Measures of Submaximal Aerobic Performance Evaluate and Predict Functional Response to Growth Hormone (GH) Treatment in GH-Deficient Adults*

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ABSTRACT
The impact of GH on functional performance in GH-deficient adults is not well understood. To investigate the effects of GH on skeletal muscle, physical, and functional capacity, we randomized 28 GH-deficient adults to receive 3 months of recombinant human GH [rhGH: somatotropin, 6.25 μg/kg lean body mass (LBM) for 1 month, 12.5 μg/kg LBM thereafter] in a double-blind placebo-controlled cross-over trial. We measured muscle fiber type, size, and insulin-like growth factor I messenger RNA, aerobic capacity [maximal oxygen uptake (VO2max), ventilation threshold (VeT)], isokinetic strength, isokinetic strength, oxygen-cost-of-walking at normal and fast speeds, and fatigue determined by the profile of mood states questionnaire. As expected, GH treatment decreased body fat, increased LBM, increased muscle fiber size, and increased muscle insulin-like growth factor-I messenger RNA 5-fold; however, muscle strength remained unchanged. At baseline, VeT occurred at a high percentage of maximal VO2max (73.3% ± 2.6) because of low VO2max (1.74 ± 0.1 L/min or 20.7 ± 1.3 mL/kg-min). Walking required high oxygen consumptions representing from 83 ± 4% of VeT at normal speeds to 120 ± 5% of VeT at fast speeds. After rhGH, there was a significant (% increase in VeT (18%), compared with placebo. This was paralleled by a nonsignificant rise in VO2max. Functionally, rhGH treatment decreased the oxygen cost of walking, relative to VeT, at normal (14% decrease, P = 0.019) and fast (21% decrease, P = 0.004) SPW speeds. A 3-variable model (baseline fast SPW speed, VeT/VO2max, and VeT) accounted for 39% of the variance of change in self-reported fatigue. These data indicate that GH-deficient adults require a high fraction of VeT for daily activities, explaining the perception of increased fatigue and impaired physical performance. The actions of rhGH on muscle fiber size translate into physiological improvement in submaximal aerobic capacity and result in functional improvement in walking ability but do not necessarily alter strength. Thus, measures of effort-independent submaximal aerobic performance provide novel objective determinants of functional impairment and fatigue and can be used to evaluate and predict response to GH treatment. (J Clin Endocrinol Metab 84: 4570–4577, 1999)

THE AVAILABILITY of recombinant human growth hormone (rhGH) facilitated investigation of the effects of GH in postpubertal life. Of particular interest are selected populations with relative reduction in circulating GH: the aging population, those with hypothalamic-pituitary disorders, and patients with acute diseases associated with enhanced tissue breakdown, including AIDS, malnutrition, postoperative wounds, infections, bony fractures, and burns (1–3). GH deficiency in adults is associated with excessive fatigue, diminished physical performance, diminished productivity, and social isolation (4–8). Administration of GH increases circulating levels of its target insulin-like growth factor-I (IGF-I), skin thickness, and bone mineral content and reduces fat mass (9). A major unresolved question, however, is the impact of this treatment on functional performance. Previous studies have emphasized muscle strength and maximal performance but have failed to clarify the mechanism of rhGH action in adults.

We used adult-onset GH deficiency as a model to investigate the mechanisms of in vivo GH action on skeletal muscle. In contrast to previous studies, we assessed submaximal (as well as maximal) physical performance to determine how the effects of rhGH treatment translate into enhanced physiological function and/or reduced perception of fatigue.

Subjects and Methods

Subjects
Thirty subjects (18–68 yr old) with GH deficiency were enrolled. GH deficiency was defined by peak GH ≤ 3 μg/L during an insulin tolerance test with adequate hypoglycemia (10, 11). All participants had adult-onset GH deficiency caused by pituitary mass lesions, had no other concurrent systemic illnesses, and had not previously received exogenous GH. Replacement of other pituitary-controlled hormones was established at least 6 months before this trial and was maintained throughout.

