Growth Hormone Has Anabolic Effects in Glucocorticosteroid-Dependent Children With Inflammatory Bowel Disease: A Pilot Study

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The present studies were designed to determine whether recombinant human growth hormone (rhGH) can counteract some of the catabolic effects of glucocorticosteroid therapy in children chronically treated with glucocorticosteroids. Whether rhGH can safely improve short-term linear growth was also investigated. The effect of rhGH on disease activity was also assessed. Ten children (6 boys, 4 girls) with inflammatory bowel disease (IBD) on oral prednisone for at least 4 months prior to these studies were recruited (mean ± SE, 11.9 ± 0.9 years). Leucine and glucose isotope studies, body composition, substrate oxidation and energy expenditure rates, and growth factors were measured at baseline (D1) and at 4 months after treatment with rhGH (0.05 mg/ kg · d subcutaneously [SC]) while continuing oral prednisone. Dual-emission x-ray absorptiometry (DEXA) and calcium kinetic analysis ($^{42}Ca/^{46}Ca$) were performed also. rhGH was continued for 6 months to assess linear growth in all 10 subjects, 7 of whom continued rhGH for 12 months. Body composition changed favorably with increased fat free mass (+3 kg, $P = .001$) and decreased percent fat mass (−3.5%, $P = .001$) after 4 months of treatment. Rates of whole body protein turnover, oxidation, and synthesis remained invariant, with no changes in substrate oxidation or resting energy expenditure rates. Linear growth velocity increased from 3.5 ± 0.4 cm/yr when the patients were treated with prednisone only, to 7.7 ± 0.9 after 6 months of combined prednisone/rhGH ($P = .001$). The growth velocity was sustained in the 7 patients treated with rhGH for 12 months. Plasma insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) concentrations also increased significantly while on rhGH treatment. No changes in calcium absorption were observed but there was a significant increase in kinetic rates of bone calcium accretion ($P = .045$) as well as in bone-specific alkaline phosphatase concentrations, a measure of bone formation ($P = .03$). Fasting and 2-hour postprandial glucose concentrations, fasting insulin levels, and HbA_{1C} were invariant during combined rhGH/prednisone treatment. The Crohn's disease activity score was unchanged with rhGH therapy. In summary, rhGH treatment of corticosteroid-dependent patients with IBD was associated with positive changes in body composition, bone metabolism, and linear growth, without deterioration of carbohydrate tolerance or intermediate metabolism of substrates. We conclude that treatment with rhGH has beneficial effects in prednisone-dependent growing children. Larger studies will be needed to assess the long-term safety and efficacy of this approach.

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CHRONIC THERAPY with pharmacological doses of glucocorticosteroids (henceforth also called "steroids") is associated with a variety of side effects which negatively impact the prolonged use of these potent anti-inflammatory agents. Even during short periods of exposure (5 days), high-dose glucocorticosteroid treatment has deleterious effects on whole body protein metabolism, increases the oxidation of at least one essential amino acid (leucine), and impairs leucine balance in humans.[1] Chronic glucocorticosteroid therapy is associated with protein wasting, poor tissue healing, and an increase incidence of infection.[2-5] In addition, bone loss is accelerated with extended steroid use.[6-9] Carbohydrate metabolism can also be affected by prolonged steroid administration, causing insulin resistance to both hepatic and peripheral tissues, leading at times to frank carbohydrate intolerance.[10-12]

When administered to growing children these side effects of glucocorticosteroid treatment are compounded further by a potent and significant suppression of linear growth.[13] Glucocorticosteroids affect both the release and actions of growth hormone (GH). Steroids decrease the growth hormone–releasing hormone (GHRH)-mediated GH release and increase somatostatin tone,[14,15] and in primary rat cell chondrocyte culture, dexamethasone inhibits cell growth and inhibits the transcription of the GH receptor, effects that are abolished or reverted by the coadministration of GH.[16]

Previous investigations have reported that GH therapy can improve nitrogen balance in burned patients,[17] during severe illness,[18] in obese volunteers,[19,20] in patients receiving total parenteral nutrition,[21] and in healthy volunteers.[22] Both recombinant human GH (rhGH) and recombinant human insulin-like growth factor I (rhIGF-I) can mitigate the protein wasting effects of prednisone administration in humans.[22,23] In addition, GH and IGF-I are highly anabolic in bone, increasing the recruitment of osteoblasts, and trabecular bone formation, and partially preventing bone loss in normal rats.[24-27] IGF-I has also been shown to enhance Type I collagen formation in hydrocortisone-treated human osteoblasts.[28] GH (through IGF-I) significantly enhances linear growth, thus, in states of "functional" GH deficiency, such as that observed in chronic steroid use, GH may also have a potentially beneficial effect. The prevention, or amelioration of some of these adverse events associated with chronic glucocorticosteroid use could have important implications in the management of a wide variety of patients.

