CLINICAL STUDY

Hyperproinsulinemia is not a characteristic feature in the offspring of patients with different phenotypes of type II diabetes

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Abstract

Objective: The purpose of this work was to study whether there are differences in plasma proinsulin levels and proinsulin-to-specific insulin ratio in the offspring of patients with different phenotypes of type II diabetes.

Design: Eleven glucose-tolerant offspring of type II diabetic patients with deficient insulin secretion phenotype (IS group), nine glucose-tolerant offspring of patients with insulin-resistant phenotype (IR group), and fourteen healthy control subjects without a family history of diabetes were studied.

Methods: Plasma specific insulin, plasma proinsulin, and plasma C-peptide levels were measured during a 2-h oral glucose tolerance test and during hyperglycemic clamp.

Results: Plasma proinsulin levels during the oral glucose tolerance test and the hyperglycemic clamp did not differ among the study groups. The IR group had a lower fasting plasma proinsulin-to-specific insulin ratio (10.3 ± 1.7%) than the control group (15.4 ± 1.4%; P < 0.05) and the IS group (18.6 ± 2.7%; P < 0.05). Furthermore, the IR group had lower plasma proinsulin-to-specific insulin ratio at 30, 60 and 90 min after the oral glucose load than the IS group. However, there were no significant differences in proinsulin-to-C-peptide ratio during the oral glucose tolerance test among the study groups. In stepwise multiple regression analysis, hepatic specific insulin extraction in the fasting state (β = 0.65; P < 0.001) and fasting blood glucose (β = 0.32; P < 0.05) together explained 52% of the variation in fasting plasma proinsulin-to-specific insulin ratio.

Conclusions: Hyperproinsulinemia is not a characteristic finding in glucose-tolerant offspring of type II diabetic probands with deficient insulin secretion or insulin-resistant phenotype. The differences in proinsulin-to-specific insulin ratios were most likely explained by different hepatic extraction among the study groups.

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Introduction

Hyperproinsulinemia is commonly present in type II diabetes (1–10) and according to most studies also in subjects with impaired glucose tolerance (7–12). The reasons for hyperproinsulinemia are, however, still unknown but several explanations have been proposed (13). Hyperproinsulinemia could be due to a primary defect in the β cell, leading to increased secretion of incompletely processed insulin precursors, i.e. impaired proinsulin processing. Indeed, in some individuals, hyperproinsulinemia has been shown to be present without hyperglycemia (14–17), suggesting that an impairment in proinsulin processing could represent an early defect in the development of type II diabetes.

Alternatively, other studies have suggested that hyperproinsulinemia is a secondary defect in subjects developing type II diabetes, and could be due to increased demand placed on the β cell by hyperglycemia and/or insulin resistance (7–8, 18). Because insulin-resistant individuals often have hyperinsulinemia, it is not clear whether hyperproinsulinemia per se is a marker of β cell distress. Therefore, disproportionally increased serum concentrations of proinsulin relative to insulin may be a surrogate marker of the failing pancreas (4), a phenomenon which has been reported in subjects with impaired glucose tolerance (8, 11, 19). However, the response of the normal β cell to increased demand has been hypothesized to result in enhanced processing of proinsulin into insulin (4). Insulin resistance has,
Indeed, been associated with low proinsulin-to-insulin ratio in normoglycemic subjects (20), whereas the study by Wang et al. (21) could not find this association. However, no previous study has taken into account the variations in hepatic extraction of insulin.

Previous studies aiming to investigate the role of hyperproinsulinemia as an early defect in type II diabetes have included individuals at high risk of developing type II diabetes, i.e. the first-degree relatives of patients with type II diabetes (9, 14, 16, 22). However, these studies have given contradictory results. Three studies have shown that the first-degree relatives of type II diabetic patients have elevated proinsulin levels (14, 16, 22), whereas in one study parental diabetes was related neither to fasting proinsulin levels nor to proinsulin-to-insulin ratio (9). As type II diabetes is a heterogeneous disorder (23), the contradictory results in previous studies may be due to different phenotypes of type II diabetes in the probands. We have recently shown that defects in insulin secretion and insulin action are inherited, and either of them could represent primary defects in the development of type II diabetes (24).