Study design
This study received ethics approval from the University of Toronto. Participants gave written informed consent. Thirty GH-deficient adults were studied in a double-blind, placebo-controlled cross-over trial. The two-period, two-group trial was an A/B B/A design. After a 1-month single-blind run-in on placebo, participants were randomized by computer-generated sequence to receive either drug A, active treatment [rhGH: somatotropin, 6.25 μg/kg lean body mass (LBM; max 0.5 mg/day or 1.5 IU/day) for 1 month, 12.5 μg/kg LBM (max 1.0 mg/day or 3 IU/day thereafter) or drug B, placebo for 3 months (period 1). After a 1-month washout, participants crossed over to the alternative treat-
ment for 3 months (period 2). Participants randomized to group 1 received drug A (rhGH) in period 1 and drug B (placebo) in period 2 (A/B); the treatment order in group 2 was B/A. All measures were made at baseline and after each treatment period. Participants were asked not to alter their level of habitual activity during the study period. Self-reports of activity levels were monitored monthly using a validated, standardized questionnaire (Canada Fitness Survey: CFS) (12) to assess compliance.

Circulating IGF-I

Serum levels of GH (Quest Diagnostics Inc., San Juan Capistrano, CA) and IGF-I (Diagnostics Systems Laboratories, Inc. Webster, TX) were measured using immunoassays, according to the manufacturers’ protocols.

Body composition measures

Measures of body composition included weight, height, body mass index (BMI = weight/height^2), and waist and hip circumferences. Skinfold thicknesses were measured at the biceps, triceps, subscapular, supra iliac, and medial calf sites, using Harpenden Skinfold Calipers (British Indicator Ltd., London, England). The subscapular and supra iliac values were summarized to give a total for the trunk skinfolds (SOTs); and all five sites were summed for a total sum of skinfolds (SOS) measure. Whole-body bioelectrical impedance measures of reactance and resistance were made using a single-frequency (800 μA at 50 KHz) BIA 101 Body Composition Analyzer (RJL Systems, Inc., Detroit, Michigan). Calibration of the device was checked monthly using a 500-ohm resistor supplied by the manufacturer. The coefficient of variation for the resistance, measured in five GH-deficient adults at baseline over six trials in a 2-day period (three trials per day), ranged from 0.8–1.5%. BMI, fat mass, and total body water (TBW) were calculated using manufacturer’s software. To assess the validity of the manufacturer’s equation for GH-deficient adults, body fat percentages calculated from the sum of four skinfolds (biceps, triceps, subscapular, and supra iliac), using Durnin and Womersley’s equation (13), were correlated to those obtained using BIA at baseline. The Pearson product correlation coefficient was significant (P = 0.00003) at r = 0.74.

Muscle tissue studies

A subset of 19 subjects (group 1, n = 9; group 2, n = 10) consented to needle biopsies of vastus lateralis muscle of their dominant leg (14) at baseline and after each treatment period. After extraneous connective tissue and fat were removed, samples were divided for analyses. Samples for histochemistry were oriented for cross-section, embedded in OCT compound (10.4% polyvinyl alcohol and 4.26% polyethylene glycol), immersed in isopentane that had been cooled in liquid nitrogen, and frozen sections were oriented for cross-section, embedded in OCT compound (10.4% polyvinyl alcohol and 4.26% polyethylene glycol), immersed in isopentane that had been cooled in liquid nitrogen, and stored at −80°C for batch analysis.

Muscle fiber type and size

Transverse 10-μm cryostat sections were fixed in Guth and Sahama fixative (15) for 5 min at 4°C, then incubated in lead adenosine triphosphatase (Pb-ATPase) medium for 60 min. This myosin ATPase stain was used to simultaneously distinguish type I and II fibers, as well as capillaries (16). Tissue limitations precluded identification of type II fiber subtypes. We counted type I and II fibers in duplicate and calculated distributions. Individual fiber diameters were measured at each of the three time points for each specimen sampled.

