Interleukin-15 responses to aging and unloading-induced skeletal muscle atrophy

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1Laboratory of Muscle Biology and Sarcopenia, Division of Exercise Physiology, West Virginia University School of Medicine, Morgantown, West Virginia; and 2Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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Pistilli EE, Siu PM, Alway SE. Interleukin-15 responses to aging and unloading-induced skeletal muscle atrophy. Am J Physiol Cell Physiol 292: C1298–C1304, 2007. First published November 29, 2006; doi:10.1152/ajpcell.00496.2006.—Interleukin-15 (IL-15) mRNA is constitutively expressed in skeletal muscle. Although IL-15 has proposed hypertrophic and anti-apoptotic roles in vitro, its role in skeletal muscle cells in vivo is less clear. The purpose of this study was to determine if skeletal muscle aging and unloading, two conditions known to promote muscle atrophy, would alter basal IL-15 expression in skeletal muscle. We hypothesized that IL-15 mRNA expression would increase as a result of both aging and muscle unloading and that muscle would express the mRNA for a functional trimeric IL-15 receptor (IL-15R). Two models of unloading were used in this study: hindlimb suspension (HS) in rats and wing unloading in quail. The absolute muscle wet weight of plantaris and soleus muscles from aged rats was significantly less when compared with muscles from young adult rats. Although 14 days of HS resulted in reduced muscle mass of plantaris and soleus muscles from young adult animals, this effect was not observed in muscles from aged animals. A significant aging times unloading interaction was observed for IL-15 mRNA in both rat soleus and plantaris muscles. Patagialis (PAT) muscles from aged quail retained a significant 12% and 6% of stretch-induced hypertrophy after 7 and 14 days of unloading, respectively. PAT muscles from young quail retained 15% hypertrophy at 7 days of unloading but regressed to control levels following 14 days of unloading. A main effect of age was observed on IL-15 mRNA expression in PAT muscles at 14 days of overload, 7 days of unloading, and 14 days of unloading. Skeletal muscle also expressed the mRNAs for a functional IL-15R composed of IL-15Rα, IL-2/15Rβ, and -γc. Based on these data, we speculate that increases in IL-15 mRNA in response to atrophic stimuli may be an attempt to counteract muscle mass loss in skeletal muscles of old animals. Additional research is warranted to determine the importance of the IL-15/IL-15R system to counter muscle wasting.

Interleukin-15 (IL-15) is a recently discovered cytokine (20) that belongs to the four α-helix bundle family of cytokines, that also include IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9. The roles of IL-15 within the immune system have been shown to include proliferation and survival of CD8+ T cells (6), the activation of natural killer cells (11), and proliferation of B cells (5). IL-15 mRNA is also constitutively expressed in a wide variety of cell and tissue types, including placenta, skeletal muscle, liver, epithelial cells, and activated macrophages (20).

IL-15 and IL-2 have redundant roles resulting from similar receptor composition for these two cytokines. The IL-15 and IL-2 receptors (IL-15R, IL-2R, respectively) are trimeric structures composed of two identical chains, the IL-2R/IL-15R β-chain (IL-2Rβ) and the common γ (γc)-chain, along with specific α-chains (18). The IL-15Rα exhibits a high affinity of binding for IL-15 protein, with a Kd of 10 pM (14). In addition to paracrine actions, IL-15 can be expressed in trans, in which the cytokine is either bound to cell-surface IL-15Rα or anchored to the cell membrane. In this manner, mature IL-15 can be presented to neighboring cells that express IL-2Rβ and γc (7). Although both IL-15 (27) and IL-15Rα (19) mRNA is expressed in skeletal muscle, it is not known if the mRNA for a functional trimeric IL-15R is also expressed in skeletal muscle, which would allow for trans presentation of IL-15 by muscle cells.

