Growth Hormone Supplementation Increases Skeletal Muscle Mass of Old Male Fischer 344/Brown Norway Rats

Gregory D. Cartee,¹ Erika E. Bohn,¹ Brian T. Gibson,² and Roger P. Farrar²

¹Biodynamics Laboratory, University of Wisconsin, Madison.
²Department of Kinesiology, University of Texas, Austin.

Growth hormone (GH) supplementation can increase the body weight of old rats, but the individual tissues affected were previously unidentified. Therefore, the masses of the heart, spleen, kidney, epididymal fat pads, and five skeletal muscles were assessed in male Fischer 344/Brown Norway rats (9, 20, 31 months) injected with recombinant human GH (0.7 mg/kg) or vehicle twice daily for 10 days. Muscle composition (fiber type, protein concentration, dry weight/wet weight ratio, citrate synthase activity) was also evaluated. Muscle mass was increased with GH treatment, and this increment was undiminished in old age. Fiber type, protein concentration, and dry weight/wet weight ratio were unaffected by GH. Citrate synthase activity declined in the plantaris and increased in the soleus with GH treatment. GH supplementation elevated heart and spleen mass, but not fat pad or kidney weight. The data demonstrate that the capacity for GH-induced hypertrophy of skeletal muscle, myocardium, and spleen is retained during old age.

METHODS

Treatment of rats. — Male Fischer 344/Brown Norway F1 hybrid (F344/BN) rats of three ages (8–9, 19–20, or 30–31 months) were obtained from Charles River Breeding Laboratories. The age groups will be hereafter referred to as the 9-, 20-, or 31-month-old groups. Upon arriving at Madison, rats were housed in a facility isolated from contact with other animals, and their caretakers did not come into contact with any other rodents. The animal room was maintained at 23–24°C, with a relative humidity of 54–55% and a light-on/light-off schedule of 6 a.m./6 p.m. Rats were provided with NIH-31M rodent diet (Agway Inc., Syracuse) and water ad libitum until 4 p.m. on the day before they were killed, when they were limited to 5 g of food. After equilibrating with their new environment for 10–20 days, rats were randomly assigned to vehicle control or GH-treated groups (before GH supplementation, body weights of age-matched control and treated groups were not significantly different). The rats received subcutaneous injections twice daily, at 8 a.m. and at 3 p.m., for 10 consecutive days. The GH group was injected with recombinant-derived human GH (rhGH; Genentech, South San Francisco, CA; 0.7 mg/kg body weight per injection) dissolved in sterile water containing 0.9% benzyl alcohol (Abbott Laboratories, Chicago); the vehicle control group was injected with an equivalent volume of sterile water. Body weight was determined on the day after the last injection when the rats were killed. Food intake during the experimental period was evaluated by measuring the food provided to the rats during the first 9 days of treatment (i.e., not including the final day where food intake was restricted) and subtracting the amount of food not eaten. On the day following the last GH injection, animals were anesthetized with pentobarbital sodium (60 mg/kg).
Several skeletal muscles (gastrocnemius, soleus, plantaris, extensor digitorum longus, and brachioradialis), as well as the heart, kidney, spleen, and epididymal fat pads, were dissected out and weighed.

**Determination of muscle protein concentration.** — Portions of the gastrocnemius and soleus muscles were homogenized in ice-cold buffer containing 20 mM Hepes, 1 mM EDTA, 250 mM sucrose (pH 7.4) at a 1:19 (wt:vol) ratio using a Kontes Duall glass-glass tissue grinder. Protein concentration in the homogenates was determined using the spectrophotometric bicinchoninic assay (Smith et al., 1985).

