Recombinant Human Growth Hormone and Recombinant Human Insulin-Like Growth Factor I Diminish the Catabolic Effects of Hypogonadism in Man: Metabolic and Molecular Effects*

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ABSTRACT

Severe gonadal androgen deficiency can have profound catabolic effects in man. Hypogonadal men develop a loss of lean body mass, increased adiposity, and decreased muscle strength despite normal GH and insulin-like growth factor I (IGF-I) concentrations. We designed these studies to investigate whether GH or IGF-I administration to male subjects with profound hypogonadism can diminish or abolish the catabolic effects of testosterone deficiency. Moreover, we also examined the nature of the interactions among GH, IGF-I, and androgens in specific genes of the im system. A group of 13 healthy subjects (mean age, 22 ± 1 yr) was studied at baseline (D1) and 10 weeks after being made hypogonadal using a GnRH analog (GnRHa; D2). At 6 weeks from baseline they were started on either recombinant human (rh) IGF-I (60 μg/kg, sc, twice daily) or rhGH (12.5 μg/kg, sc, daily) for 4 weeks. On each study day subjects had infusions of L-[13C]leucine; indirect calorimetry; isokinetic dynamometry of the knee extensors; determination of body composition (dual energy x-ray absorptiometry) and hormone and growth factor concentrations, as well as percutaneous muscle biopsies. Their data were compared with those of previously studied male subjects who received only GnRHa.

Administration of rhIGF-I and rhGH to the hypogonadal men had similar effects on whole body metabolism, with maintenance of protein synthesis rates, fat oxidation rates, and fat-free mass compared with the eugonadal state, preventing the decline observed with hypogonadism alone. This was further amplified by the molecular assessment of important genes in muscle function. During rhIGF-I treatment, im expression of IGF-I declined, and IGF-binding protein-4 increased, similar to the changes during GnRHa alone. However, rhGH administration was associated with a marked increase in IGF-I and androgen receptor messenger ribonucleic acid concentrations in skeletal muscle with a reciprocal decline in IGF-binding protein-4 expression in the hypogonadal men. The gene expression for myostatin did not change. These effects were accompanied by a much greater increase in plasma IGF-I concentrations after rhIGF-I (225 ± 32 vs. 768 ± 117 μg/L) compared with the concentrations achieved during rhGH (217 ± 20 vs. 450 ± 19 μg/L). We conclude that 1) rhGH and rhIGF-I both may be beneficial in preserving lean body mass and sustaining rates of protein synthesis during states of severe androgen deficiency in man; 2) GH may affect the im IGF system via an a


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GH, INSULIN-LIKE growth factor-I (IGF-I), and androgens all share a variety of anabolic actions in man. GH potently and selectively stimulates nitrogen retention, increases measures of whole body protein synthesis, and stimulates accretion of lean body mass in both GH-deficient (GHD) and GH-sufficient subjects (1–3), similar to the effects of IGF-I (4–9). GH and IGF-I also promote linear growth (10, 11). Both aromatizable and nonaromatizable androgens enhance measures of protein anabolism similar to those affected by GH and IGF-I. Testosterone and oxandrolone, a nonaromatizable androgen, have been shown to markedly increase measures of skeletal muscle protein synthesis in elderly and young subjects (12, 13). Treatment with testosterone increased measures of whole body protein synthesis and protein degradation with a net anabolic effect in prepubertal boys (14), whereas suppression of the GnRH axis in young men with a GnRH analog (GnRHa) markedly diminished measures of protein synthesis (15), the latter independently of any changes in the GH/IGF-I axis.

The effects of these hormones on lipid and carbohydrate metabolism are more divergent however. GH or IGF-I treatment of GHD subjects increases lean body mass and reduces fat mass (3); however, these effects of IGF-I on body composition are different from those of GH, as there are no IGF-I receptors in adipose tissue (16). GH is more potent than IGF-I in increasing lipid oxidation and decreasing the percent fat mass (3). Whereas GH therapy is associated with a subtle, but significant, increase in insulin concentrations and hence relative insulin resistance (17), IGF-I has insulin-like effects, lowering glucose concentrations despite suppression of circulating insulins (18, 19). These effects of IGF-I, which are
clearly dose dependent, are probably mediated through either the insulin receptor or its own IGF-I receptor (20, 21). Androgenic compounds, on the other hand, have been found to have synergistic effects with GH on lipolysis (22). Whether IGF-I has similar synergy of effects with testosterone on lipid metabolism has yet to be studied, however.