IGF-I Messenger RNA (mRNA) analysis

IGF-I mRNA was quantified by RT/competitive PCR (RT-PCR). Total RNA was extracted by the guanidinium isothiocyanate method, DNase treated, and quantified. One microgram was reverse transcribed using 2.5 U/mL Murine Leukemia Virus reverse transcriptase, 2.5 mM MgCl₂, 1 mM deoxynucleotide triphosphate, 2.5 mM random hexamers, and 1 U/mL RNase inhibitor. Competitive PCR used a MIMIC approach (CLONTECH Laboratories, Inc., Palo Alto, CA). Reaction mixtures included 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate, 0.1 mM upstream and downstream composite primers, 0.5 ng/mL neutral DNA fragment, and 0.25 U Taq polymerase (Perkin-Elmer Corp., Norwalk, CT). The ratio of IGF-1 band intensity (determined by densitometry) was plotted to that of IGF-I MIMIC, against the reciprocal of the molar amount of IGF-I MIMIC(17). Competitive RT-PCR was normalized for the housekeeping gene PGK-1.

Strength measures

Strength was measured isometrically (hand grip), isotonically (arm curl, leg, and bench press), and isokinetically (knee flexion/extension at three velocities). Isometric hand grip was measured to the nearest kilogram using a Lafayette hand dynamometer (Lafayette, IN). Each hand was measured alternately, and the best of three trials was recorded. One-repetition maximums (IRM) were measured isotonically using a Universal DVR Weight Resistance System (Cedar Rapids, IA) for arm curl, leg, and bench press to the nearest kg. These were obtained by asking the participant to lift the weight the participants could safely lift with proper body mechanics and progressing in small increments until their limit was achieved. Concentric strength of knee flexors and extensors was measured as mean peak torque in Newton-meters (N 160 cm) for five maximal isokinetic repetitions on a Lido Active Multijoint dynamometer (Loredan, Davis, CA) (18). Measures were made on the dominant leg through a knee range of motion from 20° to 90° and 2° flexion in the sagittal plane at velocities of 60, 120, and 180°/sec. Subjects were seated with hips flexed to 110°. Torque curves were accepted only when the coefficient of variation between repetitions was less than 10%.

Local muscular endurance (LME)

LME was measured both isotonically (arm curl, leg and bench press) and isokinetically (knee flexion/extension). Participants lifted a load equivalent to 66% of their 1 RM for the arm curl and bench press and 80% of their 1RM for the leg press paced at a rate of 30 repetitions/min for a maximum of 3 min. Pace was set using an electronic metronome. Isokinetic LME was evaluated by measuring total work done in Joules (J) during 30 isokinetic repetitions at a velocity of 180°/sec. The position and test procedures were similar to those for isokinetic strength testing.

Aerobic fitness

Aerobic fitness was measured during a continuous, progressive, pseudo-ramp treadmill protocol to symptom-limited maximum [American College of Sports Medicine guidelines (19)]. The initial treadmill work rate, based on the level of physical activity and fitness of each subject, was set low to ensure accurate estimation of VeT. This was a walking protocol for all subjects. Gas exchange was measured on a breath-by-breath basis using a turbine for ventilation and mass spectrometer (Case Airspec 2000, Biggin Hill, UK) for gas exchange. The algorithms of Beaver et al. (20) were employed to calculate alveolar gas exchange using corrections for change in lung volume and average gas concentration. Measures included maximum oxygen uptake (VO₂max) and ventilation threshold (VeT). VO₂max was the highest oxygen uptake achieved. Objective criteria for ascertaining VO₂max were that VO₂ and heart rate plateaued despite further increase in work rate.

A non-invasive method was used to estimate VeT from ventilatory equivalents for oxygen (Ve/VO₂) and carbon dioxide (Ve/VCO₂) as previously described (21, 22). VeT was identified as VO₂ at the point of inflection where Ve/VO₂ was lowest then increased progressively with further increments in treadmill work rate while Ve/VCO₂ plateaued or declined. The modified V-slope method where VCO₂ was plotted against VO₂ (20) was used to support the estimate of VeT by ventilatory equivalents. Two blinded investigators were able to clearly identify ventilation threshold (VeT) for 26/28 subjects at each of the three time points. Discrepancy in VeT between investigators was less than 10% in all cases.

