Comparison of the Metabolic Effects of Recombinant Human Insulin-like Growth Factor-I and Insulin

Dose–Response Relationships in Healthy Young and Middle-aged Adults

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Abstract

The actions of recombinant human insulin-like growth factor-I (rhIGF-I) and insulin were compared in 21 healthy young (24±1 yr) and 14 healthy middle-aged (48±2 yr) subjects during 3-h paired euglycemic clamp studies using one of three doses (rhIGF-I 0.2, 0.4, and 0.8 μg/kg·min and insulin 0.2, 0.4, and 0.8 mU/kg·min, doses chosen to produce equivalent increases in glucose uptake). In younger subjects, rhIGF-I infusions suppressed insulin by 19–33%, C-peptide by 47–59% and glucagon by 33–47% (all, $P < 0.02$). The suppression of C-peptide was less pronounced with insulin than with rhIGF-I ($P < 0.007$). The metabolic responses to rhIGF-I and insulin were remarkably similar: not only did both hormones increase glucose uptake and oxidation in a nearly identical fashion, but they also produced similar suppression of glucose production, free fatty acid levels, and fat oxidation rates. In contrast, rhIGF-I had a more pronounced amino acid-lowering effect than did insulin ($P < 0.004$).

In middle-aged subjects, basal IGF-I levels were 44% lower ($P < 0.0001$) whereas basal insulin and C-peptide were 20–25% higher than in younger subjects. Age did not alter the response to rhIGF-I. However, insulin-induced stimulation of glucose uptake was blunted in older subjects ($P = 0.05$). Our data suggest that absolute IGF-I and relative insulin deficiency contribute to adverse metabolic changes seen in middle age. (*J. Clin. Invest.* 1994. 93:1131–1139.) Key words: aging • glucose • insulin • insulin-like growth factor-I • metabolism

Introduction

IGF-I, the putative mediator of growth hormone's somatotropic effects (1), shares considerable structural homology with insulin (2, 3). The synthesis of IGF-I by recombinant biosynthetic techniques has provided the opportunity to test whether the hormone also has functional similarities to insulin. In vivo studies in normal rats demonstrated that intravenous infusions of recombinant human (rh)IGF-I stimulated peripheral glucose uptake and inhibited muscle protein breakdown but, unlike insulin, such infusions were unable to suppress hepatic

glucose production or circulating FFA levels (4). In humans, the scope of IGF-I's metabolic actions more closely resembles that of insulin (5). Its plasma glucose-lowering effects are not due solely to its capacity to stimulate peripheral glucose uptake, but also result from a pronounced inhibitory effect on hepatic glucose production. In contrast to observations in the rat, rhIGF-I unexpectedly suppressed circulating FFA concentrations in humans and, much like insulin, also reduced amino acid, glucagon, and C-peptide levels. Turkalj et al. (6) have shown that, as the magnitude of the infusion dose of IGF-I increased from 0.08 to 0.5 μg/kg·min, the rate of glucose disposal increased as determined by the rate of exogenous glucose infusion required to maintain euglycemia. However, the dose-response effects of IGF-I on glucose kinetics were not determined nor has the dose–response pattern generated by IGF-I been compared to that produced by insulin in humans.

The present study was undertaken to examine the scope of the metabolic and hormonal responses to graded intravenous infusions of rhIGF-I and to compare these responses to those obtained with insulin doses that were standardized to produce equivalent increases in peripheral glucose uptake in healthy young adult volunteers. We also sought to determine whether the metabolic and physiologic actions of rhIGF-I are impaired in healthy middle-aged as compared to young adult subjects, as has been observed with insulin (7, 8). This issue is particularly important because, unlike insulin, basal IGF-I levels progressively fall with aging in healthy adults (9, 10).

Methods

Subjects. Two groups of nondiabetic subjects were recruited based on their age: group 1 consisted of subjects ranging in age from 20 to 34 yr ($n = 21$) and those in group 2 ranged in age from 40 to 65 yr ($n = 14$). Clinical characteristics of the two study groups are summarized in Table I. The subjects were all within 20% of ideal body weight except for 3 of the 14 older subjects who were between 120% and 130% ideal body weight ($P = $ NS, younger vs. older subjects). None of the subjects had any history of diabetes or impaired glucose tolerance or clinical evidence of medical illness except for one of the older subjects who had mild hypertension treated with a low-dose β-blocker and a diuretic. This subject discontinued his medications at least 3 d before each study. Informed, written consent was obtained before participating in the study which was approved by the Human Investigation Committee of Yale University.

Materials. rhIGF-I (kindly provided by Genentech, Inc., South San Francisco, CA), regular U100 human insulin (Novo-Nordisk, Princeton, NJ) and [6-³H]glucose (Dupont Research Products, Boston, MA) were each individually diluted with sterile normal saline. The insulin infusate was prepared by adding a small quantity of each subject's whole blood to inhibit binding of insulin to the plastic syringe and tubing.

Procedures. Younger subjects received rhIGF-I (0.2 [$n = 8$], 0.4 [$n = 8$], or 0.8 [$n = 7$] μg/kg·min) as well as a matching dose of insulin

Table I. Clinical Characteristics

	Group 1	Group 2
n	21	14
Gender (M/F)	17/4	9/5
Mean age (*yr*)	24±1	48±2
BMI	24.0±0.5	25.6±0.7
%IBW	106±0.02	112±0.03

Abbreviations: BMI, body mass index weight (kg)/height (m^2); %IBW, percent ideal body weight as determined from the 1959 Metropolitan Life Tables. Data are reported as means±SEM.

