Atrophy responses to muscle inactivity. I. Cellular markers of protein deficits

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Haddad, F., R. R. Roy, H. Zhong, V. R. Edgerton, and K. M. Baldwin. Atrophy responses to muscle inactivity. I. Cellular markers of protein deficits. J Appl Physiol 95: 781–790, 2003. First published April 25, 2003; 10.1152/japplphysiol.00317.2003.—The goal of this study was to use the model of spinal cord isolation (SI), which blocks nearly all neuromuscular activity while leaving the motoneuron muscle-fiber connections intact, to characterize the cellular processes linked to marked muscle atrophy. Rats randomly assigned to normal control and SI groups were studied at 0, 2, 4, 8, and 15 days after SI surgery. The slow soleus muscle atrophied by ~50%, with the greatest degree of loss occurring during the first 8 days. Throughout the SI duration, muscle protein concentration was maintained at the control level, whereas myofibrillar protein concentration steadily decreased between 4 and 15 days of SI, and this was associated with a 50% decrease in myosin heavy chain (MHC) normalized to total protein. Actin relative to the total protein was maintained at the control level. Marked reductions occurred in total RNA and DNA content and in total MHC and actin mRNA expressed relative to 18S ribosomal RNA. These findings suggest that two key factors contributing to the muscle atrophy in the SI model are 1) a reduction in ribosomal RNA that is consistent with a reduction in protein translational capacity, and 2) insufficient mRNA substrate for translating key sarcomeric proteins comprising the myofibril fraction, such as MHC and actin. In addition, the marked selective depletion of MHC protein in the muscles of SI rats suggests that this protein is more vulnerable to inactivity than actin protein. This selective MHC loss could be a major contributor for the previously reported loss in the functional integrity of SI muscles. Collectively, these data are consistent with the involvement of pretranslational and translational processes in muscle atrophy due to SI.

myosin heavy chain messenger ribonucleic acid; actin messenger ribonucleic acid; poly(A) messenger ribonucleic acid; ribonucleic acid; deoxyribonucleic acid; protein translation

FOR THE LAST 30 YEARS, there has been a keen interest in the role of weight-bearing and neuromuscular activity in regulating the structural, functional, biochemical, and molecular properties of skeletal muscle (for reviews, see Refs. 9, 26, 31, 33). At least four primary models have been used extensively to manipulate the neuromuscular system. These include the following: 1) limb immobilization in which the target muscles are immobilized in a fixed position by either casting or pinning the joints, resulting in the muscle being maintained in either a neutral, shortened, or lengthened position (11); 2) spaceflight/hindlimb unloading, which eliminates ground reaction (weight-bearing) forces while enabling the muscles to remain functionally active (8, 24, 31); 3) denervation, which disrupts nerve muscle connections, weight-bearing activity, and any trophic influences of the motoneuron or nerve acting on the muscle; and 4) spinal cord transection, whereby the spinal cord is cut at a strategic level to eliminate higher center control on the spinal neuromotor control centers below the lesion (although the muscles still can be activated by the neural pattern generators located in the spinal cord) (10). The characteristic features of each of these models include a marked atrophy of slow and fast extensor muscles and a transformation of muscle fibers from a slow to a fast phenotype, especially in muscles that intrinsically express a slow phenotype. All of these models share a common feature in minimizing the amount of mechanical loading-induced activity performed by the muscle (32). With the exception of denervation, which interrupts the normal nerve-muscle connections, the other three models maintain varying amounts of nerve-muscle interaction, as demonstrated by chronic electromyogram recordings (2, 3, 15).

Recently, Grossman and coworkers (16) have used a novel approach to render the muscles virtually electrically silent while maintaining a functionally and anatomically intact neuromuscular connectivity. This procedure is referred to as spinal cord isolation (SI) and was initially described by Tower (34). This intervention (SI) creates a unique model that eliminates all weight-bearing and neural activity-dependent influences on the muscles but maintains neural activity-independent influences, such as trophic interactions between the motoneurons and nerves and muscles. Thus the SI model is suitable for establishing a baseline of zero electrical activity and weight bearing for defining the atrophy process.

