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Aging-sensitive cellular and molecular mechanisms associated with skeletal muscle hypertrophy

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Haddad, Fadia, and Gregory R. Adams. Aging-sensitive cellular and molecular mechanisms associated with skeletal muscle hypertrophy. *J Appl Physiol* 100: 1188–1203, 2006. First published December 22, 2005; doi:10.1152/jappphysiol.01227.2005.—Sarcopenia is an age-related loss of muscle mass and strength. The aged can increase various measures of muscle size and strength in response to resistance exercise (RE), but this may not normalize specific tension. In rats, aging reduces the hypertrophy response and impairs regeneration. In this study, we measured cellular and molecular markers, indicative of muscle hypertrophy, that also respond to acute increases in loading. Comparing 6- and 30-mo-old rats, the aims were to 1) determine whether these markers are altered with age and 2) identify age-sensitive responses to acute RE. The muscles of old rats exhibited sarcopenia involving a deficit in contractile proteins and decreased force generation. The RNA-to-protein ratio was higher in the old muscles, suggesting a decrease in translational efficiency. There was evidence of reduced signaling via components downstream from the insulin/insulin-like growth factor (IGF)-I receptors in old muscles. The mRNA levels of myostatin and suppressor of cytokine signaling 2, negative regulators of muscle mass, were lower in old muscles but did not decrease following RE. RE induced increases in the mRNAs for IGF-I, mechano-growth factor, cyclin D1, and suppressor of cytokine signaling 3 were similar in old and young muscles. RE induced phosphorylation of the IGF-I receptor, and Akt increased in young but not old muscles, whereas that of S6K1 was similar for both. The results of this study indicate that a number of components of intracellular signaling pathways are sensitive to age. As a result, key anticatabolic responses appear to be refractory to the stimuli provided by RE.

insulin responsive sequence-1; insulin-like growth factor I; gastrocnemius; translation

AGE-RELATED DECREASES IN MUSCLE STRENGTH result in decrements in performance that negatively impact health and the quality of life in aged humans (e.g., Refs. 46, 70). The term sarcopenia has been adopted to describe the loss of muscle mass that occurs as a result of the aging process. It has been estimated that the direct healthcare costs of sarcopenia were \$18 billion in the United States in 2000 (42).

SARCOPENIA

The results of cross-sectional studies suggest that sarcopenia is a major determinant of aging-associated decrements in strength (e.g., Refs. 26, 70). Sarcopenia involves significant alterations in the architecture of human muscle that stem from a loss of some myofibers and the remodeling of those that remain (64, 84). Postmortem studies of whole muscle cross sections (vastus lateralis) have found that sarcopenia results

from both a loss of myofibers and a decrease in type II fiber size (49). In humans, age-related myofiber loss and myofiber atrophy generally involve type IIA and IIB fibers, with a greater impact seen in the IIB fibers (62, 84).

The loss of skeletal muscle with aging appears to be a universal finding in mammals (62). McKiernan et al. (56) recently reported that, in rats, both fast and slow muscles experience sarcopenia. As in studies of aged humans, many animal studies have reported that, in commonly studied anti-gravity muscles [vastus lateralis, gastrocnemius, plantaris (PLN), soleus (Sol)] of Fisher 344/Brown Norway F1 cross (F/BN) rats, sarcopenia is most pronounced in fast muscles (14, 25, 56).

Some studies have also found that the muscles of aged rats appear to be more sensitive to unloading, have a lesser ability to recover following unloading, or exhibit a lesser hypertrophy response to increased loading (13, 17, 20, 60). In contrast, Mercier et al. (58) found that Sol muscles of older rats experienced the same degree of atrophy as that of younger animals in response to denervation.

MECHANISMS OF SARCOPENIA

A number of potential causes may be contributing to sarcopenia. It has been reported that there is a decreased rate of muscle protein synthesis with aging (74). In particular, Welle et al. (87, 88) reported that the synthesis of myofibrillar proteins was slower in older individuals and that this was not simply a function of reduced physical activity. In F/BN rats, Flukey et al. (25) reported that, in older animals, insulin-induced increases in protein synthesis were of a lesser magnitude compared with the younger rats.

The phosphorylation of the ribosomal p70 S6 kinase (S6K1) is increased following increased muscle loading, as well as when either the insulin or insulin-like growth factor (IGF)-I receptors are bound by their ligands (receptor ligation), and is thought to be a key regulatory step leading to an increase in translational capacity (1, 79). Morris et al. (60) reported that reloading-induced S6K1 phosphorylation was attenuated in old vs. young F/BN rats. This age-related decrease in S6K1 activity might be expected to limit muscle anabolic processes. Taken together, these studies indicate that, for a given stimulus, the anabolic response of aged muscle is blunted.

Kadi et al. (44) reported that the number of satellite cells per myofiber was found to be lower in the aged. A reduction of the number of satellite cells could indicate a depletion of the pool of satellite cells available for the repair or regeneration of damaged myofibers. Studies of human satellite cells have

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indicated that aging is associated with a reduced replicative potential and/or ability to differentiate (84). However, a number of studies have demonstrated that this is not a characteristic inherent to the satellite cells but rather a reflection of environment in which they reside (17, 84).

SARCOPENIA AND RESISTANCE EXERCISE

A number of studies have demonstrated that both aged women and men, even the very old, can increase various measures of muscle size and strength in response to resistance exercise (68). Lexell et al. (50) reported that increased strength following resistance exercise in 70- to 77-yr-old subjects was correlated with increased proportional area of type II myofibers.

Comparing the response to resistance exercise in young and old subjects, Welle et al. (89) found that the relative increase in muscle size for the knee extensors was similar for both age groups. However, in that study, resistance exercise did not result in a return of specific tension to a value similar to that seen in younger subjects in that study.

Studies using F/BN rats have found that aging is associated with either an absent or a reduced hypertrophy response to increased loading compared with younger adult animals (13, 20). Similarly, muscle regeneration appears to be impaired with age (30).

In previous studies (1–4, 11, 12, 33), our laboratory has identified a panel of cellular and molecular markers that are associated with muscle hypertrophy and that demonstrate detectable sensitivity to acute increases in loading state in muscles of both humans and rats. The hypothesis underlying the present study was that some cellular and molecular markers of muscle hypertrophy would be sensitive to age. Accordingly, the present study had two primary aims: 1) to determine whether these hypertrophy-related cellular and molecular markers are altered with age and 2) to identify responses to acute resistance exercise that are age sensitive. Our results indicate that there are age-specific differences in measurements of some of these cellular and molecular markers at rest and that the responses of these systems to an acute bout of resistance exercise differs significantly in the muscles of old vs. young rats.

METHODS

Animals

Male F/BN were purchased at 6 ($n = 12$, body mass 373 ± 10 g) and 30 ($n = 12$, body mass 564 ± 10 g) mo of age via the National Institutes of Health. All animals were housed in standard vivarium cages and allowed food and water ad libitum. Rats were acclimatized to the vivarium for a minimum of 1 wk before any treatments or data collection. All procedures were approved by the institutional animal care and use committee at the University of California Irvine.

Unilateral Muscle Exercise Protocol

For each training bout, the rats were anesthetized with ketamine-xylazine-acepromazine (30:4:1 mg/kg). Stimulation electrodes consisting of Teflon-coated stainless steel wires introduced into the subcutaneous region adjacent to the popliteal fossa via 22-gauge hypodermic needles. The needles were then withdrawn, leaving the wire in place. Before electrode insertion, a small section of Teflon coating was removed to expose the wire. Wire placement was lateral and medial of the location of the sciatic nerve allowing for field

stimulation of the nerve, resulting in muscle contraction. The stimulation wires were attached to the output poles of a Grass stimulus isolation unit interfaced with a Grass S8 stimulator. The rats were positioned in a specially built training platform described previously (15). The right leg was positioned in a foot plate attached to the shaft of a Cambridge model H ergometer. The stimulation voltage (~ 20 V) and frequency (56 ± 1 Hz) were adjusted to produce maximal isometric tension. Previous studies have demonstrated that this approach results in reproducible force production within and between rats over multiple training sessions (2). The contralateral leg was not subjected to any treatment such as sham electrode placement.

For the exercise bouts, the stimulation parameters were three maximal isometric contractions per minute, 4-s duty cycle and 16 s of rest, for 30 min. The training protocols were controlled by computer via a digital-to-analog board (DDA-06, Keithley Instruments) used to control foot plate excursion and to trigger the stimulus. A separate analog-to-digital board (DAS-16) was used to acquire force measurements (100-Hz acquisition). Data acquisition and control of stimulus triggering was programmed using LabTech Notebook (Laboratory Technologies). Data analysis was conducted using AcqKnowledge software (Biopac Systems). Force output was monitored in real time on the computer screen during each contraction. The ergometer foot plate was set such that the foot position was $\sim 44^\circ$ relative to the tibia. For this study, the ergometer foot plate was not allowed to move during the exercise.

Rats completed two consecutive bouts of resistance exercise at the beginning of their standard light cycle with 24 h of rest between the two bouts. An isometric training protocol was chosen to minimize the potential for muscle injury. Isometric mode resistance exercise is known to result in muscle hypertrophy in humans (e.g., Ref. 43). Our laboratory has previously demonstrated that isometric training protocols are effective in inducing muscle hypertrophy or preventing muscle atrophy in rats (2, 21, 22, 34).

Tissue Collection

Subgroups ($n = 6$) from the young (6 mo old) and old (30 mo old) rat groups were killed via an injection of Pentosol euthanasia solution (Med-Pharmex) at a dose of 0.4 ml/kg ip (~ 160 mg/kg pentobarbital) at 24 and 48 h after the second exercise bout. At the cessation of heart beat, a skin incision was made, and the vastus intermedius, PLN, Sol, and medial gastrocnemius (MG) muscles of both the trained and contralateral legs were dissected free of connective tissue, weighed, and snap frozen for later analysis.

Biochemical and Molecular Analyses

Tissue samples were analyzed for total protein content as described previously (3). Myofibrillar protein content was determined as described previously (80). Total protein was diluted to a final protein concentration of 1 mg/ml in a storage buffer containing 50% glycerol, 100 mM $\text{Na}_4\text{P}_2\text{O}_7$, 5 mM EDTA, and 2 mM 2-mercaptoethanol (pH 8.8) and stored at -20°C until subsequent analyses for myosin heavy chain (MHC) and actin protein content.

