Effects of Low-Dose Recombinant Human Insulin-Like Growth Factor-I on Insulin Sensitivity, Growth Hormone and Glucagon Levels in Young Adults With Insulin-Dependent Diabetes Mellitus

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Despite recent interest in the therapeutic potential of recombinant human insulin-like growth factor-I (rhIGF-I) in the treatment of diabetes mellitus, its mechanism of action is still not defined. We have studied the effects of low-dose bolus subcutaneous rhIGF-I (40 μg/kg and 20 μg/kg) on insulin sensitivity, growth hormone (GH) and glucagon levels in seven young adults with insulin-dependent diabetes mellitus (IDDM) using a randomized double-blind placebo-controlled crossover study design. Each was subjected to a euglycemic clamp (5 mmol/L) protocol consisting of a variable-rate insulin infusion clamp (6:00 PM to 8:00 AM) followed by a two-dose hyperinsulinemic clamp (insulin infusion of 0.75 mU · kg^{-1} · min^{-1} from 8 to 10 AM and 1.5 mU · kg^{-1} · min^{-1} from 10 AM to 12 noon) incorporating [6,6 $^2H_2$]glucose tracer for determination of glucose production/utilization rates. Following rhIGF-I administration, the serum IGF-I level (mean ± SEM) increased (40 μg/kg, 655 ± 90 ng/mL, $P < .001$; 20 μg/kg, 472 ± 67 ng/mL, $P < .001$; placebo, 258 ± 51 ng/mL). Dose-related reductions in insulin were observed during the period of steady-state euglycemia (1 AM to 8 AM) (40 μg/kg, 48 ± 5 pmol/L, $P = .01$; 20 μg/kg, 58 ± 8 pmol/L, $P = .03$; placebo, 72 ± 8 pmol/L). The mean overnight GH level (40 μg/kg, 9.1 ± 1.4 mU/L, $P = .04$; 20 μg/kg, 9.6 ± 2.0 mU/L, $P = .12$; placebo, 11.3 ± 1.7 mU/L) and GH pulse amplitude (40 μg/kg, 18.8 ± 2.9 mU/L, $P = .04$; 20 μg/kg, 17.0 ± 3.4 mU/L, $P > .05$; placebo, 23.0 ± 3.7 mU/L) were also reduced. No differences in glucagon, IGF binding protein-1 (IGFBP-1), acetoacetate, or β-hydroxybutyrate levels were found. During the hyperinsulinemic clamp conditions, no differences in glucose utilization were noted, whereas hepatic glucose production was reduced by rhIGF-I 40 μg/kg ($P = .05$). Our data demonstrate that in subjects with IDDM, low-dose subcutaneous rhIGF-I leads to a dose-dependent reduction in the insulin level for euglycemia overnight that parallels the decrease in overnight GH levels, but glucagon and IGFBP-1 levels remain unchanged. The decreases in hepatic glucose production during the hyperinsulinemic clamp study observed the following day are likely related to GH suppression, although a direct effect by rhIGF-I cannot be entirely discounted.

THERE HAS BEEN considerable interest in the potential use of recombinant human insulin-like growth factor-I (rhIGF-I) in the treatment of diabetes mellitus. Recent studies indicate that rhIGF-I therapy can improve insulin sensitivity and glycemic control in patients with severe insulin resistance[1-3] and non–insulin-dependent (type II) diabetes mellitus.[4-7] The precise mechanisms for this effect remain unresolved, and although IGF-I may act through its own receptor or through the insulin receptor, with which it has some degree of affinity, indirect mechanisms of action, particularly on growth hormone (GH) or glucagon secretion, have yet to be excluded.

In recent studies of subjects with insulin-dependent diabetes mellitus (IDDM), a reduction in the insulin requirement for euglycemia[8,9] and an improvement in glycemic control[10,11] have been reported following rhIGF-I administration. Subjects with IDDM commonly have abnormalities of the GH/IGF-I axis including a low serum IGF-I level,[12,13] an abnormal level of IGF binding proteins (IGFBPs),[14] and GH hypersecretion.[15] Previous physiological studies demonstrating that rhIGF-I at relatively low doses could reduce insulin requirements in these patients have attributed these effects to the suppression of GH hypersecretion.[8,9] Nevertheless, direct measures of insulin sensitivity were not used in those studies, and alternative explanations such as changes in the IGFBPs or in glucagon levels were not explored.

To examine the relationships between IGF-I replacement and insulin sensitivity in greater detail, we have performed a placebo-controlled study examining the dose-dependent effects of rhIGF-I on the insulin level for euglycemia and on GH and glucagon levels in young adult subjects with IDDM. In addition, using a stable-isotope hyperinsulinemic clamp technique, we have investigated the effects of rhIGF-I on glucose production and glucose utilization in these subjects.

SUBJECTS AND METHODS

Subjects

Seven young adult subjects with IDDM participated in the study (four males; median age, 19.8 years; range, 17.8 to 24.6; median body mass index, 24.6 kg/m^2; range, 20.7 to 29.6. All were postpubertal and had IDDM of at least 2 years' duration (median duration, 7.8 years; range, 2.1 to 12.3; median C-peptide, 0.057 pmol/L (0.047 to 0.230) at a blood glucose level >7.0 mmol/L). All were in good health with normal renal, hepatic, and thyroid function. All subjects were under treatment with a combination of intermediate- and short-acting insulin two to four times per day (median insulin dosage, 0.84 U · kg^{-1} · d^{-1}; range, 0.64 to 1.04). The median HbA_{1c} value was 10.2% (8.0% to 11.6%). The study protocol was approved by the Central Oxford Research Ethics Committee, and informed consent was obtained from the subjects and their parents. All studies were performed at the John Radcliffe Hospital in Oxford.

