IGF-I Treatment in Adults With Type 1 Diabetes

Effects on Glucose and Protein Metabolism in the Fasting State and During a Hyperinsulinemic-Euglycemic Amino Acid Clamp

Paul V. Carroll, Emanuel R. Christ, A. Margot Umpleby, Ian Gowrie, Nicola Jackson, Susan B. Bowes, Roman Hovorka, Premila Croos, Peter H. Sönksen, and David L. Russell-Jones

Type 1 diabetes is associated with abnormalities of the growth hormone (GH)–IGF-I axis. Such abnormalities include decreased circulating levels of IGF-I. We studied the effects of IGF-I therapy (40 μg · kg^{-1} · day^{-1}) on protein and glucose metabolism in adults with type 1 diabetes in a randomized placebo-controlled trial. A total of 12 subjects participated, and each subject was studied at baseline and after 7 days of treatment, both in the fasting state and during a hyperinsulinemic-euglycemic amino acid clamp. Protein and glucose metabolism were assessed using infusions of [1-^{13}C]leucine and [6-6-$^{2}H_2$]glucose. IGF-I administration resulted in a 51% rise in circulating IGF-I levels (P < 0.005) and a 56% decrease in the mean overnight GH concentration (P < 0.05). After IGF-I treatment, a decrease in the overnight insulin requirement (0.26 ± 0.07 vs. 0.17 ± 0.06 U/kg, P < 0.05) and an increase in the glucose infusion requirement were observed during the hyperinsulinemic clamp (~67%, P < 0.05). Basal glucose kinetics were unchanged, but an increase in insulin-stimulated peripheral glucose disposal was observed after IGF-I therapy (37 ± 6 vs. 52 ± 10 μmol · kg^{-1} · min^{-1}, P < 0.05). IGF-I administration increased the basal metabolic clearance rate for leucine (~28%, P < 0.05) and resulted in a net increase in leucine balance, both in the basal state and during the hyperinsulinemic amino acid clamp (–0.17 ± 0.03 vs. –0.10 ± 0.02, P < 0.01, and 0.25 ± 0.08 vs. 0.40 ± 0.06, P < 0.05, respectively). No changes in these variables were recorded in the subjects after administration of placebo. These findings demonstrated that IGF-I replacement resulted in significant alterations in glucose and protein metabolism in the basal and insulin-stimulated states. These effects were associated with increased insulin sensitivity, and they underline the major role of IGF-I in protein and glucose metabolism in type 1 diabetes. ***Diabetes*** **49:789–796, 2000**

IGF-I shares a 40% sequence homology with human proinsulin and exhibits both insulin-like and anabolic effects (1,2). The availability of recombinant human IGF-I (rhIGF-I) has led to interest in the potential of this peptide in the treatment of a variety of disease states (3–7). Type 1 diabetes has received particular attention, because the relative portal insulin deficiency of this condition is thought to be responsible for the reduced circulating levels of IGF-I (8), which in turn, through decreased negative feedback, leads to increased secretion of growth hormone (GH) (9).

Recent studies have demonstrated improved glycemic control in patients with types 1 and 2 diabetes after IGF-I treatment (10,11). IGF-I administration has also been shown to reduce the GH hypersecretion of adolescents and adults with type 1 diabetes (12,13). In those studies, the reductions in GH secretion were associated with decreased insulin requirements, without alteration in glycemic control, which indicates an increase in insulin sensitivity.

In addition to disordered glucose metabolism, patients with type 1 diabetes have abnormal protein metabolism (14,15), and insulin exerts a net anabolic effect on protein metabolism through reducing the rate of protein breakdown (16–18). Previous studies have investigated the effects of IGF-I infusion on glucose and protein metabolism in healthy subjects (2,4,7,19–21). These studies have demonstrated that although large doses of intravenous IGF-I have effects similar to insulin on glucose metabolism, the effects on protein metabolism are different and are characterized by reductions in circulating amino acid concentrations and whole-body protein flux (19), resulting in a direct stimulation of protein synthesis only when amino acid concentrations are maintained (2).

The effects of IGF-I therapy on glucose and protein metabolism in type 1 diabetes have yet to be fully explored. By use of stable isotope techniques, we examined the effects of IGF-I therapy on protein and glucose metabolism in adults with type 1 diabetes. Subjects were studied in the

From the Division of Medicine (P.V.C., E.R.C., A.M.U., N.J., S.B.B., P.C., P.H.S., D.L.R.-J.), Guy's, King's, and St Thomas' Campus, St Thomas' Hospital; and the Centre for Measurement and Information in Medicine (I.G., R.H.), City University, London, U.K.

Address correspondence and reprint requests to Dr. Paul V. Carroll, Department of Endocrinology, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, U.K.

Received for publication 7 June 1999 and accepted in revised form 21 January 2000.

P.H.S. and D.L.R.-J. have received research funding and honoraria for speaking engagements from Pharmacia and Upjohn.

Additional information can be found in an online appendix at www.diabetes.org/diabetes/appendix.asp.

CV, coefficient of variation; GH, growth hormone; GIR, glucose infusion rate; KIC, ketoisocaproic; MCR, metabolic clearance rate; NEFA, nonesterified free fatty acid; NOLD, nonoxidative leucine disposal; OX, oxidation rate; R_a, rate of appearance; R_d, rate of disposal; REE, resting energy expenditure; rhIGF-I, recombinant human IGF-I; RIA, radioimmunoassay; RQ, respiratory quotient; TTR, tracer-to-tracee ratio.

TABLE 1
Patient demographics and characteristics

Randomization and subject number	Sex	Age (years)	Weight (kg)	HbA_{1c} (%)	BMI (kg/m^2)	Duration of diabetes (years)	Insulin requirement ($U \cdot kg^{-1} \cdot day^{-1}$)
IGF-I							
1	M	39	67	7.3	22.9	20	0.68
2	F	27	62	8.2	21.8	7	0.87
3	M	45	99.5	9.8	30.4	11	0.73
4	M	26	57.5	11.5	19	22	0.87
5	M	31	73.5	10.7	23.7	5	0.68
6	F	28	71	9.4	24.4	12	0.45
Mean ± SE		32.7 ± 3.1	71.8 ± 6.0	9.5 ± 0.6	23.7 ± 1.6	12.8 ± 2.8	0.71 ± 0.06
Placebo							
7	F	44	58.5	6.9	21	12	0.99
8	M	26	88.4	7	27.9	20	0.84
9	M	22	65.9	6.5	21.8	16	0.58
10	F	30	63	7.1	22.5	16	0.60
11	F	22	63.8	9.8	22.3	2	0.88
12	F	29	85.3	11	30.6	16	0.87
Mean ± SE		28.8 ± 3.3	70.8 ± 5.2	8.1 ± 0.76	24.4 ± 1.6	13.7 ± 2.6	0.79 ± 0.07

fasting state and during a hyperinsulinemic-euglycemic clamp. Importantly, because it has been previously demonstrated that maintenance of circulating amino acids is necessary to allow detection of an anabolic effect of IGF-I (2), the plasma amino acids were also clamped during the insulin infusion.

