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Insulin-Like Growth Factor I and Growth Hormone (GH) Treatment in GH-Deficient Humans: Differential Effects on Protein, Glucose, Lipid, and Calcium Metabolism*

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ABSTRACT

We examined the effects of recombinant human (rh) insulin-like growth factor I (IGF-I) *vs.* rhGH in a variety of metabolic paths in a group of eight severely GH-deficient young adults using an array of contemporary tools. Protein, glucose, and calcium metabolism were studied using stable labeled tracer infusions of L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose, and ^{42}Ca and ^{44}Ca; substrate oxidation rates were assessed using indirect calorimetry; muscle strength was determined by isokinetic and isometric dynamometry of the anterior quadriceps, as well as growth factors, hormones, glucose, and lipid concentrations in plasma before and after 8 weeks of rhIGF-I (60 μg/kg, sc, twice daily), followed by 4 weeks of washout, then 8 weeks of rhGH (12.5 μg/kg·day, sc); the treatment order was randomized.

In the doses administered, rhIGF-I and rhGH both increased fat-free mass and decreased the percent fat mass, with a more robust decrease in the percent fat mass after rhGH; both were associated with an increase in whole body protein synthesis rates and a decrease in protein oxidation. Neither hormone affected isokinetic or isometric measures of skeletal muscle strength. However, rhGH was more potent than rhIGF-I at increasing lipid oxidation rates and improving plasma lipid profiles. Both hormones increased hepatic glucose output, but rhGH treatment was also associated with decreased carbohydrate oxidation and increased glucose and insulin concentrations, indicating subtle insulin resistance. Neither hormone significantly affected bone calcium fluxes, supporting the concept that these hormones, by themselves, are not pivotal in bone calcium metabolism. In conclusion, rhIGF-I and rhGH share common effects on protein, muscle, and calcium metabolism, yet have divergent effects on lipid and carbohydrate metabolism in the GH-deficient state. These differences may allow for better selection of treatment modalities depending on the choice of desired effects in hypopituitarism. (*J Clin Endocrinol Metab* **85:** 1686–1694, 2000)

THE METABOLIC effects of recombinant human (rh) insulin-like growth factor I (rhIGF-I) have been extensively studied in a variety of experimental situations in man, and available evidence suggests that IGF-I has both GH-like and insulin-like effects (1). IGF-I mediates many, but not all, of the metabolic actions of GH *in vivo*. IGF-I is clearly responsible for the linear growth response to GH therapy, as demonstrated by the profoundly stunted growth of patients with GH receptor deficiency (Laron's syndrome) who have high circulating GH concentrations and markedly low IGF-I levels; these patients can grow normally with rhIGF-I treatment (2, 3). IGF-I also mediates the protein anabolic actions of GH, and a protein-anabolic response has been observed after rhIGF-I similar to that observed after rhGH therapy, with a selective increase in whole body protein synthesis and no effect on proteolysis (4, 5). Regarding carbohydrate metabolism, however, there is a clear dichotomy of effects, with a potent glucose-lowering effect despite significant suppression of circulating insulin by rhIGF-I treatment (6), whereas GH treatment can be associated with a measurable increase in insulin and glucose concentrations, indicative of mild insulin resistance (7). The GH effects on fat metabolism, the increased mobilization of free fatty acids, and increased lipolysis, on the other hand, are probably not mediated by IGF-I, as there are no functional type I IGF-I receptors in the adipocyte (8). GH also has been found to have bone anabolic actions in both experimental animals and in men (9, 10), and actually, profound GH deficiency may be associated with osteopenia (11).

Most studies directly comparing the metabolic effects of these hormones have been short term (7–10 days) in both normal volunteers and GH-deficient patients (5, 12, 13). The effects of IGF-I on bone, however, are less well studied in humans, even though animal data and short term human studies suggest a positive bone anabolic effect as well (14–16). The specific mechanisms involved in the effects of these two hormones on bone calcium metabolism are not well characterized.

Available techniques using stable isotope infusions of different natural compounds now allow us to further characterize the specific changes in the intermediate metabolism of protein, glucose, and calcium in a noninvasive, well tolerated way. Indirect calorimetry and body composition assessment also add substantial information regarding the effects of hormones and nutrients at the whole body level. We set up the

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present studies to further characterize the *in vivo* effects of GH *vs.* IGF-I after more prolonged administration (8 weeks for each study arm) in the GH-deprived state. To accomplish this we recruited eight subjects with profound GH deficiency and treated them with rhIGF-I and rhGH for 8 weeks, each with a 4-week washout period in between. Marked differences in the metabolic effects of these peptides were observed.

Subjects and Methods

Study subjects

These studies were approved by the Nemours Children's Clinic Clinical Research review committee and the Baptist Medical Center Institutional Review Board. Eight patients with clinical and biochemical evidence of GH deficiency were recruited for these studies after informed written consent was obtained. Their clinical characteristics are summarized in Table 1. Those with other pituitary hormone deficiencies were receiving replacement treatment during these studies. All but two subjects (no. 2 and 6) had childhood-onset GH deficiency. Those patients previously treated with GH had been off GH for at least 1.5 yr before these studies.

Experimental design

For 3 days before admission, subjects were encouraged to consume a weight maintenance diet consisting of approximately 30 Cal/kg and 1 g/kg protein·day; they were admitted to the Wolfson Children's Hospital Clinical Research Center the afternoon before the first study. Assessment of body composition was obtained using skin fold calipers, bioelectrical impedance analysis, as well as dual emission x-ray absorptiometry (DEXA), using a tissue bar (Hologic, Inc., Waltham, MA). Isokinetic and isometric dynamometry of the anterior quadriceps was performed in our physical therapy department using a Biodex dynamometer (Biodex Corp., Shirley, NY). After a 10-min training session and 30 min of rest, maximum work and torque measures for isometric and isokinetic tests were performed. Isometric tests were performed with 5 contractions of 5 s each, with the knee placed at 45° of flexion, and 10 s of rest between contractions. Isokinetic tests were performed for knee extension and flexion at 60°/s for 5 repetitions and at 180°/s for 21 repetitions as described previously (17).