Research Design

Each subject was studied on three occasions, each separated by a minimum of 1 week and a maximum of 4 weeks. On each occasion, either placebo, rhIGF-I 20 μg/kg, or rhIGF-I 40 μg/kg was administered subcutaneously in a randomized double-blind manner. The double-

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dummy technique ensured that both subject and investigator were blind to treatment modality, and involved two separate injections of equal volume (equivalent to 20 μg/kg) on each occasion, consisting respectively of placebo alone (placebo study), placebo and rhIGF-I (rhIGF-I 20 μg/kg study), or rhIGF-I alone (rhIGF-I 40 μg/kg study).

All intermediate-acting insulin was discontinued at least 36 hours before each study period, with the blood glucose level controlled by four daily injections of soluble insulin, the last dose given between 12:00 noon and 1:00 PM on the day of the study. Subjects were admitted to the ward at 5:00 PM, and two intravenous cannulae were inserted, one into a distal forearm vein for blood sampling and the other into an antecubital fossa vein for administration of fluids. A standardized carbohydrate meal was given between 5:30 and 6:00 PM, and subjects were thereafter fasted until 12:00 noon the next day. Baseline blood samples were taken before placebo/rhIGF-I injection subcutaneously into the anterior aspect of each thigh at 6:00 PM. The subsequent experimental protocol can be divided into two phases (Fig 1).

Phase 1. A variable-rate insulin infusion was administered overnight between 6:00 PM and 8:00 AM the next morning. The rate was adjusted according to 15-minute blood glucose measurements to maintain a blood glucose level of 5 mmol/L. No glucose was administered during this period.

Phase 2. A two-step hyperinsulinemic-euglycemic clamp was performed in all subjects between 8:00 AM and 12:00 noon the next day. At 5:00 AM, a primed ([6,6 $^2H_2$] glucose 170 mg intravenously) continuous infusion of [6,6 $^2H_2$] glucose (1.7 mg/min) was administered and maintained for the remainder of the study (5:00 AM to 12:00 noon). A 180-minute equilibration period was allowed to achieve steady-state tracer enrichment before commencing the hyperinsulinemic protocol at 8:00 AM. Thereafter, a primed "low insulin infusion" clamp (insulin bolus, 3.5 mU/kg; infusion, 0.75 mU · kg^{-1} · min^{-1}) was performed between 8:00 and 10:00 AM, followed by a primed "high insulin infusion" clamp (insulin bolus, 7.0 mU/kg; infusion, 1.5 mU · kg^{-1} · min^{-1}) between 10:00 AM and 12:00 noon. Throughout each clamp, the blood glucose level was measured at 5-minute intervals and maintained at 5 mmol/L using a variable 20% dextrose infusion. The 20% dextrose was enriched with 7 mg [6,6 $^2H_2$] glucose per gram of dextrose to prevent marked decreases in blood tracer enrichment and consequent calculation errors. Blood samples for measurement of [6,6 $^2H_2$] glucose enrichment were taken every 5 minutes during established steady-state periods (Fig 1; basal period, 7:30 to 8:00 AM; low insulin, 9:30 to 10:00 AM; high insulin, 11:30 AM to 12:00 noon).

Throughout the study period (6:00 PM to 12:00 noon), blood samples were taken at regular intervals for measurement of GH (15 minutes), free insulin (30 minutes), IGF-I, IGFBP-1, acetoacetate), β-hydroxybutyrate (60 minutes), and glucagon (120 minutes). Blood samples were taken from a continuously heparinized cannula inserted into the distal forearm, which was maintained in a heated blanket to arterialize the venous blood.[16] The blood sample volume varied between 0.1 and 5.0 mL depending on requirements, and approximately 325 mL blood was obtained from each subject during each study, which was replaced with an equivalent volume of normal saline. Subjects remained supine throughout and had similar sleep patterns during each study period.

Assay Methods

HbA_{1c} levels were measured by high-performance liquid chromatography (Diamat; BioRad Laboratories, Hemel Hempstead, UK). The intraassay coefficient of variation was 1.9% and 2.2% at HbA_{1c} levels of 6.9% and 11.5%, respectively. The interassay coefficient of variation was 2.7% and 2.3% at HbA_{1c} levels of 7.0% and 11.6%, respectively. The normal range for HbA_{1c} was 4.3% to 6.1%.

C-peptide levels were determined by a double-antibody radioimmunoassay (Diagnostic Products, Euro/DPC, Llanberis, Caernarfon, Wales). The intraassay coefficient of variation was 3.4% and 3.0% at C-peptide levels of 294 and 2,614 pmol/L, respectively, and the interassay coefficient of variation was 10.0% and 1.9% at C-peptide levels of 297 and 2,929 pmol/L, respectively. The whole-blood glucose level was measured bedside using a glucose oxidase method (YSI analyser; Clandon Scientific, Hants, UK).

