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Recombinant Human Insulin-Like Growth Factor I Has Significant Anabolic Effects in Adults with Growth Hormone Receptor Deficiency: Studies on Protein, Glucose, and Lipid Metabolism*

NELLY MAURAS, VICTOR MARTINEZ, ANNIE RINI, AND JAIME GUEVARA-AGUIRRE

Division of Endocrinology, Nemours Children's Clinic and Research Programs (N.M., A.R.), Jacksonville, Florida 32207; and the Instituto de Endocrinologia y Reproduccion (V.M., J.G.A.), Quito, Ecuador

ABSTRACT

The physiological effects of insulin-like growth factor I (IGF-I) on intermediate metabolism of substrates have been extensively studied in a variety of experimental situations in man, and its effects on linear growth of children with GH receptor mutations have proven beneficial. However, there is a paucity of data on the metabolic effects of IGF-I as replacement therapy in adults with GH receptor deficiency (Laron's syndrome). We designed these studies to investigate the *in vivo* effects of 8 weeks of therapy with recombinant human IGF-I (rhIGF-I) in a unique group of 10 adult subjects with profound IGF-I deficiency due to a mutation in the GH receptor gene (mean ± SEM age, 29.2 ± 2.0 yr; 4 males and 6 females). At baseline, patients had infusions of stable tracers, including L-[^{13}C]leucine, [$^2H_2$]glucose, and d_5-glycerol, as well as indirect calorimetry, assessment of body composition (dual energy x-ray absortiometry), and measurements of growth factor concentrations. Patients were then discharged to receive twice daily rhIGF-I (60 μg/kg, sc) for the next 8 weeks when the studies were repeated identically.

Plasma IGF-I concentrations increased during rhIGF-I treatment from 9.3 ± 1.5 μg/L to 153 ± 23 (P = 0.0001). There was no change in weight during these studies, but a significant change in body composition was observed, with a decrease in percent fat mass (P = 0.003) and an increase in lean body mass (P = 0.001). These were accompanied by increased rates of protein turnover, decreased protein oxidation, and increased rates of whole body protein synthesis, as measured by leucine tracer methods ($P < 0.01$). These results are similar to those observed in GH-deficient subjects treated with GH. All measures of lipolytic activity and fat oxidation increased during treatment, with an 18% increase in the glycerol turnover rate (P = 0.04), an increase in free fatty acid and β-hydroxybutyrate concentrations, and a significant increase in fat oxidation, as measured by indirect calorimetry (P = 0.04). There were significant decreases in insulin concentrations (P = 0.01) and a reciprocal increase in glucose production rates (P = 0.04) during rhIGF-I, yet plasma glucose concentrations remained constant, suggestive of a significant insulin-like action of this peptide. RhIGF-I was well tolerated by all patients.

In conclusion, 8 weeks of treatment with rhIGF-I had significant positive effects on body composition and measures of intermediate metabolism independent of GH. These results suggest that, similar to GH treatment of adults with GH deficiency, rhIGF-I may be beneficial as long term replacement therapy for the adult patient with Laron's syndrome. (*J Clin Endocrinol Metab* **85:** 3036–3042, 2000)

THE METABOLIC actions of insulin-like growth factor I (IGF-I) are complex, with both GH-like and insulin-like effects *in vivo* depending on the dose used and the mode of administration (1). IGF-I has been clearly shown to mediate many of the growth-promoting effects of GH (2, 3), congruent with the somatomedin hypothesis. In lower doses, it also mediates the protein anabolic actions of GH by selectively increasing whole body protein synthesis without affecting rates of proteolysis, whereas in higher doses it suppresses proteolysis, similar to an insulin-like effect (4, 5). The effects of IGF-I on carbohydrate metabolism are also dose related, resulting in a suppression of circulating insulin, yet maintenance of normoglycemia, and in higher doses it can cause marked hypoglycemia, evidence of an insulin-like effect of the peptide (4, 6). The latter effect is mediated either through the insulin receptor or through its own, type 1 IGF-I receptor (7, 8). The effects of IGF-I on lipolysis have also been observed to be dependent on the length of treatment, with both increased and decreased lipid oxidation rates observed after IGF-I administration (9, 10). IGF-I, however, does not mediate the lipolytic effect of GH, as there are no functional type 1 IGF-I receptors in the adipocyte (11). Interestingly, we observed a clear dichotomy of the effects of IGF-I *in vivo* depending on the mode of delivery. There was marked enhancement of the rates of protein synthesis when recombinant human IGF-I (rhIGF-I) was given for 5–7 days as two sc injections, yet no effect on whole body protein was observed when it was administered as a continuous sc infusion for 16 h/day (9); both delivery strategies, however, had a marked impact, enhancing carbohydrate oxidation rates, indicating the differential sensitivity to the metabolic effects of this peptide.

RhIGF-I has been successfully used to promote linear

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Address all correspondence and requests for reprints to: Nelly Mauras, M.D., Division of Endocrinology, Nemours Children's Clinic, 807 Nira Street, Jacksonville, Florida 32207. E-mail: nmauras@nemours.org.

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growth in children with GH insensitivity (Laron's syndrome) (2, 3). This condition manifests as profound IGF-I deficiency despite normal or high circulating GH concentrations due to a variety of mutations in the GH receptor gene (12–14). These patients' physical, biochemical, and linear growth responses to rhIGF-I have been extensively characterized previously both in short term metabolic studies and in long term studies assessing linear growth (2, 3, 15–19). The metabolic effects of GH in the adult with GH deficiency (GHD), the increase in lean body mass, the decrease in adiposity, and the positive cardiovascular/lipid effects, are substantial and have led to the common use of GH in adults with GHD in most western countries (20, 21). Even though the benefit of rhIGF-I treatment promoting linear growth in children with homozygous forms of GH receptor deficiency (GHRD) has been clearly established, the long term use of this peptide for purposes other than linear growth has been less well characterized in the adult (22). We designed these studies to assess the effects of rhIGF-I on protein, glucose, and lipid metabolism and on body composition in a unique population of adult subjects with a homozygous mutation in the GH receptor gene resulting in Laron's syndrome. Contemporary, stable tracer methodologies were used to study intermediate metabolism before and after more prolonged (8-week) administration of rhIGF-I.