Subjects were fed dinner at 1800 h and were given 0.3 mg/kg of a stable isotope of calcium (^{44}Ca) orally mixed with milk or juice prepared at least 12 h before equilibration. A urine collection was begun after the oral Ca tracer and continued for the next 28 h. The patients were subsequently fasted except for water *ad libitum* until the completion of the studies at 1300 h the next day. The following morning (baseline study), at 0600 h, two iv needles were placed in each antecubital vein. One was kept heated for arterialized blood sampling (18). At 0800 h (time zero), three stable isotope tracers were given. One, a primed, dose constant infusion of L-[1-^{13}C]leucine (4.5 μmol/kg; 0.07 μmol/kg·min) was begun and continued uninterrupted for the next 240 min. Concomitantly, a primed infusion of [6,6, $^{2}H_2$]glucose (33 μmol/kg; 0.33 μmol/kg·min) was begun and continued also for 240 min. At time zero, 0.15 mg/kg ^{42}Ca was also given as a slow iv push over 5 min. Frequent blood samples were collected for determination of isotopic enrichment of the different tracers in plasma as well as the concentrations of hormones, substrates, and growth factors, as detailed below. Frequent breath samples were also obtained for determination of $^{13}CO_2$ enrichment in expired breath. Indirect calorimetry was performed three times during the 4 h of isotope infusion using a mouthpiece and a CPX max indirect calorimeter (Medical Graphics, St. Paul, MN). After the isotope infusions were completed, the patients were fed lunch, and one iv line was discontinued. Subjects were subsequently free to move around, and at 1600 h another blood sample was obtained for determination of Ca enrichment. Subjects were then sent home to complete the urine collection. For the next 5 days, twice daily urine samples were obtained for determination of the Ca isotopic enrichments.

After the baseline study and all urine collections were completed, patients were started on rhIGF-I at 60 μg/kg, sc, twice daily. Subjects were instructed to monitor their blood glucose concentrations using home glucose-monitoring equipment for the first week after initiation of rhIGF-I therapy and any other time there were any symptoms of hypoglycemia. They were also instructed to take the rhIGF-I injection with their meals to avoid hypoglycemia. Blood was withdrawn 4 weeks after the initiation of treatment for determination of hormone concentrations and safety laboratories. Eight weeks from the baseline study an identical study was performed. The night before the second study, starting at 2000 h, the second dose of rhIGF-I was substituted for a continuous sc infusion of the peptide at 10 μg/kg·h, which was continued uninterrupted for the next 17 h until completion of the studies the following morning. This was done to prevent hypoglycemia during the administration of rhIGF-I while the patients were fasting, while maintaining plasma IGF-I concentrations constant. We have successfully used this strategy in similar experiments (4, 5, 19). Afterward, rhIGF-I treatment was discontinued, and a washout period of 4 weeks was observed without any treatment. Blood samples were again withdrawn at the end of the washout period. rhGH was started as a single sc injection of 12.5 μg/kg at bedtime and was continued for another 8 weeks when the studies were repeated a third time. The treatment order was randomized: D1, baseline study; D2, rhIGF-I for 8 weeks; followed by 4-week washout; and then rhGH was administered for 8 weeks, followed by D3.

Blood and breath samples

The isotopic enrichments of α-ketoisocaproic acid (^{13}C labeled), and $^{2}H_2$-glucose were measured at −20, 160, 180, 200, 220, and 240 min. The Ca isotopic enrichments were measured at 0, 5, 10, 15, 20, 30, 40, 60, 120, 180, 240, and 480 min. Plasma IGF-I, IGF-binding protein-1 (IGFBP-1), IGFBP-2, IGFBP-3, insulin, and glucose concentrations were measured three times during the 240 min of tracer infusions. Serum GH concentrations were measured at 10-min intervals for the 4 h of the studies. Serum lipids were also measured while fasting on each study day. Breath samples were obtained for the measurement of expired labeled CO_2 at −20, −10, −5, 160, 180, 200, and 220 min. A small aliquot of the urine collected during the 4 h of the morning study was used for determination of urea nitrogen concentration.

Assays

Plasma enrichments of [^{13}C]α-ketoisocaproic acid and $^{2}H_2$-glucose were determined at the Nemours metabolic core laboratory by mass chromatography mass spectrometry as previously described (20, 21). $^{13}CO_2$ was measured by isotope ratio mass spectrometer as described previously (22). Urinary Ca was determined by flame atomic absorption spectrophotometry at the laboratory of Dr. O'Brien. A dual filament

TABLE 1. Clinical and biochemical characteristics of the study subjects

Patient no.	Age (yr)	Sex	Diagnosis	Peak GH (ng/mL)
1	18.9	F	Septo optic dysplasia	0.50
2	33.2	M	Astrocytoma	0.30
3	21.3	F	Craniopharyngioma	0.06
4	19.6	M	Optic glioma	1.10
5	23.0	M	Idiopathic	1.46
6	29.6	M	Idiopathic	0.16
7	15.5	M	Idiopathic	3.00
8	27.0	M	Hypoplastic anterior pituitary	0.21
Mean ± SEM	23.5 ± 2.1			0.85 ± 0.35

thermal ionization quadrapole mass spectrometer (Model THQ, Finnigan MAT, Bremen, Germany) was used to measure the Ca isotopic enrichments as previously described (23). All insulin and lipid concentrations were determined at the immunochemical core laboratory at the Mayo Clinic General Clinical Research Center (Rochester, MN) using commercial kits. IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 concentrations were measured by radioimmunometric assays at Endocrine Sciences, Inc. (Calabassas Hills, CA), and insulin concentrations were determined by chemiluminescence assay. Serum GH concentrations were measured by a highly sensitive chemiluminescence assay at the University of Virginia Clinical Research Center core laboratory (Charlottesville, VA). Plasma glucose concentrations were measured with a glucose oxidase method using a glucose analyzer (Beckman Coulter, Inc., Palo Alto, CA) at the bedside. Serum lipids [cholesterol, triglycerides, and high and low density (LDL) lipoproteins] concentrations were measured using high performance liquid chromatography methods. Urea nitrogen was measured using a Kodak Ektakem urease method (Eastman Kodak Co., Rochester, NY).

Calculations

The reciprocal pool model was used to estimate rates of whole body protein turnover at steady state as previously described (24). The rate of appearance (Ra) of glucose, a measure of hepatic glucose output, was calculated as: $Ra = [(Ei/Ep) - 1]F$, where Ei is the isotopic enrichment of the infusate, Ep is the enrichment of glucose in plasma, and F is the infusion rate.

For the Ca kinetic analysis, the fractional Ca absorption (α) was calculated from the ratio of the cumulative excretion of the oral tracer (^{44}Ca) in urine divided by the cumulative excretion of the iv tracer (^{42}Ca) as previously described (25, 26).