Samples for GH analysis were initially kept at room temperature until the completion of each study, before centrifugation and separation for storage at −20°C. The plasma GH level was then measured by immunoradiometeric assay (NETRIA; St. Bartholomew's Hospital, London, UK) using the international reference standard 80/505. All samples from each individual were analyzed in the same batch. The intraassay coefficient of variation at GH concentrations of 2.9, 14.3, and 69.4 mU/L was 8.0%, 2.0%, and 3.4%, respectively, and the interassay coefficient of variation at GH concentrations of 3.5, 15.2, and 77.4 mU/L was 9.4%, 7.7%, and 10.5%, respectively. The assay sensitivity was 0.2 mU/L. The pulse detection program, Pulsar, was used to analyze GH profiles, as previously described in studies from our department.[15]

Plasma IGF-I levels were determined by radioimmunoassay after

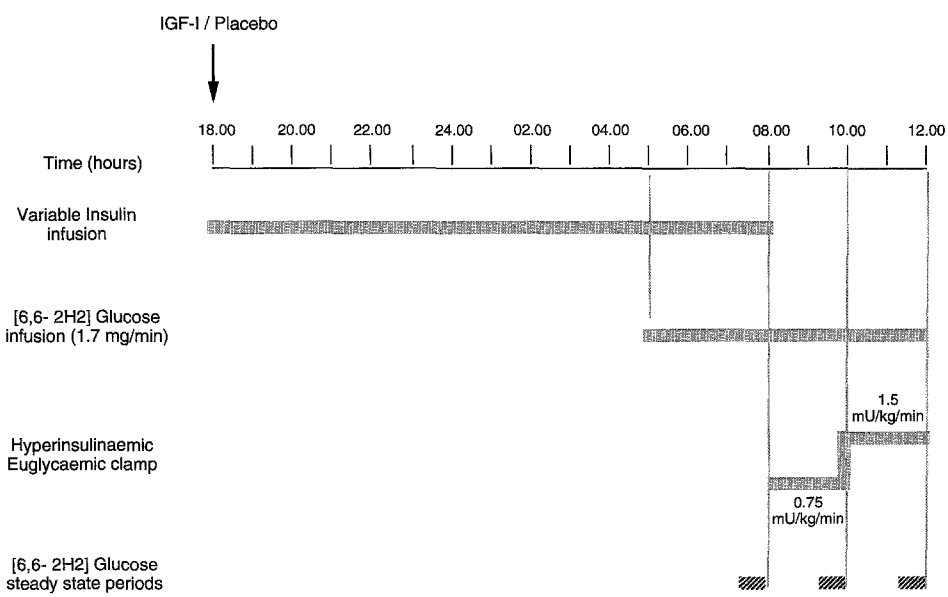

Fig 1. Schematic representation of the study protocol.

acid-ethanol extraction as previously described.[13] The intraassay coefficient of variation was 5.2% and 4.8% at analyte levels of 27.5 and 220 ng/mL, respectively. The interassay coefficient of variation was 12.7% and 10.6% at analyte levels of 77 and 242 ng/mL, respectively.

Free insulin levels were determined by first mixing 1.0 mL whole blood into a 25% solution of polyethylene glycol 6000 (Merck, Dorset, UK) to remove insulin antibodies.[17] This was then centrifuged, and the supernatant was separated and stored at −20°C until assay with a double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). The intraassay coefficient of variation was 8.2%, 4.8%, and 6.3% at 29, 106, and 328 pmol/L, respectively. The interassay coefficient of variation was 11.2%, 9.2%, and 4.7% at 30, 97, and 318 pmol/L, respectively.

The plasma glucagon level was measured by radioimmunoassay at the Radioimmunoassay Core Facility of the Diabetes Research and Training Centre, Washington University School of Medicine (St. Louis, MO). The antibody used for the assay has a high specificity for the COOH-terminal portion of the glucagon molecule. The intraassay and interassay coefficients of variation for the glucagon assay are less than 10% and less than 11%, respectively.

Serum IGFBP-1 levels were measured by a specific radioimmunoassay. Purified antigen and standards were obtained from Dr Sten Drop (Rotterdam, Holland). ^{125}I-labeled IGFBP-1 was prepared by iodination of antigen using the chloramine-T method. Antiserum was used at a final dilution of 1:10,000, which bound approximately 60% of iodinated tracer. The standards were used in a working range of 3.1 to 200 ng/mL. The intraassay coefficient of variation was 10.3% and 9.1% at 9.0 and 353 ng/mL, respectively, and the interassay coefficient of variation was 10.6% and 7.0% at 106 and 253 ng/mL, respectively.

Ketones (acetoacetate and β-hydroxybutyrate) were assayed using a standard technique[18,19] following addition of ice-cold 10% perchloric acid and separation of the samples.

[6,6 $^2H_2$] glucose was determined by gas chromatography–mass spectrometry (HP 5890; Hewlett-Packard, Woking, UK) using selected ion monitoring of a glucose acetate boronate derivative.[20] The ions monitored were of molecular mass 297 and 299 representing $[M\text{-}C^4H^9]^+$ and the corresponding fragment enriched with two deuterium atoms, respectively. The within-assay coefficient of variation for measurement of isotopic enrichment was less than 2%. The rates of glucose appearance (Ra) and disappearance (Rd) from the circulation were calculated using a modified form of the non–steady-state Steele equations,[21] which were adapted by Finegood et al[22] to account for the addition of radiolabeled tracer to the exogenous glucose infusate. The equations have been rederived from first principles for use with stable isotopes.[23]

Statistics

The data are expressed as the mean ± SEM unless otherwise stated. Data were normally distributed except for acetoacetate, β-hydroxybutyrate, and glucagon, which were analyzed after logarithmic transformation. Statistical analysis in the form of paired *t* tests was used to compare mean values for data between the rhIGF-I and placebo studies. ANOVA with repeated measures was used to search for differences with time between and within studies. SPSS (SPSS, Chicago, IL) for MS Windows (release 6.0) was used to perform all analyses. *P* values less than .05 were considered significant.