Based on these and other data we designed this pilot study to address the following questions: Can 4 to 6 months of treatment

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Table 1. Clinical Characteristics of the Study Subjects

Patient No.	Age (yr)	Sex	Weight (kg)	Height (cm)	Bone Age (yr)	GV (cm/yr)	Puberty Stage	Prednisone Dose (mg/d)	Other Meds
1	6.5	M	16.6	98.7 ± 3.9	4.5	3.8	I	15/10 (QOD)*	Vitamins
2	11.1	M	36.7	145.7 ± 0.3	10.0	5.2	I	5 (QOD)	Vitamins, mesalamine
3	14.6	M	46.6	159.7 ± 0.8	13.5	4.7	IV	10 (QOD)	Vitamins, 6MP, mesalamine
4	9.5	F	36.6	138.4 ± 0.5	9.0	2.8	I	20 (QOD)	Vitamins, 6MP
5	13.8	M	40.2	144.5 ± 2.0	12.5	2.9	I	10 (QOD)	Vitamins, 6MP, mesalamine
6	14.2	M	38.8	147.4 ± 2.0	11.6	4.2	I	20 (QOD)	Vitamins, ranitidine, olsalazine sodium, hyoscyamine
7	15.1	F	37.7	146.8 ± 2.2	12.5	4.6	II	10 (QD)	Mesalamine, 6MP, Nu-iron
8	12.6	M	44.2	145.8 ± 1.0	10.5	2.2	I	10 (QD)	Vitamins, mesalamine, zantac
9	8.9	F	22.0	119.3 ± 2.0	6.3	2.0	I	10 (QD)	Vitamins, mesalamine, iron, zantac
10	12.2	F	34.3	138.2 ± 1.9	12.0	2.4	I	10 (QOD)	Mesalamine
Mean ± SE	11.9		35.4 ± 2.9	140.2 ± 5.0	10.5 ± 1.2	4.0 ± 0.4			

Abbreviations: GV, growth velocity; QOD, every other day; 6MP, 6-mercaptopurine.

with rhGH in glucocorticosteroid-treated children improve lean body mass, decrease adiposity and decrease protein wasting? Can it improve measures of calcium absorption and bone deposition? Can it improve short-term linear growth? Can it accomplish the above without worsening of carbohydrate metabolism? To answer these questions, we chose to study a group of children with a form of inflammatory bowel disease (IBD) who had been on glucocorticosteroids for at least 4 months prior to the studies, before and after the administration of rhGH.

MATERIALS AND METHODS

These studies were approved by the Nemours Children's Clinic Clinical Research Review Committee and Baptist-Wolfson Children's Hospital Institutional Review Committee.

Subjects

Ten children (6 males, 4 females) with clinical and histologic evidence of inflammatory bowel disease (IBD) were recruited for these studies after informed written consent was obtained from their parents and assent from them. All had been diagnosed at least 1 year previously. Nine had Crohn's disease and one had a form of nonspecific IBD compatible with autoimmune enteritis. Their clinical characteristics are summarized in Table 1. All but two of the subjects (no. 3 and 7) were prepubertal during the course of the studies. Patient no. 3 had Tanner stage 3 genitals and was growing poorly (4.7 cm/yr) despite a testosterone level of 494 ng/dL at baseline. His data were included in all analyses as they followed the same trends as the prepubertal subjects and all of the studies were paired. His testosterone at 4 months was 477 ng/dL. Patient no. 7 was barely beginning to show breast buds, which remained unchanged during these studies. Her growth velocity was also very poor. All subjects had been treated with various doses of oral prednisone continuously for at least 4 months prior to these studies (~8 mg/d), and the dose was continued uninterrupted for the duration of the metabolic studies. We specifically chose to study subjects whom were expected by their gastroenterologists to remain on low doses of steroids for at least 4 months.

Experimental Design

For 3 days prior to admission subjects consumed a weight maintenance diet. Analysis of nutrition intake was performed from records of food intake kept by the parents. Patients were admitted to the Wolfson Children's Hospital Clinical Research Center (CRC). The afternoon of the patient admission, body composition analysis was performed using dual-emission x-ray absorptiometry (DEXA) for whole body and lumbar spine, using a step wedge tissue bar (Hologic 2000, Waltham, MA). The pediatric Crohn's Disease Activity Scale was administered as previously described.[29]