Within skeletal muscle, IL-15 can stimulate myosin heavy chain protein expression in differentiated myotubes (17, 34). Myotubes cultured with IL-15 also have a hypertrophic morphology when compared with control cultures that did not contain IL-15. Additionally, daily injections of IL-15 protein have been shown to reduce DNA fragmentation of gastrocnemius muscles, attenuate cancer-associated skeletal muscle loss (cachexia), and reduce the gene expression of the type I tumor necrosis factor (TNF) receptor (TNFR) in a rodent model of cancer (15). Although these results demonstrate a positive effect of exogenous IL-15 protein in myocytes, the response of endogenous intramuscular IL-15 has not been examined. Furthermore, the response of IL-15 to muscle loss that results from conditions such as aging or muscle unloading is not known.

The purpose of this study was to determine the basal responses of IL-15 mRNA expression as a result of aging and to skeletal muscle unloading, two conditions known to promote muscular atrophy. A secondary aim of this study was to determine if skeletal muscle expresses the mRNAs for a functional trimeric IL-15R. We hypothesized that skeletal muscle would respond to unloading by increasing IL-15 mRNA, with further increases as a result of age in an attempt to counter muscle loss. We used two different models of unloading to test this hypothesis. In the first approach, we induced atrophy in soleus and plantaris muscles via hindlimb suspension (HS) in rats. This model reduces plantar flexor muscle mass below control levels. In the second approach, we first induced hypertrophy in the quail patagialis (PAT) muscle via wing weighting and this was followed by wing unweight-
posed tip of the tail remained pink, indicating that HS did not interfere
with blood flow to the tail. The suspension height was monitored daily
and adjusted to prevent the hindlimbs from touching any supportive
surface, with care taken to maintain a suspension angle of ~30°. The
forelimbs maintained contact with a grid floor, which allowed the
animals to move, groom themselves, and obtain food and water freely.
HS was maintained for a total of 14 days. Control rats maintained
normal mobility, and they moved unconstrained around their cages.
Following 14 days of HS, rats were killed with an overdose of
xylazine, and the soleus and plantaris muscles from the hindlimbs
were excised.

**Wing loading/unloading.** In a second approach to study muscle
loss, Japanese Coturnix quails were hatched and raised in pathogen-
free conditions in the central animal care center at the West Virginia
University School of Medicine. The birds were housed at a room
temperature of 22°C with a 12:12-h light-dark cycle and were pro-
vided with food and water ad libitum. Twenty-four young adult birds
(2 mo) and 24 aged birds (24 mo) were examined in the present study.
The lifespan of Japanese quails is ~26–28 mo, and they are both
physically and sexually mature by 1.5 mo of age (25, 28). The PAT
muscle is flexed with the wing on the birds back at rest, but it is
stretched when the wing is extended. In our experimental stretch-
overloading model, a tube containing 10–12% of the bird’s body
weight was placed over the left humeral-ulnar joint (4). This maintains
the joint in extension throughout the period of stretch and induces
stretch at the origin of the PAT muscle. Previous studies have shown
this stretch-overloading protocol results in moderate hypertrophy of
the PAT muscles (i.e., 14-day stretch-loading induces ~35% and
~15% increases in muscle mass of young adult and aged birds,
respectively; see Ref. 4). Following 14 days of stretch overload of the
left wing, eight young and eight aged birds were killed with an
overdose of xylazine. Eight young and eight aged birds were main-
tained for a period of 7 days, in which the overloaded left wing was
unloaded. The remaining young and aged animals were killed 14 days
after the weight removal. The unstretched right PAT muscle served as
the intra-animal control muscle for each bird. PAT muscles were
dissected from the surrounding connective tissue, removed, weighed,
and frozen in isopentane cooled to the temperature of liquid nitrogen
and then stored at ~80°C until used for analyses.

**RT-PCR estimates of mRNA.** Semiquantitative RT-PCR analysis
was conducted as described in detail elsewhere (36). Frozen muscle
samples (~50 mg) were homogenized in 1 ml of TriReagent (Molecu-
lar Research Center, Cincinnati, OH) with a mechanical homoge-
nizer. Total RNA was isolated by centrifugation and washed in
ethanol according to the manufacturer’s instructions. RNA was solu-
bilized in 20 μl of RNase-free H2O. RNA was treated with DNase I
(Ambion, Austin, TX) and reverse transcribed with random primers
(Invitrogen/Life Technologies, Bethesda, MD). PCR primers were
constructed from published sequences for the rat and chicken IL-15
genes, and they are given in Table 1. Primer pairs for the gene of

