IRIS II Study: Sensitivity and Specificity of Intact Proinsulin, Adiponectin, and the Proinsulin/Adiponectin Ratio as Markers for Insulin Resistance

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ABSTRACT

Objective: This study was performed to compare the specificity and sensitivity of intact proinsulin, adiponectin, and their ratio (proinsulin/adiponectin) in the prediction of insulin resistance as assessed by the homeostasis model assessment (HOMA) score (>2 = resistant).

Research Design and Methods: Using a cross-sectional approach, 500 orally treated patients with type 2 diabetes (272 women, 238 men; mean ± SD age, 64.8 ± 11.6 years; hemoglobin A1c, 7.0 ± 1.5%; disease duration, 5.8 ± 6.1 years) were investigated. Various cutoffs for body mass index-adjusted adiponectin and proinsulin/adiponectin were compared with the established cutoff value of 10 pmol/L for fasting proinsulin.

Results: Fasting proinsulin correlated more closely with the HOMA score (r = 0.560, P < 0.001) than fasting adiponectin (r = −0.204, P < 0.001) or proinsulin/adiponectin (r = 0.355, P < 0.001). For proinsulin, specificity and sensitivity for insulin resistance in correlation to the HOMA score results were 96% and 70%, respectively. At a comparable specificity level to proinsulin, adiponectin did not reach a comparable sensitivity (14%), while the proinsulin/adiponectin ratio almost reached the same sensitivity (65%). Overall, patients with elevated proinsulin had a higher prevalence of micro- and macrovascular disease [odds ratio 1.47 (adiponectin, 1.08; proinsulin/adiponectin, 1.48) and 1.34 (adiponectin, 1.32; proinsulin/adiponectin, 1.27), respectively].

Conclusions: Elevation of fasting intact proinsulin seems to be the more specific marker for insulin resistance and increased cardiovascular risk than suppression of fasting adiponectin. Formation of the ratio does not lead to a further increase in the predictive value.

INTRODUCTION

Insulin resistance is a hallmark of type 2 diabetes. In subjects without diabetes, insulin resistance is associated with a clustering of cardiovascular risk factors1 and a high incidence of cardiovascular disease2,3 approximating the cardiovascular risk of patients with type 2 diabetes.4,5 Insulin resistance, therefore, has been proposed to be the com-

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mon link between type 2 diabetes and cardiovascular disease. Both intact proinsulin and adiponectin have been proposed to be markers of insulin resistance. Proinsulin is the precursor of insulin, which binds to the insulin receptor and has only a weak hypoglycemic effect. Mainly seen as an indicator for impaired ß-cell function, proinsulin can be detected at low concentrations (<10 pmol/L) in the blood of healthy persons but is found at higher concentrations in the blood of insulin-resistant subjects and patients with type 2 diabetes. Adiponectin is a protein expressed in adipose tissue and can be detected at concentrations of approximately 7-12 mg/L in human blood. In contrast to proinsulin, the concentration of adiponectin is suppressed in patients with type 2 diabetes and especially in patients with additional cardiovascular complications. Based on these results, both substances seem to be indicators of insulin resistance and high cardiovascular risk in patients with type 2 diabetes.

A test for insulin resistance has to provide a good specificity and sensitivity in order to base individual therapeutic decisions on the results. The sensitivity and specificity of both intact proinsulin and adiponectin have never been directly compared in a large group of patients with a wide range of insulin sensitivity. We conducted this study to investigate and compare the sensitivity and the specificity of proinsulin, adiponectin, and their ratio in diagnosing insulin resistance and also macro- and microvascular risk in patients with type 2 diabetes.

SUBJECTS AND METHODS

Patients

All patients had been diagnosed as having type 2 diabetes previously (mean duration of diabetes 5.8 ± 6.1 years) and were being treated with oral antidiabetes drugs. A detailed history was obtained in every patient to determine the prevalence of micro- and macrovascular complications. Presence of macrovascular disease was defined as a history of cerebrovascular disease, coronary heart disease, congestive heart failure, or peripheral arterial disease. Presence of microvascular disease was defined as a history of nephropathy, neuropathy, or retinopathy.