**Determination of fiber type composition.** — After dissection and weighing, soleus and plantaris muscles that were used for histochemistry were mounted on wooden holders, frozen in isopentane cooled to the temperature of liquid N₂, and stored at -80°C until processed and analyzed. Transverse sections (8 μm) were taken from the mid-belly of each muscle as previously described (Brooke and Kaiser, 1970). Frozen sections (8 μm) were taken from the mid-belly of the heart, kidney, spleen, and epididymal fat pads, were dissected out and weighed. By preincubation at pH 10.4, 4.54, or 4.50, respectively. Stained sections were projected using a Bausch and Lomb microprojector, and >1000 fibers were classified for each muscle as previously described (Brooke and Kaiser, 1970).

**Determination of muscle citrate synthase activity.** — The data were analyzed using two-way analysis of variance employing the General Linear Models Procedure (SAS; Cary, NC).

**RESULTS**

**Body weight and food intake.** — As previously reported (Cartee and Bohn, 1995), body weight (g) became progressively greater with advancing age \((p < .0001)\), and GH treatment led to a significant \((p < .0002)\) elevation in body weight (432.5 ± 4.6 vs 463.3 ± 4.9, 9-month-old; 515.1 ± 6.2 vs 545.8 ± 9.1, 20-month-old; 552.8 ± 578.7 ± 12.5, 31-month-old). The relative GH-induced increase in body weight was 7%, 6%, and 5% in the 9-, 20-, and 31-month-old rats, respectively. Food intake \((g)\) was not significantly affected by age or GH treatment \((control) vs GH: 21.9 ± 1.0 vs 22.2 ± 0.6, 9-month-old; 20.6 ± 0.7 vs 20.8 ± 1.0, 20-month-old; 24.3 ± 1.5 vs 20.8 ± 0.6, 31-month-old). The wet weights of the spleen, heart, kidney, and epididymal fat pad each increased significantly \((p < .0001)\) with advancing age (Table 1). Treatment with GH significantly increased spleen \((p < .0001)\) and heart \((p < .005)\) wet weight in each age group, compared to vehicle-treated controls, by 15–28% and 6–8%, respectively. The kidney and epididymal fat pad weights from the GH-treated groups did not differ from age-matched control groups. There were no significant effects of age or GH treatment on the relative increase above age-matched control values tended to be greatest in the oldest rats. Gastrocnemius and soleus protein concentration (Table 2) was not significantly affected by age or GH treatment.

**Muscle weight and protein concentration.** — A significant effect of age was found on the weight of every muscle studied (Table 1). The relative age-related decrease in the weight (31-month-old control vs apparent peak mass at 9- or 20-month-old controls) varied among the skeletal muscles: gastrocnemius (25%), plantaris (22%), soleus (13%), EDL (15%), and brachioradialis (10%). Treatment with GH led to a significant increase in the weight of each muscle compared to vehicle-treated controls. With the exception of the brachioradialis muscle, the magnitude of the increment above age-matched control values tended to be greatest in the oldest rats. Gastrocnemius and soleus muscle weight (31-month-old control vs apparent peak mass at 9-, 20-, and 31-month-old rats, respectively. Food intake \((g)\) was not significantly affected by age or GH treatment.

**Tissue weights and wet/dry weight ratios.** — The wet weights of the spleen, heart, kidney, and epididymal fat pad each increased significantly \((p < .0001)\) with advancing age (Table 3). Treatment with GH significantly increased spleen \((p < .0001)\) and heart \((p < .005)\) wet weight in each age group, compared to vehicle-treated controls, by 15–28% and 6–8%, respectively. The kidney and epididymal fat pad weights from the GH-treated groups did not differ from age-matched control groups. There were no significant effects of age or GH treatment on the dry weight/wet weight ratios for the gastrocnemius, soleus, heart, or kidney, demonstrating the hypertrophy was not attributable to edema (Table 4). The GH-treated groups were characterized by a statistically significant \((p < .05)\) decrease in dry weight/wet weight ratio for the spleen, but the magnitude of this decrement \((<5%)\) was quite small compared to the relative increase in spleen weight (15–28%).

