Effects of Recombinant Human Growth Hormone on Basal Metabolic Rate in Adults With Pituitary Deficiency

Kaj Stenlöf, Lars Sjöström, Lars Lönn, Ingvar Bosaeus, Henry Kvist, Jukka Tölli, Göran Lindstedt, and Bengt-Åke Bengtsson

The effect of recombinant human growth hormone (rhGH) on basal metabolic rate (BMR) was studied in a placebo-controlled, double-blind, crossover trial. Ten patients with a history of complete pituitary insufficiency were randomized for 26 weeks in each period. Three patients were excluded due to withdrawal, fever, and claustrophobia, respectively. All patients had received adrenal, thyroid, and gonadal substitution therapy for at least 1 year before the study. The dose of rhGH was 0.25 to 0.5 U/kg/wk, administered subcutaneously once a day in the evening. BMR was determined by indirect calorimetry in a computerized ventilated open-hood system. Body composition was examined using four different methods—computed tomography (CT), tritium dilution, ^{40}K determinations, and total body nitrogen (TBN) measured with neutron activation. The body composition data have previously been reported. Fat-free mass (FFM) increased and body fat (BF) decreased during the first 6 weeks of rhGH treatment, but no further changes in body composition occurred between 6 and 26 weeks. Baseline BMRs in GH-deficient (GHD) patients were in the lower part of the reference range, but BMR and the ratio between BMR and FFM (BMR/FFM) were not significantly lower than in a carefully selected control group. BMR increased between 0 and 6 weeks (mean ± SD: from 6.68 ± 1.55 to 7.75 ± 1.35 MJ/24 h, $P < .001$) and then remained unchanged between 6 and 26 weeks. The increase in BMR was closely related to the increase in FFM ($r = .91$, $P < .01$). However, the increase in BMR was not solely related to changes in FFM, since there was a significant increase in BMR/FFM at 6 weeks that was maintained at 26 weeks. Pearson correlation analysis also revealed a close association between the increase in BMR after 6 weeks of rhGH treatment and increases in a number of metabolic variables, including total 3,5,3′-triiodothyronine ([T_3] $r = .84$, $P < 0.05$), procollagen III peptide ([pIIIp] $r = .85$, $P < .05$), and free fatty acids ([FFA] $r = .95$, $P < .01$). Therefore, the increase in BMR after rhGH treatment is not simply a reflection of altered body composition, but may also involve other mechanisms including lipolysis, increased thyroxine (T_4) deiodination resulting in increased circulating T_3 concentrations, and/or increased protein synthesis as demonstrated by increased circulating pIIIp levels.

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GROWTH HORMONE (GH) has profound effects on several metabolic variables in GH-deficient (GHD) patients.[1] After 6 weeks of recombinant human GH (rhGH) treatment there is a marked change in body composition, with an increase in lean body mass (LBM) and a decrease in adipose tissue (AT).[1,2] Administration of rhGH also enhances peripheral conversion of thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3), as well as the production of insulin-like growth factor I (IGF-I)[1,3,4] and the aminoterminal propeptide of collagen III (pIIIp).[1] pIIIp, which is formed during the production of mature collagen III,[5] has been suggested as a peripheral biological marker of the metabolic effects of thyroid hormones, GH,[6,7] and basal metabolic rate (BMR).[8]

Previous long-term studies have shown that BMR increases during treatment with GH.[2,9,10] The relationship between body composition, thyroid hormones, IGF-I, pIIIp, and BMR has not been studied in detail. In a recent long-term study, BMR increased following rhGH treatment in GHD patients.[2] In that study, BMRs adjusted for fat-free mass (FFM) increased after 1 month of rhGH and then declined at 6 months. In a separate study from the same group,[11] BMR was measured in GHD patients and compared with values obtained from patients with acromegaly and healthy controls. GHD patients were found to have BMRs (adjusted for FFM) similar to those of patients with acromegaly and higher values than controls. The presence of relatively high BMRs in GHD patients was unexpected in view of the increase in BMR following rhGH treatment.

Therefore, the purpose of our study was threefold. The first aim was to confirm or rebut the previous observation of a higher BMR to FFM ratio (BMR/FFM) in GHD patients as compared with matched controls. Second, changes in BMR were related to body composition and to free fatty acids (FFA), insulin, IGF-I, thyroid hormones, and pIIIp. Finally, BMR was monitored for 6 months to assess the reasons for the decline in the metabolic rate observed by other investigators after this time interval.[2] To increase the accuracy of our data, FFM was determined with four different methods.

SUBJECTS AND METHODS

Patients

In a previously documented group of nine adults with pituitary deficiency we have reported on the effect of rhGH administration on body composition, well-being, and some metabolic variables.[1] In the current report, changes in BMR during rhGH treatment are described for this group of patients.

The patients had been GHD for more than 1 year and were included in the study according to the following criteria: (1) mean plasma GH less than 2.5 mU/L and no significant peaks in analysis of 30-minute blood samples over 24 hours; (2) maximal GH concentration less than 5 mU/L following insulin-induced hypogly-

From the Department of Medicine, Diagnostic Radiology, Radiophysics, and Clinical Chemistry, Sahlgren's Hospital, University of Göteborg, Göteborg, Sweden.

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Address reprint requests to Lars Sjöström, MD, PhD, SOS-Secretariat, Department of Medicine, Sahlgren's Hospital, S-413 45 Göteborg, Sweden.