RESEARCH DESIGN AND METHODS

Experimental subjects. A total of 12 adults with type 1 diabetes participated in this study. Their clinical and metabolic details are outlined in Table 1. The subjects were in good general health, were without advanced complications of diabetes, and had normal thyroid, renal, and hepatic function. All of the patients had a C-peptide concentration of ≤0.3 nmol/l (in the presence of a plasma glucose level ≥7 mmol/l), indicating insulin dependence. The study was approved by the Ethics Committee, Guy's and St Thomas' National Health Service Trust, and all patients provided informed written consent.

Study protocol. The study was randomized, double-blind, and placebo-controlled (Fig. 1). Patients were instructed in self-administration of IGF-I (Pharmacia and Upjohn, Milton Keynes, U.K.) and subcutaneously injected 40 $\mu g \cdot kg^{-1} \cdot day^{-1}$ of IGF-I or placebo at night (10:00 P.M.) for 7 consecutive days. This time was chosen to coincide with the usual injection time of the patients' daily isophane (NPH) insulin administration.

Identical metabolic investigations were performed before (study 1) and after (study 2) 1 week of IGF-I/placebo treatment. On each occasion, all long-acting insulin had been discontinued at least 16 h before admission. Empirically, with the first dose of IGF-I/placebo, the usual dose of evening isophane (NPH) insulin was decreased by 50%. The patients remained in daily contact with the investigators, and insulin doses were adjusted daily, based on blood glucose monitoring results.

On the days of study, the subjects were admitted to the metabolic ward at 7:00 P.M., having last eaten at 3:00 P.M. They were studied in a semirecumbent position and were allowed to drink water. An indwelling cannula was placed in a superficial vein of the antecubital fossa for administration of infusates (isotope tracers, insulin, glucose, and amino acids), and another venous cannula was placed in the contralateral arm for blood sampling. Venous blood, rather than arterial or arterialized blood, was used for the collection of the plasma hormone and substrate concentration and enrichment samples.

At 10:00 P.M., a primed (1 mg/kg) constant infusion of L-[1-^{13}C]leucine ($1 mg \cdot kg^{-1} \cdot h^{-1}$, enrichment 99%) (Tracer Technologies, Somerville, MA) was commenced and maintained until 11:30 A.M. the following day. At 6:00 A.M., a primed (107 mg) constant (102 mg/h) infusion of [6-6-$^2H_2$]glucose was commenced and continued until 11:30 A.M. A variable intravenous infusion of soluble insulin (Human Actrapid; Novo Nordisk, Copenhagen) was administered between 6:00 P.M. and 9:00 A.M. to maintain blood glucose levels between 4 and 7 mmol/l, and insulin requirements to maintain euglycemia were recorded. Between 9:00 and 11:30 A.M., a hyperinsulinemic ($1.5 mU \cdot kg^{-1} \cdot min^{-1}$), euglycemic (5 mmol/l), euleucinemic clamp was performed. Plasma glucose concentrations were maintained with an infusion of 20% glucose derived from potato starch, which is recognized to have low ^{13}C enrichment (22). The glucose infusion was spiked with 7 mg [6-6-$^2H_2$]glucose per gram cold glucose to maintain steady enrichment values. The plasma leucine was clamped at the baseline level (immediately before the beginning of the hyperinsulinemic-euglycemic clamp) by use of an infusion containing mixed amino acids (Vamin 14 EF; Pharmacia and Upjohn), according to the method of Russell-Jones et al. (2). The glucose infusion requirement to maintain euglycemia and the requirement of Vamin to maintain euleucinemia were recorded.

At 10:00 P.M., baseline blood samples were taken to measure isotope background enrichment and the plasma concentrations of glucose, amino acids, and hormones. Blood glucose concentrations were measured every 15–30 min throughout the night and every 5 min during the hyperinsulinemic-euglycemic clamp. GH concentrations were measured every 30 min from 10:00 P.M. to 7:30 A.M. Between 8:15 and 8:45 A.M., blood samples were taken for the measurements of the steady-state enrichments of α-ketoisocaproic (KIC) acid and glucose, along with amino acid concentrations. Nonesterified free fatty acid (NEFA) concentrations were measured before and every 15 min during the final hour of the hyperinsulinemic clamp. Baseline breath samples were taken at 10:00 P.M., from 8:15 to 8:45 A.M., and during the last 30 min of the hyperinsulinemic clamp for the measurement of ^{13}C enrichment of expired CO_2. Resting energy expenditure (REE), respiratory quotient (RQ), O_2 consumption, and the CO_2 production rate were assessed by indirect calorimetry at 8:00 A.M. and between 11:00 and 11:30 A.M. using a computerized open-loop gas analyzer system (Medical Graphics, St. Paul, MN).

Assays. GH was measured by a double-antibody radioimmunoassay (RIA) with a detection limit of 0.3 mU/l. The intra-assay coefficients of variation (CVs) were 10.0, 4.0, and 5.4% at 1.7, 12.1, and 22.2 mU/l, respectively. Total levels of IGF-I were measured by double-antibody RIA after acid/ethanol extraction (intra-assay CV 6%). Insulin was assayed using a double-antibody RIA with an interassay CV <9% and an intra-assay CV <6% (23). The plasma amino acid profile was measured using an Alpha Plus II automated amino acid analyzer (Pharmacia, Cambridge, U.K.). Plasma samples were deproteinized before analysis by use of 10% sulfosalicylic acid containing norleucine as an internal standard. NEFA levels were measured with an enzymatic method using a commercially available kit (Wako Laboratories, Neuss, Germany) on a Cobas Fara autoanalyzer with an interassay CV of 3.6%.

Glucose enrichment was determined by gas chromatography (Hewlett Packard 5890, Berkshire, U.K.)–mass spectrometry (VG TRIO II; Biotech, Cheshire, U.K.) using the penta-*O*-trimethylsilyl-*O*-methyloxime derivative monitoring the ions 319 and 321 for the unlabeled and D-[6-6-$^2H_2$]glucose, respectively (24). α-KIC acid enrichment and concentration were measured using ^{3}D-KIC as the internal standard as the quinoxalinol-tertbutyl dimethylsilyl derivative under selected ion monitoring by gas chromatography–mass spectrometry (MSD5971A; Hewlett Packard) at 259, 260, and 262 m/z. ^{13}C enrichment of breath CO_2 was measured on a VG SIRA series II isotope ratio mass spectrometer (VG Isotech, Cheshire, U.K.).