(0.2, 0.4, or 0.8 mU/kg · min) in random order separated by a 3–6-wk interval (two subjects participated in four studies each). The paired hormone doses were chosen, based on preliminary studies, to produce an equivalent increase in glucose uptake. Results from some of the rhIGF-I experiments at the 0.4-μg/kg · min dose have been included in a previously published report (5). The older subjects received only the two lower doses of hormones, *n* = 7 at the 0.2-μg/kg · min dose and *n* = 6 at the 0.4-μg/kg · min dose. Three subjects in this group underwent only one infusion: one each received rhIGF-I 0.2 and 0.4 μg/kg · min and insulin 0.4 mU/kg · min. The middle-aged subjects did not receive the 0.8-μg/kg · min dose of rhIGF-I. This was because of a transient, but severe, vasovagal reaction that occurred in the first subject in this age group (a 46-yr-old woman) given this dose (the high-dose studies in the young subjects were completed before this adverse reaction).

Subjects were admitted to the Yale General Clinical Research Center on the morning of the study after a 10-h overnight fast. An intravenous catheter was inserted into an antecubital vein for administration of [6-^{3}H]glucose, unlabeled glucose, and insulin (on the insulin study day) and a second catheter was inserted in a retrograde fashion into a dorsal hand vein for blood sampling. The hand was kept in a heated box (65°C) to allow for arterialization of venous blood. A third intravenous catheter was inserted only on the rhIGF-I study day for separate infusion of rhIGF-I.

At the start of the study (−180 min), a primed-continuous infusion of [6-^{3}H]glucose (25 μCi bolus, 0.25 μCi/min) was started. After a 3-h tracer equilibration period, an infusion of rhIGF-I (10, 20, or 40 μg/kg bolus given over 12 min plus a 0.2, 0.4, or 0.8 μg/kg · min continuous infusion) or regular insulin (10, 20, or 40 mU/kg bolus given over 12 min plus a 0.2, 0.4, or 0.8 mU/kg · min continuous infusion) was started and continued for an additional 3 h. Plasma glucose was maintained at ∼ 5 mM using a variable rate infusion of glucose which was adjusted based on plasma glucose measurements (in duplicate) obtained every 5 min at the bedside, as previously described (11).

Blood samples were collected at 10–60-min intervals for measurement of [^{3}H]glucose specific activity, total and free IGF-I, insulin, C-peptide, glucagon, growth hormone (GH),[1] amino acids, FFA, and β-hydroxybutyrate (β-OHB). Indirect calorimetry for measurement of resting energy expenditure, oxygen consumption and CO_2 production was performed during the last 30 min of the equilibration period and during the last 30 min of the rhIGF-I or insulin infusion. Subjects emptied their bladder just before the start of the study. Urine was then collected from time −180 to 0 min (start of the rhIGF-I or insulin infusion) and from time 0 to time +210 for measurement of nitrogen concentration.

Analytic methods. Plasma glucose concentration was measured by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma IGF-I levels, total and free, were

measured by a double-antibody radioimmunoassay after acid-ethanol extraction and size exclusion chromatographic separation of free from bound IGF-I (12). [^{3}H]glucose radioactivity was determined by a modification of the Somogyi precipitation procedure, as previously described (13). A cation-anion resin column was used to remove potentially contaminating [^{3}H]lactate and [^{3}H]alanine. Plasma insulin, C-peptide, GH, and glucagon were measured by double-antibody radioimmunoassay. Plasma concentrations of acidic and neutral amino acids were measured by cation-exchange chromatography using an automated amino acid analyzer (Dionex D-500; Dionex, Sunnyvale, CA) and FFA levels were determined using a microfluorometric assay, as previously described (14). Plasma β-OHB concentrations were measured by enzymatic assay. Indirect calorimetry was performed using a Datex Deltatrac metabolic monitor (Datex/Instrumentarium, Helsinki, Finland).

Calculations. Basal turnover of glucose was calculated by dividing the tracer infusion rate by the mean of at least three measurements of [^{3}H]glucose specific activity obtained during the last 30 min of the equilibration period. The rate of endogenous glucose production during the last hour of the rhIGF-I or insulin infusion (120–180 min) was calculated by subtracting the rate of exogenous glucose infusion from the rate of total glucose appearance determined from the tracer data (estimated using a two-compartment model of glucose kinetics [13]). Carbohydrate (CHO) and fat oxidation were calculated from standard equations: grams CHO = $4.115 \cdot VCO_2 - 2.909 \cdot VO_2 - 2.539 \cdot UN$ and grams FAT = $1.689 \cdot VO_2 - 1.689 \cdot VCO_2 - 1.943 \cdot UN$, where VCO_2 is CO_2 production in liters per day, VO_2 is oxygen consumption in liters per day, and UN is urinary nitrogen in grams per day. Data are expressed as means ±SEM. Data were analyzed using one-way and two-way analysis of variance with repeated measures or paired Student's *t* test where appropriate.

Results

Effects of rhIGF-I and insulin infusions on glucoregulatory hormones in younger subjects. Basal and steady-state (120–180 min) concentrations of IGF-I and insulin in response to infusion of the hormones are shown in Fig. 1. During the rhIGF-I infusions, there was a stepwise increase in total and free IGF-I levels with each dose (0.2, 0.4, and 0.8 μg/kg · min). Total IGF-I rose 2.6-, 3.6-, and 4.3-fold while free IGF-I was undetectable basally in all subjects and rose to 27±4, 71±6, and 147±7 μg/liter (each measurement $P < 0.0001$ vs. basal). Concentrations of insulin and C-peptide, on the other hand, were markedly suppressed (Figs. 1 and 2). Plasma insulin fell by 19%, 33%, and 32% (from $P < 0.006$ to $P < 0.02$) and C-peptide concentrations fell by 47%, 55%, and 59% at each successive increase in the rhIGF-I dose (from $P < 0.0001$ to $P < 0.003$). The insulin infusions produced a stepwise elevation of plasma insulin levels by 2.1-, 3.3-, and 6.7-fold above basal values, respectively (each $P < 0.0001$), but did not alter either total or free IGF-I levels. As shown in Fig. 2, plasma C-peptide concentrations decreased in response to the lowest (by 17%, $P < 0.02$) and highest (by 24%, $P < 0.03$) dose of insulin; however, the effect of insulin on C-peptide was less pronounced than that seen with rhIGF-I ($P < 0.0001$, ANOVA). Plasma levels of glucagon fell in response to both rhIGF-I and insulin: the three rhIGF-I infusions diminished glucagon levels by 47%, 36%, and 33%, respectively (from $P < 0.002$ to $P < 0.009$), and the three insulin infusions suppressed glucagon levels by 26%, 33%, and 22%, respectively, (from $P < 0.003$ to $P < 0.02$). Both rhIGF-I and insulin were equally effective in reducing plasma levels of glucagon (P = NS, ANOVA). There were no significant changes in GH levels during the rhIGF-I or insulin infusions (data not shown).