The aim of this study was to examine whether there are differences in proinsulin levels or in proinsulin-to-specific insulin ratio between the glucose-tolerant offspring of patients with deficient insulin secretion phenotype and insulin-resistant phenotype of type II diabetes and, furthermore, whether hyperproinsulinemia reflects an early defect in the pathogenesis of type II diabetes.

Subjects and methods

Subjects

The subjects for the present study were offspring of the patients with newly diagnosed type II diabetes who were originally studied in 1979–81 (25–28). We have followed these patients for over 10 years and performed repeated oral glucose tolerance tests (OGTT) (baseline, 5 years and 10 years). The probands were subdivided into two groups on the basis of fasting C-peptide values at the 10-year follow-up study: (i) type II diabetic patients with low fasting C-peptide level (<450 pmol/l) reflecting deficient insulin secretion capacity and (ii) type II diabetic patients with high fasting C-peptide level (>880 pmol/l) reflecting insulin resistance. Proband with glutamic acid decarboxylase and/or islet cell antibody positivity (eleven patients altogether) were excluded. Additional exclusion criteria for the selection of the offspring were: (i) diabetes mellitus in both parents or in the offspring; (ii) dyslipidemia (serum total triglycerides >2.5 mmol/l); (iii) drug treatment or any disease that could potentially disturb carbohydrate metabolism; (iv) pregnancy; (v) overt psychiatric disease; and (vi) under 30 or over 55 years of age. The formation of the study population for the present study has been described in more detail elsewhere (24). Briefly, three groups of subjects were studied.

Offspring of the probands with deficient insulin secretion phenotype (IS group) Originally, the IS group consisted of twenty subjects (fifteen women and five men) (24). The nine subjects with impaired glucose tolerance were excluded. Thus the IS group for the present study comprised eleven glucose-tolerant subjects who were offspring of eight probands.

Offspring of the probands with insulin-resistant phenotype (IR group) Originally, the IR group consisted of eighteen subjects (eleven women and seven men) (24). Likewise, the subjects with impaired glucose tolerance (nine subjects) were excluded. Thus the glucose-tolerant subjects of the IR group (nine subjects) were included in the present study and they were offspring of six probands.

Control group

The control subjects for the present study were offspring of control subjects with repeatedly normal glucose tolerance according to the World Health Organization criteria (29) determined by an OGTT (baseline, 5 years and 10 years) in the same follow-up study (25–28). The control subjects had to fulfill the following inclusion criteria: (i) age from 30 to 55 years; (ii) no diabetes; (iii) first-degree relatives without a history of diabetes; (iv) no drug treatment nor any disease that could potentially disturb carbohydrate metabolism; (v) body mass index (BMI) within the range of means ± 2 S.D. of the BMI in the IS and IR groups; and (vi) no history of hypertension. The control group consisted of fourteen glucose-tolerant offspring (five men and nine women) of eight probands.

Characteristics of the probands at the 10-year follow-up study The probands with deficient insulin secretion phenotype had mean age of 67.4 years, mean BMI of 26.7 kg/m<sup>2</sup>, mean fasting C-peptide of 335 pmol/l and mean fasting blood glucose of 13.6 mmol/l. One proband was treated with diet alone, four probands with oral hypoglycemic agents and three probands with insulin. The mean age of the probands with insulin-resistant phenotype was 66.7 years, their mean BMI was 34.7 kg/m<sup>2</sup>, their mean fasting plasma C-peptide was 951 pmol/l and their mean fasting blood glucose was 11.7 mmol/l. Three probands were treated with diet alone, three probands with oral hypoglycemic agents and none was receiving insulin treatment. The probands with the insulin-resistant phenotype had higher fasting C-peptide concentration even after correction for age, BMI and fasting blood glucose concentration. The probands of the control subjects had a mean age of 64.7 years, mean BMI of 26.0 kg/m<sup>2</sup>, mean fasting plasma C-peptide of 480 pmol/l and mean blood glucose of 5.1 mmol/l.
Study protocol
The subjects were admitted to the metabolic ward of the Department of Medicine of Kuopio University Hospital for 2 days. On the first day after 12-h fasting a 2-h OGTT was performed followed by the hyperglycemic clamp. On the second day the hyperinsulinemic euglycemic clamp was performed.

The protocol was approved by the Ethics Committee of the University of Kuopio. Every subject gave informed consent.