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Whereas muscles subjected to SI have been characterized in terms of defining the changes in muscle contractile protein phenotypes and general histological, morphological, and functional properties (16, 19, 20, 28, 29), the changes in the basic molecular makeup of the muscle have not been examined. For example, it is not clear how the total muscle protein and myofibrillar concentrations are altered during the rapid atrophy process. Also, the muscle concentrations of total RNA, total DNA, and total messenger RNA pool have not been examined, and these are important parameters impacting homeostatic protein balance of the target muscle. Therefore, the primary goals of the experiments described herein and in a companion paper (18) were to identify the basic molecular events occurring in the rapidly atrophying soleus during the first 15 days after SI intervention. In this paper, we tested two working hypotheses. First, the atrophy induced by SI occurs rapidly due to the inability of the muscle to maintain sufficient ribosomal RNA and sarcomeric protein mRNA levels necessary for translating and maintaining sufficient amounts of muscle protein to counteract the ongoing protein degradation processes. Second, in contrast to previous data from models of unloading-induced atrophy (9, 26, 31, 33), suggesting that protein translational events impact the atrophy process very briefly, we propose that processes impacting protein translation occur throughout the atrophy response, i.e., until the muscle reaches a new steady state of reduced muscle mass. To test these hypotheses, we determined the levels of the myofibrillar protein pools and the total myosin heavy chain (MHC) and actin protein, as well as the corresponding relative and total mRNA expression. In this way, we could track, as a function of time, the respective amounts of mRNA and protein pools for the two major muscle proteins, i.e., actin and myosin, which account for ~40% of total muscle protein. The temporal relationships between specific mRNAs and corresponding proteins enabled us to estimate the role of pretranslational and translational processes in contributing to the protein accretion. We also examined changes in both the concentration and content of DNA, total RNA, total mRNA, and total protein. These variables collectively are thought to be critical in determining muscle protein homeostasis.

Combined, the results of this study indicate that the muscle wasting induced in the SI model occurs to a significant extent from an inability of the skeletal muscles to maintain a normal level of sarcomeric protein accretion. This decreased sarcomeric protein accretion occurs because of two abnormalities: 1) an early and sustained loss of ribosomal RNA that is consistent with a general deficit in the translational capacity and 2) inadequate availability of key sarcomeric gene mRNAs impacting MHC to a greater extent than actin. In a companion paper (18), we demonstrate that the inadequate availability of MHC and actin mRNAs results from decreased transcriptional activity for these two genes.

METHODS

Experimental design. Approximately 70 young adult female rats weighing ~230 ± 5 g were assigned randomly to either a normal control (NC; n = 35) or a SI (n = 35) group. Animals in each experimental group subsequently were assigned randomly into subgroups (n = 7 each) and studied on the day of (time = 0) and 2, 4, 8, and 15 days after SI surgery. The rats in the SI group were anesthetized by using an intraperitoneal injection of ketamine hydrochloride (70 mg/kg body wt) and acepromazine maleate (5 mg/kg body wt) and were subjected to complete spinal cord transections at both a midthoracic and an upper sacral level plus bilateral deafferentation between the two transection sites, as described previously (16, 28). The procedures for the care and maintenance of spinal cord-injured animals have been detailed previously (16, 27). These procedures were approved by the Institutional Animal Care and Use Committee at the University of California-Los Angeles, and they conformed to the animal care procedures and standards recommended by the American Physiological Society.

Tissue processing. At each time point, animals from the appropriate subgroups were weighed, deeply anesthetized with pentobarbital sodium (100 mg/kg), and then decapitated. The soleus muscles (as well as other limb muscles not reported in this study) were removed, quickly dissected free of connective tissue, quick frozen on dry ice, and subsequently stored at −80°C until used for biochemical and molecular analyses. Analyses for all subgroups involved n = 6 or 7, as presented in the data profiles.