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1. *Abbreviations used in this paper:* GH, growth hormone; β-OHB, β-hydroxybutyrate.
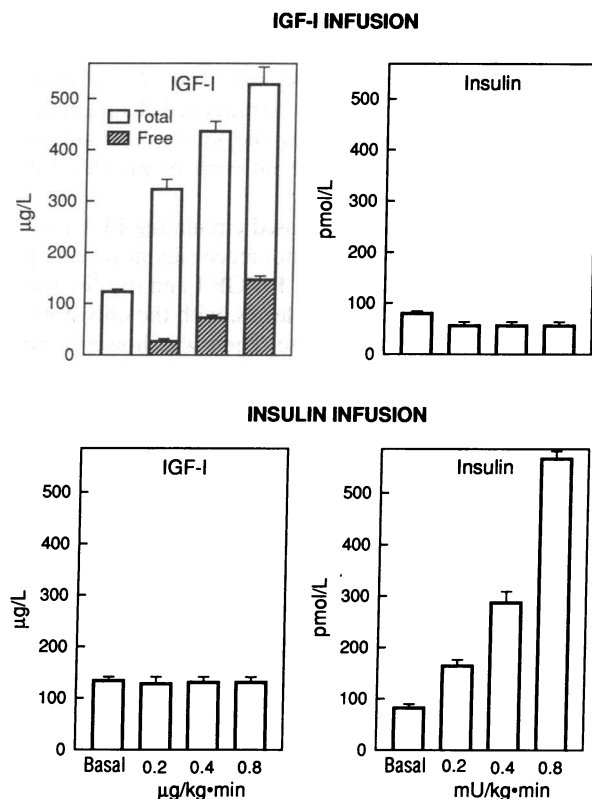
IGF-I INFUSION

Figure 1. Basal and steady-state concentrations of IGF-I, total (□) and free (▨), and insulin during rhIGF-I and insulin infusions. L, liter.
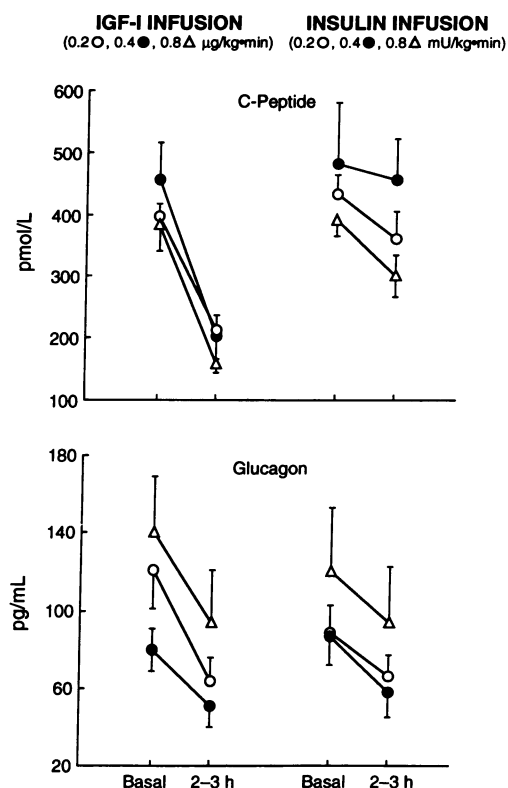

Figure 2. Basal and steady-state concentrations of C-peptide and glucagon during the three doses of rhIGF-I 0.2 (o), 0.4 (●), and 0.8 (△) μg/kg·min and matched doses of insulin 0.2 (o), 0.4 (●), or 0.8 (△) mU/kg·min. L, liter.

Effect of rhIGF-I and insulin on fuel metabolism in the younger subjects. As designed, the matched doses of rhIGF-I and insulin in the young subjects produced equivalent stepwise stimulation of glucose uptake (Fig. 3). Basal glucose uptake, 10.6 ± 0.4 μmol/kg·min, increased to 17.4 ± 2.0, 23.8 ± 2.0, and 41.1 ± 6.8 μmol/kg·min during the rhIGF-I infusions, respectively (from $P < 0.0002$ to $P < 0.005$ vs. basal), whereas with insulin, glucose uptake increased to 17.3 ± 2.7, 24.0 ± 2.2, and 48.1 ± 6.4 μmol/kg·min, respectively (from $P < 0.0006$ to $P < 0.05$ vs. basal). Both hormones also markedly suppressed hepatic glucose production to a similar degree: rhIGF-I decreased hepatic glucose production from 10.6 ± 0.4 to 5.1 ± 1.7, 1.9 ± 2.1, and 2.9 ± 2.0 μmol/kg·min (from $P < 0.0002$ to $P < 0.006$ vs. basal) and the three matched doses of insulin decreased hepatic glucose production to 5.0 ± 1.3, 1.0 ± 1.5, and 1.8 ± 2.2 μmol/kg·min, respectively (from $P < 0.0004$ to $P < 0.02$ vs. basal). There was no difference in the effect of the matched doses of rhIGF-I and insulin on hepatic glucose production (P = NS, ANOVA).

