Satellite Cell Dysfunction and Impaired IGF-1 Signaling Cause CKD-Induced Muscle Atrophy

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ABSTRACT

Muscle wasting in chronic kidney disease (CKD) begins with impaired insulin/IGF-1 signaling, causing abnormal protein metabolism. In certain models of muscle atrophy, reduced satellite cell function contributes to atrophy, but how CKD affects satellite cell function is unknown. Here, we found that isolated satellite cells from mice with CKD had less MyoD, the master switch of satellite cell activation, and suppressed myotube formation compared with control mice. In vivo, CKD delayed the regeneration of injured muscle and decreased MyoD and myogenin expression, suggesting that CKD impairs proliferation and differentiation of satellite cells. In isolated satellite cells from control mice, IGF-1 increased the expression of myogenic genes through an Akt-dependent pathway. CKD impaired Akt phosphorylation in satellite cells after muscle injury. To test whether impaired IGF-1 signaling could be responsible for decreased satellite cell function in CKD, we created an inducible IGF-1 receptor knockout mouse and found impaired satellite cell function and muscle regeneration. In addition, both CKD and IGF-1 receptor knockout mice developed fibrosis in regenerating muscles. Taken together, impaired IGF-1 signaling in CKD not only leads to abnormal protein metabolism in muscle but also impairs satellite cell function and promotes fibrosis in regenerating muscle. These signaling pathways may hold potential therapeutic targets to reduce CKD-related muscle wasting.


Complications of chronic kidney disease (CKD) including acidosis, impaired insulin/IGF-1 signaling, and excess angiotensin II or IL-6, stimulate muscle wasting by increasing protein degradation and decreasing protein synthesis.1–6 The mechanisms changing protein metabolism include impaired insulin/IGF-1 signaling, which activates caspase-3 and the ubiquitin-proteasome system to increase muscle protein breakdown.3,4,7–10

Impaired activity of muscle progenitor or satellite cells also might contribute to CKD-induced muscle atrophy as it does in other catabolic conditions.11,12 Satellite cells are located beneath the basal lamina of myofibers and have at least two functions. First, they proliferate, becoming myoblasts, and then differentiate, forming new muscle fibers to repair injured muscle.13 Second, muscle homeostasis requires proliferation and differentiation of satellite cells for normal muscle growth.9 In mice with muscle atrophy from hindlimb suspension or in rodent models of aging, myopathy, or muscle denervation, the number and activity of satellite cells were found to be reduced, indicating that satellite cells are involved in maintaining muscle mass.11,12

After muscle injury, satellite cells are activated and express the MyoD and myogenin transcription factors, leading to cell proliferation and differentiation, respectively.14–16 When these cells express embryonic myosin heavy-chain protein (eMyHC),
there is myotube formation. In muscle of mice with CKD, we found decreased expression of MyoD, myogenin, and eMyHC mRNAs, abnormalities that were corrected in muscle by chronic overloading as a model of resistance exercise. In contrast, treadmill running of mice with CKD (a model of endurance exercise) reduced muscle protein degradation but not the decrease in protein synthesis or low levels of MyoD, myogenin, or eMyHC mRNAs. The mechanisms for CKD-induced changes in satellite cell function have not been reported, so we evaluated whether defects in IGF-1 signaling impair satellite cell function because impaired insulin/IGF-1 signaling causes abnormal muscle protein metabolism. Our results identify another mechanism for the muscle atrophy that is induced by CKD.

**RESULTS**

**CKD Impairs Satellite Cell Function**

Previously, we reported that CKD reduces MyoD and myogenin expression in muscle. By counting nuclei outside the dystrophin-stained sarcolemma, it seemed that there was a significant decrease in the progenitor cells. In these experiments, we used a more rigorous method of identifying satellite cells: Positive staining with Pax-7, MyoD, and myogenin, and Myf-5 in gastrocnemius muscles of CKD mice versus values in control muscles (Figure 1A). There were, however, significantly reduced mRNAs of the myogenic markers MyoD, myogenin, and Myf-5 in gastrocnemius muscles of CKD mice versus values in control muscles (Figure 1A). We also studied isolated satellite cells from muscles of control and CKD mice. Satellite cells were identified as Pax-7– and Pax-3–positive cells (Supplemental Figure 1B). There was no difference in the terminal deoxynucleotidyl transferase–mediated digoxigenin–deoxyuridine nick-end labeling assay or activated caspase-3, indicating that apoptosis does not contribute substantially to CKD-induced satellite cell dysfunction (data not shown).

