Responsive
cell signaling pathways during the failed
15-day regrowth of aged skeletal muscle

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Various cellular signaling pathways, such as phosphatidylinositol 3-ki
nase, calcineurin, Janus kinase 2/signal transducer and activator of tran
scription 3 (STAT3), and mitogen-activated protein kinase (MAPK) have
been suggested to play an important role in skeletal muscle growth. Old
muscle, compared with young muscle, lacks the ability to completely
regrow its muscle mass after an atrophy-induced stimulus, it is hypoth
esized that defects and/or delays in the activation of specific cell signaling
pathways of aged soleus muscle limit the potential for growth. To test
this, 42 male Fischer 344 × Brown Norway rats, 30 mo old, were
hindlimb immobilized for 10 days, and their muscle samples were
compared with muscle samples analyzed from 3- to 4-mo-old rats in a
previous report (Childs TE, Spangenburg EE, Vyas DR, and Booth FW. Am J Physiol Cell Physiol: 285: C391–C398, 2003). After 10 days, the
immobilization was removed and rats were allowed to ambulate for a
series of days. Alterations in the activation or deactivation status of
specific signaling pathways were determined by comparing the phosphor
ylation (phos) and total concentration of specific signaling proteins (pan)
through Western blotting with the 10-day immobilization group. Various
cell signals and their respective time groups of the old rats were shown to
be significantly different compared with the 10-day immobilization
group. For example, peak increases during recovery from the immobili
zation were observed at 1) the third recovery day for calcineurin B-pan
and 2) the sixth recovery day for glycogen synthase kinase-3-phos, p70 S6
kinase (p70S6k)-phos and -pan, calcineurin A-pan, STAT3-phos and
-pan, p44 MAPK-pan, and p42 MAPK-pan. In contrast, Akt-pan, c-Jun
NH2-terminal kinase-phos, and p38 MAPK-phos were observed to
decrease from 10-day immobilization values to control levels. Also, Akt
phos was unchanged among all groups. In a follow-up experiment in
which muscle samples from both the present study and a previous study
(Childs TE, Spangenburg EE, Vyas DR, and Booth FW. Am J Physiol Cell Physiol: 285: C391–C398, 2003) were reanalyzed together, the
recovery-induced increase in p70S6k-phos from immobilization-atrophy
was significantly attenuated in soleus muscles of the old group.

atrophy; regulation; rehabilitation; recovery

AGING IS AN ESTABLISHED CONTRIBUTOR TO THE REDUCTION OF SKELETAL MUSCLE MASS, STRENGTH, AND FUNCTION (8, 13). THIS CONDITION, KNOWN AS SARCOPENIA, IS CHARACTERIZED BY DECELERATED PROTEIN SYNTHESIS RATES, INCREASED MITOGEN-ACTIVATED PROTEIN KINASE 3-KINASE (MAPK), AND INHIBITION OF PHOSPHATIDYLINOSITOL 3-KINASE (PI3K)/AKT SIGNALING PATHWAY. THE PI3K/AKT SIGNALING PATHWAY IS IMPORTANT FOR SKELETAL MUSCLE HYPERTROPHY. IN ADDITION, THROUGH AN INTRAMUSCULAR INJECTION OF A CONSTITUTIVELY ACTIVE AKT CONSTRUCT, MUSCLE HYPERTROPHY WAS INDUCED THROUGH DOWNSTREAM SIGNALING PROTEINS p70 S6 KINASE (p70S6K) AND EUKARYOTIC INITIATION FACTOR 4E BINDING PROTEIN (4E-BP1).

ALTERNATIVELY, INTRACELLULAR CALCIUM LEVELS ALSO PLAY A PIVOTAL ROLE IN REGULATING MUSCULAR GROWTH. CALCIUM-SENSITIVE ENZYMES SERVING AS PHOSPHATASES WITHIN THE CELL, HAVE BEEN SHOWN TO PROMOTE SKELETAL MUSCLE HYPERTROPHY THROUGH THE IGF-1 SIGNALING PATHWAY. SEMSARIAN ET AL. (27) FOUND THAT IGF-1-INDUCED MYOTUBE HYPERTROPHY THROUGH ACTIVATION OF THE CALCIUM-SENSING Ca2+ SIGNALING PATHWAY. HOWEVER, A CONTROVERSY STILL EXISTS OVER THE RELATIVE CONTRIBUTIONS FROM AKT/MTOR AND CALCIUM DURING SKELETAL MUSCLE HYPERTROPHY (14, 16). IT IS NOT UNREASONABLE TO SUGGEST THAT THE HYPERTROPHY PROCESS IN SKELETAL MUSCLE IS DEPENDENT ON THE INTEGRATED COMMUNICATION OF MULTIPLE SIGNALING PATHWAYS.