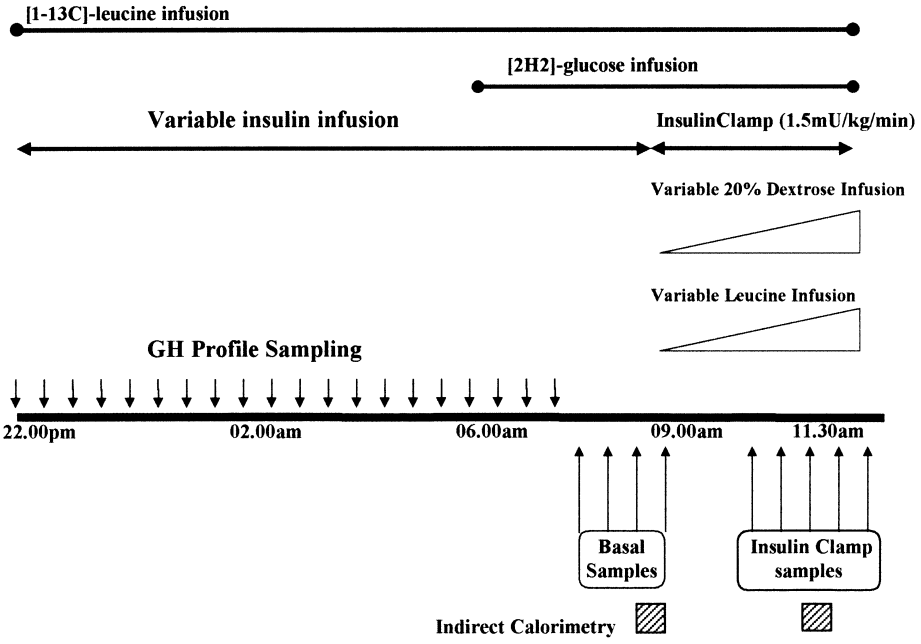

FIG. 1. The study protocol. Patients were admitted at 7:00 P.M. after a 4-h fast. Isophane (NPH) insulin had been omitted for at least 16 h. A variable insulin infusion was administered between 7:00 P.M. and 9:00 A.M. Breath samples for measurement of CO_2 were taken at the same time as plasma samples.

Calculations. Rates of leucine endogenous appearance (R_a) and leucine disposal (R_d) were calculated using the model originally proposed by Steele but modified for stable isotopes (25). Although the plasma leucine concentrations were maintained throughout the insulin clamps, we adopted the nonsteady-state equations to correct for minor variations in substrate concentration. By doing so, we reduced the possibility of introducing bias in the calculations. Reciprocal pool model calculations were adopted, and plasma KIC enrichment was used as an index for leucine intracellular enrichment. Because KIC is produced only by leucine in intracellular space, it is reasonable to assume that plasma KIC tracer-to-tracee ratios (TTRs) accurately reflect those of intracellular leucine (26). TTRs (z) were calculated from isotope ratio data and were corrected for the presence of endogenous ^{13}C and ^{3}D-KIC (used as an internal standard to calculate plasma KIC concentration); details of these calculations are included in an appendix that is available on the *Diabetes* website (www.diabetes.org/diabetes/appendix.asp) or on request from the author. The modified Steele equations (25) used for calculating leucine R_a and R_d are as follows:

$$R_{aT} = \frac{i_K}{z} - \frac{\dot{z} \cdot L \cdot p \cdot V}{z \cdot (1 + z)}$$

$$R_d = R_{aT} - \dot{L} \cdot p \cdot V + i_K$$

$$R_a = R_{aT} - i_V$$

where p denotes the ratio of effective leucine space to actual space (taken as 0.65); V denotes the volume of actual leucine space (taken as 0.20 l/kg); L denotes cold leucine concentration (μmol/l); i_K represents the infusion rate of [^{13}C]leucine (μmol · kg^{-1} · min^{-1}); i_V represents the infusion of cold leucine due to Vamin infusion to maintain the euleucinemic clamp; z denotes the TTR of KIC (unitless). R_{aT} represents the total rate of leucine appearance; R_a corrects R_{aT} for the infusion of cold leucine and represents the rate of endogenous leucine appearance from protein breakdown; R_d represents the rate of leucine disposal. Units for R_{aT}, R_a, and R_d are micromoles per kilogram per minute. To obtain values between 2 measurements and time derivatives, the following calculations were performed:

$$L = \frac{L_1 + L_2}{2} \qquad L = \frac{L_2 - L_1}{t_2 - t_1}$$

$$z = \frac{z_2 + z_1}{2} \qquad z = \frac{z_2 - z_1}{t_2 - t_1}$$

Leucine oxidation rate (OX) was calculated as

$$OX = (APE_{CO_2} \times CO_2R_a)/APE_{KIC}$$

where CO_2R_a is the production rate of CO_2 (mmol/min), and APE_{CO_2} is the enrichment of expired CO_2. Nonoxidative leucine disposal (NOLD), a measure of protein synthesis, was calculated as the difference between R_d and OX. The leucine metabolic clearance rate (MCR) was calculated as MCR = $R_d/[L]$ where [L] is the plasma concentration of leucine. Net leucine balance was calculated as NOLD – endogenous leucine R_a (expressed in micromoles per kilogram per minute).

Protein oxidation was derived from leucine oxidation assuming that 580 μmol of leucine is oxidized per gram of protein oxidation. Rates of substrate utilization were then calculated using standard equations (27).

TTRs (z_G) of glucose were calculated from isotope ratio data. Details of these calculations are included in an appendix that is available on the *Diabetes* website (www.diabetes.org/diabetes/appendix.asp) or on request from the author. Calculations for determining glucose rates of appearance (R_a) and disposal (R_d) were similar to those described previously for leucine.

Statistical analyses. Data are presented as means ± SE. Statistical analysis was performed using analysis of variance with post hoc comparison using Fisher's least squares difference test or, where appropriate, paired or unpaired t tests (two-tailed). Data that were not normally distributed were log-transformed before analysis. P values <0.05 were considered significant.

RESULTS

Clinical. The baseline clinical and metabolic characteristics were similar in the 2 groups (Table 1). All subjects tolerated IGF-I without serious side effects, and no patients were prematurely withdrawn from the study. Subject 9 was admitted to the hospital the day after study 1, after experiencing an episode of hypoglycemia. This subject had been randomized to placebo.

IGF-I. Plasma IGF-I concentration was similar in the 2 groups during study 1 (measured at 9:00 A.M.). Those subjects who received IGF-I showed a rise in circulating total IGF-I levels during study 2, whereas there were no changes in IGF-I levels in those subjects who received placebo (IGF-I group 28.2 ± 2.4 vs. 42.7 ± 3.8 nmol/l, pre- vs. posttreatment, $P < 0.004$; placebo group 34.2 ± 4.0 vs. 29.3 ± 3.2 nmol/l, pre- vs. posttreatment).

Overnight GH secretion and insulin requirements. The mean overnight GH concentration was significantly reduced after IGF-I therapy, but no change was observed after placebo (IGF-I group 23.6 ± 3.8 vs. 10.5 ± 1.3 mU/l, pre- vs. posttreatment, $P < 0.05$; placebo group 13.5 ± 1.8 vs. 14.3 ± 1.6 mU/l, pre- vs. posttreatment). The overnight insulin requirement to maintain euglycemia (5 mmol/l) was decreased after

TABLE 2
Plasma amino acid concentrations in the basal (fasting) state and at the end of the hyperinsulinemic-euglycemic clamp