When we plated the same number of satellite cells isolated from muscles of CKD and control mice, MyoD-positive cells from CKD mice were significantly lower compared with results from control mice (Figure 1B). CKD also reduced satellite cell proliferation, measured as bromodeoxyuridine (BrdU) incorporation (Supplemental Figure 1C). Differentiation of isolated satellite cells from CKD mice was decreased in cells, as identified by reduced immunostaining for eMyHC (Figure 1C). Thus, reduced MyoD expression and the lower number of eMyHC-positive cells demonstrate that CKD suppresses satellite cell activation and differentiation.

Third, we examined whether CKD impairs satellite cell activation in vivo using the standard stimulus of these cells: Cardiotoxin (CTX) injection into muscles. At 3 or 7 days after injury, isolated satellite cells from injured tibialis anterior (TA) and gastrocnemius muscles of CKD and control mice were assayed for mRNAs of myogenic genes. MyoD and myogenin mRNAs were decreased in satellite cells from muscle of CKD mice (Figure 1, D and E). To confirm that similar changes occurred in injured muscles from CKD and control mice, we examined mRNAs of MyoD and myogenin. After 3 days, mRNAs of MyoD and myogenin in injured muscles of CKD mice were significantly decreased versus results in control mice (Supplemental Figure 1, D and E). After 14 days, the trend persisted: MyoD and myogenin mRNAs in CKD muscles were significantly lower versus results from control mice.

**CKD Reduces Muscle Regeneration**

For an in vivo test of satellite cell function, we used muscle injury to determine whether CKD interfered with muscle regeneration. During the initial 72 hours, mononuclear cells accumulated in injured muscles of CKD and control mice. After 5 days, there were new myofibers (designated by central nuclei) in TA muscles of control mice, but in injured TA muscles of CKD mice, there were fewer new myofibers and more mononuclear cells versus results in control mice. At 7 days, most myofibers in injured muscles of control mice had central nuclei; mononuclear cells were found only between myofibers. In CKD mice, however, there were fewer new myofibers, myofibers were disorganized, and mononuclear cells persisted. After 14 days, control muscles were virtually normal and there were few mononuclear cells, whereas muscles from CKD mice exhibited expansion of the interstitial space, persistent mononuclear cell infiltration, and smaller new myofibers (Figure 2A). After 1 month, the size distribution of newly formed myofibers in injured muscles of CKD mice was shifted to smaller values versus results in control mice (Figure 2B). The average size of new myofibers in CKD muscles (505 μm²) was substantially smaller than in control muscles (1715 μm²; P < 0.001). Thus, CKD significantly impairs muscle regeneration.

**CKD Prolongs Inflammation in Injured Muscle**

Previously, we found that muscle injury causes infiltration of neutrophils and macrophages with increased levels of cytokines and chemokines. To study how CKD influences macrophage and neutrophil infiltration, we immunostained muscle sections with anti–Mac-2 to identify macrophages and myeloperoxidase to identify neutrophils. One day after injury, muscles from both groups were infiltrated by neutrophils and macrophages, but by day 3, the macrophage infiltration in injured CKD muscles was more intense. The pattern persisted through day 7 or 14 (Figure 2C). High mRNA levels of the macrophage marker F4/80 confirmed the Mac-2 immunostaining results (Figure 2D). We also analyzed the expression of cytokines/chemokines in injured muscle. At 3 days after injury, cytokine and chemokine mRNAs in muscle of CKD mice were higher versus results in control mice (Table 1). These responses presumably reflect the more intense infiltration of macrophages in injured muscle of CKD mice.
CKD Impairs IGF-1/Insulin Signaling in Muscle and Satellite Cells

