

# Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats

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**Lee, Sukho, Elisabeth R. Barton, H. Lee Sweeney, and Roger P. Farrar.** Viral expression of insulin-like growth factor-I enhances muscle hypertrophy in resistance-trained rats. *J Appl Physiol* 96: 1097–1104, 2004; 10.1152/japplphysiol.00479.2003.—Muscle hypertrophy is the product of increased drive through protein synthetic pathways and the incorporation of newly divided satellite cells. Gains in muscle mass and strength can be achieved through exercise regimens that include resistance training. Increased insulin-like growth factor-I (IGF-I) can also promote hypertrophy through increased protein synthesis and satellite cell proliferation. However, it is not known whether the combined effect of IGF-I and resistance training results in an additive hypertrophic response. Therefore, rats in which viral administration of IGF-I was directed to one limb were subjected to ladder climbing to test the interaction of each intervention on muscle mass and strength. After 8 wk of resistance training, a 23.3% increase in muscle mass and a 14.4% increase in peak tetanic tension ( $P_o$ ) were observed in the flexor hallucis longus (FHL). Viral expression of IGF-I without resistance training produced a 14.8% increase in mass and a 16.6% increase in  $P_o$  in the FHL. The combined interventions produced a 31.8% increase in muscle mass and a 28.3% increase in  $P_o$  in the FHL. Therefore, the combination of resistance training and overexpression of IGF-I induced greater hypertrophy than either treatment alone. The effect of increased IGF-I expression on the loss of muscle mass associated with detraining was also addressed. FHL muscles treated with IGF-I lost only 4.8% after detraining, whereas the untreated FHL lost 8.3% muscle mass. These results suggest that a combination of resistance training and overexpression of IGF-I could be an effective measure for attenuating the loss of training-induced adaptations.

adeno-associated virus; ladder climbing; detraining

HYPERTROPHY OF SKELETAL MUSCLE and its concomitant gains in power are of great interest to people from all walks of life, including the elite power athlete, patients rehabilitating from injury-induced atrophy, and the elderly who have diminished mobility due to muscular weakness. Increased load on muscle drives hypertrophy through a number of signaling pathways, which ultimately increase the synthesis of proteins and decrease protein degradation. Hypertrophy can also involve activation and fusion of satellite cells to increase the nuclear content and transcriptional activity of the individual skeletal muscle fibers (18, 34).

Hepatocyte growth factor is one of the primary effectors of satellite cell activation via the c-met receptor (2), after which these cells express receptors for insulin-like growth factor-I (IGF-I). IGF-I mediates the proliferation and subsequent dif-

ferentiation of the satellite cells, after which they fuse to existing postmitotic muscle fibers. The fusion of nascent myocytes enables the increased production of muscle proteins and/or the repair of damaged regions of muscle fibers (for review, see Ref. 12).

Due to its critical role in satellite cell proliferation and differentiation, IGF-I serves as a potential candidate for promoting skeletal muscle hypertrophy. The hypertrophic response of muscle to IGF-I has been demonstrated in several animal models in which the IGF-I gene has been overexpressed by somatic cell or germ line transmission, or the recombinant protein has been infused into the muscle bed (1, 4, 9, 21). However, this response depends heavily on the presence of an activated satellite cell population. In instances in which the satellite cell pool has been inactivated by gamma irradiation, there is only a modest hypertrophic response to IGF-I (5) or to overload conditions (22–24). In contrast, animal models that exhibit heightened satellite cell activation and muscle regeneration, such as the *mdx* mouse, display a more dramatic hypertrophic response to IGF-I (3).

Because resistance training can cause significant hypertrophy due, in part, to its activation of satellite cells, it serves as an ideal model to test if the presence of activated satellite cells allows IGF-I to increase the hypertrophic response for a given exercise regimen. However, limited investigations into the combined effects of IGF-I and resistance exercise have been performed. Previous studies have demonstrated that the combination of administration of IGF-I protein and resistance training resulted in elevated muscle-to-body weight ratios in hypophysectomized hindlimb-suspended rats compared with either treatment alone (14, 25).

The ability of adeno-associated virus (AAV) to efficiently deliver a synthetic IGF-I-producing gene raises the possibility of genetic enhancement that could result in an increased training response. At the very least, it provides a means to test whether or not the responses to resistance exercise and to IGF-I overexpression are additive. Furthermore, viral administration of IGF-I enables expression of the transgene to endure for the life of an infected nucleus. Thus it also enables one to test whether the increased muscle mass derived from resistance training can be maintained, even after the exercise regimen has ceased, in the presence of high levels of IGF-I.

To investigate the effects of IGF-I, resistance training, and their interaction in skeletal muscle, we utilized AAV expression of IGF-I in combination with a ladder-climbing exercise in young adult rats. In addition, we examined the ability of IGF-I

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expression to maintain muscle mass achieved by resistance training after exercise had stopped. The results presented here demonstrate that IGF-I expression enhances the increase in muscle mass achieved by resistance training and attenuates the loss of muscle mass during the subsequent detraining period.