True Ca absorption was calculated as $Va = Vi \times \alpha$, where Vi is the dietary Ca intake. Ca kinetic analysis was performed by measuring the isotopic enrichments of the Ca tracers in blood and urine over time using the three-pool multicompartmental model and the simulation analysis and modeling program, SAAM, as previously described (27, 28). Other absorption and kinetic terms used are: Vu, urinary Ca excretion; Vf, endogenous fecal Ca excretion (estimated as 1.5 mg/kg·day) (29); Vo^+, the rate of bone Ca deposition; and Vo^-, rate of bone Ca resorption. Substrate oxidation rates for protein, glucose, lipid, and resting energy expenditure were calculated using the rate of gas exchange (VO_2 and VCO_2) from the indirect calorimetry as previously described (30).

Fat free mass (FFM) and percent fat mass (FM) were measured using DEXA and the tissue bar as well as by the sum of skin folds as described previously (31).

Isotopes and drugs

L-[1-^{13}C]Leucine (99% enriched; Cambridge Isotopes, Andover, MA), [6,6-$^2H_2$]glucose (99.7% enriched, MSD Isotopes, St. Louis, MO), and $^{42}Ca/^{44}Ca$ (93.5% and 96% enriched, respectively; Trace Sciences International, Richmond Hill, Canada) were determined to be sterile and pyrogen free and were mixed with 0.9% nonbacteriostatic saline. rhIGF-I (10 mg/mL) and rhGH (Nutropin; 10 mg/mL) were provided by Genentech, Inc. (South San Francisco, CA).

Statistical analysis

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

Results

There was no apparent difference in the trend of any of the parameters measured depending on the treatment order of rhIGF-I or rhGH; hence, data were grouped for analysis according to the hormone received. Figure 1 shows the plasma IGF-I concentrations at baseline, after 8 weeks of rhIGF-I, after 4 weeks of washout, and after 8 weeks of rhGH.

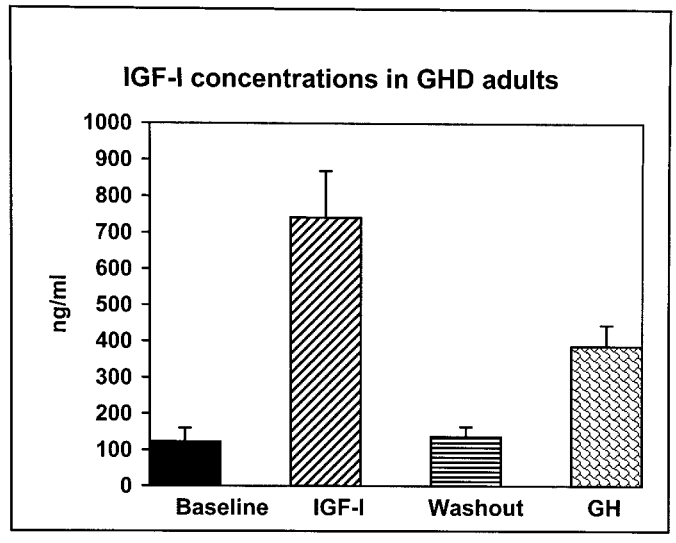

FIG. 1. Plasma IGF-I concentrations in eight GH-deficient patients studied at baseline and 8 weeks after rhIGF-I, after a 4-week washout, and after 8 weeks of rhGH treatment. There was no difference in IGF-I concentrations between baseline values and those obtained after 4 weeks of washout.

Plasma IGF-I concentrations returned to baseline levels in the study subjects after 4 weeks of discontinuation of hormone treatment.

Body composition and muscle strength (Table 2)

There was a modest increase in weight after rhIGF-I treatment, but not after rhGH. There were comparable trends in body composition using either DEXA or sum of skin folds; hence, data are shown for DEXA only. Body composition, however, changed comparably after either hormone, with a 3.1-kg (6%) increase in FFM after rhIGF-I and a 3.6-kg (6.8%) increase after rhGH ($P \leq 0.01$ for both) and decreases in %FM of 1.2% and 3% after rhIGF-I and rhGH, respectively. The decrease in %FM was greater after rhGH than after rhIGF-I ($P = 0.02$ between the two hormone treatments). There were no detectable changes in isometric or isokinetic measures of muscle strength of the leg extensors or leg flexors (data for the latter not shown).

Protein kinetics (Table 3)

There were no changes in the Ra of leucine, a measure of whole body proteolysis, after rhIGF-I, yet there was a clear trend toward higher protein turnover after rhGH administration ($P = 0.06$). Protein oxidative rates decreased comparably with both hormones (approximately −27–29%) with increases in nonoxidative leucine disposal, a measure of whole body protein synthesis, after the administration of both hormones; this was more pronounced after rhGH.

Substrate oxidation and energy expenditure rates

Clear differences in the metabolic effects of these hormones on intermediate metabolism in the GHD state were observed. Carbohydrate oxidation rates were unaltered after rhIGF-I treatment, whereas they decreased after rhGH [baseline, 18.2 ± 3.7 Cal/FFM·day; rhIGF-I, 15.3 ± 4.1 (P = NS);

TABLE 2. Changes in body composition (by DEXA) and muscle strength [by isokinetic (IK) and isometric (IM) dynamometry of the leg extensors] in GHD subjects at baseline and after treatment with rhIGF-I and rhGH for 8 weeks each

	Baseline	rhIGF-I	rhGH
Body composition			
Wt (kg)	81.3 ± 7.2	83.8 ± 7.4[a]	82.9 ± 7.9
BMI (kg/m^2)	28.2 ± 2.2	29.0 ± 2.1	28.7 ± 2.2
FFM (kg)	51.8 ± 5.4	54.9 ± 5.7[b]	55.4 ± 6.2[b]
% FM	34.7 ± 4.1	33.5 ± 4.0[b]	31.7 ± 4.5[c]
Muscle strength			
IM ext (Nm)	127.6 ± 17.3	139.6 ± 21.0	132.6 ± 22.7
IK ext 60 °/s (Nm)	152.7 ± 27.4	151.4 ± 24.0	154.2 ± 27.4
IK ext 180 °/s (Nm)	116.4 ± 17.0	111.6 ± 13.3	118.4 ± 17.9

Data from the rhIGF-I and rhGH were compared against baseline.
[a] $P < 0.05$ *vs.* baseline.
[b] $P \leq 0.01$ *vs.* baseline.
[c] $P \leq 0.001$ *vs.* baseline.

