IRIS II Study: Intact Proinsulin Is Confirmed as a Highly Specific Indicator for Insulin Resistance in a Large Cross-Sectional Study Design

ANDREAS PFÜTZNER, M.D., Ph.D., 1,2 EBERHARD STANDL, M.D., 3 CLOTH HOHBERG, M.D., 2 THOMAS KONRAD, M.D., 4 HERMANN-JOSEF STROTMANN, M.D., 5 GEORG LÜBBEN, M.D., 6 SABINE PAHLER, M.Sc., 2 MATTHIAS R. LANGENFELD, M.D., 2 JAN SCHULZE, M.D., 7 and THOMAS FORST, M.D. 2

ABSTRACT

Background: The cross-sectional IRIS-II study tried to assess the prevalence of insulin resistance and macrovascular disease in orally treated patients with Type 2 diabetes.

Methods: In total, 4,270 patients were enrolled into the study (2,146 male, 2,124 female; mean ± SD age 63.9 ± 11.1 years; body mass index 30.1 ± 5.5 kg/m²; duration of disease 5.4 ± 5.6 years; hemoglobin A1c 6.8 ± 1.3%). The study consisted of a single morning visit with completion of a standardized questionnaire and collection of a fasting blood sample.

Results: The mean intact proinsulin value was 11.4 ± 12.4 pmol/L (normal range <10 pmol/L). Homeostasis model assessment resulted in 1,147 insulin-sensitive patients (26.9%) and 3,123 patients (73.1%) with insulin resistance. Of the latter patients 1,465 (34.3% of all patients) had also elevated intact proinsulin values, while 1,658 (38.8%) had no proinsulin elevation. In contrast, 1,042 (24.4%) of the insulin-sensitive patients had normal intact proinsulin, and only 105 (2.4%) had elevated intact proinsulin concentrations (χ² test \( P < 0.0001 \)). A specificity of 93.2% (sensitivity 46.9%) was calculated for elevated intact proinsulin as an indirect marker for insulin resistance. Of the 1,451 patients treated with sulfonylurea 52% had elevated intact proinsulin values and increased prevalence of cardiovascular complications (odds ratio 1.45).

Conclusion: Type 2 patients with elevated fasting intact proinsulin values can be regarded as being insulin resistant. The results confirm that fasting intact proinsulin is a suitable measure for β-cell dysfunction and insulin resistance in type 2 diabetes and may be used to support therapeutic decisions.

INTRODUCTION

In past decades, insulin resistance and cardiovascular risk have frequently been correlated with “hyperinsulinemia” as an independent risk factor. 1-6 Insulin concentrations have also been used as a surrogate marker for determination of insulin resistance, since the use of direct methods to assess insulin resistance, such as euglycemic clamp studies or intra-
venous glucose tolerance tests (ivGTTs), are expensive, time consuming, and not suitable for routine use.7–10 However, many studies were not able to confirm the suspected negative impact of insulin on cardiovascular risk.11–15 A possible reason for these contradictive data may be found in the cross-reactivity of past insulin assays with proinsulin, which is normally secreted in low levels, but which may be elevated in the later course of type 2 diabetes.16 Increase in intact proinsulin and its specific and unspecific split products in the peripheral blood has been demonstrated to be a highly specific marker for insulin resistance17 and a possible independent risk factor of cardiovascular disease, e.g., by stimulating plasminogen activator inhibitor type-1 secretion and blocking of fibrinolysis.18–22 External substitution of proinsulin in a phase II clinical trial was associated with cardiovascular events, which led to premature termination of the study.23 On the other hand, insulin itself appears to be only a very weak risk indicator for the occurrence of cardiovascular disease in experimental and clinical studies.24,25 It may, thus, be concluded that next to hyperglycemia, hyperproinsulinemia and not hyperinsulinemia is the mediator of an increased cardiovascular risk in patients with type 2 diabetes.26 However, to support this hypothesis further epidemiological investigations need to be collected and analyzed appropriately to give evidence of the relation between impairment of β-cell function as measured, e.g., by fasting intact proinsulin secretion, insulin resistance, and cardiovascular risk.

The following analysis of the data obtained from a large cross-section epidemiological study (IRIS-II study)27 was performed to investigate whether the previously reported high specificity of intact proinsulin as a predictive marker for insulin resistance can be confirmed in a large population of orally treated patients with type 2 diabetes and to explore the potential relation of elevated intact proinsulin levels with the prevalence of macrovascular disease.

