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Short- and Long-Term Effects of Growth Hormone (GH) Replacement on Protein Metabolism in GH-Deficient Adults

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Reduced fat-free mass (FFM) in GH-deficient (GHD) adults is improved by GH replacement, but the protein metabolic changes are unclear. Using iv [$^2H_3$]leucine and oral l-[$^{13}C_1$]leucine infusions and dual emission x-ray absorptiometry, we compared leucine kinetics and body composition in eight GHD adults and eight healthy controls in the fasted and fed states, before and after 2 wk and 6 months of GH replacement. Leucine kinetics were not different between pretreatment GHD subjects and controls. After 2 wk of GH treatment, leucine oxidation decreased in the GHD subjects compared with baseline values [fasted, 41 ± 6 *vs.* 30 ± 5 μmol/kg FFM·h ($P < 0.01$); fed, 49 ± 3 *vs.* 41 ± 3.6 μmol/kg FFM·h ($P < 0.05$)], leucine balance improved [fasted, −14 ± 4 *vs.* −3.5 ± 3 μmol/kg FFM·h ($P < 0.01$); fed, 65 ± 10 *vs.* 72 ± 7 μmol/kg FFM·h ($P = 0.07$)], and protein synthesis increased [fasted, 116 ± 5 *vs.* 131 ± 6 μmol/kg FFM·h ($P < 0.05$); fed, 103 ± 6 *vs.* 116 ± 6 μmol/kg FFM·h ($P < 0.05$)]. After 6 months of GH treatment, these changes were not maintained in the fed state. The five GHD subjects with decreased FFM at baseline showed a significant increase after 6 months of GH treatment ($P < 0.05$). GH replacement in GHD acutely improves protein balance by stimulating synthesis and inhibiting catabolism. After 6 months, protein kinetics reached a new homeostasis to maintain the net gain in FFM. (*J Clin Endocrinol Metab* 88: 5827–5833, 2003)

ADULTS WITH ACQUIRED GH deficiency (GHD) generally have reduced fat-free mass (FFM) and increased fat mass (FM) compared with body mass index (BMI)-matched healthy adults (1–5). Although it has been shown that GH replacement therapy results in an increase in FFM in GHD patients (1–3), the mechanism of this protein anabolic effect has not been clearly established. For example, whereas some studies have reported that GH replacement promotes protein anabolism by restraining catabolism and stimulating synthesis in both the fasted (6–8) and fed states (8), others have shown only a transient increase (2) or no increase (9) in protein synthesis.

One possible explanation for these discrepant findings may be the fact that measurements of protein metabolism have been made at different times posttreatment, ranging from as early as 2 wk (7), when GH anabolic effect may have been maximum, to 6 months (2, 9), when FFM may have returned to normal and, hence, GH had no further anabolic effect. For example, a study by Beshyah *et al.* (9) reported no significant change in leucine kinetics after 6 months of GH replacement therapy in GHD adults. On the other hand, two different studies of fasted GHD patients reported increased nonoxidative leucine disposal, a measure of protein synthesis, and a decrease in leucine oxidation, an index of net protein catabolism, after 2 wk and 2 months, respectively, of GH treatment (6, 7).

Another factor that may confound the effects of GH replacement on protein metabolism is the prandial state of the subject during the measurement. It has been argued that the protein anabolic actions of GH require the presence of adequate insulin concentrations as well as normal insulin action (3). In the absence of insulin, the effects of GH on FFM are mainly catabolic, but in the presence of insulin the effects of GH on FFM are to restrain catabolism (3). To determine the physiological effects of GH treatment in GHD patients, it is therefore important to measure its effects on protein metabolism in both the postabsorptive state, when plasma insulin concentrations are low, and during feeding, when plasma insulin concentrations are increased. However, the majority of studies of the effects of GH replacement on protein metabolism in GHD patients have been performed in subjects in the fasted state (6, 7, 9). For these reasons it is important to determine the short- and long-term effects of GH therapy in the fed and fasted states.

There have been no previous studies of protein kinetics in GHD patients after short- and long-term treatment with GH in both the fasted and fed states. The goal of the present study was to quantify whole body and splanchnic protein metabolism in the fasted and fed states before and at two time points after GH replacement therapy. We hypothesized the following: 1) in the short term, GH treatment of GHD patients would lead to an increase in net protein synthesis in the fed state and a decrease in net protein loss in the fasted state to facilitate replenishment of FFM; and 2) after prolonged GH treatment and stabilization of body compositional changes, protein kinetics in GHD patients in the fed and fasted states would be indistinguishable from those in normal adults. To test these hypotheses, whole body leucine kinetics were measured using simultaneous infusions of an iv tracer

Abbreviations: BMI, Body mass index; FFM, fat-free mass; FM, fat mass; GHD, GH deficient; KICA, α-ketoisocaproic acid.

([$^2H_3$]leucine) and an oral tracer (l-[$^{13}C_1$]leucine) in eight GHD adult patients and in eight age-, sex-, and BMI-matched normal controls. The GHD patients were studied before starting GH replacement as well as after 2 wk and 6 months of physiological GH replacement therapy.