TABLE 3. Protein kinetic data (micromoles per kg/min) in GHD subjects

	Baseline	rhIGF-I	rhGH
Leucine Ra	1.49 ± 0.11	1.57 ± 0.13	1.86 ± 0.13[a]
Leucine oxidation	0.26 ± 0.03	0.19 ± 0.02[b]	0.19 ± 0.02[b]
NOLD	1.23 ± 0.10	1.38 ± 0.12[a]	1.68 ± 0.13[c]

Ra measures proteolysis, NOLD (nonoxidative leucine disposal) measures whole body protein synthesis rates.
[a] $P = 0.06$ *vs.* baseline.
[b] $P \leq 0.001$ *vs.* baseline.
[c] $P \leq 0.02$ *vs.* baseline.

rhGH, 13.5 ± 3.8 ($P < 0.05$ *vs.* baseline)]. Protein oxidation decreased comparably after both treatments [baseline, 3.8 ± 0.4 Cal/kg FFM·day; rhIGF-I, 2.7 ± 0.3 ($P = 0.0002$); rhGH, 2.5 ± 0.2 ($P = 0.001$ *vs.* baseline)]. Lipid oxidation rates increased only after rhGH, not rhIGF-I, treatment [14.6 ± 1.6 Cal/kg FFM·day at baseline; 15.6 ± 1.7 after rhIGF-I (P = NS); 20.1 ± 2.1 after rhGH ($P = 0.04$ *vs.* baseline)]. There were no detectable changes in resting energy expenditure (baseline, 33.4 ± 2.0 Cal/kg FFM·day; rhIGF-I, 30.3 ± 2.0; rhGH, 32.8 ± 3.0), as shown in Fig. 2.

Glucose metabolism (Table 4)

Using glucose tracer data, measures of hepatic glucose output increased comparably and significantly after the administration of both rhIGF-I and rhGH. However, circulating fasting glucose concentrations only increased after rhGH therapy, whereas insulin concentrations decreased after rhIGF-I and increased after rhGH.

Calcium absorption and kinetics (Table 5)

Complete datasets for all 3 study days were available for only five patients due to missed urine collections. Measures of fractional and total calcium absorption (α and Va, respectively) as well as kinetic measures of bone calcium deposition (Vo^+), bone calcium resorption (Vo^-), and total calcium turnover rates remained invariant during both rhIGF-I and rhGH therapy. However, measures of urinary calcium excretion were greatly increased after rhIGF-I, but not rhGH, therapy.

Hormones, growth factors, and plasma lipids (Table 6)

Both rhIGF-I and rhGH markedly increased plasma IGF-I concentrations, but the increase was substantially greater after rhIGF-I treatment. Fasting concentrations of IGFBP-1 did not change after either hormone, whereas IGFBP-2 increased only during rhIGF-I therapy, and IGFBP-3 increased only after rhGH. IGF-II concentrations plummeted after rhIGF-I and remained invariant after rhGH therapy. Total cholesterol and high density lipoprotein cholesterol concentrations were not changed during either treatment; however, a substantial decrease in LDL cholesterol was observed after 8 weeks of rhGH treatment. Circulating triglyceride concentrations were only affected by rhGH treatment with a 44% increase. A trend toward suppressed GH pulsatility was observed after rhIGF-I treatment, but it did not reach statistical significance.

Discussion

The effects of rhIGF-I were directly compared with those of rhGH in a wide array of metabolic pathways in young adults with profound GH deficiency treated with these hormones for 8 weeks each. There were significant similarities on the metabolic effects of these peptides on body composition and muscle strength, protein, and calcium metabolism for *e.g.* yet there were marked differences on lipid and carbohydrate metabolism effects, as measured with the present tools of study, again supportive of the concept that IGF-I mediates only some of the metabolic actions of GH *in vivo*.

Body composition and protein metabolism

Body composition, as measured by DEXA, was comparably affected after rhIGF-I and rhGH treatment, with measurable increases in FFM and decreased %FM even after 8 weeks of continuous therapy. This strongly suggests that long term rhIGF-I treatment may have beneficial effects comparable to those of rhGH on body composition in GH-deficient subjects, particularly as it pertains to FFM accumulation. There appears to be a more robust decline in %FM after rhGH than after rhIGF-I. Even though DEXA cannot compartmentalize if these changes were due solely to changes in lean soft tissue *vs.* water, the changes in FFM were accompanied by a 20% increase in the total rate of whole body protein synthesis, as measured by the leucine tracer studies, indicating that both hormones affect large body protein pools. These results are similar to those ob-

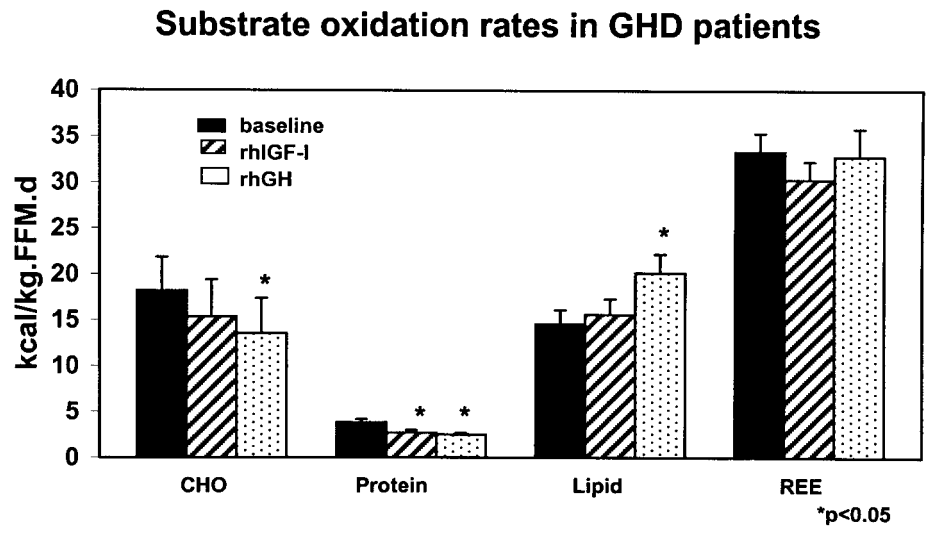

FIG. 2. Substrate oxidation rates were measured by indirect calorimetry in eight GH-deficient subjects at baseline, after 8 weeks of rhIGF-I, and after 8 weeks of rhGH, with a 4-week washout in between treatments. CHO, Carbohydrate oxidation; REE, resting energy expenditure.

TABLE 4. Glucose rate of appearance (Ra) and insulin and glucose concentrations in GHD patients during rhIGF-I/rhGH treatment

	Baseline	rhIGF-I	rhGH
Glucose Ra (mg/kg·min)	1.48 ± 0.09	1.94 ± 0.24[a]	1.78 ± 0.10[a]
Glucose (mg/100 mL)	86 ± 3	90 ± 4	94 ± 1[a]
Insulin (μU/mL)	9.2 ± 3.0	3.6 ± 1.3[a]	14.2 ± 3.5[b]

[a] $P < 0.03$ *vs.* baseline.
[b] $P < 0.001$ *vs.* baseline.