PATIENTS AND METHODS

The study was performed in accordance with Good Clinical Practice and the Declaration of Helsinki, and approval was obtained from the responsible local ethical review boards. All patients gave their written informed consent after detailed information. The data and samples from patients with type 2 diabetes with oral medication or dietary treatment were collected from 149 sites that participated in the IRIS-II study. All patients completed a standardized questionnaire, and blood samples were drawn in the fasting state. Actual therapy and prevalence of microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (stroke, coronary and peripheral artery disease, myocardial infarction) disease were assessed by the questionnaire and collection of a detailed medical history. All laboratory data were measured in a central laboratory. Hemoglobin (HbA1c) was measured by high-performance liquid chromatography (Menarini, Neuss, Germany). Glucose was determined by the use of a glucose oxidase method (SuperGL, Ruhrtal Labortechnik, Möhnesee, Germany), and lipids by dry chemical detection (Olympus, Hamburg, Germany). Insulin and intact proinsulin were determined by means of specific chemiluminescence tests: MLT Insulin [intra- and inter-assay percent coefficient of variance (CV%) 3.8% and 2.3%, respectively; reference <30 μU/mL] and MLT Intact Proinsulin [intra- and inter-assay CV% 5.2% and 8.6%, respectively; normal reference range 0–10 pmol/L; linear assay range 0.5–100 pmol/L] (Sciema Diagnostics, Mainz, Germany) as described previously.28

Homeostasis model assessment (HOMA) score

HOMA score calculation was applied as a measure for insulin resistance analysis as published previously.29 The estimate of insulin resistance by HOMA score was calculated with the following formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. As described by Hedblad et al.,30 patients with HOMA score values exceeding the 75th percentile in their population (i.e., 2.0) were considered to have insulin resistance.

Statistical analysis

Statistical analysis was performed using descriptive statistics and appropriate parametri-
cal and non-parametrical tests. Student’s t test and Mann–Whitney’s U test were used to compare the means of the measured variables. All tests were carried out two-sided. Results with \( P \) values less than 0.05 were considered statistically significant. All calculations were made with the SPSS (version 9.0; SPSS Inc., Chicago, IL) Statistical Package. Because this investigation was a descriptive cross-sectional epidemiological study, all \( P \) values are to be interpreted in an exploratory sense.

**RESULTS**

In total, 2,146 male and 2,124 female patients with type 2 diabetes without insulin therapy participated in the study [mean ± SD age 63.9 ± 11.1 years; duration of disease 5.4 ± 5.6 years, body mass index (BMI) 30.1 ± 5.5 kg/m²; waist/hip ratio 1.0 ± 0.1; HbA1c 6.8 ± 1.3%]. No significant differences in demographic data could be observed between male and female patients. Diabetes was treated using oral anti-diabetes drugs in 2,068 patients, no therapy or diet alone in 1,452, and a combination of diet and drugs in 750 patients. In most of the patients (73.5%), hypertension was documented with a mean duration of 10.0 ± 9.0 years, and with a mean blood pressure of 144:84 mm Hg as compared with 128:77 mm Hg in the non-hypertensive patients. Of the patients 66.8% were on antihypertensive medication.

The mean intact proinsulin value was found to be 11.4 ± 12.4 pmol/L, which is above the normal reference range (<10 pmol/L). HOMA resulted in 1,147 insulin-sensitive patients (26.9%) and 3,123 patients (73.1%) with different degrees of insulin resistance. Of the resistant patients 1,465 (34.3% of all patients) were also insulin resistant on the basis of elevated intact proinsulin values, while 1,658 of these resistant patients (38.8% of all patients) had no intact proinsulin elevation. In contrast, 1,042 of the insulin-sensitive patients (24.4% of all patients) had normal intact proinsulin values, and only 105 (2.4% of all patients) had elevated intact proinsulin concentrations (\( q^2 \) test \( P < 0.0001 \)). Applying these results, a sensitivity of 46.9% and a specificity of 93.2% were calculated for elevated intact proinsulin values serving as a marker for insulin resistance. The clinical data, disease prevalence, and the laboratory results of the different patient groups are presented in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Insulin sensitive</th>
<th>Insulin resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>1,042</td>
<td>1,465</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>501/538</td>
<td>69/37</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65 ± 12</td>
<td>66 ± 12</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>5.5 ± 6.1</td>
<td>7.5 ± 7.4*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 ± 4.4</td>
<td>28.4 ± 4.2*</td>
</tr>
<tr>
<td>Macrovascular disease (%)</td>
<td>25.0%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12.1%</td>
<td>19.2%</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>12.2%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>5.6%</td>
<td>8.6%</td>
</tr>
<tr>
<td>HOMA Score (mU/mL²)</td>
<td>1.38 ± 0.39</td>
<td>1.64 ± 0.29*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3 ± 0.9</td>
<td>6.9 ± 1.2*</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>6.1 ± 2.0</td>
<td>7.6 ± 2.0*</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>85 ± 22</td>
<td>99 ± 25</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>145 ± 165</td>
<td>178 ± 88*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>125 ± 33</td>
<td>125 ± 28</td>
</tr>
</tbody>
</table>

\( P \) values are to be interpreted in an exploratory sense.