Amino acid (μmol/l)	IGF-I group				Placebo group			
	Pretreatment		Posttreatment		Pretreatment		Posttreatment	
	Basal	Clamp	Basal	Clamp	Basal	Clamp	Basal	Clamp
Aspartate	5 ± 2	13 ± 2	5 ± 2	6 ± 2	7 ± 2	8 ± 5	25 ± 17	37 ± 14
Threonine	112 ± 17	163 ± 29	108 ± 12	128 ± 31	111 ± 16	189 ± 10*	109 ± 17	196 ± 37
Serine	115 ± 14	148 ± 13	112 ± 5	139 ± 15	125 ± 14	161 ± 13	134 ± 9	198 ± 32
Glutamate	69 ± 6	41 ± 7†	50 ± 9	55 ± 10	41 ± 4‡	59 ± 12	39 ± 6‡	40 ± 11
Glutamine	600 ± 54	603 ± 25	631 ± 83	617 ± 85	677 ± 72§	723 ± 51	693 ± 63§	750 ± 96
Glycine	219 ± 37	351 ± 32†	216 ± 25	372 ± 53	275 ± 46	415 ± 33	352 ± 64	530 ± 95
Alanine	184 ± 58	425 ± 43†	203 ± 24	466 ± 81‖	317 ± 51	635 ± 85*	264 ± 18	638 ± 49¶
Valine	183 ± 19	280 ± 48	151 ± 9†	234 ± 40	176 ± 23	320 ± 42*	160 ± 8	304 ± 31¶
Cysteine	51 ± 5	53 ± 4	50 ± 3	51 ± 7	49 ± 4	57 ± 2	45 ± 4	53 ± 7
Methionine	14 ± 5	66 ± 15	12 ± 4	58 ± 8‖	13 ± 4‡§	70 ± 10*	14 ± 2	72 ± 10¶
Isoleucine	33 ± 6	66 ± 18	35 ± 9	58 ± 9	27 ± 3	72 ± 12*	37 ± 5	71 ± 13¶
Leucine	137 ± 13	153 ± 26	118 ± 13†	130 ± 20	123 ± 14	172 ± 22	132 ± 8	163 ± 21
Tyrosine	23 ± 10	8 ± 7	18 ± 10	5 ± 4	26 ± 7§	26 ± 2	28 ± 4	21 ± 2
Phenylalanine	49 ± 3	110 ± 24	37 ± 12†	87 ± 17‖	39 ± 4	128 ± 20*	38 ± 5	129 ± 14¶
Ornithine	43 ± 7	47 ± 2	44 ± 3	51 ± 6	36 ± 5	49 ± 5	46 ± 10	64 ± 18
Lysine	15 3 ± 8	242 ± 39†	145 ± 6†	242 ± 37	153 ± 23	325 ± 41*	141 ± 9	307 ± 25¶
Histidine	58 ± 5	109 ± 13†	51 ± 3	101 ± 8‖	59 ± 5	148 ± 16*	56 ± 3	142 ± 8¶
Arginine	17 ± 14	126 ± 16†	14 ± 14	115 ± 15	38 ± 14	232 ± 39*	59 ± 10	170 ± 19¶

Data are means ± SE. *P < 0.01 vs. pretreatment basal (placebo); †P < 0.05 vs. pretreatment basal (IGF-I); ‡P < 0.05 vs. pretreatment basal (IGF-I); §P < 0.05 vs. posttreatment basal (IGF-I); ||P <0.05 vs. posttreatment basal (IGF-I); ¶P < 0.05 vs. posttreatment basal (placebo).

IGF-I treatment, but no change was detected after placebo treatment (IGF-I group 0.26 ± 0.07 vs. 0.17 ± 0.06 U/kg, pre- vs. posttreatment, P = 0.048; placebo group 0.22 ± 0.04 vs. 0.24 ± 0.03 U/kg, pre- vs. posttreatment). The insulin concentrations are listed in Table 3. At 9:00 A.M., the plasma insulin levels were similar during both studies in each group. The insulin levels rose throughout the hyperinsulinemic clamp, and no differences were recorded between groups or after treatment with IGF-I/placebo (Table 4).

Vamin and glucose infusions during the hyperinsulinemic clamp. The Vamin infusion requirement (average rate infused to maintain baseline plasma leucine concentration) was similar in the 2 groups during study 1. Those subjects randomized to receive IGF-I required more Vamin (P < 0.05) (Fig. 2) during the insulin clamp during study 2, whereas no change was seen in the placebo group (Fig. 2). The average glucose infusion rate (GIR) required to maintain euglycemia was similar in the 2 groups at baseline. There was no alter-

TABLE 3
Whole-body leucine kinetics after the overnight fast (basal) and during the hyperinsulinemic, euglycemic, euleucinemic clamp (insulin-stimulated) in subjects after either IGF-I (n = 6) or placebo (n = 6)

	Basal			Insulin-stimulated		
	Pretreatment	Posttreatment	P	Pretreatment	Posttreatment	P
IGF-I						
Leucine R_d (μmol · kg^{-1} · min^{-1})	1.84 ± 0.03	1.83 ± 0.04	NS	2.39 ± 0.08*	2.38 ± 0.14†	NS
Leucine R_a (μmol · kg^{-1} · min^{-1})	1.72 ± 0.04	1.71 ± 0.04	NS	1.52 ± 0.08*	1.42 ± 0.10†	NS
Leucine OX (μmol · kg^{-1} · min^{-1})	0.30 ± 0.03	0.22 ± 0.02	0.009	0.62 ± 0.07*	0.56 ± 0.04†	NS
Nonoxidative leucine R_d (μmol · kg^{-1} · min^{-1})	1.55 ± 0.06	1.61 ± 0.06	NS	1.77 ± 0.04*	1.82 ± 0.10†	NS
Leucine MCR (ml · min^{-1} · kg^{-1})	13.7 ± 0.8	17.6 ± 1.4	0.028	18.0 ± 2.4	18.2 ± 2.2	NS
Net leucine balance (μmol · kg^{-1} · min^{-1})	–0.17 ± 0.03	–0.10 ± 0.02	0.009	0.25 ± 0.08‡	0.40 ± 0.06§	0.023
Placebo						
Leucine R_d (μmol · kg^{-1} · min^{-1})	1.87 ± 0.05	1.78 ± 0.06	NS	2.40 ± 0.07‖	2.28 ± 0.06¶	NS
Leucine R_a (μmol · kg^{-1} · min^{-1})	1.75 ± 0.05	1.66 ± 0.06	0.05	1.34 ± 0.06‖	1.23 ± 0.07¶	0.009
Leucine OX (μmol · kg^{-1} · min^{-1})	0.35 ± 0.04	0.28 ± 0.02	NS	0.57 ± 0.06‖	0.63 ± 0.14¶	NS
Nonoxidative leucine R_d (μmol · kg^{-1} · min^{-1})	1.52 ± 0.07	1.51 ± 0.07	NS	1.84 ± 0.03‖	1.65 ± 0.11	NS
Leucine MCR (ml · min^{-1} · kg^{-1})	14.9 ± 1.3	14.6 ± 1.1	NS	15.6 ± 1.3	15.9 ± 1.2	NS
Net leucine balance (μmol · kg^{-1} · min^{-1})	–0.23 ± 0.04	–0.15 ± 0.02	NS	0.50 ± 0.04#	0.42 ± 0.10**	NS

Data are means ± SE. *P < 0.05 vs. basal pretreatment (IGF-I); †P <0.05 vs. basal posttreatment (IGF-I); ‡P <0.001 vs. basal pretreatment (IGF-I); §P < 0.001 vs. basal posttreatment (IGF-I); ||P < 0.05 vs. basal pretreatment (placebo); ¶P < 0.05 vs. basal posttreatment (placebo); #P < 0.001 vs. basal pretreatment (placebo); **P < 0.01 vs. basal posttreatment (placebo).