Table 1. **PCR primer information**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Accession No.</th>
<th>Sequence</th>
<th>Position</th>
<th>T&lt;sub&gt;a&lt;/sub&gt;, °C</th>
<th>Cycles</th>
<th>Product Length, bp</th>
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</thead>
<tbody>
<tr>
<td>IL-15 (rattus)</td>
<td>NM_013129</td>
<td>F: 5′-GCCGATAGCCGCCCCTTCTGATGAC</td>
<td>494–517</td>
<td>56.4</td>
<td>36</td>
<td>594</td>
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<tr>
<td></td>
<td></td>
<td>R: 5′-TGCGCGCAGGGAGGTGTATTAATAC</td>
<td>1062–1087</td>
<td></td>
<td></td>
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<tr>
<td>IL-15 (gallus)</td>
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<td>53.0</td>
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<td></td>
<td>R: 5′-AGCGATTTTTCATGCTGCTG</td>
<td>660–683</td>
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<td></td>
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<tr>
<td>IL-15Ra (rattus)</td>
<td>DQ157696</td>
<td>F: 5′-TGGCCCGAAGCCGCTATATTT</td>
<td>1–21</td>
<td>57.3</td>
<td>35</td>
<td>391</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5′-GTGGGAGGAGACCTTTCTCT</td>
<td>373–391</td>
<td></td>
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<td></td>
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<tr>
<td>IL-2Ra (rattus)</td>
<td>NM_013163</td>
<td>F: 5′-CAAGGAGATGGAGACACAGTGT</td>
<td>111–132</td>
<td>57.8</td>
<td>40</td>
<td>526</td>
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<tr>
<td></td>
<td></td>
<td>R: 5′-GGTGACCACTTATCCCCACAC</td>
<td>614–636</td>
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</tr>
<tr>
<td>IL-2Rβ (rattus)</td>
<td>NM_013195</td>
<td>5′-CAAGGCGGCTCCTGCGGATGATGATGATG</td>
<td>91–113</td>
<td>58.8</td>
<td>38</td>
<td>534</td>
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<tr>
<td></td>
<td></td>
<td>R: 5′-ACGGGCTGCAACTCCAAAGATGAGT</td>
<td>602–624</td>
<td></td>
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</tr>
<tr>
<td>γc (rattus)</td>
<td>NM_080889</td>
<td>5′-GCCTGCAAGCCGACCAACGCTCGCAC</td>
<td>251–271</td>
<td>56.7</td>
<td>36</td>
<td>440</td>
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</tbody>
</table>

T<sub>a</sub>, annealing temperature; IL, interleukin; R, receptor; γc, common γ; F, forward; R, reverse.
interest were coamplified with 18S primer pairs and competimers to the 18S primers, as an internal control, according to the manufacturer’s protocols (Ambion). The number of PCR cycles was determined for each gene to ensure analyses were done in the linear range of amplification. The signal from the gene of interest was expressed as a ratio to the 18S signal from the same PCR product to eliminate any loading errors. The cDNA from all muscle samples were amplified simultaneously for a given gene. Following amplification, 20 μl of each reaction were separated by electrophoresis on 1.5% agarose gels. Gels were stained with ethidium bromide. PCR signals were captured with a digital camera (Kodak 290), and the signals were quantified in arbitrary units as optical density × band area, using Kodak image analysis software (Eastman Kodak, Rochester, NY). As a positive control for IL-15 PCR, spleen and liver tissue were harvested from FBN rats and included in PCR analyses for IL-15 (Fig. 1A and Ref. 13). Restriction digestion of IL-15 PCR products was performed for both rat and quail IL-15 to determine primer specificity (Fig. 1B). The rat IL-15 product was cut with the AluI restriction enzyme producing bands of 466 and 128 bp. The quail IL-15 PCR product was cut with the AluI restriction enzyme, producing bands of 321 and 187 bp.