Concomitant with the increase in glucose uptake, carbohydrate oxidation rates were stimulated by both hormones; however, this effect did not reach statistical significance at the lowest dose of rhIGF-I: carbohydrate oxidation increased from 8.1 ± 1.0 μmol/kg·min to 9.7 ± 1.3 (P = NS vs. basal), 15.9 ± 1.2 ($P < 0.0002$ vs. basal), and 14.8 ± 1.7 μmol/kg·min ($P < 0.001$ vs. basal) during the three increasing doses of rhIGF-I and from 7.1 ± 0.7 μmol/kg·min to 10.3 ± 2.0, 15.8 ± 1.1, and 17.3 ± 3.7 μmol/kg·min (each measurement from $P < 0.002$ to $P < 0.03$ vs. basal) during the corresponding doses of insulin. As expected, the increase in glucose oxidation was accompanied by a fall in fat oxidation. This effect was seen only at the two higher doses of rhIGF-I, but at all three doses of insulin: basal fat oxidation with either hormone was 0.7 ± 0.1 mg/kg·min and fell to 0.6 ± 0.1 (P = NS vs. basal), 0.2 ± 0.1 ($P < 0.005$ vs. basal), and 0.3 ± 0.1 mg/kg·min ($P < 0.004$ vs. basal) during the rhIGF-I infusions and to 0.4 ± 0.2, 0 ± 0.1, and 0.1 ± 0.3 mg/kg·min (from $P < 0.009$ to $P < 0.02$ vs. basal) during the three insulin studies. Statistical analysis failed to
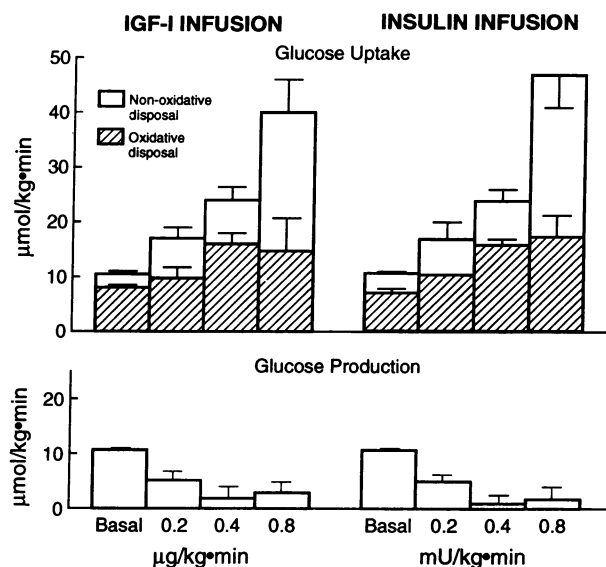

Figure 3. Basal and steady-state rates of glucose uptake, nonoxidative (□) and oxidative (▨), and glucose production during rhIGF-I and insulin infusions.

show a significant difference in overall ability of rhIGF-I and insulin to increase glucose oxidation and decrease fat oxidation.

As shown in Table II, rhIGF-I and insulin had stepwise dose-dependent ($P < 0.03$, ANOVA) suppressive effects on circulating levels of almost all of the essential and nonessential amino acids (each dose $P < 0.0007$ vs. basal). However, compared with insulin, rhIGF-I caused a significantly greater reduction in total and branched chain amino acid (each measurement, each dose $P < 0.0002$ vs. insulin, ANOVA) levels: the three increasing doses of rhIGF-I lowered total amino acid levels by 18%, 29%, and 30%, respectively and the three matched

doses of insulin lowered total amino acids by 13%, 20%, and 24%, respectively. In addition, rhIGF-I decreased circulating branched chain amino acids by 28%, 46%, and 52%, at each increased dose, whereas insulin reduced branched chain amino acid levels by 25%, 32%, and 46%. Alanine, unlike other amino acids, remained unchanged throughout each of the rhIGF-I and insulin studies.

Both rhIGF-I and insulin decreased circulating FFA concentrations, although the effect did not reach significance with the lowest dose of rhIGF-I (Fig. 4). RhIGF-I and insulin also significantly lowered blood β-OHB levels, with the only notable exception the lowest rhIGF-I dose. There was, however, no

