Growth Hormone Administration and Exercise Effects on Muscle Fiber Type and Diameter in Moderately Frail Older People

James V. Hennessey, MD,* Joseph A. Chromiak, PhD,† Shirley DellaVentura, RN, BS,* Steven E. Reinert, MS,‡ Jacqueline Puhl, PhD,§ Douglas P. Kiel, MD, MPH,¶ Clifford J. Rosen, MD,§ Herman Vandenburgh, PhD,† and David B. MacLean, MD*

OBJECTIVE: Reduced muscle mass and strength are characteristic findings of growth hormone deficiency (GHD) and aging. We evaluated measures of muscle strength, muscle fiber type, and cross sectional area in response to treatment with recombinant human growth hormone (rhGH) with or without a structured resistance exercise program in frail older subjects.

DESIGN: Placebo-controlled, randomized, double blind trial.

SETTING: Outpatient clinical research center at an urban university-affiliated teaching hospital.

PARTICIPANTS: Thirty-one consenting older subjects (mean age 71.3 ± 4.5 years) recruited as a subset of a larger project evaluating rhGH and exercise in older people, who underwent 62 quadriceps-muscle biopsies.

INTERVENTION: Random assignment to a 6-month course of one of four protocols: rhGH administered subcutaneously daily at bedtime, rhGH and a structured resistance exercise program, structured resistance exercise with placebo injections, or placebo injections only.

MEASUREMENTS: Muscle biopsy specimens were obtained from the vastus lateralis muscle. Isokinetic dynamometry strength tests were used to monitor individual progress and to adjust the weights used in the exercise program. Serum insulin-like growth factor-I (IGF-I) was measured and body composition was measured using a Hologic QDR 1000W dual X-ray densitometer.

RESULTS: The administration of rhGH resulted in significant increase in circulating IGF-I levels in the individuals receiving rhGH treatment. Muscle strength increased significantly in both the rhGH/exercise (± 55.6%, P = .0004) as well as the exercise alone (± 47.8%, P = .0005) groups. There was a significant increase in the proportion of type 2 fibers between baseline and six months in the combined rhGH treated subjects versus those not receiving rhGH (P = .027).

CONCLUSIONS: Our results are encouraging in that they suggest an effect of growth hormone on a specific aging-correlated deficit. IGF-I was increased by administrating rhGH and muscle strength was increased by exercise. The administration of rhGH to frail older individuals in this study resulted in significant changes in the proportions of fiber types. Whether changes in fiber cross-sectional area or absolute number occur with long-term growth hormone administration requires further study. J Am Geriatr Soc 49: 852–858, 2001.

Key words: older; muscle; biopsy; exercise; recombinant human growth hormone

Reduced muscle mass and strength are characteristic findings of growth hormone deficiency (GHD) and the aging process. Muscle size and strength and muscle fiber area have been noted to decrease by as much as 5% per year in growth hormone (GH)-deficient teens withdrawn from GH treatment. Muscle fiber diameters of both type 1 and type 2 (fast twitch) muscle fibers decrease to about 85% of basal values after withdrawal of active GH treatment. Compared with normal aged-matched controls, patients with GHD demonstrate reduced isometric muscle strength and muscular endurance in the quadriceps and hamstring muscle groups.

GH administration in GH-deficient adults has been demonstrated to result in an increase in lean body mass, muscle volume and isometric muscle strength. GH administration may also improve muscle strength and exercise capacity. Improvement in muscle strength may be delayed relative to an increase in lean tissue in GH-deficient adults; as shown in one study, the increase in strength of both isometric and isokinetic muscle strength first appeared...
after 12 to 24 months of treatment. Improved muscular endurance, as measured by the fatigue index, also increases from below normal (37% vs 39%) to above normal values (mean 43%) after 2 years of GH therapy.

Studies of fiber size and type following GH administration have not demonstrated consistent results, with some studies showing no change in the diameter or proportion of type 1 or type 2 fibers in GHD subjects and no change in cross-sectional surface area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in the fiber size of GH-deficient younger adults treated with recombinant human growth hormone (rhGH), another study in GH-deficient adults showed an increase in muscle cross-sectional area without an increase in fiber area or fiber type percentage.

In summary, decreases in muscle diameter, fiber cross-sectional area, and strength have been reported in adults of type 1 or type 2 fibers in GHD subjects, studies showing no change in the diameter or proportion have not demonstrated consistent results, with some in cross-sectional surface area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training. Although a recent report demonstrates a significant increase in muscle cross-sectional area in older men undergoing resistance training.
crease every 2 weeks). Resistance consisted of plastic-coated barbells or weight wraps with slide-in weights placed around ankles or wrists and secured with Velcro.