Defective insulin/IGF-1 signaling reduces Akt phosphorylation (p-Akt) in muscle, leading to CKD-induced muscle atrophy.6,26,27 In examining whether IGF-1 signaling influences satellite cell function, we isolated cells from control muscles and examined their responses to IGF-1 or its downstream product, Akt. IGF-1 increased the expression of MyoD, Myf-5, myogenin, and eMyHC in satellite cells; these responses were blocked by a phosphatidylinositol 3-kinase inhibitor, LY294002 (Figure 3). In satellite cells infected with an adenovirus expressing myristylated Akt (Akt-myr) or the inactive Akt (Akt-AAA; K179A/T308A/S473A), we found that Akt-myr mimics IGF-1 and activates myogenic gene expression. In contrast, Akt-AAA blocked this response. Thus, IGF-1 signaling activates satellite cell functions.28

To examine whether CKD suppresses IGF-1–stimulated signaling in satellite cells after injury, we injected 500 ng of IGF-1 into injured TA muscles of CKD and control mice to stimulate Akt phosphorylation. After 15 minutes, muscle sections were obtained and immunostained for both p-Akt and myogenin (a marker of differentiating satellite cells). In muscle of CKD mice, there were fewer myogenin-positive cells and a lower degree of Akt phosphorylation in myogenin-positive cells (Supplemental Figure 2A). Because most Pax-7–positive cells were myogenin positive (Supplemental Figure 2B), we conclude that CKD impairs IGF-1/insulin signaling in satellite cells.

IGF-1 Receptor Modifies Satellite Cell Activation and Myofiber Formation

Because CKD suppresses IGF-1 signaling (Supplemental Figure 2) and muscle regeneration (Figure 2), we hypothesized...
that defects in the IGF-1 receptor (IGF-1R) signaling contribute to abnormal satellite cell function and regenerative capacity. To examine this hypothesis, we created conditional IGF-1R knockout mice (IGF-1R-KO; see the Concise Methods section). By Western blotting, IGF-1R expression was decreased in gastrocnemius muscle (Figure 4A); decreased IGF-1R was confirmed by immunostaining TA muscles and isolated satellite cells (Supplemental Figure 3, A and B). MyoD expression was sharply reduced in satellite cells from IGF-1R-KO mice, indicating that IGF-1 regulates MyoD expression (Supplemental Figure 3B). Likewise, in muscles of IGF-1R-KO mice, mRNAs of other myogenic markers were suppressed (Figure 4B).

Next, we isolated satellite cells from IGF-1R-flox mice and infected them with an adenovirus expressing Cre to knockout the IGF-1R (Supplemental Figure 3C). Impaired satellite cell proliferation was measured by reduced immunostaining of Ki67 in IGF-1R-KO cells versus cells expressing IGF-1R (Ki67 is a marker of proliferation). Also, significantly fewer myotubes were formed by IGF-1R-KO satellite cells (Supplemental Figure 3D).

To examine whether IGF-1R-KO slows muscle regeneration in vivo, we injured TA muscles of these mice, and 7 days later, muscle regeneration was reduced in IGF-1R-KO mice versus results in control, IGF-1R-flox mice (Figure 4C). After 1 month, the sizes of newly formed myofibers in IGF-1R-KO muscles were significantly smaller versus control muscles (Figure 4D). Thus, satellite cells lacking the IGF-1R exhibit de-

Table 1. Cytokine and chemokine change in injured muscle of CKD and control mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MCP-1</th>
<th>IL-6</th>
<th>RANTES</th>
<th>TNF-α</th>
<th>MIP-1α</th>
<th>TCA-3</th>
<th>MIP-1β</th>
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<tbody>
<tr>
<td>No injury</td>
<td>0.70 ± 0.10</td>
<td>1.30 ± 0.23</td>
<td>1.50 ± 0.07</td>
<td>2.05 ± 0.20</td>
<td>1.24 ± 0.06</td>
<td>1.40 ± 0.10</td>
<td>1.23 ± 0.20</td>
</tr>
<tr>
<td>Injury day 3</td>
<td>6.40 ± 0.80</td>
<td>7.60 ± 0.90</td>
<td>4.20 ± 0.80</td>
<td>10.44 ± 1.10</td>
<td>2.17 ± 0.30</td>
<td>10.04 ± 0.90</td>
<td>2.50 ± 0.03</td>
</tr>
<tr>
<td>Injury day 7</td>
<td>1.50 ± 0.03</td>
<td>2.50 ± 0.20</td>
<td>2.00 ± 0.50</td>
<td>5.01 ± 0.40</td>
<td>1.70 ± 1.80</td>
<td>3.40 ± 0.60</td>
<td>1.90 ± 0.40</td>
</tr>
</tbody>
</table>

MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TCA-3, T cell activator-3.
increased expression of myogenic factors and reduced regeneration capacity, as occurs in CKD mouse muscles. These results emphasize the important role of IGF-1R signaling in the activation and function of satellite cells.