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cemia; (3) received adrenal, thyroid, and gonadal replacement therapy for at least 1 year before entering the study.

Originally, 10 patients were randomized. One patient had to be excluded from BMR measurements due to claustrophobia. After 6 weeks of rhGH treatment, another patient was withdrawn from the study due to water retention and atrial fibrillation. At the time of the BMR examination just before the start of rhGH treatment at 26 weeks, a third patient had an episode of fever. This patient was also excluded from the current report due to unreliable BMR measurements. Thus, BMR measurements were available in seven patients.

The age, sex, body mass index (BMI), and cause of pituitary deficiency for the seven patients included in the study are shown in Table 1. One subject was treated with pituitary irradiation only. The remaining six patients had been treated with pituitary irradiation after pituitary surgery.

Fourteen healthy individuals (12 men and two women) served as controls. For each individual with GHD, two subjects were matched with respect to sex, age, weight, and height (Table 2).

Basal Medication

The dose of corticosteroids was 25 mg cortisone acetate daily, except in one patient where 50 mg was divided into two daily doses. This dose was not changed during the rhGH study except in the case of intercurrent illnesses. The dose of T_4 was 0.10 to 0.15 mg daily. In two patients the dose of T_4 was increased from 0.10 to 0.15 mg daily after 6 weeks of rhGH treatment. The replacement dose of sex hormones was unchanged during the study. Three patients received bromocriptine (Pravidel; Sandoz, Basel, Switzerland) treatment (7.5 to 10 mg daily), and their doses remained unchanged throughout the study.

Study Design

The study was placebo-controlled, with a double-blind, crossover study design using rhGH (Humatrope, Eli Lilly & Co, Indianapolis, IN).

The patients were studied for a total period of 12 months and were randomized to one of two treatment groups: (1) 26 weeks of treatment with rhGH followed by 26 weeks of treatment with placebo; and (2) 26 weeks of treatment with placebo followed by 26 weeks of treatment with rhGH. Eli Lilly & Co provided the randomization codes, which were not broken until the last patient had completed the study.

The patients received both written and verbal information about the nature of the study, and their written informed consent was obtained. The study was approved by the Ethics Committee of the Medical Faculty at the University of Göteborg and by the Swedish Medical Products Agency, Uppsala, Sweden.

Table 2. Description of Controls and Patients With GDH at Baseline (mean ± SD)

	Control (n = 14)	GDH (n = 7)	*P*
Sex (M/F)	12/2	6/1	1.0
Age (yr)	46 ± 12	46 ± 9	.97
Height (m)	1.8 ± 0.08	1.8 ± 0.10	.83
Weight (kg)	90.9 ± 22.3	90.8 ± 20.7	.99
BMI (kg/m^2)	28.5 ± 5.3	28.5 ± 5.2	1.0
BF (kg)	24.5 ± 13.2	26.2 ± 5.9	.69
FFM-^{40}K (kg)	66.4 ± 13.6	64.6 ± 17.7	.82
BMR (MJ/24 h)	7.20 ± 1.30	6.68 ± 1.55	.46
BMR/FFM-^{40}K (MJ · 24 h^{-1} · kg^{-1})	0.109 ± 0.01	0.106 ± 0.01	.59
Adjusted BMR (MJ/24 h)*	7.28 ± 0.31	6.86 ± 0.36	.27

NOTE. Results are the mean ± SEM.

*Adjusted for age, BF, and FFM.

Treatment

The dosage of rhGH was 0.5 U/kg body weight per week, administered subcutaneously daily by the patient before bedtime. In two patients who experienced side effects in the form of fluid retention, arthralgia, and tinnitus, the dosage was reduced by 50%. The vials of rhGH contained 16 U (5.92 mg). The placebo vials contained the same vehicle as the rhGH vials and were visually indistinguishable. Between injections, the vials were stored for a maximum of 7 days at 5° to 12°C protected from light.

Study Protocol

Patients were studied as inpatients in the metabolic ward for 1 week before treatment with placebo or active therapy and during the sixth and 26th weeks of treatment with placebo or rhGH. The patients were also seen at the outpatient clinic at monthly intervals.

Thirty days before starting the study, during steady-weight conditions the patients kept a 4-day food record and received dietary instructions in an attempt to achieve energy balance over the study period. During the weeks at the metabolic ward, patients obtained a food intake equal to that of the 4-day food record (mean ± SD: kcal, 2,444 ± 407; protein, 17% ± 1.4%; fat, 36% ± 4.4%; carbohydrate, 46% ± 3.2%). As outpatients they were also recommended diets with the same energy and macronutrient content.

Calorimetric Methods

BMR was determined by indirect calorimetry in a computerized, ventilated, open-hood system (Deltatrac, Datex, Helsinki, Finland) before starting treatment with rhGH or placebo, as well as after 6 and 26 weeks of treatment with rhGH or placebo. The examina-

Table 1. Individual Data on Seven Patients With Pituitary Deficiency

Case No.	Age/Sex	BMI	Cause of Pituitary Deficiency	Maximum GH Level After ITT (mU/L)*	Mean GH Level (mU/L)†
1	58/M	28	Chromophobe adenoma (OP)	<0.30	<0.30
2	34/M	29	Prolactinoma (OP)	<0.30	<0.30
3	34/M	36	Prolactinoma (OP)	<0.30	<0.30
4	46/F	21	Prolactinoma (IRR)	0.97	0.33
5	52/M	31	Cromophobe adenoma (OP)	<0.30	<0.30
6	51/M	22	Cromophobe adenoma (OP)	0.44	<0.30
7	43/M	30	Prolactinoma (OP)	0.53	<0.30

Abbreviations: OP, pituitary surgery + irradiation; IRR, irradiation treatment only; ITT, intravenous insulin tolerance test.

