Growth hormone, IGF-I, and exercise effects on non-weight-bearing fast muscles of hypophysectomized rats

ELENA J. GROSSMAN,1,2 RICHARD E. GRINDELAND,1 ROLAND R. ROY,3 ROBERT J. TALMADGE,1,3 JULIANN EVANS,1 AND V. REGGIE EDGERTON2,3

1Department of Physiological Science and 2Brain Research Institute, University of California, Los Angeles, California 90095
3Life Science Division, National Aeronautics and Space Administration-Ames Research Center, Moffett Field 94035; and 3Department of Physiological Science and 4Brain Research Institute, University of California, Los Angeles, California 90095

Grossman, Elena J., Richard E. Grindeland, Roland R. Roy, Robert J. Talmadge, Juliann Evans, and V. Reggie Edgerton. Growth hormone, IGF-I, and exercise effects on non-weight-bearing fast muscles of hypophysectomized rats. J. Appl. Physiol. 83(5): 1522–1530, 1997.—The effects of growth hormone (GH) or insulin-like growth factor I (IGF-I) with or without exercise (ladder climbing) in counteracting the effects of unweighting on fast muscles of hypophysectomized rats during 10 days of hindlimb suspension were determined. Compared with untreated suspended rats, muscle weights were 16–29% larger in GH-treated and 5–15% larger in IGF-I-treated suspended rats. Exercise alone had no effect on muscle weights. Compared with ambulatory control, the medial gastrocnemius weight in suspended, exercised rats was larger after GH treatment and maintained with IGF-I treatment. The combination of GH or IGF-I plus exercise in suspended rats resulted in an increase in the size of each predominant fiber type, i.e., types I, I+IIa and IIa+IIx, in the medial gastrocnemius compared with untreated suspended rats. Normal ambulation or exercise during suspension increased the proportion of fibers expressing embryonic myosin heavy chain in hypophysectomized rats. The phenotype of the medial gastrocnemius was minimally affected by GH, IGF-I, and/or exercise. These results show that there is an IGF-I, as well as a GH, and exercise interactive effect in maintaining medial gastrocnemius fiber size in suspended hypophysectomized rats.

GROWTH HORMONE (GH) and resistive exercise have anabolic effects on skeletal muscle (10, 16, 20, 27). Spaceflight and simulated microgravity (e.g., hindlimb suspension) result in skeletal muscle atrophy (6, 20). This loss of mass with chronic absence of weight bearing may be due to a number of altered physiological stimuli such as muscle activity and loading, but some endocrine factors may also be involved. For example, pituitary GH secretion is reduced after hindlimb suspension (28) and spaceflight (14). Also, we recently reported that GH treatment can partially prevent muscle atrophy and that there is an interactive effect of exogenous GH administration and short bouts of resistive high-load exercise (ladder climbing) on maintaining muscle mass in hindlimb-suspended hypophysectomized (Hypox) rats (10). In addition to the anabolic effects of GH on skeletal muscle, there are several reports suggesting that GH may also affect the types of myosin heavy chain (MHC) isoforms expressed (2, 18).

Although GH may have a direct effect (27), insulin-like growth factor-I (IGF-I) also may mediate the actions of GH on skeletal muscle as a paracrine agent (15, 26). In addition, IGF-I can have a direct effect on skeletal muscle (12, 23). Therefore, the purposes of the present study were to determine 1) the efficacy of systemic IGF-I or GH treatment in ameliorating the suspension-induced fast muscle atrophy in Hypox rats, 2) whether short bouts of high-resistive exercise would potentiate any IGF-I or GH anabolic effect, and 3) the efficacy of systemic administration of IGF-I or GH on the expression of MHC isoforms in a fast muscle in ambulatory and suspended Hypox rats. Because suspension-induced atrophy is fiber type specific (i.e., slow fibers atrophy more than fast fibers), MHC composition and fiber size in the deep region of the medial gastrocnemius were studied. The deep region of the medial gastrocnemius was chosen because it 1) contains a mixture of fibers expressing type I, IIa, IIx, and IIb MHCs; 2) atrophies; and 3) has an increase in the percentage of fast fibers after short periods of hindlimb suspension (20). Preliminary data have been published in abstract form (11).

METHODS

Experimental animals. Male albino rats (Zivic-Miller Laboratories, Zelienople, PA) were hypophysectomized at ~240 g body weight (49 days) by the standard parapharyngeal method and arrived at Ames Research Center 5 days posthypophysectomy. Hypox rats were studied so that the effects of exogenous GH and IGF-I on skeletal muscle could be examined without uncontrolled levels of pituitary hormones or pituitary-mediated hormones. Throughout the study the rats were kept on a reversed 12:12-h light-dark cycle and maintained at 24 ± 1°C. Details of the environmental conditions have been previously described (10). Adaptation to the suspension cages and the powdered food (Purina rat Chow) began 3 days before the study started. Food and distilled water were provided ad libitum. Animal care and use were in accord with the Ames Research Center Users Guide (AHB 7180) and the NIH Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 86-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892) and were approved by the institutional Animal Care and Use Committee.