OGTT
In a 2-h OGTT (75 g glucose), samples for blood glucose, plasma specific insulin, plasma proinsulin and plasma C-peptide were drawn at 0, 30, 60, 90 and 120 min to evaluate the degree of glucose tolerance and the β cell response to an oral glucose load.

Hyperglycemic clamp
At 120 min immediately after the 2-h OGTT, blood glucose was acutely increased to 20 mmol/l by a constant 20% glucose infusion and clamped at 20 mmol/l until 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals applying the hyperglycemic clamp technique (24, 30). At 150, 165 and 180 min, samples were taken for the measurement of plasma C-peptide. In addition, at 180 min, samples for the determination of plasma specific insulin and plasma proinsulin were taken.

Euglycemic clamp
The degree of insulin resistance was evaluated with the euglycemic hyperinsulinemic clamp technique (30). A priming dose of insulin infusion (100 IU/ml; Actrapid; Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to acutely raise plasma insulin to the desired level, where it was maintained by a continuous insulin infusion of a rate of 80 mU/m² body surface area per min. Blood glucose was clamped at 5.0 mmol/l for the next 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals (mean coefficient of variation of blood glucose was <4% in both study groups and control group). The data were calculated for each 20-min interval; the mean value for the period of 120 to 180 min was used to calculate the rates of whole body glucose uptake (WBGU).

Assays and calculations
Levels of blood glucose in the fasting state, and blood glucose during the OGTT and clamp studies were measured by the glucose oxidase method (Glucose & Lactate Analyzer 2300 Stat Plus; Yellow Springs Instrument Co., Inc, OH, USA). For the determination of plasma specific insulin, plasma proinsulin and plasma C-peptide, blood was collected into EDTA tubes. After centrifugation, plasma for the determination of C-peptide was stored at −20°C until analysis. Plasma C-peptide was determined with a 125I radioimmunoassay (RIA) kit (INCSTAR Corp., Stillwater, MN, USA). Serum specific insulin was measured with a specific double-antibody RIA (human insulin-specific RIA kit, Sigma Chemical Co., St Louis, MO, USA) that has <0.2% cross-reactivity with proinsulin (31). Specificity for true insulin is achieved by the use of an insulin antibody that reacts with the free NH₂-terminal of the A-chain of insulin. Intact human proinsulin and des 31,32 human proinsulin are not reactive in this assay because the required epitope is blocked by the lysine/arginine dibasic linkage connecting insulin with C-peptide. Cross-reactivity with intact and des 31,32 proinsulin was determined to be <0.2%. Within- and between-assay coefficients of variation of the specific insulin assay ranged from 3 to 7%. Proinsulin concentrations were measured by a non-equilibrium RIA method (human proinsulin RIA kit; Linco, St Louis, MO, USA) (32). The polyclonal antibody used in this assay recognizes a proinsulin specific epitope formed by the intact A-chain–C-peptide junction. In this assay, the potency of human insulin and C-peptide is <0.1% that of proinsulin. Cross-reactivity with des 31,32 proinsulin is 95%. Because des 31,32 is the major circulating form of split proinsulin (~95%) the proinsulin RIA method reported here provides an estimate for total insulin precursor (intact + B–C-junctional cleaved forms) in plasma. The intra-assay coefficient of variation ranged from 6 to 21% using controls prepared at 5, 50 and 250 pmol/l (14). Plasma C-peptide samples were assayed in Kuopio, Finland and plasma specific insulin and plasma proinsulin were determined in San Antonio, TX, USA. The incremental glucose, specific insulin and proinsulin areas under the curve were calculated by the trapezoidal method. The acute insulin response (AIRₚₒₚ) was calculated as a ratio of the increment of plasma specific insulin (pmol/l) to that of blood glucose (mmol/l) at 30 min after the oral glucose load. Plasma C-peptide-to-plasma specific insulin ratio was calculated as an index of the hepatic extraction of specific insulin in the fasting state and during the hyperglycemic clamp. Metabolic clearance rate (MCR) of insulin was calculated from the formula: MCR = (infusion rate of exogenous insulin during the euglycemic clamp)/(mean plasma insulin concentration during the period of 120–180 min in the euglycemic clamp), expressed as μl/min per kg body weight.