YOUNG MUSCLE HAS THE ABILITY TO PROPORTIONALLY ENLARGE ITS SIZE MORE EFFECTIVELY AFTER A GIVEN STIMULUS COMPARED WITH OLD MUSCLE (13), AND THIS PROCESS MAY POTENTIALLY OCCUR THROUGH FUNCTIONING INTEGRATED COMMUNICATION EVENTS. A RECENT REPORT SHOWED THAT A SINGLE BOUT OF RESISTANCE EXERCISE CAUSED AN INCREASE IN PHOSPHORYLATION (PHOS) OF THE EXTRACELLULAR SIGNAL-RELATED KINASE 1 AND 2 (ERK1/2), P90 RIBOSOMAL S6 KINASE (p90RSK), AND MITOGEN-ACTIVATED PROTEIN KINASE-INTERACTING KINASE-1 (Mnk-1) PROTEINS IN 22-YR-OLD MALE
human subjects but decreased its ERK1/2, p90RSK, Mnk-1, p38 mitogen-activated protein kinase (MAPK), and c-Jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK) in the 79-yr-old men (33). Consequently, the responsiveness of these cell signaling pathways may continually become less efficient in response to muscle growth stimuli in aged muscle. Thus it is hypothesized that specific defects exist within a signaling pathway(s) in aged muscle and could limit potential muscle growth and contribute to a sarcopenic state.

**MATERIALS AND METHODS**

**Animals.** Forty-two male F1 generation, Fischer 344 × Brown Norway rats of 30 mo of age were received from the National Institute on Aging and acclimatized for 2 wk before beginning the study. The rats were housed one animal per cage in a 12:12-h light-dark cycle during both the immobilization and recovery phases. Rat chow and water were given ad libitum and closely monitored throughout. All experimental protocols have been approved by the Animal Use Committee of the University of Missouri-Columbia.

**Hindlimb immobilization and experimental model.** A detailed procedure for the hindlimb immobilization model has been previously described (7). Briefly, on the day of casting, rats were lightly anesthetized by using isoflurane to attach plaster of Paris casts. The plantar flexor group of both legs was maintained in a shortened, nonstretched position during the immobilization period. The immobilized rats were checked daily for damage done to casting material, which was repaired if necessary. Rats were immobilized for a period of 10 days to allow comparison to earlier studies that used the same duration (11, 12). After 10 days of immobilization, the casts were completely removed. The rats were then returned to the same cage and allowed to ambulate freely until the date that they were killed. Recovery rats from the 10-day immobilization included a control group (n = 11) and 0-day (no recovery time) (n = 7), 3-day (n = 8), 6-day (n = 7), and 15-day (n = 8) recovery groups. Each rat represented an independent observation. The animals were euthanized on these days by using a drug cocktail containing ketamine (75 mg), xylazine (3 mg), and acepromazine (5 mg). On the day that the animals were killed, the soleus muscle from both legs was carefully excised, weighed, and frozen in liquid nitrogen for storage at −80°C. In a follow-up experiment, soleus muscle samples from 3- to 4-mo-old rats employed in a previous paper (12) were reanalyzed with old muscle samples in the present study.

**Muscle protein extraction and concentration measurements.** The muscle tissue was homogenized on ice with buffer that included 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na2HPO4·H2O, 100 mM β-glycerophosphate, 25 mM NaF, 50 μg/ml leupeptin, 50 μg/ml pepstatin, 40 μg/ml aprotinin, and 1 mM NaN3·VO4. After homogenization, the samples were stored at −80°C. In a follow-up experiment, soleus muscle samples from 3- to 4-mo-old rats employed in a previous paper (12) were reanalyzed with old muscle samples in the present study.

