Alterations in Body Composition and Fat Distribution in Growth Hormone–Deficient Prepubertal Children During Growth Hormone Therapy

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Growth hormone (GH) deficiency in children results in increased body fat, reduced fat-free mass (FFM) including muscle (protein) and bone, and abdominal obesity. Thus, proper GH secretion likely has major developmental influences on later health risks including cardiovascular diseases and osteoporosis. However, the in vivo control of the development of the body composition and fat distribution by GH has not yet been accurately investigated using children with GH deficiency as a model. We determined the effect of GH therapy (GH replacement, n = 3; GH + physiologic cortisol and thyroxine replacement, n = 3) on body composition, the proportional composition of the FFM, and body fat distribution in GH-deficient prepubertal children compared with healthy control children (n = 6) not treated with GH. The GH-deficient and control children were initially matched for gender, bone age, and weight. As assessed by a 4-compartment model, GH therapy reduced percent body fat during the first 3 months of therapy but not thereafter. This change was primarily due to FFM, which increased 3-fold more in the GH-deficient group and accounted for 91.5% of the increase in body weight. Fat mass increased in the controls but was unchanged in the GH-deficient group. Therapy temporarily increased the proportional contribution of water to the FFM, decreased the proportion of mineral, and slightly increased the proportion of protein. Using magnetic resonance imaging (MRI), abdominal visceral fat was reduced in the GH-deficient group and unchanged in the controls. Abdominal subcutaneous fat measured in the same image was not changed. The abdominal and suprailiac skinfold thicknesses also were not decreased in the GH-deficient group. In conclusion, within 1 to 3 months, GH therapy accelerates lean tissue accrual, especially the water and protein components, but has a smaller effect on reducing fat mass. GH therapy has site-specific effects on reducing abdominal adiposity.

THE METABOLIC effects of growth hormone (GH), independent of the effects of sex steroids, on body composition, body fat distribution, bone mineralization, and energy expenditure can be uniquely investigated in prepubertal children with GH deficiency during the initiation of GH therapy. Several studies have described the alterations in body composition of children with GH-deficiency during GH therapy.[1-5] However, the body composition alterations have not yet been accurately determined because obtaining such data requires multicompartment body composition models.[6] Maturational and individual differences in the proportional hydration and mineralization of the fat-free mass (FFM) alter its density, violating a critical assumption of the underwater weighing method that the density of the FFM is constant. This violation also invalidates other methods such as skinfold thickness and bioelectrical impedance when the prediction models were developed using a 2-compartment underwater weighing criterion.[6] Another advantage of the 4-compartment model of body composition is that it can be used to study GH-induced alterations in the maturation (proportional contribution of water, protein, and mineral) to the FFM.

GH deficiency is associated with an accumulation of abdominal visceral fat, which is linked to reduced insulin sensitivity and increased serum lipid concentrations in children[7-9] and non–insulin-dependent diabetes mellitus and cardiovascular diseases in adults.[10-12] Understanding the site-specific lipolytic effects of GH during childhood is of great clinical interest because it is an important time for the development of the adult body fat distribution. However, the influence of GH on the amount and distribution of abdominal visceral fat has not yet been reported in children.

GH also affects the bone mineral. Children with GH deficiency have reduced bone mineral accrual,[1,13,14] as do adults with either childhood or adult-onset GH deficiency.[15,16] As a result, osteoporosis may occur more often in individuals who experienced GH deficiency during their life. Bone mineral accrual during childhood and adolescence determines the peak bone mass so these are the most important developmental periods for maximizing bone mineral content (BMC) and preventing or delaying osteoporosis.[17] Despite evidence that GH has a major anabolic effect on bone mineral accrual, a decrease in bone mineral density (BMD) has been reported the first 6 months to 1 year of GH therapy.[18] This reduction is probably due to the lengthy resorption-deposition cycle of bone. Long-term studies are necessary to determine the time course of the net anabolic influence of GH on the BMD of children, but few have been reported.[14,19]

GH therapy increases the resting energy expenditure of children even after correction for the concomitant increase in metabolically active FFM.[3,20] However, previous studies did not use accurate measures of FFM to adjust the energy expenditure. Therefore, the purpose of this study was to use criterion methods to determine the alterations in body composition, composition of the FFM, body fat distribution, and basal energy expenditure in prepubertal GH-deficient children receiving GH therapy and GH therapy plus physiologic cortisol and

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thyroxine replacement. To better understand the metabolic properties of GH, the time course of its effects in GH-deficient children were compared with normally growing children initially matched for age, biologic maturation, and body composition.

MATERIALS AND METHODS

Subjects

Six prepubertal children with GH deficiency served as the experimental group. Three of them (2 boys and 1 girl) had idiopathic GH deficiency and GH was the only hormonal medication. Another boy had idiopathic GH deficiency likely due to autoimmune hypopituitarism and was on stable doses of replacement medications including GH, thyroxine, and hydrocortisone. The remaining 2 girls had hypopituitarism secondary to craniopharyngioma and multiple surgeries. One girl received stable replacement doses of GH, thyroxine, and desmopressin acetate (dDAVP) (synthetic replacement for vasopressin) and the other girl received stable replacement doses of GH, thyroxine, dDAVP, and hydrocortisone. All children with GH deficiency received daily subcutaneous injections of recombinant human GH (~0.3 mg/kg/wk) and were studied before and 1, 3, 6, and 12 months after initiating therapy. All were prepubertal and thus hypogonadotropic hypogonadism was physiologic. Prepubertal control subjects (n = 6) were studied at baseline and at 6 and 12 months. Control subjects had normal height, weight, growth velocities, and body composition and were enrolled in a longitudinal study of the endocrine and nutritional control of growth, body composition, and body fat distribution. The controls were matched to the children with GH deficiency for gender, bone age, and weight. Control children were matched with children with GH deficiency for adiposity as closely as possible but were marginally leaner ($P = .13$, Table 1). As shown in the upper panels of Fig 1, 3 of the children with GH deficiency were obese at baseline and were not matched for adiposity because entry criteria for the controls in the longitudinal growth study included normal body weight, height, and adiposity. All subjects were prepubertal based on the criteria of Tanner.[21]

Anthropometry

The recommendations of Lohman et al[22] were followed relative to landmarks and methods. Each measure was completed 3 times and the median score used. Height was measured with a stadiometer with the head held in the Frankfurt plane. Breadths taken were the biacromial, biiliac, and elbow. Circumference measures included the natural waist, abdomen, hip, thigh, and calf. Skinfold thickness sites included the subscapular, triceps, chest, suprailiac, abdominal, thigh, and mid-calf.