All rats were conditioned to climb a 1-m ladder (inclined at 85°). Initially, the rats climbed without any load attached to the base of their tail; the load then was gradually increased by 10% increments of body weight. During this conditioning phase, the exercise regimen consisted of three climbs up the ladder performed three times per day (8:30 AM, 12:30 PM, and 4:30 PM). By the fifth day, the rats were able to climb the ladder while carrying a load equal to 70% of their body weight. After 5 days of adaptation to the ladder, rats that
refused to climb and/or showed extremes in body weight were excluded from the study.

The 10-day suspension study began 13 days posthypophysectomy. Three days before the start of the study, rats (average weight 214 g) were randomly assigned to one of nine treatment groups (n = 8/group): ambulatory plus saline (Amb+Sal); Amb plus GH (Amb+GH); Amb plus IGF-I (Amb+IGF-I); hindlimb suspended plus saline (HS+Sal); HS plus GH (HS+GH); HS plus IGF-I (HS+IGF-I); HS plus saline and exercise (HS+Sal+Ex); HS plus GH and exercise (HS+GH+Ex); and HS plus IGF-I and exercise (HS+IGF-I+Ex). The ambulatory groups were included to verify that GH and IGF-I were biologically active. An additional group of pituitary-intact rats (n = 10) were age and weight matched to the Hypox rats on the day of hypophysectomy and served as a Non-hypox control group. The 10-day duration of the suspension study was chosen because within this time period the medial gastrocnemius shows a significant amount of atrophy (6, 20) and is less active (based on chronic intramuscular electromyogram recordings) than normal (1). The specifics of the tail suspension procedure, the suspension cages, and the climbing ladder have been described in detail (10).

The exercise regimen consisted of the rats performing five climbs up a ladder while carrying a load equal to 50% of their body weight attached to the tail harness and was performed three times per day (8:30 AM, 12:30 PM, and 4:30 PM). The original plan was for the animals to climb while carrying a load equal to 70% of their body weight. However, during the second bout of exercise on the first day of the study, the Hypox rats would not climb the ladder while carrying 70% of their body weight. For that bout of exercise and for the remaining bouts of exercise, the rats carried a load equal to 50% of their body weight. The rats were exercised during the dark cycle (active period). The average climbing duration lasted ~5–10 min. Each day of the study two rats refused to climb. One rat maintained a static position on the ladder for ~30 min per session. Another rat was able to maintain a static position on the ladder for ~10 min per session with assistance. These rats were included in the statistical analyses because there were no overt differences in body or tissue weights between these two rats and the other rats in their respective group. None of the exercised suspended rats were handled on a similar time schedule but were not allowed to exert force with their hindlimbs. Every other day all rats were weighed, and the loads for the exercised rats were adjusted accordingly. Suspended rats were weighed while suspended.

The rats in the GH and IGF-I treatment groups received subcutaneous injections of recombinant human GH (22 kDa) or IGF-I (7.5 kDa), respectively. GH and IGF-I were produced in Escherichia coli and were extensively characterized by Genentech (South San Francisco, CA) and found to be identical to the major in vivo forms. GH and IGF-I were injected in three aliquots, ~30 min before each exercise session, for a total dosage of 1 mg·kg⁻¹·day⁻¹. These doses stimulated body weight gain in Hypox rats in pilot studies. The dose of IGF-I used, on a molar basis, was three times the dose of GH used. A similar volume (0.5 ml) of saline was injected at the same intervals in rats of the corresponding control groups.

The rats were killed by decapitation, and trunk blood was collected ~16–20 h after the last injection and bout of exercise. Completeness of hypophysectomy was verified by examination of the sella turcica and by determination of adrenal and testes weights. The adrenal glands and testes were removed bilaterally, cleaned of fat and connective tissue, and weighed. From each rat, 11 muscles (i.e., the medial and lateral gastrocnemii; soleus; adductor longus; plantaris; tibialis anterior; rectus femoris; extensor digitorum longus; and vastus intermedius, lateralis, and medialis) were dissected bilaterally, cleaned of excess fat and connective tissue, and weighed (wet weight). The medial gastrocnemius from one side was quick frozen in liquid nitrogen and stored at −70°C until used for protein determinations. The medial gastrocnemius on the contralateral side was stretched gently, mounted on a piece of cork with embedding media, and frozen in Freon-12-cooled by liquid nitrogen. A block, ~5 mm thick, was taken from the midbelly of the frozen medial gastrocnemius, mounted on cork such that the fibers were perpendicular to the cork surface, and stored at −70°C until used for immunohistochemical analyses. Wet weights of the predominantly fast muscles are presented in this paper, i.e., the medial and lateral gastrocnemii and plantaris (plantar flexors), tibialis anterior and extensor digitorum longus (dorsiflexors), and rectus femoris, vastus lateralis, and vastus medialis (knee extensors). Results from the slow muscles have been reported recently (21).