RESULTS

One subject failed to complete the hyperinsulinemic-euglycemic clamp protocol on two occasions due to technical difficulties, and has therefore been excluded from analysis during this phase. All remaining subjects completed the entire protocol as described, and none experienced any side effects following rhIGF-I administration.

The mean IGF-I level at baseline was similar for each study period (Fig 2A). Following injection of rhIGF-I, the level increased, peaking at 12:00 midnight and remaining elevated for the remainder of the study. The mean peak IGF-I level was 258 ± 51 ng/mL after placebo administration, compared with 472 ± 67 and 655 ± 90 ng/mL after rhIGF-I 20 μg/kg ($P < .001$) and 40 μg/kg ($P < .001$), respectively. The IGF-I level for the entire 18-hour study period was significantly higher following rhIGF-I, with a mean level of 419 ± 64 and 559 ± 63 ng/mL after rhIGF-I 20 μg/kg ($P < .001$) and 40 μg/kg ($P < .001$), respectively, compared with a placebo level of 255 ± 42 ng/mL.

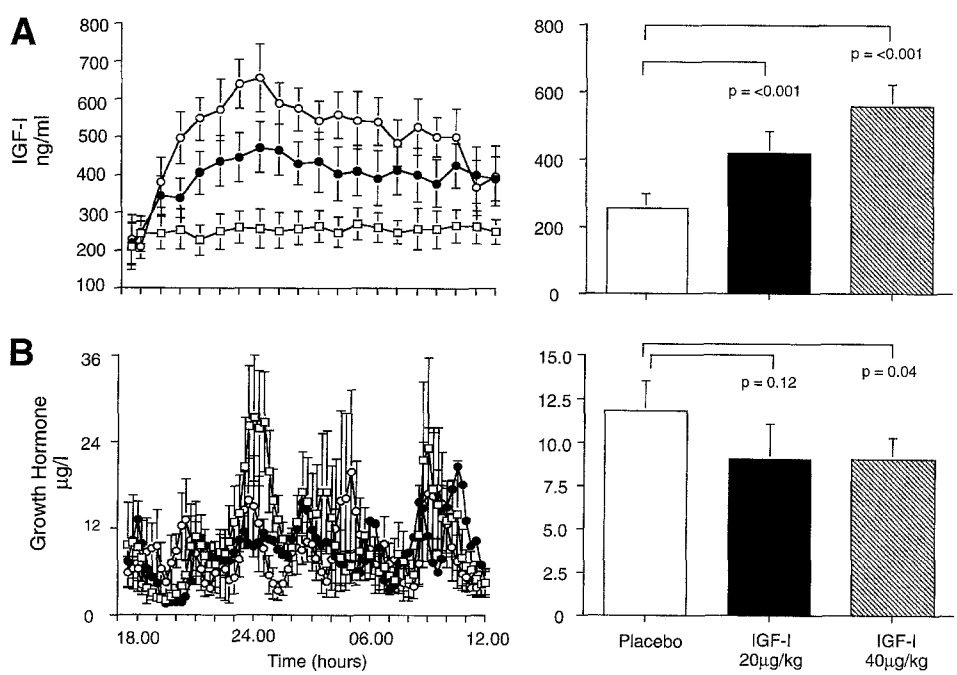

Fig 2. Serum (A) IGF-I and (B) GH levels following placebo (□), rhIGF-I 20 μg/kg (●), and rhIGF-I 40 μg/kg (○). Left, profile (mean ± SEM) between 18.00 and 12.00 h; right, mean ± SEM value for period 18.00-12.00 h.

Table 1. Overnight (8:00 PM to 8:00 AM) GH Pulse Characteristics

Parameter	Placebo		RhIGH-I 20 μg/kg			RhIGF-I 40 μg/kg		
	Mean ± SEM	Range	Mean ± SEM	Range	P*	Mean ± SEM	Range	P*
Baseline (mU/L)	3.5 ± 0.7	1.5-6.9	3.4 ± 1.1	0.9-7.8	.91	1.9 ± 0.4	0.9-3.6	.04
Peaks (n)	4.7 ± 0.4	3-6	4.4 ± 0.4	3-6	.18	4.6 ± 0.4	3-6	.79
Peak amplitude (mU/L)	23.0 ± 3.7	10.7-42.5	17.2 ± 3.4	8.7-34.9	.20	18.8 ± 2.9	9.2-33.2	.04
Peak length (min)	96 ± 6	78-120	86 ± 6	63-114	.33	88 ± 4	77-105	.25
Interpeak interval (min)	159 ± 118	101-235	161 ± 12	124-206	.80	160 ± 10	124-195	.96

*P v placebo.

The mean serum IGF-I level was also significantly different after rhIGF-I 20 μg/kg versus rhIGF-I 40 μg/kg ($P = .002$).

The greatest reduction in the mean GH level over the 18-hour study period was observed following administration of the higher rhIGF-I dose (40 μg/kg), with a mean level of 9.1 ± 1.4 mU/L compared with 11.3 ± 1.7 mU/L on placebo ($P = .04$; Fig 2B). The level after rhIGF-I 20 μg/kg was also reduced at 9.6 ± 2.0 mU/L ($P = .12$), but did not differ significantly versus the placebo studies. The mean overnight GH level (8:00 PM to 8:00 AM) showed the same dose relationship, with 12.1 ± 1.7 mU/L after placebo versus 9.7 ± 2.1 mU/L after rhIGF-I 20 μg/kg ($P = .107$) and 9.2 ± 1.4 mU/L after rhIGF-I 40 μg/kg ($P = .002$). GH pulse characteristics for the period from 8:00 PM to 8:00 AM are summarized in Table 1. Following rhIGF-I 40 μg/kg, significant reductions in both the baseline GH level ($P = .04$) and pulse peak amplitude ($P = .04$) were observed compared with placebo. A reduction in peak amplitude was also evident with rhIGF-I 20 μg/kg; however, due to the greater variability in this group, it was not statistically significant ($P = .20$). No other statistically significant differences were observed in any pulse parameters at either rhIGF-I dose compared with placebo.