Muscle MHC and Actin Protein Determination

Skeletal muscle MHC and actin proteins were separated on acrylamide gels (10% T, 2.5% C) using a standard SDS-PAGE technique as described previously (35). Briefly, 2.5 μg of total denatured protein were loaded per lane. The gels were run at constant current (30 mA) for ~ 2.5 h at 22°C . At the completion of electrophoresis, the gels were stained with Brilliant Blue G 250 (Sigma Chemical), destained, and then scanned using a Molecular Dynamics laser scanning personal densitometer (Sunnyvale, CA). The MHC and actin bands were identified based on their molecular weight and comparisons with purified protein. The intensity of the bands of interest (MHC, actin) was calculated via volume integration, which is based on the sum of

pixel density within a rectangle containing the entire band with local background correction (Image Quant Software, Molecular Dynamics). Using this method, MHC and actin proteins were expressed as arbitrary units per microgram of total protein.

Muscle Total DNA Concentration

The muscle total DNA concentration was determined using a fluorometric assay using the DNA-specific fluorescent Hoechst 33258 dye (3).

Total RNA Isolation

Total RNA was extracted from preweighed frozen muscle samples using the TRI reagent (Molecular Research Center) according to the company's protocol, which is based on the method described by Chomczynski and Sacchi (18). Extracted RNA was precipitated from the aqueous phase with isopropanol and, after washing with ethanol, dried and suspended in a known volume of nuclease-free water. The RNA concentration was determined by optical density at 260 nm (using an OD260 unit equivalent to 40 $\mu\text{g/ml}$). The muscle total RNA concentration was calculated based on total RNA yield and the weight of the analyzed sample. RNA content was calculated based on the product of the observed concentration and the mass of the muscle. RNA samples were stored frozen at -80°C to be used subsequently in relative RT-PCR procedures.

Reverse Transcription

One microgram of total RNA was reverse transcribed for each muscle sample using the SuperScript II RT from Invitrogen and a mix of oligo dT (100 ng/reaction) and random primers (200 ng/reaction) in a 20- μl total reaction volume at 45°C for 50 min, according to the provided protocol. At the end of the reverse transcription reaction, the tubes were heated at 90°C for 5 min to stop the reaction and then were stored at -80°C until used in the PCR reactions for specific mRNA analyses (see below).

PCR

A relative RT-PCR method using 18S as an internal standard (Ambion, Austin, TX) was applied to study the expression of specific mRNAs for IGF-I and mechano-growth factor (MGF), a load- and stretch-sensitive splice variant transcribed from the *igf-1* gene (92). The sequence for the primers used for the specific target mRNAs have been published previously (33, 36). The IGF-I primer used for this study detects all IGF-I splice variants. The MGF variant appears as a separate band that is not integrated when the densitometry measurements are made. Primers were purchased from Operon Biotechnologies. In each PCR reaction, 18S ribosomal RNA was coamplified with the target cDNA (mRNA) to serve as an internal standard and to allow correction for any differences in starting amounts of total RNA.

For 18S amplification, we used the Alternate 18S Internal Standards (Ambion), which yield a 324-bp product. The 18S primers were mixed with competitors at an optimized ratio that could range from 1:4 to 1:10, depending on the abundance of the target mRNA. Inclusion of 18S competitors was necessary to bring down the 18S signal, which allows its linear amplification to the same range as the coamplified target mRNA (Ambion, relative RT-PCR kit protocol).

For each target mRNA, the reverse transcription and PCR reactions were carried out under identical conditions using the same reagents premix for all the samples to be compared in the study. To validate the consistency of the analysis procedures, at least one representative from each group was included in each RT-PCR run.

One microliter of each reverse transcription reaction (0- to 10-fold dilution depending on target mRNA abundance) was used for the PCR amplification. PCR reactions were carried out in the presence of 2 mM MgCl_2 using standard PCR buffer (Bioline), 0.2 mM dNTP, 1 μM specific primer set, 0.5 μM 18S primer/competitor mix, and 0.75 unit

of Biolase DNA polymerase (Bioline) in 25- μl total volume. Amplifications were carried out in a Stratagene Robocycler with an initial denaturing step of 3 min at 96°C , followed by 25 cycles of 1 min at 96°C , 1 min at $55-60^{\circ}\text{C}$ (depending on primers), 1 min at 72°C , and a final step of 3 min at 72°C . PCR products were separated on a 2–2.5% agarose gel by electrophoresis and stained with ethidium bromide, and signal quantification was conducted by laser scanning densitometry (12). In this approach, each specific mRNA signal is normalized to its corresponding 18S. For each primer set, PCR conditions (cDNA dilutions, 18S competitor/primer mix, MgCl_2 concentration, and annealing temperature) were set to optimal conditions so that both the target mRNA and 18S product yields were in the linear range of the semilog plot when the yield was expressed as a function of the number of cycles (12).

Phosphorylation State of Intracellular Signaling Proteins

The total amount and the phosphorylation state of the insulin (Y1146)/IGF-I (Y1131) receptor, insulin receptor substrate (IRS)-1 (S307, Y612), Akt/PKB (T308), S6K1 (T389), and signal transducers and activators of transcription (STAT)-3 (Y705) were examined by immunoblotting using pan and phospho-specific antibodies. All antibodies used were from Cell Signaling Technology except for IRS-1 (Y612), which was from Biosource (catalog no. 44-819G), and the total IRS-I antibody, which was from Santa Cruz Technology (catalog no. sc-7200).

Muscle samples were extracted by homogenization in seven volumes of ice-cold buffer A (50 mM Tris·HCl, pH 7.8, 2 mM potassium phosphate, 2 mM EDTA, 2 mM EGTA, 50 mM β -glycerophosphate, 10% glycerol, 1% Triton X-100, 1 mM DTT, 3 mM benzamide, 1 mM sodium orthovanadate, 10 μM leupeptin, 5 $\mu\text{g/ml}$ aprotinin, 200 $\mu\text{g/ml}$ soybean trypsin inhibitor, and 1 mM AEBSF) using a motor-driven glass pestle. The homogenate was immediately centrifuged at 12,000 g for 30 min at 4°C . The supernatant was immediately saved in aliquots at -80°C for subsequent use in immunoblotting. The supernatant protein concentration was determined using the Bio-Rad protein assay with BSA as standard. Approximately 50 μg of supernatant proteins were subjected to SDS-PAGE (12.5% T, according to standard protocol) then electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon-P) using 10% methanol, 1 mM orthovanadate, 25 mM Tris, 193 mM glycine, pH 8.3. Phosphorylation state-specific antibodies were detected using enhanced chemiluminescence method of detection (Amersham). Signal intensity was determined by laser-scanning densitometry (Molecular Dynamics/image Quant). For each specific antibody, all samples were run under identical (previously optimized) conditions, including the transfer on the membrane, the reaction with the first and secondary antibodies, washing conditions, enhanced chemiluminescence detection, and the film exposure. To ensure the consistency of this analysis, at least one representative sample from each group was included in each gel run and Western analysis. In addition, a positive control, provided by the antibody manufacturer, was run on each gel. To normalize the amount of protein loaded between samples, protein concentration in the muscle extract was determined in duplicate to ensure accuracy, and equal amounts were loaded onto each gel lane. To verify the accuracy of gel loading, after transfer, the gel was stained with Coomassie blue, which stains residual proteins that did not transfer. This staining was quantified via densitometry. In each case, the signal obtained demonstrated a high correlation with the initial amount of protein loaded. For each set of Western blotting and detection conditions, the detected signal was directly proportional to the amount of protein loaded on the gel over a range of 20–150 μg (data not shown).

Statistical Analysis

All values are reported as means \pm SE. For each time point, treatment effects were determined by one-way ANOVA with post hoc testing (Student-Newman-Keuls) using the Prism software package

(Graphpad). These comparisons included the evaluation of contralateral vs. treatment effects as well as between-group (age) effects. Analysis of ratio data was conducted using a Student's *t*-test following log transformation of the two data sets (e.g., young vs. old) and using Kruskal-Wallis testing with Dunn post hoc analysis for multiple time points (Prism, Graphpad). Pearson correlation analysis was used to assess the relationships changes in pairs of variables (Prism, Graphpad). For all statistical tests, the 0.05 level of confidence was accepted for statistical significance.

RESULTS

Age-Related Differences

The rats used for this study would be considered young adults (6 mo old) and old adults (30 mo old) since the age of 50% mortality in this strain is 36 mo (53, 82). At the time of data collection, the body mass of the old rats was 564 ± 10 g and that of the young animals was 373 ± 10 g.

Muscle mass and protein. The mass of the old and young Sol, vastus intermedius, and PLN muscles did not differ significantly with age, whereas that of the MG was significantly lower in the old rats (Table 1). When normalized to body weight, the mass of each of these muscles from the old rats was significantly less than that of the young animals (Table 1). Compared with the Sol, vastus intermedius, and PLN muscles, the relative mass of the MG muscle exhibited the greatest degree of sarcopenia, being 46% smaller in the old vs. young rats (Table 1). Accordingly, the cellular and molecular analyses to follow were conducted on this muscle.

The total protein concentration was similar in both the old and young MG muscles (Table 2). However, the total protein content was 21% lower in the MG of the old rats (168 ± 4 vs. 213 ± 7 mg), reflecting their smaller size. In addition to being smaller and having less protein, the MG muscles of old rats had a lower concentration of myofibrillar proteins (Table 2) such that, relative to total protein, the proportion of muscle protein represented by these contractile elements was significantly less in the old animals (Table 2). Within the myofibrillar protein pool, there was a significant, apparently disproportionate, loss of MHC protein (Table 2), whereas the proportion of actin protein did not appear to change with age (Table 2). No significant differences in the relative proportions of the four adult MHC isoforms were observed for the MG muscles of old vs. young rats. The MHC % distribution was as follows for the young: MHC I: 4 ± 1 ; MHC IIa: 7 ± 0.4 ; MHC IIx: 34 ± 2 ; MHC IIb: 55 ± 2 . For the old muscles, it was MHC I: 6 ± 0.2 ; MHC IIa: 6 ± 1 ; MHC IIx: 34 ± 1 ; MHC IIb: 54 ± 2 .

Muscle RNA and DNA. The total amount of RNA present in the MG muscle was similar for both young and old rats (Table 3). Accordingly, the smaller MG muscles of the old rats had a

Table 2. Age-related differences in contractile proteins

	Young	Old
Total protein, mg/g	249 \pm 5	237 \pm 5
Myofibrillar protein, mg/g	118.0 \pm 5.5	92.21 \pm 3.3*
Myofibrillar-to-total protein ratio	0.47 \pm 0.02	0.39 \pm 0.01*
Total MHC	554 \pm 14	489 \pm 5*
Total actin	197 \pm 4	189 \pm 5

Values are means \pm SE. MHC, myosin heavy chain. **P* < 0.05, old vs. young.

significantly greater concentration of RNA (Table 3). As a result, the ratio of protein to RNA (Table 3) was significantly lower in the MG muscles of the old rats.

The concentration of DNA was also higher in the MG muscles of old rats (Table 3) such that the DNA-to-protein ratio of these muscles was 27% greater than that seen in those of the young animals (Table 3).

