Growth hormone/IGF-I and/or resistive exercise maintains myonuclear number in hindlimb unweighted muscles

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1Department of Physiological Science and 2Brain Research Institute, University of California, Los Angeles, 90095-1527; 3National Aeronautics and Space Administration-Ames Research Center, Moffett Field, 94035-1000; and 4Genentech Incorporated, South San Francisco, California 94080

Allen, David L., Jon K. Linderman, Roland R. Roy, Richard E. Grindeland, Venkat Mukku, and V. Reggie Edgerton. Growth hormone/IGF-I and/or resistive exercise maintains myonuclear number in hindlimb unweighted muscles. J. Appl. Physiol. 83(5): 1857–1861, 1997.—In the present study of rats, we examined the role, during 2 wk of hindlimb suspension, of growth hormone/insulin-like growth factor I (GH/IGF-I) administration and/or brief bouts of resistance exercise in ameliorating the loss of myonuclei in fibers of the soleus muscle that express type I myosin heavy chain. Hindlimb suspension resulted in a significant decrease in mean soleus wet weight that was attenuated either by exercise alone or by exercise plus GH/IGF-I treatment but was not attenuated by hormonal treatment alone. Both mean myonuclear number and mean fiber cross-sectional area (CSA) of fibers expressing type I myosin heavy chain decreased after 2 wk of suspension compared with control (134 vs. 162 myonuclei/mm and 917 vs. 2,076 µm², respectively). Neither GH/IGF-I treatment nor exercise alone affected myonuclear number or fiber CSA, but the combination of exercise and growth-factor treatment attenuated the decrease in both variables. A significant correlation was found between mean myonuclear number and mean CSA across all groups. Thus GH/IGF-I administration and brief bouts of muscle loading had an interactive effect in attenuating the loss of myonuclei induced by chronic unloading.

METHODS

Experimental design. Adult 3-mo-old female Sprague-Dawley rats (Simonsen, Gilroy, CA) were used for this study. Animal care and use were in accord with the “Ames Research Center Animal Users Guide” (AHB 7180) and the guidelines of the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Ames Research Center and the Animal Research Committee at UCLA. The rats were housed in pairs and kept on a standard 12:12-h dark-light cycle in a room maintained at 24 ± 1°C. Standard rat chow and water were provided ad libitum. Twenty-five rats were assigned randomly and equally to one of five groups: 1) control, saline injected (Con+S); 2) hindlimb suspended, saline injected (HS+S); 3) hindlimb suspended, GH/IGF-I injected (HS+GH/IGF-I); 4) hindlimb suspended, exercised (HS+Ex); and 5) hindlimb suspended, GH/IGF-I injected, and exercised (HS+GH/IGF-I+Ex).

Animals were HS by using a tail cast procedure described previously (11). The suspended rats were also subjected to functional overload (22) of the soleus by ablation of the gastrocnemius and plantaris 7 days before the 14-day suspension period. Overload was induced 1 wk before suspension to allow for recovery from the initial inflammatory response occurring during the first few days after surgery (5). Functional overload was employed to enhance the loading of the soleus during the brief daily bouts of resistance exercise. Previous studies have demonstrated that mean myonuclear number is not significantly increased in the soleus after only 1 wk of functional overload (25) and that functional overload does not affect the atrophic response of the soleus during HS (19).

HS+Ex and HS+GH/IGF-I+Ex rats were subjected to brief bouts of climbing exercise three times daily (at 0800, 1200, and 1600) with the first bout starting immediately after the initiation of HS as described by Grindeland et al. (11). Briefly, during each bout of exercise, the rats climbed a 1-m wire-mesh grid (85° incline) 10 times in succession with a...
weight of \( \sim 40\% \) of body weight attached to their tail cast to increase the load on the muscle. Each rat spent a total of \( \sim 5–10 \) min/day preparing to climb or climbing. The HS+S and HS+GH/IGF-I rats were suspended continuously for the duration of the experiment.

Immediately before each exercise bout, the HS+GH/IGF-I+Ex rats were given an injection (sc) of recombinant human GH and IGF-I (Genentech, San Francisco, CA) of 1 mg/kg body weight; each hormone was dissolved in 0.85% NaCl at a concentration of 250 \( \mu \)g/ml. The Con+S and HS+S rats received the same volume of 0.85% NaCl injected at the same times. Combined GH/IGF-I injections were employed because previous studies have demonstrated that optimal elevation of serum IGF-I levels and minimal effects on insulin secretion are achieved in normal, nonhypophysectomized rats with coinjection compared with either growth factor alone (12, 16). Combined GH/IGF-I injections also result in the optimal secretion of IGF-I binding proteins from the liver (16), thus increasing the half-life of injected IGF-I.