Self-paced walk

Self-paced walking (SPW) speed (m/sec) was measured using a computerized photocell timing device at normal and fast paces (23). At each
visit, subjects walked 160 meters in response to these words of instruction: walk at a normal pace, neither fast nor slow. After a 3- to 4-min rest, they were given these words of instruction: walk rather fast, but without overexerting yourself. Oxygen-cost-of-walking was calculated using the American College of Sports Medicine equation (24).

### Fatigue

The self-report profile of mood states (POMS) questionnaire has been validated for use with exercise interventions (25) and GH deficiency (26). It evaluates 6 domains of mood state: tension, depression, anger, vigor, fatigue, and confusion. Subjects rated how they had been feeling during the previous week during their normal daily activities, on a 4-point scale, responding to each of 65 words/phrases that describe feelings. Responses were summed for each domain. Transformation into T-scores provided comparison to healthy controls by percentile.

### Statistical analyses

SigmaStat (Jandel Scientific) and Statistical Analysis Systems (SAS Institute, Inc., Cary, NC) were used. Descriptive statistics included means, sn, and SEM. Chi-square testing was used to examine gender differences by group. All variables were examined for equality of carryover effects, using a two-sample t test of subject totals for period 1 plus period 2 results at \( P < 0.10 \) (27). In situations where this test was significant, between-group comparisons \((P < 0.05)\), using independent \( t \) tests, were made using data for period 1 only. Otherwise, a within-subjects approach using repeated-measures ANOVA, was used to examine differences by group (1 or 2) and treatment (baseline, placebo, and rhGH). Post hoc comparisons were made using Student-Newman-Keuls testing. Correlations were examined using the Pearson product-moment correlation coefficient. Multiple regression analysis identified the regression model that maximized \( R^2 \) (variance explained) of change moment correlation coefficient. Multiple regression analysis identified Keuls testing. Correlations were examined using the Pearson product-

### Results

#### Patient characteristics

Thirty adults met inclusion criteria and gave informed consent to participate. Although no adverse events resulted in anyone withdrawing from the study, 2 participants withdrew before completion, 1 from each period of the trial, for personal reasons. Twenty-eight subjects (15 males, 13 females) completed the 8-month trial (Table 1). There was no significant gender difference between group 1 [treatment order A/B; 8 males (M), 5 females (F)], compared with group 2 (treatment order B/A; 7 M, 8 F).

#### Biochemical changes

Circulating IGF-I concentrations fell within the normal range \( \pm 2 \) sds for each individual examined by age. The expected range for serum IGF-I in 40–49 yr old males and females is 40–256 \( \mu g/L\). Serum IGF-I levels (mean \( \pm \) SEM) were not significantly different between group 1 (120.6 \( \pm \) 28.4 \( \mu g/L\)) and group 2 (109.8 \( \pm \) 30.3 \( \mu g/L\)) at baseline. There was a significant rise in IGF-I for both group 1 (378.9 \( \pm \) 28.4 \( \mu g/L\)) and group 2 (299.4 \( \pm \) 32.0 \( \mu g/L\)) after rhGH \((P < 0.001)\) but not after placebo treatment \((122.3 \pm 28.4\) and 115.1 \( \pm \) 30.3 \( \mu g/L\), respectively). There was no significant carryover effect for circulating IGF-I. Hematocrit, hemoglobin, liver enzymes (aparate transaminase, alanine transaminase, and alkaline phosphatase) did not change with either treatment.

#### Body composition changes

There were no significant differences in percent body fat, LBM, TBW, SOS, SOTS, or BMI between the groups at baseline. There was a significant decrease in percent body fat in both group 1 (34.1 \( \pm \) 0.5\%), \( P < 0.001 \) and group 2 (30.5 \( \pm \) 0.6\%, \( P < 0.05 \)) after rhGH but not placebo \((37.2 \pm 0.6\% and 33.5 \( \pm \) 0.6\%, respectively), compared with baseline values \((37.1 \pm 0.5\% and 33.3 \( \pm \) 0.6\%, respectively). Decreased adiposity paralleled a significant increase in LBM in both groups \((3.0 \pm 0.6, P < 0.001; and 3.1 \pm 0.6 \text{ kg}, P < 0.05; respectively)\) and TBW \((2.8 \pm 0.5, P < 0.001; and 2.1 \pm 0.6 \text{ kg}, P < 0.05; respectively)\) after rhGH. Weight, height, and BMI did not change significantly with either treatment.