Muscle mRNA. The mRNA levels (relative to 18S) of IGF-I, myogenin, and SOCS3 were significantly higher in old rat MG muscles (Fig. 1), whereas the mRNA levels for myostatin and SOCS2 were lower (Fig. 2), and those for MGF, atrogen-1, and cyclin D1 were not different (not shown).

Muscle signaling components. The total amount of IGF-I receptor protein was approximately twofold higher in the MG muscles from old rats (Fig. 3A). However, using an antibody that detects tyrosine phosphorylation of the IGF-I (IGFR1)/insulin receptor (IR), no age-associated differences in relative phosphorylation state were detected (Fig. 3B).

Total IRS-1 protein was \sim 70% lower in the MG muscle of old rats (Fig. 4A). Tyrosine phosphorylation of IRS-1 was also significantly depressed in old vs. young MG muscles (Fig. 4B). As a result of the parallel changes in protein and phosphorylation, the proportion of tyrosine phosphorylation to total IRS-1 was similar in both young and old muscles (Fig. 4C). MG muscle serine phosphorylation of IRS-1 did not differ between the two age groups (not shown). However, in contrast to tyrosine phosphorylation, this resulted in a significant increase in the proportion of the IRS-1 pool that was phosphorylated on S307 (Fig. 4D).

The total amount of Akt protein was significantly greater (67%) in the MG muscles of the old rats (Fig. 5A). However, the level of phosphorylated Akt was twofold lower in the old vs. young MG muscles (Fig. 5B). There were no age-related differences in the amount of S6K1 protein present or the level of S6K1 phosphorylation (not shown).

STAT3 protein levels were significantly higher (56%) in the MG muscles of the old rats relative to those of the young animals (Fig. 6A). Phosphorylation of STAT3 was fivefold greater in the muscles of the old vs. young rats (Fig. 6B).

Table 1. Age-related differences in muscle mass

Muscle	Absolute Mass, mg		Relative Mass, mg/g body mass	
	Young	Old	Young	Old
VI	139 \pm 8	139 \pm 8	0.37 \pm 0.02	0.25 \pm 0.01*
Sol	149 \pm 6	157 \pm 6	0.41 \pm 0.01	0.29 \pm 0.01*
PLN	350 \pm 12	324 \pm 8	0.96 \pm 0.02	0.59 \pm 0.01*
MG	827 \pm 28	649 \pm 18*	2.27 \pm 0.01	1.18 \pm 0.02*

Values are means \pm SE. VI, vastus intermedius; Sol, soleus; PLN, plantaris; MG, medial gastrocnemius. **P* < 0.05, old vs. young.

Table 3. Age-related differences in RNA and DNA

	Young	Old
RNA content, μ g	476 \pm 13	493 \pm 16
RNA concentration, mg/g	0.57 \pm 0.02	0.72 \pm 0.02*
Protein-to-RNA ratio	442 \pm 16	303 \pm 13*
DNA concentration	1.1 \pm 0.03	1.3 \pm 0.03*
DNA-to-protein ratio	0.0044 \pm 0.0001	0.0057 \pm 0.0002*

Values are means \pm SE. **P* < 0.05, old vs. young.

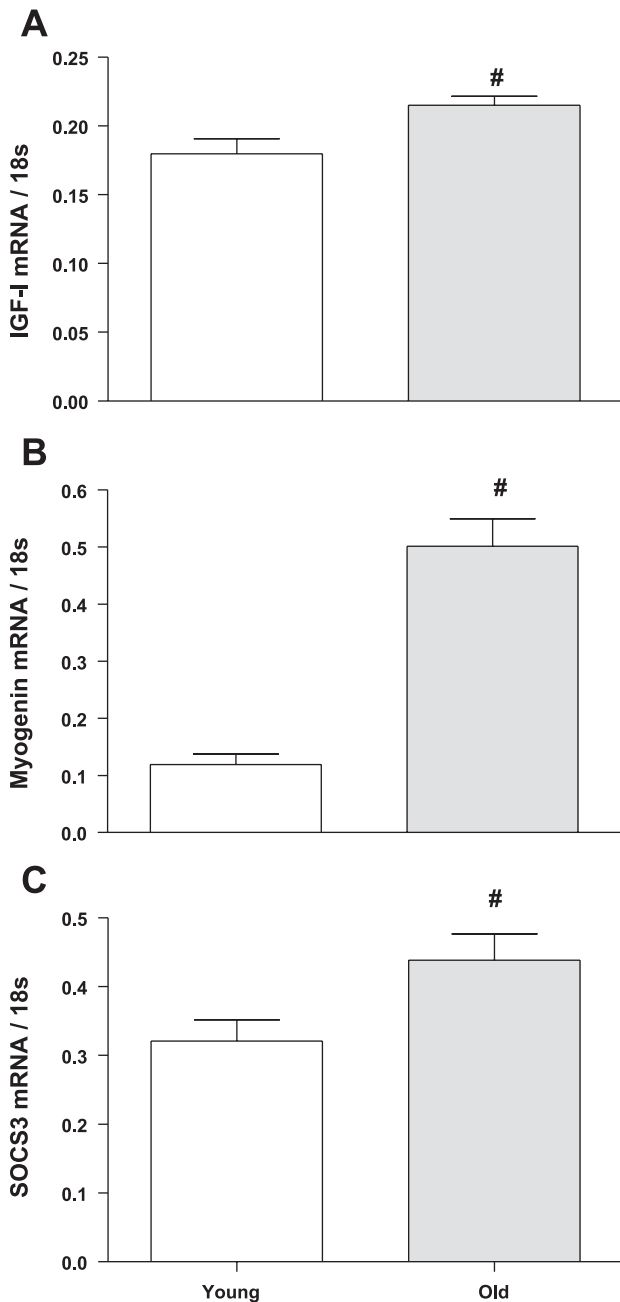


Fig. 1. Age-associated increases in the level of specific mRNAs relative to 18S rRNA. The expression and/or accumulation of the mRNA for insulin-like growth factor (IGF)-I (A), myogenin (B), and SOCS3 (C) was higher in the medial gastrocnemius (MG) muscles of old rats. # $P < 0.05$, old vs. young.

Response to Acute Exercise

Force production. Using the isometric contractile paradigm in our present study, the legs of the rats generated from ~1 to ~1.5 kg of force against the stationary foot plate (young, $1,421 \pm 49$ g; old, 929 ± 45 g). The mean integrated force produced across the two exercise bouts was 36% lower for the old vs. young rats (young, $2,810 \pm 98$; old, $1,801 \pm 96$; $P < 0.0001$). A significant force deficit was also evident after normalization to muscle mass (young, 1.97 ± 0.07 ; old, 1.39 ± 0.06 ; $P < 0.0001$).

Muscle mass and protein. The two acute bouts of resistance exercise did not result in any significant changes in muscle mass or protein concentration in either the old or young rat muscles at either time point (not shown).

Muscle RNA and DNA. The RNA content of the exercised MG muscle was significantly increased to a similar extent at 24 and 48 h after the second bout of resistance exercise in both young and old rats (Fig. 7A). The measurements of RNA, as well as all subsequent analyses, found that the results for the contralateral muscles did not change over time in this acute study. As a result, the values of contralateral legs measured at 24 and 48 h postexercise were combined for graphic representation by a single point before *time 0* on the *x*-axis (Fig. 7A). However, in each case, the statistical analyses included comparisons with the contralateral muscles harvested at each time point.

When expressed as a concentration, the change in RNA followed a different pattern with the greatest increases in the young muscles at 24 h postexercise, whereas in the old MG muscles the concentration was not significantly increased until the 48-h time point (Fig. 7B).

Acute bouts of resistance exercise did not result in significant changes in the concentration of DNA at any time point (not shown).

Muscle mRNA. The amount of IGF-I mRNA (expressed relative to 18S ribosomal RNA) present in the MG muscles of young and old rats was increased at 24 and 48 h postexercise (Fig. 8A). At 24 h postexercise, MGF mRNA levels were

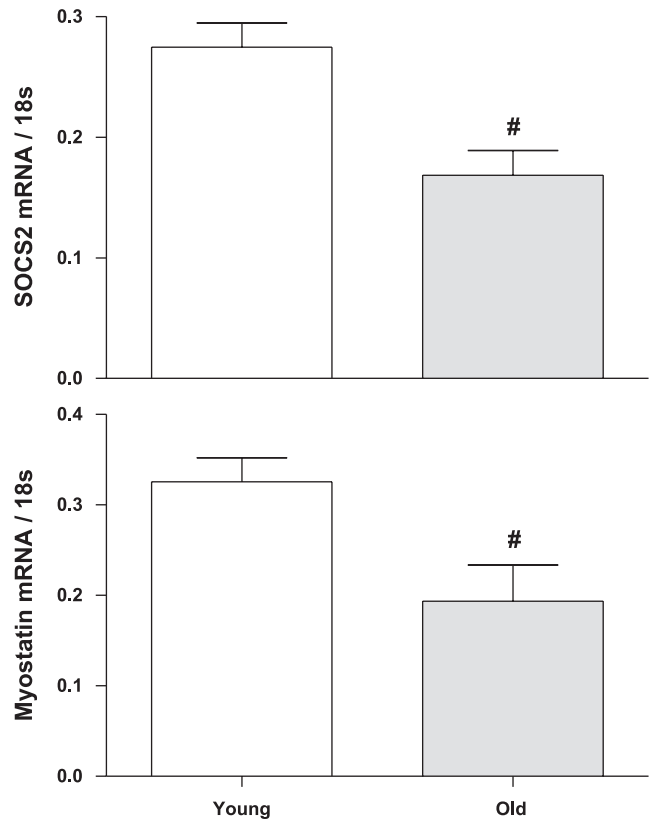


Fig. 2. Age-associated decreases in the levels of specific mRNAs relative to 18S rRNA. The expression and/or accumulation of the mRNA for SOCS2 (A) and myostatin (B) was lower in the MG muscles of old rats. # $P < 0.05$, old vs. young.