**Muscle Biopsy and Tissue Collection**

Muscle biopsy specimens were obtained from the vastus lateralis muscle using a modification of the needle biopsy technique of Bergstrom\(^4\) as previously reported.\(^6\) A 6-mm Bergstrom sampling needle was used for most procedures. Each procedure required approximately 15 minutes for initial positioning, shaving, local anesthesia, incision, sampling, and postprocedural closure and occlusion. Subjects tolerated the procedure well, with only one subject experiencing significant bruising and discomfort requiring acetaminophen and rest. Approximately eighty 125-mg samples were obtained with each biopsy.\(^6\) Muscle samples were removed from the cutting chamber with electron microscopy forceps. The tissue sample that was selected for staining was rolled lightly in talc and frozen in liquid nitrogen. Samples were stored at −80°C until further processing.

**Histochemistry and Image Analysis Techniques**

The tissue for histochemistry was warmed to −20°C and mounted on a cutting chuck. Cross-sections of 8-μm thickness were cut on a cryostat and mounted on glass microscope slides. Cross-sections were preincubated at room temperature for 15 minutes at pH 9.5, followed by staining for myosin ATPase (Brooke and Kaiser, 1970). Stained muscle cross-sections were observed under a Zeiss microscope equipped with a drawing tube attachment focused on a Numonics 2210 digitizing tablet, which was interfaced with an IBM-compatible computer. The stained cells and tablet mouse could be seen while looking through the microscope eyepieces. Morphological measurements were made at a total magnification of 100× using Sigma-Scan morphometry software (Jandel Scientific, Sausalito, CA). Two fields were located in which the fibers had been cut perpendicular to their long axis. In each field, the muscle fiber cross-sectional area of 25 Type 1 and 25 Type 2 fibers was measured, (i.e., a total of 50 fibers of each fiber type). If a total of 50 fibers of each type could not be measured from these two fields, additional fields were identified and additional fibers measured until 50 fibers of each type were measured. In a few cases, a total of 50 fibers of one fiber type could not be measured, but these samples were used as long as a minimum of 25 fibers of each type was measured. All fiber diameters from the same biopsy sample were averaged and the mean values were used for further data analysis. For determination of fiber type percentages, a minimum of 200 fibers in two or more fields was counted at a total magnification of × 100.

**Measurements of IGF-I and Body Composition**

Baseline GH values were not measured in these subjects because random sampling of GH in older individuals is invariably associated with low values.\(^2\) IGF-I was measured in a collaborator’s laboratory (CR) using previously described methods.\(^2\) IGF-I results were kept blinded to investigators and study personnel at the study site. Body composition and relative lean leg mass were measured using a Hologic QDR 1000W dual X-ray densitometer, as previously described.\(^4\)

**STATISTICAL ANALYSES**

Two statistical techniques were employed to seek differences among the experimental groups between measures (IGF-I, muscle strength 1RM, fiber type, fiber cross-sectional area) at baseline and at 6 months postintervention. First we used paired \(t\)-tests to compare the mean of each measure at baseline versus 6 months for each of the four experimental groups individually. Second, to assess for interactions between the effects of GH and exercise, we calculated for each measure a 6-month-minus-baseline difference that was incorporated as the dependent variable in a two-way analysis of variance (ANOVA) model entering main-effect terms of any growth hormone and any exercise, and an interaction term of any GH × any exercise. All statistical analyses were performed using Stata 6.0 (Stata Corp., College Station, TX).

**RESULTS**

Thirty-one patients (20 men and 11 women) who were randomly distributed to each of the four treatment groups underwent biopsies at baseline and after completing safety and efficacy testing at the 6-month visit. The administered dose of rhGH at initiation of treatment in this subgroup was 0.0037 mg/kg/d in one subject and 0.003 mg/kg/d in three subjects. As a result of a protocol change, the starting dose was reduced to 0.0025 mg/kg/d for the remaining 27 subjects and simultaneously reduced in the four who started at higher dosage. During the conduct of the study, one subject’s dose was further reduced to 0.00125 mg/kg/d as a result of fluid retention complaints. Mean doses for each group are listed in Table 1. Biopsy data on 31 subjects were available to calculate the fiber type percentages and on 29 subjects to evaluate the fiber cross-sectional diameter. Subjects’ age and gender are summarized in Table 1 for each of these four groups (7–8 subjects each).

**Response of IGF-I**

The administration of rhGH resulted in significant increases of circulating IGF-I levels in the individuals receiving rhGH treatment. Individuals in Group 1 (GH and exercise) experienced a mean 48.7% increase in IGF-I values.