Height standard deviation scores (SDS) were determined using published growth tables,[23] where height SDS = (height – 50th percentile height for sex and chronologic age)/SD of heights at that age. The 6-month height increment (height velocity) was compared to tables of incremental growth for 6-month age intervals.[24] The tables were derived from the Fels longitudinal growth study and are well suited for the study of short-term changes in growth rate due to a therapy. The height velocity SDS was also determined from these tables.

Bone Mineral/Regional Body Composition

BMC and BMD of the whole body, right hip (femoral neck + trochanteric + intertrochanteric regions), right forearm (proximal 1/3 + middistal + ultradistal regions), lumbar spine 1-4, and the tissue composition of the trunk, arms, and legs were measured by dual-energy x-ray absorptiometry (DEXA, Hologic QDR 2000, Waltham, MA) of the GH-deficient children at baseline and at 3, 6, and 12 months, and in controls at baseline and 12 months. For the whole body BMC, BMD, and tissue composition transverse scans were made with a pencil beam from head to toe at 1-cm intervals. All scans were analyzed with Hologic enhanced whole body software (v. 5.64).

Body Composition

For 5 of the 6 the subjects with GH deficiency, the body composition was estimated using a 4-compartment model[25] at baseline and at 3, 6, and 12 months, and for all controls at baseline and 12 months. We have fully described and validated this model in children.[6] For this model body density was measured by underwater weighing and corrected for residual lung volume by nitrogen washout. Total body water (TBW)

Table 1. Growth Data of the Subject Groups

	GH Deficient			Control		
	Baseline	6 Months	12 Months	Baseline	6 Months	12 Months
Age (yr)*	11.4 ± 1.3	12.0 ± 1.2	12.5 ± 1.2	10.3 ± 0.6	10.9 ± 0.6	11.3 ± 0.6
Bone age (yr)	10.0 ± 1.1			10.8 ± 0.8		
Weight (kg)*	35.7 ± 3.4	40.1 ± 3.9	44.1 ± 4.8	37.4 ± 2.6	40.1 ± 3.2	43.4 ± 3.3
Body mass index	20.2 ± 1.8	20.3 ± 2.0	20.3 ± 2.4	18.6 ± 0.9	19.0 ± 1.1	19.8 ± 1.1
GH AUC (μg/L · min)†	89 ± 49			1815 ± 413		
Height (cm)‡	133.4 ± 4.5	140.7 ± 3.7	146.2 ± 3.1	141.5 ± 2.2	144.9 ± 2.6	147.6 ± 2.7
Height SDS‡	−1.4 ± 0.5	−0.9 ± 0.5	−0.7 ± 0.5	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
Height velocity (cm/6 mo)§		6.0 ± 0.4	5.3 ± 0.5		3.0 ± 0.4	3.1 ± 0.2
Height velocity SDS§		3.3 ± 0.9	2.3 ± 0.6		0.1 ± 0.3	0.1 ± 0.2
Sitting height (cm)‡	70.9 ± 2.0	75.4 ± 1.7	76.8 ± 1.9	74.2 ± 1.1	75.6 ± 1.3	77.0 ± 1.2
Biacromial breadth (cm)‡	28.0 ± 1.2	30.4 ± 1.3	32.3 ± 1.1	29.9 ± 0.6	30.7 ± 0.7	31.8 ± 0.8
Biiliac breadth (cm)*	21.8 ± 0.8	22.7 ± 0.9	23.8 ± 0.9	22.0 ± 0.8	22.6 ± 0.7	23.7 ± 0.8
Elbow breadth (cm)‡	5.3 ± 0.1	5.6 ± 0.1	5.8 ± 0.2	5.5 ± 0.1	5.5 ± 0.1	5.7 ± 0.2
BMR (kcal · d^{-1})‡	1,098 ± 89	1,350 ± 106	1,376 ± 118	1,288 ± 74	1,280 ± 86	1,352 ± 83

NOTE. Values are means ± SE.

*Significant time effect.

†Measured only at baseline and significantly different.

‡Significant group × time interaction effect.

§Significant group effect.

Abbreviations: AUC, area under the curve; SDS, standard deviation scores; BMR, basal metabolic rate.