Table II. Basal and Steady-State Amino Acid Levels

		IGF-I infusion			Insulin infusion		
			μg/kg · min			mU/kg · min	
	·	0.2	0.4	0.8	0.2	0.4	0.8
				μM			
Ala	Basal	316±26	288±25	306±29	275±26	235±30	285±26
	Final	299±28	246±15	258±18	265±20	222±17	270±14
Asp	Basal	8±2	8±1	8±1	7±1	7±1	8±1
	Final	6±1	5±1*	6±1*	6±1	5±1*	6±1
Asn	Basal	51±3	53±4	42±4	46±3	45±3	43±3
	Final	39±2*	33±3*	26±2*	40±2*	35±2‡	33±3‡
Cit	Basal	33±4	31±3	18±2	33±3	31±3	20±2
	Final	18±3‡	15±2‡	13±2*	22±4‡	20±2‡	14±1*
Cys	Basal	60±5	55±3	55±2	56±2	53±3	54±2
	Final	57±4	45±2*	44±2*	55±3	50±3	48±2‡
Gln	Basal	502±23	473±27	448±21	475±19	468±33	442±32
	Final	433±36*	350±21‡	296±18‡	421±18*	378±29‡	368±22*
Glu	Basal	47±3	37±4	47±4	44±4	36±2	42±3
	Final	39±2*	25±2*	31±2‡	41±3	29±2‡	31±2*
Gly	Basal	219±13	241±11	177±13	208±16	212±15	174±9
	Final	194±12‡	195±14*	137±8*	206±15	200±15	160±8*
Ile	Basal	53±4	57±4	56±3	56±2	56±4	54±3
	Final	28±3‡	19±2‡	15±2‡	34±2‡	28±1‡	18±1‡
Leu	Basal	120±8	126±9	132±8	124±3	123±7	123±6
	Final	81±6‡	61±4‡	54±3‡	93±5‡	76±3‡	62±3‡
Met	Basal	23±2	27±1	22±2	23±1	26±2	22±2
	Final	15±2‡	14±1‡	10±1‡	18±1*	17±1‡	13±1‡
Phe	Basal	56±5	56±2	60±4	53±3	53±2	51±4
	Final	44±3‡	33±3‡	30±2‡	45±2‡	40±2‡	35±3‡
Ser	Basal	112±7	121±9	94±6	96±4	116±9	94±7
	Final	80±5‡	77±6‡	55±4‡	82±3‡	87±7‡	68±5‡
Taur	Basal	42±9	34±2	34±4	34±2	37±4	27±2
	Final	30±3	27±2*	24±2*	30±1*	31±4*	25±3
Thr	Basal	138±10	140±11	111±10	128±6	128±9	111±10
	Final	108±9‡	87±9‡	64±6‡	105±4‡	93±7‡	76±7‡
Tyr	Basal	62±4	56±5	63±4	55±3	53±3	59±4
	Final	47±4*	30±3*	33±4*	43±4*	36±2*	35±3*
Val	Basal	197±15	206±15	216±11	229±19	280±30	209±8
	Final	155±9*	127±7‡	126±8‡	185±11‡	210±27*	134±6‡
BCAA	Basal	369±25	390±28	404±20	409±21	460±34	386±17
	Final	264±15‡	207±12‡	195±11‡	309±20‡	300±25‡	214±9‡
Total	Basal	2016±80	1974±79	1863±73	1929±53	1973±93	1832±62
	Final	1647±71‡	1356±34‡	1195±50‡	1691±50‡	1532±62‡	1399±50‡

* $P < 0.05$ vs. basal. ‡ $P < 0.001$ vs. basal.

IGF-I INFUSION
(0.2 O, 0.4 ●,
0.8 △ µg/kg•min)

INSULIN INFUSION
(0.2 O, 0.4 ●,
0.8 △ mU/kg•min)

IGF-I INFUSION
(0.2 O, 0.4 ●,
0.8 △ µg/kg•min)
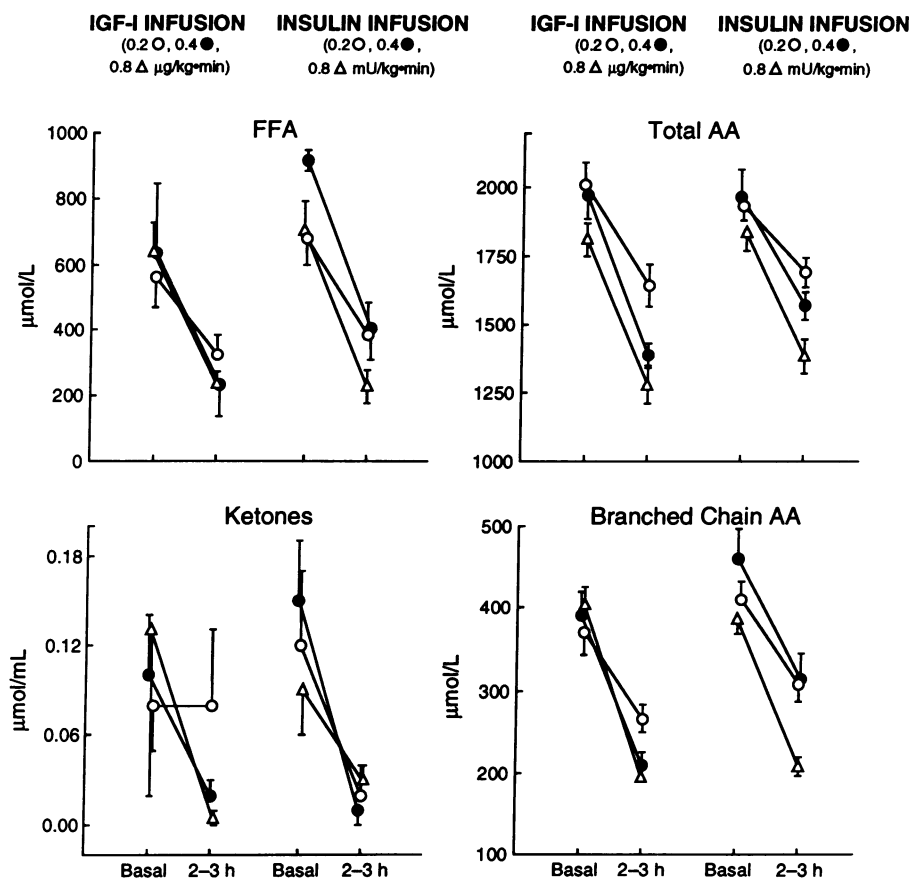
INSULIN INFUSION
(0.2 O, 0.4 ●,
0.8 △ mU/kg•min)

Figure 4. Basal and steady-state levels of FFA, ketones, total amino acids (AA), and branched chain amino acids (BCAA) during rhIGF-I and insulin infusions. L, liter.

significant difference in the overall FFA and β-OHB response to rhIGF-I as compared to insulin.