Subjects and Methods

The study was approved by the institutional review board of Baylor College of Medicine. Written informed consent was obtained from all subjects before their entry into the study. Eight adults (six women and two men) with panhypopituitarism, taking stable, physiological, replacement doses of glucocorticoid, thyroid hormone, and sex steroids, were recruited from the Neuroendocrine Clinic at Ben Taub General Hospital (Houston, TX). All patients had had panhypopituitarism for 3 yr or more. GH deficiency was defined as a peak plasma GH concentration below 2.5 ng/ml during an insulin tolerance test or an iv arginine (10% arginine hydrochloride) stimulation test. Of eight subjects with GHD, three were diagnosed by the insulin hypoglycemia test and five by the arginine stimulation test. The peak GH concentration in response to both stimulation tests was 1.1 ng/ml.

The starting dose of GH was 2.5 μg/kg·d (0.0075 IU/kg·d) in women and 2 μg/kg·d (0.006 IU/kg·d) in men, administered as a single sc injection in the evening between 2000–2100 h. The dose was doubled after 1 wk (the GH product used in the study was Humatrope, Eli Lilly & Co., Indianapolis, IN). Thereafter, the serum IGF-I concentration was measured every 6–8 wk, and the dose was titrated upward with the goal of maintaining the plasma concentration of IGF-I in the midnormal range for age and gender, up to a maximum daily dose of 4 μg/kg (0.012 IU/kg) in men and 5 μg/kg (0.015 IU/kg) in women. Female subjects receiving oral estrogen therapy were switched to transdermal estradiol (0.05 mg once a week) 4 wk before commencing GH therapy. GHD patients received oral glucocorticoid replacement according to the following regimen: 20 mg hydrocortisone at 0800 h and 10 mg at 1400 h throughout the period of the study, including the day of the metabolic studies in the General Clinical Research Center. Each metabolic study was performed 1 wk after a testosterone injection in GHD men receiving testosterone replacement and in midcycle in GHD women with normal menstrual cycles.

Controls were recruited by screening healthy volunteers for evidence of hypothalamic or pituitary disease by history and physical examination. All control subjects had the following normal laboratory tests: complete blood counts, liver and kidney function tests, free T_4, TSH, LH, FSH, PRL, testosterone or estradiol, IGF-I, and morning cortisol.

Materials

Stable isotope studies of protein metabolism were performed on each subject at baseline before GH treatment and were repeated 2 wk and 6 months after the start of GH treatment. Control subjects underwent the same protocol only once. For each study the subject was admitted to the Adult General Clinical Research Center of Baylor College of Medicine after 2 d of stabilization on a standard diet (30 kcal and 1 g protein/kg·d). After a 12-h overnight fast, iv catheters were placed in the superficial veins of both arms. At 0800 h, a primed-constant iv infusion of [$^2H_3$]leucine (prime, 7.5 μmol/kg; infusion, 7.5 μmol/kg·h) was begun and continued for 8 h. Simultaneously, a primed-constant oral administration of l-[$^{13}C_1$]leucine (prime, 7.5 μmol/kg; infusion, 7.5 μmol/kg·h) was begun and continued for 8 h. The oral tracer was administered in boluses (2.5 ml) at 15-min intervals. During the first 4 h of the infusions, the subject remained fasting (fasted study). From 4–8 h, the subject consumed a mini-meal every 30 min (fed study). Each mini-meal consisted of 1/24th of the daily caloric and protein requirement. Serial blood and breath samples were collected at baseline and every 15 min during the last hour of the fasted (h 3–4) and fed (h 7–8) studies. Indirect calorimetry was performed for 30 min, once during the fasted study (h 2.5–3) and once during the fed study (h 6.5–7).

Laboratory analyses

The blood samples were drawn into prechilled tubes containing Na_2-EDTA. They were centrifuged immediately at 4 C, and the plasma was removed and stored at −70 C for later analyses. Plasma leucine was isolated by ion exchange (Dowex 200X) chromatography and was converted to its heptafluorobutyramide derivative. The tracer/tracee ratio was measured by negative chemical ionization gas chromatography-mass spectrometric analysis by selectively monitoring ions at m/z ratios 349–352 as previously described (10). The plasma α-ketoisocaproic acid (KICA) tracer/tracee ratio was measured by negative chemical ionization gas chromatography-mass spectrometry of its pentafluorobenzyl derivative (10). The breath samples were analyzed in duplicate for ^{13}C abundance in carbon dioxide by gas isotope ratio mass spectrometry (Europa Scientific, Crewe, UK), monitoring ions at m/z ratios of 44 and 45.