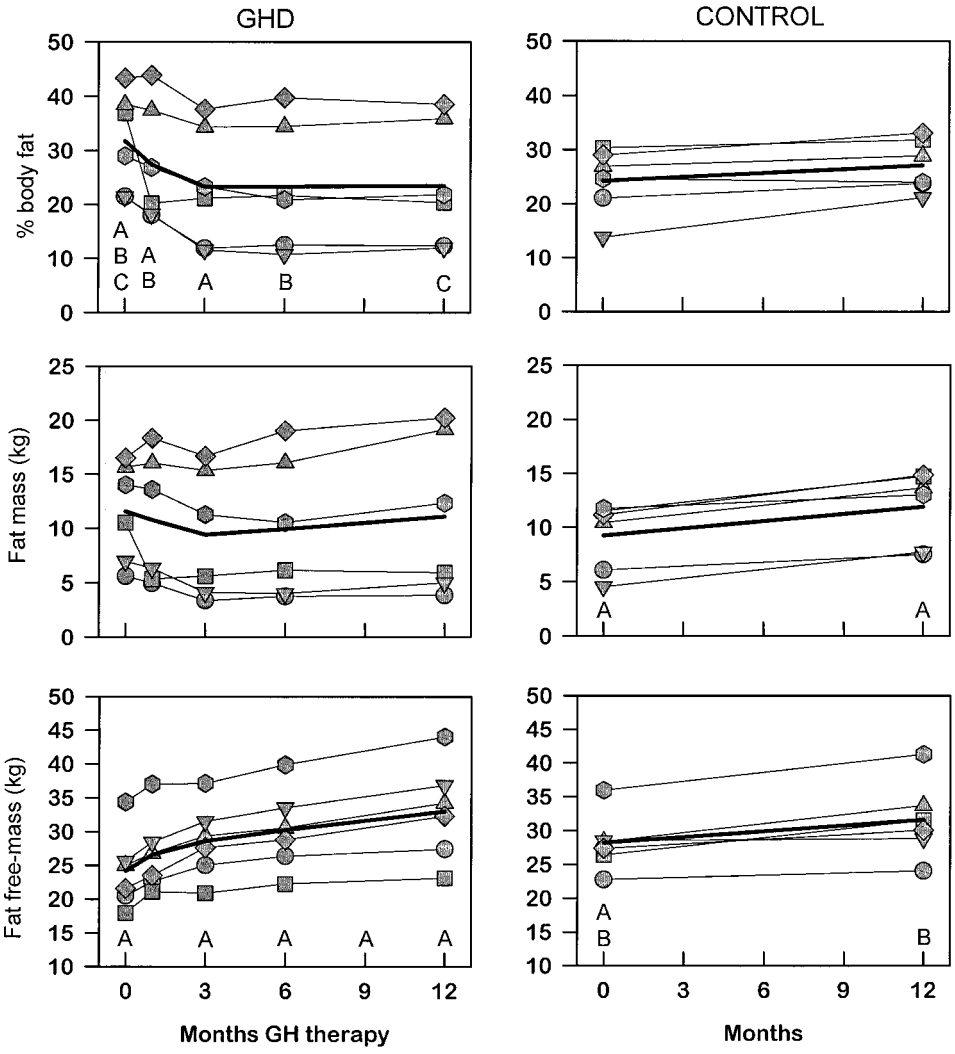

Fig 1. Body composition changes in the children receiving GH therapy (left panels) and controls (right panels). Symbols connected by lines represent repeated measures on the same child. Thick line represents mean value for each group. Like letters indicate significant differences between means.

was measured by deuterium oxide dilution. Urine samples were collected at 4 and 5 hours and the sample with the greatest enrichment used to calculate TBW. The 4-hour sample had a greater enrichment in 17 of the 36 TBW studies. A factor of 1.04 was used to correct for the incorporation of deuterium into nonaqueous tissues. The TBW was converted from liters to kilograms by dividing by 0.9937, the density of water at body temperature. As described above, BMC was determined by DEXA and divided by 0.88 to correct for fractional lowering of the volume of BMD by absorptiometry compared with the volume of bone ash mineral density. The BMC was then converted to total mineral (MIN) by dividing by 0.824.[6] The protein component (PRO) of the FFM was determined by subtraction (PRO = FFM - TBW - MIN). The proportional contribution of water (TBW/FFM), mineral (MIN/FFM), and protein (PRO/FFM) to the FFM was calculated by dividing the TBW, MIN, and PRO by the FFM. At the 1-month visit for the children with GH deficiency, the body composition was estimated using a 3-compartment water-density model, which can be used as a criterion method because it provides similar data as the 4-compartment model.[6] One child with GH deficiency did not assent to the underwater weighing procedure so her body composition was estimated each test date using age- and gender-appropriate constants for the proportional hydration of the FFM.[25] Excluding the data of this subject from statistical analysis did not change the results of the body composition data.

Magnetic Resonance Imaging

Subcutaneous (SAT) and visceral adipose tissue (VAT) at the level of the L4-L5 intervertebral space was measured with magnetic resonance imaging (MRI) using a Siemens (Iselin, NJ) Vision 1.5T scanner as previously described.[26,27] SAT and VAT were measured at baseline and at 3, 6, and 12 months for the children with GH deficiency and at baseline and 12 months for the control children.

Energy Expenditure

The basal metabolic rate (BMR) was measured for 30 minutes via indirect calorimetry (Deltatrac, SensorMedics, Yorba Linda, CA). Subjects were assessed upon waking after an overnight stay at the General Clinical Research Center. We have previously reported the within-day test-retest reliability (intraclass correlation = 0.98) of this measure in our laboratory.[26]

Blood Sampling and Assays

Spontaneous nocturnal GH release was measured at the baseline visit as previously described.[27,28] Briefly, after admission to the General Clinical Research Center at 8 AM, a catheter was inserted into a forearm vein at 4 PM and kept patent with a heparin lock. Serial blood sampling

(every 10 minutes) was initiated at 6 PM and continued until 6 AM. Activity was limited to walking and rest with the lights out after 10 PM. The subjects consumed meals and snacks constant for energy (kcal/kg), and the percentage of fat, protein, and carbohydrate. The Nichols Luma Tag human chorionic gonadotropin (hGH) chemiluminescence assay (San Juan Capistrano, CA) was used to measure serum GH concentrations. Use of the assay in our laboratory, its sensitivity and intra-assay and interassay coefficients of variation have been previously described.[27,28] GH area under the curve (AUC) was assessed by the model-free Cluster algorithm version 6.01.[29]

Serum leptin concentration was measured as previously described,[26] as was serum insulin-like growth factor-I (IGF-I) concentrations after acid-ethanol extraction.[27] Serum total cholesterol, triglyceride, phosphorus, calcium, and glucose concentrations were determined using an Olympus (Olympus Optical, Tokyo, Japan) automated chemistry analyzer, as were urine calcium and creatinine concentrations, which were measured from an aliquot of a 24-hour urine collection.

Statistics

One-way analysis of variance (ANOVA) was used to test for group differences in physical characteristics at the baseline measure. ANOVA (2 group × 3 time [baseline, 6 months, 12 months]) with repeated measures (RMANOVA) was used to test for group changes in anthropometric, blood biochemistry, energy expenditure, and physical activity variables. RMANOVA (2 group × 2 time [baseline, 12 months]) was used to test for changes in multicompartment body composition, DEXA and MRI variables. RMANOVA analyses were followed by a priori Student-Newman-Keuls pairwise multiple comparisons. The effects of GH therapy were shown by significant interaction effects, which are the focus of the analysis. To better describe the initial rapid changes in body composition, the measurements were made more frequently in the GH-deficient group (baseline, 1, 3, 6, and 12 months) than in the control group (baseline and 12 months). To utilize more of the data regarding the body composition and its proportional composition in the GH-deficient group, one-way RMANOVA analyses were completed (see later). A Bonferroni correction was made to adjust the alpha level for multiple (2) comparisons of the body composition data. Although a specific alpha level was not assigned for significance, a P value of approximately .025 (~.05/2) should be considered the criterion for a significant difference between means for these data. A slope analysis was performed to determine if there was a change in the slope of the adiposity-leptin relationship between baseline and 12 months.

