Lack of Effect of Recombinant Human Growth Hormone (GH) on Muscle Morphology and GH-Insulin-Like Growth Factor Expression in Resistance-Trained Elderly Men*

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ABSTRACT
Vastus lateralis muscle samples were obtained by needle biopsy from 18 healthy elderly men (65–82 yr) participating in a double blind, placebo (PL)-controlled trial of recombinant human GH (rhGH) and exercise and assessed for muscle morphology and skeletal muscle tissue expression of GH and insulin-like growth factors (IGFs). Subjects initially underwent progressive resistance training for 14 weeks and were then randomized to receive either rhGH (0.02 mg/kg BW-day, sc) or PL while undertaking a further 10 weeks of training. Muscle samples were obtained at baseline and at 14 and 24 weeks. The mean (±SEM) cross-sectional areas of type I and II fibers were similar (type I, 3901 ± 167 μm²; type II, 3985 ± 200 μm²) at baseline and increased (P < 0.01) by 16.2 ± 4.1% and 11.8 ± 3.8%, respectively, after the initial 14-week training period. After treatment (weeks 14–24), two-way repeated measures ANOVA revealed a main effect of time for type I (P < 0.01) and type II fibers (P < 0.05), but no group effect or interaction. The increase in cross-sectional area for the PL group was significant (P = 0.01) for type I (11.5 ± 3.6%) and approached significance (P = 0.06) for type II fibers (11.1 ± 5.9%). For rhGH, the change in type I (6.3 ± 5.9%) and II (7.1 ± 5.2%) fiber area was not significant. No apparent change in tissue GH receptor, IGF-I, IGF-I receptor, IGF-II, or IGF-II receptor messenger ribonucleic acids occurred as a result of exercise after the 14-week pretreatment period or after treatment with rhGH or PL. These results indicate that rhGH administration in exercising elderly men does not augment muscle fiber hypertrophy or tissue GH-IGF expression and suggests that deficits in the GH-IGF-I axis with aging do not inhibit the skeletal muscle tissue response to training. (J Clin Endocrinol Metab 81: 421–425, 1996)

IT HAS BEEN proposed that reduced activity of the GH-insulin-like growth factor I (GH-IGF-I) axis with normal aging may underlie characteristic somatic changes, including decreased lean and skeletal mass and increased adiposity (1, 2). These somatic changes resemble those observed in adults with complete GH deficiency and are reversed with GH replacement (3–5). Treatment of GH-deficient adults with recombinant human GH (rhGH) modestly improved muscle strength and maximal exercise capacity (3, 6, 7), suggesting that GH deficits may also contribute to age-related declines in muscle strength. However, we have recently shown that the maximal strength response of elderly men engaged in a program of resistance exercise was not enhanced by coadministration of rhGH (8). It remains possible that subtle changes in skeletal muscle may have occurred that were not detected by assessment of maximal isotonic strength. Studies in rats have shown that the expression of IGF-I messenger ribonucleic acid (mRNA) is dependent on GH (9), and increased diameter of type I and II muscle fibers results from rhGH administration (10). To assess the possible effects of resistance exercise and rhGH on muscle fiber morphology and mRNA expression of relevant GH-IGF genes, we took sequential percutaneous biopsies of the vastus lateralis muscle from the subjects in our previously reported study (8).

Subjects and Methods

The characteristics of the study group and exercise protocol have been previously described (8). Briefly, 18 healthy men, aged 65–82 yr (mean ± SEM, 70.3 ± 0.9 yr) completed the 24-week randomized placebo (PL)-controlled trial. Height and body weight were 176.2 ± 2.0 cm and 81.8 ± 2.9 kg, respectively. All volunteers were apparently healthy, having successfully completed a comprehensive screening procedure that included a health history questionnaire, physical examination, multiphasic laboratory profile, and maximal exercise stress test. The protocol was approved by the human subjects committee of Stanford University, and all subjects provided written consent.