Fibrosis Is Increased in Injured Muscle of CKD or IGF-1R-KO Mice

Others have reported that TGF-β1 is expressed during the development of fibrosis in injured muscle.29,30 We found that CKD increased TGF-β1 mRNA in injured muscle to a greater degree versus results from control mice. The difference was detected at 1 day after injury, and maximum values were present at day 3 followed by a gradual decrease to day 14 (Figure 5A). Injured muscles of CKD mice also had a sharp increase in collagen (Figure 5B).

Muscle injury of IGF-1R-KO mice increased TGF-β1 mRNA two- to three-fold versus results in control mice (Figure 5C). At 1 month after injury, muscle collagen in IGF-1R-KO mice was sharply increased compared with control results (Figure 5D); therefore, muscle injury in CKD or IGF-1R-KO decreases satellite cell function and muscle regeneration but increases TGF-β1 expression and stimulates fibrosis.

DISCUSSION

Previously, we found that CKD impairs muscle protein metabolism, producing atrophy. Impaired insulin/IGF-1 cell signaling and activation of proteolytic pathways caused these results.2-3 We now uncover a new mechanism for CKD-induced muscle atrophy: Impaired satellite cell function linked to decreased IGF-1R signaling. Others reported that activation, proliferation, and differentiation of satellite cells are necessary for maintenance of muscle mass11,12; our results show that CKD impairs these functions, reducing satellite cell proliferation and differentiation. We also identified how impaired IGF-1 signaling causes the abnormalities in satellite cell functions.
Finally, CKD and IGF-1R-KO both increase TGF-β1 mRNA and fibrosis in injured muscles. Our results suggest that improving satellite cell function in CKD could be useful in strategies designed to ameliorate muscle atrophy.

We found that CKD reduced the expression of MyoD and myogenin transcription factors in intact and injured muscle (Figure 1A; Supplemental Figure 1, D and E). Reportedly, these transcription factors are present in myonuclei. To determine that our results reflect events in satellite cells, we isolated Pax-7–positive cells from muscle of CKD mice; they had depressed MyoD expression (Figure 1B). CKD also suppressed the mRNAs of myogenic factors in satellite cells obtained from injured muscle (Figure 1, D and E). This is relevant because muscle injury markedly stimulates MyoD and myogenin expression in satellite cells, leading to their cellular proliferation/differentiation. Thus, CKD depresses satellite cell function.

We examined the influence of CKD on satellite cells in vivo and in vitro. First, the expression of myogenic markers was reduced in muscle of CKD versus control mice (Figure 1A). Second, Pax-7–positive satellite cells isolated from muscles of CKD and control mice exhibited decreased proliferation on the basis of BrdU incorporation (Supplemental Figure 1C). These cells also exhibited reduced MyoD expression and delayed differentiation into myotubes (Figure 1). Third, CKD suppressed MyoD and myogenin mRNAs in injured muscles and in satellite cells isolated from injured muscles (Figure 1, D and E; Supplemental Figure 1, D and E). Finally, CKD limited satellite cell regeneration, indicated by the smaller sizes of new myofibers (Figure 2, A and B).

Why does CKD suppress satellite cell function in regenerating muscle? Inflammation or CKD complications (e.g., high levels of angiotensin II or IL-6) could play a role. This is suggested because our results show that CKD prolongs mononuclear cell accumulation in muscle while increasing cytokine and chemokine expression (Figure 2B, Table 1). The increased F4/80 mRNA found in injured muscles of CKD mice confirmed the greater degree of macrophage infiltration (Figure 2D). Suppressed muscle regeneration and inflammation responses in injured CKD muscle could be related to impaired insulin/IGF-1 signaling because local expression of muscle IGF-1 was shown to downregulate proinflammatory cytokines and promote muscle regeneration selectively. Indeed, we found that CKD impairs IGF-1 signaling in satellite cells, leading to impaired muscle regeneration.