#### Muscle tissue analysis

Of 19 subjects who agreed to undergo repeated muscle biopsies, we obtained adequate samples for histochemical analyses for 14 subjects \((\text{group, } n = 6 \text{ M; group 2, } 6 \text{ M and } 2 \text{F})\) and IGF-I mRNA analyses for 10 subjects \((\text{group 1, } n = 4; \text{ group 2, } n = 6)\) across the 3 treatments. Mean fiber areas at baseline were not significantly different between the groups for either type I fibers \((3.6 \pm 0.6 \text{ mm}^2)\) and type II fibers \((31.8 \pm 0.6 \text{ mm}^2\) respectively). The level of significance was \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (n = 15)</th>
<th>Group 2 (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>39 \pm 3</td>
<td>40 \pm 4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.8 \pm 2.5</td>
<td>169.9 \pm 3.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.2 \pm 5.5</td>
<td>87.7 \pm 5.6</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>33.4 \pm 1.7</td>
<td>30.4 \pm 1.2</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.89 \pm 0.03</td>
<td>0.86 \pm 0.02</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>37.4 \pm 2.7</td>
<td>33.9 \pm 2.2</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>136.4 \pm 11.4</td>
<td>140.8 \pm 8.1</td>
</tr>
<tr>
<td>Sum of trunk skinfolds (mm)</td>
<td>70.4 \pm 5.5</td>
<td>69.5 \pm 3.0</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>31 \pm 3</td>
<td>32 \pm 5</td>
</tr>
</tbody>
</table>

All values are means \( \pm \) SEM. \( a \) Significant difference \((P < 0.05)\) in mean values between the groups.

\( a \) Calculated as body weight in kilogram divided by the square of height in meters.

\( b \) Calculated as the sum of the biceps, triceps, subcapular, suprailiac and medial calf skinfolds.

\( c \) Calculated as the sum of the subcapular and suprailiac skinfolds.
rhGH (59 ± 4% and 63 ± 4% for group 1 and 2, respectively) or placebo (54 ± 4% and 62 ± 3% for group 1 and 2, respectively). Both groups had a significantly higher percentage of type I, compared with type II fibers at baseline and after treatment with rhGH or placebo ($P < 0.05$). Skeletal muscle IGF-I mRNA (n = 10) increased 5-fold (4.91 ± 0.93, $P = 0.004$) after rhGH but not placebo (0.96 ± 0.3). Circulating levels of serum IGF-I in this same subset of participants did not change from baseline (123.9 ± 18.2 μg/L) to placebo (94.6 ± 9.3 μg/L) but rose significantly after rhGH (315.4 ± 46.5 μg/L; $P < 0.0001$) (Fig. 2) and were strongly correlated to IGF-I fold increase in tissue ($r = 0.88; P < 0.0001$).

**Muscle strength and LME**

There was no significant group or treatment effect for any of the measures of isometric, isotonic or isokinetic strength, or LME. As an example, mean peak knee extensor torques remained essentially unchanged from baseline values of $124 ± 4$, $108 ± 4$, and $90 ± 4$ Nm for group 1 and $113 ± 5$, $102 ± 5$ and $89 ± 4$ Nm for group 2 at 60, 120 and 180 degrees/sec, respectively. LME also remained unchanged from baseline values of $1475 ± 86$ Joules (J) for group 1 and $1591 ± 92$ J for group 2, after either placebo or rhGH treatment.

**Aerobic performance**

There was no significant difference in VeT between groups 1 and 2 at baseline. VeT as a percentage of VO$_2$max was 67.7 ± 3% in group 1 and 75.3 ± 3% in group 2. Similar to muscle histochemistry results, there was a significant ($P = 0.04$) carryover effect for VeT when totals for periods 1 and 2 were compared between group 1 and group 2. Independent $t$ tests revealed a significantly higher mean VeT ($P = 0.04$) for group 1 after rhGH treatment, compared with that of group 2 who received placebo during period 1. Maximal oxygen consumption (VO$_2$max) was not significantly different between the groups ($P = 0.49$) (Fig. 3). Although we have presented the data for the between-groups comparison here because of the carryover effect, VeT increased in both groups after rhGH treatment. In fact, the rise in VeT for group 2 was double (22%) that of group 1 (11%). These results were the same whether VeT was examined in absolute terms (L/min) or relative to body weight (mL/kg·min). These changes occurred despite no significant change in activity level (frequency, duration, or intensity) for either group, as measured using the CFS questionnaire.