**SDS-PAGE, Western blotting, and immunodetection.** Homogenates of the muscle were solubilized at a concentration of 1.25 mg/ml in loading buffer (2.5 mM Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, 5% β-mercaptoethanol, and 0.025% bromophenol blue) and boiled at 98°C for 5 min. A 100-μl aliquot of total protein was loaded (100 μg/sample) onto 10% SDS-PAGE gels with the following exceptions: 25 μg/sample for GSK-3β and a 15% SDS-PAGE gel for calcineurin B. All gels were run at 150 mV for 30 min and at 200 mV for 1.5 h to separate proteins. The gels were then transferred onto nitrocellulose membranes (Osmotics, Westborough, MA) at 51 V for 2.5 h at 4°C in transfer buffer (25 mM Tris-base, 192 mM glycine, and 20% methanol). To confirm successful transfer of protein and equal loading of lanes, which occurred in all cases, the membranes were stained with Ponceau S (data not shown). After successful transfer, the membrane was placed in blocking buffer [5% nonfat dry milk in Tris-buffered saline-0.1% Tween-20 (TBS-T)] for 1 h at room temperature, serially washed (3 × 5 min), and incubated with primary antibody in dilution buffer (5% BSA in TBS-T) overnight at 4°C. After another serial wash with TBS-T (1 × 10 min, 4 × 5 min), the membranes were incubated with both a horseradish peroxidase (HRP)-conjugated secondary antibody and a HRP-linked anti-biotin antibody in blocking buffer for 1 h followed by another serial wash with TBS-T (1 × 10 min, 4 × 5 min). Enhanced chemiluminescence reagent (Perkin Elmer Life Sciences, Boston, MA) was used to detect the HRP activity by exposure to Kodak-XAR5 autoradiographic film for the appropriate durations to keep the integrated optical densities (IODs) within a linear and nonsaturated range for all bands of each membrane. The IODs were quantified by using ImageQuant densitometry software (Molecular Dynamics; renamed Amersham Biosciences, Sunnyvale, CA). To correct the sample IODs for various exposure graphs where statistical difference occurred. The same quality control sample was loaded onto every gel. Representative blots are shown only under signaling response graphs where statistical difference occurred.

**Antibodies.** The primary antibodies Ser397/Akt-phos (1:500 dilution), Akt (1:1,000), Ser473-GSK3β-phos (1:1,000), GSK-3β (1:2,500), Thr183/Tyr185-SAPK/JNK-phos (1:500), p70S6K-phos (1:500), Thr202/Tyr204 (p44/p42 MAPK) or ERK1/2 (1:1,000), p44/42 MAPK or ERK1/2 (1:1,000), Thr183/Tyr185-SAPK/JNK-phos (1:500), SAPK/JNK (1:1,000), Thr180/Tyr182-p38 MAPK-phos (1:1,000), p38-MAPK (1:1,000), Tyr705/725 signal transducer and activator of transcription 3 (STAT3)-phos (1:1,000), STAT3 (1:1,000), and anti-biotin antibody (1:4,000) were purchased through Cell Signaling Technologies (Beverly, MA). Calcineurin A (1:10,000) and calcineurin B (1:4,000) were purchased through Sigma-Aldrich (St. Louis, MO) and Affinity Bioreagents (Golden, CO), respectively. Anti-rabbit and anti-mouse secondary antibodies (1:7,500) were purchased from Amersham Biosciences (Piscataway, NJ). Antibody specificity was verified by molecular weight, positive controls [GSK-3β-phos, p70S6K-total concentration of specific signaling proteins (pan), calcineurin B-pan, and p38-phos], blocking peptides (GSK-3β-phos and p38-phos), and absence of immunoreactivity from secondary antibody signal in the absence of the primary antibody (all molecules).

**Statistical analysis.** All data are expressed as means ± SE. Muscle mass data was analyzed by using a one-way analysis of variance and a Tukey’s post hoc test. The Western blotting values were analyzed nonparametrically by a Kruskal-Wallis test, and a post hoc analysis was done by using a Dunn’s multiple-range test with P < 0.01 as statistical marker of significant difference. The baseline comparison among experimental groups was selected to be the group at the end of 10 days of hindlimb immobilization and followed by varying days of voluntary ambulatory recovery. Values are means ± SE. *Statistical difference from the control group (−10 days), P < 0.05.
the 10 days of hindlimb immobilization because this value allowed two statistical comparisons to be made; one for atrophy (preimmobilization control vs. 10-day immobilization) and the second for recovery [10-day immobilization (which also was the 0-day recovery) value vs. 3, 6, and 15 recovery days].