Subjects and Methods

Study subjects

These studies were approved by the clinical research review committee at the Nemours Children's Clinic, the General Clinical Research Center (CRC) advisory committee at the Mayo Clinic (Rochester, MN), the institutional review board at Baptist Medical Center/Wolfson Children's Hospital (Jacksonville, FL) where these studies were conducted, and the institutional review board at the Institute for Endocrinology and Reproduction (Quito, Ecuador). Subjects were recruited after informed written consent was obtained verbally and in writing in Spanish, the subjects' native language. A group of 10 adult subjects was recruited by Dr. Guevara-Aguirre in Ecuador, all of whom had been previously identified to be homozygous for the alternative splice site mutation of codon 180 of exon 6 of the GH receptor gene and profound short stature. The genetic, biochemical, and physical characteristics of this patient population have been extensively studied previously (12, 13, 17, 19). Subjects were all in good health, were taking no chronic medications, and had not received any rhIGF-I for at least 3 yr before these studies. Their clinical characteristics are summarized in Table 1.

Study design

Subjects traveled in two groups of five with their physicians from Quito to Jacksonville twice over the 10 weeks of these experiments. A full physical exam was performed, and routine blood chemistries and cell counts were made upon arrival at our research center. For 3 days before admission to the Wolfson Children's Hospital CRC they consumed a weight maintenance diet based upon the dietary histories obtained by the research dietitian consisting of about 30 Cal/kg and 1 g/kg protein·day.

TABLE 1. Clinical characteristics of the study subjects

Subject No.	Age (yr)	Sex	Ht (cm)	BMI (kg/m^2)
1	18.3	F	134	27.0
2	24.8	F	128	19.5
3	22.3	M	127	22.9
4	37.1	F	120	31.7
5	35.2	F	110	26.1
6	26.3	F	116	23.3
7	27.1	M	116	17.8
8	30.8	M	126	20.4
9	33.9	F	116	28.4
10	36.6	M	116	26.4
Mean	29.2		121	24.4
±SEM	2.0		2	1.4

The afternoon before the first study (D1) the subjects were admitted to the Wolfson Children's Hospital CRC. Assessment of body composition was obtained using skinfold calipers, bioelectrical impedance analysis, as well as dual emission x-ray absorptiometry (DEXA), using a tissue bar (Hologic 2000, Hologic, Inc., Waltham, MA). Dinner was served at 1800 h; subsequently, the patients were fasted except for water *ad libitum* until the completion of the studies at 1300 h the next day. The next morning (baseline study) at 0600 h, two antecubital veins were cannulated after numbing the skin with an anesthetic cream (EMLA, AstraZeneca, Wilmington, DE). One cannula was kept heated for arterialized blood sampling (23). At 0800 h (time zero), three stable isotope tracers were given. One, a primed, dose constant infusion of L-[1-^{13}C]leucine (4.5 μmol/kg; 0.07 μmol/kg·min) was begun and continued uninterrupted for the next 240 min. Concomitantly, a primed infusion of [6,6-$^2H_2$]glucose (33 μmol/kg; 0.33 μmol/kg·min) was begun and also continued for 240 min. One hundred and twenty minutes into the leucine and glucose tracer infusions, a primed, dose constant infusion of [1,1,2,3,3 $^2H_2$]glycerol (d_5-glycerol) was begun, piggybacked into the same iv line and was continued for 120 min (1.4 μmol/L/kg; 0.1 μmol/L/kg·min). Frequent blood samples were collected for determination of the isotopic enrichment of the different tracers in plasma as well as the concentrations of hormones, substrates, and growth factors, as detailed below. Frequent breath samples were also obtained for determination of $^{13}CO_2$ enrichment in expired breath. Indirect calorimetry was performed three times during the 4 h of isotope infusion using a mouth piece and a CPX max indirect calorimeter (Medical Graphics, St. Paul, MN). After the isotope infusions were completed, the patients were fed lunch and discharged from the unit.

After the baseline study, all subjects returned to Ecuador and were begun on rhIGF-I at a dose of 60 μg/kg, sc, twice daily; they were closely monitored for the first 24 h by the physician team in Ecuador. They were then sent home and continued to take twice daily injections of rhIGF-I. Subjects were instructed to monitor their blood glucose concentrations using home glucose monitoring equipment for the first week after initiation of rhIGF-I therapy and any other time there were any symptoms of hypoglycemia. They were also instructed to take the rhIGF-I injection with their meals to avoid hypoglycemia.

After 8 weeks, the subjects flew back to the CRC in Jacksonville where the studies were repeated identically as at baseline (D2). The only difference was that the night before the second study, starting at 2000 h, the second dose of rhIGF-I was substituted for a continuous sc infusion of the peptide at 10 μg/kg·h using an insulin delivery pump (MiniMed, Inc., Sylmar, CA), which was continued uninterrupted for the next 16 h until the completion of the studies the following morning. This was performed to prevent hypoglycemia during the administration of rhIGF-I while the patients were fasting, while maintaining plasma IGF-I concentrations constant. We have successfully used this strategy in similar experiments previously (4, 9, 24). Each patient served as his/her own control.