IL-15Rα sequencing. PCR amplification of the IL-15Rα sequences from rat and quail cDNA produced a PCR product ~100 bp larger than expected. The corresponding PCR products were gel purified using a commercially available gel extraction kit according to the manufacturer’s instructions (QIAQuick Gel Extraction Kit; Qiagen Sciences) and sent for direct sequencing (SeqWright DNA Technology Services, Houston, TX). The newly acquired cDNA sequence information was then compared with the computer-predicted sequence originally used for the design of PCR primers (XM_577598).

Statistical analysis. Statistical analyses were performed using the SPSS software package, version 10.0. Data were analyzed using a 2 × 2 ANOVA to examine the main effects of aging and unloading and the age × unloading interaction. Data are presented as means ± SE with significance set at P < 0.05. Relationships between given variables were examined by computing the Pearson correlation coefficient.

RESULTS

Body weight: rodent HS. There were significant main effects for age (F = 91.7, P < 0.001) and unloading (F = 22.6, P < 0.001) on rodent body weight. The aging × unloading interaction was not significant (F = 0.006, P = 0.941). Fourteen days of HS significantly reduced body weights in both young adult (control: 374.7 ± 25.2 g; HS: 295.2 ± 13.3 g; −80%) and aged (control: 538.8 ± 15.9 g; aged: 456.7 ± 11.4 g; −15%) rats. The body weight of the aged rats was 44% greater than the young adult rats (young adult: 374.7 ± 25.2 g; aged: 538.8 ± 15.9 g).

Muscle characteristics: rodent HS. Muscle wet weights have been reported previously (32), and absolute muscle wet weights and the muscle weight normalized to body weight are presented in Table 2. Following 14 days of HS, the soleus muscle wet weight was 43% less in young adult rats when compared with controls. In contrast, the wet weight of aged soleus was unchanged following HS. Control soleus wet weight was 17% less in aged vs. young rats. The aging × unloading interaction was significant in the rat soleus (F = 15.0, P < 0.001). Following 14 days of HS, the plantaris wet weight was 20% less in young adult rats compared with controls. In contrast, the wet weight of the aged plantaris was unchanged following HS. Plantaris muscle wet weight was 22% less in aged compared with control plantaris muscles.

PAT muscle: quail wing unloading. The changes in PAT muscle mass following stretch overload and subsequent unloading have been reported previously (37). Fourteen days of stretch overload increased PAT wet weight ~35% in young quail and ~15% in aged quail. Young PAT muscles retained 15% hypertrophy, and aged PAT muscles retained 12% hypertrophy after 7 days of unloading compared with intra-animal

![Figure 1](http://ajpcell.physiology.org/) Interleukin (IL)-15 primer specificity. A: RT-PCR was performed for IL-15 in cDNA from rat skeletal muscle (SM), with cDNA from rat spleen (SP) and liver (LV) tissue used as positive controls. Thirty four PCR cycles at a calculated annealing temperature (T_a) of 56.6°C produced bands of 594 bp in all three tissue types. B: Restriction digestion of IL-15 PCR products from rat and quail skeletal muscle with the AluI restriction enzyme. Incubation of PCR products at 37°C for 1 h produced the predicted fragments. PCR, full PCR product; RD, restriction enzyme-digested PCR products.
control muscles. Following 14 days of unloading, young PAT muscles returned to baseline. However, aged PAT muscles retained 6% of stretch-induced hypertrophy after 14 days of unloading when compared with intra-animal control muscles.

**IL-15 transcriptional responses.** Following 14 days of HS in rodents, a significant aging × unloading interaction was observed in both the soleus (F = 5.8, P = 0.024), 7 days of unloading (F = 97.8, P < 0.001), and 14 days of unloading (F = 61.9, P < 0.001). IL-15 mRNA was not affected by 14 days of stretch overload in either young or aged birds (Fig. 3). In contrast, IL-15 mRNA was 25 and 19% greater in unloaded young and aged PAT muscles, respectively, relative to the intra-animal control muscles following 7 days of unloading (Fig. 3). Following 14 days of unloading, IL-15 mRNA returned to baseline in PAT muscles of young and aged birds (Fig. 3).