In order to investigate intact proinsulin and adiponectin over a wide range of insulin resistance, 10 groups of 50 patients each with incremental homeostasis model assessment (HOMA) scores from 0.21 to 67.32 were randomly chosen out of a 4,265-person cohort by a researcher who was blinded to the intact proinsulin and adiponectin data. Group 1 consisted of patients with the lowest HOMA score of the 4,265-person cohort; all following groups were chosen at incremental HOMA scores (Table 1). The critical range of the HOMA score is around 2.0, which is accepted as the cutoff value for insulin resistance. Therefore, one group of 50 patients was selected with a HOMA score just below 2.0, and another group of 50 patients with a HOMA score just above 2.0. All data were provided by the Clinical Department of the Institute for Clinical Research and Development (IKFE GmbH, Mainz, Germany). The collection of data was performed in accordance with Guidelines for Good Clinical Practice and after approval by the responsible ethics review board. Patients gave written informed consent for the blood draws and proinsulin and adiponectin measurements.

Measurements

All blood samples were taken in the morning with the patient fasting from midnight onwards. Blood samples were centrifuged, and plasma and serum samples were kept at -20°C until laboratory testing, which occurred within 3 months in every case.

Glucose was measured using a standard reference method (glucose oxidase method). Serum insulin was determined by chemiluminescence (Insulin Assay, Sciema, Mainz). Serum intact proinsulin was also measured by chemiluminescence as published previously (MLT Intact Proinsulin Assay, Sciema). Plasma adiponectin was measured by radioimmunoassay (human Adiponectin RIA Kit, Linco Research, Inc., St. Charles, MO).

Insulin resistance was calculated from the fasting insulin and glucose values by means of
### Table 1. Characteristics of All Patients Stratified According to HOMA Score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
<th>Group 9</th>
<th>Group 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA Score</td>
<td>0.55 ± 0.12</td>
<td>1.04 ± 0.02</td>
<td>1.53 ± 0.04</td>
<td>1.93 ± 0.01</td>
<td>2.03 ± 0.02</td>
<td>4.04 ± 0.03</td>
<td>6.14 ± 0.08</td>
<td>8.21 ± 0.12</td>
<td>10.49 ± 0.31</td>
<td>28.65 ± 10.19</td>
</tr>
<tr>
<td>Range (years)</td>
<td>67.1 ± 11.4</td>
<td>62.6 ± 12.1</td>
<td>69.6 ± 12.1</td>
<td>69.5 ± 10.5</td>
<td>65.8 ± 11.5</td>
<td>65.0 ± 9.5</td>
<td>64.4 ± 10.4</td>
<td>62.8 ± 11.5</td>
<td>60.3 ± 12.4</td>
<td>64.0 ± 12.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 32</td>
<td>26.9 ± 38</td>
<td>27.4 ± 41</td>
<td>28.3 ± 40</td>
<td>29.1 ± 41</td>
<td>30.0 ± 40</td>
<td>32.8 ± 57</td>
<td>32.7 ± 52</td>
<td>35.0 ± 73</td>
<td>32.3 ± 79</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>7.1 ± 6.7</td>
<td>5.9 ± 7.6</td>
<td>7.6 ± 9.4</td>
<td>5.1 ± 4.1</td>
<td>5.6 ± 5.3</td>
<td>6.2 ± 6.1</td>
<td>49 ± 3.8</td>
<td>43 ± 41</td>
<td>60 ± 51</td>
<td>57 ± 5.8</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.4 ± 11</td>
<td>6.1 ± 0.7</td>
<td>6.6 ± 0.9</td>
<td>6.4 ± 0.8</td>
<td>6.6 ± 0.9</td>
<td>7.3 ± 2.0</td>
<td>76 ± 16</td>
<td>76 ± 18</td>
<td>77 ± 14</td>
<td>7.8 ± 1.7</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>2.9 ± 0.9</td>
<td>5.0 ± 0.9</td>
<td>6.6 ± 1.3</td>
<td>8.3 ± 1.5</td>
<td>8.5 ± 1.5</td>
<td>14.4 ± 4.1</td>
<td>196 ± 44</td>
<td>265 ± 70</td>
<td>313 ± 7.8</td>
<td>487 ± 24.3</td>
</tr>
<tr>
<td>Intact iPi (pmol/L)</td>
<td>3.3 ± 2.0</td>
<td>3.4 ± 1.5</td>
<td>5.3 ± 2.8</td>
<td>6.9 ± 4.3</td>
<td>7.6 ± 4.3</td>
<td>13.4 ± 9.3</td>
<td>203 ± 13.8</td>
<td>231 ± 14.2</td>
<td>240 ± 16.2</td>
<td>402 ± 33.6</td>
</tr>
<tr>
<td>BMI-adjusted iPi (pmol/mg)</td>
<td>0.2 ± 0.3</td>
<td>0.3 ± 0.4</td>
<td>0.6 ± 0.8</td>
<td>1.3 ± 1.0</td>
<td>2.6 ± 3.0</td>
<td>3.0 ± 3.5</td>
<td>3.5 ± 3.5</td>
<td>4.8 ± 5.1</td>
<td>7.9 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>84 ± 21</td>
<td>88 ± 18</td>
<td>98 ± 22</td>
<td>97 ± 20</td>
<td>100 ± 18</td>
<td>125 ± 48</td>
<td>134 ± 33</td>
<td>137 ± 48</td>
<td>144 ± 36</td>
<td>181 ± 63</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Ad, adiponectin; HbA1c, hemoglobin A1c; iPi, proinsulin.