Blood and breath samples. The isotopic enrichments of α-ketoisocaproic acid ([^{13}C]KIC), and [$^2H_2$]glucose were measured at −20, 160, 180, 200, 220, and 240 min. The isotopic enrichments of d_5-glycerol were measured in plasma samples obtained at 60, 190, 200, 210, 220, 230, and 240 min. Plasma IGF-I, IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-2, IGFBP-3, insulin, and glucose concentrations were measured three times at 0, 120, and 240 min during the tracer infusions. Serum GH concentrations were measured at hourly intervals for the 4 h of the studies. β-Hydroxybutyrate and free fatty acid concentrations were measured in plasma samples at −20 and 120 min. Serum lipids were also measured while fasting on each study day. Breath samples were obtained for the measurement of expired labeled CO_2 at −20, −10, −5, 160, 180, 200, and 220 min. A small aliquot of the urine collected during the 4 h of the morning study was used for determination of the urea nitrogen concentration.

Assays. Plasma enrichments of [^{13}C]KIC, [$^2H_2$]glucose, and d_5-glycerol were determined at the Nemours metabolic core laboratory by gas chromatography mass spectrometry as previously described (25, 26). $^{13}CO_2$ was measured by isotope ratio mass spectrometer as described previously (27). All insulin and lipid concentrations were run at the immunochemical core laboratory at the Mayo Clinic General Clinical Research Center (Rochester, MN) using commercial kits; IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, and IGF-II concentrations were measured by radioimmunometric assays. β-Hydroxybutyrate concentrations were measured at the Nemours biochemical analysis laboratory by spectrophotometric enzymatic determination using kits from Sigma (St. Louis, MO), and free fatty acid concentrations were measured by calorimetric methods using kits purchased from Roche Molecular Biochemicals (Indianapolis, IN). Serum GH concentrations were measured by a highly sensitive chemiluminescence assay at the University of Virginia CRC core laboratory (Charlottesville, VA). Plasma glucose concentrations were measured by a glucose oxidase method using a glucose analyzer (Beckman Coulter, Inc., Palo Alto, CA) at the bedside. Serum lipids [cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL)] concentrations were measured by high performance liquid chromatography methods. Urea nitrogen was measured using a Kodak Ektakem urease method (Eastman Kodak Co., Rochester, NY).

Calculations. The reciprocal pool model was used to estimate rates of whole body protein turnover at steady state as previously described (28). The rate of appearance (Ra) of glucose, a measure of hepatic glucose output, as well as that of glycerol were calculated as: $Ra = [(Ei/Ep) - 1]F$, where Ei is the isotopic enrichment of the infusate, Ep is the enrichment of glucose or glycerol in plasma, and F is the infusion rate.

Substrate oxidation rates for protein, glucose, and lipid and resting energy expenditure were calculated using the rate of gas exchange (VO_2 and VCO_2) from indirect calorimetry as previously described (29).

Fat-free mass (FFM) and percent fat mass (FM) were measured using DEXA and the tissue bar as well as by the sum of skin folds as described previously (30).

Isotopes and drugs. L-[1-^{13}C]Leucine and d_5-glycerol (both 99% enriched; Cambridge Isotopes, Andover, MA) and [6,6-$^2H_2$]glucose (99.7% enriched; MSD Isotopes, St. Louis, MO) were determined to be sterile and pyrogen free and were mixed with 0.9% nonbacteriostatic saline. rhIGF-I (10 mg/ml) was provided by Genentech, Inc. (South San Francisco, CA).

Statistical analysis. Results are expressed as the mean ± SE. Paired Student's *t* test was used to estimate differences between baseline studies and rhIGF-I and rhGH treatments for all parameters tested. Wilcoxon signed ranks test was used for those parameters with results not normally distributed. Significance was established at $P < 0.05$.

Results

Hormones and growth factors

Table 2 summarizes the changes in circulating plasma growth factors before and after rhIGF-I in the 10 subjects with GH receptor mutations. There was a significant increase in plasma IGF-I concentrations during rhIGF-I treatment and a reciprocal decrease in circulating GH concentrations. Even though most patients raised their plasma IGF-I concentrations to the normal range (normative data: males, 155–432 μg/L; females, 87–368), 3 did not restore it fully (male subject, from 5 to 133; male, 6 to 113; female, 5 to 76; Fig. 1). IGFBP-1 and -2 concentrations increased, IGFBP-3 did not change, and IGF-II concentrations decreased during treatment.

TABLE 2. Hormones and growth factors of subjects with GH receptor deficiency before (D1) and after 8 weeks of rhIGF-I administration (D2)

Normal concentrations for adult subjects are shown in (*brackets*) under the given growth factor. The range of GH concentrations observed in these subjects is given in *parentheses* under columns D1 and D2.

	D1	D2	*P* value
IGF-I (μg/L) [155–432 M, 87–368 F]	9.3 ± 1.5	153 ± 23	0.0001
IGFBP-3 (mg/L) [2.2–4.2]	1.3 ± 0.1	1.3 ± 0.1	NS
IGFBP-1 (μg/L) [10–150]	49 ± 10	83 ± 13	0.02
IGFBP-2 (μg/L) [215–518]	552 ± 84	1115 ± 123	0.0003
IGF-II (IGF-II μg/L) [288–736]	188 ± 23	63 ± 5	0.0001
Mean GH (μg/L)	9.5 ± 3.2	0.3 ± 0.1	0.02
Peak GH (μg/L)	23.5 ± 8.8 (0.3–82.4)	0.6 ± 0.2 (0.1–2.5)	0.03

Body composition and protein metabolism

Table 3 summarizes the changes in body composition and protein kinetics observed during treatment. There were no changes in body weight during rhIGF-I treatment. There were, however, subtle, yet significant, changes in body composition as measured by DEXA, with a 1-kg increase in FFM ($P = 0.001$) and a corresponding decrease in percent FM after 8 weeks of treatment ($P = 0.003$).