Biochemical and molecular analyses. A preweighed portion of each muscle sample was homogenized in 20 volumes of a homogenization buffer, which contained 250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 10 mM Tris base. Myofibrillar proteins were quantitatively extracted from a known volume of the total homogenate by a modification of the original procedure described by Solara et al. (30) and were suspended into a known volume of 100 mM KCl, 10 mM Tris, and 1 mM EDTA, pH 7.4.

Muscle total DNA concentration calculation was based on total DNA concentration in the total homogenate, as was determined by using a fluorometric assay (21). Muscle total protein and myofibrillar protein concentration was based on protein concentration of the muscle whole homogenate and myofibrillar suspension, respectively, as determined by the Bio-Rad protein assay. The whole homogenate was diluted to a final protein concentration of 1 mg/ml in a storage buffer containing 50% glycerol, 100 mM Na4P2O7, 5 mM EDTA, and 2 mM 2-mercaptoethanol (pH 8.8) and stored at −20°C until subsequent analyses for MHC and actin protein content.

MHC and actin protein analysis. Skeletal muscle MHC and actin proteins were separated on acrylamide gels (10% T, 2.5% C) by using a standard SDS-PAGE technique (22). Protein samples were denatured by placing 10 μg of sample in 30 μl of sample buffer (5% β-mercaptoethanol, 100 mM Tris base, 5% glycerol, 4% SDS, and 0.05% bromophenol blue, pH 6.8) and then heating the solution for 2 min at 100°C. Ten microliters of the denatured protein solution (equivalent to 2.5 μg of total muscle protein) were loaded per lane, and the gels were run at constant current (30 mA) for ~2.5 h at 23°C. In each gel run, 0.5 μg of purified myosin and 0.5 μg of purified α-skeletal actin (Sigma Chemical) were also denatured and processed on the same gel to serve as a reference for the migration level of MHC and actin bands in the total protein samples. The gels were stained with brilliant blue G 250 (Sigma Chemical), destained, and then
scanned by using a Molecular Dynamics (Sunnyvale, CA) laser scanning personal densitometer (see Fig. 1A) for an illustration of a sample gel). The MHC and actin bands were identified on the digitized image, and their intensity was calculated via volume integration of density within a rectangle containing the entire band with local background correction. We have validated this method by loading different amounts of total proteins (0.5- to 4-µg range) on the gel (Fig. 1A); and MHC and actin band intensities were directly proportional to the amount of protein loaded, whereas the actin-to-MHC ratio remained constant over the entire range (Fig. 1B). With the use of this method, MHC and actin proteins were expressed as arbitrary units (AU) per microgram of total protein, and the amount of MHC and actin protein content was calculated based on muscle total protein content.

Total RNA isolation. Total RNA was extracted from preweighed frozen muscle samples by using the TRI reagent (Molecular Research Center, Cincinnati, OH), according to the company’s protocol, which is based on the method described by Chomczynski (13). Extracted RNA was precipitated from the aqueous phase with isopropanol and, after being washed with ethanol, was dried and suspended in a known volume of nuclease-free water. The RNA concentration was determined by optical density at 260 nm (using an optical density 260-unit equivalent to 40 µg/ml). The muscle total RNA concentration was calculated based on total RNA yield and the weight of the analyzed sample. The RNA samples were stored frozen at −80°C and were subsequently in determining total mRNA [poly(A)], total MHC mRNA, and α-skeletal actin mRNA expression by using slot-blotting procedures.