Tibial growth plate measurements. The tibia on one side was removed, and the epiphyseal widths were measured according to Greenspan et al. (9). Briefly, the tibia was split longitudinally and stained with AgNO₃. By using an ocular micrometer, 10 readings were taken across the proximal growth plate (silver line) and averaged for each rat.

Muscle protein determinations. A small piece from the midbelly of each medial gastrocnemius muscle was removed and homogenized in glass-distilled water (2.5 mg tissue/ml) for 10 s at high speed by using a Polytron homogenizer. Noncollagenous protein was determined by using the bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL) and recrystallized bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard (25).

Muscle immunohistochemical procedures. Serial cross sections (10 μm thick) were cut in a cryostat maintained at −20°C and mounted on gelatin-coated slides. Serial sections were stained by an indirect immunoperoxidase technique by using monoclonal antibodies reacting with specific MHCs. Briefly, the tissue sections were incubated with the antibodies overnight at 4°C. A Vectastain ABC kit (Vector Laboratories, Burlingame, CA) was used to amplify the antigen-antibody complex, which was then visualized by treatment with either 5-bromo-4-chloro-3-indolyl phosphate-nitroblue tetrazolium solution (Sigma Chemical) or 3,3′-diaminobenzidine (Vector Laboratories), depending on the antibody. The MHC composition of a representative (~80–90 fibers) sample of muscle fibers in the deep region of the medial gastrocnemius was determined by using a battery of antibodies. Antibodies anti-slow, anti-fast, and anti-developmental (Vector Laboratories) were used to identify slow, fast, and embryonic isoforms of MHCs, respectively. Antibodies SC-71, BF-35, BF-G6, RT-D9, and BF-F3 (generously donated by Dr. S. Schiaffino, Padova, Italy) were also used in this study. The specificity of these antibodies has been previously described (22). The fiber cross-sectional areas of the typed fibers were manually outlined in an unstained section by using an image-processing system.

Serial cross sections of the medial gastrocnemius in saline-treated ambulatory and suspended rats (n = 4/group) were stained with antibody S220 (donated by D. Fambrough, Baltimore, MD) for identification of the sarcoplasmic reticulum Ca²⁺-adenosinetriphosphatase (ATPase) fast isoform (SERCA1).

Statistics. Independent t-tests were used to determine the effect of hypophysectomy on body weight, muscle weights, fiber type composition, and fiber cross-sectional areas. A one-way analysis of variance was used to determine the overall effects of hindlimb suspension, exercise, GH, and/or
IGF-I administration on Hypox rats. Comparisons between selected treatment groups were evaluated by Fisher's least-significant difference test. To address the specific purposes of the study, all treatment groups were compared with the Amb+Sal group, the HS-treated groups were compared with the HS+Sal group, and the GH+IGF-I+Ex or IGF-I+Ex groups were compared with the GH or IGF-I alone groups in HS rats. Statistical significance was determined at P < 0.05.

RESULTS

Effects of hypophysectomy. The effects of hypophysectomy were determined from comparisons between Non-hypox and Hypox Amb+Sal rats. The mean weights of the predominantly fast muscles were ~40–55% smaller in Amb+Sal compared with Non-hypox rats (see Table 2). In the medial gastrocnemius, hypophysectomy resulted in a decrease in the percentage of fibers expressing only type IIa MHC (from 16 to 1%) or only type IIx MHC (from 25 to 9%) and an increase in fibers coexpressing type I+IIa MHC (from 8 to 22%; see Fig. 2). Embryonic MHC was contained in 2 and 12% of the fibers sampled in Non-hypox and Amb+Sal rats, respectively (see Fig. 3). The mean cross-sectional area of each fiber type in the medial gastrocnemius was larger in Non-hypox compared with Amb+Sal rats (see Fig. 4).

Body weights. The rats weighed ~240 g at the time of hypophysectomy, and by the start of the experiment (13 days later) the rats had lost ~13% body weight, weighing on the average 208 ± 1 g (Table 1). There was no suspension effect on body weight. Compared with Amb+Sal values, body weights of Amb+GH, HS+GH, and HS+GH+Ex groups were significantly increased by 31, 24, and 26%, respectively. Body weights of the Amb+IGF-I, HS+IGF-I, and HS+IGF-I+Ex groups had a 9, 6 (P > 0.05), and 7% increase in body weight compared with Amb+Sal. Exercise alone had no effect on body weight. In addition, there was no interaction effect between either GH or IGF-I and exercise on body weight.

Adrenal weights. Compared with Amb+Sal values, adrenal weights (absolute) were significantly increased in ambulatory and suspended rats treated with GH. However, there was no difference in the relative adrenal weight in GH-treated rats (data not shown), suggesting that the increases in absolute adrenal weight were due to body and adrenal growth and not to incomplete hypophysectomy.