At 6 AM, a mixture of a stable isotope of calcium (^{46}Ca, 0.5 μg/kg) was consumed mixed with juice or milk and a urine collection started for the measurement of isotopic enrichments and continued for the next 38 hours. The calcium mixture was prepared 8 to 12 hours before consumption for isotopic equilibration of tracer and tracee. The evening meal was consumed at 6 PM and the subjects were maintained fasting except for water until noon the next day. The following morning, a topical anesthetic cream (EMLA, Astra Pharmaceuticals, Westborough, MA) was applied to the intravenous injection site for at least 90 minutes to decrease the discomfort of venipuncture. At 7 AM 2 intravenous access lines were established and secured as heparin locks, one in an antecubital vein for subsequent infusion of tracers, and the other in a contralateral hand vein for frequent blood sampling using the heated hand technique.[30] At 8 AM (time 0), primed, dose-constant infusions of L-[1-^{13}C]-leucine (~4.5 μmol/kg; ~0.07 μmol kg · min) and [6, 6, $^{2}H_2$] glucose (33 μmol/kg; 0.33 μmol/kg · min) were begun and continued uninterrupted for 240 minutes. Concomitantly, an infusion of 0.08 mg/kg of ^{42}Ca was given intravenously over 3 minutes. Arterialized blood samples were collected at 10- to 20-minute intervals as detailed below as well as breath and urine samples. Indirect calorimetry was performed 3 times during these studies using a PX-MAX Calorimeter (Medical Graphics Corp, St. Paul, MN), with a mouthpiece. After the isotope infusions were completed, subjects ate lunch and were free to move around. An additional blood sample was collected at 4 PM and subjects were discharged home to complete a urine collection through 8 AM the next morning. Following discharge, twice-daily urine samples were obtained for measurement of the enrichment of the calcium tracers in the urine for the next 5 days.

After the baseline study (D1) was completed, all subjects were begun on daily injections of rhGH (Genotropin, kindly provided by Pharmacia and Upjohn, Kalamazoo, MI), at a dose of 0.05 mg/kg · d subcutaneously [SC] daily. Sixteen weeks after D1, a second study identical to that at baseline was performed (4 months). Each subject served as their own control. In order to assess linear growth, rhGH was continued for an additional 2 months and patients were remeasured after 6 months of rhGH therapy. Adequate height measurements were available in all subjects for approximately 1year prior to study participation. An x-ray of the left hand and wrist was obtained at baseline and again at 12 months in those that continued treatment for the determination of skeletal maturation according to the standards of Greulich and Pyle.[31] All other medications, including prednisone, were kept uninterrupted

during the study. At the end of 6 months of treatment, all subjects were offered rhGH therapy for another 6 months for a total of 12 months of rhGH . Seven subjects completed 12 months of treatment.

Blood, Breath, and Urine Samples

Arterialized blood samples were obtained at –5, 120, 160, 180, 200, 220, and 240 minutes to determine the enrichments of alpha-ketoisocaproic acid (α-KIC) and [$^2H_2$] glucose. At times 0, 5, 15, 30, 60, 120, 240, and 480 minutes, the enrichment of the calcium tracers was measured in blood samples. Time 0 represents the initiation of the tracer infusions. Blood was withdrawn at 10- to 20-minute intervals for determination of GH concentrations. Serum insulin and plasma IGF-I, insulin-like growth factor binding protein (IGFBP)-3, IGFBP-2, osteocalcin, procollagen peptide type 1 (PICP), bone-specific alkaline phosphatase, and plasma amino acid profiles were measured at baseline and during each study day. Urine collected between time 0 and 240 minutes was aliquoted for determination of urea nitrogen excretion. Urinary N-telopeptide and deoxypiridinoline crosslinks were also measured. Plasma glucose was measured fasting and 2 hours postprandially on D1 and at 4 months, as was glycosylated hemoglobin ($HgbA_{1C}$). Breath samples were collected at –10, 160, 180, 200, and 220 minutes for the measurement of expired labeled $^{13}CO_2$ and V_{CO_2}.

Assays

The enrichments of [^{13}C]-KIC and [$^2H_2$] glucose were measured at the Nemours biomedical analysis core laboratory by gas chromatography/mass spectroscopy as previously described.[32] The calcium isotopic enrichment of blood and urine samples was measured by thermal ionization mass spectrometry (Finnigan MAT 261, Bremen, Germany) at the US Department of Agriculture Agricultural Research Service Children's Nutrition Center (Houston, TX) by Dr Steven Abrams as previously described.[33,34] Samples were analyzed for the ratio of ^{46}Ca/^{43}Ca and ^{42}Ca/^{43}Ca with an intraassay variability of less than 0.15%. Plasma amino acid concentrations were measured by ion exchange chromatography using an amino acid analyzer (Beckman 6300, Palo Alto, CA).

IGF-I, IGFB-2, IGFBP-3, and insulin concentrations were measured by standard radioimmunoassay (RIA) at Endocrine Sciences Laboratories (Calabasas Hills, CA). Glucose was measured using a glucose oxidase method (Beckman Instruments). Osteocalcin (RIA), N-telopeptide (enzyme-linked imunosorbent assay [ELISA]), and deoxypiridinoline crosslinks (DPD) were measured at the Endocrine Sciences Laboratories. GH was measured by the Nichols Chemiluminescence Assay kits at the Virgina General Clinical Research Center Core Laboratory. The sensitivity of that assay is 0.003 μg/L. PICP was measured by RIA at Corning Nichols Institute (San Juan Capistrano, CA). Urea nitrogen excretion was measured by a Kodak (Rochester, NY) Ektrachem urease method. Glycosylated hemoglobin was measured by Latex agglutination inhibition and a specific monoclonal antibody against $HgbA_{1C}$ using a DCA 2000 (Bayer, Elkhart, IN).