FFM and FM were measured using dual emission x-ray absorptiometry in all subjects at baseline, and measurements were repeated in the GHD subjects after 6 months of GH treatment. Based on daily measurements over a 12-month period, the dual emission x-ray absorptiometry instrument at our institution has a precision of less than 2% for bone mineral density for a whole body phantom. For duplicate measurements in 10 subjects examined over a 5-d period, the precision was 3% for the soft tissue composition. Serum IGF-I assays were performed by chemiluminescent immunoassay at Arup Laboratories (Salt Lake City, UT).

Calculations

Carbon dioxide flux (Ra CO_2) was calculated from the steady state equation:

$$RaCO_2 = \left[\frac{E_{Inf}}{E_b} - 1\right] \times i$$

where E_{Inf} and E_b are the isotopic enrichments (atom percent excess) of bicarbonate in the infusate and carbon dioxide in the expired breath at isotopic steady state, and *i* is the rate of infusion of the tracer in micromoles per kilogram per hour.

At isotopic steady state, the fraction of oral tracer that enters the systemic circulation (sampled compartment) is given by the plasma tracer/tracee ratio of the intragastric tracer to the plasma tracer/tracee ratio of the iv tracer normalized for their infusion rates. This fraction was calculated as:

$$\left[\frac{E_{pIG}}{E_{pIV}} \times \frac{i_{IV}}{i_{IG}}\right]$$

where Ep_{IG} is the plateau tracer/tracee ratio of the IG tracer, Ep_{IV} is the plateau tracer/tracee ratio of the IV tracer, i_{IV} is the rate of the IV tracer infusion × the enrichment of the IV tracer, and i_{IG} is the rate of the IG tracer infusion × the enrichment of the IG tracer. Thus the percentage of IG tracer (and of dietary leucine) extracted by the splanchnic tissues was calculated by:

$$\%LEU_{splan} = \left[1 - \frac{E_{pIG}}{E_{pIV}} \times \frac{i_{IV}}{i_{IG}}\right] \times 100$$

Splanchnic leucine utilization (LEU_{splan}) was calculated as the product of the fraction of dietary leucine extracted by the splanchnic tissues and enteral leucine intake:

$$LEU_{splan}(\mu mol/kg \cdot h) = \left[1 - \left(\frac{E_{pIG}}{E_{pIV}} \times \frac{i_{IV}}{i_{IG}}\right)\right] \times [\text{enteral leu intake}],$$

Total leucine flux (Q) was calculated as:

$$Q(\mu mol/kg \cdot h) = \frac{i_{iv}}{Ep_{^{13}C\text{-}\alpha\text{-}KICA}}$$

where E_p ^{13}C-α-KICA is the plateau enrichment of α-KICA derived from the l-[$^{13}C_1$]leucine tracer.

Leucine oxidation (LEU_{ox}) was calculated as follows: LEU_{ox} (μmol/kg·h) = Ra $^{13}CO_2/E_{p\ 13C\text{-}\alpha\text{-}KICA}$, where Ra $^{13}CO_2$ is the rate of production of labeled carbon dioxide (obtained from the product of Ra CO_2 and the plateau isotopic enrichment of expired CO_2 during the l-[$^{13}C_1$]leucine infusion).

Leucine used for protein synthesis (LEU_{syn}) was calculated as leucine flux minus leucine oxidation: LEU_{syn} (μmol/kg·h) = Q-LEU_{ox}.

Leucine derived from protein breakdown (LEU_{brk}) was calculated as the difference between leucine flux and all sources of leucine intake: LEU_{brk} (μmol/kg·h) = Q − (diet Leu + IG Leu + IV Leu).

Leucine balance (Leu_{bal}) was calculated as the difference between leucine intake and leucine oxidation: Leu_{bal} (μmol/kg·h) = (diet Leu + IG Leu + IV Leu) − LEU_{ox}.

The efficiency of utilization of leucine (Leu_{EU}) was calculated as: Leu_{EU} (%) = (Leu balance/diet Leu + IG Leu + IV Leu) × 100.

All kinetic data are expressed per kilogram of FFM.

Statistical analysis

A sample size of eight was selected based on Dr. Jahoor's previous data on leucine kinetics (11): leucine flux, 167 ± 10 μmol/kg·h (coefficient of variation, 6%); leucine oxidation, 37 ± 4 μmol/kg·h (coefficient of variation, 11%). Based on these data, a sample size of eight should detect a SD difference of 1.5 with a power of 0.80, assuming a correlation of at least 0.5 between repeated measures.

The nonpaired *t* test was employed for statistical comparison of baseline data between GHD patients and controls, and repeated measures one-way ANOVA was used to compare the data obtained at the three time points in the GHD patients: baseline, 2 wk, and 6 months. If the ANOVA was significant, pairwise comparisons were made with the Bonferroni *post hoc* test. Results are presented as the mean ± SEM, with significance at $P < 0.05$.