The rhGH and exercise protocol consisted initially of 14 weeks of progressive resistance training, with 3 sessions/week. Each session consisted of a circuit of 10 exercises involving major upper and lower body movements. The program was designed to increase muscular strength and aerobic exercise capacity during the pretreatment period. Each subject underwent 24 weeks of resistance exercise and received either rhGH (0.02 mg/kg BW-day, sc) or placebo. After treatment (weeks 14–24), two-way repeated measures ANOVA revealed a main effect of time for type I (P < 0.01) and type II fibers (P < 0.05), but no group effect or interaction. The increase in cross-sectional area for the PL group was significant (P = 0.01) for type I (11.5 ± 3.6%) and approached significance (P = 0.06) for type II fibers (11.1 ± 5.9%). For rhGH, the change in type I (6.3 ± 5.9%) and II (7.1 ± 5.2%) fiber area was not significant. No apparent change in tissue GH receptor, IGF-I, IGF-I receptor, IGF-II, or IGF-II receptor messenger ribonucleic acids occurred as a result of exercise after the 14-week pretreatment period or after treatment with rhGH or PL. These results indicate that rhGH administration in exercising elderly men does not augment muscle fiber hypertrophy or tissue GH-IGF expression and suggests that deficits in the GH-IGF-I axis with aging do not inhibit the skeletal muscle tissue response to training. (J Clin Endocrinol Metab 81: 421–425, 1996)
muscle groups. Subjects performed 3 sets of 8 repetitions for each exercise at an initial intensity equal to 75% of their individual 1 repetition maximum (1-RM) values. After 14 weeks of training, subjects were randomly assigned to receive daily injections of either 0.02 mg/kg BW rhGH (Somatropin, Genentech, South San Francisco, CA; n = 10) or an equal volume of PL (Genentech; n = 8) while continuing to train for an additional 10 weeks. Subjects were instructed to maintain customary activity and dietary patterns throughout the 24-week training program.

Baseline plasma IGF-I concentrations were similar in both groups (106 ± 9 ng/mL), but doubled in the rhGH group after 10 weeks of rhGH treatment. In addition, IGFBP-3 increased in the rhGH group. Concentric muscle strength increased for all exercises, but there was no difference in muscle strength gain between rhGH and PL groups (8).

**Muscle biopsy**

Muscle samples of the left vastus lateralis m. were obtained using the needle biopsy technique (11). Specimens taken at 14 and 24 weeks were from the same location as that used at baseline, and two samples were obtained from each subject at each time point. The biopsy at the conclusion of the study was taken within 24 h of the last exercise bout and rhGH injection. One sample was oriented for transverse sectioning, mounted on a cork block in tragacanth gum, frozen in isopentane cooled by liquid nitrogen, and stored at −80°C for histochemical analysis. The second sample was placed in a plastic tube, frozen in liquid nitrogen, and stored at −80°C until analysis.

**Histomorphometry**

Transverse sections (10 µm) were cut in a cryostat (Histostat, model 975C, Cambridge Instruments, Buffalo, NY) at −20°C, stained for myofibrillar actomyosin adenosine triphosphatase, and classified as type I or II (12). No attempt in this study was made to analyze fiber subtypes. Biopsy samples from an individual were analyzed simultaneously to avoid interassay variances. The muscle fiber cross-sectional area (CSA) was determined from manual planimetry using the Bioquant Digitizing Morphometry program on a personal computer at a magnification of ×660 (R and M Biometrics, Nashville, TN). The areas of 40 type I and 40 type II muscle fibers were measured from each cross-section by the same investigator, who was blinded to group assignment and sample time point. Fibers selected were those without artifacts, with true transverse orientation and distinct cell borders, and away from the periphery of the sample (13). The coefficients of variation for repeated fiber area assessment (n = 10 biopsies) were 3.2% and 3.4% for types I and II, respectively.

