Growth Hormone Increases Muscle Mass and Strength but Does Not Rejuvenate Myofibrillar Protein Synthesis in Healthy Subjects over 60 Years Old*

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ABSTRACT
The rate of synthesis of myofibrillar proteins is slower in muscle of healthy subjects over 60 yr old than it is in young adults. Previous research suggests that reduced activity of the GH-insulin-like growth factor-I system could be a determinant of this slowing of protein synthesis. To test the hypothesis that GH could rejuvenate the rate of myofibrillar protein synthesis, we studied healthy subjects over 60 yr old, after a single injection (0.03 mg/kg, sc) of recombinant human GH (n = 6 males/2 females) or placebo (n = 6 males/2 females), or after 3 months of either GH (0.03 mg/kg, sc, 3 x/week, n = 5 males) or placebo (n = 5 males) treatment. Myofibrillar protein synthesis and whole-body protein metabolism were evaluated with the tracer L-[1-13C]leucine. GH reduced whole-body leucine oxidation by 36% (P < 0.01) in the single injection study. There was no effect of GH on whole-body protein breakdown or synthesis, or on myofibrillar protein synthesis in the quadriceps, either acutely or after 3 months of treatment. GH treatment for 3 months increased lean body mass (3.3 ± 0.7 kg, P < 0.01, as evaluated by 40K counting), muscle mass (3.3 ± 1.1 kg, P < 0.02, as evaluated by ura creatinine excretion), and thigh strength (14 ± 5%, P < 0.05, as evaluated by isokinetic dynamometry). We conclude that GH can increase muscle mass and strength in healthy men over 60 yr old, but does not restore a youthful rate of myofibrillar protein synthesis. (J Clin Endocrinol Metab 81: 3239-3243, 1996)

THE RATE of synthesis of myofibrillar proteins is significantly slower in muscle of healthy men and women over 60 yr old than it is in muscle of young adults (1–3). There are several reasons to suspect that the reduced GH secretion in older people (4, 5) contributes to the slowing of myofibrillar protein synthesis. GH was found to reverse the slowing of protein synthesis in the diaphragm of old rats (6). Both whole-body (7–10) and muscle protein synthesis (11, 12) have been reported to increase in human subjects given GH. We have observed that the rate of myofibrillar protein synthesis in healthy men over 60 yr old correlates with the plasma concentration of insulin-like growth factor-I (IGF-I) (13), which is primarily under the control of GH secretion. However, other evidence does not support an important role for GH as a determinant of muscle protein synthesis in humans (10, 14–16). The goal of the present study was to determine whether or not GH, in a dose that has been shown to increase lean body mass (17), would stimulate myofibrillar protein synthesis in healthy subjects over 60 yr old.

Materials and Methods
The subjects were men and women over 60 yr old (range 62-74) who were recruited by newspaper advertisements. All were healthy according to a physical examination, resting electrocardiogram, chest x-ray, and laboratory tests (glucose tolerance test, serum electrolytes, TSH, thyroxine, albumin and total protein, complete blood count, liver enzymes, creatine kinase, hematocrit, blood clotting profile, creatinine, and urea nitrogen). All were nonsmokers. The men involved in the 3-month trial all had normal prostate specific antigen levels and a negative digital rectal exam. Written consent was obtained from all subjects after the nature and risks of the study were explained verbally and in a written consent form. The study was approved by the University of Rochester Research Subjects Review Board.

The effect of a single injection of GH on myofibrillar protein synthesis was examined by comparing eight GH-treated subjects with eight placebo-treated subjects (six men and two women in each group). Myofibrillar synthesis after 3 months of treatment was compared in five GH-treated and five placebo-treated men. The effect of treatment on body composition and strength was also observed in the 3-month trial. Two of the men who received GH in the 3-month trial and two who received placebo had protein synthesis studies done on both the first and last days of GH or placebo administration. These men are included in the both the single-dose and the 3-month studies.

The dose of GH (Nutropin, generously supplied by Genentech, South San Francisco, CA) used in all subjects was 0.03 mg/kg. The hormone was given subcutaneously 4 h before the measurement of myofibrillar protein synthesis was initiated, except in two men in the 3-month trial in whom the final GH injection was administered the day before the myofibrillar protein synthesis study. Placebo was also provided by Genentech, and was given sc in the same volume as the GH. In the acute studies, the injections were single blind. In the 3-month trial the injections were given in a double-blind fashion three times per week (generally Monday, Wednesday, and Friday). All injections were given by nurses at the University of Rochester General Clinical Research Center. Subjects were not engaged in any regular exercise program before or during the study, and were asked to maintain their usual diets and activities during the study.