RESULTS

Initially the groups were matched for age ($P = .46$), bone age ($P = .61$), body weight ($P = .69$), and height ($P = .14$) (Table 1). The 4-compartment estimated percent body fat ($P = .13$), fat mass ($P = .33$), and FFM ($P = .20$) were also not different at baseline. As expected, GH AUC measured at baseline was 20-fold greater ($P = .004$) in the control group (Table 1).

Longitudinal growth measures are shown in Table 1. Body weight increased ($P < .001$) at the 6- and 12-month visits. The 12-month percentage increase in body weight for the controls and children with GH deficiency was 16.0% and 23.5%, respectively. The percentage change of the GH-deficient group is approximately what would occur in a girl or boy at the 75th percentile and about 50% greater than that for the 50th percentile. There were group-time interactions for height ($P < .001$), sitting height ($P = .01$), biacromial breadth ($P = .04$), and elbow breadth ($P = .008$). For each of these variables, the GH-deficient group had a greater increase than the controls between baseline and 6 months, baseline and 12 months, and 6 and 12 months. The baseline height velocity and height velocity SDS could not be accurately calculated because height measurements taken before the baseline measurement were not obtained by research-trained observers. Comparisons at 6 and 12 months showed a greater height velocity ($P = .05$) and height velocity SDS ($P = .09$) for the GH-deficient group. The natural waist ($P = .03$), abdominal ($P = .01$), hip ($P = .04$), thigh ($P = .001$), and calf ($P < .001$) girths increased over time, but there were no significant group-time interactions (data not shown). The BMR increased in the GH-deficient group from baseline to 12 months but was not changed in the controls (group–time interaction: $P = .004$). There were no significant main or interaction effects after adjusting the BMR for the increase in metabolically active FFM.

As shown in Fig 1, group-time interactions occurred for 4-compartment percent body fat ($P < .001$), fat mass ($P = .05$), and FFM ($P = .002$). The percent body fat was reduced in the GH-deficient group from baseline to 12 months (Fig 1) and was unchanged in the controls. The fat mass increased in the control group from baseline to 12 months and was unchanged in the GH-deficient group. The FFM increased between the baseline and 12-month visits for both groups, but increased 3-fold more (36.5%) in the GH-deficient group than in controls (11.8%). Within the GH-deficient group, the percent body fat was reduced ($P < .001$) during the first 3 months of therapy, but not thereafter; fat mass was not changed, but the FFM increased ($P < .001$) between all 5 test dates (Fig 1). TBW increased for both groups from baseline to 12 months but more so for the GH-deficient group (32.9%) than for the controls (12.1%, group-time interaction: $P = .005$, Fig 2). TBW/FFM was not changed ($P = .57$) between baseline and 12 months in either group (Fig 3). TBW/FFM of the GH-deficient group was modestly greater ($P = .10$) at the 1 month visit than at baseline, 6 months, and 12 months (Fig 3). There was a significant ($P < .001$) time effect for MIN. From baseline to 12 months, MIN increased similar amounts (346 g *v* 417 g) and percentages (23.6% *v* 29.1%) in the GH-deficient and control groups (Fig 2). MIN/FFM decreased by 7.9% in the GH-deficient group but increased by 10.7% in the control group (Fig 3, group-time interaction: $P = .02$). MIN was greater ($P < .001$) in the GH-deficient group at the 12-month measure than at baseline, 3 months, and 6 months (Fig 2) and MIN/FFM was greater ($P = .002$) at baseline than at 3, 6, and 12 months (Fig 3). PRO increased more in the GH-deficient group (42.7%) than in the control group (8.5%) between baseline to 12 months (group-time interaction: $P < .001$) but PRO/FFM was not changed (Figs 2 and 3, respectively). Within just the GH-deficient group, PRO increased ($P < .001$) between each test date except 3 and 6 months (Fig 2), while PRO/FFM was unchanged (Fig 3).

Similar to when using the multicompartment models, DEXA estimates of the total percent body fat ($P = .01$) and percent fat of the arms ($P = .03$), legs ($P = .003$), and trunk ($P = .04$) were reduced in the subjects with GH deficiency from baseline to 12 months, but unchanged in the controls (Table 2). The changes in DEXA-estimated total body and regional percent fat were due to nonsignificant reductions in fat mass and significant increases in FFM of the subjects receiving GH therapy (data not shown). The abdominal visceral fat measured from