To investigate the nature of these hormonal interactions we designed the present studies with the following aims. 1) Can GH or IGF-I administration affect measures of protein and lipid metabolism and alter body composition in the absence of testosterone in males? 2) Can GH or IGF-I treatment decrease or abolish the catabolic effects of androgen deficiency in man? 3) What are the interactions of GH/IGF-I, in the absence of testosterone, in the molecular environment of skeletal muscle? To accomplish this, a group of young eugonadal men was rendered hypogonadal pharmacologically and studied before and after treatment with recombinant human (rh) GH or rhIGF-I.

**Subjects and Methods**

**Subjects**

These studies were approved by the Nemours Children’s Clinic clinical research review committee and the Baptist Medical Center institutional review committee. A group of 13 healthy young males participated in these studies after informed written consent was obtained. Eight received the combined treatment of GnRHα/rhIGF-I (mean ± sem age, 22.2 ± 0.8 yr), and 5 received GnRhα/rhGH (mean ± sem age, 22.1 ± 1 yr). They were all within 5% of ideal body weight (Metropolitan Life Insurance Tables).

**Study design**

For 3 days before admission to our Clinical Research Center, each subject consumed a weight maintenance diet consisting of approximately 35-40 Cal/kg and 1.7 g/kg protein-day. Subjects were instructed to keep the same pattern of weekly exercise during these studies.

The afternoon before the isotope tracer studies, isokinetic dynamometry of the left knee extensors and flexors was performed using a Biodex Dynamometer (Biodex Corp., Shirley, NY). After a 10-min training session, followed by 30 min of rest, maximum torque production and work measures were recorded for isometric and isokinetic tests. Isometric tests were performed with 5 contractions for 5 s each, with 10 s of rest between contractions, with the knee placed at 45° of flexion. Isokinetic tests were performed for knee extension and flexion at 60°/s for 5 repetitions, and at 180°/s for 21 repetitions. Subjects also underwent body composition analysis using sum of skinfolds measurements and dual emission x-ray absorptiometry (DEXA; model 2000, Hologic, Inc., Waltham, MA).

On the morning of the first study (D1), after a 14-h overnight fast, two iv heparin locks were placed, one in the antecubital vein for the infusion of isotopes and another in a contralateral hand vein kept heated for 180°/s for 21 repetitions. Subjects also underwent body composition tests, with the knee placed at 45° of flexion. Isokinetic tests were performed with 5 contractions for 5 s each, with 10 s of rest between contractions. Isometric tests were recorded for isometric and isokinetic tests. Isometric tests were performed with 5 contractions for 5 s each, with 10 s of rest between contractions, with the knee placed at 45° of flexion. Isokinetic tests were performed for knee extension and flexion at 60°/s for 5 repetitions, and at 180°/s for 21 repetitions.

**Blood and breath samples**

During the isotopic infusion, blood samples were withdrawn to measure the isotopic enrichments of α-ketosioacapric acid of leucine ([13C]KIC) at −20, 160, 180, 200, and 240 min. Testosterone, free testosterone, IGF-I, IGF-binding protein-3 (IGFBP-3), insulin, and glucose concentrations were measured in blood samples withdrawn three times during the 240 min of the tracer infusions. Serum GH concentrations were measured at 10 min intervals during the 4-h studies. Breath samples were obtained for the measurement of expired labeled CO2 at −20, −10, −5, 160, 180, 200, and 220 min. A small aliquot of the urine collected during the 4-h morning study was used for determination of urea nitrogen concentration.

**Assays**

Plasma enrichment of [13C]KIC was determined at the Nemours metabolic core laboratory by mass chromatography mass spectrometry as previously described (24, 25). [13C]O2 was measured by isotope ratio mass spectrometer as described previously (26). All insulin, testosterone, free testosterone, IGF-I, IGF-binding protein-3 (IGFBP-3), insulin, and glucose concentrations were measured in blood samples withdrawn three times during the 240 min of the tracer infusions. Serum GH concentrations were measured at 10 min intervals during the 4-h studies. Breath samples were obtained for the measurement of expired labeled CO2 at −20, −10, −5, 160, 180, 200, and 220 min. A small aliquot of the urine collected during the 4-h morning study was used for determination of urea nitrogen concentration.