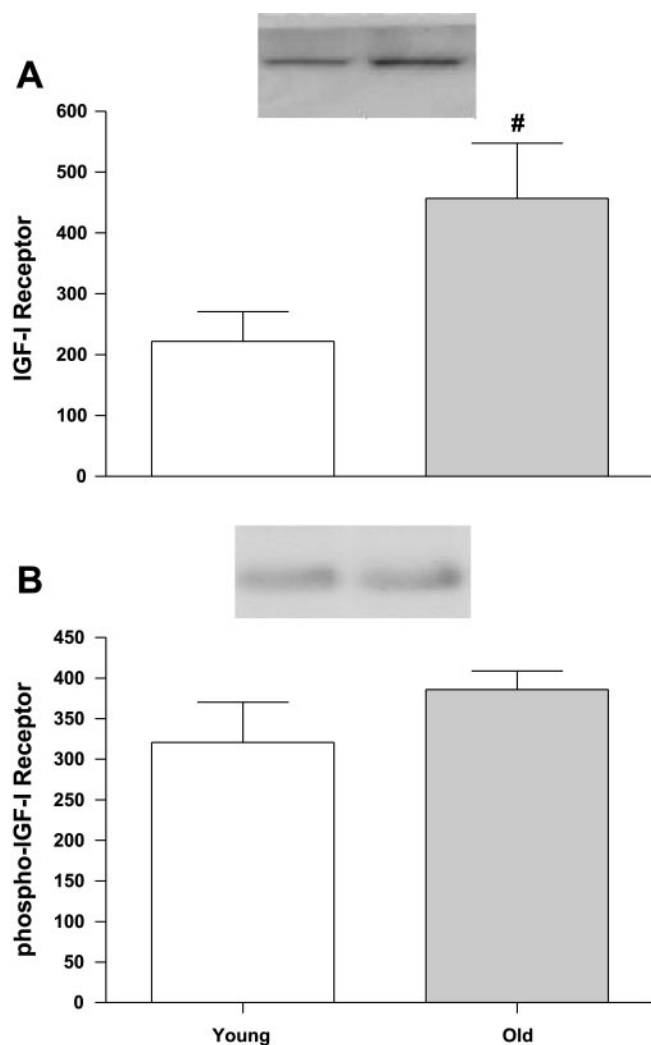


Fig. 3. Age and the muscle IGF-I receptor. The total amount of IGF-I receptor (IGFR1) protein was significantly increased in the MG muscles of old rats (A). The level of phosphorylation of the IGFR1/insulin receptor (IR) was similar in the muscles of both young and old rats (B). Insets: representative immunoblots from individual muscles in each group corresponding (i.e., young, old) to the mean data ($n = 6$ /group). The phosphorylation-specific antibody used in this study detected tyrosine phosphorylation of both the IGF-I and IR. [#] $P < 0.05$ old vs. young.

increased by six- and ninefold in the muscles of old and young rats, respectively, and remained elevated by approximately sixfold at the 48-h time point (Fig. 8B). The pattern of changes in cyclin D1 mRNA appeared to mirror those of IGF-I (Fig. 8C). The greatest increase in cyclin D1 mRNA was seen at 24 h postexercise in the MG of young rats (Fig. 8C). At 48 h postexercise, cyclin D1 mRNA had also increased in the old MG muscles. In contrast to IGF-I and MGF, acute resistance exercise increased the mRNA for myogenin only in young MG muscles and only at the 24-h time point (Fig. 8D).

There was an increase in the synthesis and/or accumulation of SOCS3 mRNA in the MG muscles of both the young and old rats that was similar at each time point (Fig. 9A). Resistance exercise resulted in a decrease in the abundance of SOCS2 mRNA at both 24 and 48 h posttraining in the MG muscles of young but not old rats (Fig. 9B). The mRNA for atrogenin-1 exhibited a modest increase (42%) in the MG mus-

cles of the young rats 48 h after the acute bout of resistance exercise (Fig. 9C). Exercise resulted in a significant decline in the amount of myostatin mRNA present in the MG muscles of the young but not old rats at 24 and 48 h (Fig. 9D).

Muscle signaling components. Acute exercise resulted in a progressive decline in IRS-1 protein that reached significance at the 48-h time point postexercise in the MG from young rats but not in the old rats (Fig. 10). No other significant changes in the total amount of a measured signaling protein were observed in response to exercise (not shown).

There was a significant increase in the phosphorylation of the IGFR1/IR of the young but not old rats at 24 h postexercise (Fig. 11A). The phosphorylation of S6K1 was significantly increased in both the young and old MG muscles at 24 h postexercise (Fig. 11B). However, at 48 h postexercise, S6K1 phosphorylation was still elevated in the old muscles, whereas in the young MG this value had returned to baseline levels (Fig. 11B). Similar to the IGF-I receptor, Akt phosphorylation was increased at 24 h postexercise in the young but not old muscles (Fig. 11C). The phosphorylation of STAT3 was increased significantly at both 24 and 48 h postexercise in the MG muscles of the young and old rats (Fig. 11D).

DISCUSSION

Sarcopenia

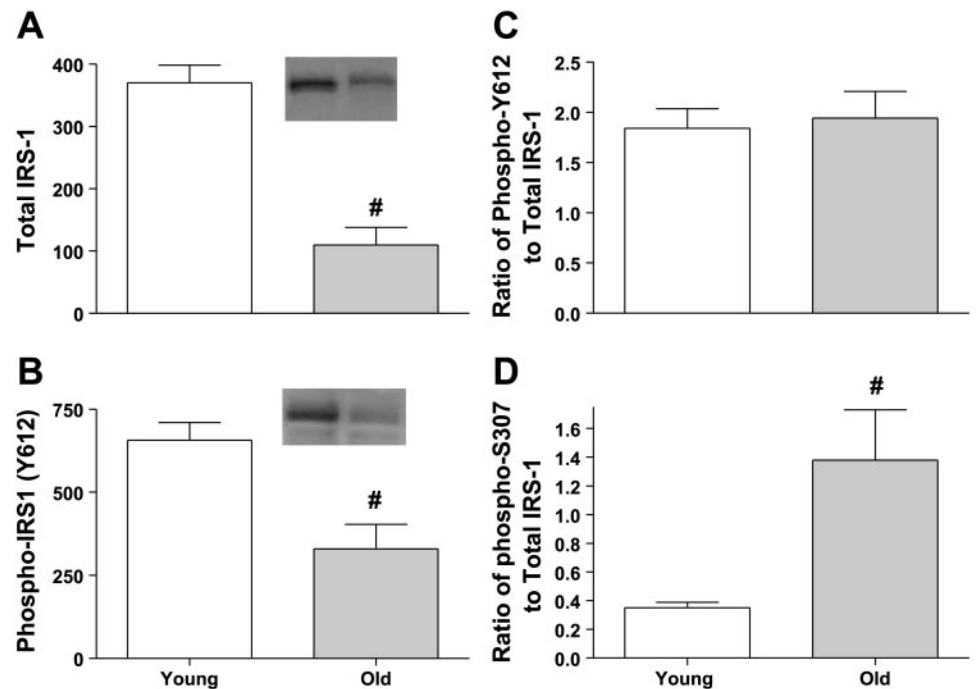
As reported by others (e.g., Refs. 38, 66), relative to body mass, the mass of the Sol, PLN, and MG muscles was significantly lower in the old vs. young rats in the present study. In addition to the overall decreased muscle size seen with aging, the MG muscles of the old rats exhibited an exaggerated loss of contractile proteins (Table 2). The disproportionate loss of contractile proteins appeared to manifest, at least partially, as a loss of MHC protein (Table 2). These findings are in general agreement with the results from a number of human studies that have found a dramatic decrease in mixed muscle- and specifically MHC-protein synthesis with age (63, 93). The present results indicate that the muscles of the old rats may be at a further disadvantage with regard to the ability to develop force than would be expected based simply on muscle mass.

Several groups have reported an age-related loss of myofiber or muscle-specific tension (41, 61). In the present study, force production normalized to muscle mass was ~30% lower in the MG muscles of the older rats. This result would tend to support observations of age-related reductions in specific tension.

Cellular and Molecular Indicators of Hypertrophy and Sarcopenia

We have previously found that prolonged application of resistance exercise training protocols, similar to that used in this study, results in muscle hypertrophy (Ref. 2 and unpublished data). As in previous studies, the rationale for the present study was to collect data following acute bouts of resistance exercise to capture cellular and molecular events associated with the initiation of a hypertrophy response (11, 12, 33). Because a number of studies have indicated that the muscles of old rats demonstrate a reduced hypertrophy response to increased loading, we hypothesized that we would be able to detect differential cellular and molecular responses to acute resistance exercise that would be indicative of the aging-sensitive components of hypertrophy-related mechanisms (13, 20).

Fig. 4. Age and muscle insulin receptor substrate-1 (IRS-1). *A*: total amount of IRS-1 protein was significantly lower in the MG muscles of old rats. *B*: phosphorylation of tyrosine 612 (Y612) on IRS-1 (activating) was significantly lower in old muscles. *C*: ratio of phospho-Y612-IRS-1 to total IRS-1 was similar in both young and old muscles. *D*: ratio of serine 307 (S307) phosphorylation (inhibitory) to total IRS-1 protein was significantly greater in the MG muscles of old rats. *Insets*: representative immunoblots from individual muscles corresponding to the mean data (i.e., young, old). $\#P < 0.05$, old vs. young.



Translational efficiency. In the present study, we found indications that the efficiency of protein translation may be impaired in older rat muscles. The majority of the cellular RNA pool consists of ribosomal RNA, and therefore total RNA measurements can be taken to roughly reflect the translational capacity of the muscle (37). In the muscles of old rats, we found that there was more RNA but that the ratio of protein to RNA was significantly lower (Table 3). This could be taken to indicate that the efficiency of protein translation is lower in the MG muscles of the old rats. Similar to the present results, Prod'homme et al. (69) previously reported that, relative to those from 12-mo-old Wistar rats, the muscles from 26-mo-old rats have decreased protein concentrations, have increased RNA concentrations, and demonstrate a decreased ribosomal efficiency for protein synthesis.

With regard to the response to resistance exercise, assuming that the preexercise RNA levels were sufficient for the maintenance of muscle protein levels, it is reasonable to assume that an increase in protein synthesis would include, and possibly be preceded by, the synthesis of more ribosomal components. In support of this assumption, acute resistance exercise resulted in an increase in total RNA (content and concentration) in the MG muscles from both groups (Fig. 7). In fact, the concentration of RNA present in the old muscles actually increased to a greater degree than in the young muscles (Fig. 7B). This indicates that, although the old muscles had a lesser protein-to-RNA ratio before the resistance exercise, further increases in RNA would apparently be necessary to support adaptation to a chronic increase in loading.

Anabolic Signaling Potential

IGF-I. With regard to growth factors, Severgnini et al. (76) reported that aging (7 vs. 30 mo old) is associated with decreased circulating IGF-I and IGFBP3 protein levels in F/BNF rats. In that study, the level of mRNA for IGF-I in the

gastrocnemius muscle increased with age, suggesting an attempt at a compensatory response. In agreement with Severgnini et al., we found that there was a slightly greater (20%) level of IGF-I mRNA present in the muscles of old rats at baseline, i.e., in the unexercised muscles (Fig. 1A).