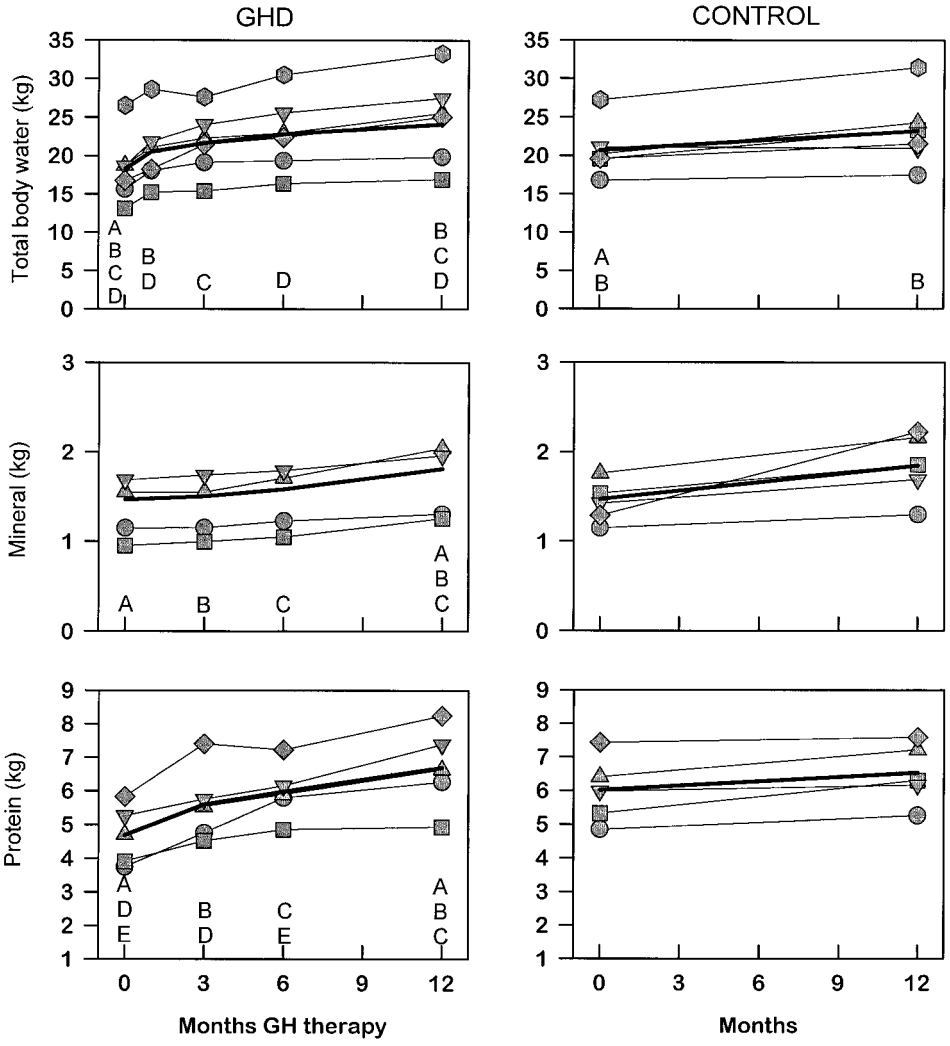

Fig 2. Composition of the FFM of children receiving GH therapy (left panels) and controls (right panels). Symbols connected by lines represent repeated measures on the same child. Thick line represents mean value for each group. Like letters indicate significant differences between means.

MRI was reduced ($P = .02$) in the GH-deficient group and unchanged in the controls from baseline to 12 months (Table 2). The abdominal subcutaneous fat measured in the same MRI was not changed in either group. The abdominal subcutaneous fat as measured by skinfold thickness also was not changed in either group (Table 2). Another truncal skinfold, the suprailiac thickness, was significant for the interaction ($P = .007$) due to an increase among controls from baseline to 12 months, while the suprailiac thickness of the GH-deficient group was unchanged. The upper body skinfold thicknesses (subscapular [$P = .003$], triceps [$P = .01$], chest [$P = .06$]) decreased in the GH-deficient group between the baseline and 6-month visit and were significant for the group-time interaction. The lower body skinfold thicknesses (thigh, medial calf) were not significantly changed in either group.

Bone mineralization data are shown in Table 3. The total body BMC ($P < .001$), total body BMD ($P < .001$), and forearm BMC ($P < .001$) increased equally in both groups from baseline to 12 months. The forearm BMD was not changed. Group-time interactions occurred for lumbar vertebrae BMC ($P = .03$) and BMD ($P = .04$), and for hip BMC ($P = .006$) and BMD ($P = .007$). The GH-deficient group experienced an increase in lumbar vertebrae BMD and hip BMC from baseline to the 12-month visit, while these variables were unchanged in the controls. The lumbar spine BMC and hip BMD increased from baseline to 12 months in both groups, but the GH-deficient group had greater increases.

Serum concentrations of hormones, lipids, and other metabolites are shown in Table 4. The serum IGF-I concentration increased each test date in the GH-deficient group, but was unchanged in the control group (group–time interaction: $P = .002$). The two-sided 95% confidence intervals for mean IGF-I concentration of the GH-deficient group at baseline, 6 months, and 12 months were 57.5 to 161.1 ng/mL, 226.5 to 510.1 ng/mL, and 287.7 to 672.9 ng/mL, respectively. The 2-sided 95% confidence intervals for mean IGF-I concentration of the control group at baseline, 6 months, and 12 months were 112.2 to 331.8 ng/mL, 138.9 to 383.9 ng/mL, and 194.0 to 391.6 ng/mL, respectively. Serum leptin concentrations decreased 51% in the GH-deficient group and increased 58% in the control group from baseline to 12 months (group-time interaction: $P = .08$). The slope of the sum of skinfolds–$\log_{(10)}$leptin

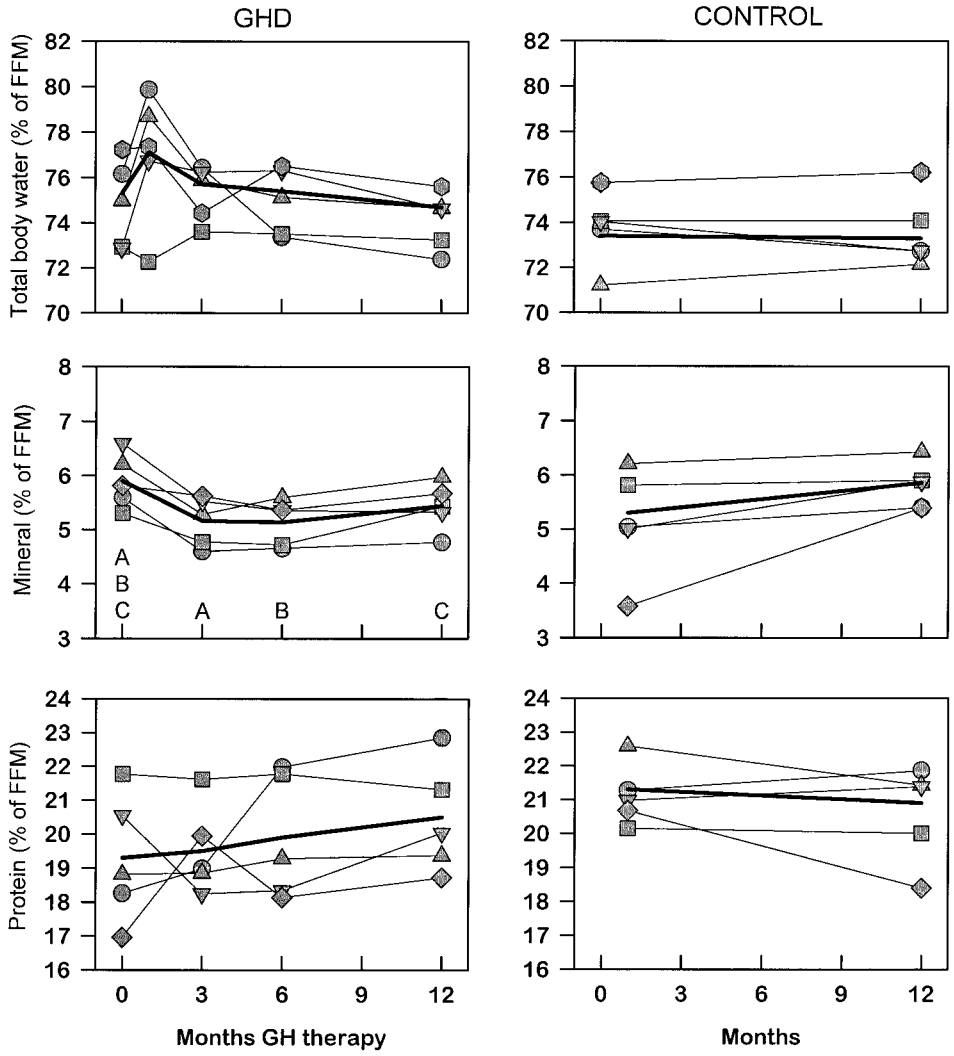

Fig 3. Proportional composition of the FFM of children receiving GH therapy (left panels) and controls (right panels). Symbols connected by lines represent repeated measures on the same child. Thick line represents mean value for each group. Like letters indicate significant differences between means.