RESULTS

Soleus weights after hindlimb immobilization. After the tenth day of immobilization, the soleus muscle mass decreased 19.2% from control values at the start of immobilization (Fig. 1). For all time points during the 15 days of recovery, old soleus muscle weights were significantly less than control values and had not regained any of the total atrophied soleus mass. Normalized soleus weights to body weights showed similar trends (data not shown).

Phosphorylation levels and total protein expression of various signaling proteins in atrophying and postimmobilization old soleus muscles. The number of rats per group is equal to the number of independent observations made for every signaling protein except for the 10-day immobilized group, which had

Fig. 2. Alterations in Akt, glycogen synthase kinase-3β (GSK-3β), and p70 S6 kinase (p70S6K) molecules that are downstream of the phosphatidylinositol-3 kinase pathway during muscle atrophy and recovery from muscle atrophy in aged animals. Values are means + SE. Integrated optical densities (IOD) are arbitrary units corrected for background and plotted on the y-axis. Time (days) of the experimental design is shown on the x-axis. Control animals are 10 days. Day 0 is the last day of the 10-day hindlimb immobilization; thus the time from −10 days to day 0 represents the 10-day immobilization (immob) period. Because day 0 also represents the start of the recovery period after cessation of hindlimb immobilization, the period from day 0 to day 15 represents recovery days. For statistical analysis, day 0 (or 10-day immobilization group) was designated as the reference time point. Recovery days from the 10 days of immobilization are 3, 6, and 15 days. For all data concerning phosphorylation status (phos; A–C), statistical differences (P < 0.01) are symbolized as follows: A, statistical difference from the control group; C, statistical difference from the 3-day recovery group; D, statistical difference from the 6-day recovery group; and E, statistical difference from the 15-day recovery group. For all data concerning the total concentration of the signaling protein (pan; 1–5), statistical differences (P < 0.01) are symbolized as follows: 1, statistical difference from the control group; 3, statistical difference from the 3-day recovery group; 4, statistical difference from the 6-day recovery group; and 5, statistical difference from the 15-day recovery group. Representative blots are shown underneath figures only in the cases where statistical differences were detected (ctrl, control; 0, 3, 6, and 15 days of recovery). A: alterations in Akt-phos (Ser473) and -pan. B: alterations in the GSK-3β-phos (Ser9) and -pan. C: alterations in p70S6K-phos (Thr389) and -pan.
only six rats instead of seven for p38, STAT3, calcineurin A, and calcineurin B measurements.

**PI3-kinase pathway.** Significant differences in Akt-pan included the preimmobilization control group, which was 52% lower than the 10-day immobilization group (Fig. 2A). This was followed by a 56 and 65% decrease in the 6-day and 15-day recovery groups, respectively. Akt-phos was not significantly different among any of the groups. GSK-3β-phos (Ser9) increased by 91% only in the 6-day recovery group (Fig. 2B) relative to the 10-day immobilized group. Also, p70S6k-phos (Thr^{389}) increased by 3,030 and 1,940% in the 6-day and 15-day recovery time points, respectively, compared with the 10-day immobilization group (Fig. 2C). p70S6k-pan values were significantly higher in the preimmobilization controls and the sixth day of recovery by 102 and 195%, respectively, compared with the 10-day immobilization group.

**Calcineurin A and B.** Calcineurin A-pan was higher than the 10-day immobilization group by 180, 463, and 219% in control, 6-day, and 15-day groups, respectively, compared with the 10-day immobilization group (Fig. 3A). Calcineurin B-pan values were 114% higher at 3 days and 57% higher at 6 days of recovery (Fig. 3B) compared with the 10-day immobilization group.

**JAK2-STAT3 pathway.** STAT3-phos (Tyr^{705}) increased at the sixth day of recovery and was significantly different from control and fifteenth day of recovery by 69 and 68%, respectively (Fig. 4). STAT3-pan was increased by 90 and 87% in the 6-day and 15-day recovery groups, respectively, compared with the 10-day immobilization group.

**MAPK pathways.** The level of JNK-phos (Thr^{183}/Tyr^{185}) decreased by 96% in the 15-day recovery group compared with the 10-day immobilization group (Fig. 5A). ERK1-pan (p44 MAPK) increased 114% after 6 days of recovery compared with the 10-day immobilization group (Fig. 5B). Also, ERK2-pan (p42 MAPK) increased by 56 and 40% in the 6-day and 15-day recovery groups, respectively (Fig. 5C). Compared with 10-day immobilization group, p38 MAPK-phos (Thr^{180}/Tyr^{182}) was 85% lower in controls and 66% lower at 15 days of recovery (Fig. 5D). p38 MAPK-pan was 27% lower in the control group.