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**Table 1. Clinical and Laboratory Results of the Different Patient Groups**

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sults of the four different groups are given in Table 1. The majority of the patients with elevated intact proinsulin values were treated with sulfonylurea alone or in combination therapy (1,451 patients, 34% of all patients). The mean level of intact proinsulin in the sulfonylurea-treated patients was significantly higher than the proinsulin levels of patients without sulfonylurea treatment ($n = 3,219$) ($15.1 \pm 15.0$ pmol/L vs. $9.5 \pm 10.1$ pmol/L, $P < 0.001$), and they had an increased prevalence of cardiovascular complications as compared with the patients treated without drugs (odds ratio 1.45). The mean concentrations of intact proinsulin in the groups with the 10 most frequently observed therapeutic regimens in our study population are given in Figure 1 together with their odds ratio for prevalence of macrovascular disease.

DISCUSSION

By using a specific assay for intact proinsulin, it has recently been demonstrated that a deterioration of $\beta$-cell secretion indicated by increased fasting intact proinsulin serum concentrations is linked to the prevalence of insulin resistance as measured by ivGTT and Minimal Model analysis. It was concluded that increased intact proinsulin is an indirect but highly specific predictor of insulin resistance and might be used in daily practice as pathophysiological marker for selection of a differentiated anti-diabetes treatment. When the same patient group was analyzed by means of the HOMA insulin resistance score, a small percentage of patients with increased intact proinsulin levels was wrongly classified as insulin sensitive by HOMA insulin resistance. A comparable percentage of "sensitive" patients with increased intact proinsulin was also seen in the actual study population. It is speculated that this group may represent again the imprecision of the HOMA insulin resistance analysis in comparison with the ivGTT/Minimal Model analysis, and that these patients may also be regarded as insulin resistant. Insulin resistance describes a state where there is reduced bio-

![Figure 1](5678_06_p478-486_5/18/05_12:43_PM_Page_481.png)

FIG. 1. Intact proinsulin values and odds ratios for prevalence of macrovascular disease [OD (MVD)] in the most commonly prescribed treatment groups. Metf., metformin; SU, sulfonylurea; GI, $a$-glucosidase inhibitor.
logical effect of any given insulin concentration. Beside its effect on glucose metabolism, insulin resistance is a strong predictor for coronary vascular disease and atherosclerosis (see, e.g., Howard et al.,32,33 Bonora et al.,31 and Hanley et al.34). However, the increase in cardiovascular disease may not be entirely due to increases in glucose levels per se, because in many studies, including the UK Prospective Diabetes Study, the magnitude of association was only modest. Indeed, insulin resistance may be responsible for the increased cardiovascular risk even in the "prediabetic" state.30,33 Subcutaneous application of proinsulin, on the other hand, was associated with an increased risk for cardiovascular events33 in a phase II clinical study. The same risk may be introduced by increased secretion of endogenous proinsulin forced by insulin resistance. The β-cell secretion disorder resulting in increased proinsulin secretion followed by increased plasminogen activator inhibitor type-1 levels and an impaired fibrinolysis18 may at least partially contribute to the observed correlation between insulin resistance and cardiovascular disease. It has to be pointed out, however, that the assessment of cardiovascular disease was only performed by patient history and a patient questionnaire, which is a weak methodological approach.

In a prospective Japanese comparator study, effective treatment of insulin resistance with pioglitazone led to a decrease in proinsulin levels, while they remained unchanged when sulfonylurea drugs were applied although the same improvement in HbA1c was achieved.34 Another prospective parallel study comparing pioglitazone with glimepiride treatment revealed also a comparable improvement in glucose control for both drugs. However, peroxisome proliferator-activated receptor-γ activation led to an additional overall improvement of insulin resistance and the metabolic situation (decrease in insulin, intact proinsulin, increase in adiponectin), as well as in a significant improvement of several macrovascular risk factors (including a decrease in intima-media thickness and serum high-sensitivity C-reactive protein).35,36 It may therefore, next to improvement of long-term glucose control, be of importance to diagnose the secretion state of the β-cell, and to improve insulin resistance and β-cell dysfunction by selection of appropriate therapeutic measures as discussed below. This may result in an additional improvement of the patient’s macrovascular risk profile. Based on our findings and on the information that can be found in the literature, intact proinsulin measured by a non–cross-reactive assay can serve as a marker to investigate β-cell function, and allows for a secretion-oriented pathophysiological staging of type 2 diabetes as we recently proposed.18 In our actual study population, 1,042 patients were in proposed stage I, 1,763 patients were in stage II, and already 1,465 patients were in stages IIIa and IIIb (Fig. 2). It is remarkable that known duration of disease was not correlated with these different stages of type 2 diabetes, and no other demographic parameter could be identified as another means of differentiation. However, when the two groups with elevated proinsulin were combined, insulin resistance also correlated with BMI, and the prevalence of macrovascular disease was slightly higher. It is not uncommon that type 2 diabetes is diagnosed at later stages, and many patients suffer already from secondary complications when the disease is recognized for the first time.37