## METHODS

**Animal care and experimental design.** Thirty-one female Sprague-Dawley rats, 2 mo of age, were obtained from the Animal Resource Center, the University of Texas at Austin, Texas. All experiments involving animals were approved by the University of Texas at Austin's Animal Care and Use Committee. Twenty-four of these rats were injected with recombinant AAV harboring rat IGF-I cDNA (rAAVIGF-I) into the posterior compartment of their right lower leg, targeting the flexor hallucis longus (FHL) muscle (see below), whereas a sham group ( $n = 7$ ) received an injection of the same volume of PBS solution.

Two months after injection, viral-injected rats were randomly assigned into three groups ( $n = 8$  each condition): control (Con), resistance training (RT), and detraining (DT). All animals were allowed water ad libitum and were weighed once every week. The RT and DT groups received resistance training, which is described below. Animals in the Con group remained in their cages and experienced only normal in-cage activity. Food intake of Con animals was moderately restricted to balance their weight to match their RT counterparts. This was achieved by providing the animals with matched calories per day per body weight. After completion of 8 wk of resistance training, the animals from RT and Con groups were utilized for assessment of contractile function of the FHL. The remaining animals (DT group) were placed in their cages for an additional 12 wk, after which they were also utilized for FHL contractile function.

**Viral construction and injection.** A recombinant AAV plasmid (pSUB201) was constructed that contains the myosin light chain 1/3 promoter/enhancer, rat IGF-IA cDNA, and simian virus 40 polyadenylation signal for viral production, as previously described (4). Recombinant AAV serotype 1 (rAAV-2/cap1: AAV-2 genomes pseudopackaged into AAV-1 capsids) was prepared by the Institute for Human Gene Therapy Vector Core (Philadelphia, PA) following published procedures (13).

Rats ( $n = 24$ ) were anesthetized using pentobarbital sodium (50 mg/kg), and an incision was made in the medial aspect of the lower limb to expose the muscles of the posterior compartment under aseptic conditions. Six hundred microliters of 10% glycerol/PBS containing  $\sim 10^{10}$  recombinant AAV particles were injected into the posterior compartment targeting the FHL muscle. Preliminary experiments with vital dye (0.1% Evans blue) injections into the same compartment confirmed that the injected solution could be targeted to the FHL (data not shown). The incision was sutured after the procedure using 4-0 silk. Sham Con ( $n = 7$ ) were injected in the same manner with 600  $\mu$ L of 10% glycerol/PBS solution. On recovery from anesthesia, the animals were returned to the animal facility.

**Resistance-training protocol.** The rats in the RT and DT groups were subjected to one training period per day every third day for 8 wk. Training was accomplished utilizing a 1-m ladder with 2-cm grid steps and inclined at 85°. Initially, rats were familiarized with the ladder by practicing climbing the ladder from the bottom to the top cage for 3 days, after which the resistance-training regimen started.

A cylinder containing weights was attached to the base of the tail with foam tape (3M Concan) and a Velcro strap. The initial weight attached to each animal was 50% of its body weight. Rats were positioned at the bottom of climbing apparatus and motivated to climb the ladder by touching the tail. When the rats reached the top of the ladder, they were allowed to rest in a simulated home cage for 2 min. After the rest period, additional weights were placed in the cylinder, and the rats were returned to the bottom of the ladder for subsequent climbs. Rats climbed the ladder with 50, 75, 90, and 100% of maximal

load from the previous exercise session. If a rat was able to climb the ladder with these loads, additional weights were placed in the cylinder in 30-g increments for each subsequent climb. This procedure was repeated until eight climbs were achieved or until the rat failed to climb the entire length of the ladder. An electrical shock (0.2–0.3 mA) was used to motivate the rats as necessary. The training session was stopped when the rat refused to climb up the ladder after three successive shocks to the tail.

**In situ contractile properties of FHL.** At the end of 8 wk of training or 12 wk of detraining, rats from the respective groups were anesthetized initially using intraperitoneal injection of pentobarbital sodium (80 mg/kg). Additional injections were administered subcutaneously every 40 min to ensure anesthesia. The distal tendon of the FHL muscle was isolated and connected to the lever of a dual-mode servo galvanometer (model 3005 B, Cambridge Technologies). The muscle was stimulated to contract utilizing a Grass S88 stimulator with leads applied to the sciatic nerve. The muscle was kept wet in mineral oil, and the temperature was maintained between 36.5 and 37.5°C with a radiant heat lamp and monitored on the muscle surface with a thermometer. The muscle length was adjusted to optimal muscle length with a micrometer, while maximal twitch tension was determined using stimulation at 0.5 Hz and 7 V. The muscle was stimulated at 150 Hz and 14 V for peak tetanic tension ( $P_o$ ). After each contraction, the muscle was allowed to rest for 1 min. The galvanometer was interfaced with the computer (Macintosh Quadra 840 AV) and equipped with a National Instruments analog-to-digital board. The data were stored and analyzed using LabView software (version 3.0, National Instruments, Austin, TX). The resting muscle length was measured at the end of measurement. At the completion of contractile measurements, the FHL was excised immediately from the leg, cleaned of excess fat and external connective tissue, and weighed. The muscle was divided into four parts from the proximal portion for 1) gene expression detection, 2) protein and myosin heavy chain (MHC) composition, 3) citrate synthase (CS) activity, and 4) morphology. The portion for morphology was mounted in embedding compound (Tissue-Tek) and rapidly frozen in isopentane cooled in liquid nitrogen and stored at -80°C. The remaining portions were rapidly frozen in liquid nitrogen and stored for subsequent analysis. Specific tension was calculated by dividing  $P_o$  by the total muscle cross-sectional area, where cross-sectional area ( $\text{mm}^2$ ) = muscle mass (mg)  $\times$  [1.06 (mg/mm<sup>3</sup>)  $\times$  muscle fiber length (mm)]<sup>-1</sup>. Muscle fiber length of the FHL was determined using muscles from the sham group by nitric acid digestion (7).