TABLE 5. Absorption and kinetics of Ca using stable tracer data in GHD subjects

	Baseline	rhIGF-I	rhGH
Vi	1238 ± 275	1286 ± 241	1179 ± 237
α	0.38 ± 0.03	0.43 ± 0.09	0.48 ± 0.05
Va	492 ± 135	469 ± 81	499 ± 89
Vu	110 ± 25	253 ± 39[a]	167 ± 39
Vo^+	1068 ± 185	1353 ± 196	1274 ± 45
Vo^-	908 ± 218	1279 ± 219	1056 ± 140

All units are milligrams per day except α, which has no units.
Vi, Dietary Ca intake; α, fractional Ca absorption; Va, total Ca absorption; Vu, urinary Ca excretion; Vo^+, bone Ca deposition; Vo^-, bone Ca resorption.
[a] $P < 0.005$.

served by us and others in both healthy volunteers and volunteers made catabolic with either glucocorticosteroid treatment or during caloric deprivation treated with rhGH, rhIGF-I, or both (4, 5, 12, 32). However, contrary to the results found in the normal subjects, where administration of both of these hormones was associated with a selective increase in whole body protein synthesis with no effects on proteolysis, in the GH-deficient state studied here, rates of proteolysis were also markedly increased after rhGH, but not rhIGF-I, treatment, indicating a differential effect of GH in GH-depleted states. The latter may be secondary to the relative insulin resistance typically observed after GH therapy, particularly in GH-deficient states (7) as insulin inhibits proteolysis (33); hence, in insulin resistance states increased proteolytic rates would be expected (34). Previous reports in GH-deficient subjects studied with comparable isotope tracer methods showed diminished rates of whole body leucine turnover compared to those in healthy controls (35). Other reports, also in GH-deficient patients in whom leucine turnover was measured before and after GH therapy, showed a selective increase in protein synthesis after GH treatment (36) similar to that observed in healthy controls treated with GH (32). This difference is probably related to dose, as in the study of GH-deficient subjects (36) the highest dose of GH administered was 3.3 μg/kg·day, almost a fourth of the dose used in the present studies performed in younger patients; again, the higher dose was associated with greater insulin resistance.

Muscle strength

Skeletal muscle has few GH receptors, yet type I IGF-I receptors are rather ubiquitous in skeletal tissue, suggesting that IGF-I is the main mediator of the actions of GH in myocytes (37). In the present studies we raised plasma IGF-I concentrations to levels observed in the midst of puberty, particularly after rhIGF-I, yet there were no detectable changes in skeletal muscle strength in these individuals, as neither isokinetic nor isometric measures of strength were altered by rhGH or rhIGF-I treatment. These results parallel those observed in elderly subjects treated with rhGH (38) and in GH-deficient subjects treated with rhGH for up to 6 months where similar measures of muscle strength were used (39, 40). Even though the length of treatment (8 weeks each) might arguably not be enough to detect an effect on muscle strength, in similar experiments conducted by us in healthy subjects rendered intentionally hypogonadal pharmacologically, we detected significant decreases in muscle strength after only 10 weeks of sustained hypogonadism (17). The latter suggests that sex steroids may play a more pivotal role than the GH/IGF-I axis in enhancing skeletal muscle strength in humans. There are, to our knowledge, no published results to date on similar trials using rhIGF-I examining muscle strength; however, the present results suggest that neither rhIGF-I nor rhGH *per se* affects muscle strength in the absence of significant exercise training. The use of these

TABLE 6. Hormones, growth factors, and plasma lipid concentrations in GHD patients

	Baseline	rhIGF-I	rhGH
IGF-I (ng/mL)	121 ± 38	742 ± 127[a]	386 ± 58[a]
IGFBP-1 (ng/mL)	21 ± 9	30 ± 11	17 ± 12
IGFBP-2 (ng/mL)	340 ± 78	813 ± 160[a]	241 ± 48
IGFBP-3 (mg/L)	2.23 ± 0.33	2.30 ± 0.42	3.45 ± 0.34[a]
IGF-II (ng/mL)	408 ± 88	106 ± 15[b]	411 ± 39
Mean GH (ng/mL)	0.47 ± 0.18	0.24 ± 0.15	1.61 ± 0.55[c]
Peak GH (ng/mL)	1.00 ± 0.49	0.14 ± 0.07	2.62 ± 0.53[c]
Total cholesterol (mg/100 mL)	177 ± 14	188 ± 21	175 ± 11
LDL (mg/100 mL)	112 ± 13	135 ± 21	89 ± 10[c]
HDL (mg/100 mL)	39 ± 4	37 ± 4	39 ± 3
Triglycerides (mg/100 mL)	126 ± 19	131 ± 27	182 ± 33[d]

[a] $P < 0.002$.
[b] $P < 0.01$.
[c] $P < 0.03$.
[d] $P < 0.05$.

agents as ergogenic agents thus appears unsubstantiated by the available data.

Calcium flux

Calcium tracer studies have been extensively used in a variety of experimental situations in humans and offer the advantage of allowing the assessment of bone calcium fluxes in a noninvasive way (23–26, 29, 41, 42). Using these tools, measures of calcium absorption (Va), bone calcium deposition (Vo^+), and bone calcium resorption (Vo^-) did not change after rhIGF-I or after rhGH therapy, suggesting that these peptides do not significantly affect bone calcium metabolism in the short term. Previous studies of the effects of GH on bone have yielded somewhat conflicting results, some showing no effect of GH on bone mass after 6 months of therapy, and others showing an actual decrease in bone mineral density after treatment (40). Histomorphometric analysis of transiliac bone biopsies in GH-deficient patients treated with rhGH for 1 yr showed that only cortical thickness, not trabecular bone, increases after GH therapy (43). It is now clear that GH increases bone formation in GH-deficient states only after prolonged treatment (>18 months) (9). IGF-I, on the other hand, has been shown to have potent bone anabolic actions *in vitro* and *in vivo* in the short term, stimulating collagen synthesis (15) as well as increasing the proliferation of osteoblast precursors (14, 15). In short term (6- to 7-day) experiments, the administration of rhIGF-I to normal women fasted for 10 days (16), to women with anorexia nervosa (44), or to healthy young adult volunteers (45) was associated with increased serum markers of bone formation. There is, however, a paucity of data on the effects of rhIGF-I on bone mineralization after long term exposure. The results presented here after 8 weeks of rhIGF-I or rhGH each contrast with the marked effects on calcium kinetics observed after 4 weeks of testosterone administration in boys (41) and 4 weeks of oral estrogen treatment in hypogonadal girls (42), where calcium absorption and bone calcium deposition were increased using identical calcium tracer tools. Selective suppression of gonadal steroids in normal young men was also associated with marked decreases in bone calcium fluxes (Vo^+) and profound urinary calcium losses after even 4 weeks of sustained hypogonadism (46), indicating that the length of treatment in the present studies, *i.e.* 8 weeks of each hormone, might not be the main factor influencing the lack of effect, but, rather, it could be the fact that GH and IGF-I are not as potent as gonadal steroids in influencing calcium absorption and bone calcium deposition in the short term in the GH-deficient state. The observed increase in urinary calcium excretion with rhIGF-I is probably related to the increased renal filtration typically observed after rhIGF-I treatment (47). The physiological relevance of this observation would require further study, but chronic rhIGF-I treatment may need careful assessment of calcium balance.

