Beneficial metabolic effects of insulin-like growth factor I in patients with severe insulin-resistant diabetes type A

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Severe insulin resistance type A is due to mutations in the insulin receptor gene and is characterized by glucose intolerance or diabetes mellitus, despite extreme hyperinsulinemia, virilization and acanthosis nigricans. At present, there is no therapy for this condition. Recently, we showed that glucose levels in three such patients are promptly lowered by an iv bolus of recombinant human insulin-like growth factor I (rhIGF-I). In the present study, we investigated two of these rare patients again and determined fasting and postprandial glucose, insulin, C-peptide, proinsulin and lipid levels during five control, five treatment and three wash-out days while on a constant diet. Treatment consisted of 2 × 150 µg rhIGF-I/kg sc per day, which elevated total IGF-I levels 4.5-fold above the control. Fasting glucose levels (days 1–5) in the two patients were 9.6 ± 1.3 and 9.2 ± 1.2 mmol/l, respectively, and fell to 4.4 ± 0.4 and 5.1 ± 0.5 mmol/l on treatment days 8–10. Fasting insulin (2950 ± 450 and 690 ± 125 pmol/l), C-peptide (2217 ± 183 and 1317 ± 235 pmol/l) and proinsulin control levels (125 ± 35 and 66 ± 0 pmol/l) also decreased by ~65% during rhIGF-I treatment, as did the respective postprandial levels. Lipid levels hardly changed at all. In conclusion, IGF-I appears to correct partially some metabolic sequelae of severe insulin resistance and may, hence, be used as a new therapeutic agent.

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Severe insulin resistance type A has been described first by Kahn et al. in women with glucose intolerance or frank diabetes, despite extreme hyperinsulinemia, virilization and acanthosis nigricans (1). Several mutations of the insulin receptor gene have been detected in patients with insulin resistance type A (2). Insulin-like growth factor I (IGF-I) interacts with the type I IGF receptor and, with lesser affinity, with insulin receptors (3). An iv bolus of recombinant human (rh)IGF-I in healthy subjects leads to a marked transitory increase of free IGF-I levels followed by hypoglycemia (4, 5). The hypoglycemic effects of IGF-I are mediated via the insulin and the type I IGF receptor. Maximal effects of IGF-I on glucose uptake are similar or even greater than those of insulin in healthy and moderately insulin-resistant diabetic animals (6, 7). Thus, the effect of an iv bolus of rhIGF-I was investigated in patients with severe insulin resistance type A (8) and in a patient with Mendenhall’s syndrome, also characterized by severe insulin resistance (9). Intravenous rhIGF-I lowered plasma glucose and decreased insulin levels in these patients but the fall of glucose was slow (8, 9) compared to the rapid hypoglycemic effect of rhIGF-I in healthy subjects. It must, therefore, be assumed that in patients with insulin receptor defects rhIGF-I acts exclusively via the type 1 IGF receptor.

As these effects of iv rhIGF-I may be helpful therapeutically, we investigated in this study the effects of sc administered rhIGF-I in two of the patients with severe insulin resistance type A who responded to an iv bolus of rhIGF-I (8) and measured fasting and postprandial glucose levels, insulin secretion and lipid levels. We found that rhIGF-I normalized fasting plasma glucose and improved meal tolerance in the face of markedly reduced insulin secretion.

Subjects and methods

Subjects

Two otherwise healthy patients (MR and AS) with severe insulin-resistant diabetes mellitus gave informed and written consent before participating in this study, which was approved by the Ethics Committee of the Department of Internal Medicine of the University Hospital at Zurich. The medical history of the patients has been reported previously (8) (MR = case 1 and
AS = case 2) and their actual clinical characteristics are shown in Table 1. AS had a wedge-resection of the ovaries at age 16. Severe insulin-resistant diabetes was diagnosed on the basis of the clinical symptoms, an iv insulin tolerance test (Fig. 1), the exclusion of anti-insulin and anti-insulin receptor antibodies and the demonstration of insulin receptor defects (10). The weight of the patients was constant prior to the study.