relationship was not changed ($P = .64$) between the baseline (slope = 0.0050 $\log_{(10)}$ng · dL^{-1} · mm^{-1}) and 12-month (slope = 0.0037 $\log_{(10)}$ng · dL^{-1} · mm^{-1}) test dates. The GH-deficient group had a greater fasting serum insulin concentration ($P = .04$). The serum total cholesterol concentration was reduced in the GH-deficient group from baseline to 6 months and unchanged in the controls (group–time interaction: $P = .08$). The triglyceride concentration was unchanged. The serum phosphorus concentration of the GH-deficient group increased more than that of the control group from baseline to 12 months (group–time interaction: $P = .005$). The serum calcium and glucose concentrations and the urinary calcium/creatinine ratios were not different between groups nor significantly changed over time.

DISCUSSION

We used prepubertal GH-deficient children, appropriate pair-matched controls, and criterion methods to investigate the metabolic effects of GH therapy on body composition, body fat distribution, bone mineralization, and energy expenditure over a 1-year period. Frequent testing of the children at the initiation of GH therapy allowed for a more accurate determination of the time course of the alterations in growth and metabolism. All of the children had hypogonadotropic hypogonadism which is physiologic for this age group and which makes the interpretation easier because of the known effects of sex steroid on body composition and fat distribution.[30] Three of the 6 patients had idiopathic GH deficiency and required only GH replacement. The remaining 3 patients required additional replacement doses of thyroxine and/or hydrocortisone (see Methods). Replacement doses were used because we cannot mimic in vivo intermittent pulsatile release. These between-patient differences in hormonal replacement requirements and our inability to mimic in vivo patterns of release of thyroxine and cortisol may have made the response to GH therapy more variable. Both hormones have specific effects on body composition. Thyroxine deficiency increases adiposity, while excessive in vivo release or over replacement reduces adiposity. Excessive endogenous release or replacement doses of corticosteroids increase adiposity. The possible confounding effects of replacement doses of thyroxine and corticosteroids should be kept in mind when interpreting the results of this study. However,

Table 2. Body Fat Distribution Data of the Subject Groups

	GH-Deficient			Control		
	Baseline	6 Months	12 Months	Baseline	6 Months	12 Months
% fat 4C model*	31.8 ± 3.8	23.3 ± 4.8	23.5 ± 4.7	24.3 ± 2.5		27.1 ± 2.0
FM 4C model (kg)*	11.5 ± 1.9	9.9 ± 2.6	11.1 ± 3.0	9.2 ± 1.3		11.9 ± 1.4
FFM 4C model (kg)	24.2 ± 2.3	30.2 ± 2.5	33.0 ± 3.0	28.2 ± 1.8		31.5 ± 2.3
% body fat DEXA*	30.0 ± 4.8	25.5 ± 5.0	25.3 ± 5.2	26.4 ± 2.3		29.2 ± 3.6
FM DEXA (kg)	11.1 ± 2.4	10.5 ± 2.7	12.3 ± 3.5	9.8 ± 1.2		12.7 ± 2.0
Arm % fat*	41.4 ± 7.1	30.3 ± 6.4	31.4 ± 6.3	33.3 ± 2.9		34.2 ± 3.8
Leg % fat*	38.4 ± 4.3	32.9 ± 5.1	31.4 ± 4.7	37.0 ± 2.7		37.9 ± 3.0
Trunk % fat*	29.8 ± 7.2	19.5 ± 5.5	20.1 ± 5.8	17.8 ± 3.2		22.1 ± 4.9
AVF (cm^2)*	96.8 ± 14.6	72.6 ± 10.8	59.1 ± 11.0	53.4 ± 11.8		59.1 ± 7.1
ASF (cm^2)	128.7 ± 39.5	114.4 ± 42.8	120.2 ± 41.2	119.4 ± 22.3		144.6 ± 33.3
Subscapular (mm)*	21.6 ± 6.6	13.8 ± 3.9	13.8 ± 3.9	10.3 ± 1.8	11.0 ± 2.6	12.2 ± 2.6
Triceps (mm)*	18.8 ± 3.5	13.5 ± 3.4	13.0 ± 3.1	15.3 ± 1.9	14.8 ± 1.5	16.5 ± 1.9
Chest (mm)*	13.9 ± 3.3	9.8 ± 2.0	8.9 ± 2.7	7.1 ± 0.7	6.7 ± 1.0	7.2 ± 0.9
Suprailiac (mm)*	24.5 ± 7.1	20.1 ± 6.3	22.3 ± 6.7	13.1 ± 2.5	17.4 ± 4.1	21.1 ± 4.9
Abdominal (mm)	24.4 ± 8.1	23.6 ± 6.7	24.1 ± 6.7	20.1 ± 2.7	19.5 ± 3.6	24.2 ± 4.4
Thigh (mm)	20.4 ± 3.2	16.7 ± 3.4	18.7 ± 5.5	19.6 ± 2.0	19.9 ± 1.7	21.5 ± 2.5
Calf (mm)	16.5 ± 3.0	17.0 ± 4.8	18.1 ± 5.7	15.4 ± 1.5	14.7 ± 1.9	16.3 ± 2.0

NOTE. Values are mean ± SE.

*Significant group-time interaction.

Abbreviations: 4C, 4-compartment; FM, fat mass; ASF, abdominal subcutaneous fat; AVF, abdominal visceral fat.

many group-time statistical interactions existed because the replacement therapy was as close to "physiologic" as possible and a control group and a matched-pairs design were utilized. The individual data are shown and each of the patients and controls responded in the same way.