Substrate oxidation rates and energy expenditure

rhIGF-I and rhGH had divergent effects on other pathways of intermediate metabolism, as measured by gas exchange and indirect calorimetry. Carbohydrate oxidation rates were decreased after rhGH, but not rhIGF-I, administration in these subjects. This is probably secondary to the relative insulin resistance caused by rhGH therapy, whereas the insulin-like effect of rhIGF-I compensated for the suppressed insulin production during rhIGF-I treatment. Hepatic glucose production (glucose Ra), on the other hand, was increased after both hormones, indicating that the hepatic sensitivity to insulin was diminished during both treatments, albeit possibly by different mechanisms: during rhGH by increased insulin resistance, and during rhIGF-I by diminished portal insulin secretion. This increase in hepatic glucose production after both rhGH and rhIGF-I is comparable to that observed after short term (7-day) rhIGF-I treatment in healthy volunteers reported by us previously (19). However, the present results contrast with those observed in GH-deficient subjects treated with both hormones reported previously (13), in whom there was no detectable effect of either hormone on carbohydrate oxidation when measured by the same tools, and hepatic glucose output was increased only after rhGH. The latter difference mostly likely relates to the length of treatment, as the present model of treatment for 8 weeks each was substantially longer than the 7 days previously reported, allowing the changes in insulin sensitivity to become apparent. rhIGF-I has been clearly shown to increase insulin sensitivity in healthy subjects (48). These results sug-

gest that rhIGF-I may be advantageous over rhGH as a replacement therapy in GH-deficient subjects who also have carbohydrate intolerance. This observation would require further study.

Protein oxidation rates decreased comparably in both treatment arms, similar to the results observed in the leucine tracer studies. This suggests that both hormones affect protein metabolism comparably.

Lipid oxidation rates were increased only after the administration of rhGH, not rhIGF-I. This contrasts with the report by Hussain *et al.* (19) in GH-deficient subjects treated with both hormones, in whom lipid oxidation rates increased significantly after both. Again, the significantly more prolonged treatment scheme in the present studies probably explains these differences, as there are no type I IGF-I receptors in adipocytes (8); hence, any short term increase in lipid oxidation observed after 7 days of treatment was attributed to the decrease in insulin production caused by rhIGF-I with the consequent increase in lipolysis and presumably lipid oxidation (19). In the present treatment paradigm, rhGH had a more pronounced effect than rhIGF-I on lipid oxidation and a modest, but significantly greater, decrease in %FM. The latter suggests that rhIGF-I may affect body composition differently than rhGH by promoting protein/muscle accretion and causing a nutrient shift, from adipose to lean body tissue, whereas GH acts in both adipocytes and whole body protein directly. Resting energy expenditure was unchanged after long term treatment with either hormone, underscoring the shift in nutrient utilization with preservation of total energy used.

The difference between the effects of rhIGF-I reported here and those observed in normal volunteers might also be due to the differences in the pharmacokinetics of IGF-I in the GH-deficient state. We recently reported our data from pharmacokinetic studies in GH-deficient patients treated with rhIGF-I, several of whom also participated in the present study, and showed that GH deficiency is associated with normal absorption and distribution of IGF-I, yet faster elimination kinetics than normal subjects (49). However, the higher IGF-I plasma concentrations after rhIGF-I observed here throughout the 8 weeks of the study make the consideration of these differences as being secondary to increased clearance of the peptide unlikely.

Hormones, growth factors, and plasma lipids

rhIGF-I and rhGH had divergent effects on the circulating concentrations of IGFBPs and GH, as previously observed (50); after rhIGF-I treatment, IGF-I concentrations were more increased than after rhGH as well as IGFBP-2 administration, whereas IGF-II concentrations were decreased only after rhIGF-I treatment, and IGFBP-3 levels were increased only after rhGH treatment. Interestingly, only prolonged rhGH, not rhIGF-I, had a significant lowering effect on the LDL concentration in these GH-deficient subjects.

We believe the washout period to be adequate to allow the effects of the two hormones to be detected. Even though the baseline study was not repeated before the initiation of the second hormone cycle, plasma IGF-I concentrations were measured and had clearly returned to baseline in the study subjects after 4 weeks of washout. Previously published studies assessing the effects of both of these hormones have used similar or shorter washout periods. Kupfer *et al.* (12), when studying healthy, GH-sufficient, calorically deprived volunteers, treated these subjects with either rhIGF-I alone or rhGH and rhIGF-I combined in random order with a 3-week washout in between and observed enhancement of the combination treatment on nitrogen balance studies. Moreover, Hussain *et al.* (13) evaluated intermediate metabolism in GH-deficient adults using a paradigm without any washouts, *i.e.* 7 days of observation, followed by 7 days of daily rhIGF-I, then 7 days of rhGH and 7 days of combined treatment. These investigators found divergent effects on lipid and glucose metabolism produced by the two treatment modalities. Also, Lucidi *et al.* (36), while investigating the relative dose response for rhGH in GH-deficient adults, used a paradigm of 1 week of treatment, 2–3 weeks of washout, and then another dose of rhGH. Differential effects of the two doses on protein metabolism were observed. In addition, Chapman et. al. (51) examined the temporal relation between discontinuation of rhIGF-I treatment and resumption of normal GH production in normal volunteers and found a rebound in GH production within 5–7 days after discontinuation. We have studied GH-deficient subjects, who show a marked decrease in the GH-dependent proteins, IGFBP-3 and acid-labile subunit during rhIGF-I administration and have shown normal absorption and distribution of IGF-I, yet faster elimination kinetics than normal subjects (49); hence, one would expect an even faster clearance of IGF-I in GH-deficient subjects after discontinuation of treatment.