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<tr>
<th>Table 1. Age and Gender of Subjects in the Four Treatment Groups Who Consented to Paired Biopsies</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>GH and exercise</td>
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<tr>
<td>GH only</td>
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<td>Exercise only</td>
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<tr>
<td>Control</td>
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Notes: Mean doses of study drug dispensed to individuals participating over the 6-month period between baseline and follow-up biopsy. Differences in dosing reflect minor variances in basal dose as explained above and adjustments to dose made in response to symptoms. Doses dispensed to those in the exercise only and control groups reflect extrapolated values based on volume injected. SD = standard deviation; rhGH = recombinant human growth hormone; GH = growth hormone.
Muscle Function/Morphology with GH and Exercise

Muscle Strength

Muscle strength of the right quadriceps muscle increased significantly in both the GH/exercise (52.8–80.5 lbs, +55.6%, P = .0004) and the exercise alone (40.9–61.4 lbs, +47.8%, P = .0005) groups between baseline and 6 months (see Figure 1). There was a trend toward increased quadriceps strength with rhGH alone (38.9–47.1 lbs, +21.3% P = .098) and a decrease among the control subjects (42.5–37.0 lbs, −16.2% P = .096) although neither of these changes reached statistical significance. In the multivariate model (n = 30, F = 14.9, P < .00005) the change in 1RM was significantly different for both the GH and exercise main effects (P = .007, P < .00005, respectively). The interaction of those effects was not significant (P = .26).

Fiber Type

There was no significant difference from baseline to 6 months in the change in percentage of type 2 fibers in any of the four experimental groups: GH and exercise (baseline 47.0%, +14.9% P = .37), GH alone (baseline 51.7%, +13.5% P = .12), exercise only (baseline 54.8%, −8.7% P = .14) nonexercise receiving placebo injections (baseline 53.5%, −5.5% P = .57) (Figure 2). Paired t testing indicated a statistically significant increase in the proportion of type 2 fibers from baseline to 6 months in the combined GH treated subjects (groups 1 and 2). The two-way ANOVA model, however, incorporating any GH and any exercise main effects, was not significant (n = 31, F = 1.85, P = .16).

Fiber Cross-Sectional Area

The cross-sectional area of the type 1 and 2 muscle fibers both increased approximately 4% in the GH-treated exercisers, 1.6 and 7.8%, respectively, in those treated with GH alone, and 5.8 and 15.9%, respectively, in the exercisers randomized to placebo injections (Table 2). None of these changes were statistically significant; nor were changes in combined groups (GH-treated subjects versus those not

(127.7–189.9 ng/mL, P = .0357), whereas those in Group 2 (GH only) increased 61.7% (111.6–180.4 ng/mL, P = .0039). No significant change was observed in those not exposed to rhGH: Group 3 (exercise only) increased 1.5% (108.6–110.3, P = .86), Group 4 (Control) decreased 10.1% (141.5–127.3, P = .12). The two-way ANOVA model incorporating the change in IGF-I levels as the independent variable was significant overall (n = 31, F = 7.3, P = .001) and for the main effect of any GH (P = .0001). The main effect of any exercise and the interaction of GH and exercise were not significant (P = .77 and 0.48, respectively).

Figure 1. Strength assessment of right knee extension as measured by the percentage change of 1RM. Increases in 1RM were statistically significant (P < .0005). Each bar represents the mean ± SD of determinations in each group, from left to right, Group 1 (n = 8), 2 (n = 7), 3 (n = 8), 4 (n = 8). 1RM = 1 repetition maximum; SD = standard deviation; GH = growth hormone; EX = exercise; PBO = placebo.
receiving GH; Type 1 ANOVA n = 29, F = 0.20, P = .89; Type 2 ANOVA n = 29, F = 0.70, P = .56).

DISCUSSION

We demonstrated a significant increase in the percentage of type 2 fibers in older subjects who received daily administration of low-dose rhGH; this was accompanied by a trend toward increasing muscle strength. The exercise intervention significantly increased quadriceps strength in this group, but there were no significant changes in muscle fiber diameter.