**Body composition**

Fat-free mass (FFM) and percent fat mass were measured using DEXA and the tissue bar as well as by the sum of skin folds as described previously (27).
Assessment of gene expression in muscle biopsy samples

Total ribonucleic acid (RNA) was collected from muscle biopsy samples using RNAzol B as previously described (15). Total RNA from the muscle biopsy sample was incubated with reverse transcriptase (Reverse Transcription System, Promega Corp., Madison, WI) to produce complementary DNA. A standard qualitative RT-PCR method was used for the measurement of transcripts for the androgen receptor, IGF-I, IGFBP-4, and myostatin with glyceraldehyde phosphate dehydrogenase (GAP) serving as the internal control. PCR products were run on an agarose gel, blotted to Nytran filters (Schleicher & Schuell, Inc., Keene, NH) and hybridized with a 32P-labeled oligonucleotide contained in the DNA fragment. Table 1 summarizes the sense and antisense primers, DNA fragment size, and cycle number for each gene. These cycle numbers were chosen from previous titration assays that showed both GAP and the gene in question to be on the linear phase of the PCR assay. Actin and myosin were measured by a ribonuclease protection assay as previously described (15). For the rhIGF-I group there were six subjects available, and for the rhGH group there were five. The myostatin assay in the rhGH group was only available in four subjects.

Calculations

Leucine kinetics. Standard isotope dilution methods using the essential amino acid leucine were used in these experiments. Plasma enrichments of [1-13C]KIC were used as the index of intracellular enrichment of leucine using the reciprocal pool model (24, 28). All estimates were made at near steady state, between 160–240 min of infusion. The rate of substrate oxidation rates. Combustion equations calculate the oxidation of substrates (sugars, lipids, and proteins) from the rates of O2 and CO2 exchanged and total nitrogen excretion in the urine as previously described (29).

Body composition. DEXA scan data were used to estimate body composition changes. FFM represents the sum of nonfat mass plus bone mineral content as calculated using the tissue composition reference bar of Hologic, Inc.

Comparisons with subjects treated with GnRHa alone or rhGH/rhIGF-I alone. The responses of the hypogonadal subjects treated with rhIGF-I or rhGH were compared with those of eight healthy adult male subjects (mean ± SEM age, 23.2 ± 0.5 yr; body mass index, 23.8 ± 0.8) treated solely with GnRHa for 10 weeks, some of whom have been reported previously (15). The effects of rhIGF-I or rhGH treatment in hypogonadal males in this study were also compared with those of eight GH-deficient adult subjects (six men and two women; mean ± SEM age, 23.5 ± 1.1 yr; body mass index, 28.2 ± 2.2) treated with similar doses of rhGH (12.5 μg/kg/day, sc) or rhGH-I (60 μg/kg, sc, twice daily) for 8 weeks, each reported previously (3). Data for the hypogonadal and GHD subjects were gathered identically to those in the present study.

Isotopes and drugs

L-[1-13C]Leucine (99% enriched; Cambridge Isotopes, Andover, MA) were determined to be sterile and pyrogen free and were mixed with 0.9% sterile and nonpyrogenic sodium chloride. rhIGF-I (10 mg/mL) and rhGH (Nutropin; 5 mg/mL) were provided by Genentech, Inc. Lupron was provided by TAP Pharmaceuticals, Inc.

Statistical analysis

Results are expressed as the mean ± SEM. Paired Student’s t test was used to estimate differences between baseline studies and rhGH-I or rhGH treatments for all parameters tested. The statistical analysis used to test for treatment differences was ANOVA with repeated measures on one factor. Significance was established at P = 0.05. Significance levels less than 0.05 were followed up with appropriate post-hoc comparison procedures using Bonferroni corrections. Two series of analyses were conducted: one for the current study with two groups, and a similar analysis using the data from a third group previously studied by us (15).