**Functional performance**

At baseline, walking at a normal pace of $1.33 ± 0.02$ m/sec for group 1 and $1.38 ± 0.02$ m/sec for group 2 required
oxygen consumptions that represented 103 ± 4% and 83 ± 4% of their respective ventilation thresholds (VO₂/VE₉). Although group 1 walked significantly faster (P = 0.02) at normal pace after rhGH (1.40 ± 0.02 m/sec), they did so with a significantly reduced (P = 0.02) VO₂/VE₉. Conversely, there was no significant (P = 0.168) increase in walking speed at normal pace (1.42 ± 0.02 m/sec) in group 2 after placebo and no associated decline in VO₂/VE₉, which remained unchanged from baseline (89 ± 4%). At baseline, walking at fast pace (1.67 ± 0.02 m/sec and 1.64 ± 0.02 m/sec for groups 1 and 2, respectively) required oxygen (O₂) consumption that exceeded VE₉ in group 1 (120 ± 5%), and levels were close to maximal in group 2 (93 ± 5%). In both groups, fast SPW pace remained unchanged with either treatment. Despite no significant increase in walking speed at fast pace, there was a significant decline in VO₂/VE₉ in group 1 after rhGH, down to 99.8 ± 0.5%.

**Fatigue**

Of the POMS subscales, only anger remained unchanged from baseline after placebo and rhGH. All five other subscales improved with rhGH. The mean fatigue score for both groups declined significantly (P < 0.001) from 14.8 ± 1.1 to 10.3 ± 1.4 (57th to 49th percentile, compared with normals) after rhGH. This significant reduction in fatigue score was the dependent variable used to investigate which baseline measure of biochemical markers, body composition, muscle strength, aerobic capacity, and walking performance best predicted decreased fatigue in patients with rhGH.

**Prediction of change in fatigue**

Multiple regression analyses indicated that baseline measures of fast SPW, VE₉/VO₂max and VE₉ explained 39% of the variance of change in fatigue with treatment (Table 2). This suggests that baseline measures of aerobic performance and walking ability predict which patients will experience a reduction in fatigue with rhGH.

**Adverse events**

Side effects included minor joint arthralgia (7 on rhGH, 1 on placebo), peripheral edema (4 during rhGH, 1 on placebo), and headaches (1 patient, both periods).

### Table 2. Prediction of change in fatigue with rhGH treatment

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Coefficient</th>
<th>Partial R²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline fast SPW speed</td>
<td>9.68</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Baseline VE₉/VO₂ (%)</td>
<td>-0.16</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>Baseline VE₉</td>
<td>-3.99</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>Total R²</td>
<td>0.39</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Equation: VE₉ = -2.79 - 3.99 [baseline VE₉ (L/min)] - 0.16 [VE₉/VO₂ (%)] + [baseline SOS (mm)]

Determinants of change in fatigue identified by multiple regression analysis from baseline measures of: fast self-paced walk (SPW) speed, ventilation threshold as a percentage of maximal oxygen consumption (VE₉/VO₂ (%)), and baseline ventilation threshold (VE₉).  

- Change in fatigue was calculated as the difference in fatigue score between rhGH treatment and baseline using the POMS questionnaire.
- Measures the increase in percentage of the variance explained by the indicated variable when last added into the regression analysis.
- The P values displayed refer to the significance of the indicated explained variance (R²) in the full regression.