**Detection of gene expression.** Reverse transcription-PCR was used to detect the presence of viral IGF-I transcripts in injected muscles. Total RNA isolated from frozen tissue (TRIZol reagent, GIBCO BRL) was subjected to reverse transcription and PCR (GeneAMP, Applied Biosystems) to specifically detect viral IGF-I expression. Oligonucleotides were used that recognized rat IGF-I and the SV40 polyadenylation signal (TCCGCTGAAGCCTACAAAGTC, sense primer; GAAGGAAAGTCCTGGGGTC, antisense primer). Primers that amplified GAPDH (TGAAGGTGGAGTCAACGGATTGGT, sense primer; TGTGGGCCATGAGGTCCACCA, antisense primer) served as a positive control for the procedure.

**Presence of central nuclei.** Frozen cross sections (10  $\mu\text{m}$ ) of the FHL were subjected to immunohistochemistry to assess the proportion of central nuclei in the muscle fibers. Rabbit anti-laminin (Neo-markers, Labvision, Fremont, CA) and rhodamine-conjugated donkey anti-rabbit IgG (H+L) (Jackson Immunoresearch Laboratories, West Grove, PA) were used to outline the muscle fibers. The sections were counterstained with 4,6-diamidino-2-phenylindole to visualize the myonuclei. Microscopy was performed on a Leitz DMR microscope (Leica Microsystems, Bannockburn, IL). Image acquisition and analysis was carried out using a MicroMAX digital camera system (Princeton Instruments, Trenton, NJ) and imaging software (OpenLab, Improvision). Four high-powered fields acquired from four different muscles subjected to each condition were utilized for calculating the

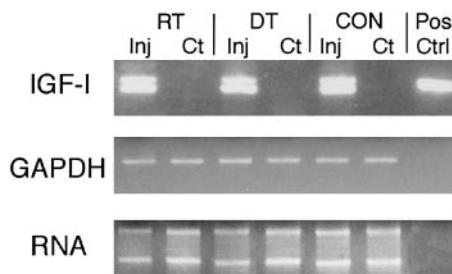


Fig. 1. Persistence of viral insulin-like growth factor-I (IGF-I) expression shown by reverse transcription-PCR. Flexor hallucis longus (FHL) muscles injected with recombinant adeno-associated virus IGF-I (rAAVIGF-I) (Inj lanes) displayed IGF-I expression from the viral transgene, which was not detectable in noninjected muscles (Ct lanes). Expression persisted throughout the study, which was 9 mo long. GAPDH expression was utilized to validate the reverse transcription procedure. Electrophoresis of total RNA extracted from the FHL muscles confirmed that the RNA was intact. The assay was performed on FHL muscles ( $n = 4$ ) from each group. Representative lanes for each condition are displayed. Con, control group; RT, resistance-trained group; DT, detrained group; Pos Ctrl, positive control plasmid containing viral IGF-I construct.

proportions of fibers containing central nuclei. Approximately 800 fibers were analyzed per muscle cross section.

**Muscle protein content and MHC composition.** Total protein and myofibrillar protein content were determined following the methods of Taylor and Kandarian (33). Briefly, muscles were homogenized with a polytron blade homogenizer (maximal speed) in a solution (pH 6.8) containing 8.5% sucrose, 50 mM KCl, 5 mM EGTA, and 100 mM MgCl<sub>2</sub>. An aliquot of this homogenate was used to determine total protein concentration using a Bradford assay (Bio-Rad). An aliquot of the remaining homogenate was centrifuged at 2,500 g for 15 min at 4°C. The supernatant was discarded and the pellet resuspended in a solution (pH 6.8) consisting of 100 mM KCl, 5 mM EGTA, 5 mM MgCl<sub>2</sub>, and 0.1% Triton X-100. The suspension was then centrifuged at 2,500 g for 10 min, and the supernatant discarded. This step was repeated two additional times. The pellet was then washed twice in a medium (pH 6.8) containing 100 mM KCl and 5 mM EDTA and centrifuged at 2,500 g for 10 min. The myofibrillar pellet was resuspended in 150 mM KCl and 50 mM tris(hydroxymethyl)aminomethane (pH 7.4). This myofibrillar suspension was used for determination of myofibrillar protein concentration. A Bradford assay was used for the determination of the myofibrillar protein concentration using the same procedure as described for total protein concentration.