**Comparison of young vs. old.** The increase in p70S6k-phos was significantly attenuated at the third recovery day in the soleus muscle from old compared with young rats (Fig. 6). No significant differences for GSK-3β-phos, STAT3-phos, or p38-pan during recovery were found between young and old soleus muscles.

**DISCUSSION**

Gastrocnemius muscle in aged animals lacked the ability to regrow after 77 days of recovery from hindlimb immobilization (11). The goal of this study was to characterize the

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**Fig. 3.** Alterations in the protein concentrations of calcineurin A (CaN A; A) and calcineurin B (CaN B; B) during muscle atrophy and recovery from muscle atrophy. The format of the presentation of the data is the same as described in Fig. 2 legend.

**Fig. 4.** Alterations in the phos (●) (Tyr^{705}) and protein (○ - ○) of STAT3 during muscle atrophy and recovery from muscle atrophy. The format of the presentation of the data is the same as described in Fig. 2 legend except for STAT3-phos, which used the 6-day recovery group as the reference point for statistical analysis because 0 and 6 days were not significantly different from one another. Numbers indicate significant differences for pan antibodies, and letters indicate significant differences for phosphorylation-specific antibodies (See Fig. 2 legend for more detail.)
responsiveness of multiple cell signaling pathways in skeletal muscle from aged animals during reloading from an atrophied state. Multiple changes in the phosphorylation status and total protein concentration occurred in various signaling proteins, and the findings are in partial agreement with the hypothesis that a specific defect in p70S6k was acquired within a signaling pathway(s) in aged muscle.

After 10 days of immobilization, the soleus muscle had atrophied by 19.2% in the old animals. This percentage of atrophy is much less than the 38.5% previously observed in young rats (12). The lower percentage of muscle atrophy observed in the old soleus muscle after the immobilization period suggests the muscle had already lost a substantial amount of mass over time. Previously, Chakravarthy et al. (11) documented that gastrocnemius muscle from old rats failed to show any significant regrowth of the gastrocnemius muscle for 77 days after a limb immobilization-induced stimulus. Here, we confirm a similar finding in the soleus muscle, the major load-bearing muscle of the hindlimb. We have also confirmed that, even after 30 days of recovery in 30-mo-old rats, no significant muscle mass was regained (25). This unexpected observation is in agreement with reports that aged animals have attenuated (2, 13, 10, 24) or cannot increase their muscle mass after mechanical overload produced by surgical ablation (4). Specifically, 15 days after the removal of the immobilization material, the soleus muscle had failed to show any regrowth in the old animals. However, our laboratory previously found that after 6 and 15 days of reload in the soleus muscle of young...
animals that mass had recovered by 37 and 40%, respectively, after the same bout of atrophy (12). These data indicate that soleus muscle does not regrow at the same rate in old animals during the 15-day recovery period from limb immobilization as it does in young animals. This observation has potential clinical relevance because the reduction of muscle growth in old animals may be contributing to the inability to recover protein synthesis.

Interestingly, Akt phosphorylation did not increase at any time point in the reloaded, old muscle. Using direct gene injection, Bodine et al. (5) found that Akt is an important regulator of muscle hypertrophy in skeletal muscle. In addition, Bodine et al. found that Akt-phos was significantly elevated after 14 days of compensatory hypertrophy in young animals. Akt is thought to contribute to muscle hypertrophy through activation of mTOR-p70S6K (5) and/or deactivation of the GSK-3β (5). Therefore, the lack of change in Akt phosphorylation postimmobilization may limit the regrowth of old soleus muscle.