**Discussion**

GH deficiency in adulthood is associated with functional deficits and reduction in quality of life. Treatment with rhGH normalizes biochemical parameters, substrate metabolism, and body composition; effects on physical capacity (muscle strength, aerobic capacity) and quality of life measures have been described. However, it remained unclear whether these findings reflected changes in skeletal muscle tissue and their relationship, if any, with physical and functional capacity. We show, for the first time, that administration of rhGH alters skeletal muscle morphology, ameliorates the depressed VE₉ (a measure of submaximal aerobic function), VO₂max (a measure of maximal aerobic capacity), and reduces the physiological load of performing activities of daily living (such as walking) and self-reported fatigue associated with GH-deficiency. Moreover, we have proposed a pathophysiological basis of the functional deficits and their relationship to self-reported fatigue in GH-deficiency.

GH regulates DNA, RNA, and protein synthesis in most tissues (28). GH stimulates tissue growth through the action of its target, IGF-I (29, 30). We demonstrate, for the first time, that human skeletal muscle IGF-I mRNA increases in response to systemic rhGH administration. This response of muscle is similar to that indicated in data obtained in GH-treated rodents (30). The increase in IGF-I was associated with increased muscle fiber size, primarily involving type I fibers.

In hypophysectomized rats, administration of GH increased and restored the proportion of the fatigue-resistant type I muscle fibers (31); however, two studies of GH-deficient humans failed to demonstrate this effect (32, 33). Despite evidence of muscle atrophy (7–8%) from body composition measures and computerized tomographic studies, previous work failed to demonstrate a consistent change in fiber-type cross-sectional areas or proportions in GH-deficient patients, compared with age- and gender-matched controls (32). The authors concluded that the differences were not significant, because of a lack of power, given the small sample size. However, data reported in that study indicate that type I fibers were larger than II fibers before and after 6 months of rhGH treatment. Our analysis confirms this. Type II muscle fibers are recruited in high-intensity exercise and are usually larger than type I fibers in normal subjects (34). While the cross-sectional diameter of muscle correlates with isometric strength, the specific contributions of type I (compared with type II) fibers to isokinetic strength are not clear. Our previous findings indicate that the similar fiber areas in GH-deficient adults are attributable to significant reduction in type II fiber size and that type I fiber area better correlates with impaired muscle strength in this population (35).

Adults with GH deficiency frequently complain of muscle weakness and fatigue (36). They demonstrate muscle strength deficits in isometric and isokinetic contractions (37–39). Isometric quadriceps force per unit body weight was 35% lower in GH-deficient adults, compared with age-matched controls, suggesting decreased intrinsic muscle strength (40). Studies evaluating the effects of rhGH treatment on the relationship between thigh muscle mass and muscle strength...
in GH-deficient adults, using computerized tomographic (37, 41), magnetic resonance imaging (42), and muscle biopsies (32), have largely reported that the increase in muscle mass is only partially accompanied by an increase in strength of the quadriceps extensor mechanism. Our findings (of no significant increase in ability to generate maximal knee extensor torque during isokinetic tasks) are similar to those previously reporting no significant increase, or slight decrease, in isometric or isokinetic quadriceps strength with rhGH treatment trials of 6 months or less in duration (37, 38, 41). Only those trials exceeding 6 months in duration have shown an increase in isokinetic quadriceps strength (38, 42). This suggests that early modest changes in muscle fiber sizes may not translate into significant improvement in maximal strength. This may be a result of increase in muscle mass in the absence of peripheral or central neural adaptations in motor unit recruitment and/or muscle energy stores necessary to operationalize peak force production. Also, intrinsic muscle strength is lower in those muscles that contain relatively more slow, type I than fast, type II muscle fibers (43, 44). Animal models have shown that neither GH, nor IGF-I, nor exercise alone has been as effective in reducing muscle atrophy as a combination of exercise and growth factors (45–47) and that muscle loading and GH/IGF-I factors have interactive effects in maintaining muscle fiber size (48). The optimal approach to improve muscle function in GH-deficient adults may be to combine exercise with GH treatment to improve neural activation and enzymatic processes, in addition to the increase in muscle mass that occurs, to enable maximum force production.

Aerobic capacity (VO2max) in GH-deficient patients is reduced to levels comparable with those observed in congestive heart failure (49, 50). Reduced muscle mass might account for this aerobic deficit. However, given the minor differences in muscle tissue that we report, it seems that other factors are implicated. The mechanisms underlying improvement in aerobic function have not been fully explored. Fatigue and poor exercise capacity in GH-deficient adults may relate not only to reduced skeletal muscle mass but also to cardiovascular impairment (9). Indeed, cardiac muscle performance and diminished plasma volume are key deficits in this patient population (51, 52).