The Ra of leucine, a measure of proteolysis, was increased in these subjects after rhIGF-I treatment, leucine oxidation rates decreased, with an overall increase in the nonoxidative leucine disposal (NOLD), a measure of whole body protein synthesis. These findings were also observed when the data were expressed per kg FFM (data not shown).

Fat metabolism

All measures of lipolytic activity and lipid oxidation were increased after 8 weeks of rhIGF-I therapy. There was an 18% increase in the Ra of glycerol, a measure of whole body lipolysis, from 2.51 ± 0.13 μM/kg·min on D1 to 2.96 ± 0.21 on D2 ($P = 0.06$) or, when expressed per kg FM, from 5.77 ± 0.58 μmol/kg FM·min on D1 to 7.06 ± 0.79 on D2 ($P = 0.04$). Fasting plasma free fatty acid concentrations increased after rhIGF-I administration from 0.34 ± 0.04 mmol/L to 0.42 ± 0.06 ($P = 0.06$), and β-hydroxybutyrate from 0.50 ± 0.20 mg/dL to 1.70 ± 0.50 ($P = 0.02$). Using gas exchange mea-

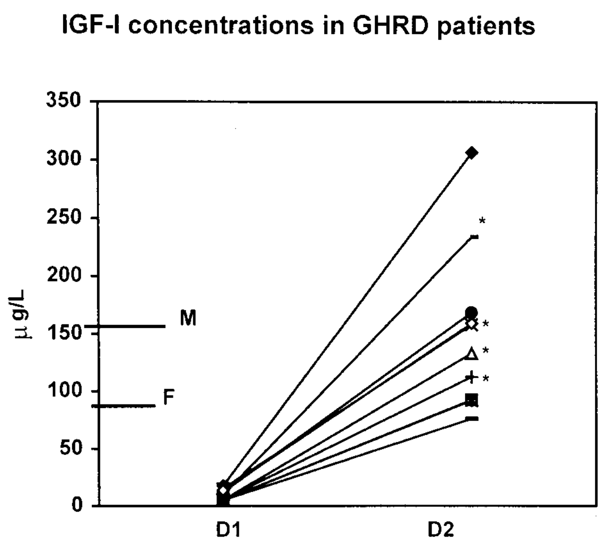

FIG. 1. Plasma IGF-I concentrations in GHRD patients before (D1) and after (D2) 8 weeks of twice daily sc rhIGF-I. The *thick bars* on the *y*-axis represent the lower limits of normal for male (M) and female (F) subjects.

surements via indirect calorimetry, lipid oxidation rates increased from 19.2 ± 3.8 Cal/FFM·day to 27.6 ± 4.4 ($P = 0.04$; Fig. 2). Plasma lipid concentrations remained invariant during rhIGF-I treatment: total cholesterol, 179 ± 11 mg/dL on D1 and 176 ± 11 on D2; HDL, 47 ± 3 mg/dL on D1 and 46 ± 4 on D2; LDL, 112 ± 10 mg/dL on D1 and 109 ± 8 on D2; triglycerides, 100 ± 15 mg/dL on D1 and 106 ± 24 on D2 (P = NS for all comparisons).

Glucose metabolism

There was maintenance of normoglycemia despite suppression of circulating insulin concentrations in the fasted state after rhIGF-I therapy: plasma glucose concentrations: D1, 88 ± 2 mg/dL; D2, 88 ± 4 (or D1, 4.9 ± 0.1 mmol/L; D2, 4.9 ± 0.2); insulin concentrations: D1, 5.4 ± 1.5 μU/mL; D2, 2.4 ± 0.7 ($P = 0.01$; or D1, 32.4 ± 9.0 pmol/L; D2, 14.4 ± 4.2). Glucose Ra, a measure of hepatic glucose production, was increased after rhIGF-I therapy from 1.95 ± 0.15 mg/kg·min to 2.36 ± 0.18 ($P = 0.00001$). Glucose oxidation rates were decreased from 25 ± 4 Cal/FFM·day to 17 ± 5 ($P = 0.04$) after chronic rhIGF-I therapy.

TABLE 3. Changes in body composition, as measured by DEXA, and whole body protein kinetics, as measured by [^{13}C]leucine infusions (micromoles per kg/min), in 10 adult patients with GHRD

	D1	D2	*P* value
Body composition			
Wt (kg)	35.5 ± 2.3	35.7 ± 2.2	NS
Fat-free mass (kg)	17.4 ± 0.9	18.4 ± 0.9	0.001
% Fat mass	47.1 ± 3.1	45.7 ± 3.1	0.003
Protein kinetics			
Leucine Ra	1.24 ± 0.12	1.39 ± 0.10	0.004
Leucine oxidation	0.28 ± 0.04	0.22 ± 0.03	0.01
NOLD	0.95 ± 0.09	1.16 ± 0.08	0.0001

Comparison with GHD and normal subjects

Some of the responses of the subjects with GHRD to rhIGF-I were compared with those of a group of 8 GHD adults treated with rhGH (12.5 μg/kg·day, sc) and rhIGF-I (60 μg/kg twice daily, sc) for 8 weeks, each reported previously [mean age, 23.5 ± 2.1 yr; 6 males and 2 females; body mass index (BMI), 28.2 ± 2.2 kg/m^2] (31). Data were also compared with those of a group of 10 healthy males (mean age, 23.7 ± 0.5 yr; BMI, 25.2 ± 0.9 kg/m^2) who participated in similar studies, some of which have been reported previously (32). The data from the experiments were gathered identically as in the present GHRD subjects. Figure 3, *left panel*, shows the changes in IGF-I concentrations in the 3 groups of subjects, the *right panel* shows the fasting insulin concentrations, and Fig. 4 shows the rates of nonoxidative leucine disposal, a measure of whole body protein synthesis.