Statistical analysis
All calculations were performed with the SPSS for Windows program (SPSS Inc., Chicago, IL, USA). Data
are shown as means ± S.E.M. The differences among the three groups were tested by one-way analysis of variance (ANOVA) for continuous variables and by Mantel–Haenszel’s test for dichotomized variables. Only if the P value was < 0.05 were the two groups compared with the Mann–Whitney non-parametric test for unpaired samples or with the χ²-test when appropriate. Determinants of plasma proinsulin levels and proinsulin-to-specific insulin ratio were evaluated by multiple linear regression analysis.

Results

Clinical and biochemical characteristics of the study groups

Table 1 shows the clinical and biochemical characteristics of the study groups. The groups were comparable with respect to age and gender. The offspring with the insulin-resistant phenotype (IR group) had a higher BMI and lower rates of WBGU than the control group (P < 0.05 and P < 0.05 respectively) or the offspring with the deficient insulin secretion phenotype (IS group) (P < 0.01 and P < 0.05 respectively). The IR group tended to have a lower MCR of insulin as compared with that of the IS group (P < 0.05; ANOVA: P = 0.16). Fasting blood glucose levels did not differ among the study groups. The IR group had higher fasting plasma specific insulin levels as compared with those of the control group (P < 0.05) or the IS group (P < 0.05). Fasting plasma proinsulin and C-peptide levels did not differ among the study groups. The IR group had a lower fasting plasma proinsulin-to-fasting plasma specific insulin ratio as compared with that of the control group (P < 0.05) or the IS group (P < 0.05), and it persisted after adjustment for BMI or WBGU. However, no difference was found in fasting plasma proinsulin-to-plasma C-peptide ratio among the study groups. Furthermore, the hepatic extraction of specific insulin in the fasting state tended to be lower in the IR group than in the control group (P = 0.07) or in the IS group (P = 0.08). Interestingly, the differences in fasting plasma proinsulin-to-fasting plasma specific insulin ratios between the study groups disappeared after the adjustment for hepatic extraction of specific insulin.

OGTT

Figure 1 demonstrates the blood glucose and plasma specific insulin response during the OGTT. There were no statistically significant differences in blood glucose levels at any time-points measured after the oral glucose load. However, the blood glucose response expressed as an incremental glucose area under the curve (AUCgsuc) tended to be higher in the IS group and IR group than in the control group (P = 0.058 and P = 0.18 respectively; ANOVA: P = 0.09). The IR group had higher plasma specific insulin levels at 60, 90 and 120 min after an oral glucose load than the control group (P < 0.05, P < 0.05, P < 0.01 respectively). In addition, the IR group tended to have higher plasma specific insulin levels at 30, 60 and 90 min than the IS group (P = 0.15, P = 0.11 and P = 0.08 respectively). The plasma specific insulin response expressed as an incremental specific insulin area under the curve was higher in the IR group (705.1 ± 114.3 pmol/l×h) than in the control group (411.3 ± 61.0 pmol/l×h; P < 0.05) and in the IS group (454.1 ± 101.2 pmol/l×h; P < 0.05) even after adjustment for AUCgsuc (ANOVA: P = 0.052). The IS group had lower specific AIRgl at 30 min during the OGTT (88.3 ± 14.9 pmol specific insulin/mmol glucose) than the control group or the IR group (159.4 ± 25.6 pmol specific insulin/mmol glucose; P < 0.05 and 159.8 ± 27.8 pmol specific insulin/mmol glucose; P < 0.05 respectively; ANOVA: P = 0.060).

Table 1 Characteristics of the study subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 14)</th>
<th>IS group (n = 11)</th>
<th>IR group (n = 9)</th>
<th>P value (ANOVA/χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.1 ± 1.5</td>
<td>41.9 ± 1.7</td>
<td>41.7 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/9</td>
<td>4/7</td>
<td>4/5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 1.0</td>
<td>24.5 ± 0.7</td>
<td>28.8 ± 1.1†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WBGU (µmol/kg per min)</td>
<td>62.2 ± 4.4</td>
<td>56.6 ± 2.4</td>
<td>45.5 ± 3.7†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCR (µl/kg per min)</td>
<td>88.0 ± 7.0</td>
<td>88.6 ± 3.1</td>
<td>72.9 ± 4.9</td>
<td>= 0.16</td>
</tr>
<tr>
<td>Fasting state</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma specific insulin (pmol/l)</td>
<td>43.1 ± 4.3</td>
<td>46.8 ± 6.6</td>
<td>69.6 ± 10.8†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma proinsulin (pmol/l)</td>
<td>6.18 ± 0.53</td>
<td>7.67 ± 1.06</td>
<td>7.07 ± 1.40</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma C-peptide (pmol/l)</td>
<td>470 ± 30</td>
<td>514 ± 52</td>
<td>602 ± 73</td>
<td>NS</td>
</tr>
<tr>
<td>Proinsulin-to-specific insulin ratio (%)</td>
<td>15.4 ± 1.4</td>
<td>18.6 ± 2.7</td>
<td>10.3 ± 1.7†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Proinsulin-to-C-peptide ratio (%)</td>
<td>1.31 ± 0.11</td>
<td>1.49 ± 0.13</td>
<td>1.20 ± 0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Hepatic extraction of specific insulin</td>
<td>11.75 ± 1.02</td>
<td>12.77 ± 1.92</td>
<td>9.06 ± 0.48</td>
<td>= 0.15</td>
</tr>
</tbody>
</table>