Epiphyseal widths. After suspension, the widths of the tibial epiphyseal plate were slightly (7%) but significantly decreased compared with Amb+Sal values (Table 1), suggesting that suspension inhibited autonomous bone growth. Exercise alone did not prevent this decrease in epiphyseal width. GH treatment increased the epiphyseal width by 40 and 30% in ambulatory and suspended rats, respectively, compared with the values of the Amb+Sal rats. Exercise had an interactive effect with GH on epiphyseal width. The increase (41%) in epiphyseal plate thickness of GH+Ex suspended rats was similar to the increase in GH-treated ambulatory rats. Compared with the values of the Amb+Sal rats, the epiphyseal plate was 20 and 15% wider in ambulatory and suspended rats treated with IGF-I. Thus IGF-I alone had about one-half the effect of GH on epiphyseal width. No interactive effect between exercise and IGF-I on epiphyseal width was observed.

Muscle weights. Compared with Amb+Sal rats, suspension resulted in a 13% decrease in the weight of the medial gastrocnemius (Fig. 1). GH administration increased the medial gastrocnemius weight in ambulatory rats and maintained the weight in suspended rats compared with Amb+Sal rats. In contrast, IGF-I treatment in ambulatory or suspended rats had no effect on the weight of the medial gastrocnemius. However, exercise did show an interactive effect with either GH or IGF-I treatment. The medial gastrocnemius weight was increased by 13% in the HS+GH+Ex group and was maintained in the HS+IGF-I+Ex group compared with the Amb+Sal group. In addition, the medial gastrocnemius weight in the GH- and IGF-I-treated suspended groups was larger than in the HS+Sal group.

The two other plantar flexor muscles, the lateral gastrocnemius and plantaris, were not significantly affected by suspension, showing a 9 and 8% atrophy (P > 0.05), respectively (Table 2). Both muscles were larger after GH treatment in both ambulatory and suspended rats compared with the appropriate controls. IGF-I treatment was effective in increasing muscle

Table 1. Body and adrenal weights and tibial epiphyseal widths

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt</th>
<th>Adrenal Wt</th>
<th>Epiphyseal Width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial, g</td>
<td>Final, g</td>
<td>%Δ from initial</td>
</tr>
<tr>
<td>Non-hypox</td>
<td>317</td>
<td>376 ± 9*</td>
<td>+19</td>
</tr>
<tr>
<td>Amb+Sal</td>
<td>206 ± 4</td>
<td>202 ± 4</td>
<td>−2</td>
</tr>
<tr>
<td>Amb+GH</td>
<td>206 ± 3</td>
<td>264 ± 4*</td>
<td>+28</td>
</tr>
<tr>
<td>Amb+IGF-I</td>
<td>206 ± 2</td>
<td>220 ± 2*</td>
<td>+7</td>
</tr>
<tr>
<td>HS+Sal</td>
<td>214 ± 7</td>
<td>200 ± 1</td>
<td>+4</td>
</tr>
<tr>
<td>HS+GH</td>
<td>210 ± 3</td>
<td>251 ± 3*</td>
<td>+20</td>
</tr>
<tr>
<td>HS+GH+Ex</td>
<td>208 ± 3</td>
<td>215 ± 4*</td>
<td>+3</td>
</tr>
<tr>
<td>HS+Sal+Ex</td>
<td>208 ± 4</td>
<td>196 ± 5</td>
<td>−6</td>
</tr>
<tr>
<td>HS+GH+Ex</td>
<td>209 ± 4</td>
<td>255 ± 4*</td>
<td>+22</td>
</tr>
<tr>
<td>HS+IGF-I+Ex</td>
<td>210 ± 2</td>
<td>271 ± 4*</td>
<td>+3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Δ%, percent change; NA, data not available; Non-hypox, non-hypophysectomized; Amb, ambulatory; Sal, saline; HS, hindlimb suspended; GH, growth hormone; IGF-I, insulin-like growth factor I; Ex, exercise; for further description of groups see METHODS. Significantly different at P < 0.05: *Amb + Sal vs. each group; †Amb + Sal vs. each HS group; ‡HS + GH vs. HS + GH + Ex.
Exercise, HS plus IGF-I, and Exercise. Significantly increased the weight of all three muscles in ambulatory and suspended rats. In contrast, IGF-I treatment affected only the rectus femoris in ambulatory rats. Exercise alone had no effect on the weights of the knee extensor muscles. In addition, exercise did not significantly potentiate the effect of GH or IGF-I treatments in any knee extensor muscle of suspended rats.

Protein concentration. The mean noncollagenous protein concentration in the medial gastrocnemius was 18.9% of wet weight with a range from 18 to 19.9% across treatment groups (data not shown). The only significant difference was that the protein concentrations were higher for both GH- and IGF-I-treated suspended groups than for both growth factor-treated ambulatory groups.

Fiber types. Many fibers in the medial gastrocnemius of Hypox rats colabeled for multiple adult isoforms of MHC: in some fibers as many as three adult isoforms were expressed (Fig. 2). IGF-I treatment of ambulatory rats resulted in a decrease in the percent of fibers expressing only type I MHC with an increase in fibers coexpressing type IIa MHC compared with Amb+Sal rats. In HS+Sal rats there was an increase in the percentage of fibers expressing type IIx MHC compared with Amb+Sal rats. In general, there were no consistent trends in MHC isoform profiles with the administration of GH or IGF-I with or without exercise in suspended rats.