RNA slot blotting. One microgram of total RNA was placed in 20 µl of denaturing buffer (10% formaldehyde, 67% formamide, and 0.5 × MOPS, pH 7) at 60°C for 15 min. Samples were brought up to 100 µl volume with 6 × SSC and were applied onto a positively charged nylon membrane (GeneScreen plus, NEN) by using a slot-blot apparatus (Schleisher and Schuell). After UV fixation, these membranes were hybridized with three different probes consecutively as follows. 1) An antisense α-skeletal actin mRNA probe was used to determine α-skeletal actin mRNA expression, or a common antisense MHC mRNA probe was used to determine the total MHC mRNA expression. The MHC probe is complementary to the coding region ∼500 nucleotides upstream from the stop codon of type I MHC mRNA. This region is 100% identical in all of the MHC isoforms, and the obtained signal corresponds to the total population of MHC mRNA expressed in the muscle. 2) An oligo(dT) probe (12- to 18-mer, Life Technology) was used to detect poly(A) RNA (total mRNA population). 3) An antisense 18S ribosomal RNA probe, whose signal is directly proportional to the amount of total RNA, was used to normalize for possible variability in the amount of loaded RNA per slot. Probes were 5’ end labeled with P32 by using γ-ATP and T4 polynucleotide kinase. Hybridization and washing procedures were carried out, as described previously (17). Hybridization signals were detected and analyzed by using a PhosphorImager and Image Quant analysis software (Molecular Dynamics). For each sample, the MHC mRNA, actin mRNA, and dT [poly(A)] signals were normalized to the corresponding 18S signal. The slot-blot hybridization signal for these probes was strongly correlated with the amount of loaded total RNA, ranging from 0.25 to 2 µg/slot. The sequence of oligonucleotides probes used for hybridization is as reported in Adams et al. (1). Total MHC mRNA, total α-skeletal actin mRNA, and total mRNA were expressed either as relative to 18S or as content per muscle in AU. This latter value is obtained based on total RNA content and the specific signal generated relative to corresponding 18S per 1 µg total RNA.

Statistical analyses. All values are reported as means ± SE. For each time point, treatment effects were determined by ANOVA with Student-Newman-Keuls post hoc testing by using the Prism software package (Graphpad). For certain

Fig. 1. Validation of the myosin heavy chain (MHC) and actin protein quantification. A: separating gel after Coomassie blue staining depicting MHC and actin bands when total muscle protein was loaded in increased amounts from 0.5 to 4 µg. S is the standard, which consists of a mixture of purified myosin and actin (0.5 µg each), and is used as a reference to identify these proteins in the total homogenate. Each band density was determined by integrating the volume of pixel values within the rectangle containing the entire band, corrected to local background (see rectangle position in the 2.5-µg lane). B: relationships between the density of either MHC or actin bands vs. the amount of loaded protein. Lines were generated by linear regression analysis. Each data point is the average of 4 separate samples of the plantaris muscle from control rats. The correlations for both actin and MHC were significant (r = 0.99, P < 0.05), and the slopes of the regression lines were significantly different from zero, with the slope of the MHC line being ∼2.5 times that of the actin line. Despite the difference in the slope of these 2 lines, the actin-to-MHC ratio (actin/MHC; %) was maintained at ∼40% (average of 41 ± 2%) over the entire range. Regression analysis determined that this ratio line was significantly linear but that the slope was not different from zero, i.e., it remained constant with different amounts of loaded protein. AU, arbitrary unit.
variables, to assess the time course response to SI, regression analysis (Graphpad, Prism) was performed, whereby $x$ was the SI duration and $y$ the specific variable analyzed. These data were analyzed in terms of the linearity of the curve, the slope of the line, and whether there was a difference between NC and SI for these parameters. For all statistical tests, the 0.05 level of confidence was accepted for statistical significance.

RESULTS

Body and absolute and relative soleus muscle weights. The mean body weight of the NC group demonstrated a typical increase over the 15-day experimental period, whereas there were small reductions in body weight of the SI group during the first 8 days after SI surgery. This response was followed by weight gains equivalent to that seen for the NC group (Fig. 2A). The SI rats were significantly smaller than NC rats at all time points, except after 2 days of SI. Absolute soleus muscle wet weight progressively decreased to 43% relative to NC groups during the 15-day experimental period, with the greatest loss occurring during the first 8 days after SI (Fig. 2B). Normalizing the data to body weight reflected the same pattern, i.e., the soleus muscle was significantly smaller in the SI than NC groups after 4, 8, and 15 days of SI (Fig. 2C).