* P < 0.05 vs controls; † P < 0.01 vs IS group; † P < 0.05 vs IS group; NS, not significant.

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Figure 2 shows the plasma C-peptide and plasma proinsulin response during the OGTT. There were no differences in plasma proinsulin levels among the study groups. The plasma proinsulin response expressed as an incremental proinsulin area under the curve tended to be higher in the IS group (50.9 ± 8.5 pmol/l×h) and the IR group (49.4 ± 4.9 pmol/l×h) as compared with the control group (34.5 ± 5.9 pmol/l×h; P = 0.06 and P < 0.05 respectively; ANOVA: P = 0.15). These differences, however, disappeared after adjustment for AUCgluc. There were no differences in plasma C-peptide levels among the study groups. The incremental plasma C-peptide area under the curve (AUCC-peptide), however, tended to be higher in the IR group (2974 ± 145 pmol/l×h) than in the control group (2237 ± 182 pmol/l×h, P < 0.05; ANOVA: P = 0.15) but this difference disappeared after the adjustment for AUCgluc. The AUCC-peptide for the IS group was 2895 ± 461 pmol/l×h.

Figure 3 shows the plasma proinsulin-to-plasma specific insulin ratio and plasma proinsulin-to-plasma C-peptide ratio during the OGTT. The IR group had a lower plasma proinsulin-to-plasma specific insulin ratio in the fasting state (P < 0.05) than the control group. Furthermore, the IR group had a lower plasma proinsulin-to-plasma specific insulin ratio in the fasting state, and at 30 min, 60 min and 90 min than the IS group (P < 0.05 in the fasting state, and at 30 min and 90 min; P < 0.01 at 60 min). In addition, the IS group had higher plasma proinsulin-to-plasma specific insulin ratio at 30 min and 90 min as compared with that in the control group (P < 0.05 for both comparisons). There were, however, no differences in plasma proinsulin-to-plasma C-peptide ratio during the OGTT among the study groups (Fig. 3, lower part).

Hyperglycemic clamp study

Table 2 shows the plasma specific insulin, plasma proinsulin and plasma C-peptide levels during the hyperglycemic clamp. The IR group had higher plasma specific insulin levels than the IS group (P < 0.05). The groups were comparable with respect to plasma proinsulin and C-peptide levels and, further, to the ratios of plasma proinsulin-to-specific insulin and proinsulin-to-plasma C-peptide. The hepatic extraction of specific insulin did not differ among the study groups.

Multiple linear regression analyses

In the stepwise multiple linear regression analysis, hepatic specific insulin extraction in the fasting state was the strongest determinant of the variation of fasting plasma proinsulin-to-specific insulin ratio (r² = 0.42, P < 0.001) (Table 3). Furthermore, fasting blood glucose was the second strongest determinant of the variation of fasting plasma proinsulin-to-specific insulin ratio, explaining, together with hepatic specific insulin extraction, 52% of the variation of fasting plasma proinsulin-to-specific insulin ratio (P < 0.05). On the other hand, fasting blood glucose and WBGU explained 35% of the variation of fasting plasma proinsulin levels (P < 0.05) (Table 3).