Results

GHD patients were receiving stable hormone replacement for hypopituitarism with hydrocortisone, thyroid hormone, and sex steroids as appropriate (Table 1). GHD patients were matched with control subjects for age, sex, and BMI. Both groups had normal liver and kidney function, and fasting glucose and hemoglobin A_{1c} values (Table 2). Subjects 1 and 4 had normal menstrual periods and therefore did not require sex steroid replacement. Subjects 2 and 3 had mildly elevated PRL levels; subject 2 had a small residual prolactinoma, and subject 3 had a small nonfunctioning pituitary tumor; both tumors had been stable in terms of size and hormone production for more than 3 yr. The mean GH dosage was 0.41 ± 0.15 mg/d. The mean IGF-I concentration before starting GH was 71 ± 16 ng/ml (9.2 ± 2 nmol/ml), and it increased to 222 ± 46 ng/ml (28.9 ± 6 nmol/ml) after 6 months of GH therapy ($P < 0.01$; Fig. 1). Six of the eight patients tolerated the GH treatment protocol without any side-effects. One patient developed mild edema and arthralgia requiring temporary dosage reduction. Another patient developed fasting hyperglycemia; hence, her GH dose was reduced, and she was maintained on 2.5 μg/kg·d throughout the remainder of the study, without recurrence of hyperglycemia. Serum cortisol and free T_4 concentrations measured at 1000 h at each study visit were within normal limits in all GHD patients. There was no significant change in mean fasting plasma glucose [83 ± 0.7 *vs.* 88 ± 4 mg/dl (4.6 ± 0.04 *vs.* 4.8 ± 0.2 mmol/liter)], insulin [7.6 ± 2.5 *vs.* 8.3 ± 1.9 μU/ml (54 ± 18 *vs.* 59 ± 14 pmol/liter)], or hemoglobin A_{1c} (5.5 ± 0.1% *vs.* 5.6 ± 0.3%) values after 6 months of GH therapy.

Protein kinetics

There were no significant differences in the rates of leucine oxidation and leucine balance between GHD patients and controls in the fasted and fed states (Table 3). After 2 wk of GH therapy, while GH dosage and plasma IGF-I levels were still subphysiological, there were significant decreases in the rates of leucine oxidation in GHD patients in both the fasted and fed states ($P < 0.01$ and $P < 0.05$, respectively; Table 3).

TABLE 2. Biochemical and hormonal characteristics of controls and GHD subjects

	Controls	GHD
Age (yr)	48.5 ± 1.2	50.8 ± 4.5
BMI (kg/m^2)	33.8 ± 8.7	34.3 ± 2.7
Sex (M:F)	2:6	2:6
Hemoglobin (g/dl)	14.3 ± 0.3	13.2 ± 0.1
BUN (mg/dl)	13.1 ± 0.9	14.3 ± 0.2
Creatinine (mg/dl)	0.85 ± 0.04	0.9 ± 0.1
Free thyroxine (ng/dl)	1.2 ± 0.1	1.3 ± 0.1
Testosterone (ng/dl)	463 ± 297	467 ± 179
AM cortisol (μg/dl)	11.8 ± 1.2	15.5 ± 6.5
Fasting glucose (mg/dl)	88 ± 4.1	83 ± 0.7
$HbA1_C$ (%)	5.3 ± 0.1	5.5 ± 0.1

All values are mean ± SEM. BUN, Blood urea nitrogen; AM, morning; $HbA1_C$, glycosylated hemoglobin. To convert serum free thyroxine from ng/dl to nmol/liter, multiply by 12.87. To convert serum testosterone from ng/dl to nmol/liter, multiply by 0.0347. To convert plasma cortisol from μg/dl to nmol/liter, multiply by 27.59. To convert plasma glucose from mg/dl to nmol/liter, multiply by 0.055.

TABLE 1. Clinical profile of subjects with GH deficiency

Subject no.	Age (yr)	Sex	Duration of PHP (yr)	Serum IGF-I (ng/ml)	BMI (kg/m^2)	Serum PRL (ng/ml)	Cause of PHP	Hormone replacement therapy
1	50	F	4	128	43.5	17	Sheehan's syndrome	HC, LT4
2	45	F	5	52	26.3	60	Resection of pituitary macroadenoma	HC, LT4, E/P
3	58	F	6	77	32.0	51	Resection of pituitary macroadenoma	HC, LT4, E/P
4	37	F	4	132	31.0	19	Resection of pituitary macroadenoma	HC, LT4
5	67	F	10	46	32.0	19	Resection of pituitary macroadenoma	HC, LT4, E/P
6	32	F	15	65	49.2	5	Resection of craniopharyngioma	HC, LT4, E/P, DDAVP
7	67	M	6	105	29.2	4	Pituitary apoplexy	HC, LT4, T
8	50	M	3	119	28.0	9	Lymphocytic hypophysitis	HC, LT4, T

HC, Hydrocortisone; LT4, thyroid hormone; E/P, estrogen and progesterone; T, testosterone; DDAVP, desmopressin; PHP, panhypopituitarism; F, female; M, male. To convert serum IGF-I from ng/ml to nmol/ml, multiply by 0.13. To convert serum PRL from ng/ml to μg/liter, multiply by 1.0.