Rats were killed 2 wk after the initiation of suspension; control rats were killed at the same time. The soleus muscles were removed, wet weighed, frozen in isopentane cooled in liquid nitrogen, and stored at \(-70\)°C.

Confocal microscopy. Myonuclear number and fiber CSA were determined by using confocal microscopy of fluorescently stained isolated single fiber segments as described previously (3, 4). Four muscles, one each from the Con+S, HS+S, HS+GH/IGF-I, and HS+Ex, provided too few fibers and were excluded from the statistical analyses. Single muscle fiber segments were mechanically dissected, placed on gelatin-coated slides, and stained for 5 min each with 54 \( \mu \)M acridine orange and with \( 1.5 \times 10^{-7} \) M propidium iodide, then rinsed with phosphate-buffered saline and mounted in 100% glycerol with coverslips with "struts" of hardened nail polish in the corners to minimize fiber compression. All fibers were treated in an identical fashion to avoid differences caused by specimen preparation. Although slight osmotic changes in fiber size may have occurred in response to rinsing and staining, relative differences between groups should be unaffected (4). Fibers were analyzed on a Sarastro 2000 confocal microscope (Molecular Dynamics, Sunnyvale, CA) by using the filter sets for acridine orange fluorescence as described previously (3, 4).

Single-fiber gel electrophoresis. After confocal analysis, fibers were unmounted, rinsed in phosphate-buffered saline, and dehydrated for 5 min in 50% ethanol. Fibers were scraped from the slide by using a clean razor blade and were placed in 8–15 \( \mu \)l electrophoresis sample buffer (17) and then stored at \(-5\)°C. Single-fiber protein samples were run on a Protean II Minigel apparatus (Bio-Rad, Richmond, CA) by using the technique of Talmadge and Roy (26) to separate the adult myosin heavy chains (MHC). Gels were stained with Rapid Coomassie, as per the supplier’s instructions (Diversified Biotech, Boston, MA), and evaluated for MHC expression as described previously (3, 4). A previous study (4) demonstrated that, after 2 wk of spaceflight, mean myonuclear number is significantly decreased only in type I MHC-expressing soleus fibers. Thus only fibers expressing type I MHC, either alone or in combination with any type II MHC, were analyzed.

Statistical procedures. Data are expressed as means \( \pm SE \). For statistical evaluation of differences among groups, a one-way analysis of variance was performed, followed by the Fisher’s protected least squares difference post hoc test. The Pearson product correlation was calculated for the relationship between myonuclear number and fiber CSA. An alpha level of 0.05 was set as the limit for statistical significance.

RESULTS

Muscle wet weight. Compared with Con+S rats, the soleus muscle wet weight was 23% smaller in HS+S rats (Fig. 1). Daily injection of GH/IGF-I alone had no effect on the soleus wet weight of suspended rats, and the mean muscle wet weight in HS+GH/IGF-I rats was lower than in control. Exercise alone, in contrast, partially attenuated the loss in soleus muscle wet weight such that the mean muscle wet weight of HS+Ex rats was similar to that in controls. The mean muscle wet weight of HS+GH/IGF-I+Ex rats was restored to Con+S levels and was larger than that of HS+S and HS+GH/IGF-I groups.

Fiber CSA. Compared with Con+S rats, the mean fiber CSA was 55, 49, 44, and 23% smaller in HS+S, HS+GH/IGF-I, HS+Ex, and HS+GH/IGF-I+Ex rats, respectively (Table 1, Fig. 2). The HS+GH/IGF-I+Ex rats had a larger mean fiber CSA than that of the HS+S, HS+GH/IGF-I, and HS+Ex rats (Table 1).

Myonuclear number. Mean myonuclear number was lower in HS+S, HS+GH/IGF-I, and HS+Ex rats than in Con+S rats (Table 1). However, the mean myonuclear number in HS+GH/IGF-I+Ex rats was not significantly different from control. In addition, the mean myonuclear number was higher in HS+GH/IGF-I+Ex rats than in HS+S and HS+GH/IGF-I rats.

Relationships between myonuclear number and fiber size or muscle wet weight. Because mean fiber CSA area decreased to a greater extent than mean myonuclear number, the mean cytoplasmic volume per myonucleus was significantly decreased in HS+S, HS+GH/IGF-I,
and HS+Ex rats (Table 1). In contrast, the mean cytoplasmic volume per myonucleus in HS+GH/IGF-I+Ex rats was not significantly different from that in Con+S rats and was significantly higher than that of HS+S rats.