The patients' prior treatment had consisted only of a diet.

**Experimental procedure**

A dietary history served to calculate the sucrose-free diet during the study. The patients received 37 (MR) and 30 kcal/kg body weight (AS) (50% carbohydrates, 30% fat, 20% protein). The diet was given as breakfast (08.00 h), lunch (12.00 h) and dinner (18.30 h), each containing 25% of the total calories, and as snacks at 10.00, 16.00 and 21.30 h, with 10, 10 and 5% of the total calories, respectively. Breakfast was identical for each patient throughout the study. Initially, an insulin tolerance test was performed with an iv bolus of 0.45 IU insulin/kg body weight at times 0 and 120 min. Blood was drawn at regular intervals and analyzed for glucose, insulin and C-peptide levels. One to two weeks later, the patients were hospitalized for five control days (days 1–5), five subsequent rhIGF-I treatment days (days 6–10) and three wash-out days (days 11–13). As rhIGF-I has prolonged effects after its discontinuation (11), the control and treatment period were not randomized. Instead, the patients were

### Table 1. Characteristics of two patients (MR and AS) with severe insulin resistance type A.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MR</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>19.9</td>
<td>27.6</td>
</tr>
<tr>
<td>HbA1c (%) normal 4.0–6.4</td>
<td>12.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>9.4</td>
<td>1</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>125</td>
<td>55</td>
</tr>
<tr>
<td>Menstrual cycle (days)</td>
<td>Amenorrhoic</td>
<td>30</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Enlarged clitoris</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

![Figure 1](image-url)

**Fig. 1.** Plasma glucose (●), insulin (⊲) and C-peptide levels (▲) in MR (top panel) and AS (bottom panel) before and following the iv injection of 0.45 IU insulin/kg at time 0 and again at 2 h.
followed during the wash-out days. A higher rhIGF-I dose was used in this study compared to that in a trial with type 2 diabetes (2 × 120 µg rhIGF-I/kg per day (11), 150 µg rhIGF-I/kg body weight (provided by Ciba-Geigy AG, Basle, Switzerland) was dissolved in distilled water (25 g/l) and injected sc on days 6–10 into the thigh at 07.00 h and 18.30 h. The injection at 18.30 h on day 10 was omitted. Fasting blood was drawn every morning except on days 2 and 3 for the analysis of total IGF-I and -II, free IGF-I, growth hormone (GH), glucose, insulin, C-peptide, proinsulin, lipid levels and routine chemistry. Plasma glucose also was determined before each main meal. Postprandial levels of glucose, insulin, C-peptide and proinsulin were determined during meal tolerance tests on days 4, 8, 9, 11 and 12. On these days, the snack at 10.00 h was eaten with the breakfast between 08.00 and 08.30 h and contained 1.6 (MR) and 1.3 g carbohydrate/kg (AS), respectively.

**Analytical methods**

Venous blood was allowed to clot before centrifugation and the overlying serum was decanted and stored in aliquots at −20°C until assayed. The methods have been described in detail previously (11–13). Briefly, plasma glucose was determined on a glucose analyzer (Model 2, Beckman Instruments, Inc., Fullerton, CA), and insulin, C-peptide, GH (normal fasting 95% CV < 7 µg/l) and lipoprotein(a) were determined with commercial RIAs. Total IGF-I and -II (mean normal 26 ± 7.7 and 87 ± 17 nmol/l) were measured with RIAs after extraction of 250 µl of serum with Sep-Pak C18 (Waters Associates, Milford MA, USA) to dissociate IGFs from IGF-binding protein (IGFBPs) (12). For free IGF-I (mean normal 1.3 ± 0.9 nmol/l), 100 µl serum samples were chromatographed on a Sephadex G-50 fine column (equilibrated with 0.15 mol/l NH4HCO3/0.02% NaN3/0.02% HSA, pH 7.8) to separate free UGFs from IGFs’ bound to IGFBPs. Fractions between 52 and 77% bed volume were pooled, lyophilized and dissolved for analysis of IGF-I by RIA (12). Total and fractionated lipoprotein triglyceride and cholesterol levels were measured enzymatically with kits (Boehringer Mannheim GmbH, Mannheim, Germany). Serum (150 µl) was centrifuged at 140 000g for 150 min and lipid levels of very low density lipoprotein (VLDL) were measured in the overlying 40 µl. High-density lipoprotein (HDL) was separated from the infranatant by phosphoric–wolfram acid precipitation (13). Proinsulin was determined with an enzyme-linked immunoabsorbant assay (14) (normal 95% CV < 1.2–13 pmol/l). All samples of one patient were analyzed in duplicate at one to three dilutions within one or two assays.