*Maximum GH concentration after ITT.

†Mean of 24-hour GH profile (48 samples).

tions were performed according to standardized conditions in the morning after an overnight fast and after 30 minutes' rest on the bed used for examinations. Respiratory data were then collected each minute for 30 minutes. BMR was calculated as the mean energy expenditure over the 30 minutes and expressed as megajoules per 24 hours.

The mathematical procedures used were those described by Ferrannini.[12] Protein oxidation was calculated from urinary nitrogen excretion over 24 hours, and carbohydrate and lipid oxidations were calculated from the nonprotein respiratory quotient. The oxygen and CO_2 analyzers were calibrated before and after each experiment using oxygen/CO_2 mixtures of known composition. The overall standard error of a single determination[13] was 4%, as calculated from two determinations on consecutive days in 20 healthy subjects.

The technical error of the system, checked in intervals by ethanol combustion experiments, was approximately 3%.

Reference values for BMR, taking sex, age, height, and weight into account, were obtained from the equations suggested by Schofield.[14]

Body Composition

The body weight with patients dressed in underwear was determined to the nearest 0.1 kg on a calibrated scale. Height was determined to the nearest centimeter. Body composition was determined by assessment of total body potassium, total body water, and total body nitrogen (TBN), and computed tomography (CT). CT examinations were performed at 0 and 26 weeks. Else, body composition assessments were performed before and after 6 and 26 weeks in each period.

The total body potassium level was measured in a 3π whole-body counter.[15] FFM-^{40}K was estimated from total body potassium by assuming that 1 kg FFM contains 64.7 mmol potassium in men[16] and 62.0 mmol in women.[17] The 6-week total body potassium examinations were available only in five patients due to ^{137}Cs contamination from the Chernobyl catastrophy. Later in the study, techniques had been developed to correct for ^{137}Cs.[15]

Total body water content was measured by an isotope dilution technique using tritiated water as a tracer.[18] The equilibration time was 3 hours, plasma water was collected by sublimation in vacuum, and the specific activity was determined by liquid scintillation counting. No correction for loss of label due to nonaqueous hydrogen exchange (2% to 4%) was performed. One kilogram FFM as determined with tritiated water was assumed to contain 0.725 L water.

TBN content was measured by in vivo neutron activation.[19] A ^{252}Cf source was used to produce the neutrons. The method is based on characteristic emission of photons after capture of low-energy (thermal) neutrons by ^{14}N nuclei.[19,20]

AT and muscle volumes were determined by CT using a Philips Tomoscan 310 (Eindhoven, The Netherlands) as described previously.[15-17,21-22] In short, the patients were examined in the supine position with arms stretched over their heads. Twenty-two transsectional scans were obtained at each investigation. In each scan the area of all picture elements (pixels) in the attenuation interval −190 to −30 Hounsfield Units (HU) was defined as AT,[17,21,22] and −29 to +151 HU was defined as areas of muscle tissue plus skin or visceral organs.[21,22] The two latter types of areas were separated by various cursor techniques.[22] The distances between scans were measured from frontal scanograms to the nearest millimeter. Total tissue volumes ([V] AT, "muscle," or visceral organs) were calculated as

$$V = \sum_{1}^{23} \frac{a(b + c)}{2},$$

where a is the distance between two adjacent scans, and b and c are tissue areas of these two scans. In these calculations, the tip of the toes and the tip of the fingers were considered as two scans with tissue areas equal to zero. Including "zero scans," 24 scans were thus available, and the total volumes of AT, muscle, or visceral organs were obtained by summing 23 volumes located between all adjacent scans. As calculated from double determinations, the error of the CT method was 0.4% for AT, 0.3% for muscle, and 0.7% for visceral organs.[21,22] Tissue and organ volumes determined with CT were converted to weights by multiplying by corresponding organ densities.[21,22] Lean body mass (ie, the non-AT mass) was calculated as body weight minus AT in kilograms.

Biochemical Assays

Methods for the determination of serum concentrations of GH, thyroid-related hormones, pIIIp, and FFA have been described in detail previously.[1] In short, plasma GH concentrations were determined by an immunoradiometric assay (Pharmacia, Uppsala, Sweden); IGF-I by an immunoradiometric assay after formic acid–ethanol extraction (Byk-Sangtec Diagnostica, Dietzenbach, Germany); serum free T_4 and free T_3 by ligand analog radioimmunoassays ([RIAs] Amerlex M, Amersham International, Amersham, Bucks, England); serum total T_4 by a single-antibody RIA (Farmos Diagnostica, Turku, Finland); total T_3 by a polyethylene glycol–assisted double-antibody RIA (Diagnostic Products, Los Angeles, CA); pIIIp by an immunoradiometric assay (Hoechst-Behringwerke, Marburg, Germany); FFA with a commercially available kit (NEFAC, Wako, Neuss, Germany); and insulin with the Pharmacia Insulin RIA, a secondary-antibody solid-phase RIA (Pharmacia). Blood samples were drawn in the morning after an overnight fast. All samples from each patient were run in the same assay and in complete blocks.