The percentage of fibers coexpressing embryonic MHC in addition to adult MHC isoforms was 12–14% in all ambulatory Hypox rats and increased to 17–19% in all exercised suspended groups (Fig. 3). A much lower percent was found in the suspended-nonexercised groups (4–7%). In all groups, the embryonic isoform was colabeled primarily in fibers also expressing type IIa MHC (data not shown). No fibers expressed only the embryonic MHC isoform. Fibers that expressed the embryonic isoform were included in the statistical analyses for the percentage of fibers expressing adult MHC isoforms (Fig. 2) and for the fiber cross-sectional areas (Fig. 4).

Suspension had no effect on the expression of SERCA1 in Hypox rats (data not shown). Fibers that stained

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**Table 2.** Effect of growth hormone, IGF-I, and exercise on muscle wet weight in ambulatory and hindlimb-suspended rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LG</th>
<th>Plt</th>
<th>EDL</th>
<th>TA</th>
<th>RF</th>
<th>VL</th>
<th>VM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-hypox</td>
<td>1.052 ± 25*</td>
<td>423 ± 6*</td>
<td>209 ± 3*</td>
<td>730 ± 21*</td>
<td>1,168 ± 21*</td>
<td>1,385 ± 26*</td>
<td>444 ± 13*</td>
</tr>
<tr>
<td>Amb+Sal</td>
<td>516 ± 20</td>
<td>204 ± 7</td>
<td>107 ± 3</td>
<td>354 ± 9</td>
<td>525 ± 14</td>
<td>714 ± 25</td>
<td>255 ± 13</td>
</tr>
<tr>
<td>Amb+GH</td>
<td>658 ± 13*</td>
<td>262 ± 6*</td>
<td>138 ± 3*</td>
<td>448 ± 13*</td>
<td>647 ± 16*</td>
<td>843 ± 24*</td>
<td>292 ± 11*</td>
</tr>
<tr>
<td>Amb+IGF-I</td>
<td>571 ± 12*</td>
<td>222 ± 7</td>
<td>119 ± 3*</td>
<td>397 ± 8*</td>
<td>592 ± 16*</td>
<td>779 ± 29</td>
<td>269 ± 10</td>
</tr>
<tr>
<td>HS+Sal</td>
<td>470 ± 19</td>
<td>187 ± 5</td>
<td>106 ± 3</td>
<td>341 ± 9</td>
<td>508 ± 18</td>
<td>655 ± 28</td>
<td>235 ± 13</td>
</tr>
<tr>
<td>HS+GH</td>
<td>569 ± 21†</td>
<td>223 ± 8†</td>
<td>128 ± 4†</td>
<td>438 ± 12†</td>
<td>613 ± 22†</td>
<td>805 ± 29†</td>
<td>277 ± 10†</td>
</tr>
<tr>
<td>HS+IGF-I</td>
<td>500 ± 17</td>
<td>196 ± 7</td>
<td>118 ± 2†</td>
<td>391 ± 7†</td>
<td>542 ± 16</td>
<td>715 ± 18</td>
<td>247 ± 7†</td>
</tr>
<tr>
<td>HS+Sal+Ex</td>
<td>482 ± 15</td>
<td>183 ± 6*</td>
<td>100 ± 3</td>
<td>344 ± 15</td>
<td>489 ± 18</td>
<td>633 ± 27*</td>
<td>214 ± 7*</td>
</tr>
<tr>
<td>HS+GH+Ex</td>
<td>622 ± 24††</td>
<td>234 ± 6†</td>
<td>140 ± 4††</td>
<td>458 ± 12††</td>
<td>629 ± 16††</td>
<td>836 ± 29††</td>
<td>285 ± 11††</td>
</tr>
<tr>
<td>HS+IGF-I+Ex</td>
<td>533 ± 10†</td>
<td>201 ± 5</td>
<td>115 ± 3†</td>
<td>377 ± 6†</td>
<td>550 ± 8</td>
<td>717 ± 12</td>
<td>228 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. LG, lateral gastrocnemius; Plt, plantaris; EDL, extensor digitorum longus; TA, tibialis anterior; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis. Significantly different at P ≤ 0.05: *Amb+Sal vs. each group; †Amb+Sal vs. each HS group; ‡HS+GH vs. HS+GH+Ex.
positive for type II MHC also stained positive for SERCA1.