As a result of the reduction in leucine oxidation, leucine balance, representing the leucine used for protein synthesis, improved in both the fasted and fed states ($P < 0.01$ and $P = 0.07$, respectively; Table 3).

After 6 months of GH treatment, when the plasma IGF-I concentration was stable in the midnormal range, the rates of leucine oxidation and balance diverged in the fasted and fed states. In the fasted state, the leucine oxidation rate remained significantly suppressed ($P < 0.01$), and leucine balance was significantly improved ($P < 0.01$). However, in the fed state, both oxidation and balance returned to pretreatment values (Table 3).

There were no significant differences in the rates of leucine released from protein breakdown or leucine used for protein synthesis between GHD patients and controls in the fasted and fed states (Table 4). After 2 wk of GH therapy, there was an increase in leucine used for protein synthesis in both the fed and fasted states ($P < 0.05$ for both; Table 4). After 6 months of GH treatment, whereas leucine used for protein synthesis remained higher compared with the pretreatment value ($P < 0.05$), there was no difference in the fed state. GH therapy had no significant effect on leucine flux from protein breakdown (Table 4). The percentage of enteral leucine taken up by the splanchnic bed was almost identical in controls and GHD subjects, and GH treatment had no significant effect on splanchnic leucine kinetics (Table 5).

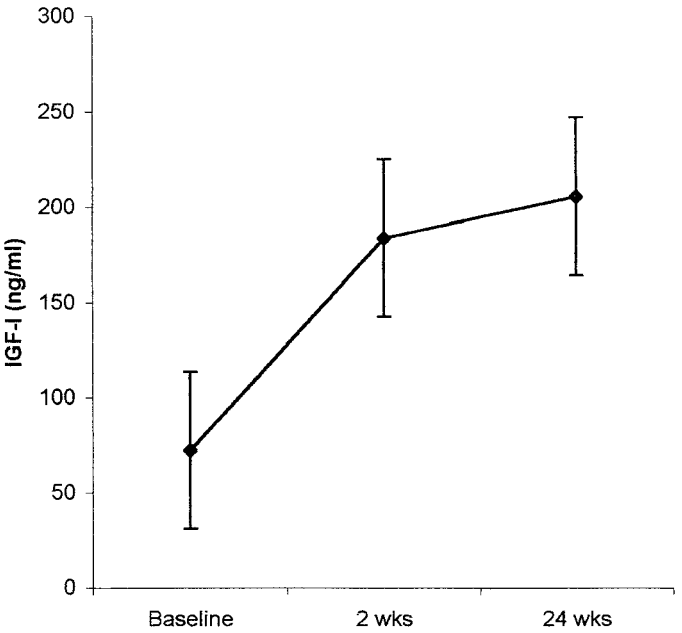

FIG. 1. IGF-I response to GH therapy in GHD patients. To convert serum IGF-I from ng/ml to nmol/ml, multiply by 0.13.

Body composition

Body composition was measured in controls once and in GHD subjects before and after 6 months of GH therapy. Both FFM and FM were modestly lower in GHD patients than controls, but the difference was not significant (Table 6). The ratio of FFM to body weight (0.59 ± 0.4) in the GHD subjects was almost identical to that in the controls (0.61 ± 0.04). After 6 months of GH replacement, FFM increased in five of the eight GHD subjects, showed no change in one, and decreased in two. Hence, although there was an overall mean increase in FFM of 1 kg, this change was not significantly different from pretreatment values. A closer examination of the data of individual GHD subjects, however, showed that five of the eight GHD subjects had a lower pretreatment FFM/body weight ratio (0.54 ± 0.4) compared with the other three GHD subjects (0.66 ± 0.06) and to the controls (0.61 ± 0.04). For this subset of five subjects, the gain in FFM was 3.5 kg, which was significantly greater than the pretreatment value ($P < 0.05$), and their FFM/body weight ratio increased significantly to 0.57 ± 5 ($P < 0.05$).

Discussion

The aims of this study were to compare the leucine kinetics of GHD subjects to those of healthy controls and to determine the short- and long-term effects of GH replacement therapy on leucine kinetics and body composition in the GHD subjects. There were no differences in any measure of leucine kinetics between the GHD subjects and controls in both the fasted and fed states. There was a decrease in leucine oxidation and an improvement in leucine balance in the GHD subjects after 2 wk of GH treatment in both the fed and fasted states. Concomitantly, leucine flux to protein synthesis increased. These changes were maintained after 6 months of treatment in the fasted state, but not in the fed state, as FFM trended higher. These results suggest that GH replacement therapy in GHD patients elicits an acute improvement in protein balance due to an increase in synthesis and a decrease

TABLE 3. Whole body leucine oxidation rate and balance in controls and GHD subjects