Statistical Methods

Statistical calculations were performed with the Minitab statistical program, version 9.[23] Baseline and 6- and 26-week data, including percentage changes, were checked for normal distribution using the Shapiro-Wilk statistic and were then analyzed using paired *t* tests with Bonferroni correction for the planned number of comparisons, as well as Pearson's product-moment correlation coefficient and Pitman's test.[24] Adjusted least-squares means of BMR were calculated with the general linear model using age, body fat (BF), and FFM as covariates and type of group (controls or GHD) as factors.

In the three subjects starting with placebo, observations at 0, 6, and 26 weeks did not change with respect to FFM as determined with tritiated water, FFM-^{40}K, LBM-CT, or BMR. On the other hand, there was a carryover effect in these respects to the second period for the four patients who started the study with rhGH. This has been shown previously by others.[25] Since a washout period was not included in the design, a formal crossover analysis therefore could not be performed. To analyze the effect of rhGH treatment, both groups were pooled using values obtained in the week preceding the initiation of active treatment as pretreatment values.

RESULTS

Baseline Observations

According to the matching procedures, controls and GHD patients were very similar with respect to age, height, weight, and BMI (Table 2). FFM, BMR, BMR/FFM, and BMR adjusted for age, BF, and FFM tended to be lower and BF higher in GHD patients, but compared with controls, the differences did not reach statistical significance (Table 2).

Taking sex, age, weight, and height into account, the mean BMR in the GHD group was in the lower part of the calculated reference range[14] before treatment (Fig 1). In fact, four of seven patients had BMRs below the reference range.

Before treatment, BMR was positively related to body weight ($r = .94$, $P = .002$), TBN, FFM-^{40}K, FFM–tritiated water, muscle-CT, and LBM-CT in GHD patients (Table 3). Furthermore, baseline BMR was positively related to the baseline free T_3 to free T_4 ratio and negatively related to free T_4, whereas no significant correlations were found between BMR and total T_3, IGF-I, pIIIp, FFA, or insulin (Table 3).

In contrast, in the control group BMR was positively related to total T_3 (mean ± SD, 1.82 ± 0.18) and tended to be positively related to serum pIIIp (mean ± SD, 3.46 ± 0.68). As expected, BMR was closely related to FFM in controls (Table 3).

Effects of GH treatment

Body composition. FFM estimated from tritium or ^{40}K increased by approximately 5 kg between 0 and 6 weeks, whereas no significant changes were observed between 6 and 26 weeks. The differences between 0 and 26 weeks remained significant (Table 4). The increase in LBM-CT between 0 and 26 weeks was approximately 3 kg. BF changed in the opposite direction, ie, there was a reduction between 0 and 6 weeks and no change between 6 and 26 weeks (Table 4).

BMR. BMR increased by 18% from 6.68 ± 1.55 to 7.75 ± 1.35 MJ/24 h ($P < .001$) between 0 and 6 weeks (Fig 1). At 26 weeks BMR was essentially unchanged (7.60 ± 1.36 MJ/24 h) as compared with 6 weeks (NS), and was still significantly higher than at baseline ($P < .05$; Fig 1).

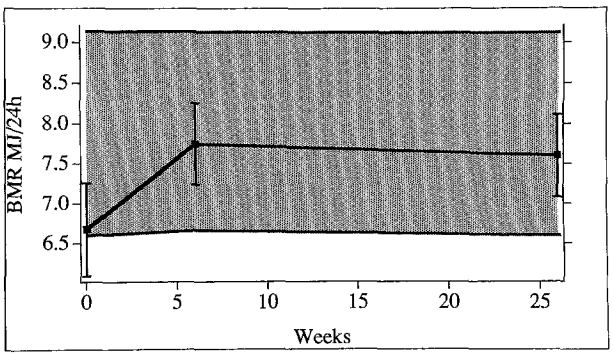

Fig 1. Mean ± SEM BMR at baseline and after 6 and 26 weeks of treatment with rhGH. (▨) Mean ± SD reference range (adjusted for age, sex, height, and weight[14]) at the three examinations.

Table 3. Correlation Coefficients Between BMR and Indicated Variables

	BMR			
		GHD		
	Controls	Pretreatment	0 to 6 Weeks	6 to 26 Weeks
LBM-CT		.91‡		.72* (0-26 wk)
Muscle-CT		.91‡		.74* (0-26 wk)
AT total-CT		.75*		−.20 (0-26 wk)
FFM-^{40}K	.92§	.88‡	.97‡	.28
FFM-tritiated water		.94‡	.91‡	.21
TBN		.85†	.86†	−.20
FT_4	.24	−.79†	−.37	.56
Total T_3	.59†	−.01	.84†	.95‡
FT_3/FT_4	.39	.79†	.52	−.10
IGF-I		.18	−.39	.34
pIIIp	.52*	.45	.85†	.51
FFA		.44	.95‡	−.17

NOTE. At 6 and 26 weeks, correlations are given for percentage changes in BMR *v* percentage changes of indicated variables.

*$P < .1$.

†$P < .05$.

‡$P < .01$.

§$P < .001$.

The increase in BMR between 0 and 6 weeks was strongly related to the increase in FFM ($r = .91$, $P < .01$; Table 3, Fig 2A). However, the expanding FFM was not the sole explanation for the increasing energy expenditure between 0 and 6 weeks, since BMR increased also when expressed per kilogram FFM (Fig 3). Furthermore, BMR/FFM remained elevated between 6 and 26 weeks (Fig 3) in the absence of a further increase in FFM (Table 4). BMR/LBM-CT increased significantly between 0 and 26 weeks (Fig 3).