TABLE 4
REE, RQ, carbohydrate, fat and protein oxidation, NEFA, and insulin levels in the basal and insulin-stimulated states before and after IGF-I or placebo therapy

	Basal			Insulin-stimulated		
	Pretreatment	Posttreatment	*P*	Pretreatment	Posttreatment	*P*
IGF-I						
REE (kcal · kg^{-1} · day^{-1})	28.8 ± 2.5	25.1 ± 2.0	NS	35.3 ± 2.3*	36.5 ± 1.8†	NS
RQ	0.83 ± 0.03	0.77 ± 0.01	NS	0.84 ± 0.04	0.88 ± 0.05	NS
Carbohydrate oxidation (mg · kg^{-1} · min^{-1})	1.83 ± 0.86	1.26 ± 0.74	NS	2.61 ± 1.41	2.96 ± 1.49†	NS
Fat oxidation (mg · kg^{-1} · min^{-1})	0.71 ± 0.25	0.95 ± 0.18	NS	0.85 ± 0.18	0.63 ± 0.52	NS
Protein oxidation (mg · kg^{-1} · min^{-1})	0.50 ± 0.05	0.38 ± 0.03	0.01	0.98 ± 0.11*	0.95 ± 0.08†	NS
NEFA (mmol/l)	1.07 ± 0.20	0.85 ± 0.16	NS	0.22 ± 0.05*	0.13 ± 0.09†	0.02
Plasma insulin (mU/l)	33.2 ± 7.2	31.0 ± 7.4	NS	183 ± 32*	159 ± 27†	NS
Placebo						
REE (kcal · kg^{-1} · day^{-1})	31.1 ± 1.7	29.8 ± 1.8	NS	36.8 ± 4.1	35.4 ± 5.7	NS
RQ	0.80 ± 0.02	0.78 ± 0.01	NS	0.82 ± 0.03	0.83 ± 0.03	NS
Carbohydrate oxidation (mg · kg^{-1} · min^{-1})	1.70 ± 0.37	0.74 ± 0.19	NS	1.96 ± 0.46	2.31 ± 0.99	NS
Fat oxidation (mg · kg^{-1} · min^{-1})	0.83 ± 0.14	1.06 ± 0.13	NS	0.70 ± 0.12	0.72 ± 0.24	NS
Protein oxidation (mg · kg^{-1} · min^{-1})	0.60 ± 0.06	0.46 ± 0.03	NS	0.96 ± 0.11‡	1.07 ± 0.24§	NS
NEFA (mmol/l)	1.02 ± 0.14	0.95 ± 0.13	NS	0.15 ± 0.06‡	0.18 ± 0.10	NS
Plasma insulin (mU/l)	40.4 ± 7.0	28.2 ± 6.4	NS	161 ± 19‡	155 ± 20§	NS

Data are means ± SE. NEFA levels are an average of 4 values taken at 15-min intervals during the last 60 min of the clamp. *P < 0.05 vs. pretreatment basal (IGF-I); †P < 0.05 vs. posttreatment basal (IGF-I); ‡P <0.05 vs. pretreatment basal (placebo); §P < 0.05 vs. posttreatment basal (placebo).

ation in the GIR after placebo, but the GIR was increased after IGF-I treatment (P < 0.05) (Fig. 2).

Glucose kinetics. Both glucose R_a and R_d were similar in both groups during study 1 and remained unchanged after both placebo and IGF-I treatment in the basal state (Fig. 3*A* and *B*). The hyperinsulinemic-euglycemic clamp resulted in a decrease in glucose R_a and an increase in glucose R_d in the baseline study in both patient groups (Fig. 3*A* and *B*). Placebo therapy did not alter glucose kinetics, but, although the insulin-mediated suppression of glucose R_a was unaltered, a significant increase in glucose R_d was observed in the IGF-I–treated patients during the clamp (P < 0.05) (Fig. 3*B*).

Amino acids. The plasma amino acid concentrations (including leucine) were similar between the 2 groups at baseline and were maintained during the insulin clamp studies. Significant reductions in the circulating plasma concentrations of the essential amino acids (i.e., valine, leucine, phenylalanine, and lysine) were recorded after IGF-I therapy (P < 0.05) (Table 2). There were no changes in the fasting amino acids in the subjects who received placebo (Table 2). The amino acid concentrations, leucine levels in particular, were well maintained during the insulin infusions, with similar profiles obtained on each study occasion (Table 2). Increases were observed in several of the amino acids during the Vamin infusion throughout the hyperinsulinemic clamp, including the amino acids alanine, valine, methionine, lysine, histidine, and arginine, as shown in Table 2.

Leucine kinetics. In the basal (fasted) state, the rate of NOLD, leucine R_a, and the MCR of leucine were similar in the 2 groups (Table 3). The decreased plasma leucine concentration after IGF-I treatment was attributed to an increase in the leucine MCR (P < 0.05) with no change in the rate of leucine R_a (protein breakdown) (Table 3). A decrease in leucine oxidation was observed in the subjects randomized to IGF-I treatment (P < 0.01) (Table 3), resulting in an increase in basal net leucine balance in the IGF-I–treated patients but not in the placebo-treated patients (Table 3).

The insulin infusion altered leucine kinetics, but the basal plasma leucine concentration was successfully maintained in both placebo- and IGF-I–treated groups during each study (Table 2). In both groups, the combination of the hyperinsulinemic clamp and amino acid infusion increased leucine oxidation, decreased endogenous leucine R_a (proteolysis), and maintained NOLD (protein synthesis) (Table 3). IGF-I administration resulted in a further decrease in endogenous leucine R_a during the hyperinsulinemic clamp, but no changes were observed in the subjects who received placebo. These changes resulted in an increase in net leucine balance in the IGF-I–treated patients but not in the placebo-treated patients during the insulin infusion (with provision of amino acids) (Table 3). The rates of leucine oxidation, NOLD, and MCR were similar in the 2 groups during the insulin clamp.

Substrate utilization. REE, RQ, and the rates of carbohydrate and fat oxidation were similar at baseline in the 2 groups (Table 4). Insulin infusion, combined with adminis-

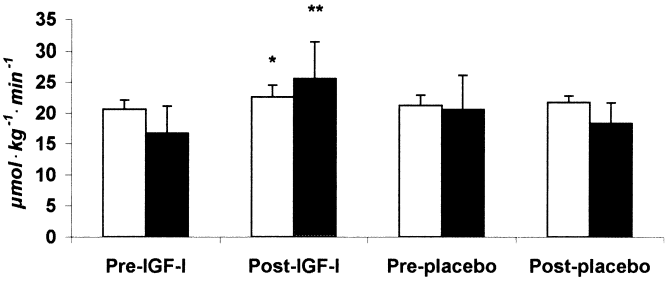

FIG. 2. Amino acid mixture (Vamin) and glucose infusion requirements before and after IGF-I/placebo treatment throughout the entire hyperinsulinemic-euglycemic amino acid clamp (150 min). Vamin (□); glucose (■).*P < 0.05, **P < 0.05 compared with pre–IGF-I.