Leucine (μmol/kg FFM · h)	Controls	GHD		
		Baseline	2 wk post GH	6 months post GH
Fasted State				
Isotope infusions	24 ± 1.1	26.3 ± 2	26.2 ± 1.5	26.2 ± 1.5
Oxidation rate	34 ± 6.1	41 ± 6	30 ± 5[a]	30 ± 5[a]
Balance	−10 ± 3.5	−14 ± 4	−3.5 ± 3.5[a]	−3.8 ± 3.6[a]
Fed State				
Total exogenous intake (meal + infusions)	105 ± 17	113 ± 7.6	113 ± 6.6	111 ± 18
Oxidation rate	47 ± 7.6	49 ± 3.2	41.0 ± 3.6[b]	48.0 ± 4
Balance	58 ± 11	65 ± 10	72 ± 7.3[c]	65 ± 7
Balance as % of intake	54 ± 8	55 ± 9	63 ± 4	57 ± 3.8

All values are mean ± SEM.
[a] $P < 0.01$; [b] $P < 0.05$; [c] $P = 0.07$ compared to baseline.

TABLE 4. Whole body leucine kinetics in controls and GHD subjects

Leucine flux (μmol/kg FFM·h)	Controls	GHD		
		Baseline	2 wk post GH	6 months post GH
Fasted state				
Total	149 ± 8	151 ± 4	156 ± 7	157 ± 9
From protein breakdown	125 ± 7	126 ± 5	134 ± 6	130 ± 9
To protein synthesis	115 ± 5	116 ± 5	131 ± 6[a]	127 ± 11[a]
% of total flux	78 ± 2	77 ± 3	81 ± 1	81 ± 3
Fed state				
Total	144 ± 7.0	152 ± 6	157 ± 10	159 ± 7
From protein breakdown	46 ± 3	39 ± 8	44 ± 8	47 ± 5
To protein synthesis	98 ± 10	103 ± 6	116 ± 6[a]	112 ± 7
% of total flux	67 ± 5	68 ± 2	74 ± 2	70 ± 3

All values are mean ± SEM.
[a] $P < 0.05$ compared to baseline.

TABLE 5. Splanchnic leucine kinetics in controls and GHD subjects

Leucine (μmol/kg FFM · h)	Controls	GHD		
		Baseline	2 wk post GH	6 months post GH
Enteral intake	93.0 ± 7.6	100.0 ± 7.3	100 ± 5.6	99.2 ± 5.4
Splanchnic uptake	26.6 ± 3.9	32.3 ± 5.7	28.8 ± 1.8	27.0 ± 4.2
As % of enteral intake	29	31.4	28.9	27.2
Enteral flux to extrasplanchnic tissues	66.4	67.7	70.9	72.2

All values are mean ± SEM.

TABLE 6. Body composition of controls and of GHD subjects at baseline and after 6 months of GH treatment

	Controls	GHD	
		Pre-GH	6 months post GH
Group A	(n = 8)	(n = 8)	(n = 8)
BWt (kg)	99 ± 10	92 ± 8	92 ± 8
FM (kg)	39.3 ± 7.1	34.9 ± 5.6	34.8 ± 5.6
FFM (kg)	58.5 ± 3.9	52.5 ± 3.9	53.5 ± 3.9
FFM/BWt	0.61 ± 0.04	0.59 ± 0.04	0.59 ± 0.04
Group B	(n = 5)	(n = 5)	(n = 5)
BWt (kg)	108 ± 14	97 ± 12	99 ± 11
FM (kg)	47 ± 9	41 ± 8	42 ± 7
FFM (kg)	60 ± 5	51 ± 3	54.4 ± 4[a]
FFM/BWt	0.57 ± 0.04	0.54 ± 0.04	0.57 ± 0.5[a]

All values are mean ± SEM. BWt, Body weight. Group A shows data for controls and the entire group of 8 GHD subjects. Group B shows data for the 5 GHD subjects who had low FFM at baseline and their matched controls. See text for details.
[a] $P < 0.05$ compared to baseline.

in catabolism. After 6 months of treatment, protein kinetics reached a new homeostasis to maintain the net gain in FFM.

Our observation of a decrease in leucine oxidation and an increase in the rate of utilization of leucine for protein synthesis in GHD subjects after 2 wk of GH therapy suggest that GH replacement improves protein balance by inhibiting amino acid oxidation, thereby making more amino acids available for the synthesis of protein. This finding corroborates the earlier findings of Lucidi *et al.* (7) in fasted GHD subjects studied at 12 and 36 h after 2 wk of GH therapy on alternating days. They reported a 30% decrease in the leucine oxidation rate and a 13% increase in nonoxidative leucine disposal (that is, leucine used for protein synthesis), results almost identical to our present findings in the fasted state. Our results also corroborate the findings of Russell-Jones *et al.* (6, 8) in fasted GHD subjects after 2 months of GH therapy. They reported a decrease in leucine oxidation and an increase in nonoxidative leucine disposal (an index of protein synthesis) in a GH-treated group compared with a placebo-treated group of GHD subjects. Together, our present findings and those of previous studies suggest that in the fasted state, GH replacement therapy exerts a protein anabolic effect in GHD patients primarily by inhibiting oxidation of amino acids and stimulating protein synthesis.