BMR expressed per kilogram TBN increased between 0 and 6 weeks ($P < .01$) and tended to decrease between 6 and 26 weeks ($P = .090$; Fig 3).

Lipid oxidation. FFA and lipid oxidation increased between 0 and 6 weeks, but these variables did not change significantly between 6 and 26 weeks (Table 4). The changes in BMR and FFA between 0 and 6 weeks were strongly correlated ($r = .95$, $P < .01$; Table 3), whereas no correlation was found for changes between 6 and 26 weeks ($r = .17$, NS).

Thyroid hormones. Total T_3 and the free T_3/free T_4 ratio (index of deiodination) increased between 0 and 6 weeks and decreased between 6 and 26 weeks (Table 4). Increases in BMR and total T_3 between 0 and 6 weeks were positively correlated ($r = .84$, $P < .05$; Table 3, Fig 2B), as were the decreases in the same variables between 6 and 26 weeks ($r = .95$, $P < .001$; Table 3).

pIIIp. pIIIp increased between 0 and 6 weeks and decreased between 6 and 26 weeks (Table 4). The increases in BMR, BMR/FFM, and pIIIp between 0 and 6 weeks were positively related (ΔBMR/FFM *v* ΔpIIIp: $r = .86$,

Table 4. Body Composition, BMR, and Serum IGF-I, Thyroid Hormones, pIIIp, and FFA at Baseline and 6 and 26 Weeks of GH Treatment (mean ± SD)

	n	Baseline	n	6 Weeks	n	26 Weeks
Body weight (kg)	7	90.8 ± 20.7	7	91.6 ± 19.8	7	90.5 ± 20.6
LBM-CT (kg)	7	64.1 ± 16.5			7	67.1 ± 15.2^{D}
FFM-^{40}K (kg)*	5	65.1 ± 21.5	5	69.8 ± 21.6^{AA}	5	68.4 ± 20.5^{d}
FFM-Tri (kg)	7	67.7 ± 19.0	7	73.0 ± 17.2^{AA}	7	71.7 ± 18.3^{D}
BF-Tri (kg)	7	23.2 ± 3.0	7	18.6 ± 5.0^{AA}	7	18.8 ± 4.0^{D}
TBN (kg)	7	2.03 ± 0.67	7	2.18 ± 0.62^{AA}	7	2.24 ± 0.60^{BDD}
BMR (MJ/24 h)	7	6.68 ± 1.55	7	7.75 ± 1.35^{AAA}	7	7.60 ± 1.36^{DD}
Lipid ox (mg/min)*	5	57.9 ± 20.8	5	92.8 ± 10.2^{A}	5	75.8 ± 20.9
Total T_3 (nmol/L)	7	1.7 ± 0.3	7	2.3 ± 0.5^{AA}	7	2.0 ± 0.5^{bD}
FT_3/FT_4	7	0.41 ± 0.14	7	0.73 ± 0.15^{AAA}	7	0.56 ± 0.15^{BDD}
FT_3 (pmol/L)	7	5.0 ± 1.3	7	6.8 ± 1.4^{AA}	7	6.3 ± 1.9^{d}
FT_4 (pmol/L)	7	12.8 ± 3.6	7	9.6 ± 2.8^{AA}	7	11.5 ± 3.4
IGF-I (kU/L)	7	84 ± 33	7	520 ± 181^{AAA}	7	553 ± 190^{DDD}
pIIIp (μg/L)	7	0.76 ± 0.39	7	2.25 ± 0.30^{AAA}	7	1.75 ± 0.60^{BDD}
FFA (mmol/L)*	6	363 ± 48	6	537 ± 159^{a}	6	580 ± 212

NOTE. *P* values were adjusted according to the Bonferroni inequality technique.

Abbreviation: Tri, as determined using tritiated water; ox, oxidation.

*For the sake of comparison, only patients available at all examinations are reported.

$^{a}P < .05$, $^{A}P < .025$, $^{AA}P < .01$, $^{AAA}P < .001$: 0 to 6 weeks.

$^{b}P < .05$, $^{B}P < .025$; $^{BB}P < .01$, $^{BBB}P < .001$: 6 to 26 weeks.

$^{d}P < .05$, $^{D}P < .025$, $^{DD}P < .01$, $^{DDD}P < .001$: 0 to 26 weeks.

$P < .05$), whereas the changes between 6 and 26 weeks were not (Table 3).

IGF-I. IGF-I increased dramatically between 0 and 6 weeks, but did not change significantly between 6 and 26 weeks (Table 4). Changes in BMR and IGF-I between 0 and 6 weeks or between 6 and 26 weeks were not correlated (Table 3).

Insulin. Fasting insulin concentrations did not change significantly during treatment. No significant relationship could be found between BMR and insulin levels in untreated GHD patients or between changes in these two variables during rhGH treatment (not shown).

DISCUSSION

This study has demonstrated that GHD patients have a BMR in the lower part of the normal range but not significantly different from that of healthy controls in this small study group. This was true whether the absolute BMR was used or BMR was expressed per kilogram FFM or adjusted for age, BF, and FFM. rhGH treatment increased BMR, FFM, and also BMR/FFM. Contrary to earlier reports, there was no decline in BMR/FFM after 6 months of treatment. The rhGH-induced increment in BMR was positively related to increases in FFM, TBN, total T_3, pIIIp, and FFA, but not IGF-I.