One portion of each FHL muscle was weighed and then homogenized in Laemmli buffer (20). Total protein in each homogenate was determined by a Bradford assay (Bio-Rad) and normalized to total tissue wet weight, and equal protein amounts were then separated using SDS-PAGE (31). Resolved gels were stained with Coomassie blue R-250 for 45 min followed by destaining for 2 h in 50% methanol-7% acetic acid. Images of the gels were captured with a high-performance digital camera (Kodak), and quantification of the MHC bands was performed with associated software (Kodak 1D, Eastman Kodak, Rochester, NY).

**Oxidative enzymes.** CS activity, a mitochondrial enzyme, was measured from whole muscle homogenates by using spectrophotometry (27).

**Statistical analysis.** All data were expressed as means  $\pm$  SE. A one-way analysis of variance was used to compare all variables among groups. Fisher's least significant difference was used to test for group differences. A two-way analysis of variance was used to test for interaction between activity and rAAVIGF-I injection. A significance level of  $P < 0.05$  was used for all comparisons.

## RESULTS

**IGF-I promotes muscle hypertrophy in normal rat skeletal muscle.** To assess the effects of viral administration of IGF-I in rat muscle, Con animals were examined 4 mo postinjection of rAAVIGF-I. RT-PCR analysis confirmed the expression of viral IGF-I (Fig. 1) in all muscles treated with rAAVIGF-I, compared with uninjected or sham-injected Con. Viral IGF-I expression promoted a significant increase in muscle mass and strength (Fig. 2, Table 1) in young growing rats. We observed a 14.8% increase in muscle mass and 16.6% increase in P<sub>o</sub> in the FHL muscles treated with rAAVIGF-I. The results are similar to those of previous studies using a similar technique in mouse muscle (4, 5). Rats in the sham group displayed no significant difference in muscle mass between saline-injected and uninjected limbs ( $610 \pm 10$  and  $596 \pm 10$  mg, respectively), confirming that the increase in muscle mass was due to the expression of IGF-I and not to the injection procedure.

**Resistance training promotes significant hypertrophy in rat skeletal muscle.** A second group of animals was subjected to resistance training to test the ability of the ladder-climbing exercise to increase muscle hypertrophy (RT group). After 8 wk of resistance training, the animals could carry 1,400 g up the ladder, which was over three times their body weight. The training protocol promoted a significant increase in muscle mass and force generation (Fig. 2, Table 1). FHLs in the RT group showed a 23.3% increase in muscle mass and a 14.4% increase in P<sub>o</sub> compared with that in the Con group. Although there was a modest decrease of specific force in the RT group, the difference was not significant compared with the Con values (Fig. 2, Table 1). These results help to validate that the ladder-climbing exercise regimen can cause muscle hypertrophy similar to other resistance-training models.

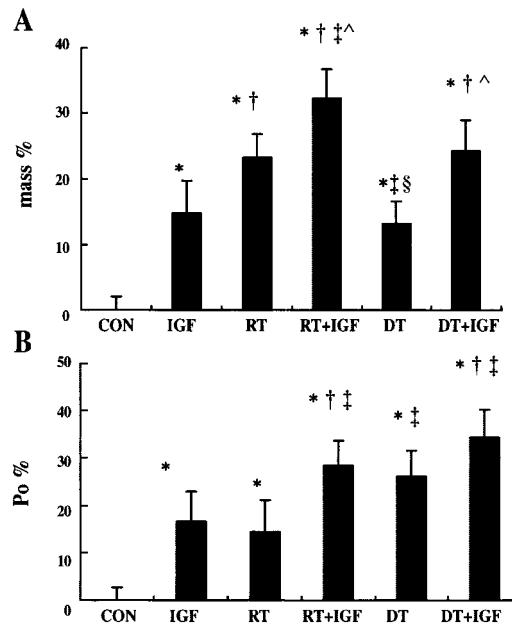


Fig. 2. Effect of resistance training and IGF-I expression on FHL muscle mass and force production ( $n = 7$  each group). A: percent change in muscle mass compared with Con FHL muscles. B: percent change of maximum tetanic force production (P<sub>o</sub>) compared with Con muscles. Values are means  $\pm$  SE. Significantly different from \*Con, †IGF, ‡RT, §RT+IGF, and ^DT:  $P < 0.05$ .

Table 1. Contractile properties of *flexor hallucis longus*

	Con	IGF	RT	RT + IGF	DT	DT + IGF
Body weight, g	402 ± 1.2	402 ± 1.2	403 ± 21.2	403 ± 21.2	403 ± 24.5	403 ± 24.5
Muscle mass, mg	566 ± 12.4	650 ± 18.7 <sup>a</sup>	698 ± 10.4 <sup>a,b</sup>	749 ± 17.1 <sup>a,b,c</sup>	640 ± 8.4 <sup>c</sup>	713 ± 18.5 <sup>a,b,c</sup>
CSA, mm <sup>2</sup>	59.3 ± 1.3	67.7 ± 1.9 <sup>a</sup>	72 ± 1.2 <sup>a,b</sup>	77.1 ± 1.3 <sup>a,b,c</sup>	66.3 ± 1.0 <sup>a,c</sup>	72.3 ± 5.0 <sup>a,b,d,e</sup>
P <sub>o</sub> , mN	15,050 ± 424	17,549 ± 622 <sup>a</sup>	17,218 ± 665 <sup>a</sup>	19,323 ± 476 <sup>a,b,c</sup>	18,971 ± 517 <sup>a,c</sup>	20,216 ± 624 <sup>a,b,c</sup>
SP <sub>o</sub> , N/cm <sup>2</sup>	25.3 ± 0.5	25.9 ± 0.6	23.9 ± 0.9	25.1 ± 0.4	28.9 ± 0.9 <sup>a,c</sup>	28.1 ± 1.2 <sup>a,d</sup>