**Sequencing of rattus IL-15Ra.** A computer-predicted mRNA sequence from GenBank for the rattus IL-15Ra chain was used to construct PCR primers (XM_577598). The PCR product had a predicted size of 325 bp following amplification. However, following PCR amplification and subsequent gel electrophoresis, the PCR product was closer to 400 bp, with no multiple bands observed (Fig. 4A). The PCR product was gel purified, sequenced, and compared with the computer-predicted sequence from GenBank. The newly sequenced cDNA was identical to the predicted sequence at the 5’- and 3’-ends, with a unique sequence of 103 bases contained in the middle (Fig. 4B). Following verification by DNA sequencing and restriction digestion with HindIII (Fig. 4A), the new sequence was submitted to GenBank (accession no.: DQ157696).

**IL-15R expression in skeletal muscle.** Primers specific for the three IL-15R chains (α, β, and γ) and the IL-2Rα chain were constructed to examine mRNA expression in rat skeletal muscle. mRNA isolated from rodent spleen tissue was used as a positive control for each of these primers (Fig. 5A). As shown in Fig. 5B, rat skeletal muscle expresses mRNA for each of the IL-15R chains. Additionally, mRNA for the IL-2R-specific α-chain was detected in the spleen and in skeletal muscle.

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**Table 2. Rodent muscle characteristics**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Young Adult Animals</th>
<th>Aged Animals</th>
<th>Age-induced Difference in Control Muscles (Young vs. Aged)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young control</td>
<td>Aged HS</td>
<td>Difference HS vs. control, %</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Soleus, mg</td>
<td>171.2 ± 9.8</td>
<td>97.9 ± 6.0</td>
<td>−43*</td>
</tr>
<tr>
<td>Plantaris, mg</td>
<td>400.8 ± 22.2</td>
<td>319.2 ± 17.5</td>
<td>−20*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. HS, hindlimb suspension; BM, body mass. Characteristics of 20 young adult (5–7 mo) and 18 senescent (33 mo) male Fischer 344 × Brown Norway rats. NS, not significant. *P < 0.05.

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**Fig. 2.** IL-15 transcriptional changes following hindlimb suspension (HS). A: IL-15 mRNA expression in soleus muscles following HS. B: IL-15 mRNA expression in plantaris muscles following HS. A significant aging × unloading interaction was observed in both skeletal muscles, indicating age influenced the response of IL-15 mRNA. Data are expressed as means ± SE with significance at *P < 0.05, significant unloading effect (*) and significant age effect (**).
IL-15 and skeletal muscle unloading. The first report on the effects of IL-15 in skeletal muscle demonstrated its ability to increase the myosin heavy chain protein content in differentiated mouse C2C12 myotubes in vitro (34). These results were subsequently supported by data in primary human skeletal muscle cell cultures (17). This effect of IL-15 was independent of the hypertrophic effects of insulin-like growth factor-I (33), which may become important with aging when anabolic hormone levels typically decrease (40). In this study, IL-15 mRNA was greater in all aged skeletal muscles examined and, in general, increased as a result of unloading. We propose that this is an age-related adaptation of skeletal muscle to counter muscle loss in response to atrophic stimuli. Future studies should address the efficacy of IL-15 in sparing muscle mass in aged animals and in response to conditions that promote muscle atrophy.

The greatest effects of IL-15 may be seen under conditions of stress, such as that invoked by aging and disease. This is suggested, in part, because the changes in IL-15 mRNA in the current study were less dramatic in the muscles from young animals than in the aged. Furthermore, an increase in systemic IL-15 levels in vivo increases the force output of diaphragm muscles from mdx mice (21), which is a model for muscular dystrophy that has a high turnover of contractile protein as a result of degeneration/regeneration. In this same study, IL-15 promoted muscle regeneration within the first 6 days after a myotoxic injury as evidenced by an increase in fiber cross-sectional area (21). Elevated IL-15 also spares muscle mass and decreases the rate of protein degradation in young tumor-bearing rats (10). Collectively, these data suggest that IL-15 can act as an anabolic agent for skeletal muscle during periods of injury and/or periods of muscle wasting. This may explain, in part, why a significant interaction of aging and loading/unloading was not observed in the quail model used in this study. As noted above, the quail model of unloading allows previously hypertrophied muscle to atrophy, but muscle mass does not go below that of control contralateral muscles. This contrasts to the HS model, where muscle mass is reduced well below control levels after unloading. Thus it is possible that the underlying mechanisms leading to atrophy of