Discussion

The novel finding of this study was that the glucose-tolerant offspring of type II diabetic patients with deficient insulin secretion or insulin-resistant phenotype had normal plasma proinsulin levels in the fasting state, after an oral glucose load and during the
hyperglycemic clamp. Moreover, the offspring of probands with insulin-resistant phenotype had low fasting proinsulin-to-specific insulin ratio as has been shown previously (20), but our results suggest that this may be due to reduced hepatic extraction of specific insulin in the fasting state. Likewise, the differences in plasma proinsulin-to-specific insulin ratios between the study groups after the glucose load were likely due to different hepatic extraction of specific insulin.

The role of hyperproinsulinemia in disturbances of glucose metabolism has remained unclear. Increased secretion of incompletely processed insulin precursors, i.e. impaired processing of proinsulin, could be due to a primary, inherent abnormality of the β cell machinery itself or could be secondary to external factors acting on the β cell or a combination of these two (13). Several possibilities of inherent β cell defect(s) leading to hyperproinsulinemia have been proposed, including, for example, various alternative defects in endopeptidase (PC2 and/or PC3) function (33) and/or extracellular accumulation of islet amyloid polypeptide in β cells (4). Similar defects have, however, been proposed to develop secondarily as a consequence of increased secretory demand of the β cell, especially due to hyperglycemia (4, 33). Although increased proinsulin levels are a recognized feature of type II diabetes, it is unclear at which point this dysfunction develops. Some studies with glucose-tolerant individuals at high risk of developing type II diabetes, i.e. first-degree relatives and individuals with a history of gestational diabetes, have shown that impaired processing of proinsulin could be an early defect in the pathogenesis of type II diabetes.
(14–17). On the other hand, other studies including first-degree relatives of type II diabetic patients have shown that hyperproinsulinemia is not present before the development of clinical hyperglycemia (9, 22), suggesting that hyperproinsulinemia is a secondary phenomenon. As type II diabetes is a heterogeneous disorder (23), the controversy among the previous studies can be explained by a lack of classification of

![Graph](image)

**Figure 3** Plasma proinsulin-to-plasma specific insulin and plasma proinsulin-to-plasma C-peptide ratio during the OGTT in the control group (○), the IS group (△) and the IR group (■).

$^* P < 0.05$ (IR group vs control group); $^§ P < 0.05$ (IS group vs control group); $^\ddagger P < 0.05$ and $^\ddagger\ddagger P < 0.01$ (IR group vs IS group).

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n = 14$)</th>
<th>IS group ($n = 11$)</th>
<th>IR group ($n = 9$)</th>
<th>$P$ value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma specific insulin</td>
<td>1023 ± 155</td>
<td>779 ± 124</td>
<td>1434 ± 232$\ddagger$</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma proinsulin (pmol/l)</td>
<td>64.1 ± 4.7</td>
<td>65.1 ± 4.9</td>
<td>74.3 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma C-peptide (pmol/l)</td>
<td>3823 ± 270</td>
<td>4243 ± 520</td>
<td>4895 ± 547</td>
<td>NS</td>
</tr>
<tr>
<td>Proinsulin-to-specific insulin ratio (%)</td>
<td>8.0 ± 1.2</td>
<td>9.8 ± 1.2</td>
<td>7.7 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Proinsulin-to-C-peptide ratio (%)</td>
<td>1.69 ± 0.07</td>
<td>1.62 ± 0.08</td>
<td>1.59 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Hepatic extraction of specific insulin</td>
<td>4.75 ± 0.76</td>
<td>5.98 ± 0.52</td>
<td>4.74 ± 1.27</td>
<td>NS</td>
</tr>
</tbody>
</table>

$\ddagger P < 0.01$ vs IS group; NS, not significant.

Table 2 Plasma-specific insulin, proinsulin and C-peptide levels in the hyperglycemic clamp. The results are given as means ± S.E.M. of $n$ (%).
Since C-peptide accurately reflects the especially in hepatic insulin extraction, in many differ-
ent physiological (37) and pathological conditions (38). Since C-peptide accurately reflects the β cell secretory capacity (39) and, similarly to proinsulin, the hepatic extraction of C-peptide is negligible (40, 41), the ratio of proinsulin-to-C-peptide may be considered as a better indicator of distressed β cells than is the ratio of proinsulin-to-insulin. The offspring of probands with insulin-resistant phenotype had, indeed, normal proinsulin-to-C-peptide ratio in the fasting state and after an oral glucose load. Moreover, they had low fasting C-peptide-to-fasting specific insulin molar ratios, an index of hepatic extraction of specific insulin (42) and, in the linear multiple regression analysis, the hepatic specific insulin extraction was the strongest determinant of the variation of proinsulin-to-specific insulin ratio. Consequently, it is likely that low proinsulin-to-specific insulin ratio in the fasting state in this group was due to impaired hepatic extraction of specific insulin. Furthermore, under hyperglycemic conditions during the hyperglycemic clamp the off-
spring of probands with the insulin-resistant pheno-
type had normal proinsulin-to-specific insulin ratios. These results indicate that de facto there are no apparent defects in the processing of proinsulin in the offspring of type II diabetic patients with insulin-resistant phenotype.