Results

Body composition and muscle strength (Table 2)

There was a significant weight increase in hypogonadal subjects treated with rhIGF-I (2 kg; P = 0.007), but no weight change after rhGH (P = 0.93); the latter is similar to what we observed during GnRHa treatment alone. FFM did not change after rhIGF-I or rhGH treatment of hypogonadal subjects contrary to the decrease in FFM observed during hypogonadism alone (P < 0.01 for GnRHa vs. GnRHa/rhIGF-I, by ANOVA; Fig. 2). This also contrasts with the increase in FFM observed after rhIGF-I or rhGH treatment in GHD subjects (3). The percent fat mass was significantly increased despite rhGH-I treatment (P = 0.003), and a similar trend was observed after rhGH (P = 0.07).

There was a comparable decline in muscle strength of the quadriceps as measured by the knee extension peak torque at 60 or 180°/s with rhGH and rhGH-I treatment, yet it only achieved statistical significance in the rhGH-I group. This may be due to the great variability in the data and may or not be biologically significant. A greater number of subjects will need to be studied to better assess this issue.

Protein kinetics (Table 3)

Enrichments of the plasma samples for [13C]KIC were: in the rhIGF-I group, 4.610 ± 0.220 mole % enrichment on D1 and 4.454 ± 0.320 on D2; and in the rhGH group, 4.911 ± 0.360 on D1 and 5.019 ± 0.280 on D2. The 13CO2 enrichments were: in the rhIGF-I group, 7.861 ± 0.519 atom % excess on D1 and 8.828 ± 0.453 on D2; and in the rhGH group, 7.858 ± 0.438 on D1 and 8.909 ± 0.728 on D2. There were no significant changes in rates of proteolysis (leucine rate of appearance) and protein synthesis (nonoxidative leucine disposal)

### TABLE 1. rt-PCR primers, DNA fragment sizes, and cycle number for each gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Antisense primer</th>
<th>Sense primer</th>
<th>DNA fragment size (bp)</th>
<th>Cycle no.</th>
</tr>
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<tbody>
<tr>
<td>Androgen receptor</td>
<td>CCGCGAAATGATCTATCATGAC</td>
<td>GATGCTCTAGCTGCCCCCCAGA</td>
<td>627</td>
<td>26</td>
</tr>
<tr>
<td>IGF-I</td>
<td>CTCCTGGGCTTTGGCAAGG</td>
<td>AAATCGAACCTTTGAGGAC</td>
<td>395</td>
<td>25</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>TGGAGTTGCCGGCTCTTGG</td>
<td>CCATCCAGGAAAGCCTGGCA</td>
<td>310</td>
<td>25</td>
</tr>
<tr>
<td>Myostatin</td>
<td>TAGAGGTAAACGACACATC</td>
<td>ATCAATGGAAACATGCAAT</td>
<td>860</td>
<td>30</td>
</tr>
<tr>
<td>GAP 1*</td>
<td>GTTATGCGGATTTCATGGT</td>
<td>GGATCAGCCGATTGGTT</td>
<td>206</td>
<td>25</td>
</tr>
<tr>
<td>GAP 2</td>
<td>TCCACCACTCTGCTGCTGTA</td>
<td>GGTATACCTGGAAAGAGCTCAT</td>
<td>473</td>
<td>26, 30</td>
</tr>
</tbody>
</table>

* We used two different sets of primers for GAP, so that DNA fragment sizes would be sufficiently different to separate on agarose gel from the gene being tested. For the larger DNA fragments, AR, and myostatin, we used the larger GAP (473 bp). For IGF-I and IGFBP-4 we used the smaller GAP (206 bp).
Evaluate the effects of growth hormone (GH) and IGF-I on body composition, muscle strength, and hormonal changes in hypogonadal subjects treated with GnRHa/GnRHa/rhIGF-I or GnRHa/rhGH.

**Substrate oxidation and energy expenditure rates (Table 4)**

There were no changes in the rates of carbohydrate, protein, and lipid oxidation as measured by indirect calorimetry after rhGH treatment, whereas protein oxidation rates increased 43% (P = 0.001) in rhIGF-I-treated hypogonadal males together with an increase in resting energy expenditure. These data are in contrast with the decreased lipid oxidation rates observed after 10 weeks of induced hypogonadism and the increased lipid oxidation rates after rhGH treatment of GHD patients (3, 15).

**Circulating hormones, growth factors, and substrates (Table 5)**

GnRHa therapy induced a reduction of testosterone and free testosterone levels to less than 15% of baseline levels in both treatment groups. rhGH and rhIGF-I treatments of hypogonadal subjects were associated with a significant increase in plasma IGF-I levels, but the increase was greater after rhIGF-I. After both rhGH and rhIGF-I treatments, IGFBP-3 increased significantly (46% and 31%, respectively). This contrasts with observations in GHD subjects, in whom IGFBP-3 increased after rhGH, but not rhIGF-I, treatment (3).