Blood glucose profiles during each study night were identical, with a stable steady state achieved by 1:00 AM. The steady-state blood glucose level between 1:00 and 8:00 AM did not differ between placebo studies (5.3 ± 0.1 mmol/L) and studies with rhIGF-I 20 μg/kg (5.3 ± 0.1 mmol/L) and rhIGF-I 40 μg/kg (5.3 ± 0.1 mmol/L). The insulin infusion rate required for maintenance of euglycemia was slightly lower following rhIGF-I, but not statistically different from the placebo (placebo, 0.25 ± 0.04; rhIGF-I 20 μg/kg, 0.22 ± 0.04; and rhIGF-I 40 μg/kg, 0.23 ± 0.03 mU/kg/min). However, the mean plasma free-insulin level during this steady-state period was significantly reduced after both rhIGF-I doses compared with the placebo (Fig 3). A dose-response relationship was observed, with a mean level of free insulin on placebo studies of 72 ± 8 pmol/L compared with 48 ± 5 pmol/L ($P = .01$) and 58 ± 8 pmol/L ($P = .03$) following rhIGF-I 40 μg/kg and 20 μg/kg, respectively. In contrast, glucagon profiles were similar between all three studies (Fig 4). The baseline glucagon level was 65 ± 8, 67 ± 6, and 70 ± 10 ng/L on placebo and rhIGF-I 20 and 40 μg/kg studies, respectively. The glucagon level declined steadily during each study, with a lower level after 18 hours but not statistically different from baseline. The mean level for the entire 18-hour period was not different versus the placebo (54 ± 6 ng/L) following either rhIGF-I 20 μg/kg (58 ± 2 ng/L) or 40 μg/kg (55 ± 8 ng/L). Similarly, the steady-state euglycemia level for IGFBP-1 and the ketone bodies acetoacetate and β-hydroxybutyrate was not statistically different between any of the studies (Table 2).

Blood glucose and free insulin levels achieved during each step of the hyperinsulinemic clamp study are shown in Table 3. No statistically significant differences were observed between the three groups. The 20% dextrose infusion rate required to maintain euglycemia during the low insulin infusion phase was higher following rhIGF-I 40 μg/kg (3.75 ± 0.85 mg · kg^{-1} · min^{-1}), although this was not significantly different versus either the placebo (3.45 ± 0.46 mg · kg^{-1} · min^{-1}) or rhIGF-I 20 μg/kg (3.30 ± 0.59 mg · kg^{-1} · min^{-1}). During the high insulin infusion condition, the glucose infusion rate was higher after both rhIGF-I doses (7.62 ± 0.83 mg · kg^{-1} · min^{-1} after rhIGF-I 20 μg/kg and 8.03 ± 0.57 mg · kg^{-1} · min^{-1} after rhIGF-I 40 μg/kg), although again these were not statistically different from placebo (7.11 ± 1.13 mg · kg^{-1} · min^{-1}).

The glucose Ra, (hepatic glucose production) calculated during steady-state periods decreased with an increasing insulin infusion rate (Fig 5). Although glucose Ra values were lower after rhIGF-I administration during the low-dose insulin infusion compared with placebo (0.73 ± 0.07 and 0.75 ± 0.16 mg · kg^{-1} · min^{-1} after rhIGF-I 20 and 40 μg/kg, respectively, *v* 0.82 ± 0.15 after placebo), this did not reach statistical significance. However, during the high insulin infusion, glucose Ra values were significantly lower after rhIGF-I 40 μg/kg compared with placebo (1.03 ± 0.13 mg · kg^{-1} · min^{-1} for placebo *v* 0.64 ± 0.37 mg · kg^{-1} · min^{-1} for rhIGF-I 20 μg/kg, $P > .05$, and 0.21 ± 0.29 mg · kg^{-1} · min^{-1} for rhIGF-I 40 μg/kg, $P = .04$; Fig 5).

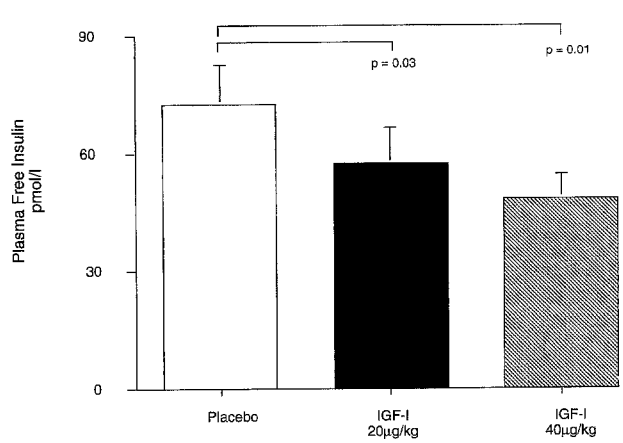

Fig 3. Plasma free insulin levels (mean ± SEM) following administration of placebo, rhIGF-I 20 μg/kg, and rhIGF-I 40 μg/kg during steady-state euglycemia (1 AM to 8 AM).