Effects of rhIGF-I and insulin infusions in older subjects and comparison with younger subjects. Fig. 5 shows basal IGF-I, insulin, and C-peptide levels in the older and younger subjects. Basal levels of IGF-I were strikingly lower in the older as compared with the younger subjects (72 ± 6 vs. 129 ± 4 µg/liter, $P < 0.0001$), whereas basal insulin tended to be (98 ± 11 vs. 81 ± 4 pM, $P = 0.097$) and C-peptide was significantly higher (548 ± 51 vs. 426 ± 17 pM, $P < 0.032$) in the older subjects. The increment in total and free IGF-I levels on the other hand, was virtually identical in the older and younger subjects during each dose of rhIGF-I (Table III). Furthermore, the rhIGF-I infusions caused similar suppression of insulin and C-peptide levels in both age groups. During the insulin infusions, there were no changes in IGF-I levels and steady-state insulin levels were comparable in both age groups at each dose. As seen in the younger subjects, rhIGF-I induced a greater fall in C-peptide levels than did insulin ($P < 0.0001$), however, no effect of age on this parameter was demonstrable. Basal glucagon levels were similar in both older and younger subjects and fell equally in response to either hormone. No consistent changes in growth hormone levels were seen in either group to any dose of either hormone (data not shown).

As shown in Fig. 6, basal glucose uptake was similar in the older as compared to the younger subjects and rhIGF-I infusion produced a virtually identical increase in glucose uptake in both groups. In contrast, the overall stimulatory effect of both doses of insulin on glucose uptake was reduced by ~ 20% in the older subjects ($P = 0.056$). On the other hand, age had no

effect on the ability of rhIGF-I or insulin to suppress hepatic glucose production in either group (Fig. 7) nor could an age effect be identified for the response of plasma FFA or β-OHB levels to both hormones, ($P = $ NS, ANOVA).

Discussion

The present study demonstrates that rhIGF-I shares many of the metabolic actions of insulin, but highlights several specific differences. In order to facilitate such comparisons, low to moderate doses of insulin were selected to probe the low end of the insulin dose–response curve and they were matched with infusions of rhIGF-I designed to increase glucose uptake in a similar, dose-responsive manner in healthy young adult subjects. Because IGF-I, unlike insulin, is protein bound (15) and it is
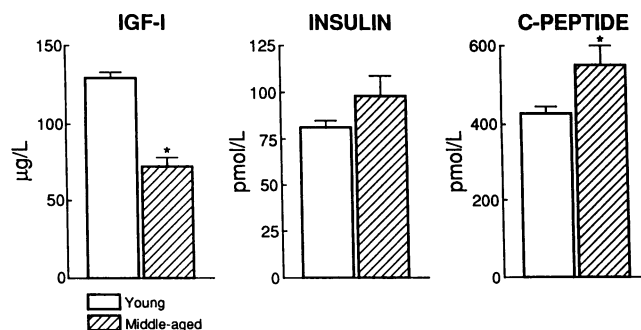

Figure 5. Basal levels of IGF-I, insulin, and C-peptide in healthy young (□) as compared to middle-aged (▨) subjects, $P < 0.04$. L, liter.

Table III. Basal and Steady-State Levels of Glucoregulatory Hormones during rhIGF-I and Insulin Infusions in Young Compared to Middle-aged Subjects

	IGF-I (0.2 μg/kg·min)		Insulin (0.2 mU/kg·min)		IGF-I (0.4 μg/kg·min)		Insulin (0.4 mU/kg·min)	
	Basal	Final	Basal	Final	Basal	Final	Basal	Final
Total IGF-I (μg/liter)								
Young	130±10	324±18*	136±12	129±10	119±11	437±28*	130±11	129±12
Older	63±6‡	237±9*‡	79±12‡	73±9‡	76±10‡	359±30*	73±16‡	66±18‡
Free IGF-I (μg/liter)								
Young	<2	27±4*	<2	<2	<2	71±6*	<2	<2
Older	<2	31±10*	<2	<2	<2	69±8*	<2	<2
Insulin (pM)								
Young	70±6	57±5*	78±7	166±11*	83±12	56±5*	85±12	284±25*
Older	99±17	58±6*	103±21	193±15*	99±19	63±12*	102±23	283±35*
C-peptide (pM)								
Young	398±20	212±25*	433±31	361±45*	455±60	203±37*	482±49	455±67
Older	613±76‡	328±56*	623±109	523±67	472±59	256±52*	546±98	416±59
Glucagon (ng/liter)								
Young	121±20	64±13*	89±14	66±11*	80±11	51±11*	87±15	58±13*
Older	138±15	93±10*	136±16	100±19*	197±75	151±73*	242±113	195±108*

* $P < 0.05$ vs. basal. ‡ $P < 0.05$ vs. young.

the free level of IGF-I or that fraction complexed with the small IGF binding proteins that is thought to be the biologically active component (16, 17), the doses of rhIGF-I and insulin were selected according to their observed biological effects (i.e., their capacity to stimulate glucose uptake) rather than on a molar basis. This provided a means of adequately raising free IGF-I and insulin in the face of protein binding. Therefore, while the doses of insulin and rhIGF-I were bioequivalent with respect to stimulation of glucose uptake, it should be noted that, on a molar basis, 18-fold more rhIGF-I was required to achieve similar increases in glucose disposal in this study. Similar results
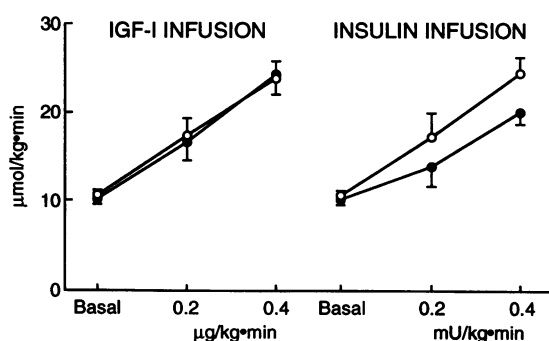

Figure 6. Comparison of rhIGF-I- and insulin-stimulated glucose uptake in young (○) as compared to middle-aged (●) subjects.

were obtained by Guler et al. (18) when they compared the glucose-lowering effect of intravenous boluses of the hormones. By standardizing the doses in this manner, we were able to examine relative differences in potency of rhIGF-I and insulin on other insulin-sensitive substrates, hormones, and metabolic processes.