Insulin levels were significantly decreased after rhIGF-I treatment (−48%; P = 0.002) and were significantly increased after rhGH (114%; P = 0.02), similar to those in the hypogonadal state (15).

**Muscle messenger RNA (mRNA) expression of growth factors**

There was a significant decrease in mRNA gene expression for im IGF-I in the testosterone-deficient subjects treated with rhIGF-I, similar to that observed in the GnRHa-treated subjects reported previously (15). However, there was a substantial increase in the expression of IGF-I mRNA after rhGH treatment of similar subjects (Figs. 3 and 4). mRNA expressions of the androgen receptor, IGFBP-4, myostatin, actin, and myosin are summarized in Table 6. The expression of the androgen receptor was not significantly different from baseline in the hypogonadal men treated with rhIGF-I, yet it was increased during treatment with rhGH. IGFBP-4, on the other hand, was significantly increased in the rhIGF-I-treated group, similar to that in the GnRHa-treated group (15), yet it did not increase during rhGH treatment. The expressions of myostatin, actin, and myosin message were unchanged during systemic rhIGF-I or rhGH treatment in these testosterone-deficient men.
TABLE 3. Whole body leucine kinetic rates (micromoles per kg/min), including the rate of appearance (Ra; a measure of proteolysis), oxidation, and nonoxidative leucine disposal (NOLD; a measure of protein synthesis) in healthy males before (D1) and after GnRHa/rhIGF-I or GnRHa/rhGH treatment (D2)

<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>D1 (mean ± SD)</td>
<td>D2 (mean ± SD)</td>
<td>D1 (mean ± SD)</td>
</tr>
<tr>
<td>Ra</td>
<td>2.15 ± 0.23</td>
<td>1.87 ± 0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oxidation</td>
<td>0.40 ± 0.06</td>
<td>0.35 ± 0.09</td>
<td>0.43</td>
</tr>
<tr>
<td>NOLD*</td>
<td>1.74 ± 0.20</td>
<td>1.52 ± 0.19</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Data from men treated with GnRHa only, published previously, are included for comparison (15).

\( P < 0.04 \) for differences between the GnRHa group vs. GnRHa/rhIGF-I and GnRHa/rhGH groups (by ANOVA).

TABLE 4. Substrate oxidation and resting energy expenditure (REE) rates at baseline (D1) and after rhIGF-I or rhGH treatment (D2) of hypogonadal men (GnRHa-induced)

<table>
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</thead>
<tbody>
<tr>
<td></td>
<td>D1 (mean ± SD)</td>
<td>D2 (mean ± SD)</td>
<td>D1 (mean ± SD)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.1 ± 0.8</td>
<td>7.3 ± 2.1</td>
<td>0.30</td>
</tr>
<tr>
<td>Oxidation</td>
<td>6.7 ± 0.5</td>
<td>5.8 ± 0.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Lipid oxidation</td>
<td>18.5 ± 1.6</td>
<td>12.8 ± 1.6</td>
<td>0.05</td>
</tr>
<tr>
<td>REE</td>
<td>30.1 ± 2.0</td>
<td>27.4 ± 1.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

All units are kilocalories per kg FFM/day (FFM by DEXA). Data from men treated with GnRHa only, published previously, are included for comparison (15).

TABLE 5. Changes in circulating hormones and substrates in men with GnRHa-induced hypogonadism at baseline (D1) and after rhIGF-I treatment or rhGH (D2)

<table>
<thead>
<tr>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1 (mean ± SD)</td>
<td>D2 (mean ± SD)</td>
<td>D1 (mean ± SD)</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>535 ± 141</td>
<td>31 ± 9</td>
<td>0.02</td>
</tr>
<tr>
<td>Free testosterone (pg/mL)</td>
<td>23.1 ± 3.1</td>
<td>2.1 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>IGF-I (µg/L)</td>
<td>227 ± 44</td>
<td>291 ± 60</td>
<td>0.08</td>
</tr>
<tr>
<td>IGFBP-3 (mg/L)</td>
<td>3.3 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fasting insulin (µU/mL (pmol/L))</td>
<td>4.8 ± 1.3</td>
<td>5.8 ± 1.2</td>
<td>0.14</td>
</tr>
<tr>
<td>Fasting glucose [mg/100 mL (mmol/L)]</td>
<td>88 ± 4</td>
<td>88 ± 2</td>
<td>(29 ± 8) (35 ± 7)</td>
</tr>
</tbody>
</table>

Systeme International units for insulin and glucose are given in parentheses. Data from men treated with GnRHa only published previously, are included for comparison (15).