Whereas most studies of the protein anabolic effect of GH have been conducted in the fasted state, very few studies have investigated the response in the fed state, especially in GHD patients. The effect of feeding is important because of the possible synergistic effects of meal-induced hyperinsulinemia, known to restrain protein breakdown rate (12), and GH replacement therapy, known to stimulate protein synthesis (6–8). In one of their studies, Russell-Jones *et al.* (8) determined the effect of 2 months of GH therapy on leucine kinetics in GHD subjects immediately after a meal. Using nonsteady state formulas to calculate leucine kinetics, they reported increases in both leucine oxidation and nonoxidative disposal rates in GH-treated compared with placebo-treated subjects. However, the increase in the nonoxidative leucine disposal rate was greater than the increase in the oxidation rate, indicating that GH also promotes protein balance in the fed state by stimulating protein synthesis. In the present study we mimicked a steady state by feeding mini-meals every 30 min for 4 h and made the same observations reported by Russell-Jones *et al.* (8). That is, although meal ingestion resulted in a marked increase (37%) in the leucine oxidation rate compared with the fasted value, leucine balance and the rate of leucine utilization for protein synthesis were increased relative to pretreatment rates. This was due to the fact that GH treatment attenuated leucine oxidation, that is, protein catabolism, despite the known stimulatory effects of a meal on amino acid oxidation (12). In another experiment using an almost identical feeding sched-

ule (hourly feeds) as that in the present study, but with a different tracer method to measure protein kinetics after 1 month of GH therapy, Binnerts *et al.* (2) reported essentially the same finding, that is, increases in protein balance and synthesis compared with pretreatment values. Together the combined findings of these studies suggest that in the short-term, GH replacement therapy promotes protein anabolism in GHD subjects by inhibiting amino acid catabolism and stimulating protein synthesis in both the fed and fasted states.

After 6 months of treatment, the improvements in leucine balance and protein synthesis observed after 2 wk of GH therapy were maintained in the fasted state, but not in the fed state. This was due to the fact that leucine oxidation in the fed state returned to the pretreatment value, suggesting an inability of GH to restrain the meal-induced increase in amino acid oxidation. The same finding was reported for fed subjects after 6 months of GH therapy in the study by Binnerts *et al.* (2). Both protein balance and synthesis reached a new steady state while maintaining an increase in FFM. In another study, Beshyah *et al.* (9) looked at the effect of 6 months of GH therapy on leucine kinetics in GHD subjects in the fasted state. In agreement with our present results in the fasted state, they reported a modest, although not significant, improvement in protein synthesis. However, unlike our present finding in fasted GHD subjects, they did not observe a decrease in the leucine oxidation rate. Another variant finding reported by Beshyah *et al.* (9) was their observation of lower rates of protein synthesis and breakdown in patients with hypopituitarism compared with healthy controls, whereas our present data show no significant differences in leucine kinetics in GHD patients and controls. Nevertheless, our overall findings suggest that after 6 months of GH therapy, protein metabolism reaches a new steady state to maintain the net gain in FFM.

Our present finding that GH treatment had no effect on the protein breakdown rate corroborates the previous findings of Russell-Jones *et al.* and Lucidi *et al.* (6–8) and suggests that the protein anabolic action of GH may not be mediated exclusively through the action of either insulin or IGF-I, both of which have been shown to promote protein anabolism primarily through their inhibitory effects on proteolysis (13, 14). Furthermore, in the present study the hyperinsulinemia that accompanies feeding did not cause a further improvement in leucine balance after 2 wk of GH therapy. Actually, the GH-induced improvement in leucine balance of 10.5 μmol/kg FFM·h in the fasted state was better than the 7 μmol/kg FFM·h in the fed state. Finally, the maximum protein anabolic effect of GH was elicited after 2 wk of GH therapy, when the plasma IGF-I concentration was still in the subphysiological range.