BMR and BMR/FFM of untreated GHD patients have previously been examined by Salomon et al.[11] They observed BMRs (expressed as % of predicted) in the low-normal range similar to our findings, but also reported that BMR expressed per kilogram FFM (called LBM in their report) was higher in GHD patients than in normal subjects. This finding is at variance with our results. One possible explanation for this is that their control group was younger (33 ± 3 *v* 38 ± 2 years), had a lower BMI (22.7 ± 1.3 *v* 28.3 ± 1.0 kg/m^2), and contained a smaller fraction of females (29% *v* 33%). In any case, mean BMRs were not statistically adjusted for these inequalities.[11] Another possibility is that some inaccuracy may have been introduced into their data with the use of total body potassium for FFM measurements. It is known that the potassium content per unit muscle tissue is elevated in acromegalic patients and is normalized after successful treatment.[26] Therefore, the potassium content per unit muscle tissue may well be reduced in GHD patients, resulting in an underestimation of FFM and overestimation of BMR/FFM if standard assumptions about potassium content per kilogram FFM are used. Despite this theoretical risk, BMR/FFM of GHD patients was not elevated above values of carefully matched controls in the current study, and if anything, tended to be lower. The fact that BMR/FFM is increased by rhGH treatment in GHD patients (see below) makes it less likely that GHD untreated patients should have higher BMR/FFM than controls with normal GH secretion.

In accordance with a previous study,[2] we observed an increase in both BMR and BMR/FFM after 6 weeks' rhGH treatment. Other studies in patients without endocrine disturbances undergoing elective surgery have shown that even 5 days of rhGH treatment increases energy expenditure.[27] However, bolus injections[28] and 4-hour infusions[29] of rhGH do not alter the metabolic rate in healthy volunteers despite changes in other metabolic parameters. The effects of rhGH on energy expenditure thus seem to require some days of treatment to become detectable.

However, it has been reported that the increase in BMR/FFM measured at 1 month declines when remeasured at 6 months, although no explanation was offered for this finding.[2] In contrast to this report, we found that BMR

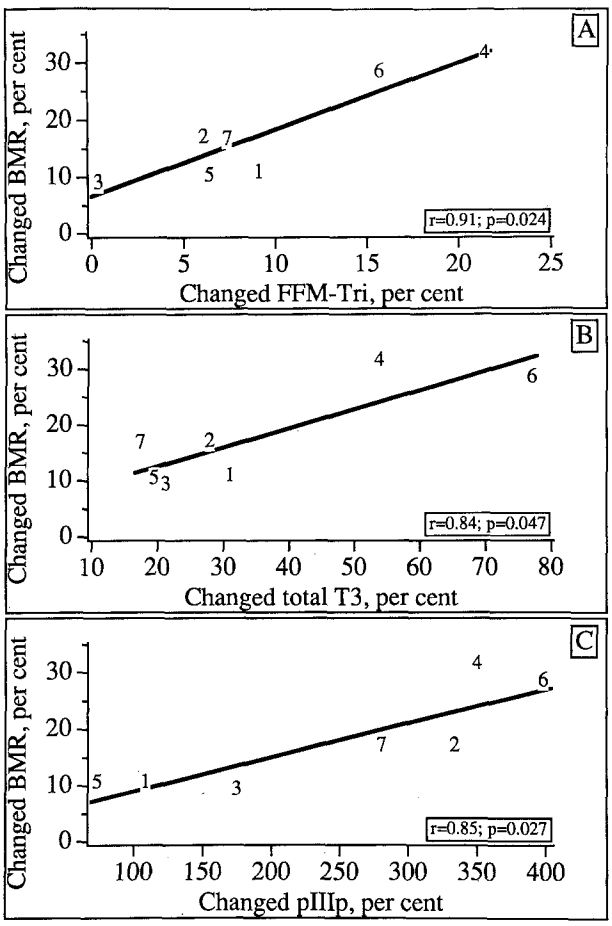

Fig 2. Relationships between changes in BMR and (A) FFM (determined from tritiated water [FFM-tri]), (B) total T_3, and (C) pIIIp in seven patients with GHD after 6 weeks of treatment with rhGH. Case numbers are as in Table 1. Pearson correlation coefficients are shown. The significance of the relationships was calculated with Pitman's nonparametric test.

and BMR/FFM were similar at 6 and 26 weeks. This was the case both when FFM was estimated from ^{40}K and from total body water. Although LBM-CT was not measured at 6 weeks, BMR/LBM-CT and BMR/FFM-^{40}K were very similar both at baseline and at 6 months. Therefore, results based on three different body composition techniques suggest that BMR/FFM is not reduced between 6 and 26 weeks of rhGH treatment in GHD patients.

The tendency toward a reduction of BMR/TBN between 6 and 26 weeks was due to a continued increase in TBN over this period. Since total body potassium, mainly located intracellularly, did not change between 6 and 26 weeks, the nitrogen accumulation after 6 weeks mainly represent the extracellular, nonrespirating protein matrix.

Treatment with rhGH resulted in a reduction of BF and an increase of FFM under unchanged energy intake conditions. This implies that carbon atoms are transferred from lipids in AT to proteins in FFM, a process that requires energy. Therefore, it was not surprising that the change in FFM (ΔFFM) was strongly related to the change in BMR.

