-reactive protein (CRP) is the main acute phase protein and elevated CRP levels are an indicator of systemic inflammation. Mild elevations of CRP levels, even when within the clinically normal range, are independently predictive of CHD (4, 5).

Experimental studies have shown that hyperglycemia stimulates the release of the inflammatory cytokine interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) from various cell types and results in the induction and secretion of acute-phase reactants by adipocytes (6, 7). Although hyperglycemia milieu potentially promotes the production of inflammatory mediators, the relation between glucose homeostasis and markers of subclinical inflammation is controversial (8–12), and few studies have directly examined the association between CRP levels and fasting insulin, fasting glucose, and insulin resistance.

Various indices of insulin sensitivity have been proposed based on the interrelations between the levels of insulin, glucose, and other parameters obtained either in the fasting state or during the oral glucose tolerance test and the data obtained during a hyperinsulinemic euglycemic clamp (13). The homeostasis model assessment of insulin resistance (HOMA-IR) (14), one of the indirect indices for the assessment of insulin resistance, correlates well with euglycemic clamp measures in men and women, younger and older
adults, and obese and non-obese individuals (15–18). HOMA-IR is currently being proposed by investigators as a useful index of insulin sensitivity, particularly in epidemiological studies (13, 15–19). In the present cross-sectional study of apparently healthy Japanese, we examined the associations of serum CRP with blood levels of insulin and glucose and insulin resistance estimated by the HOMA-IR.

**Methods**

**Subjects**

We studied 2,108 Japanese individuals (907 men and 1,201 women) aged 40 to 69 years who were participants in the Minoh Study performed in 2003. The Minoh Study was designed to clarify risk factors for major diseases, including hypertension, dyslipidemia, and diabetes among Japanese. The study protocol was approved by the Institutional Review Board of the Osaka University Graduate School of Medicine. We considered the return of self-administered questionnaires signed by the subjects to imply their consent to participate in the study. Of 2,108 potential participants, 484 (23.0%) were excluded: 163 (7.7%) had type 2 diabetes (a fasting glucose level of ≥7.0 mmol/l or receipt of hypoglycemic medication), 271 (12.9%) were receiving anti-hypertensive medication, 142 (6.7%) had medications for dyslipidemia, 29 (1.4%) had a past history of either CHD or stroke, and 3 (0.1%) had CRP levels >10 mg/l because of chronic disorders of the joints and connective tissues. The final study population for analysis therefore consisted of 652 men and 972 women.

**Data collection**

The participants were asked to fast for at least 10 hours and to avoid heavy physical activity for more than 2 hours before the examinations. An interviewer assessed the usual weekly intake of alcohol in units of ‘go’ (a traditional Japanese unit of measurement, by volume, corresponding to 23 g of ethanol), which were converted to grams of ethanol per day. One ‘go’ is 180 ml of sake, and it corresponds to one bottle (663 ml) of beer, two single shots (75 ml) of whiskey, or two glasses (180 ml) of wine. Subjects who reported consuming ≥0.3 go per week were regarded as current drinkers. The questionnaire also asked about smoking habits (never, past, or current smoker); past or current smokers were asked about the number of cigarettes smoked per day. Subjects who reported smoking at least 1 cigarette per day during the year before the examination were classified as current smokers. Body mass index (BMI), calculated as weight divided by the square of height in meters, was used as an index of relative weight. A BMI of ≥25 kg/m², the newly proposed Japanese BMI cutoff for obesity (20), was used to define obesity. Blood samples were drawn from an antecubital vein. Serum total cholesterol level, triglyceride level, high-density lipoprotein (HDL) cholesterol level, CRP level, glucose level, and insulin level were determined by the Hitachi7170 autoanalyzer in the laboratory of Mihoh Medical Health Center. Total cholesterol, triglycerides, and HDL cholesterol were measured enzymatically. CRP levels were measured with nephelometry, a latex particle-enhanced immunoassay (Nittobo, Tokyo, Japan), with the interassay and intraassay coefficients of variation (CVs) of <2.0 % and <1.9 %, respectively. The assay is sensitive enough to detect 0.05 mg/l of CRP. Undetectable CRP values were recorded as 0.025 mg/l. Glucose was measured by a hexokinase-glucose dehydrogenase method [Sysmex, Kobe, Japan: interassay and intraassay coefficients of variation (CVs), <0.9 % and <0.6%, respectively]. Insulin levels were determined with a radioimmunoassay kit (Sysmex: interassay and intraassay CVs, <3.5 % and <3.0%, respectively). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: fasting insulin (µU/ml) X fasting glucose (mmol/l)/22.5 (14).