Soleus muscle total protein and myofibrillar protein concentration and content. To analyze the temporal changes in total protein and myofibril concentration, regression lines were generated between the 4- and 15-day time points. The slopes of the regression lines for protein concentration for both the NC and SI groups were not different from zero (NC slope, $1.6 \pm 0.8$; SI slope, $1.6 \pm 0.8$) (Fig. 3A), indicating no significant change in protein concentration during the 15-day experimental period. In contrast, the regression lines for myofibril concentration were significantly different between NC and SI groups after 4, 8, and 15 days of SI (Fig. 3B).
between the two groups (Fig. 3B). The slope of the regression line for the SI group was $-5.9 \pm 0.8$ and was significantly different from zero, whereas the slope of the regression line for the NC group was not significantly different from zero. These data demonstrate that the 4- to 15-day interval after SI is associated with a gradual and selective loss of myofibrillar proteins. When the protein data were expressed as the total content per muscle, i.e., mg/g x g/muscle (wt) = mg protein/muscle, there was a progressive decrease in the protein content of the SI relative to the NC group that closely mimicked the pattern of muscle weight loss (compare Fig. 3C with Fig. 2, B and C). These data indicate that the loss in muscle mass resulted from a proportional loss of water and total protein in the muscle. Total protein loss in the soleus muscle of SI rats averaged $60 \pm 5\%$ relative to NC at the 15-day time point. When the myofibril data were expressed as the content per muscle, there was a progressive loss in myofibril content in the SI group throughout the experimental period (Fig. 3D). This myofibril loss averaged $65 \pm 5\%$ in the SI relative to the NC group at 15 days.

**Total MHC and actin protein concentration and content.** Total MHC protein and actin fractions were separated by gel electrophoresis (see METHODS). Densitometric analysis of the MHC and actin bands showed that the SI muscles were associated with a significant decrease in the MHC protein fraction relative to total protein at the 8- and 15-day time points (Fig. 4A). In contrast, the actin portion relative to total protein was maintained constant throughout the experimental period (Fig. 4B). These changes resulted in a significant increase in the actin-to-MHC ratio at the longer time point.

![Fig. 4. Soleus muscle total MHC and α-skeletal (Sk) actin protein expression as determined by denaturing gel electrophoresis and laser scanning densitometry. Total MHC (A) and α-Sk actin (B) protein were corrected to 1 µg of total muscle protein and expressed in arbitrary scan units (AU). C: % ratio of actin to MHC for both the NC and SI groups. D: representative SDS gel used for these analyses. C: NC group; S: SI group. E and F: total MHC and actin protein content per muscle, respectively. Values are means ± SE; n = 6 or 7/group for each time point. \*P < 0.05 vs. NC.](image)
points (Fig. 4C), a parameter that is relatively constant over a wide range of the amount of protein loaded in the assay system (Fig. 1B). Both total MHC and actin protein content were significantly depleted in the SI relative to the NC muscles when expressed on a per-muscle basis at the 4-day time point and thereafter (Figs. 4, E and F). MHC protein isoform composition was examined by using methods described previously (32) and confirmed previous findings (19), i.e., the atrophied muscles of SI rats are transformed to a faster phenotype (data not shown). For example, after 15 days of SI, the percent type I MHC protein composition relative to the total MHC pool had decreased by 15%, and the muscle content was ~80% lower in SI relative to NC muscles. In contrast, the percent type IIx MHC protein composition of the soleus muscle increased from 1–2% in NC to ~18% (P < 0.05) in SI rats. When corrected to whole muscle, the type IIx MHC content increased from 47 ± 19 to 104 ± 30 AU (P > 0.05). These data suggest that, whereas there was a net loss in the total MHC protein pool, this loss was selective to slow MHC isoforms (types I and IIa), whereas other factors operating at the transcriptional and pretranslational levels most likely impacted the enhanced expression of the type IIx MHC isoform, which becomes the major MHC isoform in the soleus muscle after 90 days of SI (19).