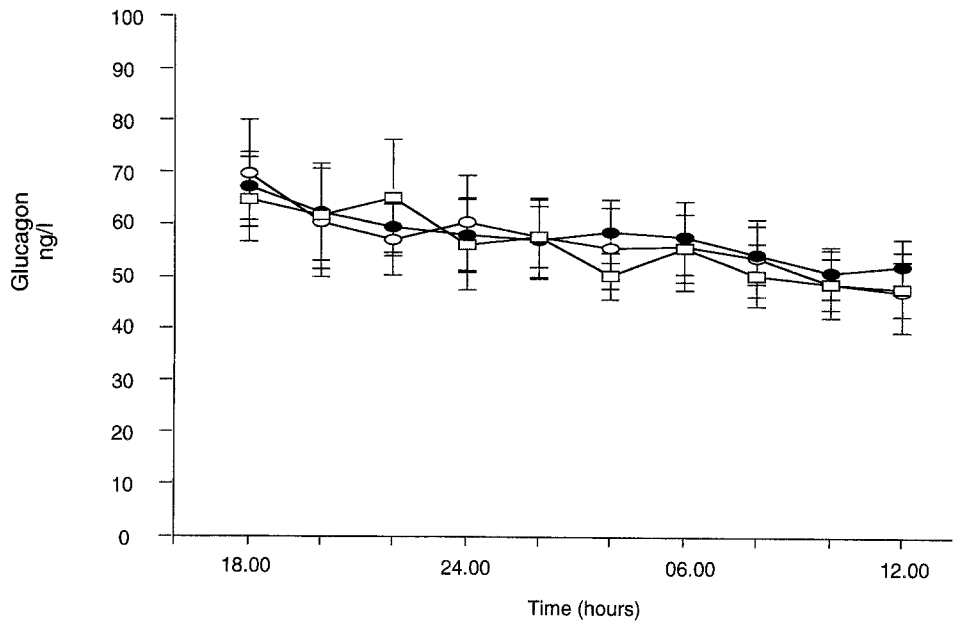

Fig 4. Plasma glucagon profiles (mean ± SEM) following administration of placebo (□), rhIGF-I 20 μg/kg (●), and rhIGF-I 40 μg/kg (○).

The glucose Rd (peripheral glucose utilization) from the circulation increased with an increasing insulin infusion rate. No statistical difference in the Rd glucose was observed following rhIGF-I administration during either low or high insulin infusion conditions (Fig 5). Glucose Rd values during the low insulin infusion phase of the clamp were 4.19 ± 0.28, 3.90 ± 0.32, and 4.24 ± 0.66 mg · kg^{-1} · min^{-1} following placebo, rhIGF-I 20 μg/kg, and rhIGF-I 40 μg/kg, respectively. At high insulin infusion rates, Rd values were higher but not statistically different between studies (placebo, 9.46 ± 0.85 mg · kg^{-1} · min^{-1}, *v* 9.21 ± 0.89 and 7.83 ± 1.23 mg · kg^{-1} · min^{-1} after rhIGF-I 20 and 40 μg/kg, respectively).

IGFBP-1, acetoacetate, and β-hydroxybutyrate levels during the hyperinsulinemic clamp decreased as insulin infusion rates increased. However, no statistical differences in the levels were found between any of the studies, with similar values at each phase of the hyperinsulinemic clamp (data not shown).

DISCUSSION

Serum IGF-I levels measured before administration of rhIGF-I and also during each placebo study were low compared with the normal range for postpubertal adolescents and young adults (255 ± 42 *v* 445 ± 18 ng/mL).[13] Following rhIGF-I administration, IGF-I increased in a dose-dependent manner into the normal and upper-normal range with the 20- and 40-μg/kg dose, respectively. IGF-I levels were maintained for the duration of the study, reflecting the long half-life of subcutaneously injected rhIGF-I.[24] The insulin level required to maintain euglycemia during the period from 1:00 to 8:00 AM also responded in a dose-dependent manner. Mean plasma free insulin levels were significantly reduced by 20% and 33% following injection of rhIGF-I 20 and 40 μg/kg, respectively, compared with placebo. This suggests a significant improvement in insulin sensitivity and, despite being of lower magnitude, is consistent with recent physiological studies performed by Cheetham et al[8] and Bach et al.[9] In their respective studies, rhIGF-I as a single subcutaneous injection (40 μg/kg) or as a subcutaneous infusion (100 to 200 μg/kg/d for 3 consecutive days) resulted in a reduction in plasma insulin levels of approximately 50% and 80%. Our observation that a dose of rhIGF-I as low as 20 μg/kg could improve insulin sensitivity is in keeping with findings from recently reported clinical trials of rhIGF-I therapy in subjects with NIDDM where similar doses were used.[6,7] Nevertheless, during steady-state euglycemia, we did not observe any statistically significant changes in the insulin infusion rate following rhIGF-I, raising the possibility that changes in the plasma free insulin level may have been the result of changes in the underlying insulin clearance rate. Metabolic clearance rates for insulin have been reported to be either reduced or be unchanged following rhIGF-I administration to healthy subjects,[25,26] but have never been directly determined in studies involving subjects with diabetes mellitus. Both Cheetham et al[8] and Bach et al[9] noted significant reductions in the insulin infusion requirement during euglycemia that correlated with the reduced

Table 2. IGFBP-1, Acetoacetate, and β-Hydroxybutyrate Values (mean ± SEM) During Overnight Steady-State Euglycemia Conditions (1:00 to 8:00 AM) (mean ± SD)