**Statistical Analyses**

Data on characteristics of the participants are reported as means SD and percentages except when the distribution was strongly skewed; in that case medians and interquartile ranges are given. Pearson’s correlation coefficients between continuous variables of interest were calculated. Mean values of fasting insulin, fasting glucose, and HOMA-IR were compared across the different levels of CRP. Categories of CRP were defined by the following tertiles: <0.25 mg/l, 0.25–0.59 mg/l, and ≥0.60 mg/l. In each comparison, the group with the lowest tertile of CRP served as the reference group. Multiple linear regression was used to adjust for covariates of interest, including age, cigarette smoking, alcohol consumption, and BMI. Mean values of fasting insulin, fasting glucose, and HOMA-IR were also compared for participants grouped by CRP level and obesity, after adjustment for the covariates of interest. When parametric procedures were used, CRP, insulin, and HOMA-IR were transformed into natural logarithms to reduce the skewness of their distributions.

Data were analyzed by using the SPSS/PC statistical package (SPSS, Chicago, IL, USA). All reported P values are two-tailed, and those less than 0.05 were considered to be statistically significant.

**Results**

Characteristics of the participants according to gender are shown in Table 1. Tests for differences in characteristics across genders were significant except for age. The percentages of current smokers and current drinkers and the mean values of BMI, systolic and diastolic blood pressures, and fasting glucose were significantly higher among men than among women. On the other hand, the mean values of total cholesterol and HDL cholesterol were significantly higher among women than among men. The median values of triglycerides, CRP, fasting insulin, and HOMA-IR were higher among men than among women, and the differences in log-transformed values of these variables were significant.

Pearson’s correlation coefficients for study variables of
interest according to gender are shown in Table 2. In both men and women, there was a statistically significant positive association between CRP and age, BMI, fasting insulin, fasting glucose, and HOMA-IR. CRP was also significantly associated with the number of cigarettes smoked per day in men.

The mean values of fasting insulin, fasting glucose, and HOMA-IR according to serum CRP and gender are shown in Table 3. Elevated CRP levels were significantly associated with increased fasting insulin levels, fasting glucose levels, and HOMA-IR in both men and women. With the adjustment for age, cigarette smoking, and alcohol consumption, similar associations were also observed between CRP levels and fasting insulin levels, fasting glucose levels, and HOMA-IR. Additional adjustment for BMI largely reduced the associations of CRP level with fasting insulin level, fasting glucose level, and HOMA-IR in both sexes, and the differences in the means of fasting insulin level, fasting glucose level, and HOMA-IR across CRP levels became much smaller.

The mean values of fasting insulin, fasting glucose, and HOMA-IR according to serum CRP, obesity and gender are shown in Table 4. Obesity status was associated with increased levels of fasting insulin, fasting glucose, and HOMA-IR in both men and women. Obese individuals had higher levels of fasting insulin, fasting glucose, and HOMA-IR than did non-obese individuals at the same level.

---

Table 1. Characteristics of the Participants, According to Sex

<table>
<thead>
<tr>
<th></th>
<th>Men (n=652)</th>
<th>Women (n=972)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.9±8.4</td>
<td>55.8±7.8</td>
<td>0.810</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>38.8</td>
<td>7.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current drinking (%)</td>
<td>77.0</td>
<td>41.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.5±2.7</td>
<td>21.9±2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117.9±17.1</td>
<td>111.4±17.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>74.8±10.5</td>
<td>69.3±9.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.53±0.82</td>
<td>5.90±0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.23 (0.88–1.84)</td>
<td>0.86 (0.64–1.15)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.53±0.40</td>
<td>1.91±0.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)†</td>
<td>0.51 (0.27–1.00)</td>
<td>0.32 (0.19–0.61)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>6.8 (4.6–9.9)</td>
<td>6.3 (4.3–8.9)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.73±0.47</td>
<td>5.37±0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.71 (1.15–2.60)</td>
<td>1.47 (1.01–2.20)</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