Calculations

Leucine tracers. The reciprocal pool model, which uses [^{13}C]-KIC as a measure of the intracellular enrichment of leucine, was employed to calculate the rates of appearance of leucine (leucine Ra), oxidation, and nonoxidative leucine disposal (NOLD, an estimate of whole body protein synthesis) at steady state as previously described.[35,36]

Glucose tracers. The rate of appearance of glucose (glucose Ra) was calculated as Ra = F[Ei/Ep) - 1], where F is the infusion rate of the tracer, Ei is the enrichment of the infusate and Ep is the enrichment of glucose in plasma at steady state.

Calcium tracers. Analysis of calcium kinetics was performed using a 3-pool compartmental model as previously described,[33] with the aid of the simulation analysis and modeling program (SAAM).[33] The enrichments of the tracers in urine were measured over 5 days. Vo^+ represents the rate of flow of calcium into the final pool (bone) and Vo^- the rate of bone resorption. Endogenous fecal calcium was estimated as 1.5 mg/kg · d as previously described.[37] Vu represents urinary calcium excretion and Vbal total calcium balance.

The true fractional absorption of calcium was calculated as the ratio of the accumulated oral versus intravenous tracer in the urine 24 hours after its administration:

$$\alpha = \int_0^t \frac{^{46}Ca}{^{42}Ca} \text{ in urine}$$

Va (true dietary calcium absorption) is calculated as Vi (calcium intake) · a.

Body composition and substrate oxidation rates. Body composition was calculated using DEXA. Substrate oxidation and resting energy expenditure rates were estimated using O_2 and CO_2 expired flow rates measured by calorimetry, utilizing gas exchange equations as described by Ferranini.[38]

Statistical Analysis

Data are presented as means ± SEM. Paired Student's *t* tests were performed to calculate significance of the rhGH effect for all parameters measured in the study. A 2-tailed analysis was used unless otherwise indicated. Significance was established at $P < .05$.

Isotopes

L-[^{13}C]leucine, 99% enriched (Cambridge Isotopes, Andover, MA) and D-[6,6-$^2H_2$]glucose, 99.7% enriched (MSD Isotopes, St Louis, MO), were determined to be sterile and pyrogen-free (limulus lysate assay), and prepared using 0.9% bacteriostatic saline. ^{42}Ca and ^{46}Ca were purchased from Trace Sciences International (Richmond Hill, Canada), and prepared by the National Institutes of Health Pharmacy as calcium chloride salts.

RESULTS

Both pubertal subjects in the cohort were included in all data analysis (N = 10) as they were growing poorly before treatment despite the presence of puberty and they behaved similarly to the prepubertal children in all parameters of study, including growth. However, when the 2 pubertal subjects are excluded from analysis, there is no change in the level of significance observed in any of the parameters measured below. This is indicated in parenthesis as N = 8, ie, excluding the 2 pubertal subjects.

Body Composition

There were no changes in the body mass index (D1, 18.0 ± 0.5 kg/m^2; 4 months, 18.4 ± 0.7; P = not significant [NS]). There was, however, a significant increase in fat free mass (D1, 25.1 ± 2.0 kg; 4 months, 28.1 ± 2.3; $P = .001$; $P = .003$, N = 8). While the total fat mass did not change significantly (D1, 9.3 ± 1.2 kg; 4 months, 8.6 ± 1.3; P = NS), since the patients were growing, when the data are expressed as a percentage of the total weight, there was a significant decrease in percent fat mass as measured by DEXA scanning after rhGH therapy (D1, 26.0% ± 2.2%; 4 months, 22.5% ± 2.2%; $P = .001$; $P = .006$, N = 8). These data are summarized in Fig 1.