Values are means ± SE for  $n = 7$  observations in each condition. Con, control; IGF, insulin-like growth factor; RT, resistance training; DT, detraining; CSA, cross-sectional area; P<sub>o</sub>, peak tetanic tension; SP<sub>o</sub>, specific peak tetanic tension. Significantly different from <sup>a</sup>Con, <sup>b</sup>IGF-I, <sup>c</sup>RT, <sup>d</sup>RT + IGF-I, and <sup>e</sup>DT:  $P < 0.05$ .

**IGF-I enhances the hypertrophic effect of resistance training.** One limb of the RT animals was treated with rAAVIGF-I to test whether there was an additive response between increased IGF-I expression and resistance training. The results show that there was a significant increase in the amount of hypertrophy after resistance training in the presence of high IGF-I expression (RT + IGF-I, Fig. 2, Table 1). There was a 7.3% increase in muscle mass and a 12.2% increase in P<sub>o</sub> between the RT + IGF-I and the RT-only FHL muscles. Overall, there was a 31.8% increase in muscle mass and a 28.3% increase in P<sub>o</sub> with the combined interventions compared with Con muscles. Therefore, the combination of resistance training and increased IGF-I expression produced significantly greater hypertrophy than either treatment alone. The observed increases were due to independent effects of resistance training and of increased IGF-I expression and not due to interaction of the two conditions, as assessed by two-way analysis of variance.

**The loss of muscle mass with detraining is attenuated by IGF-I expression.** A third group of animals that had been treated with rAAVIGF-I in one limb was subjected to 8 wk of resistance training followed by 12 wk of sedentary activity to test whether the loss of muscle mass after resistance training was prevented by IGF-I expression (DT and DT + IGF-I groups). IGF-I expression minimized the loss of FHL muscle mass after 12 wk of detraining (Fig. 2, Table 1). DT muscles lost 8.3% mass compared with muscles in the RT group. Muscles in the DT + IGF-I group lost only 4.8% mass after detraining, which was not significantly different from the muscle mass observed in the RT + IGF-I group. In the DT animals, muscle mass was 11.4% higher in the FHL muscles treated with rAAVIGF-I compared with the uninjected limbs. The difference in muscle mass was not significantly different from that observed between the Con and Con + IGF-I groups.

**Protein content in response to IGF-I and resistance training.** Total protein and myofibrillar protein content were determined from muscle homogenates to test whether the differences in muscle mass were correlated to differences in protein content. Measurements were normalized to tissue wet weight. The results are shown in Table 2. Muscles that underwent resistance training in the absence of rAAVIGF-I had a significant

decrease in total protein and myofibrillar protein compared with control muscles. This difference disappeared in resistance-trained muscles that had been injected with rAAVIGF-I. Total protein increased in concert with increases in muscle mass such that there was no significant difference in relative protein content between muscles. Muscles from both detraining groups exhibited no significant differences in protein content compared with Con muscles.

**Adaptational response to IGF-I and resistance training.** To assess whether there was a shift in fiber properties associated with resistance training or IGF-I expression, MHC content and CS activity were measured. MHC isoforms were differentiated in the FHL by SDS-PAGE. Separation of 1, 2b, and 2a/x was achieved based on protein samples from muscles with known compositions. Figure 3 displays representative samples from each condition. The FHL was composed predominantly of MHC<sub>2a/x</sub>, with a small percentage of MHC<sub>2b</sub> (Table 2). MHC<sub>2b</sub> content fell below the level of detection in the FHL from RT and DT animals. Viral expression of IGF-I did not affect the proportions of myosin isoforms in any condition.

The aerobic capacity of whole muscle homogenate, as determined by CS activity, showed no changes in the IGF or RT group alone (Table 2). However, CS activity significantly increased in the RT + IGF muscles compared with either the Con or IGF groups ( $P < 0.05$ ). Twelve weeks of detraining resulted in no alteration in CS activity (Table 2). Therefore, the aerobic capacity of muscle increased only with the combined treatment of IGF-I and resistance training.