Dose	IGFBP-1 (ng/mL)	Acetoacetate (μmol/L)	β-Hydroxybutyrate (μmol/L)
Placebo	57.91 ± 9.89	100 ± 20	100 ± 20
RhIGF-I 20 μg/kg	68.84 ± 12.45	80 ± 10	110 ± 30
RhIGF-I 40 μg/kg	66.99 ± 10.60	120 ± 40	190 ± 80

Table 3. Hyperinsulinemic Clamp Blood Glucose and Free Insulin Levels

	Low Insulin Step (0.75 mU/kg/min)		High Insulin Step (1.5 mU/kg/min)	
	Blood Glucose (mmol/L)	Free Insulin (pmol/L)	Blood Glucose (mmol/L)	Free Insulin (pmol/L)
Placebo	5.1 ± 0.1	237 ± 27	5.1 ± 0.1	517 ± 65
RhIGF-I 20 μg/kg	5.2 ± 0.1	232 ± 69	5.0 ± 0.1	472 ± 66
RhIGF-I 40 μg/kg	5.2 ± 0.1	216 ± 73	4.9 ± 0.1	473 ± 40

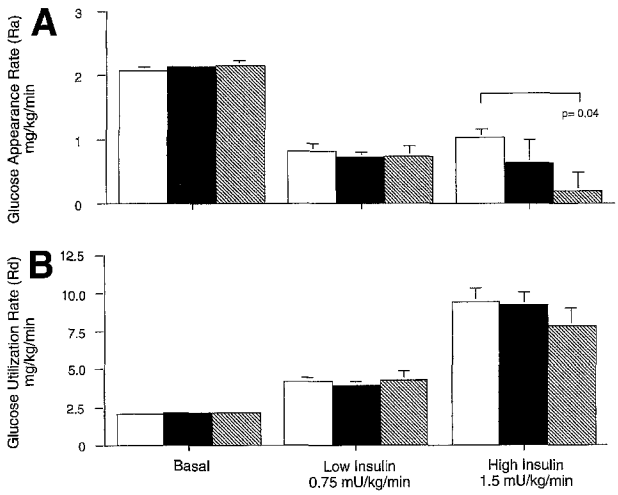

Fig 5. Glucose (A) Ra and (B) Rd values following placebo (□), rhIGF-I 20 μg/kg (■), and rhIGF-I 40 μg/kg (▨) during hyperinsulinemic-euglycemic clamp (8 AM to 12 noon). Mean ± SEM value during hyperinsulinemic steady-state periods: basal (7:30 to 8 AM), low insulin infusion (0.75 mU/kg/min, 9:30 to 10 AM), and high insulin infusion (1.5 mU/kg/min, 11:30 AM to 12 noon).

insulin levels, and changes in the insulin clearance rate were not suspected. While GH[27] and changes in the GH level may also have a role in determining the insulin clearance rate, the relatively small number of subjects in our study may be one reason for the lack of a statistically significant change in the insulin infusion rate following rhIGF-I.

Reductions in the insulin level for euglycemia were accompanied by decreases in the mean overnight (8:00 AM to 8:00 PM) GH level, which, compared with placebo, was reduced on the order of 24% and 19% after rhIGF-I 40 and 20 μg/kg, respectively. Cheetham et al,[8,28] under similar study conditions, reported a larger reduction in the mean overnight GH level on the order of 40% following rhIGF-I 40 μg/kg, whereas Bach et al[9] achieved a reduction on the order of 60% in diabetic subjects. The larger magnitude of GH suppression may explain why a larger reduction in the euglycemic steady-state insulin level was found in their subjects compared with ours, although other differences such as the mean IGF-I level achieved (Cheetham et al,[8] 359 ± 26 ng/mL; Bach et al,[9] 900 to 1,500 ng/mL), the younger age range (median age, 16 years (range, 14 to 18) and 14 to 20 years, respectively), and the higher pretreatment mean GH level in their subjects could all combine to account for the differences.

Our observations following rhIGF-I administration are consistent with the hypothesis that reduced insulin requirements resulting from an improvement in insulin sensitivity are mediated by suppression of GH secretion.[29] IGF-I is known to suppress GH secretion from the hypothalamus/pituitary[30-32] and can indirectly reduce the insulin antagonistic effects exerted by GH. GH alters insulin sensitivity by increasing hepatic glucose production and decreasing peripheral glucose utilization.[33,34] The magnitude, time of onset, and duration of these anti-insulin effects are regulated by changes in GH pulse amplitude rather than changes in baseline GH secretion.[35,36] Fowelin et al[35] showed that GH pulses similar to those typically found in IDDM subjects exert anti-insulin effects that are maximal after 4 to 5 hours and last for up to 7 hours following a pulse. Confirmation of this effect can also be found from studies of the dawn phenomenon in IDDM, where characteristic surges in nocturnal GH secretion correlate with early morning insulin resistance and increasing insulin requirements.[37-39] Administration of rhIGF-I 40 μg/kg to young IDDM subjects has been shown to reduce GH pulse amplitude and baseline secretion, with little change in any other GH pulse characteristic.[8,28] In our study, reductions in GH pulse amplitude were also observed following administration of both rhIGF-I 20 and 40 μg/kg, with no change in either the GH pulse frequency, duration, or pulse interval, consistent with these previous observations and with the mechanism by which improvements in insulin sensitivity could occur. Recently, a direct relationship between insulin requirements and GH levels both before and after rhIGF-I administration has been noted in a much larger cohort of subjects with IDDM.[40]