DISCUSSION

The data from this study demonstrate that the IL-15 gene is responsive to skeletal muscle aging and unloading, two conditions known to promote muscle atrophy. IL-15 mRNA increased as a result of both unloading and aging in the predominantly slow-muscle-containing soleus muscle, supporting previous microarray studies in skeletal muscle (29, 38). In contrast, the fast-muscle-containing plantaris muscle had increases in IL-15 mRNA only in the aged unloaded samples. The quail model of unloading used in this study differed from the HS model in that wing unloading allowed regression of previously hypertrophied muscle, whereas, during HS, the plantar flexor muscles atrophy relative to control muscles. The interaction of aging and loading/unloading on IL-15 mRNA expression was not significant in the quail model, indicating the age of the quail did not influence the adaptive response. Despite this difference, the main effect of age on IL-15 mRNA expression was evident in PAT muscles, as was the case in the soleus and plantaris muscles following HS. These data indicate that aging is a significant stimulus for increases in IL-15 mRNA in skeletal muscles. The evidence for this is that IL-15 expression was greater in aged muscles of differing fiber type composition (i.e., soleus vs. plantaris) and before unloading was initiated (i.e., previously hypertrophied PAT vs. basal soleus and plantaris). The differences in IL-15 expression observed in this study support the observations from previous studies demonstrating that skeletal muscles from aged animals respond to atrophic conditions differently than muscles from young animals (24, 32).

IL-15 and skeletal muscle. The first report on the effects of IL-15 in skeletal muscle demonstrated its ability to increase the myosin heavy chain protein content in differentiated mouse C2C12 myotubes in vitro (34). These results were subsequently supported by data in primary human skeletal muscle cell cultures (17). This effect of IL-15 was independent of the hypertrophic effects of insulin-like growth factor-I (33), which may become important with aging when anabolic hormone levels typically decrease (40). In this study, IL-15 mRNA was greater in all aged skeletal muscles examined and, in general, increased as a result of unloading. We propose that this is an age-related adaptation of skeletal muscle to counter muscle loss in response to atrophic stimuli. Future studies should address the efficacy of IL-15 in sparing muscle mass in aged animals and in response to conditions that promote muscle atrophy.

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previously hypertrophied muscles to basal levels, compared with atrophy below control levels, may differ. Another possibility is that prior loading in the quail muscle may have invoked expression of anti-apoptotic proteins that reduced the severity of muscle unloading following loading. For example, our laboratory has previously demonstrated that previously hypertrophied PAT muscles from aged quail retain the loading-induced increase of the anti-apoptotic molecule XIAP during periods of subsequent unloading (37). However, XIAP is not increased in unloaded rat muscles after HS compared with control muscles (35). Additionally, other anti-apoptotic changes were noted in 14-day-unloaded PAT muscles of aged quails, such as increased Bcl-2 and decreased Bax protein levels (36). This is in contrast to the increased Bax mRNA expression and protein content following 14 days of HS in the aged rat plantaris (32) and medial gastrocnemius muscles (35). We hypothesize that an anti-apoptotic adaptation of previously hypertrophied muscle may take place in aged quail during extended periods of unloading as muscle returned to basal levels. Similar adaptations may not take place during periods of HS-induced muscle atrophy where muscle mass can be considerably less compared with muscles from control animals.

**IL-15 and apoptosis.** A role for IL-15 in the attenuation of apoptosis is suggested by data showing that exogenous IL-15 protein inhibits death pathway-associated apoptotic signaling. Multisystem apoptosis initiated in mice via treatment with an anti-Fas antibody was suppressed with injection of a long-lasting IL-15-IgG3, fusion protein (9). In addition, IL-15 transgenic mice are resistant to a lethal dose of *Escherichia coli* (23). IL-15 administration in control mice also reduces the death rate from a lethal challenge of *E. coli*. The data further show that administration of IL-15 to isolated peritoneal cells in vitro prevented TNF-α-induced apoptosis (23).