One weakness is that our data were obtained from a cross-sectional and not a prospective study. However, the majority of patients taking secretion-stimulating drugs (sulfonylurea and glinides) showed elevated intact proinsulin levels, while no or only minor elevation of this marker was seen in patients treated with drugs known to have insulin-sensitizing effects (metformin, thiazolidinediones). It also has been shown that effective treatment of insulin resistance can reduce the endogenous insulin secretion needs and may result in a restoration of appropriate insulin processing in the β-cell.34-36 It is possible that the contradictory data about the potential negative impact of sulfonylurea drugs on cardiovascular complications in type 2 patients that had been published over the past years39 can simply be explained by differences in the investigated populations with regard to their β-cell secretion stage. Further prospective studies will, however, be required to evaluate whether elevation in intact proinsulin concentration is a harmless epiphe-
nomenon of sulfonylurea treatment or contributes to an increased cardiovascular risk.

In our study population, patients with increased intact proinsulin levels also presented with an increased prevalence of macrovascular complications and lower high-density lipoprotein values. The breakdown into different therapeutic groups led to fairly small groups, but the relation remained highly significant and consistent. Therapeutic combinations with known positive effects on insulin resistance were correlated with lower intact proinsulin levels and lower prevalence of vascular complications. It has to be indicated that because of this cross-sectional study design no conclusion can be drawn about a real causal relationship among insulinotropic treatment, insulin resistance, and macrovascular risk. However, early measures of resistance treatment like diet, exercise, and metformin monotherapy have been reported to decrease insulin resistance, cardiovascular risk, and also proinsulin levels in patients with type 2 diabetes. Even more impressive results have been observed in clinical studies using troglitazone, pioglitazone, and rosiglitazone. Blood proinsulin levels showed no change or were increasing in patients treated with sulfonylurea.

Based on the results of the study presented here and the available past and most recent published literature reporting about interventional study results with different drugs on β-cell secretion, insulin resistance, and cardiovascular risk, the following pathophysiological treatment recommendations for the different treatment approaches can be provided under consideration of fasting intact proinsulin secretion:

1. If fasting intact proinsulin is below the upper reference limit (<10 with the MLT chemiluminescence assay or <11 pmol/L with the Linco intact proinsulin enzyme-linked immunosorbent assay, stages I and II in Fig. 2), the diabetes treatment can be performed with all available therapeutic means (lifestyle, diet, sulfonylurea, metformin, sensitizers, and insulin).

2. If fasting intact proinsulin is above the normal reference range (stages IIIa and IIIb in Fig. 2), intervention with sulfonylurea drugs may not be beneficial, and treatment should focus on means of β-cell protection and reduction of insulin resistance (e.g., exercise, thiazolidinediones, insulin).
In the case of sulfonylurea and/or sole dietetic intervention, it is advisable to monitor intact proinsulin levels on a regular basis (e.g., once every 6 months) to identify the time point when a more intensive β-cell protective treatment approach may be feasible to prevent further deterioration of β-cell dysfunction and macrovascular impairment.

In conclusion, it has been confirmed by this study that the vast majority of Type 2 patients with elevated fasting intact proinsulin values can be regarded as being insulin resistant. The intact proinsulin assay may be a valuable addition to routine diagnostic procedures as a specific marker for insulin resistance and to monitor treatment approaches. Although performed in a large patient group, this study certainly has the limitation of the cross-sectional approach. However, prospective studies that have been initiated to explore the potential effects of different oral anti-diabetes drugs on β-cell function and cardiovascular risk in patients with type 2 diabetes support the findings reported here.

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Address reprint requests to:
Prof. Dr. Andreas Pfützner
Institute for Clinical Research and Development
IKEF GmbH
Parcusstrasse 8
D-55116 Mainz, Germany
E-mail: AndreasP@ikfe.de
This article has been cited by:


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