Olwino et al. (65) found that the overloading (gastrocnemius tenotomy) of PLN muscles for 5 days induced increases in the expression of MGF mRNA that were greatly attenuated in older SD rats (3 vs. 24 mo old), suggesting that MGF-related responses to loading are dampened with aging. In the present study, we found that the response of IGF-I mRNA (all transcripts except MGF) to acute resistance exercise was less robust in the MG muscles of old rats (Fig. 8A). However, in contrast to the results of Olwino et al., we observed a very large increase in MGF mRNA that was essentially the same in both young and old rat MG muscles (Fig. 8B). Although the changes in MGF mRNA appear to be particularly dramatic (i.e., 6- to 9-fold increases), the greater magnitude of this change is in part due to the very low levels seen at baseline (Fig. 8B). With regard to the differing results between the present study and that of Olwino et al. (65), there were study-related differences in rat strain and overloading model. An additional possible explanation for the divergence in result is the analysis of the PLN (65) vs. MG muscle (present study).

IGF-I receptor. Willis et al. (91) reported that the mRNA for IGF1R was significantly higher in the gastrocnemius muscles of very old (24 mo old) C57B1/6 mice. In general agreement with this finding, the amount of IGF1R protein was higher in the old vs. young muscles of rats in the present study (Fig. 3A). In contrast, the amount of receptor phosphorylation was essentially the same for both the young and old MG muscles (Fig. 3B). These results could be taken to suggest that there is an age-related deficit in IGF-I activation in the muscles of old rats. However, there are two technical caveats associated with these results.

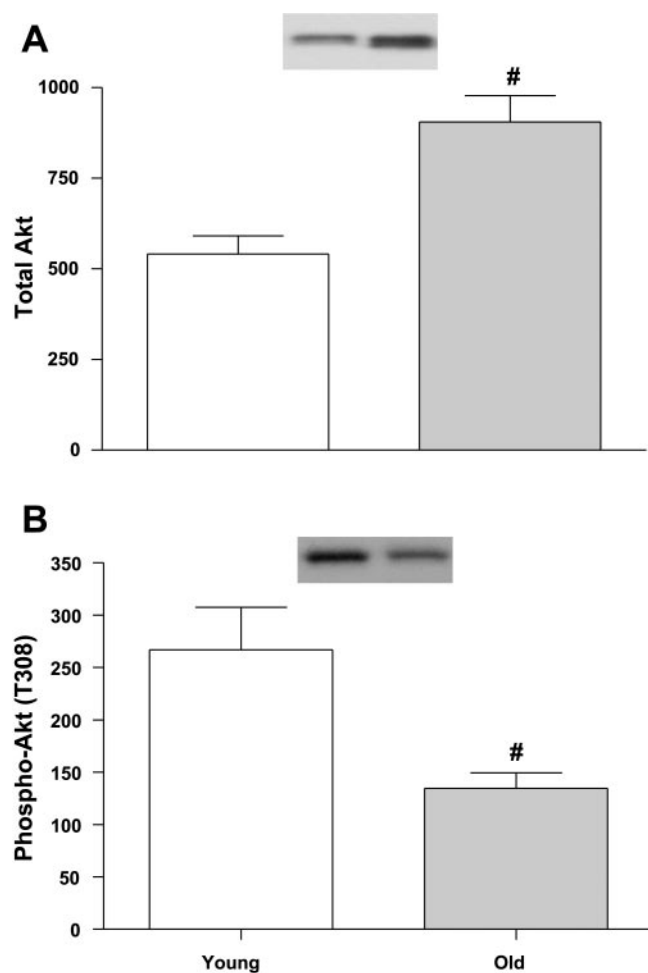


Fig. 5. Age and muscle Akt. *A*: aging was associated with a significant increase in the total amount of Akt protein present in old MG muscles. *B*: level of phosphorylation of threonine 308 (T308) on Akt (activating) was significantly lower in the old muscles. *Insets*: representative immunoblots from individual muscles corresponding to the mean data (i.e., young, old). $^{\#}P < 0.05$, old vs. young.

First, the antibody used for the measurement of receptor phosphorylation is known to react with both the IGF-I (i.e., IGFR1) and insulin (IR) receptors. Zhu et al. (95) recently reported that, in F344 rats, aging was not associated with alterations in the total amount of IR in muscle. However, these authors did find that the insulin-stimulated phosphorylation of the IR was very significantly blunted in old rats. Therefore, one interpretation of the present results would be that the lesser level of receptor phosphorylation (e.g., the same level with increased IGFR1 protein) may be entirely the result of decreases in phospho-IR.

Second, IGFR1 protein was detected using an immunoblot and, therefore, represents both intracellular as well as cell surface protein.

Ligation of the insulin or IGF-I receptors has been shown to have powerful anabolic effects in skeletal muscle (5, 9, 81). With regard to the exercise response, in the MG muscle of young rats, tyrosine phosphorylation of the IGFR1/IR, indicative of receptor activation, was significantly increased 24 h postexercise (Fig. 11A). However, there was no significant change in this parameter in the old muscles. This indicates that

the muscles of the old rats were in some way refractory to the exercise stimulus in this regard. In this instance, the data suggest that the time frame was probably sufficient to capture a response, in that the IGFR1/IR phosphorylation data trended downward between the 24- and 48-h time points in both the young and old muscles. Based on our laboratory's previous findings and changes seen in IGF-I mRNA in the present study, we tend to assume that exercise-induced alterations in IGFR1/IR phosphorylation reflect primarily autocrine/paracrine IGF-I signaling. However, as noted above, the technical limitations imposed by using an phospho-IGFR1/IR antibody preclude definitive conclusions in this matter.

IRS-1. The IRS-1 protein is immediately downstream of the insulin and IGF-I receptor and links these receptors to a number of signaling cascades. The muscles of 30-mo-old rats had less IRS-1 protein than those of the young animals (Fig. 4). These findings are in accord with those of Arias et al. (8), who reported that aging was associated with a decrease in the concentration of IRS-1 protein in rat skeletal muscles.

Phosphorylation of IRS-1 at tyrosine 612 is generally associated with an increase in signaling via IRS-1-stimulated PI3-kinase activity (31). We found that, in addition to a decrease in the amount of IRS-1 present in the old MG muscles, less of that

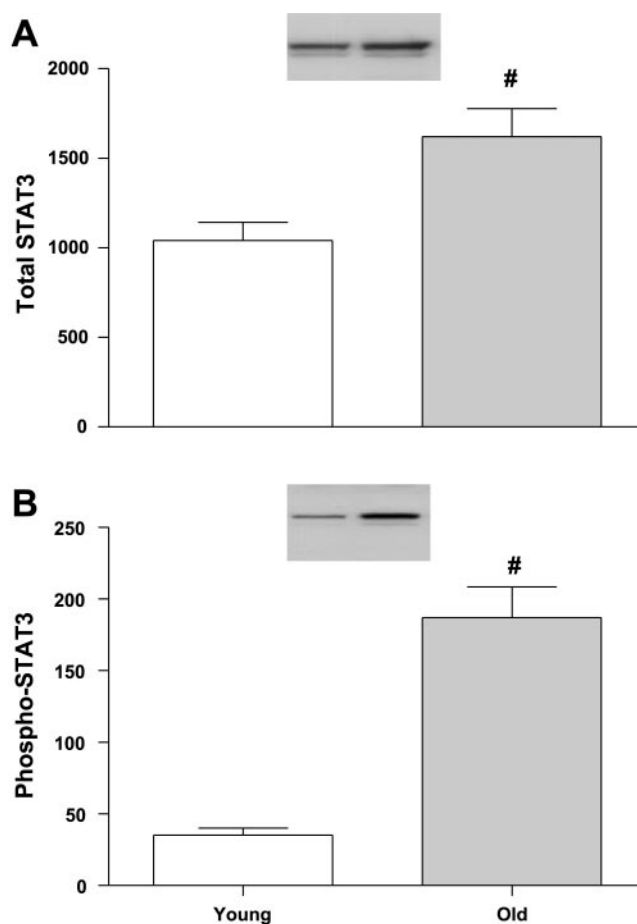


Fig. 6. Age and muscle STAT3. *A*: total amount of STAT3 protein present in MG muscles was significantly greater in the old rats. *B*: level of phosphorylation of tyrosine 705 on STAT3 (activating) was significantly higher in the muscles of old rats. *Insets*: representative immunoblots corresponding to the mean data (i.e., young, old). $^{\#}P < 0.05$, old vs. young.

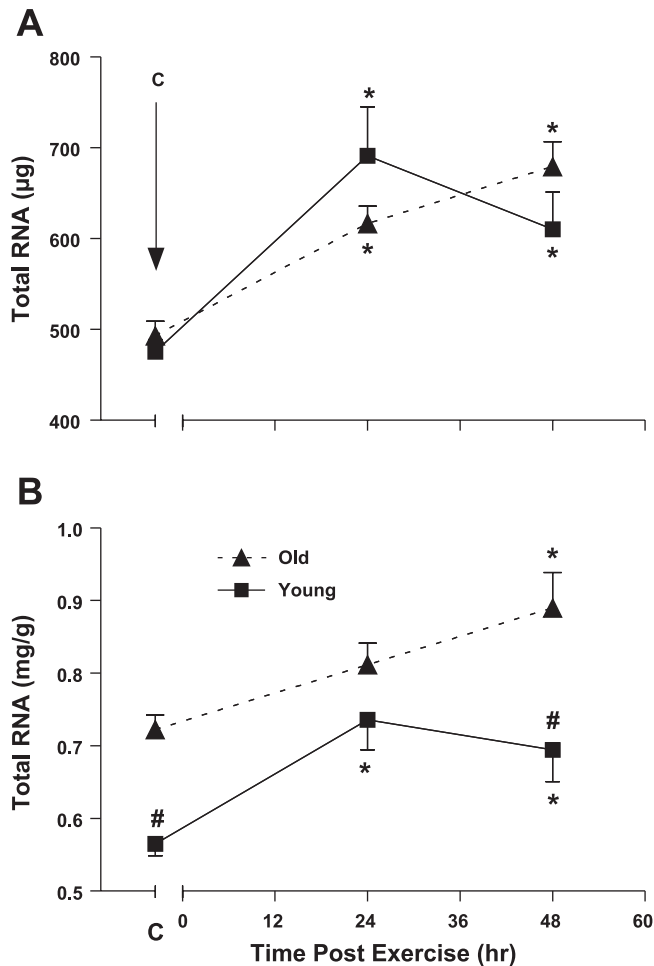


Fig. 7. Acute resistance exercise induced changes in muscle total RNA. *A*: RNA content of the MG muscles of both young and old rats increased following resistance exercise. *B*: concentration of RNA (mg/g muscle) also increased in MG muscles as a result of resistance exercise. In the contralateral MG muscles, there were no time-dependent changes in RNA or any other parameter measured for this study. Accordingly, the contralateral data were combined to simplify the graphical presentation. These data are presented to the left of *time 0* on the *x*-axis, as illustrated by the C designation in Fig. 12A, and a C on the *x*-axis for all subsequent plots. It is important to note that the contralateral data were not combined for the statistical analyses. * $P < 0.05$, resistance exercise vs. contralateral. # $P < 0.05$, old vs. young.

pool was phosphorylated at tyrosine 612 (activation) (Fig. 4B). Normalizing Y612 phosphorylation to the amount of IRS-1 protein present eliminates the differences between the young and old muscles (Fig. 4C). However, this normalization would mask the dramatic decrease in the relative amount of signaling via IRS-1 that would be occurring in the young vs. old muscles. Even accounting for the decrease in muscle mass in the old rats, there would still be an ~65% deficit in amount of total IRS-1 present. Although the phosphorylation of IRS-1 is not driven by mass action, it is possible that the low levels of protein present may become rate limiting for this process.