Fiber size. In general, fiber sizes were somewhat larger in ambulatory rats treated with either GH or IGF-I compared with Amb+Sal. However, the differences were significant only for type I+IIa+IIx MHC, type I+IIa+IIx MHC, and type IIa+IIx MHC for the Amb+GH rats. The cross-sectional area of types I, I+IIa, and IIa+IIx MHC fibers decreased by 25, 20 (%P<0.05), and 14% (%P<0.05), respectively, after suspension (Fig. 4). Neither GH, IGF-I, nor exercise alone had any significant effect on fiber cross-sectional area in suspended rats. Consistent with the effect on muscle weight, however, either GH or IGF-I treatment had a strong interactive effect with exercise on fiber cross-sectional area. The combination of either GH or IGF-I treatment plus exercise in suspended rats resulted in an increase in the mean cross-sectional area of each of the predominant fiber types, i.e., types I, I+IIa, and IIa+IIx (see Fig. 2), compared with HS+Sal rats. The cross-sectional areas of the predominant fiber types were significantly greater in the GH- or IGF-I treatment plus exercise groups than in HS+Sal group. GH+Ex and IGF-I+Ex in suspended rats increased the MHC type I

Fig. 2. Mean (±SE) percentage of fibers of medial gastrocnemius expressing adult myosin heavy chains for each experimental group. Significantly different at P<0.05: *Amb+Sal vs. each group; Amb+Sal vs. each HS group.

Fig. 3. Mean (±SE) percentage of fibers in medial gastrocnemius expressing embryonic myosin heavy chain for each experimental group. Significantly different at P<0.05: *Amb+Sal vs. each group; Amb+Sal vs. each HS group; HS+GH vs. HS+GH+Ex; HS+IGF-I vs. HS+IGF-I+Ex.
fiber cross-sectional area by 53 and 52% compared with HS+Sal rats and by 15 and 14% compared with Amb+Sal rats. For fibers expressing type I+IIa MHC, fiber cross-sectional area increased by 21 and 16% over Amb+Sal and by 52 and 46% over HS+Sal after treatment with GH or IGF-I, respectively, in combination with exercise. For fibers expressing type IIa+IIx MHC, fiber cross-sectional area increased by 21 and 28% over Amb+Sal and by 41 and 49% over HS+Sal after treatment with GH or IGF-I, respectively, in combination with exercise. Fibers expressing type IIx+IIb MHC and type IIb MHC were not affected by suspension. The cross-sectional area of fibers expressing type IIb MHC was 39 and 33% larger in HS+GH+Ex and HS+IGF-I+Ex rats, respectively, compared with Amb+Sal rats.

**DISCUSSION**

Muscle weight and fiber size adaptations. For fast muscles, there was an –42–55% decrease in wet weight in Hypox rats compared with Non-hypox rats. As was reported by Grindeland et al. (10), treatment of Hypox ambulatory and suspended rats with GH resulted in an increase in the weight of fast hindlimb muscles compared with the appropriate control. The increases in weight found in the present study were larger than those reported by Grindeland et al., most likely because of the animals being treated with GH for 3 additional days (10 vs. 7 days). In the present study, each of the slow muscles of ambulatory rats studied (i.e., soleus, adductor longus, and vastus intermedius) responded to GH treatment, whereas only the soleus and vastus intermedius of suspended rats responded to GH treatment (21). IGF-I treatment in suspended rats had an anabolic effect on the fast dorsiflexors but not the fast plantar flexors and on only one (vastus intermedius) of the three slow plantar flexors studied (21). These differential responses to IGF-I treatment may reflect an interactive effect between IGF-I and the level of muscle activation. For example, the tibialis anterior
becomes hyperactive immediately after suspension and remains hyperactive for at least 10 days of suspension, whereas both slow (soleus) and fast (medial gastrocnemius) plantar flexors are less active for the first 10 days of suspension than are those of ambulatory rats (1). The failure of the plantar flexor muscles to respond to IGF-I treatment may also reflect lower or less persistent levels of IGF-I than in GH-treated suspended rats. In these same rats, serum IGF-I levels in GH-treated rats were approximately sixfold higher than in IGF-I-treated rats (21).

The mean cross-sectional areas of each fiber type in the medial gastrocnemius were significantly smaller in all Hypox groups compared with the Non-hypox group. Generally, GH or IGF-I treatment of ambulatory and suspended Hypox rats increased the size of all fiber types relative to their respective controls, although the effect was significant only for fibers containing type I+IIaIlx MHC or IIx MHC. In most cases, the increases in muscle weight were greater than the increases in fiber cross-sectional areas. This apparent discrepancy may be the result of fibers being sampled only in the deep region of the medial gastrocnemius.

Two of the purposes of the present study were to determine 1) the efficacy of systemic IGF-I or GH treatment in ameliorating the suspension-induced fast muscle atrophy in Hypox rats and 2) whether short bouts of high-resistive exercise would potentiate any IGF-I or GH anabolic effect. In this case, absolute muscle weights were deemed to be more informative than muscle weights expressed relative to body weight. For example, expression of the data as relative weights assumes that muscle weight is directly proportional to body weight. This assumption is not necessarily valid, particularly in a GH- or IGF-I-treated Hypox rat (with and without exercise) in which body composition (i.e., fat and/or lean body mass) may be changing. This contention is supported by the observation that gastrocnemius weight increases similarly in GH-treated Hypox rats that are fed ad libitum or pair fed compared with control, whereas the body weight is significantly lower in the pair-fed compared with freely eating rats (3).