**Muscle fiber type composition.** — There was no significant effect of age or GH treatment on the fiber type composition of the soleus (Table 5). In the plantaris, a significant

### Table 1. Effect of Age and Growth Hormone Treatment on Skeletal Muscle Weight

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>9 Months</th>
<th>20 Months</th>
<th>31 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Growth</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>2287 ± 38</td>
<td>2349 ± 55</td>
<td>2209 ± 27</td>
</tr>
<tr>
<td>Plantaris</td>
<td>464 ± 6</td>
<td>465 ± 13</td>
<td>457 ± 6</td>
</tr>
<tr>
<td>Soleus</td>
<td>195 ± 5</td>
<td>203 ± 6</td>
<td>214 ± 5</td>
</tr>
<tr>
<td>EDL</td>
<td>206 ± 3</td>
<td>210 ± 7</td>
<td>205 ± 9</td>
</tr>
<tr>
<td>Brachioradialis</td>
<td>323 ± 6</td>
<td>361 ± 8</td>
<td>345 ± 8</td>
</tr>
</tbody>
</table>

**Notes:** Values (means ± SE) are expressed as mg; \(n = 6–10\) muscles per group. EDL = extensor digitorum longus. n.s. = nonsignificant \((p > .05)\).
alter the distribution of muscle water between the extracellular and intracellular compartments as determined by mannitol space (Cartee and Bohn, 1995). The GH-induced increase in muscle mass in old rats, along with no decrement in protein concentration or dry weight/wet weight ratio, is consistent with the observation that 8 days of GH supplementation significantly enhances the rate of protein synthesis in skeletal muscle from old male rats (Sonntag et al., 1985). Assuming that the remainder of the musculature responded in a fashion similar to the muscles that we studied, skeletal muscle would account for a substantial portion of the GH-induced body weight gain.

Two earlier studies reported no effect of rhGH on muscle mass of older female rats. Ullman and coworkers (1990) found no increase in EDL mass of 26-month-old female Sprague-Dawley rats treated with rhGH (4 IU injected daily for 10 weeks). Carmeli et al. (1993) observed no rhGH-induced (0.6 mg/kg, subcutaneously injected on alternate days for 3 weeks) change in muscle (gastrocnemius and plantaris) or body weight of 26-month-old female Wistar rats. Carmeli and colleagues did find that GH administration attenuated the muscle weight loss in immobilized (casted) hindlimbs muscles of the old rats. The absence of young controls undergoing identical GH supplementation in each...
of the earlier studies prevents an unambiguous evaluation of the influence of age on GH-induced changes in muscle mass. However, in an earlier study, Ullman and Oldfors (1989) reported a 24% increase in the EDL weight of 5-month-old female Sprague-Dawley rats injected with rhGH (4 IU daily for 36 days). Unlike our results, these previously published findings suggested that, with advancing age, skeletal muscle becomes resistant to the growth promoting effects of GH.

The explanation for the absence of a GH effect on the muscle mass of older rats in earlier studies is uncertain. Gender might be a factor because female rats were used in most of the earlier studies. Unlike our results, these previously published studies reported a 24% increase in the EDL weight of 5-month-old rats (Jansson et al., 1982). For this reason, the rats in this study were injected twice daily, in the morning and the afternoon. It is notable that Sonntag et al. (1985) studied male rats injected twice daily with GH when they documented a GH-induced enhancement in muscle protein synthesis during old age.

Previously, we reported that the serum insulin-like growth factor-I (IGF-I) concentration did not change with age in control animals used for this study (controls: 9-month = 4.24; 20-month = 4.40; 31-month = 4.25 U/ml), and IGF-I levels were approximately doubled by GH supplementation at every age (GH: 9-month = 9.73; 20-month = 9.52; 31-month = 8.98 U/ml) (Cartee and Bohn, 1995). Since skeletal muscle expresses receptors for both GH and IGF-I, muscle hypertrophy could potentially be directly mediated by GH or indirectly mediated by IGF-I. However, Guler et al. (1988) demonstrated that infusion of rhIGF-I does not stimulate muscle growth in hypophysectomized rats, suggesting that the hypertrophy depends on the presence of GH.