*Linear regression analysis. NS, not significant.
HOMA analysis, in which a HOMA score = [insulin (in mU/L) × glucose (in mmol/L)]/22.5, with a value of >2 classified as insulin resistant.14

Statistics
Since adiponectin values have been shown to correlate with body mass index (BMI),17 corresponding adjustment was performed for all adiponectin values before further analysis. Differences between mean values were tested for significance using Student’s t test. All values are given as mean ± standard deviation, and statistical significance was inferred at a two-sided value of \( P < 0.05 \).

RESULTS
In total, samples of 500 male and female patients (mean age 64.8 ± 11.6 years) with type 2 diabetes mellitus were investigated. An overview about the group characteristics and the laboratory results is given in Table 1. Insulin sensitivity as measured by the HOMA score ranged from 0.6 ± 0.1 in Group 1 to 28.7 ± 10.2 in Group 10. Fasting intact proinsulin concentrations ranged from 3.3 ± 2.0 (Group 1) to 40.2 ± 33.6 (Group 10) pmol/L. Fasting adiponectin concentrations ranged from 15.7 ± 7.9 (Group 1) to 7.9 ± 5.5 (Group 10) mg/L. The relationship of intact proinsulin, adiponectin, and their ratio with insulin resistance determined as determined by the HOMA score is shown in Figure 1. Overall, intact proinsulin \( (r = 0.560, P < 0.0001) \) correlated more closely with insulin resistance than adiponectin \( (r = -0.204, P < 0.0001) \) or the proinsulin/adiponectin ratio \( (r = 0.355, P < 0.001) \).

At a cutoff value of 10 pmol/L proinsulin, specificity for predicting insulin resistance was 96%, while sensitivity was 70%. Specificity and sensitivity calculated for various cutoff values for adiponectin were always lower than for proinsulin. When different cutoff values for optimum sensitivity and specificity were calculated for proinsulin/adiponectin, these values did not exceed sensitivity and specificity for proinsulin alone (Table 2).

Patients who had proinsulin of >10 pmol/L had a higher incidence of macro- and microvascular disease. The odds ratio for macrovascular disease was 1.34 (microvascular disease, 1.47) for patients with elevated intact proinsulin concentrations.

FIG. 1. Intact proinsulin, adiponectin, and their ratio in relation to HOMA score.
proinsulin concentrations of $>10$ pmol/L. Neither adiponectin nor the proinsulin/adiponectin ratio, regardless of various cutoff values, reached the odds ratio of proinsulin (Table 2). In comparison, patients with a HOMA score of $>2.0$ had an odds ratio for macrovascular disease of 1.44 and of 1.27 for microvascular disease.

**DISCUSSION**

A fasting intact proinsulin concentration of $>10$ pmol/L predicts the presence of insulin resistance in patients with type 2 diabetes mellitus at a very high specificity and high sensitivity. The significance of elevated fasting intact proinsulin concentration so far has been mainly described as a risk factor for development of type 2 diabetes and as a marker of impaired $\beta$-cell function in established type 2 diabetes. However, previous studies found a correlation of intact proinsulin with insulin sensitivity in subjects without diabetes and in patients with newly diagnosed type 2 diabetes. In our study with 500 patients with established type 2 diabetes, who were specifically chosen for a wide range of insulin sensitivity, we found a close correlation of fasting intact proinsulin with insulin resistance. Further analysis revealed that a concentration of fasting intact proinsulin of $>10$ pmol/L (upper limit of the normal reference range) predicts insulin resistance with 96% specificity and 70% sensitivity and much more accurately than adiponectin concentration at any given cutoff.