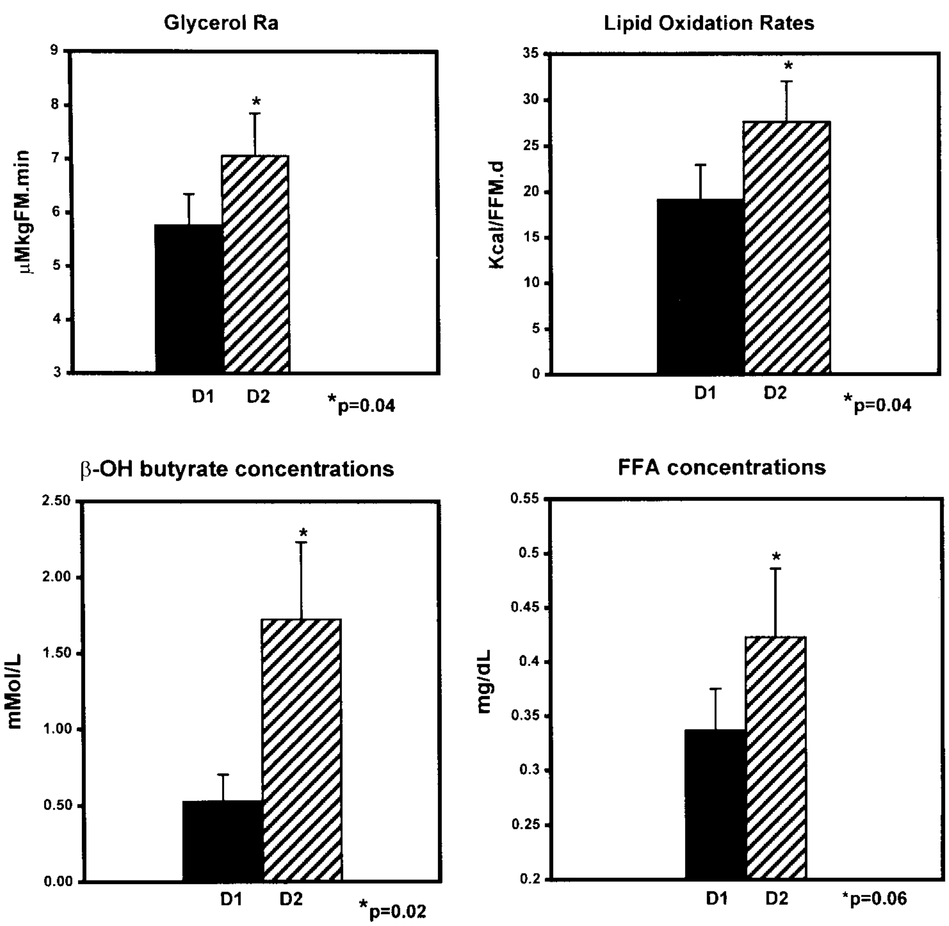

FIG. 2. The *upper left panel* shows the rates of glycerol turnover, a measure of lipolysis, before (D1) and after (D2) 8 weeks of rhIGF-I in 10 subjects with Laron's syndrome as measured by d_5-glycerol tracer studies. The *upper right panel* shows rates of lipid oxidation, as measured by indirect calorimetry; the *lower left panel* shows the changes in free fatty acid concentrations, and the *lower right panel* shows the changes in β-hydroxybutyrate during the same experiments.

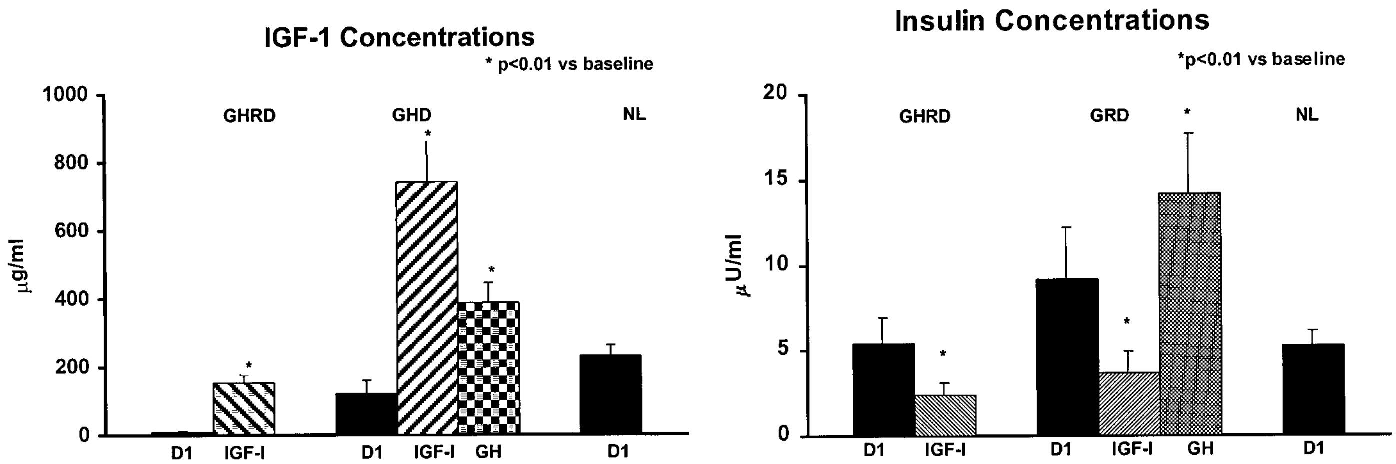

FIG. 3. Comparison of plasma IGF-I (*left panel*) and plasma insulin (*right panel*) concentrations in adults with GHRD before and after 8 weeks of rhIGF-I treatment (n = 10), in adult patients with GHD before and after 8 weeks of rhIGF-I treatment and 8 weeks of rhGH treatment (n = 8), and in healthy controls (n = 10). The comparisons *vs.* baseline within each group for IGF-I are: GHRD, $P = 0.001$; GHD after rhIGF-I, $P = 0.002$; and GHD after rhGH, $P = 0.0001$. For insulin concentrations the within-group comparisons are: GHRD, $P = 0.01$; GHD after rhIGF-I, $P = 0.02$; and GHD after rhGH, $P = 0.001$. To covert insulin concentrations to picomoles per L multiply by 6.