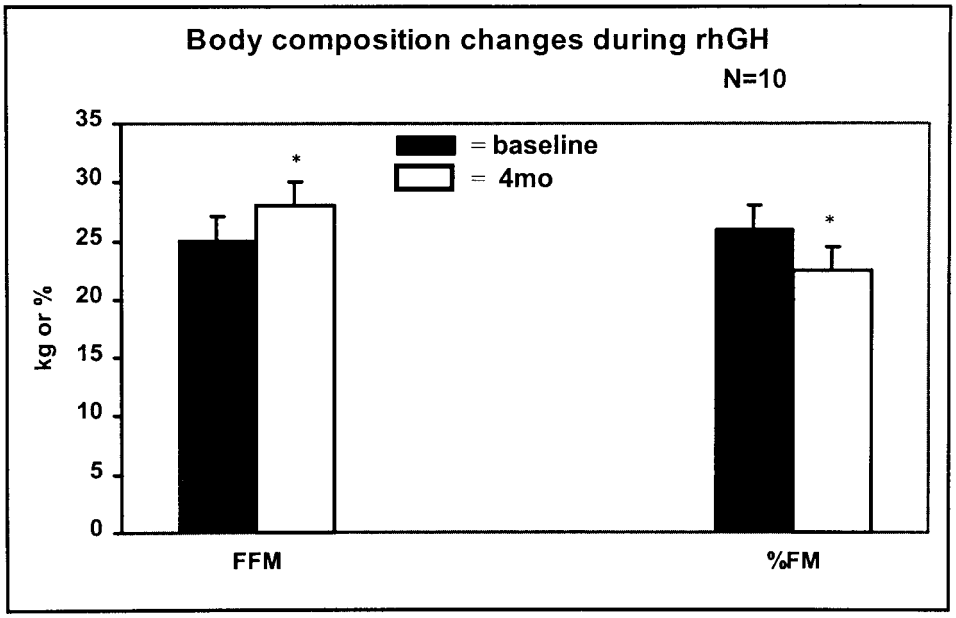

Fig 1. Body composition analysis by DEXA performed at baseline and 4 months after rhGH treatment in 10 prednisone-dependent children with IBD. Fat free mass (FFM) and % fat mass (%FM) changed significantly.

Protein Kinetics/Plasma Amino Acids/Substrate Oxidation Rates

Rates of whole body proteolysis (leucine Ra), protein oxidation, and estimates of whole body protein synthesis (NOLD) remained invariant during the 4 months of the studies (leucine Ra, D1: 2.48 ± 0.13 μmol/kg · min, 4 months: 2.49 ± 0.18; leucine oxidation, D1: 0.32 ± 0.03, 4 months: 0.28 ± 0.03; NOLD, D1: 2.16 ± 0.12, 4 months: 2.21 ± 0.16, P = NS for all comparisons). Fasting plasma amino acid concentrations remained unchanged during rhGH therapy (data not shown).

Urinary nitrogen excretion was measured to calculate the substrate oxidation rates (baseline, 1.4 ± 0.3 g/4 h; 4 months, 1.1 ± 0.2). The rates of lipid, glucose, and protein oxidation did not change significantly during rhGH treatment, nor did the rates of energy expenditure (lipid oxidation, D1: 19 ± 4 kcal/FFM · d, 4 months: 26 ± 2; glucose oxidation, D1: 17 ± 4 kcal/FFM · d, 4 months: 15 ± 2; protein oxidation, D1: 8.6 ± 0.5 kcal/FFM d, 4 months: 5.4 ± 0.5; resting energy expenditure: D1: 44.4 ± 3.7 kcal/FFM · d, 4 months: 45.0 ± 3.6, P = NS for all comparisons).

Linear Growth/Growth Factors

Linear growth was measured after 6 months of continuous rhGH/glucocorticosteroid treatment and compared with pretreatment growth velocities during therapy with steroids alone. There was a variable, yet significant improvement in linear growth in all subjects studied (growth velocity: 3.5 ± 0.4 cm/yr pretreatment, 7.4 ± 1.1 after 6 months of rhGH, P = .001; $P <$ .05, N = 8) (Fig 2). Seven of these 10 patients continued rhGH therapy beyond 6 months for a total of 12 months of treatment. Three were tired of taking injections and stopped the rhGH after 6 months. The growth velocity of those 7 subjects increased from 3.6 ± 0.5 cm/yr to 7.4 ± 0.8 at 12 months (P = .001; P = .003, no pubertal subjects) and their height standard deviation score (SDS) increased from −1.7 ± 0.5 at baseline to −1.2 ± 0.6 at 12 months (P = .03, 1-tailed, N = 7). The 12-month bone age advanced 1 year in a chronological year.

During the metabolic portion of the studies, circulating growth factors were also measured. IGF-I concentrations increased from 227 ± 38 μg/L on D1 to 375 ± 53 at 4 months (P = .001; P = .004, N = 8), IGFBP-3 rose from 2.5 ± 0.2 mg/L to 3.5 ± 0.3 (P = .001, P = .003, N = 8). IGFBP-2 concentrations decreased from 558 ± 92 μg/L on D1 to 377 ± 82 (P = .05) at 4 months. Mean GH concentrations at baseline were 1.2 ± 0.2 μg/L during frequent sampling.

Bone/Calcium Metabolism

Using stable tracers of calcium, measurements of fractional (α) and total (Va) calcium absorption and urinary calcium excretion did not change after 4 months of combined rhGH/glucocorticosteroid treatment (Table 2). However, Vo^{+}, a kinetic measure of the rate of calcium deposition into deep bone increased significantly (Fig 3). Serum markers of bone formation and resorption were not significantly affected by rhGH treatment during continued treatment with glucocorticosteroids, except for a significant increase in bone-specific alkaline phosphatase, a marker of bone formation (Table 2 and Fig 3). Bone mineral density of the lumbar spine was significantly low as compared to age- and sex-matched control data in all the subjects studied at baseline (bone marrow density, lumbar spine: 0.57 ± 0.03 gm/cm^2; Z score: -2.36 ± 0.33; range, −0.67 to −4.07).