Fiber type adaptations. After 23 days of hypophysectomy, there was an ∼15 and ∼18% decrease in fibers expressing only type IIa MHC and only type IIx MHC, respectively, with an ∼8 and ∼14% increase in fibers expressing only type I MHC and type I+IIa MHC, respectively. The proportion of medial gastrocnemius fibers expressing only type I MHC was increased in only the Amb+Sal group compared with the Non-hypox group. In contrast, in the soleus, all Hypox groups had increased proportions of fibers expressing only type I MHC (21). These data are consistent with previous observations. For example, Shorey et al. (24) reported an increase in type I fibers in the gastrocnemius of 120-day-old rats that were hypophysectomized at 60 days. In addition, a decrease in type II fibers and an increase in hybrid fibers identified by myosin ATPase staining were observed in the extensor digitorum longus 3 wk after hypophysectomy (2). However, Loughna and Bates (18) reported decreases in the mRNA for type I and IIa MHC and an increase in the mRNA for type IIb MHC in the rat gastrocnemius after 3 wk of hypophysectomy.

In the present study the single-fiber MHC profiles in the medial gastrocnemius of Hypox rats did not change in response to 10 days of GH treatment. Similarly, GH treatment of Hypox rats for 7 or 11 days had no effect on the fiber type distribution of the extensor digitorum longus, although 15 days of GH treatment increased the proportion of type I fibers (2). In contrast, 7 days of GH treatment of Hypox rats has been shown to increase the transcript levels of type IIa MHC and decrease the transcript levels of type IIb MHC (18). The results of GH treatment on fiber types are also conflicting in Non-hypox rats. For example, GH treatment (2 wk or 6 mo) of male Non-hypox, barrier-protected, specific-pathogen-free Fischer 344 rats aged 8, 16, and 24 mo did not alter the fiber type proportions in the extensor digitorum longus (7). In contrast, Aylings et al. (2) reported an increase in the proportion of type I fibers in the extensor digitorum longus in Non-hypox rats after GH treatment for 7 and 11 days. Thus one can only surmise from these studies that the dose of GH, the period after hypophysectomy, and the age or strain of the animal may contribute to the apparently conflicting conclusions regarding the effect of hypophysectomy and/or GH treatment on fiber type adaptations.

The mean proportion of fibers containing the embryonic isoform was ∼4% for the nonexercised HS groups. This proportion was ∼12% (P > 0.05) and ∼17% in the ambulatory and suspended-exercised groups, respectively (Fig. 3), suggesting that weight support stimulated the expression of this MHC. No fibers expressed only the embryonic MHC, and the mean sizes of the fibers expressing embryonic MHC were not different from fibers of the same adult MHC type but not expressing this developmental isoform. These data suggest that new fibers were not being formed but rather that there was a reexpression of this developmental isoform in some fibers. Exogenous GH and IGF-I did not affect the percentage of fibers expressing embryonic MHC in ambulatory, suspended, or suspended-exercised rats, suggesting that the reexpression of embryonic MHC was not the result of deficient GH or IGF-I levels. In contrast to our findings, a decrease in embryonic MHC mRNA and an increase in neonatal MHC mRNA levels were found after 21 days of hypophysectomy in the rat gastrocnemius (18). The reason(s) for this apparent difference is (are) unknown. The percentage of fibers in the soleus expressing embryonic MHC increased after hypophysectomy but did not appear to be enhanced by loading or to be affected by GH or IGF-I.

GH or IGF-I plus exercise interactions on muscle weight and/or fiber size. The combination of GH or IGF-I treatment and exercise could have either an additive (growth factor effect + exercise effect = growth factor-exercise effect) or an interactive (growth factor effect + exercise effect < growth factor-exercise effect) effect. The weights of all predominantly fast (present paper) and the slow soleus and adductor longus (21)
muscles in this 10-day study of Hypox rats were significantly larger in HS+GH+Ex than in HS+Sal rats and showed an interactive effect between GH and exercise. In contrast, there was an interactive effect of IGF-I and exercise only for the medial and lateral gastrocnemius and extensor digitorum longus (present paper), and none of the slow muscles showed an interactive effect between IGF-I and exercise (21). Similarly, there was an interactive effect between GH and exercise for the medial and lateral gastrocnemius, tibialis anterior, and soleus, but not for the plantaris, in Hypox rats suspended for 7 days and exercised at a lower intensity than in the present study. Thus all of these data clearly show a strong interactive effect between GH and exercise on muscle weight in suspended Hypox rats and support the view that GH and IGF-I act, in part, independently.