**Presence of central nuclei.** Immunohistochemistry was utilized to analyze the proportion of central nuclei in the FHL fibers after the various treatments, as an indicator of muscle fiber regeneration and satellite cell activation (Fig. 4, Table 3). In the Con, RT, and DT groups, <1% of the fibers contained central nuclei in the uninjected muscles. In the presence of IGF-I (Con + IGF-I), the proportion of centrally nucleated fibers increased to 2.4%, but the change was not significant. The most dramatic increase was observed in muscles that had undergone resistance training in the presence of IGF-I expression, where 21.5 ± 3.9% (mean ± SE) of the fibers in a given field had central nuclei. After detraining, the level of centrally

Table 2. MHC composition, protein content, and citrate synthase activity of the *flexor hallucis longus* muscle

	Con	IGF	RT	RT + IGF	DT	DT + IGF
MHC <sub>2a/x</sub> , %	97.0 ± 1.7	93.2 ± 3.2	100 ± 0*	100 ± 0*	100 ± 0*	100 ± 0*
MHC <sub>2b</sub> , %	5.9 ± 0.5	8.5 ± 3.1	0*	0*	0*	0*
Citrate synthase, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	15.4 ± 0.7	14.1 ± 0.8	16.0 ± 0.9	17.7 ± 0.9*†	16.9 ± 0.6	16.2 ± 0.4
Total protein, mg/g tissue	355.6 ± 20.4	329.3 ± 40.3	271.5 ± 10.5*	298.7 ± 46.4	350.1 ± 18.0	293.4 ± 37.6
Myofibrillar protein, mg/g tissue	46.2 ± 5.8	57.4 ± 12.9	27.3 ± 6.0*	38.5 ± 3.6	54.1 ± 9.7	45.5 ± 4.6

Values are means ± SE for  $n = 7$  muscles for each condition. MHC, myosin heavy chain. Significantly different from \*Con and †IGF:  $P < 0.05$ .

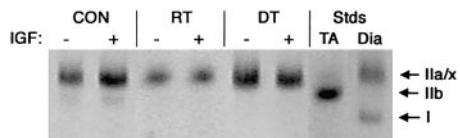


Fig. 3. Myosin heavy chain (MHC) content of the FHL by SDS-PAGE. The FHL was composed predominantly of MHC<sub>2a/x</sub> (IIa/x) with a small percentage of MHC<sub>2b</sub> (IIb) (see Table 2 for measurements). MHC<sub>2b</sub> content fell below the level of detection in the FHL from resistance-trained and detrained animals. Viral expression of IGF-I did not affect the proportions of myosin isoforms. Standards (Stds) included tibialis anterior (TA) and diaphragm (Dia) from rat to identify the MHC isoforms 2a/x, 2b, and 1 (I). + and -, Presence or absence, respectively, of IGF-I expression by adeno-associated virus injection.

nucleated fibers in the IGF-I-treated muscles fell, but was still significantly higher than that in the Con + IGF-I muscles.

## DISCUSSION

This study tested whether the combined effect of resistance training and IGF-I overexpression on muscle mass and strength was the sum of either treatment alone. The results show that each intervention produces significant hypertrophy in the targeted muscle, and the increase in muscle mass is significantly greater in muscles that experienced resistance training in the presence of IGF-I. While the combined treatments do not produce an additive response, there is a significant enhancement of hypertrophy compared with either treatment alone. The study also addressed the effects of detraining on muscle mass and tested whether IGF-I could prevent the decrease of mass associated with sedentary activity. We found that high IGF-I expression attenuated the loss of muscle mass that arises from the cessation of resistance training. The proportional increase in muscle mass observed in the presence of IGF-I during resistance training was maintained during the detraining phase.

Resistance training was achieved by using regular bouts of ladder climbing. This resulted in a 23.3% increase in absolute muscle mass of FHL. Ladder-climbing regimens have been found to produce a 5–26% increase in muscle mass (11, 16, 19, 26, 32). Several muscle groups can be affected, including the soleus, plantaris, and gastrocnemius, depending on the duration and specifications of the training protocol (11, 16, 19, 26, 32). The ladder-climbing regimen utilized in this study is relatively modest in intensity compared with other study designs, in that the number of repetitions per session and frequency of exercise sessions are significantly less than those in other protocols. In a previous study, it was found that the mass of other muscle groups was not affected by this resistance-training protocol, whereas the FHL displayed a significant increase in muscle mass (17). We postulate that the selective hypertrophy of FHL is due to its eccentric mode of action during climbing. We observed that rats place their toes on the ladder, and the initial phase is a lengthening of the FHL and flexor digitorum longus, which share common tendinous attachments. The rat then goes through plantar flexion and knee extension in concentric con-

tractions. Thus the FHL undergoes both eccentric and concentric phases of activity. The eccentric exercise has been shown to be more effective than concentric exercise for improving muscle mass and strength (6, 15, 35), presumably due to the increased damage/repair cycles experienced after eccentric contraction (10). While not directly tested in this study, it is likely that the inclusion of eccentric contractions is a significant factor in the specific hypertrophic response in the FHL.

Food intake for the Con animals was restricted to ensure that body weights were similar in the trained and untrained groups. When ad libitum-fed rats undergo resistance-training regimens, body weight decreases compared with age-matched sedentary Con (17). To determine absolute changes in muscle mass with IGF-I expression or resistance training, food intake of sedentary and exercising animals was matched to provide equal calories per day per body weight.