Actin and total MHC mRNA. The mRNA relative to 18S of both total MHC (Fig. 5A) and actin (Fig. 5B) mRNA markedly decreased during the first 8 days after SI. The total MHC mRNA normalized to 18S returned to the control level between day 8 and day 15. The total MHC and actin mRNA expressed relative to the total RNA per muscle were significantly decreased after 4 days of SI and thereafter (Fig. 5, C and D). Thus there was a critical loss of these two specific sarcomeric mRNAs that could have limited their availability for protein translation in the inactive muscles. The fact that both total MHC mRNA and actin mRNA decreased to a similar extent (Fig. 5, A vs. B), whereas only total MHC protein was decreased relative to total muscle protein (Fig. 4, A vs. B), may indicate that, during the SI-induced atrophy process, the MHC and actin are lost at different rates. The MHC loss is either faster or greater than that of the actin, and this could be the result of the former possessing a higher turnover rate than the latter. Alternatively, these findings could indicate that the SI induces some degradation enzymes (for example, a specific ubiquitin E3 ligase selective for slow MHC) that more specifically target the MHC component of the sarcomeric protein for degradation.

RNA, mRNA, and DNA concentration and content. In view of the rapid muscle atrophy in response to SI, we hypothesized that translational processes were likely contributing to the severe loss in both total and myofibrillar proteins. We examined the total cellular RNA pool, total mRNA population, and total DNA in NC and SI muscles as general markers for the status of translational processes. Total RNA concentration in the SI group rapidly decreased relative to the NC group during the early time points (from day 2 to day 4, P < 0.05 at day 4) and then gradually returned to control levels (Fig. 6A). However, the RNA content per muscle progressively decreased up to 8 days and then remained stable at ~55% below control levels thereafter (Fig. 6B). Combined, these data suggest that there was a significant reduction in the RNA pool during the timeframe when the muscle was going through its most rapid state of protein loss. Because ~85% of the total RNA pool is ribosomal, it appears that there is a significant loss of the key machinery necessary for protein translation in the soleus muscles of SI rats.

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**Fig. 5.** Soleus muscle total MHC mRNA and α-Sk actin mRNA expression as determined by slot-blot analysis. Total MHC mRNA corrected to the 18S subunit (A) and expressed relative to the total RNA was content per muscle (C). B and D: same analyses, respectively, for actin mRNA as noted for the MHC. Values are means ± SE; n = 6 or 7/group for each time point. *P < 0.05 vs. NC.
These data also indicate that the loss in RNA occurs very early in the atrophy process.

The total mRNA [poly(A)] mRNA levels relative to 18S tended to be slightly higher in the SI relative to NC muscles (P<0.05 at 8 days) (Fig. 6C). This unexpected observation occurred at a time when total MHC and actin mRNA to 18S were being reduced by ~80%. A loss in total mRNA was observed only when the total mRNA was expressed as the content per muscle (Fig. 6D) and simply reflected the muscle atrophy. These observations suggest that the general catabolic state in the SI muscles is not associated with a reduction in the overall availability of the total mRNA pool for translation.

In contrast to total RNA, there was a significant increase in DNA concentration in the SI relative to the NC group after 8 and 15 days of inactivity (Fig. 6E). An increased DNA concentration reflects a greater loss of protein (cytoplasmic mass) than of the number of nuclei, resulting in “cells” becoming smaller in volume and cross-sectional area (16). However, when the DNA was expressed on a per-muscle basis, there actually was a significant net loss of DNA during the atrophy process at all time points, except at 2 days (Fig. 6F). This observation is consistent with the net loss of nuclei in atrophying muscles (4–7).