Subjects stayed at the Clinical Research Center the night before the protein synthesis studies, where they received a standard meal and were not allowed to engage in any strenuous activities. The schedule of procedures for the protein metabolism determinations is shown in Table I. Subjects rested in bed and were not fed during the protein synthesis studies. Myofibrillar protein synthesis was determined by incorporation of the tracer L-[1-13C]leucine, using 13C enrichment of plasma ketocaprope as an index of intramuscular 13C-leucyl-transfer RNA enrichment, as described previously (1–3). Whole-body leucine appear-
ance from proteolysis, leucine oxidation, and leucine incorporation into proteins were determined as described previously (1–3).

Plasma concentrations of GH and IGF-I were determined at 2-h intervals during the single-injection studies. The early morning, fasting plasma IGF-I was determined before and after treatment in the 3-month study. Commercial kits were used for immunoradiometric assays of GH (Nichols Institute, San Juan Capistrano, CA) and IGF-I (Diagnostic Systems Laboratories, Webster, TX). Early morning plasma testosterone concentrations of the men were determined on the day of each protein synthesis study with a RIA kit (Diagnostic Products Corp., Los Angeles, CA).

Strength, lean body mass, urinary creatinine excretion, and urinary 3-methylhistidine excretion were measured before the first GH or placebo injection, and during the final week of GH or placebo injections. Strength of left and right knee extensors and knee flexors was assessed with a Cybex II isokinetic dynamometer. Strength was defined as the peak torque during five maximal contractions, measured at angular velocities of 60, 120, and 240 °/sec. Lean body mass was determined from total body potassium (18). The ratio of 3-methylhistidine to creatinine was determined in a 24-h urine collection the day before the protein synthesis studies. This ratio is an index of the fractional myofibrillar degradation rate (19). The subjects consumed a meat-free diet for 2 full days before the urine collection for 3-methylhistidine was initiated, and on the day of the measurement. Creatinine excretion was measured for 3 days during which the subjects consumed the meat-free diet both before and at the end of treatment, and was used as an index of muscle mass by assuming that 1 g/day of creatinine excretion is equivalent to 20 kg of muscle (20). Urinary 3-methylhistidine was determined by high performance liquid chromatography (21). Creatinine excretion was determined with an autoanalyzer method (22).

The statistical significance of differences in mean values between the placebo- and GH-treated groups was evaluated by Student's t tests. Within-subject treatment effects were evaluated with paired t tests. Because it was hypothesized a priori, based on previously published results, that GH would increase lean body mass, muscle mass, strength, and myofibrillar protein synthesis, and would reduce the rate of whole-body leucine oxidation, one-tailed significance levels were used for these comparisons. Data are presented as the mean ± one se.

Results

Effects of a single dose of GH

The mean age, height, total body weight, and lean body mass of the GH-treated subjects (66 ± 1 yr, 173 ± 3 cm, 82 ± 2 kg, 57 ± 3 kg, respectively) were similar to those of the placebo-treated subjects (66 ± 1 yr, 176 ± 2 cm, 79 ± 5 kg, 57 ± 3 kg, respectively). Testosterone levels were similar in the GH-treated men (19 ± 4 nmol/L) and the placebo-treated men (22 ± 5 nmol/L).

Mean plasma GH and IGF-I levels during the protein synthesis study are shown in Fig. 1. The only effect that GH had on protein metabolism was a 36% reduction in the whole-body rate of leucine oxidation (Table 2). There were no significant differences between GH and placebo groups in the fractional rate of myofibrillar protein synthesis, whole-body leucine appearance rate, or whole-body incorporation of leucine into proteins (Table 2 and Fig. 1).

Effects of 3 months of GH

Before the start of the 3-month trial of GH or placebo injections, the placebo group had a slightly higher mean height (178 ± 2 vs. 172 ± 3 cm), weight (83.5 ± 4.5 vs. 74.6 ± 4.3 kg), lean body mass (59.3 ± 2.2 vs. 56.7 ± 3.2 kg), and muscle mass (30.2 ± 2.9 vs. 27.8 ± 1.9 kg) than the GH group, but none of these differences was statistically significant. The mean age of the placebo group (67 ± 2 yr) was similar to that of the GH group (66 ± 2 yr). Initial plasma IGF-I concentrations were 15.1 ± 2.9 nmol/L in the GH group and 16.2 ± 1.4 nmol/L in the placebo group. There was no baseline difference in mean isokinetic strength of either the knee flexors or the knee extensors (Table 2).