Safety

Treatment with either rhGH or rhIGF-I was well tolerated by the study subjects. There was evidence of mild fluid retention shortly after rhIGF-I initiation, which improved as the treatment progressed. One subject had a hypoglycemic reaction after a dose of rhIGF-I when he skipped breakfast after the injection. Some subjects had transient tachycardia after the first few doses of rhIGF-I. Overall, there were no significant side-effects after hormone treatment. All subjects completed the 10-week studies.

Discussion

Under these controlled experimental conditions we were able to assess the effects of both rhIGF-I and rhGH on a multiplicity of metabolic pathways without the confounding effects of androgenic steroids. Our results show that even though neither IGF-I nor GH had the potent protein-anabolic effects observed in normal and GHD subjects (2, 3, 6, 9), the administration of these hormones nonetheless diminished the protein-wasting effects and helped preserve body composition (decreasing the loss of lean tissue) in severely hypogonadal men. In profound testosterone deficiency we previously observed marked changes in body composition, with decreased lean body mass, increased adiposity, decreased lipid oxidation rates, and decreased rates of whole body protein synthesis (15). These negative effects were markedly diminished in the present study in which these men were treated with rhGH or rhIGF-I, with better preservation of lean body mass and protein synthesis rates when they were treated with either of these peptides. However, we did find a dichotomy of the effects of these hormones when we examined skeletal muscle. There was a marked difference in the specific effects of these hormones on the expression of IGF-1, IGFBP-4, and the androgen receptor, suggesting that IGF-I and GH have differential effects on skeletal muscle.

Body composition and protein metabolism

Induction of hypogonadism in men with GnRHa results in a decrease in lean body mass and an increase in fat mass (15). These effects were independent of any change in systemic GH production or in IGF-I concentrations, suggesting that androgens, per se have potent anabolic actions in man. When similar hypogonadal men were concomitantly treated with rhGH, there were no changes in body weight, yet a slight, but
A significant increase in weight was observed after rhIGF-I administration. Even though hypogonadism resulted in the expected increase in adiposity (as measured by percent fat mass), there was better preservation of FFM, as measured by DEXA, after treatment with either rhIGF-I or rhGH.

Even though we did not measure changes in total body water after treatment, it is unlikely that the differences observed are related solely to different water contents. Both GH and IGF-I have been observed to cause fluid retention and even mild edema, especially shortly after initiation of treatment (10, 30, 31). The data available in humans regarding the accrual of lean body mass after GH treatment do not fully separate how much of the change is due to changes in total body water vs. lean soft tissue. However, data in experimental animals treated with GH demonstrate that the net gain in lean body mass after GH treatment is in large part in skeletal muscle tissue. For instance, food-restricted rats treated with GH showed an increase in total muscle protein and an increase in skeletal muscle protein after GH (32). Castrated pigs showed marked accretion of both visceral and skeletal protein after 42 days of GH treatment (33), and ewes thus treated for 56 days showed a 22% decline in fat tissue mass and a 36% in protein accretion (34). Taken in aggregate, these data suggest that the changes in lean body mass are probably in part associated with an increase in lean soft tissue separate from body water. Further studies using labeled water and other body composition tools will need to be performed to fully characterize these changes.