However, the increased BMR was not solely explained by changes in body composition, since BMR adjusted for FFM (BMR/FFM) also increased between 0 and 6 weeks. Furthermore, BMR and BMR/FFM remained elevated between 6 and 26 weeks despite no further changes in BF and FFM over the latter period.

Other mechanisms not directly related to body composition may thus produce this increase in BMR adjusted for FFM. rhGH has a lipolytic effect[30] that may have resulted in the increased FFA levels at 6 weeks, and on average, in even higher FFA levels at 26 weeks. Several studies suggest an obligatory coupling between FFA levels and lipid oxidation.[10,31] However, in the current study the increase in FFA after 6 weeks was significantly associated with the increase in BMR, but not with the increase in BMR/FFM or lipid oxidation. Another possible cause of the increase in BMR/FFM was the change in deiodination of T_4 to T_3,[1,3,4]

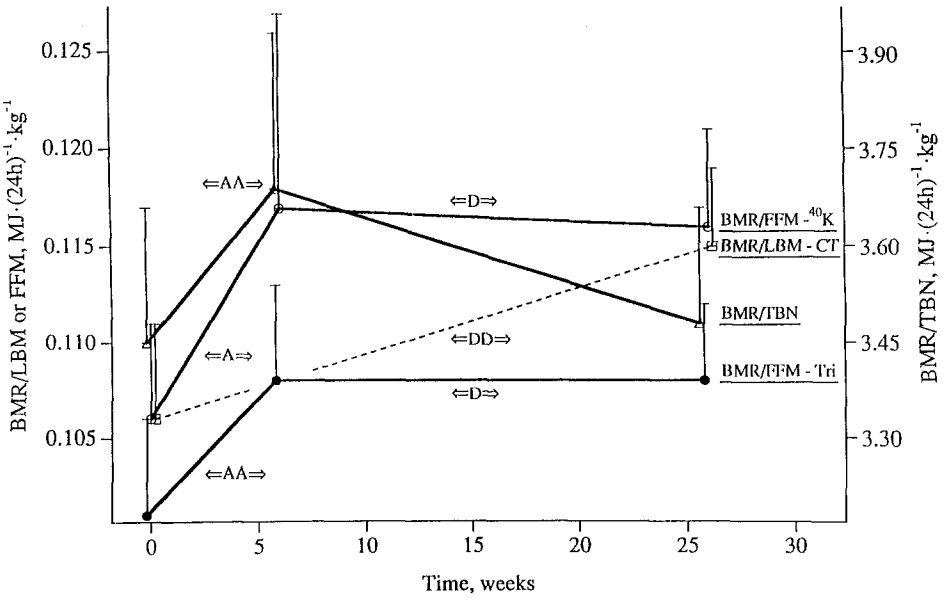

Fig 3. Mean ± SEM BMR, standardized for LBM, FFM, or TBN, at baseline and after 6 and 26 weeks of treatment. LBM was measured with CT, FFM was estimated by ^{40}K measurements or tritium dilution (Tri), and TBN was measured with neutron activation. Letters indicate degree of significance as in Table 4.

resulting in increased circulating T_3 levels, which were closely related to the increase in BMR. Moreover, a negative relationship between free T_4 and BMR has been observed in euthyroid subjects, and it has been suggested that this relationship is explained by a shift of free T_4 from the extracellular to the intracellular space in euthyroid subjects with a relatively high BMR.[8] In this study free T_4 was also negatively related to BMR in the baseline condition, and there was a decrease in free T_4 during the first 6 weeks of treatment that occurred in parallel with the increase in BMR.

IGF-I was used as an indicator of the rhGH effect, and during rhGH treatment IGF-I increased sixfold. Unexpectedly, neither basal IGF-I and BMR nor changes in these two variables were correlated. To understand the relationships between IGF-I and BMR, it might be necessary to take the binding proteins of IGF-I into account.[32] These protein levels were not measured in the current study.

The increased BMR may also be related to increased protein synthesis or protein turnover, as reflected by the elevated levels of circulating pIIIp. Changes in pIIIp were related to rhGH-induced changes in BMR and BMR/FFM. The correlation between pIIIp and the change in BMR/FFM may indicate that pIIIp reflects currently ongoing protein metabolism over and above previously experienced changes in FFM. pIIIp has previously been suggested as an indicator of rhGH[6] and thyroid hormone[7] metabolic effects and as a peripherally synthesized biological marker of energy expenditure.[8] The correlation between changes in pIIIp and changes in BMR provides support for the latter suggestion.

In conclusion, this study has demonstrated that rhGH induces an increase in BMR that persists at 6 months and is not only related to changes in FFM but also to a number of other metabolic factors. Due to the low number of patients, the interrelationships between these techniques could not be examined with multivarate techniques. Studies in progress are needed to better understand the complex relationships between BMR, body composition, GH, and other hormones.