There were no serious adverse effects of GH treatment, but four subjects had mild symptoms of carpal tunnel syndrome, as noted in previous studies (16, 23, 24). Two of these subjects had experienced similar symptoms to a lesser extent before the study. After 3 months of injections, there was not a significant change in total body weight in either group (±0.7 ± 1.3 kg placebo group, +1.0 ± 1.0 kg GH group). Lean body mass did not change in the placebo group (−0.4 ± 1.5 kg), but increased (+3.3 ± 0.7 kg, P < 0.01) in the GH group. There was not a statistically significant change in fat mass (total weight minus lean body mass) in either the GH (−2.3 ± 1.1 kg) or placebo-treated groups (+1.1 ± 1.3 kg). Mean muscle mass, as indicated by creatinine excretion, did not significantly change in the placebo group (+1.3 ± 3.3 kg) and increased (+3.3 ± 1.1 kg, P < 0.02) in the GH group. Early morning plasma IGF-I concentrations 3 months after injections were higher (P < 0.05) in the GH group (22.8 ± 2.6 nmol/L) than in the placebo group (16.6 ± 1.0 nmol/L). Testosterone levels were not different in the GH (24 ± 5 nmol/L) and placebo groups (23 ± 3 nmol/L). Strength tended to improve more in GH-treated than in placebo-treated subjects, but a significant treatment effect was not observed for all muscle group/angular velocity categories (Table 3). However, when the percent changes from individual categories were averaged as an index of the overall change in thigh strength, there was a significant increase (14 ± 5%, P < 0.05) in the GH group but not in the placebo group (Table 3). GH increased knee flexion strength (18 ± 5%, averaged over all velocities and both legs) more than knee extension strength (10 ± 5%, P < 0.02), although the level of statistical significance relative to the placebo effect was no greater for knee flexion (P = 0.07) than it was for knee extension (P = 0.05).

After 3 months of injections, mean whole-body leucine appearance, leucine oxidation, and leucine incorporation into proteins were similar in GH-treated and placebo-treated...
GROWTH HORMONE EFFECTS ON MUSCLE

Fig. 1. Mean ± SEM plasma concentration of GH and IGF-I, 2–10 h after a single injection of GH (0.03 mg/kg, sc) or placebo. The GH group is represented by circles and solid lines; the placebo group by squares and broken lines. The GH group had significantly (P < 0.01) higher GH levels at every time point and had significantly (P < 0.01) higher IGF-I levels at 8 and 10 h compared with the placebo group.

TABLE 2. Whole-body and myofibrillar protein metabolism after a single injection of GH or placebo

<table>
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<tr>
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<th>GH</th>
<th>Placebo</th>
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<th>GH</th>
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<td>Protein → leucine</td>
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<td>152 ± 8</td>
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<td>(μmol ⋅ h⁻¹ ⋅ kg LBM⁻¹)</td>
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<td>42 ± 4</td>
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<td>Leucine oxidation</td>
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<td>123 ± 5</td>
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<td>(μmol ⋅ h⁻¹ ⋅ kg LBM⁻¹)</td>
<td>0.044 ± 0.003</td>
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Values are mean ± one se. LBM, lean body mass.

* P < 0.01, GH group significantly less than placebo group.

men (Table 4). There also was no significant difference in the mean fractional rate of myofibrillar protein breakdown (3-methylhistidine to creatinine ratio) or the mean postabsorptive fractional rate of myofibrillar synthesis between GH-and placebo-treated subjects (Table 4 and Fig. 2).

Discussion

The present study confirms that lean body mass is increased in men over 60 yr old who receive 0.03 mg GH three times weekly (17), even though subjects were not specifically selected for having low GH or IGF-I concentrations. Studies in GH-deficient adults indicate that increased skeletal muscle mass accounts for at least some of the increase in lean body mass associated with GH treatment (25–28). The increase in creatinine excretion and improvement in strength in the GH-treated subjects in the present study also are consistent with increased muscle mass. In previous studies, GH was ineffective in promoting strength gains in older subjects involved in a strength training program (16, 29). However, the strength improvement we observed is consistent with the modest increases in strength associated with GH treatment in GH-deficient adults (25, 27, 30), although changes in strength have not always achieved statistical significance (27).

For reasons outlined in the introduction, we had hypothesized that administration of GH would stimulate myofibrillar protein synthesis in skeletal muscle of older subjects. The hypothesis was not supported. Previous studies of the effect of GH on muscle protein synthesis in humans have produced varying results. Fryburg et al. (11, 12) reported a large increase in the incorporation of phenylalanine into forearm tissues in response to an infusion of GH in healthy young volunteers. The incorporation of phenylalanine into the forearm tissues should reflect mostly muscle protein synthesis. Copeland and Nair (10) did not find an increase in strength.
the incorporation of phenylalanine into leg tissues during a single iv infusion in healthy young volunteers. The most likely explanation for these discrepant results is that Fryburg et al. infused the GH for a longer period of time (6 h) than Copeland and Nair (3.5 h), and achieved higher GH levels during the infusions (30-35 ng/mL vs. 12-20 ng/mL). The GH levels achieved in the present study also were somewhat lower than those obtained by Fryburg et al. Yarasheski et al. also had lower GH levels than we did.