**GH-IGF expression**

Total RNA was isolated from biopsy samples (3–10 mg tissue) using the RNA STAT-60 kit (Tel-Test B, Friendswood, Texas) and quantified by determining absorbance at 260 nm. We routinely obtained 2–10 µg total RNA. Levels of mRNAs of GH receptor (GHR), IGF-I, IGF-I receptor (IGF-IR), IGF-II, and IGF-II receptor (IGF-IR) were analyzed by RT-PCR. Total RNA (100 µg/mL) was reverse transcribed using random hexamers and murine leukemia reverse transcriptase at 37°C for 30 min, at 42°C for 10 min, and at 47°C for 5 min, and then heat inactivated at 99°C for 5 min. The complementary DNA (cDNA) samples were diluted 10-fold and used for PCR amplification, as we previously described (14-16). The cDNAs were amplified in a 5-µL PCR reaction mixture containing 50 µM dNTP, 1.0 mM/L primer, 0.1 µCi [α-32P]dCTP (Amersham Corp., Arlington Heights, IL), 0.125 µU T3 (or Tα) DNA polymerase. The DNA polymerase and reaction mixture was added at 65°C (hot start PCR procedure). The cDNA from IGF-II mRNA was amplified for 25 cycles at 94°C for 30 s and at 65°C for 60 s, followed by a 3-min extension at 72°C. The cDNAs as for IGF-I, IGF-IR, IGF-IR, and GHR were amplified for 30 cycles, whereas 18S ribosomal RNA (rRNA) was amplified concurrently for 11 cycles as an internal RT-PCR control. The primers were designed from different exons to amplify specific cDNA products that could be distinguished from those derived from genomic DNA contamination. The sizes of the expected PCR products were 165 bp (IGF-I), 265 bp (IGF-IR), 201 bp (IGF-II), 22 bp (IGF-IR), 185 bp (GHR), and 140 bp (18S ribosomal RNA). The PCR products were run on a 5% polyacrylamide-urea gel and analyzed by quantitative autoradiography.

The oligonucleotide primers used were as follows: GHR: 5'-primer in exon 9, 5'-AAC GGA TTT CGT CAT TTC CAA AGG-3'; 3'-primer in exon 10, 5'-ATC GCT TAG AAG TCT GCT GCT TGG TCA-3'; IGF-I: 5'- primer in exon 3, 5'-GGA ATT CCG CGG TCA GCT GGT GG-3'; 3'-primer in exon 4, 5'-GGA ATG TCT CTT AGA TCA CAG CTC CCG AAG-3'; IGF-II: 5'-primer in exon 5, GGA ATG TCT CTT ACC CCC CCA G-3'; 3'-primer in exon 8, 5'-GGA AGC TTA GTA CTT CTC CAG GGC GC-3'; IGF-IR: 5'-primer in exon 6, 5'-GAATG CTT GTA GAG ATG CTA TT-3'; 3'-primer in exon 7, 5'-CTG AGT CAC ATG AGG AGA GCG CAC GCA TGG T-3'; and 18S ribosomal RNA: 5'-primer, 5'-GGG ATC TCC TGC CAG TAG CAT AAT C G-3'; 3'-primer, 5'-GGG AAC TTA GAG GAG CGA GCG AAC AAA GG-3'. Mixed bases for both human and rat (mouse) are in parentheses. Underlined bases are restriction enzyme sites.

**Statistical analysis**

Data were analyzed with a statistical software package (Statview II, Abacus Concepts, Berkeley, CA). Analysis included standard descriptive statistics, linear regression, Student's t test, one-factor ANOVA, and two-way (group × time) repeated measures ANOVA. All tests were two-tailed, and an α level of 0.05 was adopted for significance. All values are expressed as the mean ± SEM.

**Results**

Although 18 men completed the study, biopsy specimens from 2 members of the rhGH group were unsuitable for histomorphometry, and these subjects have been deleted from the results. There was no difference in age, height, weight, type I or II fiber CSA, or muscle mRNA levels of GH-IGF axis gene products (GHR, IGF-I, IGF-IR, IGF-II, and IGF-IR) between rhGH (n = 8) and PL (n = 8) groups at baseline or before initiation of rhGH treatment.