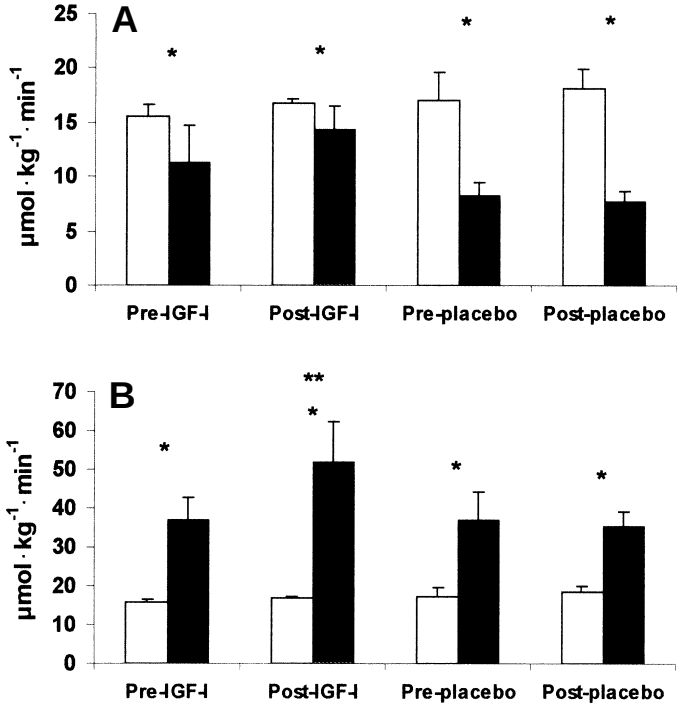

FIG. 3. Whole-body glucose kinetics in the basal (fasting) state and during the hyperinsulinemic-euglycemic clamp in patients before and after IGF-I/placebo administration. *A*: Glucose R_a in patients studied in the fasting (basal) state (□) and in patients studied during the hyperinsulinemic-euglycemic amino acid clamp (stimulated state) (■). *P < 0.01 for within-group comparisons between the basal and stimulated states. No significant differences between the groups in response to hyperinsulinemia were recorded. *B*: Glucose R_d in patients studied in the fasting (basal) state (□) and in patients studied during the hyperinsulinemic-euglycemic amino acid clamp (stimulated state) (■). *P < 0.01 for within-group comparisons between the basal and stimulated states. No significant differences in response to hyperinsulinemia were recorded between groups, except for the change in R_d before and after IGF-I treatment (P < 0.05).**

tration of both glucose and amino acids, resulted in a rise in REE, with an increase occurring in protein oxidation (Table 4). IGF-I therapy was not associated with alterations in fat or carbohydrate oxidation in either the basal or insulin-stimulated states; however, the basal protein oxidation was decreased after IGF-I treatment (P = 0.01) (Table 4).

NEFAs. NEFA concentrations were similar in both groups during study 1, and hyperinsulinemia resulted in decreased plasma NEFA levels on each study occasion in both the IGF-I– and placebo-treated patients (Table 4). Basal NEFA levels were unchanged by IGF-I treatment, but the levels observed during hyperinsulinemia were further lowered after IGF-I treatment (P < 0.02) but not after placebo treatment (Table 4).

DISCUSSION

This study demonstrated that IGF-I administration increased systemic IGF-I levels, resulting in reduced GH secretion and improved insulin sensitivity in adults with type 1 diabetes. Stable isotope techniques indicated that IGF-I treatment resulted in alterations in both glucose and protein metabolism with augmented insulin-mediated peripheral glucose uptake, increased utilization of amino acids in the basal state, and increased net protein anabolism in both the basal state and during a hyperinsulinemic amino acid clamp. Protein oxidation was reduced, and increased insulin-mediated decreases in NEFA levels were observed after IGF-I administration.

Several studies have demonstrated that both GH and insulin are required for appropriate hepatic production of IGF-I (8,28,29). IGF-I treatment in the current study (40 μg · kg^{-1} · day^{-1}) restored circulating total IGF-I levels to the upper part of the aged-matched normal range (12.8–62.2 nmol/l). This therapy could thus be considered replacement of the IGF-I deficiency of type 1 diabetes. Earlier studies in both adolescents and adults have demonstrated that IGF-I administration reduces nocturnal GH secretion and the requirement for insulin in type 1 diabetes (12,13). In the present study, there was a marked reduction in both GH secretion (~56%) and the overnight insulin requirement (~35%) after IGF-I therapy. Larger doses of IGF-I (50 μg/kg twice a day) have resulted in higher IGF-I levels but in only marginally greater reductions (~70%) in GH secretion, suggesting that the consequences of reduced GH levels may be observed with lower doses of IGF-I than previously used.

Although the overnight insulin requirements were decreased after IGF-I treatment, the basal glucose production and peripheral glucose uptake were unchanged. Assuming that the glycemic potency of IGF-I is 8% of that of insulin (21), the dosage used in this study (40 μg · kg^{-1} · day^{-1}) is equivalent to ~0.06 U · kg^{-1} · day^{-1} of insulin for the patients in this study. The reduction in usual insulin dose was 0.11 U · kg^{-1} · day^{-1}, which indicates that hypoglycemic action was not solely due to insulin-like activity. It is likely that improved insulin sensitivity as a result of decreased GH secretion accounts for this finding. An increased GIR was observed during the hyperinsulinemic clamp after IGF-I but not after placebo administration. This occurrence was associated with an increase in glucose utilization (with unaltered insulin-mediated suppression of glucose production), indicating that this dose of IGF-I altered glucose kinetics by increasing insulin-stimulated peripheral glucose uptake. It is probable that the decreased NEFA levels during the hyperinsulinemic clamp, after IGF-I administration, made a significant contribution to the increased glucose utilization observed through their competition with glucose via the glucose-NEFA (Randle) cycle (30). Reduced drive for lipolysis, as a result of IGF-I–induced decreased GH secretion, may explain this effect. However, recent evidence suggests that maintenance of GH levels during rhIGF-I treatment does not abolish the effects of IGF-I on reducing insulin requirements, suggesting that mechanisms independent of GH levels may also influence insulin sensitivity (31).

Basal leucine metabolism was altered by IGF-I administration. These changes included reduced circulating levels of leucine and leucine oxidation and the increased leucine MCR and net leucine balance. Similar findings have been observed after IGF-I administration in healthy subjects and GH-deficient adults (32–35) and in patients with Cushing's syndrome after GH therapy (36). Evidence suggests that GH therapy results in upregulation of amino acid transporter systems (37), increasing the efficiency of cellular amino acid uptake. The results from this study indicate that IGF-I may share this action or mediate this effect, resulting in the observed increased leucine MCR, in turn contributing to the observed reduced plasma leucine concentration. The decreased leucine oxidation is likely to be a direct result of the reduced plasma leucine concentration (38) that is brought about by

increased insulin sensitivity (16). Since type 1 diabetes is associated with an abnormal protein metabolism characterized by increased plasma amino acid concentrations and flux (14), the increase in leucine balance, as observed in the current study, indicates that correction of the relative IGF-I deficiency of type 1 diabetes may result in net gain in lean tissue, which, in the longer term, may favorably influence metabolic control.

It is difficult to successfully clamp amino acid concentrations during insulin infusions using commercial mixers, such as the one used in this study. The primary objective was to prevent a decrease in the circulating leucine concentration to detect a potential anabolic effect of IGF-I (2). This was successfully achieved, but as a result, increases were observed in several of the amino acid concentrations throughout the clamp protocol. These changes may be physiologically important, since hyperaminoacidemia has been shown to increase protein synthesis (38). In addition, combined hyperinsulinemia with hyperaminoacidemia has been demonstrated to further increase anabolism in both isolated-limb and whole-body studies (39). In this study, hyperinsulinemia was observed during the clamp protocol, but importantly, the plasma insulin concentrations and amino acid levels were similar on each study occasion in both groups, indicating that the changes in leucine metabolism were attributable to IGF-I.