Previous studies have reported decreased FFM and increased FM in GHD adults compared with controls (1–5), and that GH therapy results in a gain in FFM (1–4). Hence, it was rather unexpected that 6 months of GH therapy did not cause a significant gain in FFM in the GHD group as a whole. Actually, the mean ratio of FFM to body weight (0.59 ± 0.4) for the entire GHD group was almost identical to that of the control group (0.61 ± 0.04); hence, it is not surprising that 6 months of GH therapy did not cause any significant overall gain in FFM. The loss of FFM in GHD patients is known to correlate positively with the duration of GHD, and the mean duration of GHD in our subjects was 6.6 yr, with a range of 3–15 yr. Furthermore, our GHD patients had multiple pituitary hormone deficiencies, and despite clinically and biochemically adequate replacement with glucocorticoids, sex hormones, and T_4, it is possible that variations in the dosage of or responses to these hormones might have caused variability in FFM among individuals in the GHD group. Hence, some individuals in the GHD group did not have significantly lower FFM than the controls before starting GH replacement therapy. A closer examination of the data of individual GHD subjects revealed that five of the eight GHD subjects had a lower ratio of FFM to body weight (0.54 ± 0.4) compared with the other three GHD subjects (0.66 ± 0.06) and to the controls. The three subjects who did not have low FFM to body weight ratio compared with their matched controls included the two men receiving testosterone replacement therapy, suggesting that the anabolic effect of testosterone might have played a role in maintaining their FFM at a higher level. In the subset of five GHD subjects with low baseline FFM, the mean gain in FFM was 3.5 kg, which was significantly greater than pretreatment values ($P < 0.05$). Moreover, their posttreatment FFM/body weight ratio increased to 0.57 ± 5, a value very similar to that in the control group. This gain of 3.5 kg FFM after 6 months of GH replacement in this subgroup of five GHD patients is comparable to the 4.0 kg reported by Binnerts *et al.* (2) after 6 months of treatment. The attainment of a normal FFM in all GHD subjects after 6 months of GH treatment may explain, in a teleological fashion, why GH lost its protein anabolic effect, as it was physiologically unnecessary to promote protein anabolism any further. The lack of further FFM accretion despite continued GH administration suggests the presence of a negative feedback mechanism from FFM regulating the anabolic effects of GH.

We are not aware of any other study of the effect of GH treatment on splanchnic leucine metabolism. The percentage of enteral leucine extracted by the splanchnic bed (~30%) was remarkably similar in the two groups of subjects and to previously reported values for adult humans (14). Although there was a trend toward a decrease in both the absolute and the percent uptake of leucine during GH treatment, these changes were not significant.

In summary, we have demonstrated that short-term GH replacement therapy increases the efficiency of amino acid utilization, decreases protein catabolism, and enhances net protein synthesis in GHD patients in both the fasted and fed states. After 6 months of GH replacement, FFM normalizes and protein kinetics reach a new homeostasis. Finally, the anabolic actions of GH do not seem to be mediated exclusively through the actions of either insulin or IGF-I.

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References

1. **Salomon F, Cuneo R, Hesp, R, Sonksen PH** 1989 The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 321:1797–1803
2. **Binnerts A, Swart GR, Wilson JH, Hoogerbrugge N, Pols HA, Birkenhager JC, Lamberts SW** 1992 The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf) 37:79–87
3. **Salomon F, Cuneo R, Sonksen PH** 1991 Growth hormone and protein metabolism. Horm Res 36(Suppl 1):41–43
4. **Vance ML, Mauras N** 1999 Growth hormone therapy in adults and children. N Engl J Med 341:1206–1216
5. **Carroll PV, Christ ER** 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. J Clin Endocrinol Metab 83:382–395
6. **Russell-Jones DL, Weissberger AJ, Bowes SB, Kelly JM, Thomason M, Umpleby AM, Jones RH, Sonksen PH** 1993 The effects of growth hormone on protein metabolism in adult growth hormone deficient patients. Clin Endocrinol (Oxf) 38:427–431
7. **Lucidi P, Laureti S, Santoni S, Lauteri M, Busciantella-Ricci N, Angeletti G, Santeusanio F, De Feo P** 2000 Adminstration of recombinant human growth hormone on alternate days is sufficient to increase whole body protein synthesis and lipolysis in growth hormone deficient adults. Clin Endocrinol (Oxf) 52:173–179
8. **Russell-Jones DL, Bowes SB, Rees SE, Jackson NC, Weissberger AJ, Hovorka R, Sonksen PH, Umpleby AM** 1998 Effect of growth hormone treatment on postprandial protein metabolism in growth hormone-deficient adults. Am J Physiol 274:E1050–E1056
9. **Beshyah SA, Sharp PS, Gelding SV, Halliday D, Johnston DG** 1993 Whole-body leucine turnover in adults on conventional treatment of hypopituitarism. Acta Endocrinol (Copenh) 129:158–164
10. **Reid M, Badaloo A, Forrester T, Heird WC, Jahoor F** 2002 Response of splanchnic and whole-body leucine kinetics to treatment of children with edematous protein-energy malnutrition accompanied by infection. Am J Clin Nutr 76:633–640
11. **Jahoor F, Shangraw RE, Miyoshi H, Wallfish H, Herndon DN, Wolfe RR** 1989 Role of insulin and glucose oxidation in mediating the protein catabolism of burns and sepsis. Am J Physiol 257:E323–E331
12. **Nissen S, Haymond MW,** Changes in leucine kinetics during meal absorption: effects of dietary leucine availability. Am J Physiol 13:E695–E701
13. **Fukagawa NK, Minaker KL, Rowe JW, Goodman MN, Matthews DE, Bier DM, Young VR** 1985 Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in postabsorptive men. J Clin Invest 76:2306–2311
14. **Elahi D, McAloon-Dyke M, Fukagawa NK, Sclater AL, Wong GA, Shannon RP, Minaker KL, Miles JM, Rubenstein AH, Vandepol CJ** 1993 Effects of recombinant human IGF-I on glucose and leucine kinetics in men. Am J Physiol 265:E831–E838