The proportions of fiber types observed at baseline were similar to those observed in younger adults with frank GH deficiency, older but physically fit men, and frail older people with significant physical impairments. This suggests that the GH secretory decline associated with aging, referred to as the somatopause, may contrib-

Table 2. Change in Fiber Cross-Sectional Area (Mean Value (μm² + SD)) Measured After 6 Months of Treatment and Compared with Baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>Type 1 Fibers</th>
<th>Type 2 Fibers</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>% Change</td>
</tr>
<tr>
<td>GH and exercise</td>
<td>6564 ± 863</td>
<td>+3.9</td>
</tr>
<tr>
<td>GH only</td>
<td>5548 ± 230</td>
<td>+1.6</td>
</tr>
<tr>
<td>Exercise only</td>
<td>5786 ± 800</td>
<td>+5.8</td>
</tr>
<tr>
<td>Control</td>
<td>5317 ± 951</td>
<td>−0.4</td>
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SD = standard deviation; GH = growth hormone.
ute to the recognized decline in total muscle mass and decreased number of type 2 fibers.25 The baseline biopsies in this population were not consistent with an increased percentage of type 2 fibers that has been predicted in young adults with GH deficiency.6 Our results were more in line with those observed in studies of older people.26 The apparent decrease of type 1 fibers and increase in type 2 fibers following 6 months GH administration is also different from the reported effect of GH in hypophysectomized rats.27 Although Grossman demonstrated no consistent change in proportions of fiber types in hypophysectomized rats, they demonstrated an increase in fibers expressing type I and IIa myosin and a decrease in fibers expressing type I myosin in ambulatory control rats treated with GH.28 Overall, these refined studies in animals may demonstrate the mechanisms for phenotype changes in response to GH treatment.

Our results in older people also differ from published small studies of humans with GH deficiency that have failed to demonstrate similar changes in the proportion of type 1 versus type 2 fibers.10,12,13,29 In a study of 23 adult patients with GH deficiency completing 6 months of rhGH therapy or placebo, no change was noted between the two treatment groups in fiber percentages, but the authors comment that the limited number of subjects may account for the apparent lack of effect.13 In a similar manner, the recently published results from Woodhouse et al. demonstrated no change in the proportion of type 1 and type 2 fibers among the 14 (much younger, GH-deficient) subjects biopsied after higher-dose rhGH treatment or placebo.10

The administration of rhGH to our frail older subjects did not result in significant changes in the cross-sectional area of the examined muscle fibers. This finding is consistent with results reported previously.11,13 At baseline, biopsies from our subjects demonstrated type 1 and type 2 fiber areas similar to those reported in young GH-deficient adults with a mean age of 39 to 4013,30 and older men with a mean age of 69 and 80 years.26 Our findings are somewhat at variance with those of Woodhouse et al. in younger GHD patients.10 In their study, using a larger dose of rhGH (6.25 μg/kg), a significant increase in muscle fiber size of approximately 35% to 45% was noted, regardless of fiber type.10

Muscle fiber areas of both type 1 and type 2 muscle cells are reported to decrease to about 85% of basal values after withdrawal of active GH treatment in GH-deficient individuals1 and type 2 fiber atrophy is reported in sarcopenic frail older people.24 Direct prospective evidence of a therapeutic GH effect on muscle diameter and cross-sectional area is mixed, with negative results reported in two small studies conducted in GHD patients.11,13 An apparent effect in the limited number of subjects reported by Woodhouse et al.10 and no data demonstrating increases in aging populations.12 The findings of Grossman et al. in hypophysectomized rats studied for 10 days with hind limb suspension, similar to those of Woodhouse et al., demonstrate some increase in both type 1 and type 2 fiber size in rats treated with either IGF-I or GH.28 There are several notable differences in the Woodhouse study10 that make direct comparison with our results difficult, including the dose of GH, the age of subjects at baseline, and the underlying degree of GH deficiency in the GHD population versus the aging population. These findings point to the multiple factors that may contribute to GH response, including age of host and duration of treatment. It is possible that longer duration of treatment may be required for phenotypic changes in fiber type to occur in these younger GH-deficient subjects. More studies are clearly required to resolve these issues.

Similar to the exercise effect noted in the resistance training of older people,24 we noted significant increase in the strength of the quadriceps muscles in both of our exercising groups. However, we were able to demonstrate only a trend toward type 2 fiber enlargement, most prominent in those exercising and receiving placebo injections. Although the magnitude of increase was consistent with that expected for subjects of this age,12,24 the lack of statistical significance may be due to the relatively large baseline areas, which are consistent with values published elsewhere,1,23 or the relatively small number of subjects for whom data were available.

During aging, there is a specific loss of type 2 fibers that may be associated with the loss of muscle power required to rapidly correct changes in position or gait. Our results are encouraging in that they suggest an effect of GH on a specific aging-correlated deficit. Furthermore, the results indirectly suggest that the relative GHD associated with aging may be causally associated with changes in muscle composition or function. In conclusion, the administration of rhGH to frail older individuals in this study resulted in a significant change in muscle fiber type. Because there is not likely to be an increase in absolute fiber numbers, our results suggest that enzyme or other compensational shifts in muscle fibers might occur in response to GH supplementation. Whether changes in fiber diameter or absolute number occur with longer-term or higher-dose GH administration will require further study.

REFERENCES