HDL: indicates high-density lipoprotein, HOMA-IR: homeostasis model assessment of insulin resistance. Data are mean±SD, percentages, or median (interquartile range) for variables with skewed distributions. †Test of significance was based on log-transformed values.

Table 2. Pearson’s Correlation Coefficients* between Variables of Interest, According to Sex

<table>
<thead>
<tr>
<th></th>
<th>C-reactive protein (mg/l)†</th>
<th>Fasting insulin (µU/ml)†</th>
<th>Fasting glucose (mmol/l)</th>
<th>HOMA-IR†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (n=652)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.117</td>
<td>-0.154</td>
<td>0.037</td>
<td>-0.143</td>
</tr>
<tr>
<td>Cigarette smoking (cigarettes/day)</td>
<td>0.169</td>
<td>0.070</td>
<td>0.037</td>
<td>0.072</td>
</tr>
<tr>
<td>Alcohol consumption (g/day of ethanol)</td>
<td>-0.030</td>
<td>-0.068</td>
<td>0.107</td>
<td>-0.052</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.227</td>
<td>0.530</td>
<td>0.106</td>
<td>0.524</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)†</td>
<td>0.214</td>
<td>0.100</td>
<td>0.219</td>
<td></td>
</tr>
<tr>
<td><strong>Women (n=972)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.179</td>
<td>0.061</td>
<td>0.198</td>
<td>0.086</td>
</tr>
<tr>
<td>Cigarette smoking (cigarettes/day)</td>
<td>-0.004</td>
<td>0.025</td>
<td>-0.026</td>
<td>0.020</td>
</tr>
<tr>
<td>Alcohol consumption (g/day of ethanol)</td>
<td>-0.055</td>
<td>-0.110</td>
<td>0.120</td>
<td>-0.101</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.347</td>
<td>0.442</td>
<td>0.235</td>
<td>0.448</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)†</td>
<td>0.251</td>
<td>0.193</td>
<td>0.264</td>
<td></td>
</tr>
</tbody>
</table>

HOMA-IR: indicates homeostasis model assessment of insulin resistance. *Test of significance, p<0.05 for correlation coefficient (r, absolute value) ≥0.077 in men and r ≥0.063 in women, p<0.01 for r ≥0.101 in men and r ≥0.082 in women, and p<0.001 for r ≥0.125 in men and r ≥0.103 in women. †Calculations of correlation coefficients were based on log-transformed values.
In this sample of apparently healthy subjects, elevated CRP levels were associated with increased fasting insulin levels, fasting glucose levels, and HOMA-IR in both men and women. With the adjustment for BMI in addition to all the other covariates, the differences in the means of fasting insulin level, fasting glucose level, and HOMA-IR according to CRP level became smaller, but elevated CRP levels were associated with increased insulin levels and HOMA-IR. Stratified analyses by CRP level and obesity showed that obese individuals had much higher fasting insulin levels, fasting glucose levels, and HOMA-IR than did those who were not obese, even though their CRP levels were at the same level.

Low-grade inflammation is one of the novel risk factors implicated in the development of atherosclerosis (3, 21). A growing body of evidence implicates adipose tissue in general, and visceral adiposity in particular, as a key regulator of inflammation. Inflammatory cytokines, such as IL-6 and...
TNF-α, have been shown to be released by adipose tissue and to affect insulin resistance (22, 23), and the release of these cytokines is greater in obese subjects (24, 25). IL-6 increases postprandially, in parallel to glucose and insulin concentrations in the interstitial fluid of subcutaneous adipose tissue (22). TNF-α and another inflammatory cytokine, leptin, produce insulin resistance by influencing the function of insulin receptor or impairing insulin action and inhibiting insulin secretion (26, 27). TNF-α may also alter β-cell function of non-esterified-fatty-acid production (28, 29). Leptin, aside from possibly having a proinflammatory role, impairs insulin action and inhibits insulin secretion (27). Because adipose tissue produces IL-6 and TNF-α and the synthesis of CRP, mostly under the control of IL-6 and TNF-α, stimulates the production of CRP (30, 31), the associations of CRP levels with fasting insulin, fasting glucose, and HOMA-IR could be due to the presence of a subclinical inflammatory reaction.