Resistance training alone produced disproportionate increases in muscle mass and force (Table 1, Fig. 2) in that mass increased ~10% more than muscle force. Although this did not result in significantly different specific forces from the Con group, the results suggest that the ladder-climbing regimen caused damage, such that all fibers were not contributing to force production, and that the damage was not adequately repaired. Alternatively, some proportion of the observed increase in muscle mass could have been due to a change in water content. The decrease in total protein content (Table 2) supports the hypothesis that there was an increase in water content in the RT animals. Other factors leading to decreases in muscle force, such as increased fibrosis and damaged sarcolemmas, were not determined in this study and so remain as potential contributors to the disparity between muscle mass and strength. In other models of resistance training, there was no indication of lower specific forces (18, 25); however, the function of the FHL had not been examined in previous studies. The presence of IGF-I in resistance-trained muscles eliminated any disparity between mass and force gains, and the drop in protein content and myofibrillar content also disappeared. This suggests that protein synthetic processes were increased in the presence of IGF-I. In the *mdx* mouse diaphragm, which displays significant fibrosis and a high proportion of damaged muscle fibers (29), transgenic expression of IGF-I significantly reduces the number of damaged fibers, assessed by Evans blue dye uptake, and virtually eliminates fibrosis (3). It is possible in resistance-trained rat muscle that IGF-I-mediated repair processes inhibit fibrotic infiltration, but this was not directly tested in this study.

Even though this exercise regimen is sufficient to cause significant hypertrophy (and possibly damage) in the FHL, it was not severe enough to cause satellite cell fusion to existing fibers. The lack of central nuclei in the RT group suggests that satellite cells were not the cause of hypertrophy, and, instead, that an increase in muscle fiber protein synthesis was the source of increased mass via IGF-I binding to its receptor

Table 3. Presence of central nuclei

	Con	IGF	RT	RT + IGF	DT	DT + IGF
Central nuclei, %	0.1 ± 0.05	2.4 ± 0.39	0.4 ± 0.15	21.5 ± 3.9 <sup>a,b,c,e</sup>	0.5 ± 0.2	8.1 ± 2.4 <sup>a,b,c,d,e</sup>

Values are means ± SE for  $n = 4$  high-powered fields from  $n = 4$  muscles from each condition. Significantly different from <sup>a</sup>Con, <sup>b</sup>IGF-I, <sup>c</sup>RT, <sup>d</sup>RT + IGF-I, and <sup>e</sup>DT:  $P < 0.05$ .