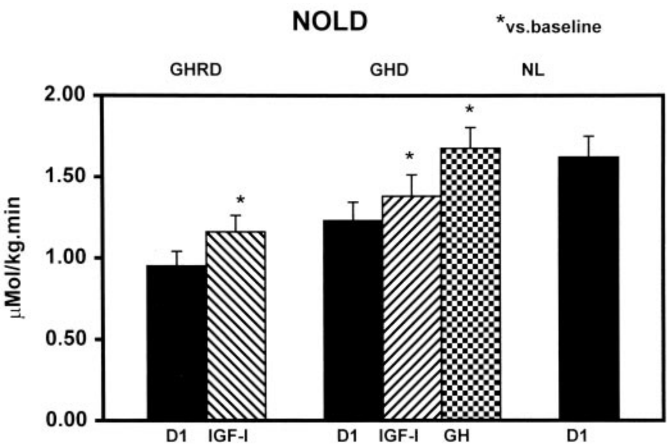

FIG. 4. Comparison of NOLD, a measure of protein synthesis, in the same three groups as those shown in Fig. 3. The within-group comparisons *vs.* baseline are: GHRD, $P = 0.001$; GHRD after rhIGF-I, $P = 0.06$; and GHRD after rhGH, $P = 0.02$.

Safety profile

These doses of rhIGF-I were well tolerated in all subjects. After the initiation of treatment there were complaints of occasional headaches, tachychardia, and soft tissue swelling, the latter most likely secondary to fluid retention. These symptoms were all transient and well tolerated. No patient discontinued the medication because of them. General chemistries, blood counts, thyroid profile, and sex steroid concentrations remained invariant during these studies.

Discussion

Relatively short term (8-week) daily administration of rhIGF-I had substantial, positive effects in IGF-I-deficient adults with GH receptor mutations. Rates of protein synthesis increased significantly; body composition changed with increased lean body mass and decreased adiposity without changes in total body weight. Rates of lipolysis and lipid oxidation also increased after rhIGF-I, suggestive of a nutrient shift and a net protein anabolic effect. Glucose production rates increased with long-term treatment, yet there was no hyperglycemia despite persistent insulinopenia. The expected increase in glucose resulting from this increase in hepatic glucose production was probably negated by increased glucose transport, suggestive of a compensatory increase in insulin-like sensitivity during treatment.

These patients with GH receptor mutations had GH concentrations that were significantly suppressed during rhIGF-I treatment, whereas the GH-dependent growth factors were unaltered; hence, they represent a unique biological model that allows the measurement of IGF-I effects without the confounding effects of GH. Treatment with rhIGF-I resulted in normalization of the plasma IGF-I concentrations in most of the subjects studied and a near-normalization in the others, without any measurable alteration in IGFBP-3 concentrations and a marked decline in circulating GH concentrations. These data are similar to previously reported results after short-term treatment with this peptide and after prolonged treatment for linear growth purposes (3, 33).

The observed changes in body composition, with increased lean body mass and decreased adiposity, were not accompanied by total body weight changes and are remarkable considering that subjects were treated for only 8 weeks. These changes were similar to those observed in GHD patients treated with rhGH and rhIGF-I for 8 weeks, each at similar doses reported by us recently (31) and not dissimilar from those observed after more prolonged treatment with rhGH in adults with GHD (21). Five adults treated with rhIGF-I at twice the dose used here were also reported to have decreased adiposity, as measured by skinfold thickness, after 6–9 months (22). Interestingly, rhIGF-I treatment of Laron's syndrome subjects resulted in increased protein breakdown and protein synthesis rates, with a net protein anabolic effect. These results differ from the selective increase in protein synthesis rates observed after rhIGF-I in healthy individuals (4) and GHD subjects (31) and may be secondary to the chronic relative insulinopenia observed during treatment, as insulin administration typically suppresses proteolysis (34).

The effects of 8 weeks of chronic rhIGF-I treatment on all measures of fat metabolism in this cohort of GHRD subjects is perhaps the most intriguing. Using stable isotopes of glycerol, measurement of glycerol turnover allows the estimation of rates of lipolysis at the whole body level. The breakdown of stored triglyceride in the adipocyte results in the release of both free fatty acids and glycerol, and as there is no glycerol kinase in the adipocyte (hence, reesterification of the released glycerol is not possible within the fat cell), the measurement of the rate of appearance of glycerol is used as a measure of whole body lipolysis. Using this tool, lipolytic rates were increased during rhIGF-I treatment, and this difference was most obvious when the data were expressed per kg FM, highlighting the sensitivity of adipose tissue to the antilipogenic effects of IGF-I. There was minimal change in FFA concentrations, yet the glycerol Ra increased, and as glycerol concentrations did not change, it may be that some of the effect of the treatment was to increase the effectiveness of FFA utilization, such that despite greater release of the products of lipolysis the utilization was increased so as to maintain the same concentration.

Measures of gas exchange and indirect calorimetry in these subjects also revealed a significant increase in the oxidation of lipids; there was also an increase in the concentrations of ketone bodies (β-hydroxybutyrate), which, taken in aggregate, suggest that not only is there mobilization and breakdown of stored lipids, but there is also increased oxidation of the broken fat. As there appear to be no functional type 1 IGF-I receptors in the adipocyte (11), and both GH and insulin concentrations are suppressed during rhIGF-I treatment, these data suggest that these effects on fat metabolism are secondary to chronic relative insulin deficiency during rhIGF-I treatment. This is congruent with recent data in rats that show that IGF-I reduces fat mass via inhibition of the lipogenic capacity of adipocytes and inhibition of insulin secretion (35). Even though the plasma lipid profiles remained unchanged in these subjects during treatment, the beneficial effects of IGF-I replacement on fat metabolism deserve further long-term study.