As expected, the GH-deficient group had greater increases in height and height SDS than the control group (Table 1). The GH-deficient group also had a greater height velocity and height velocity SDS. The incremental growth tables of Baumgartner et al[24] were used to determine the growth velocity and growth velocity SDS. These tables were specifically developed to assess short-term growth in response to a therapy and their tabulated data allow for a more accurate determination of the growth velocity than from a growth chart. The mean annualized height velocity of 10 to 12 cm/yr of the GH-deficient group is similar to previous investigations.[5,31] The mean height velocity SDS of the control group of 0.1 cm confirms the appropriateness of the control group. Other bone growth measures, including sitting height, biacromial breadth, and elbow breadth, also increased more in the GH-deficient group than in the control group.

The body weight of the GH-deficient and control groups increased 12.4% and 7.2% from baseline to 6 months and 23.4% and 16.0% from baseline to 12 months, respectively, but the group-time interaction was not significant. Gains in FFM accounted for 91.5% of the increase in body weight for the GH-deficient group and 55.5% of the weight increase of the controls. The most rapid increase in FFM occurred during the first month of therapy. Previous studies[2-5,20] of children could not determine to what extent these initial rapid increases in FFM were due to catch-up growth or transient increases in TBW caused by GH therapy.[32,33] TBW increased most rapidly between baseline and the 1-month visits (Fig 2) and a transient increase ($P = .10$) in TBW/FFM occurred in all but one of the children in the GH-deficient group at the 1-month visit (Fig 3), suggesting that the initial rapid increase in FFM was due, in

Table 3. Total Body and Regional Bone Mineralization Data of the Subject Groups

	GH-Deficient			Control	
	Baseline	6 Months	12 Months	Baseline	12 Months
Total body BMC (g)*	987 ± 125.6	1,101 ± 126	1,260 ± 150	1,125 ± 70	1,323 ± 97
Total body BMD (g/cm^2)*	0.83 ± 0.04	0.85 ± 0.04	0.88 ± 0.04	0.86 ± 0.02	0.89 ± 0.01
Forearm BMC (g)*	5.9 ± 0.8	6.5 ± 0.9	7.6 ± 1.1	5.6 ± 0.6	7.1 ± 0.7
Forearm BMD (g/cm^2)	0.41 ± 0.02	0.42 ± 0.03	0.43 ± 0.03	0.41 ± 0.01	0.41 ± 0.02
L_1-L_4 BMC (g)†	20.8 ± 2.7	25.1 ± 2.7	28.7 ± 2.6	22.7 ± 1.1	26.7 ± 1.7
L_1-L_4 BMD (g/cm^2)†	0.58 ± 0.05	0.66 ± 0.04	0.71 ± 0.05	0.63 ± 0.02	0.67 ± 0.03
Hip BMC (g)†	14.6 ± 2.8	17.1 ± 2.5	21.0 ± 3.1	16.9 ± 1.3	19.6 ± 2.0
Hip BMD (g/cm^2)†	0.70 ± 0.07	0.75 ± 0.06	0.82 ± 0.07	0.72 ± 0.02	0.76 ± 0.03

NOTE. Values are means ± SE.

*Significant time effect.

†Significant group-time interaction.

Table 4. Hormonal and Metabolic Data of the Subject Groups

	GH-Deficient			Control		
	Baseline	6 Months	12 Months	Baseline	6 Months	12 Months
IGF-I (ng/mL)*	109.3 ± 20.1	368.3 ± 55.2	480.3 ± 74.9	222.0 ± 42.7	261.4 ± 47.7	292.8 ± 38.4
Prolactin (ng/mL)	8.74 ± 2.47	8.28 ± 2.66	4.88 ± 1.58	5.21 ± 0.56	7.52 ± 0.64	8.71 ± 2.36
Leptin (ng/mL)	16.8 ± 4.4	8.7 ± 2.6	8.2 ± 3.2	7.6 ± 1.3	8.9 ± 2.4	12.0 ± 3.8
Insulin (μIU/mL)†	18.1 ± 2.7	32.0 ± 12.1	38.0 ± 13.2	5.4 ± 0.7	7.1 ± 1.9	8.7 ± 1.4
Glucose (mg/dL)	86.5 ± 5.1	97.8 ± 8.7	88.4 ± 3.3	84.7 ± 3.9	91.5 ± 6.0	87.4 ± 3.4
Total cholesterol (mg/dL)*	176.0 ± 12.5	153.5 ± 13.1	151.5 ± 10.6	148.2 ± 6.2	153.7 ± 6.5	158.8 ± 10.0
Triglycerides (mg/dL)	212.5 ± 39.1	170.0 ± 30.6	191.7 ± 39.4	119.7 ± 22.2	121.2 ± 32.0	149.3 ± 32.4
Phosphorous (mg/dL)*	5.02 ± 0.16	5.63 ± 0.19	5.95 ± 0.18	4.82 ± 0.23	4.47 ± 0.25	5.08 ± 0.18
Calcium (mg/dL)	9.63 ± 0.13	9.45 ± 0.15	9.55 ± 0.14	9.48 ± 0.16	9.47 ± 0.16	9.63 ± 0.2
Calcium/creatinine‡	0.15 ± 0.04	0.20 ± 0.05	0.21 ± 0.06	0.21 ± 0.02	0.16 ± 0.02	0.18 ± 0.02

NOTE. Values are means ± SE.

*Significant group-time interaction.

†Significant group effect.

‡From 24-hour urine collection ([mg/dL]/[mg/dL]).

part, to GH-induced edema. GH treatment in GH-deficient adults increases TBW/FFM primarily due to an expansion of extracellular water including plasma volume,[33] and this probably also occurs in children.[34] The TBW/FFM may not remain elevated in children as long as in adults because the natural maturation of the FFM results in a reduction in TBW/FFM.[6] The maturational effect in adults, if any, would be in the opposite direction of children (increased TBW/FFM) due to aging-related losses in protein and bone mineral.