If the metabolic effects observed after both hormones had been in parallel, then there could be legitimate concern about the adequacy of the washout period, but in the present work there were marked differences in specific metabolic paths after 8 weeks of each hormone, making the findings perhaps more compelling. There were clear differences in carbohydrate metabolism. There were no detectable effects on carbohydrate oxidation after rhIGF-I, yet a significant decrease after rhGH. Lipid oxidation did not change after rhIGF-I, yet it increased after rhGH. Insulin concentrations decreased after rhIGF-I, yet they increased after rhGH. IGFBP-2 increased only after rhIGF-I, not after rhGH. IGFBP-3 increased only after rhGH, not after rhIGF-I. LDL cholesterol decreased only after rhGH and not after rhIGF-I treatment. Taken in aggregate, the observed dichotomy of effects of both hormones is probably the result of the specific hormone treatment and not due to the carryover effect of the previous hormone administered.

Summary and conclusions

A direct comparison of the metabolic effects of IGF-I and GH in GH-deficient subjects shows that both hormones, in the doses administered, decrease the %FM and increase FFM; however, rhGH had a more potent effect in decreasing adiposity. Both are associated with an increase in whole body protein synthesis rates and a decrease in protein oxidation. Neither hormone

affected isokinetic or isometric measures of skeletal muscle strength. However, rhGH was more potent than rhIGF-I at increasing lipid oxidation rates and improving plasma lipid profiles. Both hormones increased hepatic glucose output, but rhGH treatment was also associated with decreased carbohydrate oxidation and increased glucose and insulin concentrations, indicating subtle insulin resistance. Neither hormone significantly affected bone calcium fluxes, supporting the concept that these hormones by themselves are not pivotal in bone calcium metabolism. In conclusion, rhIGF-I and rhGH share common effects on protein, muscle, and calcium metabolism, yet have divergent effects on lipid and carbohydrate metabolism in the GH-deficient state. These differences may allow for better selection of treatment modalities depending on the choice of desired effects in hypopituitarism.

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References

1. **Mauras N, Haymond MW.** 1996 Metabolic effects of recombinant human insulin-like growth factor I in humans: comparison with recombinant human growth hormone. Pediatr Nephrol. 10:318–323.
2. **Guevara-Aguirre J, Rosenbloom AL, Vasconez O, et al.** 1997 Two-year treatment of growth hormone (GH) receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and to GH-treated GH deficiency. J Clin Endocrinol Metab. 82:629–633.
3. **Rosenbloom AL, Guevara-Aguirre J, Rosenfeld RG, Pollock BH.** 1994 Growth in growth hormone insensitivity. Trends Endocrinol Metab. 5:296–303.
4. **Mauras N, Beaufrere B.** 1995 rhIGF-I enhances whole body protein anabolism and significantly diminishes the protein-catabolic effects of prednisone in humans, without a diabetogenic effect. J Clin Endocrinol Metab. 80:869–874.
5. **Mauras N.** 1995 Combined recombinant human growth hormone and recombinant human insulin-like growth factor I: lack of synergy on whole body protein anabolism in normally fed subjects. J Clin Endocrinol Metab 80:2633–2637.
6. **Guler HP, Zapf J, Froesch ER.** 1987 Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. N Engl J Med. 317:137–140.
7. **Fowelin J, Attvall S, Lager I, Bengtsson BA.** 1993 Effects of treatment with recombinant human growth hormone on insulin sensitivity and glucose metabolism in adults with growth hormone deficiency. Metabolism. 42:1443–1447.
8. **DiGirolamo M, Eden S, Enberg G, et al.** 1986 Specific binding of human growth hormone but not insulin-like growth factors by human adipocytes. FEBS Lett. 205:15–19.
9. **Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Slootweg MC.** 1998 Growth hormone and bone. Endocr Rev. 19:55–79.
10. **Bravenboer N, Holzmann P, DeBoer H, Lips P.** 1997 The effect of GH treatment on bone remodeling in GH-deficient men. Endocr Rev. 4:8.
11. **Rose NT, Wilhelmsen L, Landin-Wilhelmsen K, Lappas G, Bengtsson BA.** 1997 Increased fracture rates in adults with GH deficiency. Eur J Endocrinol. 137:240–245.
12. **Kupfer SR, Underwood LE, Baxter RC, Clemmons DR.** 1993 Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. J Clin Invest. 91:391–396.
13. **Hussain MA, Schmitz O, Mengel A, et al.** 1994 Comparison of the effects of GH and IGF-I on substrate oxidation and on insulin sensitivity in GH-deficient humans. J Clin Invest. 94:1126–1133.
14. **McCarthy TL, Centrella M, Canalis E.** 1989 IGF and bone. Connect Tissue Res. 20:277–282.
15. **McCarthy TL, Centrella M, Canalis E.** 1989 Regulatory effects of IGF-I and II on bone collagen synthesis in rat calvarial cultures. Endocrinology. 124: 301–309.
16. **Grinspoon SK, Baum HB, Peterson S, Klibanski A.** 1995 Effects of rhIGF-I administration on bone turnover during short-term fasting. J Clin Invest. 96:900–906.
17. **Mauras N, Hayes V, Welch S, et al.** 1998 Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength and adiposity. J Clin Endocrinol Metab. 83:1886–1892.
18. **Copeland KC, Kenney FA, Nair KS.** 1992 Heated dorsal hand vein sampling for metabolic studies: a reappraisal. Am J Physiol. 263:E1010–E1014.
19. **Mauras N, Martha PM, Quarmby V, Haymond MW.** 1997 rhIGF-I administration in man: differential sensitivity to the metabolic effects of subcutaneous (SC) bolus vs. continuous delivery. Am J Physiol. 272:E349–E355.
20. **Horber FF, Horber-Feyder CM, Krayer S, Schwenk WF, Haymond MW.** 1989 Plasma reciprocal pool specific activity predicts that of intracellular free leucine for protein synthesis. Am J Physiol. 257:E385–E399.
21. **Schwenk WF, Berg PJ, Beaufrere B, Miles JM, Haymond MW.** 1984 Use of *t*-butyldimethyl silyation in gas chromatographic mass spectrometric analysis of physiologic compounds found in plasma using electron impact ionization. Anal Biochem. 141:101–109.
22. **Schoeller DA, Klein PD.** 1979 A microprocessor controlled mass spectrometer for the fully automated purification and isotopic analysis of breath CO_20. Biomed Mass Spectrom. 6:350–355.
23. **Yergey AL, Abrams SA, Vieira NE, Eastell R, Hillman LS, Covell DG.** 1990 Recent studies of human calcium metabolism using stable isotope tracers. Can J Physiol Pharmacol. 68:973–976.
24. **Schwenk WF, Beaufrere B, Haymond MW.** 1985 Use of reciprocal pool specific activities to model leucine metabolism in humans. Am J Physiol 249:E646–E650.
25. **Abrams SA, Esteban NV, Vieira NE, Yergey AL.** 1991 Dual tracer stable isotopic assessment of calcium absorption and endogenous fecal excretion in low birth weight infants. Pediatr Res. 29:615–618.
26. **Abrams SA, Esteban NV, Vieira NE, Sidbury JB, Specker BL, Yergey AL.** 1992 Developmental changes in calcium kinetics in children assessed using stable isotopes. J Bone Miner Res. 7:287–293.
27. **Neer R, Berman N, Fisher L, Rosenberg R.** 1967 Multicompartmental analysis of calcium kinetics in normal adult males. J Clin Invest. 46:1364–1379.
28. **Berman M, Weissberger AJ.** 1990 SAAM manual (simulation, analysis, and modeling). Bethesda: NIH.
29. **Abrams SA, Sidbury JB, Muenzer J, Esteban NV, Vieira NE, Yergey AL.** 1991 Stable isotopic measurement of endogenous fecal calcium excretion in children. J Pediatr Gastroenterol Nutr. 12:469–473.
30. **Ferranini E.** 1988 The theoretical bases of indirect calorimetry: a review. Metabolism. 37:287–301.
31. **Durnin JV, Womersley J.** 1974 Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr. 32:77–97.
32. **Horber FF, Haymond MW.** 1990 Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J Clin Invest. 86:265–272.
33. **Fukagawa NK, Minaker KL, Rowe JW, et al.** 1985 Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in post-absorptive men. J Clin Invest. 76:2306–2311.
34. **Mauras N, Welch S, Rini A., Haymond MW.** 1998 Ovarian hyperandrogenism is associated with insulin resistance to both peripheral carbohydrate and whole body protein metabolism in post pubertal young females: a metabolic study. J Clin Endocrinol Metab. 83:1900–1905.
35. **Hoffman DM, Pallasser R, Duncan M, Nguyen TV, Ho KK.** 1998 How is whole body protein turnover perturbed in growth hormone-deficient adults? J Clin Endocrinol Metab. 83:4344–4349.
36. **Lucidi P, Lauteri M, Laureti S, et al.** 1998 A dose-response study of growth hormone (GH) replacement on whole body protein and lipid kinetics in GH-deficient adults. J Clin Endocrinol Metab. 83:353–357.
37. **Florini JR, Ewton DZ, Coolican SA.** 1996 GH and the IGF system in myogenesis. Endocr Rev. 17:481–517.
38. **Yarasheski KE, Zachwieja JJ, Campbell JA, Bier DM.** 1995 Effect of GH and resistance exercise on muscle growth and strength in older men. Am J Physiol. 000:E268–E276.
39. **Cuneo RC, Salomon F, Wiles CM, Hesp R, Sonksen PH.** 1991 Growth hormone treatment in growth hormone-deficient adults. I. Effects on muscle mass and strength. J Appl Physiol. 70:688–694.
40. **Carroll PV, Christ ER, Bengtsson BA, et al.** 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab. 83:382–395.
41. **Mauras N, Haymond MW, Darmaun D, Vieira NE, Abrams SA, Yergey AL.** 1994 Calcium and protein kinetics in prepubertal boys. Positive effects of testosterone. J Clin Invest. 93:1014–1019.
42. **Mauras N, Vieira NE, Yergey AL.** 1997 Estrogen therapy enhances calcium