There was a clear interactive effect between GH or IGF-I and exercise on the fiber sizes in the deep region of the medial gastrocnemius of suspended rats. The mean cross-sectional areas of fibers expressing type I+IIa MHC were 125, 100, and 144 µm² larger in HS+GH, HS+IGF-I, and HS+Sal+Ex rats, respectively, compared with the HS+Sal group. The mean cross-sectional areas of fibers expressing type I+IIa MHC were 391 and 347 µm² larger in HS+GH+Ex and HS+IGF-I+Ex rats, respectively, indicating an ~44% interactive effect between exercise and either growth factor. In addition, there was an ~15% interactive effect between GH or IGF-I and exercise on the cross-sectional area of fibers expressing only type I MHC. DeVol et al. (5) have shown that after functional overload in Hypox rats the IGF-I mRNA levels increased in the overloaded muscles. Furthermore, hindlimb muscle IGF-I mRNA and protein increased in GH-deficient rats after training (29). These findings suggest that the increased muscle load or exercise resulted in locally produced IGF-I. As observed with muscle weight, the combination of exercise-induced local IGF-I production and the administration of IGF-I may have contributed to the additive effect in fiber cross-sectional area in the medial gastrocnemius. Our findings on the mean fiber cross-sectional areas for the predominant fiber types are consistent with an increase in the tension capability of the medial gastrocnemius of rats treated with exercise and either growth factor.

Comparison of GH-exercise interaction effects in Hypox and Non-hypox rats. In a previous study (17) of Non-hypox suspended rats, GH and exercise (ladder climbing) appeared to have an additive effect on the gastrocnemius and plantaris muscle weights. These results differ from the interactive effect between GH and exercise observed in almost all fast muscles of Hypox rats (present study; Ref. 10). In addition, the weights of the planter flexors in Hypox (present study; Ref. 10), but not Non-hypox (17), rats treated with GH and exercise were maintained near or above the levels observed in ambulatory control rats. The different response to grid climbing in suspended Hypox and Non-hypox rats could be related to several factors, e.g., the duration of the experimental period and the relative intensity of the exercise performed. Compared with the present study, the study by Linderman et al. (17) using Non-hypox rats was of a shorter duration (10 vs. 5 days) and involved relatively low-intensity exercise (50% body weight in both studies) because Non-hypox rats can carry heavier loads than that used, e.g., 75% body weight and for more repetitions (13). Another possible explanation for the difference in the response of Hypox and Non-hypox rats could be that rats become more responsive to exogenous growth hormone after hypophysectomy. Thus the lack of a GH effect in the study of Linderman et al. may be due to the Non-hypox rats suppressing its own GH, thereby rendering the exogenous GH less effective.

Limitations of the study. Although the results of the present study clearly show an interactive effect of GH or IGF-I with exercise in ameliorating the loss of muscle mass in Hypox suspended rats, it does not demonstrate what the relative effects would be over a range of doses, i.e., the efficaciousness of the exercise, IGF-I, or GH and the interaction effects at different dosages. For example, the relative effectiveness of the dosages of IGF-I or GH used in the present study may reflect the shorter half-life of IGF-I compared with GH and/or the relative effectiveness of the dose of GH or IGF-I administered. The half-life of injected IGF-I in Non-hypox rats is ~4 h, whereas the half-life of IGF-I in Hypox rats is ~30 min (30). The shorter half-life of IGF-I in Hypox rats probably reflects the stimulation of the binding protein complex (IGF, IGF binding protein-3, and the acid-labile subunit). Although GH and IGF-I induce IGF binding protein-3 in Hypox rats (4), the acid-labile subunit of the complex forms only in Hypox rats treated with GH (4). The dose (1 mg/kg body wt) of GH used stimulates significant growth (one-half the rate of intact rats) in 100-g Hypox rats in weight gain assays (R. E. Grindeland, unpublished observations; also see Table 1). The dose (1 mg/kg body wt) of IGF-I used also stimulates growth in Hypox rats but to a lesser extent than did GH (Table 1; V. Mukku, personal communication).

Comparisons between GH and IGF-I also are confounded by GH having both direct (IGF-I independent) and indirect (mediated by IGF-I in the muscle) effects on skeletal muscle (8, 19). For example, Hypox rats treated with GH have increased serum IGF-I levels (21) and increased liver and muscle IGF-I levels (3). Thus the effects of systemically administered GH may partly reflect an autocrine and/or paracrine role of locally produced IGF-I in the muscle (26). The present results do show, however, that treatment with GH, and to a lesser extent IGF-I, in combination with exercise can be used as an effective countermeasure to suspension-induced atrophy.

Perspective. The present results together with previous studies (10, 17, 21) demonstrate that GH or IGF-I in combination with short bouts of resistive exercise can ameliorate skeletal muscle atrophy induced by non-weight bearing in Hypox and, to a lesser degree, Non-hypox rats. Thus, from a clinical point of view, the
present results suggest that the interactive potential between GH and IGF-I and resistive exercise in preventing skeletal muscle atrophy or in enhancing recovery from atrophy should be recognized in any treatment strategy. From a more basic perspective, the present study represents a unique demonstration that either brief or prolonged weight bearing in Hypox rats facilitates the expression of an embryonic MHC isoform rarely observed in skeletal muscles of adult Non-hypox rats, again emphasizing the potential importance of weight bearing in the regulation of genes in skeletal muscle fibers.

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Address for reprint requests: R. R. Roy, Brain Research Institute, UCLA School of Medicine, Center for the Health Sciences, 10833 Le Conte Ave., Los Angeles, CA 90095-1761.

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