The well-characterized cell death pathway initiated by the binding of TNF-α to the type I TNFR (i.e., extrinsic apoptotic pathway) can be altered with increases in IL-15 protein. For example, daily injections of IL-15 protein for 7 days in a rodent model of cancer resulted in significant decreases in the gene expression of both the type I and type II TNFR (15). Furthermore, incubation of fibroblasts with IL-15 in vitro attenuates apoptosis induced by TNF-α (8). The TNF-α apoptotic pathway was disrupted when the cytoplasmic signaling molecule TRAF2, which normally mediates the downstream apoptotic signal from the TNFR, was recruited to the cytoplasmic side of the IL-15Rα. Interestingly, this recruitment of TRAF2 to IL-15Rα was only observed when both TNF-α and IL-15 protein were present in the culture media (8). Thus IL-15 seems to function, at least in part, to inhibit apoptosis by blocking the signaling downstream of the TNFR. This is relevant in aging muscle because the extrinsic apoptotic pathway is very active in aged skeletal muscle (31). We speculate that the changes in IL-15 mRNA observed in the current study may represent an attempt to counter the pro-apoptotic environment typically observed in aged skeletal muscle.

Another potential means for IL-15 to function in an anti-apoptotic role may be as a result of its association with the anti-apoptotic protein Bcl-2 (30, 41). There is a reduction in the percentage of CD8+ T cells in IL-15Rα−/− mice, and this is due in part to a reduction of Bcl-2 expression (41). Exogenous IL-15 upregulates Bcl-2 levels in these cells and contributes to a reduction in cell death upon activation (41). Additionally, HIV-specific CD8+ T cells were shown to exhibit reduced levels of Bcl-2. When these cells were cultured with IL-15, Bcl-2 expression increased, and this was associated with an attenuation of apoptosis of CD8+ T cell cultures (30). The mRNA expression and protein content of Bcl-2 has been shown to increase in aged skeletal muscles and in response to atrophic stimuli (32, 35). Although these results do not show a direct Bcl-2-mediated anti-apoptotic role for IL-15, this possibility warrants further investigation.

**HS in rodents.** The HS model of unloading has been widely used in rodents to study the effects of unloading on bone (22) and muscle (2, 3, 16, 38). In the current study and others (3, 24, 32), HS has been used to examine the interaction of aging and unloading. The aging-associated loss of muscle mass and strength (i.e., sarcopenia) is exacerbated with inactivity (39). Muscle mass declines by ~40% between the ages of 20 and 60, with strength declining by 20–40% (reviewed in Ref. 12). The current study is consistent with previous findings showing that aged skeletal muscle responds differently to unloading compared with young adult skeletal muscle (24, 32, 35).

The results of this study differ from previous reports from our laboratory that have shown greater muscle loss in aged FBN rats than in young adult rats after HS (3). Variability in animal responses to HS can occur, even in the same laboratory (16). For example, Fitts et al. (16) reported variability in soleus atrophy and peak isometric tetanic tension in response to 1 and 2 wk of HS. The authors speculated that variability in these data may be induced by diverse responses in animal movements or environmental disturbances that result in random muscular contractions. The HS technique results in limb unloading with muscular innervation left intact, which allows the hindlimbs to move freely in space. Initially, EMG activity decreases, but it returns to baseline levels as soon as 3 days after HS initiation (1). In our study, animals were checked two times daily after the induction of HS, random hindlimb muscular contractions were observed, and this may have contributed to our current results.

In conclusion, IL-15 mRNA is constitutively expressed in skeletal muscle, and it is responsive to both muscle aging and limb unloading. Our data indicate that aging is a significant
stimulus for increased IL-15 mRNA expression, since main effects of age were observed in all muscles examined using two models of aging and in two different animal species. Additionally, skeletal muscle expresses mRNA for a functional trimeric IL-15R, which would allow for trans presentation of IL-15 by muscle cells. It is possible that skeletal muscle responds to atrophic stimuli by increasing IL-15 levels to be secreted as a traditional cytokine or by presenting IL-15 on the sarcolemma bound to the IL-15R. Future experiments should examine the direct effects of modulating the IL-15/IL-15R system in response to atrophic stimuli as a means to spare muscle mass with aging and during periods of disuse or muscle injury/disease.

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