absorption and retention and diminishes bone turnover in young girls with Turner's syndrome: a calcium kinetic study. Metabolism. 46:908–913.
43. **Bravenboer N, Holzmann P, de Boer H, Roos JC, van der Veen EA, Lips P.** 1997 The effect of growth hormone (GH) on histomorphometric indices of bone structure and bone turnover in GH-deficient men [published erratum appears in J Clin Endocrinol Metab 1997 Jul;82(7):2238]. J Clin Endocrinol Metab. 82:1818–1822.
44. **Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Klibanski A.** 1996 Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. J Clin Endocrinol Metab. 81:3864–3870.
45. **Mauras N, Doi SQ, Shapiro JR.** 1996 Recombinant human insulin-like growth factor I, recombinant human growth hormone, and sex steroids: effects on markers of bone turnover in humans. J Clin Endocrinol Metab. 81:2222–2226.
46. **Mauras N, Hayes V, Yergey AL.** 1999 Profound hypogonadism has significant negative effects on calcium balance in males: a calcium kinetic study. J Bone Miner Res. 14:577–582.
47. **Hirschberg R, Brunori G, Kopple JD, Guler HP.** 1993 Effects of insulin-like growth factor I on renal function in normal men. Kidney Int. 43:387–397.
48. **Hussain MA, Schmitz O, Mengel A, et al.** 1993 Insulin-like growth factor I stimulates lipid oxidation, reduces protein oxidation, and enhances insulin sensitivity in humans. J Clin Invest. 92:2249–2256.
49. **Mauras N, Quarmby V, Bloedow DC.** 1999 Pharmacokinetics of insulin-like growth factor-1 in hypopituitarism: correlation with binding proteins. Am J Physiol. 277:E579–E584.
50. **Jones JI, Clemmons DR.** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev. 16:3–34.
51. **Chapman IM, Hartman ML, Pieper KS, Skiles EH, Pezzoli SS, Hintz RL, Thorner MO.** 1998. Recovery of growth hormone release from suppression by exogenous insulin-like growth factor I (IGF-I): evidence for a suppressive action of free rather than bound IGF-I. J Clin Endocrinol Metab. 83:2836–2842.

Erratum

In the article "Normocortisolemic Cushing's syndrome initially presenting with increased glucocorticoid receptor numbers" by Ron S. Newfield, George Kalaitzoglou, Teresa Licholai, David Chilton, Javed Ashraf, E. Brad Thompson, and Maria I. New (*The Journal of Clinical Endocrinology & Metabolism* **85:**14–21), the authors regret that acknowledgement of the extensive editorial assistance of Laurie Vandermolen was inadvertently omitted. The authors wish to express their appreciation for her help in the publication of this manuscript.