Although aging has repeatedly been shown not to alter the fiber type composition of the soleus or EDL (Eddinger et al., 1988; Florini and Ewton, 1989; Brown et al., 1992), skeletal muscle hypertrophy could potentially be directly mediated by GH or indirectly mediated by IGF-I. However, Guler et al. (1988) demonstrated that infusion of rhIGF-I does not stimulate muscle growth in hypophysectomized rats, suggesting that the hypertrophy depends on the presence of GH.

### Table 5. Effect of Age and Growth Hormone Treatment on Skeletal Muscle Fiber Type Composition

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>Growth Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantaris*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>9.4 ± 1.3</td>
<td>7.9 ± 0.9</td>
</tr>
<tr>
<td>Type IIA</td>
<td>15.3 ± 1.5</td>
<td>14.2 ± 1.9</td>
</tr>
<tr>
<td>Type IIB</td>
<td>75.1 ± 1.2</td>
<td>77.9 ± 2.6</td>
</tr>
<tr>
<td>Soleus*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>93.5 ± 2.1</td>
<td>95.8 ± 0.4</td>
</tr>
<tr>
<td>Type IIA</td>
<td>6.3 ± 1.9</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Type IIB</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

Notes: Values (means ± SE) are expressed as percentage of fibers. n.s. = nonsignificant (p > .05).

### Table 6. Effect of Age and Growth Hormone Treatment on Skeletal Muscle Citrate Synthase Activity

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>Growth Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Months</td>
<td>27.45 ± 2.02</td>
<td>24.72 ± 1.42</td>
</tr>
<tr>
<td>20 Months</td>
<td>27.07 ± 1.39</td>
<td>22.58 ± 1.49</td>
</tr>
<tr>
<td>31 Months</td>
<td>25.32 ± 1.99</td>
<td>21.06 ± 1.91</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Months</td>
<td>28.71 ± 1.53</td>
<td>29.68 ± 1.67</td>
</tr>
<tr>
<td>20 Months</td>
<td>29.41 ± 1.75</td>
<td>35.84 ± 1.96</td>
</tr>
<tr>
<td>31 Months</td>
<td>23.57 ± 0.99</td>
<td>28.38 ± 1.42</td>
</tr>
</tbody>
</table>

Notes: Values (means ± SE) expressed as μmol•min⁻¹•g⁻¹; n = 6–10 rats per group. n.s. nonsignificant (p > .05).
month-old control groups. However, plantaris CS activity was not significantly affected by age in F344/BN rats.

Few studies have evaluated the influence of GH on the activity of mitochondrial enzymes in skeletal muscle. Administration of rhGH (556 μg/day by osmotic minipumps for 10 days) to male Sprague-Dawley rats (35 days old) did not affect succinate dehydrogenase activity of the soleus (Jiang et al., 1993); this protocol also did not influence soleus mass. Unexpectedly, the GH supplementation elicited divergent effects on the CS activity in the two muscles that we studied: an increase was observed for the soleus and a decline occurred in the plantaris. Although the explanation for these findings is uncertain, it is apparent that GH treatment can alter the metabolic profile of muscles, even when fiber type composition and total protein concentration are unaffected. Along these lines, we recently reported that GH treatment regimen used in this study can reduce the glucose transport activity of skeletal muscle (Cartee and Bohn, 1995).