In the old muscle, GSK-3β phosphorylation significantly increased 91% at the sixth day of recovery compared with the 10-day immobilization group. [The 10-day immobilization/0-day recovery value was selected for baseline comparison because it allowed statistical comparisons for atrophy (preimmobilization control vs. the baseline of 10-day immobilization group) and recovery (baseline vs other recovery groups).] GSK-3β is a downstream signaling target in the PI3-K pathway known to affect various cell functions such as metabolism, gene transcription, and cell cycle regulation (18). Hardt and Sadoshima (18) also described GSK-3β as a novel regulator of cardiac hypertrophy. Active or nonphosphorylated GSK-3β phosphorylates a transcription factor, nuclear factor of activated T cells (NFAT), and initiates NFAT removal from the nucleus. Vyas et al. (30) recently found that active GSK-3β negatively regulated skeletal myotube hypertrophy by potentially reducing NFAT transcriptional activity. However, after phosphorylation of GSK-3β at Ser9 by Akt, GSK-3β is inactivated and potentially allows NFAT to remain active within the nucleus to induce the transcription of necessary hypertrophy genes. Therefore, the phosphorylation of GSK-3β might be a crucial signaling mechanism for skeletal muscle regrowth, and its function appeared intact in the old rats. However, an alternative signaling pathway besides Akt may be regulating the activity of GSK-3β due to Akt’s lack of phosphorylation during muscle recovery.

In the present study, p38MAPK phosphorylation in the 10-day immobilization group of old rats was significantly higher than controls and the 15-day recovery groups. Because the soleus muscle was shown to significantly atrophy during the 10-day immobilization time period, we speculate that the activation of p38 MAPK could be promoting the loss of muscle tissue. Childs et al. (12) observed a similar rapid increase of p38-phos during atrophy of the young soleus muscle. p38 is a member of the SAPK cascade of the MAPK pathway and has been shown to significantly increase in phosphorylation after bouts of exercise and muscle overload (22). Carlson et al. (9) demonstrated that p38 MAPK-phos was elevated for 24 h after a functional overload in the soleus muscle of young rats. p38 may also act to prevent or retard muscle atrophy; however, it is clearly not an effective hypertrophy agent under immobilization conditions on the basis of the present study and our laboratory’s previously published data (12). It is concluded that p38 MAPK could act to stimulate soleus muscle atrophy during disuse, and the sustained activation of p38MAPK in old muscle recovery could inhibit the regrowth process.

STAT3 phosphorylation increased after 6 days of reloading in the old animals, which suggests its signaling may be functional. As a member of the Janus kinase 2 (JAK2)/STAT3 pathway, STAT3 is known to regulate the intracellular actions of various growth factors and also induce satellite cell proliferation in vitro (29). STAT3 is a transcription factor known to homodimerize and translocate to the nucleus after phosphorylation (Tyr705) by an upstream kinase, JAK2.

We hypothesized that one or more cell signaling pathways may become defective during the reloading of skeletal muscle in aged animals. This could provide a potential molecular mechanism for lack of muscle regrowth observed in the old animals. To test this possibility, soleus muscle samples from the 3- to 4-mo-old rats in a previous report of Childs et al. (12) were reanalyzed by loading them on the same gel with samples from old rats. Those signaling proteins chosen for follow-up experimentation were qualitatively assessed as having a differential response between age groups. Each gel consisted of samples for all five time points for both ages. The increase in p70S6k-phos was significantly attenuated at the third recovery day in the soleus muscle from old compared with young rats (Fig. 6). p70S6k is a downstream signaling component of the PI3-kinase pathway and is thought to be a significant contributor to protein synthesis (28). The smaller upregulation of
IGF-I-1Eb mRNA (local muscle IGF-I) expression observed in the overloaded plantaris muscle of old vs. young rats by Owino et al. (24) may contribute to lesser p70S6K-phos observed in the nonregrowing soleus muscles of older rats in the present study. Baar and Esser (3) have previously reported a tight correlation between the activation of p70S6K and the long-term increase in muscle mass. It has been suggested that the phosphorylation of p70S6K may serve as an important marker for the phenotypic thesis during the recovery time period. As a result, this specific signaling pathway designates a potential translational defect for skeletal muscle growth in aged animals.

Limitations of the present study include the following: 1) later time points beyond 15 days of recovery may reveal regrowth and additional changes in signaling molecules, and 2) no intervention was provided for the rats to facilitate muscle regeneration. However, because many older humans undergo no exercise training after periods of physical inactivity, the aim of the present study was to provide baseline data.

In conclusion, aged skeletal muscle exhibits an attenuated increase in the phosphorylation of p70S6K. With the development of this adverse modification in a cell signaling network, skeletal muscle may not effectively communicate the introduction of external stimuli (i.e., workloads) in old skeletal muscle. In the future, new strategies for reducing or eliminating the progression of sarcopenia will transpire from improving the efficacy of a dysfunctional signaling pathway or factor.

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