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REFERENCES

1. Bengtsson BÅ, Edén S, Lönn L, et al: Treatment of adults with growth hormone deficiency with recombinant human growth hormone. J Clin Endocrinol Metab 76:309-317, 1993

2. Salomon F, Cuneo RC, Hesp R, et al: 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, 1989

3. Jörgensen JOL, Pedersen SA, Laurberg P, et al: Effects of growth hormone therapy on thyroid function of growth hormone–deficient adults with and without concomitant thyroxine-substituted central hypothyroidism. J Clin Endocrinol Metab 69:1127-1132, 1989

4. Grunfeld C, Sherman BM, Cavalieri RR: The acute effects of human growth hormone administration on thyroid function in normal men. J Clin Endocrinol Metab 67:1111-1114, 1988

5. Kühn K: The classical collagens: types I, II and III, in Magne R, Burgeson RE (eds): Structure and Function of Collagen Types. New York, NY, Academic, 1987, pp 1-42

6. Lindstedt G, Wejkum L, Lundberg PA, et al: Serum procollagen III as indicator of therapeutic effect in children treated for somatotropin deficiency. Clin Chem 30:1879-1880, 1984

7. Nyström E, Caidahl K, Fager G, et al: A double-blind cross-over 12-month study of L-thyroxine treatment of women with 'subclinical' hypothyroidism. Clin Endocrinol 29:63-76, 1988

8. Stenlöf K, Sjöström L, Fagerberg B, et al: Thyroid hormones, procollagen III peptide, body composition and basal metabolic rate in euthyroid individuals. Scand J Clin Lab Invest 53:793-803, 1993

9. Henneman PH, Forbes AP, Moldawer M, et al: Effects of human growth hormone in man. J Clin Invest 39:1223-1238, 1960

10. Bray GA: Calorigenic effect of human growth hormone in obesity. J Clin Endocrinol Metab 29:119-122, 1969

11. Salomon F, Cuneo RC, Hesp R, et al: Basal metabolic rate in adults with growth hormone deficiency and in patients with acromegaly: Relationship with lean body mass, plasma insulin level and leucocyte sodium pump activity. Clin Sci 83:325-330, 1992

12. Ferrannini E: The theoretical bases of indirect calorimetry: A review. Metabolism 37:287-301, 1988

13. Dahlberg G: Statistical Methods for Medical and Biological Students. London, UK, Allen & Unwin, 1948, p 122

14. Schofield WN: Predicting basal metabolic rate: New standards and review of previous work. Hum Nutr Clin Nutr 39C:5-41, 1985 (suppl 1)

15. Kvist H, Sjöström L, Chowdhury B, et al: Body fat and adipose tissue determinations by computed tomography and by measurements of total body potassium, in Yasamara S, Harrison JE, McNeill KG, et al (eds): In Vivo Body Composition Studies. New York, NY, Plenum, 1990, pp 197-218

16. Kvist H, Chowdhury B, Sjöström L, et al: Adipose tissue volume determination in males by computed tomography and ^{40}K. Int J Obes 12:249-266, 1988

17. Sjöström L, Kvist H, Cederblad Å, et al: Determination of total adipose tissue volume and body fat in women by computed tomography, ^{40}K and tritium. Am J Physiol 250:E736-E745, 1986

18. Bengtsson BÅ, Brummer RJ, Edén S, et al: Body composition in acromegaly. Clin Endocrinol 30:121-130, 1989

19. Vartsky D, Elis KJ, Cohn SH: In vivo quantification of body nitrogen by neutron capture prompt gamma-ray analysis. J Nucl Med 20:1158-1165, 1979

20. Larsson L, Alpsten M, Mattsson S: In-vivo analysis of nitrogen using a ^{252}Cf source. J Radioanal Nucl Chem 114:181-185, 1987

21. Sjöström L: A computed tomography based multicompartment body composition technique and anthropometric predications of lean body mass, total and subcutaneous adipose tissue. Int J Obes 15:29-30, 1991 (suppl 2)

22. Chowdhury B, Sjöström L, Alpsten M, et al: A multicompartment body composition technique based on computed tomography. Int J Obes 18:219-234, 1994

23. Gregory D (ed): Minitab Reference Manual (ed 9). State College, PA, Minitab, 1992

24. Bradley JW: Distribution-Free Statistical Tests. London, UK, Prentice-Hall, 1968, pp 68-86

25. Crist DM, Peake GT, Loftfield RB, et al: Supplemental growth hormone alters body composition, muscle protein metabolism and serum lipids in fit adults: Characterization of dose-dependent and response-recovery effects. Mech Ageing Dev 58:191-205, 1991

26. Landin K, Petruson B, Jakobsson KE, et al: Skeletal muscle sodium and potassium changes after successful surgery in acromegaly: Relation to body composition, blood glucose, plasma insulin and blood pressure. Acta Endocrinol (Copenh) 128:1-5, 1993

27. Ward HC, Halliday D, Sim AJW: Protein and energy metabolism with biosynthetic human growth hormone after gastrointestinal surgery. Ann Surg 206:56-61, 1986

28. Möller N, Schmitz O, Pörksen N, et al: Dose-response studies on the metabolic effects of a growth hormone pulse in humans. Metabolism 41:172-175, 1992

29. Möller N, Jörgensen JOL, Alberti KGMM, et al: Short-term effects of growth hormone on fuel oxidation and regional substrate metabolism in normal man. J Clin Endocrinol Metab 70:1179-1186, 1990

30. Sjöström L, Alpsten M, Andersson B, et al: Hormones, body composition and cardiovascular risk, in Ellis KJ, Eastman JD (eds): Methods on In Vivo Body Composition Assessment. New York, NY, Plenum, 1993: pp 233-243

31. Steinberg D, Nestlé PJ, Buskirk ER, et al: Calorigenic effects of norepinephrine correlated with plasma free fatty acid turnover and oxidation. J Clin Invest 43:167-176, 1964

32. Blum WF, Ranke MB: Use of insulin-like growth factor–binding protein 3 for the evaluation of growth disorders. Horm Res 33:31-37, 1990