The hyperinsulinemic clamp studies provide further evidence that rhIGF-I can alter insulin sensitivity in IDDM, as suggested in normal subjects.[41] We observed that hepatic glucose output (Ra) was lower after rhIGF-I administration compared with placebo, although, unexpectedly, this only reached statistical significance during the high-rate insulin infusion phase of the hyperinsulinemic clamp and not during the earlier lower-rate insulin infusion phase. This observation may be related to the actual timing of the clamp procedures, particularly if it is postulated that changes in insulin sensitivity occur maximally up to 5 to 7 hours after suppression of nocturnal GH pulses.[35] Reductions in GH secretion could affect hepatic glucose production either by reducing the direct stimulatory effect of GH on the liver or by indirectly reducing the availability of nonesterified free fatty acids (NEFAs).[42] NEFAs were not measured in this study, so an effect by GH via this route cannot be entirely discounted. It has also been suggested that in normal subjects the effect of rhIGF-I on hepatic glucose production may be due to other factors, and in particular, glucagon may be important in mediating these effects.[43] Few studies of rhIGF-I have included measurements of glucagon levels, and the results are conflicting. Studies in normal human subjects using rhIGF-I infusions by Hussain et al[41] have shown no changes in glucagon levels, whereas Boulware et al[44] reported dose-related reductions of up to 47%. In our study using a lower dose of rhIGF-I in subjects with IDDM, glucagon levels showed no significant changes during the time course of each study, and the overall mean levels were not different compared with placebo.

The effect of rhIGF-I on hepatic glucose production was not statistically significant with the lower 20-μg/kg dose. This may have been due to the smaller reduction in the mean overnight GH level and GH amplitude observed with this dose, although it is noteworthy that a significant reduction in the mean insulin level for overnight euglycemia was achieved earlier in the study. The discrepancy in these observations is intriguing, and raises the question as to whether the changes in insulin sensitivity found earlier in the study protocol were achieved by mechanisms other than those mediated by GH. There is some evidence that rhIGF-I may have a direct effect on insulin sensitivity, as recently suggested by Crowne et al[45] in a study in

which GH levels were replicated on both rhIGF-I and placebo nights in subjects with IDDM. A direct effect by rhIGF-I could account for the reductions in overnight insulin levels, but cannot explain the results found during the hyperinsulinemic clamp studies. A direct action by rhIGF-I on the liver is unlikely, as the adult liver is thought to have a relative paucity of IGF-I receptors.[46] Interaction with the hepatic insulin receptor is also unlikely at these low doses of rhIGF-I, as the affinity of IGF-I for the insulin receptor is only about 1% that of insulin.[47] IGF-I receptors are abundant in muscle, yet we observed no direct effect of rhIGF-I on peripheral glucose disposal (Rd), in contrast to the many studies in both animals[48-50] and healthy humans[26,51] in which rhIGF-I has been shown to stimulate peripheral glucose disposal either alone or in combination with decreased hepatic glucose production.[41,44,52-54] These differences may be explained by the timing of our hyperinsulinemic clamp procedures in relation to administration of rhIGF-I. Inadvertently, the design of our experimental protocol and the timing of the hyperinsulinemic clamps may not have been optimal for detecting the direct actions of rhIGF-I on peripheral glucose disposal, and we can only speculate that if the clamps had been performed earlier during the course of the study these actions may have been much more readily detected. rhIGF-I was injected at 6:00 PM, with peak levels after 6 hours, and although IGF-I levels were still elevated when the hyperinsulinemic clamp studies were commenced (14 hours later), they were already in decline and were much lower than the values achieved in many of the physiological studies in which rhIGF-I infusions were used.

IGFBP-1 levels could also have an important role in modulating IGF-I bioactivity and effects on insulin sensitivity.[55] Yet no differences in the IGFBP-1 response were noted between rhIGF-I and placebo studies, particularly during the overnight euglycemic period from 1:00 to 8:00 AM when free insulin levels were reduced following rhIGF-I administration, and this is in keeping with similar observations by Cheetham et al.[56] In contrast, Baxter et al[57] noted an increase in IGFBP-1 levels following rhIGF-I (100 μg/kg) in normal subjects, but these changes were probably related to the suppression of endogenous insulin secretion, whereas in our subjects insulin levels for euglycemia were sustained throughout. Given the reciprocal relationship between insulin levels and IGFBP-1,[58] the similar IGFBP-1 levels observed despite the reduction in insulin levels following rhIGF-I suggests that IGF-I may suppress IGFBP-1 levels directly.

In our studies, there were no differences in ketone levels despite the reduced free insulin levels during euglycemia. This may be explained by the opposing effects on ketogenesis induced by insulin and GH, with the former primarily determining fasting levels and the latter regulating overnight production rates.[59] A reduction in both GH and insulin levels caused by rhIGF-I would balance out the effects on ketogenesis induced by each, resulting in little change in circulating ketone levels. IGF-I is known to have little direct effect on ketogenesis, reflecting the low number of IGF-I receptors located on the adipocyte[47,60] and the relative resistance of adipose tissue to the actions of IGF-I.[47]

In conclusion, reductions in the insulin level for overnight euglycemia can be observed with a single subcutaneous dose of rhIGF-I as low as 20 and 40 μg/kg in subjects with IDDM. At these doses, there were no changes in either glucagon or IGFBP-1 levels, but dose-dependent reductions in the mean GH level were observed. Using direct measures of insulin sensitivity, we observed reductions in hepatic glucose production, which are compatible with an indirect effect mediated by changes in the overnight GH level. Although changes in peripheral glucose utilization were not observed, there nevertheless remains a possibility that improvements in insulin sensitivity following subcutaneous rhIGF-I administration are mediated in part by direct mechanisms of action.

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