Carbohydrate Metabolism

There was no change in either fasting or 2-hour postprandial glucose concentrations during the combined prednisone/rhGH treatment (fasting, D1: 5.1 ± 0.2 mmol/L, 4 months: 5.3 ± 0.2; 2-hour postprandial, D1: 6.7 ± 0.5, 4 months: 6.7 ± 0.4; P = NS for both comparisons). There was a trend towards higher fasting insulin concentrations during combined prednisone/

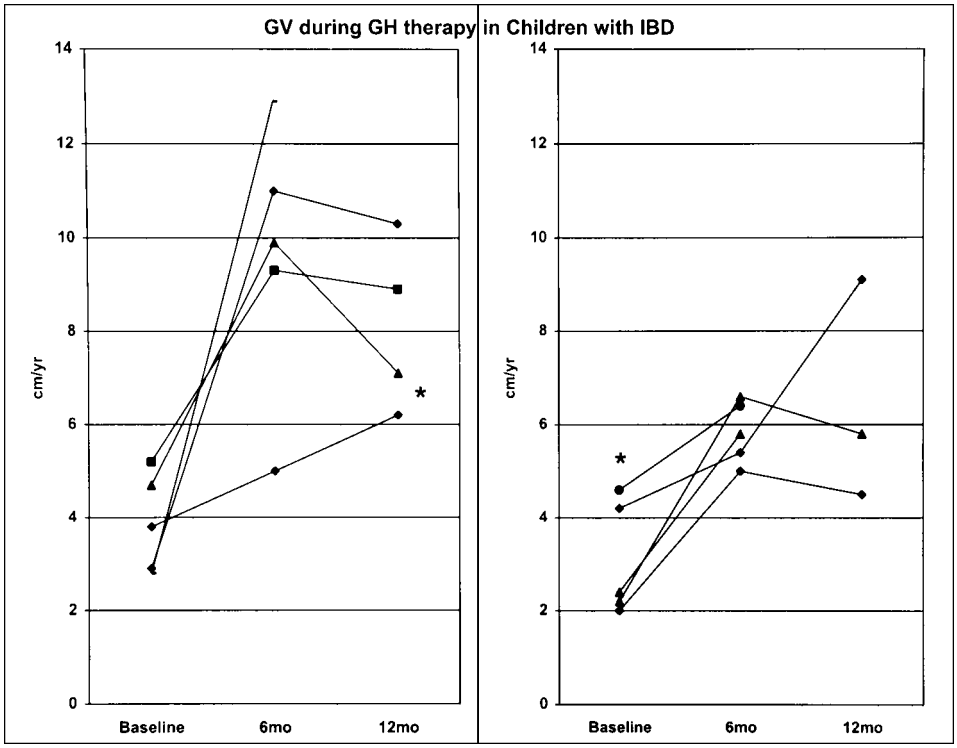

Fig 2. The 2 panels represent the growth velocity of individual subjects at baseline and after 6 months of continuous rhGH therapy in 10 patients on prednisone therapy for IBD. Seven continued treatment with rhGH for 12 months. *The 2 pubertal subjects in the cohort who were growing poorly. Two panels are shown for the ease of reading.

rhGH therapy; however, there was substantial variability and the difference did not reach statistical significance (D1, 105.6 ± 35.4 pmol/L; 4 months, 151.2 ± 48.6; $P = .41$). Glycosylated hemoglobins (HbA_{1C}) remained invariant during therapy (D1, 4.9% ± 0.1%; 4 months, 5.1 ± 0.2; $P = .17$). There was no detectable change in the measure of hepatic glucose output, glucose Ra, during rhGH therapy (D1, 20.5 ± 3.3 μmol/kg · min; 4 months, 20.5 ± 3.0).

Table 2. Calcium Absorption/Kinetics and Bone Markers During rhGH Therapy

	Baseline	4 Months	*P* Value
Calcium kinetics			
α (% absorption)	52 ± 5	51 ± 6	.42
Va (total absorption, mg/d)	501 ± 65	577 ± 98	.91
Vbal (total retention, mg/d)	310 ± 64	377 ± 93	.56
Vo^+ (accretion, mg/d)	1604 ± 197	2069 ± 294	.045*
Vo^- (resorption, mg/d)	1294 ± 166	1690 ± 242	.32
Vu (urinary excretion, mg/d)	137 ± 33	141 ± 27	.91
Bone markers			
Osteocalcin (ng/mL)	48 ± 8	65 ± 11	.12
PICP (μg/L)	317 ± 54	323 ± 25	.87
N-telopeptide (nmol BCE/μmol creatinine)	318 ± 62	406 ± 103	.35
DPD (nmol DPD/μmol creatinine)	15.3 ± 2.6	18.1 ± 2.3	.35
Bone-specific alkaline phosphatase (ng/mL)	39 ± 8	54 ± 12*	.03

Abbreviation: BCE, bone collagen equivalent.
*One-tailed *t* test.