Responses of markers of cell size and protein synthesis capacity. Based on the morphology of the muscle fibers in the soleus, as observed microscopically on transverse and longitudinal cross sections of the muscle, the majority of whole muscle proteins originates from the muscle-fiber cytoplasm (unpublished observations). Although whole muscle DNA is heterogeneous and may be significantly contaminated by nonmuscle cell DNA, it is largely accepted that whole protein-to-DNA ratio in muscle may be used as a rough measure of the myonuclear domain, which is also directly proportional to fiber cross-sectional area (12, 14). We have used the protein-to-DNA ratio as an index of muscle-fiber size (cross-sectional area). Figure 7A shows a progressive decrease in this ratio in the SI rats, an observation consistent with the reported decrease in the mean cross-sectional area of the fibers in the soleus of SI rats (16). Total RNA is used as a marker of cellular protein translation, because it consists of ~85% ribosomal RNA, which is a major component of the protein translational machinery. The RNA-to-DNA ratio, commonly used to estimate the amount of synthetic material per cell (synthetic capacity), is significantly lower in SI than NC rats at all time points (Fig. 7B). Furthermore, the RNA-to-protein ratio, which provides insight into the synthetic potential of the
exacerbated by the decline in the mRNA of key sarco-
meric proteins (actin and myosin); and 3) marked re-
ductions in both total RNA concentration and content
may have limited the capacity for protein translation.
The rapid atrophy phase in this model of muscle inac-
tivity demonstrates the existence of well-coordinated
sets of molecular events that lead to a smaller, faster,
and weaker muscle (29). Furthermore, it is apparent
that the MHC proteins, in particular the slower iso-
forms, are selected for rapid depletion.

Sarcomeric gene expression, myosin, and actin. My-
osin and actin are two of the most abundant proteins
expressed in skeletal muscle, combining to account for
~60–65% of the myofibril pool, which in turn accounts
for >50% of the total protein pool. Our findings in this
study show that, between 4 and 15 days of SI, the
soleus muscle proteins are progressively depleted of
the myofibril fraction (Fig. 3). The MHC fraction of the
total protein decreased by ~50% at 8 and 15 days
post-SI, whereas the actin fraction did not change
significantly. Interestingly, both MHC and actin
mRNAs relative to 18S ribosomal RNA were lower in
the SI than NC group. This response suggests that the
fate of MHC and actin is different, and they may be
subjected to differential processes at the translational
and/or posttranslational levels.

Furthermore, these findings raise two important
questions. The first question is: does this MHC-actin
imbalance recover to normal levels after longer dura-
tions of SI treatment, e.g., after 30, 60, or 90 days?
After 60 days of SI, the specific tension, i.e., force per
unit cross-sectional area, of the soleus muscle was 33%
lower than that for control (29). This finding suggests
an intrinsic muscle weakness that cannot be accounted
for by the decrease in fiber size alone. Thus we specu-
late that the imbalance of MHC to actin expression
and/or the myofibril deficit relative to total protein
observed at these early time points also may apply to
longer durations of inactivity.

The second question that these data raise is: do the
observed specific losses in myofibril and MHC protein
also occur in other models of muscle atrophy, such as
hindlimb unloading and denervation? In this context,
it has been reported that specific depletion of myosin
relative to actin occurs under conditions of acute quad-
riplegic myopathy, a condition attributed to the admin-
istration of nerve-blocking agents and corticosteroids
(23, 25).

Previous studies using Northern blots have found
varying levels of reduction in actin and β-MHC mRNA
in the hindlimb-unloading model during the early
stages (1–7 days) of soleus muscle atrophy, suggesting
that translational processes must dominate the reduc-
tions in protein synthesis because protein synthesis
reductions appeared to be greater than the reductions
in mRNA for MHC and actin (33). Using slot-blotting
hybridization, as well as the RT-PCR approach to de-
termine the levels of total MHC mRNA, actin mRNA,
and the various MHC mRNA isoforms, we found that
the actin and total MHC mRNA concentrations, nor-
malized to the 18S subunit of the ribosomal system,
were markedly reduced in the SI rats (Fig. 5) and to a