The correlation between mean myonuclear number and mean fiber CSA for all rats combined was 0.75 (P < 0.05; Fig. 2). The correlation between mean myonuclear number per fiber and mean muscle wet weight for all rats combined was 0.61 (P < 0.05; data not shown). Within any group, there was no apparent relationship between mean myonuclear number and mean fiber CSA or muscle wet weight.

**DISCUSSION**

In the present study, HS for 14 days resulted in 17% fewer myonuclei and a 55% decrease in mean CSA in soleus fibers. These adaptations are similar to the 16% decrease in mean myonuclear number and the 42% decrease in mean fiber CSA in rat soleus fibers after a 14-day spaceflight (4). Thus 1 wk of functional overload followed by 2 wk of HS produced cellular changes in soleus fibers that were comparable to those induced by 2 wk of spaceflight alone. In the present study, Ex alone was able to ameliorate the effects of HS on muscle wet weight but had no effect on the decrease in fiber CSA. It is possible that the 1 wk of functional overload before HS induced inflammation and edema (5) and that this was aggravated by the periodic loading experienced during the subsequent 2 wk of HS+Ex. This scenario could account for the increased muscle wet weight, whereas continued atrophy of the muscle fibers due to the HS could account for the decrease in fiber CSA. This study, however, cannot clearly differentiate the role of active climbing vs. postural weight support in maintaining muscle mass in the exercised groups. This issue needs to be addressed in future studies.

As we have suggested previously (3, 4), changes in myonuclear number may be associated with alterations in fiber size, because a loss of myonuclei would reduce the total pool of DNA available for transcription. On the other hand, it is also possible that myonuclei are eliminated, not as a mechanism for regulating fiber volume but as a means of maintaining coordinated myonuclear protein expression. For example, the large reduction in fiber volume during muscle atrophy could produce crowding and overlapping of myonuclear domains, interfering with efficient coordination and cooperation among myonuclei in regulating protein expression. In this scenario, myonuclei could be eliminated as a consequence of the reduction in fiber volume rather than as a mechanism for downregulating fiber size.

The mechanism(s) by which myonuclei were eliminated from the muscle fibers of these adult HS rats is currently unknown. One possibility is that myonuclei are eliminated by a form of apoptosis, or programmed cell death (24). The multinucleated nature of skeletal muscle fibers raises the question of how individual nuclei can be eliminated from a common cytoplasm. Studies on multinucleated heterokaryons and Tetrahymena have shown that apoptotic death can occur in a single nucleus without destroying all nuclei or the cell itself (6, 8). Indeed, we recently demonstrated that the number of myonuclei with double-stranded DNA breaks, an indication of apoptosis, is increased in the soleus muscle after HS (D. L. Allen, R. R. Roy, and V. R. Edgerton, unpublished observations).

In the present study, neither Ex nor GH/IGF-I injection alone was sufficient to prevent the decrease in mean fiber CSA associated with HS. Previous studies using only endurance (10, 13) or brief intermittent resistance exercise (14) have also demonstrated only a partial amelioration of the associated atrophy. Further-
more, injection of GH alone had no effect on attenuating the muscle atrophy accompanying a 4-day spaceflight (15). On the other hand, studies that have employed both resistance Ex and GH and/or IGF-I injection have demonstrated the greatest countermeasure effect (11, 18, 23). These and the present data provide support for the hypothesis that muscle loading and the GH/IGF-I factors have interactive effects in maintaining muscle fiber size. In the present study, it is also possible that the 1 wk of overload before the HS affected the muscle wet weight response. For example, Adams and Haddad (1) reported significant increases in the rat plantaris muscle IGF-I message and peptide and total DNA content after only 3 days of functional overload. Therefore, it is possible that augmented IGF-I secretion resulting from the 1 wk of overload could have had an effect during the 14 days of HS in the present study. However, the soleus muscles of all suspended animals were overloaded, and, even if there were a residual effect, it seems unlikely that this would differ among the experimental groups.

At least two scenarios can be envisioned as to how muscle loading and the GH/IGF-I factors could have synergistic effects to protect unloaded muscles from atrophying. First, muscle loading could stimulate secretion of other growth or transcriptional factors, augmenting the effect of the exogenously administered growth factors. Second, muscle loading could enhance GH/IGF-I receptor levels and/or receptor binding capacity, thus increasing the sensitivity of the muscle to the available levels of growth factors. The present data suggest that either or both of these mechanisms may play a role in regulating muscle volume through a modulation of myonuclear number. Whether this modulation of myonuclear number is the result of changes in the generation of new myonuclei or in the rate of loss of myonuclei remains undefined.

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REFERENCES