**Statistics**

Areas under the curve (AUC) were calculated using the trapezoidal rule.

**Results**

Both patients tolerated rhIGF-I treatment well. Resting and postural blood pressure did not change during the study. The heart rate of MR increased by 12% between days 7 and 10. Body weight increased by 0.8 (MR) and 2.0 kg (AS) by days 10 and 11, respectively, and returned to control values on day 13. The time course suggests that this change reflects changes in body water. Both patients reported mild tenderness of the parotid glands on days 7–11 (MR) and 7–9 (AS). In AS, amylase and lipase increased from 49 ± 4 and 54 ± 12 U/l during the control period to peaks of 70 and 86 U/l on days 7 and 11, respectively. A pancreas-specific isofrom of amylase did not change significantly. AS had slight headaches on days 8 and 9 and visible facial swelling on day 10. Burning at the injection site for 30–60 s was noted regularly even after reducing the rhIGF-I concentration to 10 g/l, which had never caused local irritation (11).

Total IGF-I levels were in the normal range during the control period (30.5 ± 1.0 in MR and 22.9 ± 5.0 nmol/l in AS) and reached 111 ± 8 in MR and 121 ± 7 nmol/l in AS during days 8–10 of rhIGF-I administration. The IGF-I levels returned to 38.0 (MR) and 43.6 nmol/l (AS) on day 13. Respective total IGF-II levels in MR and AS were 63 ± 14 and 88 ± 9 (control), 38 ± 4 and 27 ± 4 (treatment) and 65.2 and 72.0 nmol/l (day 13). Fasting free IGF-I levels in MR were 2.9 ± 0.4 (control), 15.4 ± 2.0 (treatment) and 2.6 nmol/l (day 13). Fasting GH levels in MR and AS were 2.0 ± 1.2 and 3.3 ± 3.3 (control), 0.7 ± 0.3 and 1.5 ± 0.9 (treatment) and 1.7 ± 0.8 and 3.3 ± 0.9 µg/l (wash-out), respectively.

Fasting plasma glucose levels (Fig. 2) fell into the normal range on day 9 or 10 and increased during the wash-out period in MR but not in AS. In MR and AS, plasma glucose levels before lunch were 15.5 ± 1.8 and 13.1 ± 1.1 (control), 10.2 ± 0.9 and 6.3 ± 1.3 (treatment) and 13.1 ± 0.7 and 7.4 ± 2.4 mmol/l (wash-out), respectively. The respective values before dinner were 12.3 ± 2.3 and 11.6 ± 0.7 (control), 9.3 ± 2.0 and 7.0 ± 1.3 (treatment) and 8.0 and 9.3 mmol/l (wash-out). Postprandial plasma glucose levels changed similarly (Fig. 3). Fructosamine levels decreased between days 6 and 10 from 430 to 377 (MR) and from 431 to 380 µmol/l (AS).