There was a modest, yet significant, increase in the glucose Ra, a measure of hepatic glucose production, in these patients. This is similar to the increase observed after rhIGF-I treatment of normal (9) and GHD subjects (31, 36) reported by us previously and strongly suggests that there is relative suppression of insulin concentrations at the portal level. The fact that glucose concentrations remained normal and there was no hyperglycemia despite an increase in glucose production rates during these 8-week experiments suggest, however, that IGF-I actively participates in glucose transport. The latter could be either through the insulin receptor (7) or through its own type 1 receptor (8). Di Cola *et al.* studied insulin receptor-deficient mice and showed that IGF-I caused a prompt and sustained decrease in plasma glucose levels, similar to that in mice with intact insulin receptors (8). They also observed that in skeletal muscle, IGF-I treatment caused phosphorylation of IGF-I receptors and increased the levels of the phosphatidylinositol-3-kinase p85 subunit, consistent with the possibility that IGF-I stimulates glucose uptake in a phosphatidylinositol-3-kinase-dependent manner. It is likely that the sustained glucose transport and persistent normoglycemia observed here reflect this action of rhIGF-I in humans through both the insulin and the IGF-I receptor. Based on these data, the long-term use of rhIGF-I in the adult with GHRD should not have deleterious effects on carbohydrate tolerance. Further studies on long-term safety in the adult are needed.

Compared with GHD subjects reported previously (31), it appears that the metabolic deficiencies observed are more severe in GHRD states. Even though there are substantial increases in rates of protein synthesis after rhIGF-I, *e.g.* compared to GHD patients treated with rhGH and rhIGF-I, the best normalization of these rates was observed in the GHD subjects treated with rhGH. It is noticeable that rhIGF-I treatment of the GHD subjects resulted in the largest increase in plasma IGF-I concentrations, followed by rhGH treatment in GHD subjects and lastly rhIGF-I treatment of GHRD patients. There are, however, comparable increases in NOLD per U increase in IGF-I for the GHRD patients treated with rhIGF-I as for the GHD patients treated with rhGH, supportive of the idea that IGF-I mediates the protein anabolic actions of GH in man (4, 24). Adult GHD subjects treated with rhIGF-I studied by us using the same doses as those reported here showed normal absorption and distribution of IGF-I, yet faster elimination kinetics than normal subjects (37), similar to results observed in GHRD subjects reported previously (38). However, the rise in plasma IGF-I concentrations observed in this cohort, even though not fully normal in three patients, is appropriate, and the metabolic effects observed are substantial, suggesting that a dose of 60 μg/kg twice daily may be appropriate for long-term studies in this patient population. Whether complete normalization of the metabolic derangement of these patients could be overcome with years of rhIGF-I therapy remains to be fully studied. However, considering the pivotal role of this hormone in a multiplicity of metabolic functions *in vivo*, the actual, albeit limited, availability of this peptide, and the standard practice of endocrinology to replace hormone-deficient states, it behooves us to perform the necessary long-term studies in the affected adults with this syndrome. The ultimate effects on the quality of life of these patients would also need to be assessed.

In conclusion, we studied intermediate metabolism of proteins, sugars, and lipids in a group of severely IGF-I-deficient subjects treated with rhIGF-I for 8 weeks. IGF-I affects protein, glucose, and lipid metabolism independent of GH and has positive anabolic effects in this condition. These results suggest that rhIGF-I may be beneficial for long-term replacement of the adult with Laron's syndrome.