At baseline, the CSAs of type I and II fibers were similar (type I, 3991 ± 167 µm²; type II, 3995 ± 200 µm²). By the end of the 14-week exercise pretreatment period, type I and II fiber CSAs increased (P < 0.01), with both fiber types displaying a similar percent change (type I, 16.2 ± 4.1%; type II, 11.8 ± 3.8%). After drug treatment (weeks 14–24), two-way repeated measures ANOVA revealed a main effect of time for type I (P < 0.01) and II fibers (P < 0.05), but no group effect or interaction (Fig. 1). Further, there was no difference between groups at week 14 or 24, or for percent change after treatment. The increase in CSA for PL group from weeks 14–24 was significant (P = 0.01) for type I fibers and approached significance (P = 0.06) for type II fibers. No significant change in GHR, IGF-I, IGF-IR, IGF-II, or IGF-IR mRNA levels occurred as a result of exercise following the 14-week pretreatment period. After randomization and treatment with rhGH or PL, no group effect, time effect, or interaction for any variable was observed (Table 1). Nevertheless, considerable variation was noted, especially for the rhGH-treated group, in IGF-I and IGF-IR from weeks 14–24. Examining this group at these time points revealed a possible trend in IGF-I (P = 0.21) and IGF-IR (P = 0.13). However, these apparent increases at week 24 were due to two individuals for IGF-I and three individuals for IGF-IR, who displayed substantial increases in respective tissue mRNA levels. The subjects who responded with augmented
IGF-I expression were not the same as those who had increased IGF-IR. Removal of these subjects resulted in identical values at weeks 14 and 24 for IGF-I (0.922 ± 0.208 vs. 0.942 ± 0.202) and IGF-IR (1.896 ± 0.299 vs. 1.855 ± 0.373).

Further, there was no relationship between plasma IGF-I and IGF-IR. Snyder et al. (36) reported that although rhGH administration initially promoted nitrogen retention and increased plasma IGF-I concentrations in diet-restricted obese subjects, the anabolic effect was lost after 5 weeks, even though plasma IGF-I concentrations remained elevated. Similarly, Henneman et al. (37) found GH-induced nitrogen retention in animals (22) and humans (23). Administration of GH in old rats has been shown to increase skeletal muscle protein synthesis (24) and enhance skeletal muscle regeneration (25). Modest increases in CSA of muscle fibers and an increase in the number of satellite cells have been reported in animals after daily GH administration (10, 26). The effect of exogenous GH on fiber CSA in man has only been reported in GH-deficient adults, and the results are mixed. Preece et al. (27) and Rutherford et al. (28) reported fiber atrophy after cessation of GH therapy for 12 months, whereas Whitehead et al. (29) and Cuneo and colleagues (30) found no effect on type I or II muscle fiber size or fiber type proportions after 6 months of rhGH therapy.

In healthy exercising adults, an augmented anabolic effect of rhGH administration has not been observed. Yarasheski et al. (31) did not find an enhanced effect of rhGH administration in exercising young men on the quadriceps muscle protein synthesis rate compared to the effect of exercise alone in 13C-leucine tracer experiments. In young power athletes (32) and experienced weight lifters (33), rhGH treatment in conjunction with training did not result in augmented muscle strength or muscle protein synthesis. Yarasheski and co-workers (31) speculated that the absence of an anabolic effect from prolonged GH treatment in resistance-trained individuals may be due to down-regulation of the muscle GHR as well as the IGF-IR, as evidenced in mouse muscle (34). It has also been observed that elevation of some IGF-binding proteins both potentiates and inhibits the interaction between IGF-I and its receptor (35). Evidence exists from several sources for tissue resistance to elevated plasma IGF-I after prolonged GH treatment, possibly due to down-regulation of IGF-IR. Snyder et al. (36) reported that although rhGH administration initially promoted nitrogen retention and increased plasma IGF-I concentrations in diet-restricted obese subjects, the anabolic effect was lost after 5 weeks, even though plasma IGF-I concentrations remained elevated.


tance exercise and that gains in fiber CSA are not dependent on augmented tissue GH-IGF axis gene expression.