The most striking finding of our study concerns the effect of rhGH treatment on submaximal aerobic measures (VeT). Ventilation threshold (VeT) is an effort-independent physiological marker of ability to perform submaximal, prolonged activity. Working at intensities above VeT results in metabolic acidosis, hyperventilation, and inability to sustain performance. The physiological basis of VeT remains uncertain, but explanations include a shift toward anaerobic (from aerobic) energy metabolism (53), or progressive recruitment of muscle fibers with less oxidative capacity (54). Despite ongoing debate about the relationship of VeT to changes in blood lactate, epinephrine, and potassium levels, its clinical utility is well-established (55).

Mean VeT at baseline for both groups was 73.3 ± 2.6% of VO2 max. This value is high, compared with that of healthy normals (45–65%) (56–60) and explains the perception of increased fatigue in GH-deficient adults. The impact of rhGH on submaximal aerobic performance (VeT) was greater than the effect on maximal capacity (VO2max). To walk at a normal pace, group 1 required an oxygen O2 consumption that was significantly higher than that of group 2, relative to their VO2max. In patients of group 1, whose daily activities (such as walking) would represent the equivalent of a heavy task to non-GH-deficient individuals, the effect of rhGH on VeT/VO2max was greater. After rhGH treatment, these individuals walked at a faster pace, with a significantly reduced physiological load. Previous studies have examined the effect of GH deficiency on VeT (49) and VO2max (49, 61). Both studies showed no appreciable improvement in VeT/VO2max with rhGH treatment. However, those studies used cycle ergometry that is limited by LME. We used treadmill testing, which better evaluates systemic cardiovascular performance (62). Further, our estimates of improvement in VeT after rhGH treatment are conservative, because we have used only period 1 data for the comparison, attributable to carryover effects on VeT, whereas the response of group 2 to rhGH treatment in period 2 was double that of group 1.

The response of VeT and SPW to rhGH treatment suggests that these measures serve as objective markers that assess physical function, reflect fatigue, and predict response to treatment in this population. VeT is an effort-independent measure that is often used to characterize a patient’s functional capacity and to assess the outcome of therapeutic modalities. VeT is reduced in conditions characterized by excessive fatigue, including cardiac failure (63), chronic fatigue syndrome (64), chronic pulmonary disease (65), and acromegaly (66). Exercise training of patients with chronic congestive heart failure increases VeT and improves functional status (50). We demonstrate that GH-deficient adults require a higher fraction of VeT for daily activities (like walking) and suggest that this explains their perception of increased fatigue and impaired physical function. Treatment with rhGH increased VeT in our patients. The decreased fraction of VeT to VO2max, used for walking at normal pace, may decrease the sense of fatigue experienced by rhGH-treated patients. The relationship between change in self-reported fatigue and baseline status supports the hypothesis that fatigue and VeT are intimately linked.

The POMS questionnaire (67) is a reliable and sensitive indicator of mood in many populations (68–71). We have documented larger reductions in fatigue with rhGH treatment in patients who have the most profound impairments and functional limitations. The association of fatigue with low VeT, and the requirement of a high proportion of VeT just to walk, support the hypothesis that impairment of submaximal aerobic function determines sense of fatigue.

In this study, we have shown that GH-deficient adults have VeT that occurs at a high percent of VO2max, caused by low VO2max. Walking requires high oxygen consumption at normal and fast speeds. GH treatment significantly increases VeT without parallel rise in VO2max. Functionally, this translates into decreased oxygen cost of walking, relative to VeT, at normal and fast speeds. Our data demonstrate increases in muscle fiber size and a rise in skeletal muscle IGF-I production in parallel with circulating IGF-I levels. Most physiologically relevant, however, is that there seems to be a preferential effect of rhGH action on type I muscle fibers, an effect that seems to be more closely associated with improved
submaximal aerobic performance should be used as objective markers of functional impairment and fatigue to evaluate and predict response to GH.

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References