Insulin is recognized to alter leucine kinetics in both healthy subjects and patients with type 1 diabetes (16–18) by reducing the leucine (R_a – protein breakdown) and, thus, plasma amino acid levels. In this study, the exogenous amino acid infusion requirement to maintain basal amino acid concentrations during the insulin clamp was increased; this reflected the fact that leucine R_d was maintained despite a decrease in endogenous leucine R_a after IGF-I treatment. These changes resulted in an increase in net leucine balance during the clamp in the IGF-I–treated patients but not in the placebo-treated patients. These data are consistent with studies in healthy subjects in whom an IGF-I infusion resulted in a direct stimulation of protein synthesis when amino acid levels were maintained with an exogenous amino acid infusion (2). Combined infusions of insulin and IGF-I have been shown to have greater effects on reducing protein degradation (40), suggesting that IGF-I has a dual action on protein metabolism, with both direct stimulation of protein synthesis and augmentation of insulin-mediated reduction in protein breakdown. We speculate that enhanced amino acid delivery to cells via IGF-I–mediated upregulation of amino acid transporters is responsible for the increased protein synthetic rate in these studies.

Basal REE and RQ were unaltered by IGF-I therapy. These findings are in contrast to previous studies in patients with type 1 diabetes and healthy subjects (13,32), in which larger doses of IGF-I were associated with an increase in REE. It has been suggested that increased fat oxidation may account for these increases (32), but no changes in fat oxidation were observed in the current study, either in the basal state or during the hyperinsulinemic clamp. It is likely that differences in the dose of IGF-I administered account for these differences, indicating that at lower replacement doses of IGF-I, less marked effects are demonstrable on substrate oxidation and energy expenditure.

Of great interest is the observation that IGF-I administration augmented the decrease in NEFA levels during hyperinsulinemia. Previous studies investigating the effects of IGF-I administration on NEFA metabolism have produced conflicting results, with decreased (19,20,41,42), increased, and unaltered (2,35) plasma NEFA levels reported by various investigators. It is likely that decreased GH-mediated lipolysis and simultaneously enhanced insulin sensitivity resulted in the lower circulating NEFA levels during the IGF-I administration. A study of IGF-I administration in GH-deficient adults also demonstrated reductions in NEFA levels (33), indicating that IGF-I may also regulate NEFA levels through mechanisms independent of GH.

These data increase our understanding of IGF-I action and indicate possible mechanisms responsible for the action of IGF-I on glucose and protein metabolism. The effects on insulin-mediated glucose uptake are associated with reduced NEFA levels, suggesting that enhanced insulin sensitivity occurs via the glucose-NEFA cycle and, in turn, is related to reductions in GH secretion. The effects of IGF-I on protein metabolism are known to be complex and are thought to involve intracellular phosphorylation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase, leading to increases in protein synthesis (43). The leucine kinetic data in this study provide evidence that, in addition to effects on intracellular signaling, IGF-I may regulate amino acid transport systems, thereby affecting delivery and utilization of amino acids. Thus, IGF-I has a dual role in increasing insulin action and sensitivity with respect to glucose and protein metabolism, with an additional role on protein metabolism in the basal non–insulin-stimulated state. Although further studies are necessary to elucidate the molecular mechanisms of IGF-I action, this in vivo study, which used a dose of IGF-I that increased the mean IGF-I concentration to the upper limit of the age-matched normal range, increases the understanding of how IGF-I regulates protein and glucose metabolism in type 1 diabetes.

ACKNOWLEDGMENTS

The Juvenile Diabetes Federation supported the research studies. P.V.C. was supported by a fellowship from the Special Trustees (Endowments) of St Thomas' Hospital. E.R.C. was supported by the Swiss National Foundation and the Margarethe and Walther Liechtenstein Foundation, Basel, Switzerland. I.G. was supported by the Economic and Social Research Council, Swindon, Wiltshire, U.K.

We are grateful to Pharmacia and Upjohn for supplying the rhIGF-I.

REFERENCES

1. Rinderknecht E, Humbel RE: The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem* 253:2769–2776, 1978
2. Russell-Jones DL, Umpleby AM, Hennessy TR, Bowes SB, Shojaee-Moradie F, Hopkins KD, Jackson NC, Kelly JM, Jones RH, Sönksen PH: Use of a leucine clamp to demonstrate that IGF-I actively stimulates protein synthesis in normal humans. *Am J Physiol* 267:E591–E598, 1994
3. Cotterill AM: The therapeutic potential of recombinant human insulin-like growth factor I. *Clin Endocrinol (Oxf)* 37:11–16, 1992
4. Mauras N, Horber FF, Haymond MW: Low dose recombinant human insulin-like growth factor I fails to affect protein anabolism but inhibits islet cell secretion in humans. *J Clin Endocrinol Metab* 75:1192–1197, 1992
5. Bach MA, Chin E, Bondy CA: The effects of subcutaneous insulin-like growth factor-I infusion in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 79:1040–1045, 1994
6. Yarwood GD, Ross RJ, Medbak S, Coakley J, Hinds CJ: Administration of human recombinant insulin-like growth factor-I in critically ill patients. *Crit*