In addition, rates of protein synthesis (as measured by stable isotopes studies) were unchanged from baseline rates after treatment with either rhIGF-I or rhGH. This is in sharp contrast to the marked decrease in rates of nonoxidative leucine disposal (a measure of whole body protein synthesis) observed after 10 weeks of sustained hypogonadism induced by GnRHa, as reported previously (15). There were, however, differences between the effects of rhIGF-I and rhGH, with no change in protein oxidation rates in the rhGH-treated subjects yet an increase in the rhIGF-I-treated ones, the latter similar to what we observed in men treated with GnRHa alone (15). The latter suggests that systemic IGF-I cannot fully overcome the protein catabolic effects of hypogonadism as well as GH. These results contrast with what we observed in eugonadal subjects; after rhGH treatment there is a 20% increase in protein synthesis rates in healthy volunteers, and there is an approximately 13% increase after rhIGF-I (2, 4, 9, 19), whereas in GHD patients the increases observed are approximately 36% and 12% after rhGH and rhIGF-I, respectively (3). Taken in aggregate, the present data suggest that IGF-I and GH can preserve lean body mass and sustain protein synthesis rates in hypogonadal individuals. The data also suggest that androgens may be needed for the full effects of GH and IGF-I on protein pools to be observed. The latter, however, would require confirmation with further studies by adding testosterone back to similarly studied patients after GnRHa/rhGH or GnRHa/rhIGF-I, for example.

### Table 6. Gene expression in muscle biopsy samples of hypogonadal mean treated with either rhGH (n = 5) or rhIGF-I (n = 6)

<table>
<thead>
<tr>
<th>Gene</th>
<th>rhIGF-I</th>
<th>rhGH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>Androgen receptor</td>
<td>6.4 ± 1.9</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>5.2 ± 0.7</td>
<td>10.0 ± 1.0*</td>
</tr>
<tr>
<td>Myostatin</td>
<td>6.0 ± 1.5</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Actin</td>
<td>4.5 ± 0.3</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Myosin</td>
<td>6.0 ± 1.8</td>
<td>7.5 ± 3.6</td>
</tr>
</tbody>
</table>

* P ≤ 0.05, by paired t test.
Carbohydrate and lipid metabolism

After 10 weeks of severe hypogonadism, otherwise healthy men showed substantial decreases in the rate of oxidation of lipids, as measured by indirect calorimetry (15). These rates, however, remained invariant in the present study when hypogonadal men were treated with either rhGH or rhIGF-I. It is likely that the mechanisms of the effects of these hormones preventing the decrease in fat oxidation differ, with a direct GH effect increasing lipid oxidation and IGF-I’s action mediated through the suppression of insulin concentrations (6, 35). These observations are different from the significant increase in lipid oxidation rates observed after rhGH therapy in GHD adults (3, 5) and suggest that, as in the rat, androgens are necessary for the full lipolytic effect of GH (22).

Measures of carbohydrate metabolism showed a trend toward greater carbohydrate oxidation rates in the rhIGF-I treated subjects, even though the trend did not reach statistical significance ($P = 0.06$). rhIGF-I has been shown both to increase carbohydrate oxidation in healthy eugonadal subjects (36) and to have no impact in GH-deficient individuals (3, 5). rhGH treatment, on the other hand, was associated with decreased carbohydrate oxidation rates in GH deficiency (3, 5). Similar to the divergent effects on lipids, IGF-I and GH work differently on glucose metabolism, with increased insulin resistance after GH (17) and an insulin-like effect after IGF-I administration in humans (18, 19). This dichotomy of effects is also evident in the present experiments in testosterone-deficient men; when treated with rhIGF-I or rhGH, their fasting glucose concentrations remained invariant, but after rhIGF-I, insulin concentrations were lower than baseline, and they were significantly higher after rhGH. Our present data indicate that androgens are probably not critical for the GH/IGF-I system to affect glucose metabolism.

Muscle strength

The administration of physiological or supraphysiological doses of testosterone has been shown to increase skeletal muscle strength in both elderly and young men (12, 37), and the induction of a hypogonadal state with GnRHa results in a quantifiable loss of muscle strength, as measured by isokinetic dynamometry (15). However, GH and IGF-I have been shown to have variable effects on altering functional muscle strength. Neither rhGH nor rhIGF-I had any effect on muscle strength in GHD subjects treated for 8 weeks (3). However, rhGH was shown to increase isometric and isokinetic muscle strength in GHD adults after 2 yr of treatment (38). rhGH did not alter muscle strength in healthy young men (39), and in elderly subjects its was associated with either no effect on strength (40) or an increase in strength without a change in myofibrillar protein synthesis rates (41). During treatment of testosterone-deficient men with rhGH or rhIGF-I, this variability in the effect on muscle strength was again apparent, with a decrease in functional measures of muscle strength after treatment with either peptide, reaching significance only after rhIGF-I, probably due to the variability in the data. A larger number of subjects will need to be studied to better ascertain the roles of these hormones in preventing the relative muscle weakness of the hypogonadal state.