IGF-1 is involved in many developmental processes, including cell proliferation, differentiation, and regeneration. Musaro and colleagues demonstrated that overexpression of locally acting IGF-1 (mIGF-1) improves muscle function in mouse models of senescence, injury, myodystrophy, or amyotrophic lateral sclerosis. In muscle and satellite cells, we found that CKD impaired IGF-1R–mediated signaling (Supplemental Figure 2, A and B), and when we suppressed the IGF-1 receptor in mice (IGF-1R-KO), satellite cell activation and muscle regeneration were reduced as in CKD mice (Figure 4). In our studies of IGF-1R-KO mice, other hormones changing in response to IGF-1R-KO could affect our results; therefore, we isolated satellite cells from muscle of IGF-1R-flox mice and knocked out IGF-1R using a Cre-expressing adenovirus. In these cells, there was reduced proliferation and differentiation, as occurred in intact muscles (Supplemental Figure 3, C and D). The results point to a critical role for IGF-1 signaling in satellite cells.

Myostatin could be another mechanism causing CKD-induced responses, because it can suppress muscle growth. Increased myostatin in muscle of CKD animals is associated with muscle atrophy, and we find that inhibiting myostatin function in CKD mice can improve their muscle weight and
protein metabolism (Zhang et al., manuscript in preparation). Besides myostatin, there is evidence that muscle repair could respond positively to endogenous stem cells.42 We have not examined whether circulating stem cells influence the maintenance of muscle mass, but this possibility would be interesting.

The development of fibrosis is a serious complication of impaired muscle regeneration. Fibrosis can fill spaces in injured muscles and connect injured muscle fragments, but it makes regaining muscle strength difficult because of impaired connective tissue contraction. Others reported that prolonged inflammatory responses can initiate fibrosis linked to increased expression of TGF-β1 and collagen deposition.43–45 In injured muscles, we found TGF-β1 mRNA increased, especially in CKD mice. Collagen deposition followed the same pattern as the TGF-β1 mRNA (Figure 5, A and B). We speculate that the collagen production in injured CKD muscles was due to the increase in TGF-β1 from macrophages accumulating in injured muscles (Figure 2C). Macrophages are profibrotic cells, and TGF-β1 is a key inducer of fibrosis in muscle and other tissues.46 Indeed, Warren et al.47 reported freeze injury of muscles in CCR2-null mice prolonged macrophage accumulation and increased fibrosis. Thus, impaired IGF-1 signaling in regenerating muscles of CKD mice led to fibrosis, because similar events occurred in IGF-1R-KO mice (e.g., increased TGF-β1 and collagen deposition in injured muscles; Figure 5). In fact, increased IGF-1 expression in injured muscle suppresses inflammation and reduces fibrosis.21,29,48 How frequently CKD causes muscle fibrosis is unknown, because only small series of patients with CKD have been tested for muscle fibrosis.49

We conclude that CKD reduces IGF-1R-mediated signaling in satellite cells, impairing their activation and contributing to CKD-induced muscle atrophy. Because catabolic conditions frequently cause impaired insulin/IGF-1 signaling, our results could apply to other conditions that cause muscle atrophy.

CONCISE METHODS

Reagent and Antibodies

CTX was obtained from Calbiochem (La Jolla, CA) and an antibody against phospho-Akt (Ser473) from Cell Signaling Technology (Beverly, MA). Antibodies against IGF-1R, MHC, and Myf-5 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The MyoD antibody was from Vector Laboratories (Burlington, CA), and antibodies against Pax-7, Pax-3, and eMyHC and myogenin were from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). The laminin antibody was from Sigma-Aldrich (St. Louis, MO), anti–Mac-2 was from Cedarlane Labs (Burlington, NC); anti-Ki67 and IGF-1 were from R&D System (Minneapolis, MN). The anti-myeloperoxidase antibody was from Abcam (Cambridge, MA). DMEM and FBS were from Cellgro Mediatech (Manassas, VA). BrdU Labeling and Detection Kit II was obtained from Roche Applied Science (Indianapolis, IN).