In contrast to tyrosine phosphorylation, phosphorylation of serine residues, such as S307, is thought to inhibit the ability of IRS-1 to interact with IR and IGFR1, resulting in inhibition of signaling via PI3-kinase (31). In the MG muscles of old rats, a greater proportion of the IRS-1 present was phosphorylated at S307 (Fig. 4D). Taken together, these results indicate that there

is most likely a significant deficit in the ability of old muscle to signal via IRS-1.

In the present study, we observed a substantial degree of age-related divergence in the effects of acute exercise on IRS-1. The muscles of the young but not old rats exhibited a decrease in the amount of IRS-1 protein present (Fig. 10). In the study by Arais et al. (8), 10 wk of treadmill running actually ameliorated the age-associated deficit in IRS-1. However, the differences in time point (acute vs. long duration) and exercise mode (resistance vs. endurance) preclude conclusions on whether the IRS-1 responses seen in the present work and those reported by Arais et al. are in conflict.

As noted above, there was a progressive decline in IRS-1 protein that reached significance at the 48-h time point postexercise in the MG from young rats (Fig. 11). Increased signaling through PI3-kinase is known to result in recurrent inhibition of signaling from upstream receptors that can be mediated via the mTOR-, S6K1-, or Erk-induced phosphorylation of several serine residues of IRS-1 (31, 72). In some instances, this process can lead to a degradation of IRS-1 (31). In the present study, we measured the phosphorylation of S307 and did not detect significant differences in the muscles of the young rats following exercise. However, a number of additional serine residues can be targeted by the various kinases, which may have mediated the observed exercise response (31).

Recent reports have suggested that activity of some of the signaling pathways discussed in this paper may be directly linked to contractile activity as opposed to being a function of receptor ligation (40). In this scenario, it would be possible to speculate that activation of signaling components, such as mTOR, downstream from IRS-1 could result in feedback-related serine phosphorylation and degradation of IRS-1 protein even in the absence of increased signaling via relevant receptors. In the present study, the absence of changes in IRS-1 abundance in the muscles of the old rats may tend to argue against this alternative, but the results do not directly speak to this issue.

Akt. Akt plays a number of roles that may be important in sarcopenia. These roles include the suppression of apoptosis and modulation of muscle-specific protein degradation via the inhibition of the expression of the ubiquitin E3 ligases atrogin-1 and MuRF-1. Akt activity also promotes protein translation via the inhibition of glycogen synthase kinase-3 and the activation of mTOR. The activation of Akt is known to be sensitive to the ligation of the insulin and IGF-I receptors. At baseline, the amount of Akt protein present in the MG muscles of the old rats was 67% greater than that in the young (Fig. 5A). This result appears to be in conflict with results published by Arais et al. (8). However, there were slight differences in the age (3 and 26 mo old), strain of rats (F344), and muscles (plantaris) used in that study that may account for the difference in result (8).

In the present study, the baseline level of Akt phosphorylation was approximately twofold lower in the muscles of old rats (Fig. 5B), a result similar to that reported in mice by Li et al. (51). If expressed as a function of the total amount of Akt protein present, the proportion of Akt that is phosphorylated would be more than threefold lower in the muscles of the old rats (0.50 ± 0.06 vs. 0.15 ± 0.01).

In a pattern similar to that of IGFR1/IR receptor phosphorylation, resistance exercise-induced phosphorylation of Akt

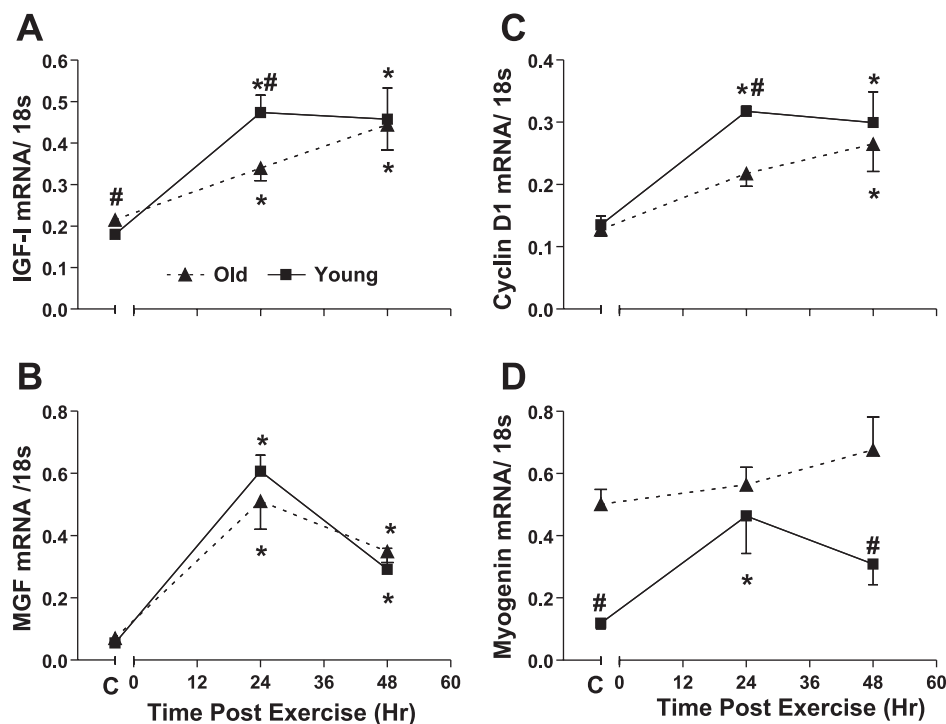


Fig. 8. Acute resistance exercise induced changes in specific muscle mRNA relative to 18S rRNA. Resistance exercise induced an increase in the expression and/or accumulation of the mRNA for IGF-1 (A), mechano-growth factor (MGF; B), and cyclin D1 (C) in both the young and old MG muscles. D: resistance exercise resulted in a significant increase in myogenin mRNA at 24 h postexercise only in the muscles from young rats. * $P < 0.05$, resistance exercise vs. contralateral. # $P < 0.05$, old vs. young.

was increased in young but not old muscles (Fig. 11C). Using the administration of desIGF-I as a stimulus, Li et al. reported that the increase in Akt phosphorylation (ser473) in the muscles of both young and old mice was similar. In the context of the results from Li et al., the present findings may indicate that the IGF-I sensitivity of the old muscles remains relatively intact but that there is a decrease in the ligation of the IGF-I receptor with age. This interpretation appears to be supported by the lack of a significant increase in tyrosine phosphorylation of the IGFR1/IR in the old MG muscles following resistance

exercise (Fig. 11A). Considering the role of Akt, this age-related dampening of exercise-induced Akt activation certainly has the potential to negatively impact nascent anabolic processes within skeletal muscle.

S6K1. The activating phosphorylation of S6K1 was similar in both young and old MG muscles (Fig. 11B). Following the acute resistance exercise, S6K1 phosphorylation was increased to a similar absolute level in both young and old muscles at 24 h. However, at the 48-h time point, the response between the old and young muscles diverged with S6K1 phosphoryla-

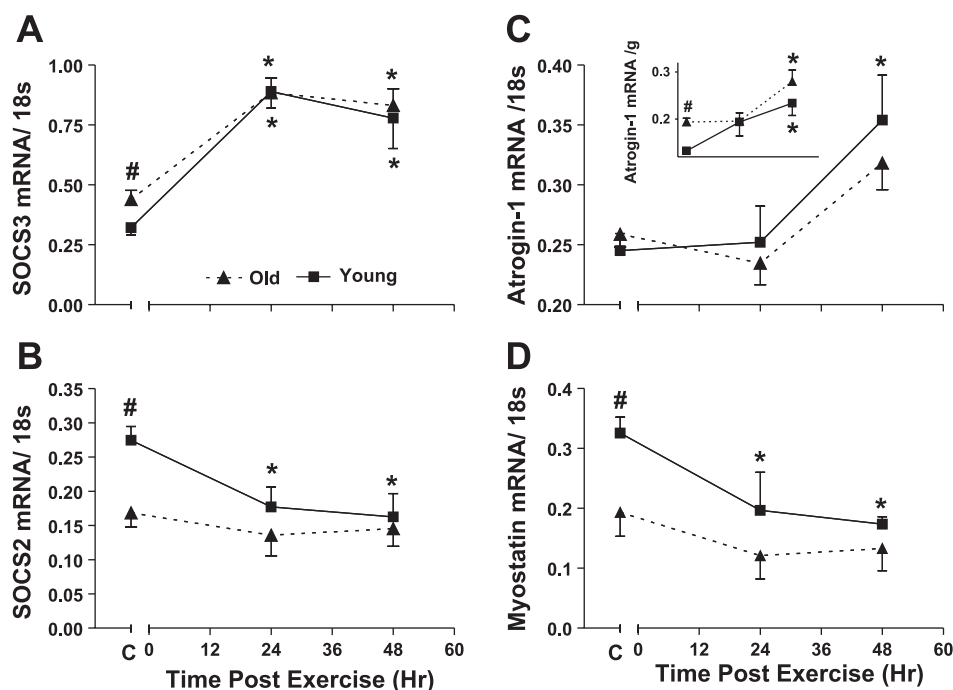


Fig. 9. Acute resistance exercise induced changes in muscle mRNA. A: following resistance exercise, the mRNA for SOCS3 increased significantly and in a similar fashion in the MG muscles of both young and old rats. B: resistance exercise induced a decrease in SOCS2 mRNA in the young but not old muscles. C: relative to 18S, the amount of atrogin-1 mRNA increased in the muscles of young but not old rats at 48 h after resistance exercise. Inset: changes in atrogin-1 mRNA expressed per gram of muscle, indicating that the concentration of this mRNA increased in both the young and old muscles postexercise. D: resistance exercise resulted in a significant decrease in myostatin mRNA in the MG muscles of young but not old rats. * $P < 0.05$, resistance exercise vs. contralateral. # $P < 0.05$, old vs. young.