Intramuscular growth factor genes

IGFBP-4 is secreted by skeletal muscle cell lines and is a negative regulator of IGF-I in muscle (42). IGFBP-4 mRNA levels are very high in proliferating myoblasts, but decrease as differentiation proceeds in the presence of IGF-I and insulin (43). A number of in vitro studies suggest that IGFBP-4 functions only as an inhibitor of IGF action, and its main function may be to protect cells from overstimulation by IGFs or to allow other signaling paths normally inhibited by IGF-I exposure to be activated (42). Data from previously reported males with severe hypogonadism showed a marked decline in the expression of IGF-I mRNA despite invariant systemic IGF-I concentrations and a reciprocal increase in IGFBP-4. The same results were observed here despite systemic rhIGF-I administration for 4 weeks to similar subjects. Interestingly, however, systemic administration of rhGH had very different effects, with a significant increase in IGF-I transcripts and a reciprocal decline in IGFBP-4. These differences were not due to greater IGF-I concentrations after rhGH, as rhIGF-I treatment resulted in higher IGF-I concentrations than those in rhGH-treated subjects. These results are the opposite of those observed in elderly men treated with rhGH, where no change in mRNA expression for in vivo IGF-I was observed after treatment with rhGH for 14 weeks (44). rhGH treatment, but not rhIGF-I, also increased the expression of the androgen receptor in the present experiments. The results after rhGH treatment in hypogonadal individuals are similar to those observed after testosterone treatment of elderly subjects (12) and suggest that GH is an important regulator of the in vivo IGF-I and androgen receptor systems, possibly through an IGF-I-independent mechanism. These data support the concept that the local paracrine production of IGF-I is more important than systemic IGF-I in mediating GH’s effects in muscle (45, 46). Neither actin nor myosin gene expression was affected by these changing hormonal milieu, suggesting that other mechanisms besides the effects in skeletal muscle proteins are operative in the development of muscle weakness during testosterone deficiency.

The lack of effects of rhGH and rhIGF-I on myostatin gene expression in the individuals studied here are indeed interesting. Myostatin, a novel regulatory protein first described by McPherron et al. (47), is expressed in both developing and adult mouse skeletal muscle, and mutations in the myostatin gene during neonatal development result in the hypermuscular phenotype (increased muscle fibers and decreased fat and bone mass) in both cows and mice (48, 49). Increased expression of the myostatin gene has been observed in human immune deficiency-infected, acquired immunodeficiency syndrome wasting patients (50), suggesting that myostatin is a negative regulator of muscle growth in humans. In the present set of experiments we observed no significant change in myostatin expression in skeletal muscle during rhGH or rhIGF-I treatment. Whether the lack of decrease in protein synthesis rates observed in these experiments in h-
prenatal men during rhIGF-I and rhGH treatment may be related to the lack of an increase in myostatin gene expression requires further study.

Summary and conclusions

In a model of severe androgen deficiency in men treated with rhGH or rhIGF-I we observed that 1) both hormones prevented the loss of lean body mass that occurs after induction of the hypogonadal state, and both preserved protein synthesis rates to baseline values; 2) measures of fat oxidation remained invariant during the hypogonadal state when subjects were treated with rhGH or rhIGF-I compared with the decreased oxidation of fat observed during the hypogonadal state; 3) rhGH was associated with increased and rhIGF-I with lower insulin concentrations, similar to what is observed in eugonadal subjects; and 4) rhIGF-I had no measurable effect on the im expression of different genes or growth factors during the hypogonadal state, yet rhGH increased the mRNA expression of IGF-I and androgen receptor and reduced that of IGFBP-4. We conclude that rhGH and rhIGF-I may both be beneficial in preserving lean body mass and sustaining rates of protein synthesis during states of severe androgen deficiency in man; GH may affect the im IGF system via a paracrine, local production of IGF-I; and androgens may be necessary for the full anabolic effect of GH/IGF-I in man. Future studies will need to determine whether rhGH or rhIGF-I plays a role in the treatment of men made hypogonadal pharmacologically or in hypogonadal men unable to take full testosterone replacement.

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References