Muscle Regeneration Model

All animal experiments and procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee. Anesthetized male mice, 6 to 10 wk old, were studied after injection of 80 μl of 10 μM CTX in saline into one TA or gastrocnemius muscle using a 27-G needle; the contralateral muscle was injected with the same volume of PBS.

Satellite Cell Isolation

Muscles were digested with 0.2% Collagenase Type II (Worthington Biochemical, Lakewood, NJ) at 37°C for 30 minutes in DMEM with 1% penicillin/streptomycin. The mixture was passed through a 100-μm filter, and satellite cells were isolated by centrifugation at the interface between 40 and 70% Percoll.40 Satellite cells on matrigel-coated plates (BD Bioscience, San Jose, CA) were cultured in DMEM with 20% FBS, 1% penicillin/streptomycin mixture, 40 μg/ml gentamicin, and 2% chicken embryo extract. To differentiate cells into myotubes, we incubated them for 5 days in DMEM plus 2% horse serum.

Reverse Transcriptase–PCR

Total RNA was extracted from muscle using Trizol, and cDNAs were synthesized using the first-strand cDNA synthesis kit with oligo DT 12 to 18 primers (Invitrogen, Carlsbad, CA); real-time PCR was performed with the Opticon Real-Time PCR (MJ Research, Waltham, MA). Primers we used will be provided if requested. Relative mRNA expression levels were calculated from cycle threshold (Ct) values using 18S as the internal control [relative expression = 2(sample Ct − 18S Ct)].

Subtotal Nephrectomy

C57/BL6 mice underwent subtotal nephrectomy in two stages: Approximately 50% of the right kidney was removed; 7 days later, the left kidney was removed. The mice were fed a 12% protein diet for 2 weeks before beginning 40% protein chow. At 1 month after second surgery, mice with a blood urea nitrogen level >80 mg/dl were studied.

Generation of Mice with Inducible IGF-1R Knockout

Transgenic mice expressing the estrogen receptor-Cre (ER-Cre) were obtained from Jackson Laboratory (Bar Harbor, ME). After breeding with mice (C57BL6 background) containing the IGF-1R-flox (exon 3), those expressing both ER-Cre and IGF-1R-flox were identified by genotyping. At 3 months of age, IGF-1R-KO and control mice (IGF-1R-flox) were administered an intraperitoneal injection of 2 mg of tamoxifen (10 mg/ml in corn oil; Sigma-Aldrich) daily for 5 days, and IGF-1R deletion was confirmed by reverse transcriptase–PCR, Western blotting, and immunohistochemistry. Others studied mice after 7 days of tamoxifen to obtain more complete IGF-1R knockout as confirmed by reverse transcriptase–PCR26; we treated mice for 5 days to reduce systemic responses to IGF-1R knockout.

Histochemical and Immunohistochemical Staining

Serial frozen (−20°C) transverse cryosections (8 μm) from the mid-belly of control and injured TA muscles were mounted on glass slides. Sections were air-dried and fixed in acetone for 10 minutes before staining with hematoxylin and eosin. Tissue collagen and fibrosis were stained using Sirius red (Sigma-Aldrich). MyoD, myogenin, and
p-Akt were examined by standard immunohistochemical techniques.5

Imaging of Muscle Sections and Analyses
Images were obtained using a Nikon 80i upright microscope (Melville, NY). Myofiber sizes were measured after immunostaining muscle with anti-laminin using NIS-Elements Br 3.0 software (Nikon); the distribution of fiber sizes was calculated as described previously.5

Statistical Analysis
Results are expressed as means ± SEM. Significance testing was performed using one-way ANOVA followed by pair-wise comparisons using the Student-Newman-Keuls test. Statistical significance was set at P < 0.05. A minimum of three replicates were performed for each experimental condition.

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DISCLOSURES
None.

REFERENCES
23. Tidus PM. Skeletal Muscle Damage and Repair, Champaign, IL, Human Kinetics, 2008
29. Sato K, Li Y, Foster W, Fukushima K, Badlani N, Adachi N, Usas A, Fu FH, Huard J: Improvement of muscle healing through enhancement of
42. Supplemental information for this article is available online at http://www.jasn.org/.