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References

1. **Mauras N, Haymond MW.** 1996 Metabolic effects of recombinant human insulin-like growth factor I in humans: comparison with recombinant human growth hormone. Pediatr Nephrol. 10:318–323.
2. **Walker J, Van Wyk JJ, Underwood LE.** 1992 Stimulation of statural growth by recombinant insulin-like growth factor I in a child with growth hormone insensitivity syndrome. J Pediatr. 121:641–646.
3. **Guevara-Aguirre J, Rosenbloom AL, Vasconez O, et al.** 1997 Two year treatment of GH receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and to GH-treated GH deficiency. J Clin Endocrinol Metab. 82:629–633.
4. **Mauras N, Beaufrere B.** 1995 Recombinant human insulin-like growth factor-I enhances whole body protein anabolism and significantly diminishes the protein catabolic effects of prednisone in humans without a diabetogenic effect. J Clin Endocrinol Metab. 80:869–874.
5. **Turkalj I, Keller U, Ninnis R, Vosmeer S, Stauffacher W.** 1992 Effect of increasing doses of recombinant human insulin-like growth factor-I on glucose, lipid, and leucine metabolism in man. J Clin Endocrinol Metab. 75:1186–1191.
6. **Guler HP, Zapf J, Froesch ER.** 1987 Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. N Engl J Med. 317:137–140.
7. **Zapf J, Schoenle E, Waldrogel M, Sand I, Froesch ER.** 1981 Effect of trypsin treatment on rat adipocytes on biological effects and binding of insulin and IGFs: further evidence for the action of IGFs through the insulin receptor. Eur J Biochem. 113:605–609.
8. **Di Cola G, Cool MH, Accili D.** 1997 Hypoglycemic effect of insulin-like growth factor-1 in mice lacking insulin receptors. J Clin Invest. 99:2538–2544.
9. **Mauras N, Martha PM, Quarmby V, Haymond MW.** 1997 rhIGF-I administration in man: differential sensitivity to the metabolic effects of subcutaneous (SC) bolus vs. continuous delivery. Am J Physiol. 272:E349–E355.
10. **Hussain MA, Schmitz O, Mengel A, et al.** 1993 Insulin-like growth factor I stimulates lipid oxidation, reduces protein oxidation, and enhances insulin sensitivity in humans. J Clin Invest. 92:2249–2256.
11. **DiGirolamo M, Eden S, Enberg G, et al.** 1986 Specific binding of human growth hormone but not insulin-like growth factors by human adipocytes. FEBS Lett. 205:15–19.
12. **Berg MA, Guevara-Aguirre J, Rosenbloom AL, Rosenfeld RG, Francke U.** 1992 Mutation creating a new splice site in the growth hormone receptor genes of 37 Ecuadorian patients with Laron syndrome. Hum Mutat. 1:24–32.
13. **Berg MA, Argente J, Chernausek S, et al.** 1993 Diverse growth hormone gene mutations in Laron syndrome. Am J Hum Genet. 52:998–1005.
14. **Amselem S, Duquesnoy P, Attree O, et al.** 1989 Laron dwarfism and mutations of the GH receptor gene. N Engl J Med. 321:989–995.
15. **Woods KA, Dastot F, Preece MA, et al.** 1997 Phenotype: genotype relationships in growth hormone insensitivity syndrome. J Clin Endocrinol Metab. 82:3529–3535.
16. **Kranzler JH, Rosenbloom AL, Martinez V, Guevara-Aguirre J.** 1998 Normal intelligence with severe IGF-I deficiency due to GH receptor deficiency: a controlled study in a genetically homogeneous population. J Clin Endocrinol Metab. 83:1953–1958.
17. **Bachrach LK, Marcus R, Ott SM, et al.** 1998 Bone mineral, histomorphometry, and body composition in adults with GH receptor deficiency. J Bone Miner Res. 13:415–421.
18. **Laron Z.** 1995 Prismatic cases: Laron syndrome (primary growth hormone resistance) from patient to laboratory to patient. J Clin Endocrinol Metab. 80:1526–153.
19. **Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J.** 1994 Growth hormone insensitivity syndrome due to primary GH receptor deficiency. Endocr Rev. 15:369–390.
20. **Growth Hormone Research Society.** 1998 Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency. J Clin Endocrinol Metab. 83:379–381.
21. **Vance ML, Mauras N.** 1999 Growth hormone therapy in adults and children. N Eng J Med. 341:1206–1216.
22. **Laron Z, Klinger B.** 1994 IGF-I treatment of adult patients with Laron syndrome. Clin Endocrinol (Oxf). 41:631–638.
23. **Copeland KC, Kenney FA, Nair KS.** 1992 Heated dorsal hand vein sampling for metabolic studies: a reappraisal. Am J Physiol. 263:E1010–E1014.
24. **Mauras N.** 1995 Combined recombinant human growth hormone and recombinant human insulin-like growth factor I: lack of synergy on whole body protein anabolism in normally fed subjects. J Clin Endocrinol Metab. 80:2633–2637.
25. **Horber FF, Horber-Feyder CM, Krayer S, Schwenk WF, Haymond MW.** 1989 Plasma reciprocal pool specific activity predicts that of intracellular free leucine for protein synthesis. Am J Physiol. 257:E385–E399.
26. **Schwenk WF, Berg PJ, Beaufrere B, Miles JM, Haymond MW.** 1984 Use of *t*-butyldimethylsilylation in gas chromatographic mass spectrometric analysis of physiologic compounds found in plasma using electron impact ionization. Anal Biochem. 141:101–109.
27. **Schoeller DA, Klein PD.** 1979 A microprocessor controlled mass spectrometer for the fully automated purification and isotopic analysis of breath CO_2. Biomed Mass Spectrom. 6:350–355.
28. **Schwenk WF, Beaufrere B, Haymond MW.** 1985 Use of reciprocal pool specific activities to model leucine metabolism in humans. Am J Physiol. 249:E646–E650.
29. **Ferranini E.** 1988 The theoretical bases of indirect calorimetry: a review. Metabolism. 37:287–301.
30. **Durnin JV, Womersley J.** 1974 Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr. 32:77–97.
31. **Mauras N, O'Brien KO, Welch S, et al.** 2000 IGF-I and GH treatment in GH deficient humans**:** differential effects on protein, glucose, lipid and calcium metabolism J Clin Endocrinol Metab. 85:1686–1694.
32. **Mauras N, Hayes V, Welch S, et al.** 1998 Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength and adiposity. J Clin Endocrinol Metab. 83:1886–1892.
33. **Hartman ML, Clayton PE, Johnson ML, et al.** 1993 A low dose euglycemic infusion of recombinant human insulin-like growth factor I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. J Clin Invest. 91:2453–2462.
34. **Fukagawa NK, Minaker KL, Rowe JW, et al.** 1985 Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in post-absorptive men. J Clin Invest. 76:2306–2311.
35. **Frick F, Oscarsson J, Vikman-Adolfsson K, Ottosson M, Yoshida N, Eden S.** 2000 Different effects of IGF-1 on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. Am J Physiol Endocrinol Metab. 278:E729–E737.
36. **Hussain MA, Schmitz O, Mengel A, et al.** 1994 Comparison of the effects of GH and IGF-I on substrate oxidation and on insulin sensitivity in GH-deficient humans. J Clin Invest. 94:1126–1133.
37. **Mauras N, Quarmby V, Bloedow DC.** 1999 Pharmacokinetics of insulin-like growth factor-1 in hypopituitarism: correlation with binding proteins. Am J Physiol Endocrinol Metab. 277:E579–E584.
38. **Grahnen A, Kastrup K, Heinrich U, et al.** 1993 Pharmacokinetics of recombinant human insulin-like growth factor I given subcutaneously to healthy volunteers and to patients with growth hormone receptor deficiency. Acta Paediatr. 391(Suppl):9–13.