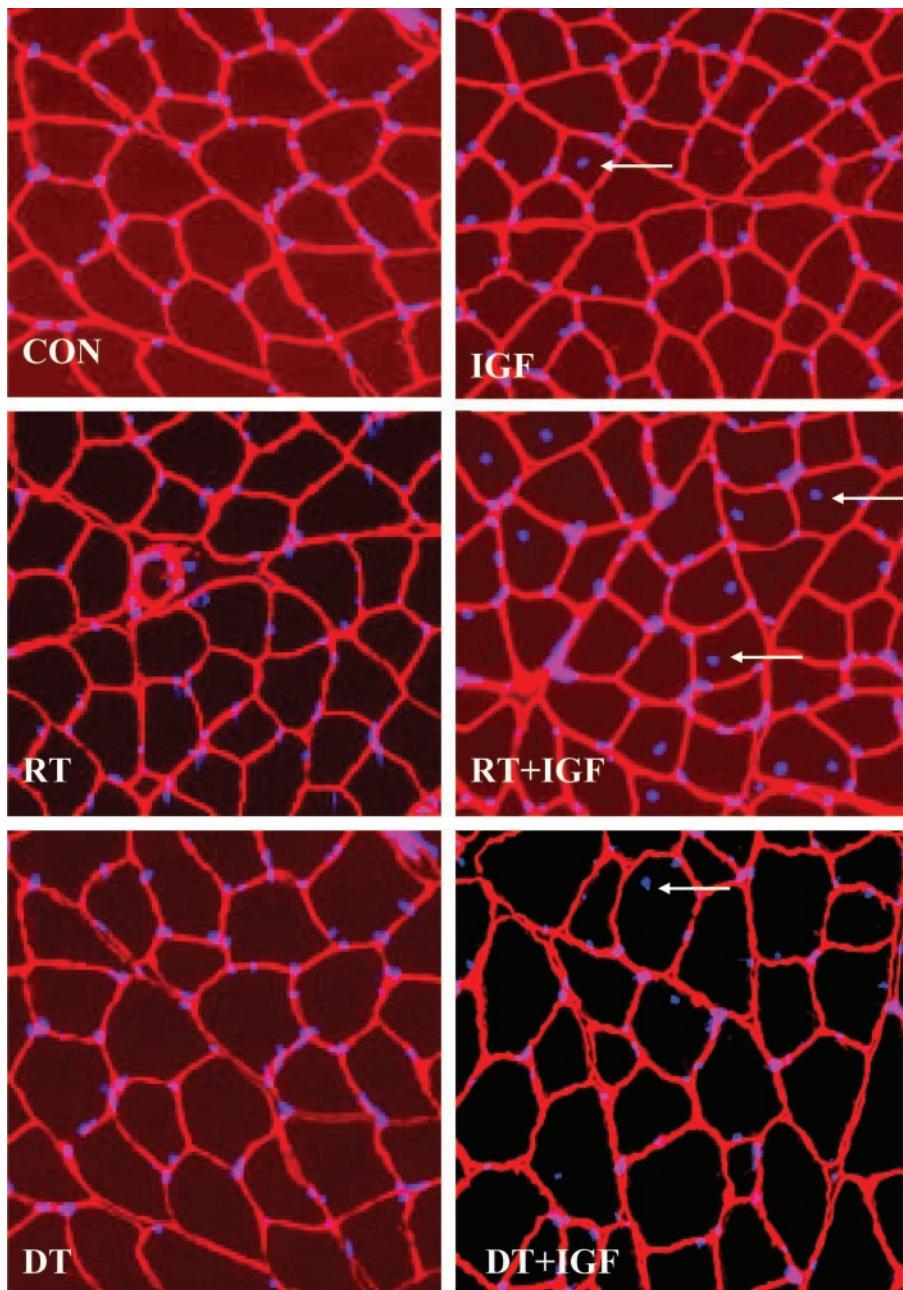


Fig. 4. Analysis of muscle fiber nucleation by immunohistochemistry. Representative images of frozen cross sections that had been stained with  $\alpha$ -laminin to delineate the sarcolemma and with 4,6-diamidino-2-phenylindole to reveal that all nuclei are shown for each experimental condition. Central nuclei (arrows) were apparent in all rAAVIGF-I-injected cross sections and were most prominent in muscles from the RT + IGF group.

located on the muscle fiber membrane. Furthermore, the modest increase in central nuclei in the Con + IGF-I muscles suggests that the activated satellite pool was minimal, possibly due to the lack of muscle growth at this rat age. However, the increase in central nuclei seen in the RT + IGF-I muscles supports the hypothesis that the exercise has activated the satellite cells and, hence, has "primed" them for IGF-I to promote their proliferation and fusion. Inactive satellite cells *in vivo* do not express receptors for IGF-I, and so this cell population must be activated, potentially by damage, to be responsive to increased levels of IGF-I (2). Although not addressed in this study, it would be important to test in the level of activated satellite cells increased with resistance training.

The presence of central nuclei after detraining in the IGF-I-treated muscles likely serves as markers for fusion events,

which occurred during resistance training, although it is also possible that muscle repair and regeneration continued during the detraining phase activity. The time course of muscle regeneration can be several weeks, and, during that time, central nuclei are thought to migrate to the periphery of the fibers (8). An investigation of the change in proportion of central nuclei during detraining in combination with an assessment of satellite cell activation would help to elucidate and contribute to the understanding of the processes of which mechanism led to the diminishment of central nuclei.

Although not investigated in this study, the results support the hypothesis that there are multiple pathways that can produce muscle hypertrophy. First, resistance training and IGF-I, in part, can promote protein synthetic pathways within the muscle fibers that increase muscle mass without the need for satellite cell fusion. In addition, IGF-I expression amplifies the

proliferation of activated satellite cells, thereby enhancing muscle hypertrophy through the fusion of these cells to existing myofibers. The boost in muscle mass with the combined treatment is likely through both pathways and suggests that the proportion of hypertrophy due to IGF-I activation of the satellite cells is ~7%, or one-half of the total increase in muscle mass by IGF-I. This finding is similar to that found in the study in which satellite cell proliferation was blocked by gamma irradiation. In the previous study, the absence of satellite cell proliferation eliminated one-half of the increase in muscle mass produced by viral expression of IGF-I (5).

To our knowledge, no study has investigated the effects of IGF-I on attenuating the reversal of training-induced adaptations in rat skeletal muscle during detraining. Detraining results in a significant reduction in resistance-training-induced muscular adaptations (27, 28) and was the case in our exercise model. After 12 wk of detraining, the proportional loss in muscle mass was attenuated by IGF-I expression. Two possible mechanisms could contribute to the attenuation of the effects of detraining. First, the increased satellite cell activation and proliferation produced by the combination of resistance training and IGF-I could help to maintain muscle mass during detraining as the cells that had been activated during the training period continue to undergo fusion. This effect would diminish over time as the signals for activation had ceased. Alternatively, the drive through protein synthetic pathways by IGF-I could maintain the gains in mass and force derived from resistance training. It is unclear what would occur over much longer periods without exercise. Previous studies in sedentary mice suggest that high levels of IGF-I can maintain muscle mass for the lifetime of the animal (4, 20), and so it is possible that, once increased muscle mass is achieved (via resistance training or IGF-I), the presence of IGF-I will prevent the significant loss of that mass. Further examination of the mechanisms underlying the attenuation of loss of muscle mass is warranted to determine whether either of the above processes are involved.

The implications of the prevention of the loss of muscle mass broach complicated issues. One can foresee great benefits derived from viral IGF-I treatment directed at the elderly population, such as maintaining strength and mobility and thereby improving the quality of life of treated individuals. However, as demonstrated in this study, the same techniques can be utilized to enhance athletic performance by increasing muscle mass and strength, and also, perhaps, prolonging the benefits of training during periods of imposed inactivity, such as after an injury.

In conclusion, we have demonstrated that the combination of resistance training and overexpression of IGF-I induces greater hypertrophy than either treatment alone. Furthermore, overexpression of IGF-I attenuates the loss of muscle mass after 12 wk of detraining. These results suggest that a combination of resistance training and overexpression of IGF-I could be an effective means for promoting muscle hypertrophy and attenuating the loss of training-induced adaptations due to various situations, including detraining and aging.

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