The acute study confirms that GH does not alter whole-body protein breakdown (7-10, 12, 14-16, 31). The lack of effect of GH on 3-methylhistidine excretion in the present study and in previous research (16, 31) also indicates that whole-body myofibrillar protein breakdown is unaltered by GH. Our acute study also confirms that GH reduces whole-body leucine oxidation (7-10, 12, 14). Most previous studies have indicated that GH increases the whole-body incorporation of labeled leucine (7-10, 16) or 15N-glycine (14, 31, 37) into proteins, but Fryburg and Barrett (12) did not find a significant effect of GH on whole-body leucine incorporation into proteins, even though amino acid incorporation into forearm tissues was markedly increased. The overall conclusion from these whole-body studies is that GH causes protein accretion by promoting amino acid incorporation into proteins over amino acid oxidation, without altering proteolysis.

The anabolic effects of GH may be mediated in part by its stimulation of IGF-I production. The circulating IGF-I concentrations approximately doubled within 10 h of a single GH injection, and early morning IGF-I concentrations increased approximately 50% in response to repeated GH injections. Fryburg (33) reported that IGF-I increased forearm protein synthesis, but the increase in IGF-I levels was much greater than that observed in the present study. Only a very large dose of IGF-I inhibited forearm proteolysis (33).

In general, it appears that a high IGF-I concentration promotes whole-body protein synthesis (9, 34, 35), although it may inhibit protein synthesis acutely if amino acid levels decrease (36-38). In acute studies, high IGF-I levels may reduce (37, 38) or not affect (34, 36) whole-body proteolysis. After several days of elevated IGF-I levels, whole-body protein synthesis is unchanged (9, 35). IGF-I can inhibit leucine oxidation (9, 35, 37) and urea excretion (39), but leucine oxidation is not always reduced by IGF-I in short-term studies (34, 36, 38). All of these whole-body studies had increases in circulating IGF-I levels of more than 2-fold. Thus the lack of effect of elevated IGF-I levels on whole-body protein synthesis in the present study probably reflects the smaller increase in IGF-I levels rather than a resistance to IGF-I in older subjects.

The present results raise the question of how there could be an increase in lean body mass and muscle mass during GH treatment without a significant reduction in proteolysis or a stimulation of protein synthesis. One potential explanation is that only a small increase in muscle protein synthesis was needed to produce the observed increase in muscle mass. A typical subject would normally synthesize approximately 4 kg of myofibrillar proteins over a 3-month period if we used a higher fractional rate of synthesis for this calculation than the value reported in the present paper because myofibrillar synthesis is faster in fed subjects (2). A gain in muscle mass of 3.3 kg, as observed in the present study, would increase the myofibrillar protein mass by about 0.4 kg. Thus a sustained 10% increase in the daily myofibrillar synthesis, in the absence of any change in protein breakdown, could have produced the observed increase in muscle mass. A 10% effect is too small to detect with the variability of the protein synthesis method. According to our previous research (1-3), complete rejuvenation of the myofibrillar protein synthesis rate in older subjects would require a 40% stimulation. Thus although we cannot exclude a small increase in protein synthesis as the mechanism of the increased muscle mass during GH treatment, we can conclude that reduced activity of the GH/IGF-I axis is an unlikely explanation for the slowing of myofibrillar protein synthesis in old age.

Acknowledgments

We thank Sangeeta Mehta, Dave Robson, and the staff of the Clinical Research Center for their technical assistance. We thank Judy Canham and Dr.Neil Gesundheit of Genentech, Inc. for providing the GH and placebo.

References


TABLE 4. Whole-body and myofibrillar protein metabolism after 3 months of either placebo or GH treatment

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<thead>
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<tr>
<td>Protein → leucine</td>
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<td>152±5</td>
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<tr>
<td>(µmol x h^-1 x kg LBM^-1)</td>
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<tr>
<td>Leucine oxidation</td>
<td>30±3</td>
<td>23±2</td>
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<tr>
<td>(µmol x h^-1 x kg LBM^-1)</td>
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<tr>
<td>Leucine → protein</td>
<td>129±4</td>
<td>128±6</td>
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<tr>
<td>(µmol x h^-1 x kg LBM^-1)</td>
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<tr>
<td>3-methylhistidine excretion</td>
<td>195±28</td>
<td>152±21</td>
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<tr>
<td>(µmol/g creatinine excretion)</td>
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<tr>
<td>Myofibrillar protein synthesis (%/h)</td>
<td>0.033±0.005</td>
<td>0.044±0.011</td>
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Values are mean ± one SE.

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<tr>
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<th>Protein + leucine</th>
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<th>3-methylhistidine excretion</th>
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<tr>
<td>(pmol * h^-1 * kg LBM^-1)</td>
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<td>(pmol * h^-1 * kg LBM^-1)</td>
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<td>(pmol/c creatinine excretion)</td>
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