The growth-promoting influence of GH supplementation varied among the tissues studied. Spleen mass of the GH-treated rats was 15–28% higher than the values for age-matched controls, greatly exceeding the relative elevation in body weight (5–7%). This observation is consistent with earlier results in younger rats (Ullman and Oldfors, 1989). GH treatment led to an increase (6–9%) in myocardial mass compared to control values, and the magnitude of the increase was roughly proportional to the effect on body weight. Comparable observations have been reported for younger rats (Ullman and Oldfors, 1989; Rubin et al., 1990). Kidney weight was unaltered in the GH-supplemented groups, a finding that differs from published data for young rats: kidney weight has been reported to increase roughly in proportion to the increases for body weight and heart weight (Greenbaum and Young, 1953; Ullman and Oldfors, 1989). IGF-1, independent of GH, can promote hypertrophy of the spleen, heart, and kidney of young rats (Clark et al., 1985; Guler et al., 1988). As described above, the circulating IGF-I levels were substantially elevated in the GH-treated rats. This protocol also did not influence soleus mass. Unexpectedly, the GH supplementation elicited divergent effects on the CS activity in the two muscles that we studied: an increase was observed for the soleus and a decline occurred in the plantaris. Although the explanation for these findings is uncertain, it is apparent that GH treatment can alter the metabolic profile of muscles, even when fiber type composition and total protein concentration are unaffected. Along these lines, we recently reported that GH treatment regimen used in this study can reduce the glucose transport activity of skeletal muscle (Cartee and Bohn, 1995).

In conclusion, we have identified several of the individual tissues (i.e., skeletal muscle, heart, and spleen) that contribute to the increased body weight in adult, middle-aged, and old male F344/BN rats after a brief period of GH administration. GH supplementation led to an increase in the mass of the skeletal muscles studied, and the magnitude of this increment was unattenuated by advancing age. The results suggest that an increase in skeletal muscle mass accounted for a large portion of the greater body weight in the GH-treated rats.

ACKNOWLEDGMENTS

This research was supported by an award from the American Federation for Aging Research and National Institute on Aging grant AG-10026 (G. D. Cartee). We are grateful for the excellent technical assistance of Carol Briggs-Tung, Mindy Huiting, Myriah Mathisen, and Deborah Nucatola. Genetech generously provided the recombinant-derived human growth hormone.

Address correspondence to Dr. Gregory D. Cartee, Biodynamics Laboratory, University of Wisconsin, 2000 Observatory Drive, Madison, WI 53706.

REFERENCES


Greenbaum, A.L.; Young, F.G. A comparison of the differences in the total nitrogen content of the muscles of the rat, resulting from treatment with growth hormone and from inanition. J. Endocrinol. 9:127–135; 1953.


Hart, I.C.; Chadwick, P.M.E.; Boon, T.C.; Langley, K.E.; Rudman, C.; Souza, L.M. A comparison of the growth-promoting, lipolytic, diabeto-
EFFECTS OF GROWTH HORMONE ON MUSCLE


Received June 28, 1995
Accepted September 19, 1995

Scheie Eye Institute
Department of Ophthalmology
University of Pennsylvania Health System
presents
THE 11TH ANNUAL SYMPOSIUM ON LOW VISION
Saturday, June 15, 1996
8:00 a.m. – 5:00 p.m.

TOPICS INCLUDE:

Current Therapeutic Trials for Macular Degeneration
Hereditary Retinal Degeneration – Present and Future
Physiatry and Vision Rehabilitation
Occupational Therapy and Vision Rehabilitation
Coordinating Low Vision Rehabilitation with Occupational Therapy
Problem Solving Techniques in Vision Rehabilitation
Evaluation and Management of Homonymous Hemianopsias
The Visually Impaired Child
The Auto-Focus Telescope – It’s Here!
What’s New and Exciting in Low Vision Rehabilitation

Workshops: Fitting and Prescribing Telescopes

COURSE FACULTY: Chairman - Janet DeBerry Steinberg, O.D.
Richard Brilliant, O.D.                                            Henry Greene, O.D.
Alexander J. Brucker, M.D.                                      Samuel G. Jacobson, M.D., Ph.D.
Abbe Dantzig, OTR/L                                             Keith Robertson, M.D.
Paul Freeman, O.D.                                               Bruce Rosenthal, O.D.

CME Credits: 6 credit hours in Category 1

Fee: $125 for practicing O.D./M.D./D.O. (includes breakfast, luncheon & coffee breaks)

Location: Scheie Eye Institute, 51 No. 39th Street, Philadelphia, PA 19104

Information: Diane Lutz, CME Coordinator [215] 662-8141