Dysproportionally increased proinsulin relative to insulin has been suggested to be an early indicator of failing β cells (4). The response of the normal β cell to increased demand, however, has been hypothesized to be enhanced processing of proinsulin into insulin (4). In normoglycemic subjects, increased β cell secretory demand in connection with insulin resistance has been associated with a low proinsulin-to-insulin ratio, which has been interpreted to be due to enhanced processing of proinsulin in these subjects (20). However, patients with type II diabetes have had markedly increased proinsulin levels relative to insulin in nearly all studies (5, 6, 9), and this has been suggested to be due to impaired β cell function and/or increased demand caused by insulin resistance and/or hyperglycemia (34). Interestingly, in the present study, the offspring of probands with insulin-resistant phenotype had low fasting proinsulin-to-specific insulin ratios. As proinsul-
in and insulin have different MCRs (35, 36), the ratio of circulating proinsulin-to-insulin is controlled by the rates of secretion and clearance of both proinsulin and insulin. Furthermore, there is considerable intra- and inter-individual variation in the clearance of insulin, especially in hepatic insulin extraction, in many differ-

Table 3 Variables associated with plasma proinsulin-to-specific insulin ratio in the fasting state and fasting plasma proinsulin levels in stepwise multiple regression analyses.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>RCa</th>
<th>Betaa</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma proinsulin-to-specific insulin ratio</td>
<td>1.02 ± 0.21</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatic specific insulin extraction in the fasting state</td>
<td>7.08 ± 2.79</td>
<td>0.32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.519</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma proinsulin</td>
<td>4.51 ± 1.55</td>
<td>0.46</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>−0.46 ± 0.18</td>
<td>−0.38</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WBGU</td>
<td>0.350</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Variables included in the model: age, gender, the rates of WBGU, fasting blood glucose, fasting plasma proinsulin-to-
specific insulin ratio, hepatic specific insulin extraction in the fasting state, MCR and the study group (control/IS/IR).

b Variables included in the model: age, gender, the rates of WBGU, fasting blood glucose, fasting plasma proinsulin, hepatic specific insulin extraction in the fasting state, MCR and the study group (control/IS/IR).

Values are the regression coefficients (RC) ± S.E. for the linear model of parameters.

Values are the standardized regression coefficients (β) for the linear model of parameters.
offspring of diabetic probands with deficient insulin secretion phenotype. The glucose-tolerant offspring of probands with deficient insulin secretion phenotype in the present study had, however, impaired specific insulin secretion at 30 min after the glucose load, in accordance with our earlier report (24) based on immunoreactive insulin levels. Interestingly, however, they did not have reduced β cell mass based on normal maximal C-peptide secretory capacity and, furthermore, had normal proinsulin levels and proinsulin-to-specific insulin ratio in the hyperglycemic clamp. Consequently, the primary defect in insulin secretion in this group could lie in defective sensing of ambient post-load glucose levels or alternatively in incapacity to secrete the required amounts of insulin in response to meals.

In the present study, the classification of different phenotypes of type II diabetes, i.e. deficient insulin secretion and insulin-resistant phenotype, was based on fasting C-peptide values of the diabetic probands measured at the 10-year follow-up study of well-characterized type II diabetic patients (25–28). This classification may be quite robust for the phenotyping of diabetic patients, but we have recently shown that fasting C-peptide level in the type II diabetic probands is related to early defects in glucose metabolism in their non-diabetic offspring (24).

In conclusion, hyperproinsulinemia is not a fundamental feature in glucose-tolerant offspring of type II diabetic probands with deficient insulin secretion or insulin-resistant phenotype. The difference in proinsulin-to-specific insulin ratio between the study groups is most likely to be due to different hepatic extraction of specific insulin rather than to differences in the processing of proinsulin.

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