Fig. 7. Relationships between muscle protein, DNA, and RNA. A:
protein-to-DNA ratio as a cellular markers of cell size. B: RNA-to-
DNA ratio as an index of synthetic material per cell. C: RNA-to-
protein ratio as a measure of protein synthetic potential. Values are
means ± SE; n = 6 or 7/group for each time point *P < 0.05 vs. NC.
much greater extent in the muscles from the SI rats compared with that reported for the hindlimb-unloading model. This observation, coupled with the finding that the ribosomal RNA pool was significantly reduced during the onset of the rapid phase of atrophy with SI (Fig. 6A), suggests that pretranslational processes could contribute to the downregulation of these two proteins in the muscles of SI rats. Importantly, in a companion paper (18), we provide evidence that transcriptional activity for both the slow, type I MHC and the actin genes is reduced by ~50% after 7 days of SI. This reduction in transcriptional activity is consistent with the marked reduction in mRNA expression for these two genes. These collective observations provide strong evidence that there is less drive in a variety of key processes that contribute to protein synthesis relative to protein degradation in the SI model. Furthermore, the effects of these processes are specific to the protein in question, i.e., fast MHC, slow MHC, and actin.

Total RNA content. One of the surprising observations in the present study was the rapid loss in the total RNA concentration of the SI muscle. Because the bulk of the RNA in different types of cells is ribosomal, it appears that this key component of protein translation was rapidly downregulated. In the context of this observation, it is interesting that the same phenomenon has been reported in other models of atrophy, such as limb immobilization and hindlimb unloading (33). Furthermore, we have observed that the total RNA is reduced in atrophying human muscle (unilateral limb suspension model; P. A. Tesch, F. Haddad, and K. M. Baldwin, unpublished observations). In contrast, when rodent muscles are functionally overloaded, one of the earliest responses to the overload stimulus is a marked increase in total RNA concentration and content (1). Thus it is becoming increasingly apparent that changes in RNA concentration and content may be key adaptive responses in both atrophic and hypertrophic processes. Clearly, more work is needed on different atrophy and hypertrophy models to determine whether the plasticity of the RNA pool is a common phenomenon for regulating muscle cell mass.

How do the present findings contribute to understanding the process of muscle atrophy? In 1990, Thomson and Booth published a working model of muscle atrophy (33). In that model, it was suggested that protein synthesis rapidly decreases during the first few days of unloading and remains essentially constant until a new atrophied steady state is reached (Fig. 4 in Ref. 33). In contrast, muscle degradation was proposed to be gradually turned on, reach a peak several days after the onset of muscle unloading, and then return to control levels as the muscle mass reaches a new steady state. The findings presented herein, although on a different atrophy model, also suggest that reductions in pretranslational events are likely to be impacting protein synthesis to a much greater extent as the atrophy process initially unfolds (hence protein loss) and reaches its steepest rate of decline (Figs. 2 and 3). This scenario is consistent with the observation that

the decline in the actin and MHC mRNA pools (Fig. 5) occurs very early, and these mRNA levels are maintained at a low level throughout the atrophy response (Fig. 5). Combined, these findings suggest that 1) the pretranslational processes impacting protein synthesis contribute to the net loss in muscle protein well into the atrophy process, i.e., beyond the early stages of unloading; and 2) the protein translational mechanisms may be playing a greater role in muscle loss throughout the atrophy process than what is traditionally thought.

Summary. The slow antigravity rat soleus skeletal muscles atrophied rapidly in response to inactivity induced by SI. The atrophy response was associated with a severe loss in muscle protein that matched the loss in muscle mass. Between 4 and 15 days of SI, we observed a gradual loss in myofibrillar concentration coupled with ~50% reduction in the MHC fraction of the total protein. Muscle atrophy and protein losses were coupled to the regulation of key mRNA substrate levels along with the machinery to translate them. These findings indicate that the rapid reduction in protein and muscle can be attributed to pretranslational, translational, and perhaps posttranslational processes. Furthermore, the present study provides important evidence that those atrophy processes that occur in the absence of weight-bearing activities, such as chronic disuse and spaceflight, are not solely regulated by protein degradation processes. Rather, they are strongly influenced by events that negatively impact on the muscle’s ability to generate protein.

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DISCLOSURES

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