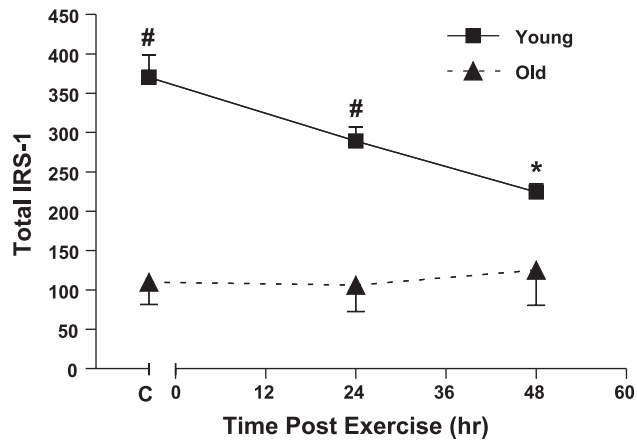


Fig. 10. Acute resistance exercise induced changes in IRS-1 protein. The total amount of IRS-1 protein was significantly decreased in the MG muscles at 48 h after resistance exercise. * $P < 0.05$, resistance exercise vs. contralateral. # $P < 0.05$, old vs. young.

tion remaining elevated in the old muscles (Fig. 11B). The patterns of the S6K1 response seen in both the young and old rat muscles appear to parallel the changes in total RNA (Figs. 7 vs. 11), and these two processes were significantly correlated (e.g., phos-S6K1 vs. RNA concentration; $r = 0.51$, $P = 0.0001$). S6K1 phosphorylation of the ribosomal S6 protein is thought to favor an increase in the translation of ribosomal proteins (71), a process that would be expected to parallel increased transcription of ribosomal RNA.

Based strictly on statistical significance, there appeared to be a divergence between the Akt and S6K1 phosphorylation responses seen in the young vs. old muscles in this study. However, it should be noted that, in terms of potential biological significance, the mean Akt phosphorylation was 2.4-fold greater (not significant) than control in the old rats, whereas in the young rats the statistically significant change (with much less variability) was just 83% at 24 h (Fig. 11C). This contrasts

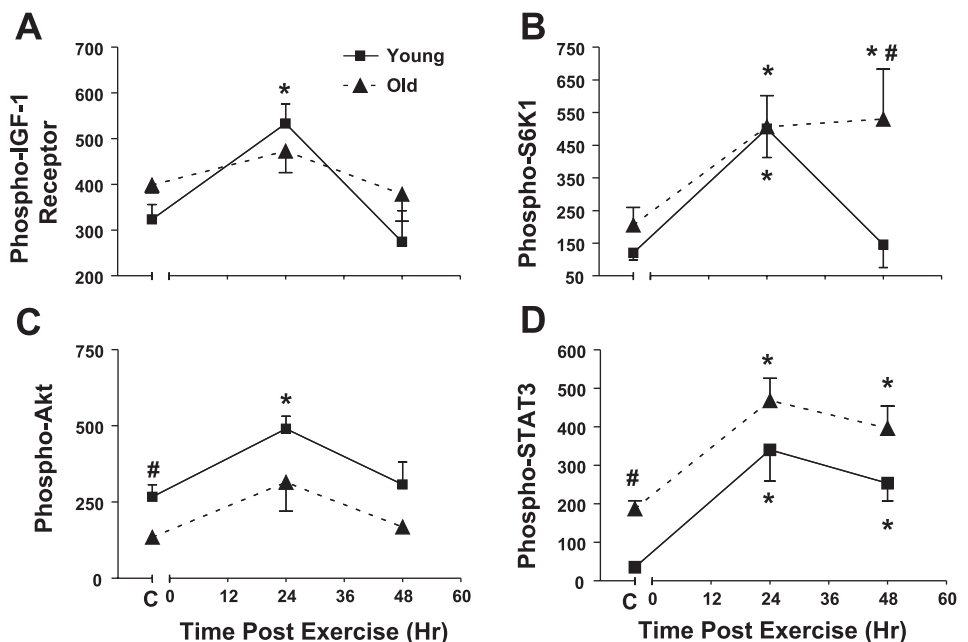
markedly with the magnitude of the changes seen in S6K1 phosphorylation, which were 2.5- and 4-fold for the old and young muscles, respectively. This suggests that the apparent divergence in phosphorylation responses between Akt and S6K1 may be a function of insufficient statistical power due to variability as opposed to a biologically significant mechanism.

Perhaps of more interest was the prolonged increase in S6K1 phosphorylation seen in the old MG muscles (Fig. 11B). In this instance, it is tempting to speculate that the prolonged increase in S6K1 phosphorylation seen in the muscles of the old rats may be related to an attempt at compensation for an overall ineffectual anabolic response. This conclusion is bolstered by the observation that significantly increased phosphorylation of S6K1 per se has been reported to be insufficient to induce increased protein synthesis in skeletal muscle in response to stretch (40). As an alternative interpretation, the prolonged S6K1 phosphorylation (Fig. 11B), and possibly the prolonged RNA response (Fig. 7), observed in the old muscles from the present study may simply be a function of an age-associated alteration in the kinetics of the response to increased loading that would not have been captured by the time points used in the present study.

In general agreement with the present results, Parkington et al. (66) found no age-related differences in S6K1 protein levels in muscles of rats of the same age and strain as in the present study. Similar to the results for the MG muscle in the present study, these authors also reported that the baseline level of S6K1 phosphorylation was the same in young and old PLN muscles. However, S6K1 phosphorylation was reported to be significantly higher in the tibialis anterior muscles of old rats (66).

Following exercise, the levels of S6K1 phosphorylation were similar for both the young and old muscles at 24 h poststimulation. However, the magnitude of the change in S6K1 phosphorylation appeared to be somewhat blunted in the muscles of the older rats (4-fold vs. ~2.5-fold at 24 h) (Fig. 11B). This result is in basic agreement with those of Parkington

Fig. 11. Acute resistance exercise induced changes in the phosphorylation of intracellular signaling intermediates. A: in response to resistance exercise, the phosphorylation of IGFR1 was significantly increased in the MG muscles of young but not old rats. B: phosphorylation of S6K1 at threonine 389 (T389) is closely correlated with the activity of this enzyme and was significantly increased following resistance exercise in both young and old MG muscles. C: phosphorylation of Akt was significantly increased only in the muscles from young rats and only at 24 h after resistance exercise. Phosphorylation of STAT3 increased in a similar manner in both the young and old muscles. * $P < 0.05$, resistance exercise vs. contralateral. # $P < 0.05$, old vs. young.



et al. (66, 67), who found that the S6K1 response to exercise was blunted in the old vs. young muscles. In a series of studies, these authors used an exercise model involving electrical stimulation to produce force development via the contraction of antagonistic muscles (e.g., no external loading) (66, 67). These authors found that S6K1 phosphorylation was elevated in tibialis anterior muscles of old rats at 0 and 6 h after stimulation (1.2- and 0.6-fold, respectively) and at 6 h (2.4-fold increase) in young muscles. In the same animals, the PLN muscle of young but not old rats exhibited increased S6K1 phosphorylation at 0 and 6 h poststimulation. The differences in response seen in these two muscles may have been a function of the lengthening (tibialis anterior) vs. shortening actions (PLN) experienced by these muscles using this model.

In contrast to the results of Parkington et al., Hornberger et al. (39) recently reported that mechanically induced S6K1 phosphorylation does not appear to be sensitive to age in mouse skeletal muscle. These authors found that passive stretch results in similar increases in S6K1 phosphorylation in muscles from both young and old mice (39). Taken together with the present results, these findings may indicate that muscle activation may have a different effect on this parameter compared with passive changes in muscle length (39, 66, 67).

In the present study, the exercise-induced increase in S6K1 phosphorylation was prolonged in the old MG muscles compared the young muscle response (Fig. 11B). In contrast, Parkington et al. (66, 67) found that the reverse, i.e., that S6K1 phosphorylation was decreased between 0 and 6 h postexercise in the old muscles but increased over that time period in the young muscles. It is most likely that the points of divergence in the present results vs. those reported by Parkington et al. are a function of the different exercise models (e.g., high-force isometric vs. concentric or eccentric contractions with no external load).

Cyclin D1 and DNA. In response to increased muscle loading, there may be a recruitment of muscle stem cells (myogenic precursor cells, satellite cells) and/or other cell types to support the hypertrophy process. Studies have indicated that aging does not appear to depress the inherent ability of satellite cells to activate in response to various perturbations (17, 19). In this relatively short-term study, we did not employ methods to directly detect cell proliferation. However, as a potential indicator of proliferative activity, we measured the mRNA levels of cyclin D1, the key regulatory mediators of cyclin-dependent kinase activity and therefore the cell cycle (77). In both the young and old muscles cyclin D1 mRNA levels increased following acute resistance exercise (Fig. 8C). However, there was a significant delay in the cyclin D1 response of the older muscles such that the increase did not attain significance until 48 h postexercise. This observation appears to be in accord with *in vitro* studies demonstrating a lag in the proliferative responses of satellite cells from aged muscles (75). As in previous studies (e.g., Ref. 33), the pattern of changes seen in cyclin D1 mRNA were very similar to those observed for IGF-I mRNA (Fig. 8, A and C), such that there was a very powerful correlation between these two variables (Fig. 12).

In the present study, we found that the concentration of DNA was higher in the muscles of old rats. This finding is similar to that of Leeuwenburgh et al. (48), who reported that the nuclear domain size is significantly smaller in the muscles of old F/BN rats. The mechanisms and causes of a decreased

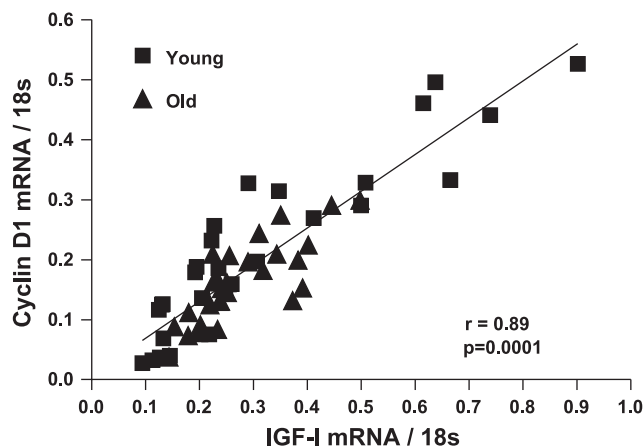


Fig. 12. Relationship between levels of IGF-I and cyclin D1 mRNA. There was a significant positive correlation between muscle levels of IGF-I and cyclin D1 mRNA.

myonuclear domain size with aging have not been clearly elucidated. In the context of the present study, the lag seen in nascent proliferative activity (i.e., increase in cyclin D1) could be the result of a lesser stimulus due to the ability of myofibers with more nuclei to hypertrophy without the need to incorporate additional nuclei from an external source. Alternatively, the smaller domain size/higher DNA concentration seen in old muscles may indicate that some myonuclei have a reduced capacity, requiring a greater density for myofiber maintenance. If this were the case, then the lag in cyclin D1 in the present study and the proliferative lag reported by Schultz and Lipton (75) most likely represent a refractory response possibly due to alterations in signaling.