The remarkable accuracy of the predictive value of fasting intact proinsulin seems to be due to two major reasons. First, a very specific test for determination of intact proinsulin was used that has a negligible cross-reactivity for des31,32-proinsulin. Des31,32-proinsulin is a partially processed by-product of the $\beta$-cell commonly secreted in later stages of type 2 diabetes and has been implicated in the development of macrovascular disease. A nonspecific proinsulin test method with cross-reactivity for proinsulin split products of methods used in previous studies could explain some confounding results. Therefore, the specific test for intact proinsulin used in our study strengthens our findings. Second, the relation between insulin resistance and fasting intact proinsulin concentrations (Fig. 1) shows a steep gradient in the area between insulin sensitivity (i.e., HOMA score $<2.0$) and insulin resistance (i.e., HOMA score $>2.0$). To maximize selectivity in this crucial area, we had chosen a group of patients with a HOMA score of just below and another group of 50 patients with a HOMA score of just above 2.0 (Table 1). As a result, we could confirm that the previously published cutoff value of a fasting intact proinsulin of 10 pmol/L predicts the presence of insulin resistance in patients with type 2 diabetes with very high specificity and high sensitivity. In contrast, fasting adiponectin concentration was a less specific and sensitive tool to diagnose insulin sensitivity. This is also apparent in the function of adiponectin concentrations in relation to insulin sensitivity, which shows a nearly flat section between HOMA scores of 2 and 4. The ratio between intact

<table>
<thead>
<tr>
<th>HOMA Cutoff</th>
<th>&lt;10</th>
<th>&lt;7</th>
<th>&lt;10</th>
<th>&lt;13</th>
<th>&gt;1</th>
<th>&gt;1.5</th>
<th>&gt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>78%</td>
<td>59%</td>
<td>37%</td>
<td>88%</td>
<td>95%</td>
<td>97%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>70%</td>
<td>48%</td>
<td>74%</td>
<td>87%</td>
<td>77%</td>
<td>65%</td>
<td>55%</td>
</tr>
<tr>
<td>MaVD</td>
<td>1.34</td>
<td>1.32</td>
<td>1.12</td>
<td>1.25</td>
<td>1.27</td>
<td>1.31</td>
<td>1.21</td>
</tr>
<tr>
<td>MiVD</td>
<td>1.47</td>
<td>1.08</td>
<td>1.01</td>
<td>1.01</td>
<td>1.48</td>
<td>1.30</td>
<td>1.22</td>
</tr>
</tbody>
</table>
proinsulin and adiponectin resulted in specificity values almost similar to that of intact proinsulin alone, but did not result in improved specificity or sensitivity for predicting insulin resistance.

It seems interesting that intact proinsulin, which is mainly seen as a marker of impaired β-cell function, should be such an impressive tool for the diagnosis of insulin resistance. We were able to substantiate this finding by showing that patients with a fasting proinsulin concentration of >10 pmol/L have a significantly higher prevalence of microvascular (47%) and macrovascular (34%) disease than patients with type 2 diabetes mellitus but normal fasting intact proinsulin concentration. In other words, an abnormal test for fasting intact proinsulin reveals patients with a high cardiovascular risk at comparable rates as the HOMA score. In a study examining newly diagnosed patients with type 2 diabetes, no correlation between proinsulin and macrovascular disease was apparent. Although our data stem from a cross sectional study and, therefore, cannot estimate future risk, it is well in agreement with previous studies showing increased microvascular and macrovascular risk in patients with impaired insulin resistance. Therefore, the accumulation of cardiovascular disease in patients with diabetes having elevated fasting intact proinsulin in our study is likely to be the result of the excellent agreement between the HOMA score and the classification according to intact proinsulin concentration. Moreover, proinsulin concentrations are correlated in clinically healthy subjects with cardiovascular risk factors such as plasminogen activator inhibitor type 1, fibrinogen, an atherogenic lipid profile, and carotid intima-media thickness. In young males without diabetes who had suffered a myocardial infarction, proinsulin concentrations were linked to the extent of coronary atherosclerosis, and in middle-aged men without diabetes, proinsulin proved to be a strong predictor of incident coronary heart disease, even independent from insulin resistance. Interestingly, therapeutic trials for the treatment of type 2 diabetes with externally administered biosynthetic proinsulin were stopped because of the increased incidence of cardiovascular events in the proinsulin treatment group. Our finding of a high prevalence of cardiovascular disease in the patients with elevated intact proinsulin strengthens the role of intact proinsulin as a marker of micro- and macrovascular risk in patients with type 2 diabetes as published recently.

In conclusion, a fasting intact proinsulin elevation proved to be a better marker than adiponectin (even after BMI adjustment) to diagnose insulin resistance in patients with type 2 diabetes. Patients with elevated intact proinsulin also had a high risk of micro- and macrovascular disease. Therefore, the specific determination of fasting intact proinsulin offers the advantage of identifying those patients with type 2 diabetes who have insulin resistance and a high cardiovascular risk and in consequence might benefit from a treatment specifically targeting insulin resistance.

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