Obesity, as described earlier, causes low-grade chronic inflammation through enhanced adipose tissue-derived cytokine expression, and inflammatory factors are thus likely in the pathway that links obesity to insulin resistance. Significant associations between CRP and BMI were found in this study (the correlation coefficient: 0.227 for men and 0.347 for women). The previous studies of various populations also found a positive association between body fat composition and CRP (12, 32, 33). Furthermore, a recent study has shown that although acute phase reaction correlates with insulin resistance and obesity in type 2 diabetic patients, its association with insulin resistance is partly independent of adiposity (34). In this study, the adjustment for BMI largely reduced the estimates of associations between CRP and the indicators of insulin resistance. We also found that fasting insulin levels, fasting glucose levels, and HOMA-IR remained higher among obese than non-obese individuals at the same level of CRP. These findings suggest a possible role of subclinical inflammation in insulin resistance and glucose intolerance, but it may only partly explain the link between obesity and impaired glucose homeostasis. Factors in addition to inflammation may also be involved in the association between obesity and impaired glucose homeostasis.

Limitations of this study should be noted: First, CRP was assessed from one blood sample only, but none of the subjects had a CRP level of greater than 10 mg/l, the cut-off point generally used to identify clinically relevant infection. Secondly, HOMA-IR was used for assessing insulin resistance. Although HOMA-IR provides a useful index to assess insulin resistance in epidemiological studies in which only fasting samples are available (13, 15–19), its reliability among Japanese elderly patients ≥70 years old with poorly controlled type 2 diabetes has been questioned (35). However, all participants in the present study were less than 70 years of age and were non-diabetics. Thus, HOMA-IR could be considered to be a reliable marker of insulin resistance. Third, we excluded the subjects who reported taking drugs for hypertension or dyslipidemia or having type 2 diabetes or a past history of cardiovascular disease. The selection of apparently healthy subjects could have had an effect on the observations. Finally, we assessed obesity with BMI instead of waist circumference. The central pattern of distribution, with its higher weighting of waist circumference, is associated with more insulin resistance than is the peripheral pattern of distribution, and individuals with the central pattern are more likely to have glucose intolerance and hyperinsulinemia resulting from insulin resistance (36, 37). Nevertheless, most physicians routinely assess BMI, whereas the value of waist measurements in clinical practice has not been thoroughly examined and may require modification for different ethnic groups. A number of investigations have also shown that BMI can predict the development of type 2 diabetes and other metabolic disturbances as robustly as waist circumference (38, 39). Moreover, the Japan Society for the Study of Obesity has recently reported that BMI can estimate visceral fat measured by computed tomography as robustly as waist circumference (20). This Society suggests that obesity is adequately specified as a BMI ≥25 kg/m² in Japan where the prevalence and degree of obesity remained mild. Further studies are needed to clarify whether CRP plays an important role in glucose homeostasis.

Despite these potential limitations, our findings indicate that increased CRP levels, even when within the clinically normal range, are associated with increased fasting insulin, fasting glucose, and HOMA-IR, suggesting the potential role of subclinical inflammation in insulin resistance and glucose intolerance.

Acknowledgements: This study was supported in part by grants-in-aid from the Japan Arteriosclerosis Prevention Fund (JAPF), Tokyo, Japan and the Ministry of Education, Culture, Sports, Science and Technology in Japan.

References
7) Guha M, Bai W, Nadler JI, Natarajan R. Molecular mechanisms of tumor necrosis factor α gene expression in mononuclear cells via hyperglycemia-induced oxidative stress-dependent and independent pathways.
C-reactive Protein and Insulin Resistance