Disease Activity Scale

The Crohn's Disease Activity Scale adapted for pediatric subjects[29] was administered at baseline and at 4 months by an experienced observer. The scale comprises variables to assess the degree of disease activity, including the number of stools per day, severity of abdominal pain, presence of an abdominal mass, use of antidiarrheal medicines, hematocrit, weight, height, extra-intestinal manifestations of the disease, and general well-being. There was no change in the disease activity score during treatment with rhGH and prednisone at 4 months versus the score during prednisone alone at baseline (D1, 23 ± 3; 4 months, 19 ± 3; $P = .21$).

Safety/Tolerability

There were no adverse events reported during the 6 months of treatment and rhGH was well tolerated. One subject (no. 1) had an acute enteritis during the first month of the studies secondary to *Clostridium difficile* bacteria, which responded to antibiotic treatment. There were no dropouts in the 6 months of the studies.

DISCUSSION

Treating a group of children with IBD on chronic glucocorticosteroid therapy with rhGH resulted in positive changes in body composition, linear growth, and bone anabolism without detrimental effects in carbohydrate metabolism or the intermediate metabolism of substrates. After 4 months of treatment, rhGH was associated with a measurable increase in fat free mass and a parallel decrease in percent fat mass without any change in body mass index. The observed changes in body

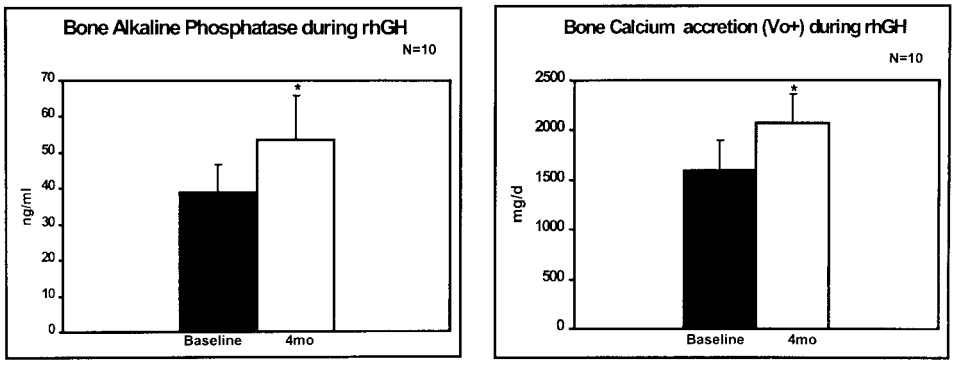

Fig 3. Bone-specific alkaline phosphatase concentrations, a measure of bone formation, as well as Vo^+ (mg/d), a kinetic measure of bone calcium accretion, increased during combined rhGH and prednisone treatment at 4 months, as compared to glucocorticosteroid treatment alone.

composition are similar to those reported during GH replacement therapy in general[39] and comparable to those reported after rhGH treatment in adults with short gut syndrome.[40] Available data suggest that steroids induce obesity mostly through an increase in energy intake as evidenced by significant increases in resting energy expenditure after 4 days of treatment.[41] In the present experiments there was a decrease in adiposity and increased growth without measurable changes in resting energy expenditure, suggestive of a shift in nutrient utilization towards lean tissue deposition. The data also suggest that GH can overcome at least part of the protein wasting and gain in adiposity associated with glucocorticosteroid therapy.

There was an acceleration in linear growth among the children studied during 6 months and even 12 months of rhGH therapy (Fig 2). These effects are promising considering the significant inhibition of linear growth caused by both chronic steroids and the primary disease process (IBD). Chronic steroids induce a decrease in circulating GH concentrations, probably via stimulation of the somatostatin tone,[42-45] and suppress GH receptor gene expression.[46] Glucocorticosteroids also suppress skeletal IGF-I synthesis by decreasing IGF-I gene transcription,[47] and they act locally to suppress longitudinal bone growth.[48] In vitro experiments using rat growth plate chondrocytes showed that there is a time-dependent decrease in DNA synthesis and cell proliferation with dexamethasone via a reduction of GH receptor expression and inhibition of both the GH and IGF-I receptor.[16] It is well known that Crohn's disease can also cause profound growth retardation independent of steroid use.[49] The acceleration in linear growth observed here is similar to that observed in a heterogeneous group of prednisone-treated children given rhGH and followed in a database reported recently where the growth velocity of children with inflammatory disease on steroids increased from 2.7 to 5.6 cm/yr after 1 year of rhGH treatment.[50] This was also observed in chronic renal failure and cystic fibrosis patients treated with rhGH.[51,52] In renal failure patients, after 5 years of rhGH the height standard deviation score was -1.6 ± 1.2, whereas in those not treated with rhGH it was -2.1 ± 1.2,[51] and in cystic fibrosis treatment with rhGH improved their growth velocity from $3.7 \pm .5$ cm/yr to 7.7 ± 1.7 after 1 year of treatment.[52] In the present study there were significant increases in circulating IGF-I and IGFBP-3 concentrations, suggestive of an IGF-I–mediated effect at the tissue level. These data suggest that a state of "functional" GH deficiency caused by chronic steroids may be overcome in children with IBD by the administration of growth hormone. Longer term studies are needed to assess increasing ultimate adult height with rhGH in this setting.