Care Med 25:1352–1361, 1997
7. Zenobi PD, Graf S, Ursprung H, Froesch ER: Effects of insulin-like growth factor-I on glucose tolerance, insulin levels and insulin secretion in healthy man. *J Clin Invest* 89:1908–1913, 1992
8. Russell-Jones DL, Rattray M, Wilson VJ, Jones RH, Sönksen PH, Thomas CR: Intraperitoneal insulin is more potent than subcutaneous insulin at restoring hepatic insulin-like growth factor-I mRNA levels in the diabetic rat: a functional role for the portal vascular link. *J Mol Endocrinol* 9:257–263, 1992
9. Dunger DB, Cheetham TD, Crowne EC: Insulin-like growth factors (IGFs) and IGF-I treatment in the adolescent with insulin-dependent diabetes mellitus. *Metabolism* 44:119–123, 1995
10. Acerini CL, Patton CM, Savage MO, Kernell A, Westphal O, Dunger DB: Randomised placebo-controlled trial of human recombinant insulin-like growth factor-I plus intensive insulin therapy in adolescents with insulin dependent diabetes mellitus. *Lancet* 350:1199–1204, 1997
11. Zenobi PD, Jaeggi-Groisman SE, Riesen WF, Roder ME, Froesch ER: Insulin-like growth factor-I improves glucose and lipid metabolism in type II diabetes mellitus. *J Clin Invest* 90:2234–2241, 1993
12. Cheetham T, Jones J, Taylor AM, Holly J, Matthews DR, Dunger DB: The effects of recombinant insulin-like growth factor-I administration on growth hormone levels and insulin requirements in adolescents with type I (insulin-dependent) diabetes mellitus. *Diabetologia* 36:678–681, 1993
13. Carroll PV, Umpleby M, Ward GS, Imuere S, Alexander E, Dunger D, Sönksen PH, Russell-Jones DL: rhIGF-I administration reduces insulin requirements, decreases growth hormone secretion, and improves the lipid profile in adults with IDDM. *Diabetes* 46:1453–1458, 1997
14. Luzi L, Castellino P, Simonson DC, Petrides AS, DeFronzo RA: Leucine metabolism in IDDM. *Diabetes* 39:38–48, 1990
15. Nair KS, Garrow JS, Ford C, Mehler RF, Halliday D: Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia* 25:400–403, 1983
16. Umpleby AM, Boroujerdi MA, Brown PM, Carson ER, Sönksen PH: The effect of metabolic control on leucine metabolism in type 1 (insulin-dependent) diabetic patients. *Diabetologia* 29:131–141, 1986
17. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. *J Clin Invest* 80:1784–1793, 1987
18. Nair KS, Ford GC, Halliday D: Effect of intravenous insulin treatment on in vivo whole-body leucine kinetics and oxygen consumption in insulin-deprived type 1 diabetic patients. *Metabolism* 36:491–495, 1987
19. Turkalj I, Keller U, Ninnis R, Vosmeer S, Stauffacher W: 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, 1992
20. Boulware SD, Tamborlane WV, Rennert NJ, Gesundheit N, Sherwin RS: Comparison of the metabolic effects of recombinant human insulin-like growth actor-I and insulin: dose-response relationships in healthy young and middle-aged adults. *J Clin Invest* 93:1131–1139, 1994
21. Guler H-P, Zapf J, Froesch ER: Short-term metabolic effects of recombinant human insulin-like growth factor-I in healthy adults. *N Engl J Med* 317:137–140, 1987
22. Scrimgeour CM, Bennet WM, Connacher AA: A convenient method of screening glucose for ^{13}C:^{12}C ratio for use in stable isotope tracer studies. *Biomed Environ Mass Spectrom* 17:265–266, 1988
23. Sönksen PH: Double antibody technique for the simultaneous assay of insulin and growth hormone. In *Hormones in Human Blood: Detection and Assay*. Antoniades H, Ed. Cambridge, MA, Harvard University Press, 1976, p. 176–199
24. Shojaee-Moradie F, Jackson NC, Jones RH, Mallet AI, Hovorka R, Umpleby AM: Quantitative measurement of 3-O-methyl-D-glucose by gas chromatography-mass spectrometry as a measure of glucose transport in vivo. *J Mass Spectrom* 31:961–966, 1996
25. Wolfe RR: *Radioactive and Stable Isotope Tracers in Biomedicine*. New York, Wiley-Liss, 1992
26. Matthews DE, Schwarz D, Yang RD, Motil KJ, Young VR, Bier DM: Relationship of plasma leucine and α-ketoisocaproate during a l-[1-^{13}C] leucine infusion in man: a method for measuring human intracellular leucine tracer environment. *Metabolism* 31:1105–1112, 1982
27. Dauncey MJ, Murgatroyd PR, Cole TJ: A human calorimeter for the direct and indirect measurement of 24 h energy expenditure. *Br J Nutr* 39:557–566, 1978
28. Boni-Schnetzler M, Schmid C, Meier PJ, Froesch ER: Insulin regulates insulin-like growth factor-I mRNA in rat hepatocytes. *Am J Physiol* 260:E846–E851, 1991
29. Wurzburger MI, Prelevic GM, Sönksen PH, Wheeler M, Balint-Peric L: Effect of recombinant human growth hormone treatment on insulin-like growth factor (IGF-I) levels in insulin-dependent diabetic patients. *Acta Diabetol* 32:131–134, 1995
30. Hall SE, Saunders J, Sönksen PH: Glucose and free fatty acid turnover in normal subjects and in diabetic patients before and after insulin treatment. *Diabetologia* 16:297–306, 1979
31. Crowne EC, Samra JS, Cheetham T, Watts A, Holly JM, Dunger DB: Recombinant human insulin-like growth factor-I abolishes changes in insulin requirements consequent upon growth hormone pulsatility in young adults with type I diabetes mellitus. *Metabolism* 47:31–38, 1998
32. Hussain MA, Schmitz O, Mengel A, Keller A, Christiansen JS, Zapf J, Froesch ER: Insulin-like growth factor-I stimulates lipid oxidation, reduces protein oxidation and enhances insulin sensitivity in humans. *J Clin Invest* 95:2249–2256, 1993
33. Hussain MA, Schmitz O, Mengel A, Glatz Y, Christiansen JS, Zapf J, Froesch ER: Comparison of the effects of growth hormone and insulin-like growth factor I on substrate oxidation and on insulin sensitivity in growth hormone deficient humans. *J Clin Invest* 94:1126–1133, 1994
34. Fryburg DA, Jahn LA, Sherita A, Oliveras DM, Barrett EJ: Insulin and insulin-like growth factor-I enhance skeletal muscle protein anabolism during hyperaminoacidaemia by different mechanisms. *J Clin Invest* 96:1722–1729, 1995
35. Elahi D, McAloon-Dyke M, Fukagawa NK, Sclater AL, Wong GA, Shannon RP, Minaker KL, Miles JM, Rubenstein AH, Vandepol CJ, Guler H-P, Good WR, Seaman JJ, Wolfe RR: Effects of recombinant human IGF-I on glucose and leucine kinetics in man. *Am J Physiol* 265:E831–E838, 1993
36. Bowes SB, Umpleby M, Cummings MH, Jackson NC, Carroll PV, Lowy C, Sönksen PH, Russell-Jones DL: The effect of recombinant human growth hormone on glucose and leucine metabolism in Cushing's syndrome. *J Clin Endocrinol Metab* 82:243–246, 1997
37. McGivan JD, Pastor-Anglada P: Regulatory and molecular aspects of mammalian amino acid transport. *Biochem J* 299:321–334, 1994
38. Tessari P, Inchiotro S, Biola G, Trevisan R, Fantin G, Marescotti MC, Iori E, Tiengo A, Crepaldi G: Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo: evidence for distinct mechanisms in regulation of net amino acid deposition. *J Clin Invest* 79:1062–1069, 1987
39. Bennet WM, Connacher AA, Scrimgeour CM, Jung RT, Rennie MJ: Euglycaemic hyperinsulinaemia augments amino acid uptake by human leg tissues during hyperaminoacidemia. *Am J Physiol* 259:E185–E194, 1990
40. Umpleby AM, Shojaee-Moradie F, Thomason MJ, Kelly JM, Skottner A, Sönksen PH, Jones RH: Effects of insulin-like growth factor-I (IGF-I), insulin and combined IGF-I-insulin infusions on protein metabolism in dogs. *Eur J Clin Invest* 24:337–344, 1994
41. Laager R, Ninnis R, Keller U: Comparison of the effects of recombinant human insulin-like growth factor-I and insulin on glucose and leucine kinetics in humans. *J Clin Invest* 92:1903–1909, 1993
42. Boulware SD, Tamborlane WV, Matthews LS, Sherwin RS: Diverse effects of insulin-like growth factor I on glucose, lipid and amino acid metabolism. *Am J Physiol* 262:E130–E133, 1992
43. Kuemmerle JF, Bushman TL: IGF-I stimulates intestinal muscle cell growth by activating distinct PI 3-kinase and MAP kinase pathways. *Am J Physiol* 275: G151–G158, 1998