Concomitant with the similar increases in glucose uptake, the infusions of rhIGF-I and insulin also caused similar, dose-dependent increases in oxidative and nonoxidative glucose disposal (i.e., glycogen storage). Interestingly, both hormones had greater stimulatory effects on nonoxidative compared to oxidative metabolic pathways. This is consistent with the data of Jacob et al. (4) and Zapf et al. (19) who showed that rhIGF-I infusions in rats effectively stimulated muscle glycogen synthesis. Unexpectedly, the inhibitory effect of both hormones on endogenous hepatic glucose production was virtually indistinguishable. Since the liver is exquisitely sensitive to insulin, it is not surprising that even the lowest dose of insulin caused a substantial suppression of hepatic glucose production that was further enhanced with the two higher doses. The similar biologic potency of even the lowest dose of rhIGF-I on hepatic glucose production is remarkable when viewed in the context of the data of Caro et al. (20) who reported that normal adult human hepatocytes are virtually devoid of IGF-I receptors. It is possible that rhIGF-I's effect on the liver is mediated via the insulin receptor, a conclusion supported by data of Hartmann et al. (21) who showed that IGF-I induced insulin-like actions in cultured adult rat hepatocytes via interaction with the insu-
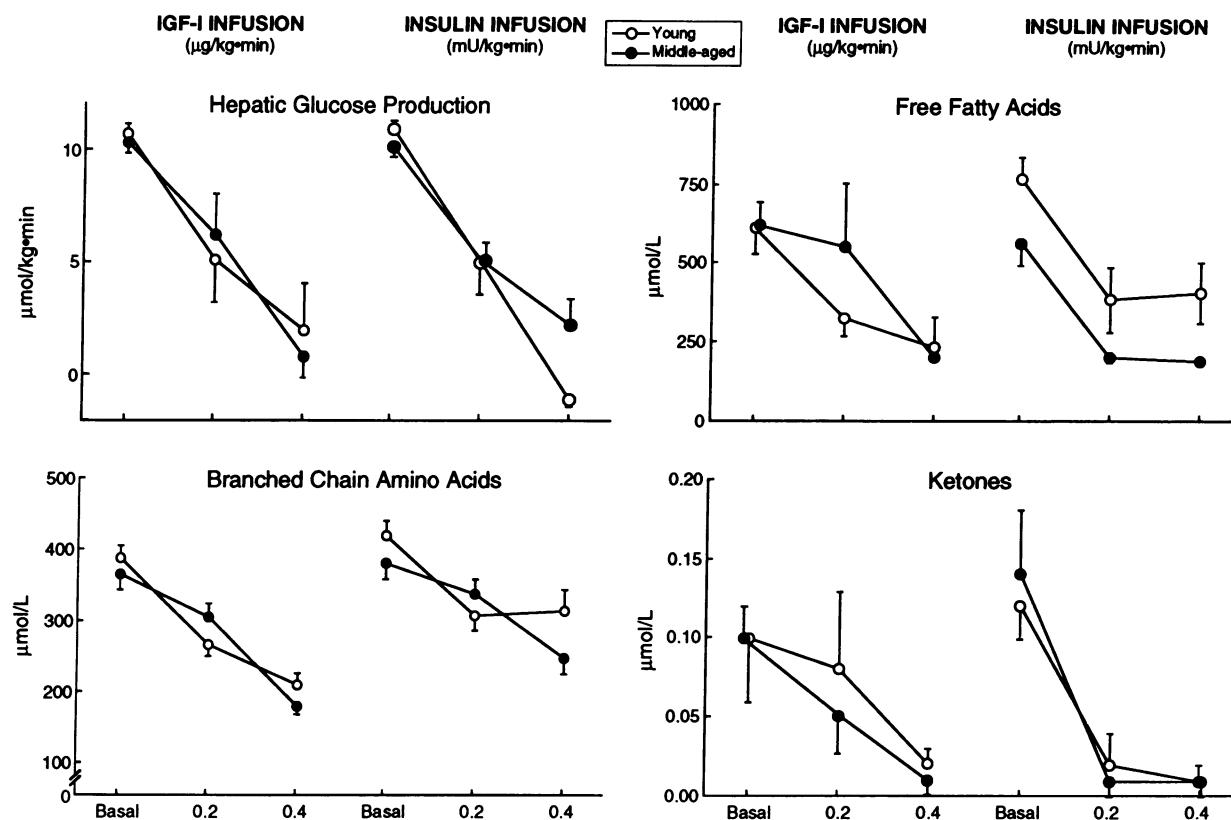
Figure 7. Basal and steady-state glucose production rates and levels of free fatty acids, branched chain amino acids, and ketones during rhIGF-I and insulin infusions in young (o) as compared to middle-aged (●) subjects. L, liter.

lin receptor. Furthermore, the concentration of IGF-I to which the insulin receptor is exposed may be higher than the measured free levels because the fenestrated capillary system of the liver allows passage of IGF-I bound to IGF binding proteins (17). Alternatively, IGF-I could be stimulating IGF-I/insulin hybrid receptors like these that have been found in HepG-2 hepatoma cells (22); however, the existence of such receptors in normal liver tissue has not been identified. Finally, it is likely that the suppression of hepatic glucose production is, at least in part, mediated by the fall in circulating glucagon (23). However, in as much as the decline in glucagon during rhIGF-I infusion, like that seen during somatostatin infusion, is accompanied by a reduction in portal insulin as well, the persistent inhibitory effect on the liver (which is not seen with somatostatin) implies that the phenomenon cannot be explained solely by glucagon deficiency. Whatever the mechanism, the dose–response patterns generated by rhIGF-I and insulin were equivalent not only in the periphery, but at the level of the liver as well.