Fasting insulin levels were 20- (MR) and 4-fold (AS) elevated during the control period compared to healthy controls and decreased by 65% in both patients during treatment, as did C-peptide levels (Fig. 2). The fasting C-peptide/insulin ratios were 0.87 ± 0.23 (MR) and 1.90 ± 0.22 (AS) during the control period and 1.03 ± 0.08 (MR) and 1.76 ± 0.34 (AS) on treatment days 8–10. Postprandial insulin secretion (Fig. 3) also was reduced during treatment but peak levels (at 10.00 h) were not delayed compared to the control period. During the wash-out period, fasting and
postprandial insulin and C-peptide levels rose more rapidly than glucose levels. The mean proinsulin levels of the two patients in the fasting state and 2 h following breakfast were 96 and 156 (days 4–5), 38 and 80 (days 8–9) and 69 and 174 pmol/l (day 12), respectively.

Fasting lipid levels are shown in Table 2. Postprandial AUCs for total triglycerides in MR and AS were 3.7 and 6.4 (control), 4.3 and 6.7 (treatment) and 4.1 and 6.7 mmol/l·h (wash-out), respectively.

Discussion
This study demonstrates that sc rhIGF-I normalizes fasting and markedly improves postprandial glucose levels in patients with severe insulin resistance type A. Some patients with this disease have been treated with up to 177 500 IU of insulin per day with little or no success (1), as expected from the absence of functional insulin receptors. Insulin and IGF-I can stimulate glucose transport and glycogen synthesis via type 1 IGF receptors (3). Thus, huge doses of exogenous insulin

Table 2. Lipid levels of two patients (MR and AS) with severe insulin resistance type A before (days 1–6), during (days 7–10) and following (days 11–13) sc rhIGF-I administration.

<table>
<thead>
<tr>
<th></th>
<th>Total triglycerides (mmol/l)</th>
<th>VLDL Triglycerides (mmol/l)</th>
<th>Total cholesterol (mmol/l)</th>
<th>LDL Cholesterol (mmol/l)</th>
<th>HDL Cholesterol (mmol/l)</th>
<th>Lipoprotein(a) (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>Days 1–6 0.9 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>3.7 ± 0.5</td>
<td>2.1 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Days 7–10 0.9 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.16 ± 0.03</td>
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<tr>
<td></td>
<td>Days 11–13 0.8 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>3.5 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>AS</td>
<td>Days 1–6 1.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Days 7–10 1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>5.1 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Days 11–13 0.9 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>0.01 ± 0.11</td>
</tr>
</tbody>
</table>

Fig. 2. Fasting plasma glucose levels (top panel), insulin (middle panel) and C-peptide levels (bottom panel) in MR (left) and AS (right) before (days 1–5) (○), during (days 6–10) (●) and following (days 11–13) (□) sc administration of 2 × 150 µg rhIGF-I/kg.
and the exceptionally high endogenous insulin levels in our patients can cross-react with type 1 IGF receptors and save these patients from extreme hyperglycemia and ketoacidosis. Similarly, iv bolus (8, 9) and sc rhIGF-I injections in the present study must have stimulated glucose uptake in muscle by binding to the type 1 IGF receptors (15), which are functionally normal in both of our patients (10). It is less likely that IGF-I lowered glucose levels by suppressing hepatic glucose production, because hepatocytes lack type 1 IGF receptors (16). On the other hand, fasting glucose levels decreased more markedly in this study than postprandial levels. This is in agreement with studies in diabetic BB/w rats in whom IGF-I did suppress hepatic glucose production but was relatively ineffective compared with insulin (6). A glucose lowering effect of IGF-I via the few insulin receptors that may be expressed in our patients is also unlikely, because they are probably saturated with the huge insulin levels. Finally, IGF-II also stimulates glucose uptake in chondrocytes cultures (17) but the IGF-II levels decreased during IGF-I administration. Fructosamine levels with a half-time of 10 days decreased by 12% during 5 days, which suggest a true decrease of the average glucose levels by approximately 25% during IGF-I administration.