There were no measurable changes in rates of whole body protein synthesis or turnover during the concomitant administration of glucocorticosteroids and rhGH in these experiments contrary to the protein-anabolic effects observed after rhGH therapy in healthy volunteers and in GH-deficient patients.[53,54] rhGH and rhIGF-I have been shown to abolish the increase in protein oxidation observed after short-term treatment with glucocorticoids.[22,23] Several explanations for our findings are plausible. First, since the subjects had been treated with steroids at least 4 months prior to the experiments, it is possible that the effect of GH on whole body protein pools was one of essentially preventing any further deterioration in protein wasting. The fact that growth velocities increased while the children were on steroids and rhGH, as compared to the velocity on steroids alone, indirectly supports that notion. Alternatively, these data could imply that GH cannot overcome the protein wasting effects of *chronic* steroid use in human patients with long-term disease. Third, it is possible that the greatest changes may have occurred in the first several weeks to months of rhGH treatment and that the balance between protein synthesis and proteolysis would have returned to a near normal steady-state condition by the time we studied them. Consistent with this speculation is the evidence of protein anabolism, ie, increase in linear growth velocity that occurred during treatment. Actually 7 of the 10 subjects studied here had leucine turnover studies performed 1 month after initiation of treatment and a substantial increase in rates of whole body protein synthesis (NOLD) was observed ($P = .001$, data not shown).

There were subtle, yet positive changes in bone metabolism as well, with an increase in Vo^+, a kinetic measure of the rate of calcium deposition into bone and in bone-specific alkaline phosphatase concentrations (Fig 3). Using the same calcium tracer tools, glucocorticosteroids have been shown to decrease bone accretion,[55] hence the observed increase in bone calcium deposition is likely the result of GH's effects. These data are congruent with those reported in glucocorticoid-treated growing rats where GH was shown to counteract the inhibition of longitudinal growth and the decrease in cancellous bone caused by these steroids.[56] There was also a striking reduction of bone mineral density at baseline in most of the children studied here,

particularly in the lumbar area (Z score, -2.36 ± 0.33). Considering that the greatest percentage of trabecular bone mass accumulates in the peripubertal years and continues to accrue into late adolescence and early adulthood,[57,58] it is clear that steroid-treated children in this age group are at a significant disadvantage to achieve normal peak bone mass and at a high risk for osteoporosis. Longer term studies with growth-promoting agents such as GH and/or bisphosphonates[59] will be needed to assess the best way to prevent further bone loss in this condition.

It is unlikely that the positive effects observed after rhGH treatment would have been observed spontaneously during continuous steroid administration. Even though we did not repeat these studies after discontinuation of rhGH therapy, data available in the literature support the notion that continued use of steroids in children has profound deleterious effects beyond the treatment phase. In a recent large study of patients with cystic fibrosis treated with alternate-day prednisone and followed through final adult height, results showed that boys thus treated have a persistent impairment of linear growth as compared to those not treated with the steroids.[60] The effects of GH on linear growth and body composition in steroid-treated children are also limited to the treatment period. In a group of 85 renal transplant glucocorticosteroid-treated children randomized to either rhGH or no treatment for 1 year, those treated with both rhGH and steroids grew substantially better than those on steroids alone.[61] In addition, the beneficial effects of 1 year of rhGH treatment on linear growth and body composition in steroid-dependent children with juvenile chronic arthritis are abolished after cessation of treatment.[62] Collectively, these data suggest that the effects observed in our present studies are the result of rhGH treatment.

rhGH was well tolerated by all children in these studies. Carbohydrate metabolism was not negatively impacted by the rhGH treatment. The latter is important considering the diabetogenic potential of chronic steroids. However, careful monitoring of measures of carbohydrate metabolism is warranted if GH and steroids are to be used in combination. Treatment of these patients with IBD with rhGH was not associated with any change of the disease activity scores. This contrasts with data reported recently in a group of adults with Crohn's disease treated with either rhGH or placebo in whom the rhGH-treated group was reported to have an improvement in the disease activity score.[63] The difference in ages (adult *v* pediatric), in experimental design (placebo-controlled *v* open-label) and the variable frequency of steroid use in the adult study may explain these differences. Even though the rhGH treatment did not worsen the underlying disease in our present studies, these data also underscore the fact that GH should not be considered a treatment for the disease itself but rather for some of its chronic complications, mostly related to steroid use.

In summary, rhGH treatment of glucocorticosteroid-treated children with IBD was associated with increased lean body mass, decreased adiposity, and increased linear growth. These changes were accompanied by marked increases in circulating IGF-I concentrations and also with anabolic effects in bone, without any deterioration in carbohydrate metabolism or in disease activity scores. We conclude that the administration of rhGH to glucocorticosteroid-treated growing children has beneficial effects on whole body metabolism and linear growth while the child remains on steroids. Larger randomized trials will be needed to determine the long-term safety and benefit of this intervention.

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