RhIGF-I and insulin also had bioequivalent effects to reduce circulating levels of FFA and β-OHB and fat oxidation in young subjects. Although we have previously reported that FFA levels were significantly decreased by rhIGF-I (5), it is surprising that the insulin-like effects of rhIGF-I on adipose tissue (reduction in plasma levels of FFA and in turn the decrease in fat oxidation) were only minimally different from matched doses of insulin. This is particularly remarkable since rhIGF-I simultaneously inhibited endogenous insulin secretion which would be expected to counteract rhIGF-I's effects on lipid metabolism. In vitro studies in rat (24–26) and more

recently human (27, 28) adipose tissue have shown that insulin was 600–1,000 times more potent than rhIGF-I in inhibiting lipolysis; however, Kern et al. (29) have shown that rhIGF-I was 40-fold more potent than insulin in inducing lipoprotein lipase activity. It is noteworthy in this regard that some investigators have been unable to show the presence of specific IGF-I binding sites in human adipocytes (27, 30), while others were able to do so (28). Nevertheless, even in those studies in which specific IGF-I binding sites were identified, it appeared that IGF-I's metabolic effects occurred via stimulation of the insulin receptor in adipose tissue (26, 27). This possibility cannot be ruled out in the present study.

Similar, but quantitatively differing effects of rhIGF-I and insulin were seen in the responses of amino acid and islet hormone levels. RhIGF-I produced a greater reduction of circulating levels of total ($P < 0.007$) and branched chain amino acid levels ($P < 0.0002$) than did insulin, despite the production of hypoinsulinemia with rhIGF-I infusion. These data suggest that rhIGF-I's effects on protein metabolism may be more pronounced than those of insulin, at least in relation to glucose metabolism. While insulin's in vivo effects on protein metabolism appear to be mediated by inhibition of protein breakdown (31–33), rhIGF-I's effects on protein metabolism have been less well studied, but appear to be induced by similar mechanisms. Clemmons, Underwood, and co-workers have shown that, even in calorically restricted adult humans, rhIGF-I can improve nitrogen balance (34) and studies using labeled leucine have found an acute, inhibitory effect of rhIGF-I on protein breakdown (4, 6, 35), whereas Mauras et al. (36) were unable to show such an effect with low-dose rhIGF-I. Our data

showing a more pronounced effect of rhIGF-I on protein metabolism than insulin is consistent with the long-held view that rhIGF-I plays a crucial role in promoting the slower growth processes, while insulin acts mainly on the more acute metabolic processes. Nevertheless, the current data imply that the importance of IGF-I as a regulator of body fuel metabolism may have been underestimated from studies in vitro and in animals.

It should be emphasized that the observed insulin-like effects of rhIGF-I on glucose, lipid, and protein metabolism occurred concomitantly with a rapid and striking dose-dependent reduction in plasma levels of insulin and C-peptide. These effects seen with rhIGF-I were significantly greater than those seen with insulin. In addition glucagon levels were also decreased by both hormones. It is not known whether the inhibitory effect of rhIGF-I on islet cells is a direct or indirect effect, however, the presence of IGF-I receptors on pancreatic islet cells (37) would suggest that a direct effect of IGF-I is certainly possible. This view is consistent with studies using cultured rat pancreatic β cells (38) and perfused rat pancreas (39) that have shown a direct effect of IGF-I to inhibit glucose-, glucose plus glucagon- and arginine-induced insulin secretion, that was significantly greater than that seen with insulin or IGF-II. Furthermore, an inhibitory effect of rhIGF-I on glucose-mediated insulin secretion (40) and hypoglycemia-induced glucagon secretion has recently been reported in humans (41). The present study, in which euglycemia is maintained, indicates that even relatively small doses of rhIGF-I are able to reduce insulin and glucagon secretion even in the absence of changes in basal plasma glucose concentrations.

Interestingly, there were essentially no differences in the metabolic effects of rhIGF-I in the older as compared to the younger subjects. It should be noted that while the responses to rhIGF-I were comparable in both age groups, IGF-I levels were strikingly lower in the older subjects. While previous studies have shown that IGF-I levels decline with age (9, 10), the populations in those reports have been older than the healthy, active middle-aged subjects in this study in which only one subject was over 60 yr old and 10 of the 14 "older" subjects were < 50 yr old. The older subjects were slightly heavier than the younger subjects. It is conceivable that mild obesity contributed, in part, to the decline in IGF-I levels seen in the older group since body mass index and percentage of ideal body weight have been shown to correlate negatively with IGF-I levels in males, although a similar effect is not seen in females (10). In contrast, basal insulin and C-peptide levels were higher in the middle-aged subjects, suggesting that insulin secretion was increased in this group. In keeping with the increased insulin levels, the response of glucose uptake to insulin was somewhat impaired in the older subjects; however, we could not detect any differences in the metabolic response of the more insulin-sensitive fuels—fat and protein. Thus our data are consistent with the idea that IGF-I and insulin, both anabolic hormones, undergo changes in circulating concentrations as well as metabolic effectiveness during even the early stages of aging: IGF-I's metabolic actions are normal, but circulating concentrations are reduced while insulin's metabolic effects are impaired but this is compensated for by increased insulin secretion. The net metabolic result is a relatively greater deficiency of IGF-I than insulin which may lead to the changes in body composition that are seen during aging.

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