The effects of GH on water distribution had the potential to confound the TBW measures (and thus body composition measures) by altering the time course for exchange equilibrium and the maximal levels attained. For example, patients with an expansion of extracellular water due to ascites have a delay in isotopic equilibration until 4 hours after the dose.[35] In anticipation of individual differences and GH-induced changes in equilibration, urine samples were collected 4 and 5 hours after oral dose administration and the sample with the greatest enrichment was used to determine TBW. In the children with GH deficiency, the 4-hour sample had a greater enrichment in approximately half (17 of 36) of the TBW studies. This sampling protocol would not have produced appreciable effects on TBW estimates of the controls subjects because in normal subjects the enrichment peaks and plateaus from about 3 to 6 hours after oral dose administration.[36] We found no differences when comparing the 4-hour (489.1 delta per mil) and 5-hour (484.8 delta per mil) enrichment data from 153 TBW studies completed in healthy children in our laboratory. Assuming that our sampling protocol produced accurate TBW estimates, the 4-compartment and 3-compartment water density models we employed have the distinct advantage of correcting for the confounding effect of alterations in TBW when estimating the body composition.[6]

Increases in mineral and protein components of the FFM during the first month of therapy could not be determined because DEXA measures were not completed. An increase in total body mineral during the first month would not be expected because it did not increase above baseline until the 12-month measure and the MIN/FFM was reduced between the baseline and 3-month measurements. An early increase in the protein component cannot be discounted because the total protein increased between baseline and 3 months and the PRO/FFM was not changed throughout the study suggesting that the rate of protein accrual kept pace with the rate of increase of the total FFM.

Consistent with several previous studies,[4,20,37,38] the most rapid change in fat mass occurred during the first months of therapy. The fat mass then gradually increased to baseline amounts by the 12th month of therapy. The temporary reduction in fat mass[1,20,39] may be due to a transitory lipolytic effect of GH therapy,[1,20] but another possible mechanism exists. After an initial 3-month catch-up period, the fat mass may begin to increase due to natural growth in fat mass which occurs in all children,[40] including the control group in the current study. After 3 months, the increase in fat mass of the GH-deficient group increased at the same rate as the control group, further suggesting that this is a physiologic growth effect. The lipolytic effect of GH is probably still present, in adults it is maintained for at least 18 months[41,42] but, in children, the physiologic response is a gradually increasing fat mass. Our hypothesis is further supported by the percent body fat data. After a reduction during the first 3 months of therapy, there was no change in percent body fat despite the gradual increase in fat mass. The percent body fat did not change because the anabolic effect of GH produced a proportional increase in the FFM. After the first 3 months of therapy, the FFM increased at a rate very similar to control children (Fig 1). The similar increases in fat mass and FFM of the control and GH-deficient groups after the catch-up period again demonstrate that the groups were well matched for their stage of pubertal maturation, another factor affecting the rate of accrual of fat mass and FFM.[43]

The data from our multicompartment body composition models were confirmed by DEXA analyses, which demonstrated that total body percent fat and regional reductions in percent fat of the arms, legs, and trunk of the GH-deficient group were due to nonsignificant reductions in fat mass and significant increases in FFM (Table 2). MRI of the abdomen and skinfold thicknesses identified specific regional lipolytic effects of GH therapy. From MRI analysis, the abdominal visceral fat was reduced but the abdominal subcutaneous fat

was not. The smaller change in truncal subcutaneous fat was corroborated by the abdominal and suprailiac skinfold thicknesses, which also were not reduced in the GH-deficient group. In normal children, the rate of change in the abdominal skinfold thickness is positive during the time of peak height velocity,[44] which coincides with the time of peak GH release. Moreover, girls have a greater GH release than boys,[27] but at similar ages, girls have a greater abdominal skinfold thickness than boys relative to the triceps skinfold thickness.[44] Thus, GH may have little effect on the subcutaneous abdominal fat of youth. Consistent with previous studies,[45-46] GH therapy reduced skinfold thicknesses of other truncal sites, including the suprailiac, chest, and subscapula, but not the peripheral lower body sites (thigh, calf).

Serum concentrations of leptin, which is secreted primarily from subcutaneous adipocytes,[26] were reduced by 51.2% while the sum of skinfolds, a measure of the total subcutaneous fat, was reduced by only 12.6%. However, a direct inhibitory effect of GH on leptin secretion[47] could not be confirmed because the slope of the leptin-sum of skinfolds relationship of the GH-deficient group did not differ between the baseline and 12-month visits. Serum leptin concentrations of the controls increased from baseline to 12 months. Prepubertal increases in leptin have been previously documented in both longitudinal[48] and cross-sectional[49-51] studies due, in large part, to maturational increases in fat mass.

The total body and regional BMC and BMD of the GH-deficient group increased during GH therapy, but only the hip and lumbar spine BMD increased at a greater rate than in the controls (Table 3). Others have reported greater increases in BMD of the lumbar spine (areal and volumetric) than the total body[1,13] in response to GH therapy. Boot et al[1] suggest this is due to the high percentage of trabecular bone in the lumbar spine compared with the total skeleton. Similar to previous studies, the serum calcium concentration remained unchanged,[1] although others have reported a sustained decrease after 3 months of therapy.[52] Serum phosphorus levels increased with GH therapy probably due to an antiphosphaturic effect of GH mediated by an increased rate of renal tubular phosphate reabsorption.[53,54]

GH therapy normalized serum IGF-I concentrations. Serum insulin concentrations also increased during GH therapy despite reductions in the percentage body fat, highlighting the well-known insulin antagonistic effects of GH on glucose metabolism.[55] Data regarding the response of lipids to GH therapy are inconclusive. As demonstrated previously,[31,56] total cholesterol was reduced toward normal with GH therapy. Although this is not a consistent finding,[1,4] reductions in a composite atherogenic index indicated an overall reduction in atherosclerosis risk.[1,4]

As confirmed in the present study, children experience an increased energy expenditure during GH therapy.[33,56] However, these data are confounded by concomitant increases in metabolically active FFM. Attempts have been made to correct the changes in energy expenditure for the change in FFM,[3,56] but criterion measures of body composition have not been used. Using criterion body composition models, we found that GH does not independently increase the metabolic rate. That is, the increase in basal metabolic rate with GH therapy could be accounted for by the increase in metabolically active FFM.

In conclusion, we have shown specific alterations in body composition and the regional distribution of body fat during hormonal replacement therapy in a group of prepubertal boys and girls with either isolated GH deficiency or GH deficiency plus thyroxine and cortisol deficiency. All children received proper replacement doses resulting in eumetabolic states and, as shown in the figures, the individual responses in body composition and body fat distribution were very similar. Using a paired control design and criterion methods of body composition and fat distribution, we have accurately defined the kinetics of the pharmacologic effects of hormonal replacement in this group of children with either single (GH) or multiple (GH + thyroxine and cortisol) hormonal deficiencies receiving physiologic replacement doses of hormones.

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