Insulin-like growth factor I inhibited insulin secretion in vitro (18). In this study, IGF-I decreased fasting insulin, C-peptide and proinsulin levels by ~65% of the control. Smaller doses of IGF-I suppressed fasting insulin secretion almost completely in healthy subjects (12) and in type 2 diabetics (11). The difference may be due to the marked hyperglycemia, which continuously stimulates insulin secretion and may lead to hyperplasia of the β-cells of the type A diabetic patients. A relatively small decrease of insulin secretion also may be seen in the peripheral blood because less insulin is cleared by binding to, and internalization of, insulin receptors on liver cells in the severe insulin-resistant patients with reduced numbers of receptors (10). This prolongs the half-time of insulin, as supported by the very low C-peptide/insulin ratio in the insulin-resistant patients. This mechanism also could explain the much slower decline of insulin levels following an iv bolus of rhIGF-I in severe insulin-resistant patients (8, 9) than in healthy subjects (4, 19). Insulin secretion also may have been reduced due to lowered glucose levels. Whether this near-normalization of glucose levels
improves \( \beta \)-cell responsiveness (glucose toxicity) cannot be excluded from this study. However, mean glucose levels during IGF-I treatment were still markedly elevated, which makes this mechanism unlikely.

Insulin-like growth factor I suppressed GH secretion in this and earlier studies (11, 12) and altered the pattern of IGFBPs in the severe insulin-resistant patients, as in type 2 diabetics (20). During IGF-I administration less IGF-I is bound by a 150-kD complex while more is bound by low-molecular-weight IGFBPs. They may cross the vascular endothelial barrier, get in contact with muscle (21) and stimulate glucose uptake. It appears that these changes account for most of the lowered glucose levels in the face of markedly reduced insulin levels.

Lipid levels hardly changed during IGF-I therapy. In type 2 diabetics, IGF-I decreased triglyceride levels clearly, with the greatest effect in those subjects with the highest control levels (13), which was probably due to concomitantly decreased substrates for triglyceride synthesis (12). The insulin-resistant patients in this study had normal control levels. Of the substrates, glucose decreased markedly but insulin and free fatty acid levels (not shown) decreased only relatively little. It is, thus, not surprising that in our patients with severe insulin resistance due to the lack of functional insulin receptors, lipid levels hardly changed.

There are clear differences between the two insulin-resistant subjects. The C-peptide/insulin ratio was almost two-fold higher in AS than in MR. Fasting insulin levels as a measure of insulin resistance were fivefold higher in MR than in AS. Glucose levels during the wash-out period increased rapidly in MR but not in AS. Similarly, during 4 weeks of sc rhIGF-I administration the glucose levels remained 30% lower compared to the control in AS and were only reduced slightly in MR (P. D. Zenobi et al., unpubl. obs.). These differences may be due to the insulin receptor defects and degree of insulin resistance. Insulin receptors are almost undetectable in AS, while the number of insulin receptors in MR is only reduced slightly. Insulin receptors of MR but not of AS carry a mutation in the tyrosine kinase region of the insulin receptor \( \beta \)-subunit gene. The defect was found on one allele so that 25% of the insulin receptors might be functional (10). Kuzuya et al. described patients with severe insulin resistance due to different insulin receptor defects who responded very heterogeneously to treatment with rhIGF-I (22). Usala et al. reported a type 1 diabetic with severe insulin resistance during ketoacidosis that was accompanied by reduced insulin but normal IGF-I responsiveness of adipocytes (23). In that study, several IGF-I boluses exerted prolonged glucose-lowering effects (23), as we found during the wash-out period in patient AS.

The side effect during rhIGF-I administration were rated as mild by both patients and did not worsen during prolonged treatment, as reported by Kuzuya et al. (22) and observed during 4 weeks in our two severe insulin-resistant patients (PD Zenobi, unpubl. obs.). Both of these studies demonstrate that beneficial metabolic effects of rhIGF-I persist, at least in some patients, over prolonged treatment periods and support the fact that insulin-resistant patients may profit from treatment with rhIGF-I.

Acknowledgments. We thank Mr A Lenz and Dr ME Roder for measuring the lipids and proinsulin, respectively. The help of the nurses on the metabolic ward also is highly appreciated. This work has been supported by the Swiss National Science Foundation (Grant No. 31-9095.87).

References


Received January 11th, 1994

Accepted May 17th, 1994