Myogenin. Myogenin is a muscle-specific transcription factor that is a member of the myogenic regulatory factor family. The mRNA level for myogenin was fourfold higher in the MG muscles of the old rats (Fig. 1B). This result is in basic agreement with that of Edstrom and Ulfhake (23), who previously reported that myogenin mRNA levels are higher in old vs. young rat Sol muscle. It is interesting to note that the muscles of spinal cord injury subjects do not exhibit a significantly higher level of myogenin transcripts, suggesting that inactivity per se does not appear to account for this high level seen in the old muscles (12).

We have previously reported that, in the muscles of both humans and rats, one of the earliest responses to an acute bout of resistance exercise is an increase in myogenin mRNA levels (11, 33). We have also observed that the myogenin response to exercise is intact in the muscles of spinal cord injury subjects (12). In the present study, myogenin mRNA increased following resistance exercise in the muscles of the young rats but did not change significantly in the old muscles (Fig. 8D).

SOCS3 and STAT3. Relative to the young MG muscles, the levels of SOCS3 mRNA were significantly higher in the old rat MG muscles (Fig. 1). However, exercise resulted in a similar accumulation of SOCS3 mRNA in the MG muscles of both the young and old rats (Fig. 9A). SOCS3 is thought to function primarily as a negative feedback agent in response to receptor signaling via the Jak/STAT pathway (16). Both IR and IGF1R have been reported to be sensitive to negative regulation by SOCS3 (59). For example, Ueki et al. (83) recently reported

that SOCS3 acts as a negative regulator of insulin signaling in adipocytes and myoblasts. In addition, the negative impact of IL-6 overexpression on growth appears to be mediated via an increase in the expression of SOCS3 (52). In the present study, we found that there was a significant, negative correlation between SOCS3 mRNA levels and the tyrosine phosphorylation of IRS-1 ($r = -0.52$, $P = 0.002$). Interestingly, there was also a significant correlation between the mRNA levels of SOCS3 and the amount of IRS-1 protein present in MG muscles ($r = -0.50$, $P = 0.047$). This suggests that upregulation of SOCS3 may participate in negative feedback impacting IRS-1 in response to resistance exercise.

The phosphorylation of STAT proteins allows for dimerization and translocation to the nucleus where they function as transcription factors (16). For example, in some tissues, STAT3 is known to target genes that regulate apoptosis, cell proliferation, and angiogenesis (7). In the present study, both the total amount of STAT3 protein and the phosphorylation of STAT3 was significantly higher in the muscles of the old rats (Fig. 6). In possible agreement with the present results on the response of STAT3 to acute exercise (Fig. 11D), Morris et al. (60) found that the phosphorylation of STAT3 was increased after 6 days of reloading in the gastrocnemius of F/BN rats.

STAT3 activity is often associated with increased SOCS3 expression (16). As noted above, SOCS3 mRNA levels were also higher in old muscles (Fig. 1). Following resistance exercise, there were parallel increases in STAT3 phosphorylation in the muscles of the young and old rats (Fig. 11D). These exercise-induced changes seen in STAT3 phosphorylation appeared to follow a pattern similar to those observed for SOCS3 mRNA (Figs. 9A and 11D) such that there was a significant correlation between these two variables ($r = 0.54$, $P = 0.0001$). This similarity in the pattern of response suggests that increased signaling via STAT3 may have mediated the change seen in SOCS3 mRNA, possibly leading to a decrease in the signaling flux through IRS-1 (PhosSTAT3 vs. PhosIRS-1, $r = -0.45$, $P = 0.009$).

SOCS2 and myostatin. Both myostatin and SOCS2 have been definitively shown to negatively regulate muscle size. SOCS2 knockout mice exhibit remarkable increases in body and muscle size as a result of a decrease in inhibition of growth hormone (GH) signaling (28, 29). Similarly, lack of myostatin expression results in large increases in body and muscle mass during development (47, 57). Myostatin also continues to regulate muscle mass in adult animals and humans (32, 54, 94).

The amount of SOCS2 and myostatin mRNA present in the muscles of the old rats was significantly lower than that seen in the younger muscles (Fig. 1, C and D). This is an interesting result in that one might expect the sarcopenic MG muscles to have higher levels of the message for these negative regulators.

In human muscles, myostatin mRNA levels have been reported to be unchanged with age (55, 85). In agreement with the present study, Baumann et al. (10) reported that myostatin mRNA expression decreased with age in the gastrocnemius muscle of rats. However, these authors found that immunoblot analyses indicated a progressive increase in myostatin protein expression with age. In contrast to the results of Baumann et al., Kawada et al. (45) did not find any increase in myostatin protein with age in the gastrocnemius muscles of mice. However, Kawada et al. reported just a single electrophoretic band for myostatin, whereas Baumann et al. (10) reported that two

separate myostatin bands were detected. If there is in fact an age-related decrease in myostatin mRNA, it may simply be a function of feedback from elevated myostatin protein levels. Unfortunately, myostatin protein was not measured in the present study, and thus our data are not useful in resolving this question.

SOCS2 regulation appears to be accomplished via the modulation of GH signaling (24). Similarly, there are reports that myostatin may also interact with the GH axis (54, 55). In the absence of information on protein levels, the age-associated differences in SOCS2 and myostatin mRNA levels seen in the present study may be interpreted as an indication of some level of GH resistance since the message for these negative regulators is low at the same time that muscle size is rather dramatically depressed.

Turning to the exercise response, the muscles of the young rats demonstrated significant decreases in both myostatin and SOCS2 mRNA in response to the acute exercise bouts (Fig. 8, B and D). In light of the negative relationship between these mediators and muscle mass, this response would be appropriate for muscles preparing to enter a hypertrophic phase to compensate for sustained increases in loading. In contrast to the response of the young muscles, the MG muscles of the old rats did not experience any change in the mRNA levels of myostatin and SOCS2. This could indicate that the mechanisms that regulate SOCS2 and myostatin mRNA levels exhibit an age-related reduction in responsiveness with regard to either magnitude or kinetics. Alternatively, the amount of mRNA expression seen in the old muscles may represent some baseline level. Subjectively, the mRNA levels seen in the young muscles following exercise appeared to assume an asymptotic approach to those seen in the muscles from old rats (Fig. 9, B and D), possibly supporting this latter supposition.

Atrogin-1. Atrogin-1 has been identified as a muscle-specific E3 ligase that appears to be generally upregulated in association with states that induce muscle atrophy (27). In humans, aging is not associated with an increase in the mRNAs for the muscle-specific E3 ubiquitin ligases atrogin-1 and muscle ring finger 1 (86, 90). Similarly, in the present study, the levels of atrogin-1 mRNA relative to 18S ribosomal RNA were essentially the same in the muscles of both young and old rats. This would suggest that upregulation of atrogin-1 mRNA does not appear to be mediating sarcopenia.

The expression of muscle E3 ligases, such as atrogin-1 and muscle ring finger 1, has been shown to be negatively regulated by IGF-I signaling via the Akt-mediated phosphorylation of FOXO transcription factors (73, 78). As noted above, the absolute and relative amount of Akt phosphorylation was significantly lower in the muscles of old rats (Fig. 5). This suggests that the accumulation of atrogin-1 mRNA is not closely linked to Akt phosphorylation. This disconnection becomes even more evident when atrogin-1 mRNA levels are expressed as a function of the muscle size. In general, the results of mRNA analyses are expressed relative to some measure of the total amount of RNA present to ensure that any changes seen are not simply a function of a bulk increase in the total RNA pool. However, in the present case, the total RNA pool in the old muscles was significantly greater than that in the young muscles (Fig. 7). Therefore, it may be instructive to take this into account when assessing the amount of atrogin-1 mRNA present. Figure 9C, *inset*, presents the atrogin-1 mRNA

data in units per gram of muscle. In contrast to the main plot, this presentation shows that the total amount of atrogin-1 mRNA present in the old muscles was actually significantly greater than that in the young muscles before the resistance exercise. This apparent disassociation between Akt phosphorylation and atrogin-1 mRNA suggests that other processes such as alterations in the stability and degradation rate of the atrogin-1 mRNA may be modulating the amount of atrogin-1 mRNA and that elevated atrogin-1 may be important in sarcopenia.

Potential Study Weaknesses

Although we have described a number of our findings as being age related, it is instructive to note that the most dramatic effects were seen in the MG muscle. In rats, the MG is generally thought to be recruited primarily for locomotor and possibly high force activities such as jumping, whereas the Sol muscle is highly active during postural maintenance (e.g., Ref. 6). Hepple et al. (38) recently reported that normal activity levels of old F/BN rats are 34% lower than those of 8-mo-old adults. This suggests that some portion of the sarcopenia seen in old F/BN rat muscles, and particularly in the MG, is most likely due to reduced muscle loading rather than some age-specific alteration in physiology. However, the resistance exercise imposed in the present study represented maximal activation of the target muscles regardless of age. As a result, differential responses seen at the cellular and molecular levels should be attributable to the differences in age.

In conclusion, the cellular and molecular mechanisms that are deranged leading to sarcopenia have yet to be definitively identified. In the present study, there are results that suggest that one of the contributing factors may be a reduction in the efficiency of protein translation. There appear to be a number of indicators of potential compensatory responses by the old muscles to this defect. These may include an upregulation in components of the intrinsic IGF-I system that include increased type 1 IGFR1 protein levels and elevated IGF-I mRNA. Similarly, the amount of Akt protein and the mRNA for myogenin are also increased in old muscles. However, these potentially positive parameters are apparently offset by deficits in their employment. For example, the activating phosphorylation of the IGFR1/IR, IRS-1, and Akt was lower in the old muscles, and, unlike the case in the young muscles, these activating events failed to increase in response to resistance exercise.

With regard to the possible mechanisms of sarcopenia, correlative results from the present study suggest that STAT3, acting via SOCS3, may negatively affect the highly anabolic IRS/PI3 kinase signaling cascade mediating a reduction in the amount of IRS-1 protein available and interfering with the activating phosphorylation of this protein.

In addition to the STAT3/SOCS3 data, the failure of the old muscles to downregulate the message for both myostatin and SOCS2 in response to resistance exercise suggests that the regulation of these powerful mediators of muscle size is clearly altered in old skeletal muscles.

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