It is well known that a sustained program of moderate to high intensity resistance exercise results in skeletal muscle fiber hypertrophy in both young (17) and older (18) adults, which was evident during the initial 14-week training period. Similar increases were observed in type I and II fiber CSA accompanied by significant improvements in muscle strength (8). However, no difference was observed between groups after the initiation of rhGH or PL treatment. Moreover, the only significant change from week 14 to 24 occurred in the PL group for type I fibers. Although exercise-induced muscle hypertrophy in the elderly results from an increase in muscle protein synthesis (19), it has been unclear whether fiber area can be enhanced in exercising adults by GH supplementation (20). The present study suggests that in elderly adults, enhanced fiber hypertrophy does not occur with replacement GH therapy. This supports the early work of Goldberg (21), who demonstrated that work-induced muscle growth is not pituitary GH dependent.

Several lines of evidence indicate that rhGH has the potential to be beneficial in older exercising adults. GH is a potent anabolic agent that stimulates protein synthesis and nitrogen retention in animals (22) and humans (23). Administration of GH in old rats has been shown to increase skeletal muscle protein synthesis (24) and enhance skeletal muscle regeneration (25). Modest increases in CSA of muscle fibers and an increase in the number of satellite cells have been reported in animals after daily GH administration (10, 26). The effect of exogenous GH on fiber CSA in man has only been reported in GH-deficient adults, and the results are mixed. Preece et al. (27) and Rutherford et al. (28) reported fiber atrophy after cessation of GH therapy for 12 months, whereas Whitehead et al. (29) and Cuneo and colleagues (30) found no effect on type I or II muscle fiber size or fiber type proportions after 6 months of rhGH therapy.

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tention to be lost after 1 month of daily injections of human GH, but the anabolic effect was restored after cessation of treatment for 2 weeks, and Mandel et al. (38) reported down-regulation of erythrocyte IGF-I receptor after 6 months of rhGH treatment in GH-deficient children.

To date, it has been unclear whether IGF-I expression is enhanced in human muscles undergoing exercise-induced hypertrophy (20). Although considerable variation was apparent in our study cohort, it appears that tissue IGFs and GH and IGF receptors are not consistently affected by resistance exercise alone or exercise combined with rhGH administration. The lack of tissue change may partially account for the absence of an anabolic effect in the rhGH-treated group, preventing autocrine/paracrine action of IGF-I, which has been proposed to have an important role in local tissue hypertrophy (39).

Recent studies in rats suggest that skeletal muscle IGF-I mRNA is activated by synergistic overload (40), treadmill running (41), and stretch-induced hypertrophy (22), and that this response is independent of GH status. Species differences, the experimental protocol employed to induce hypertrophy, and the age of the subjects may contribute to the divergent results obtained in the present study and those of DeVol et al. (40), Zanconato et al. (41), and Czerwinski et al. (42). Nonetheless, it is possible that up-regulation of IGF gene expression did occur earlier in the time course of treatment in the present study. Further, as the message half-lives for IGF-I and their receptors are unknown, local production of IGF may have increased without any detectable change in mRNA at our sampling time point. It has also been suggested that the GH-induced increase in GHBP prevents GH from binding to its target tissue receptor (43); however, GH-binding protein was not measured in the present study. Additionally, this PCR assay can detect a change of approximately 50% in mRNA abundance, so it is possible that small changes in muscle mRNAs were not detected.

In conclusion, administration of the trophic agent rhGH did not augment the skeletal muscle response to physical exercise in terms of muscle strength (8), morphology, or muscle GH-IGF mRNA expression. These results raise doubts concerning the potential role that rhGH supplementation may have in addressing atrophy of skeletal muscle and decline of strength with aging.

References