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### Permalink

<https://escholarship.org/uc/item/7647m3h0>

### Journal

Journal of Applied Physiology, 102(1)

### ISSN

8750-7587 1522-1601

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### Publication Date

2007

### DOI

10.1152/jappphysiol.00776.2006

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Peer reviewed

## Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise

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Submitted 13 July 2006; accepted in final form 11 September 2006

**Garma T, Kobayashi C, Haddad F, Adams GR, Bodell PW, Baldwin KM.** Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. *J Appl Physiol* 102: 135–143, 2007. First published September 28, 2006; doi:10.1152/jappphysiol.00776.2006.—The present study was undertaken to test the hypothesis that the contraction mode of action [static-isometric (Iso), shortening-concentric (Con), or lengthening-eccentric (Ecc)] used to stress the muscle provides a differential mechanical stimulus eliciting greater or lesser degrees of anabolic response at the initiation of a resistance training program. We performed an acute resistance training study in which different groups of rodents completed four training sessions in either the Iso, Con, or Ecc mode of contraction under conditions of activation and movement specifically designed to elicit equivalent volumes of force accumulation. The results of this experiment indicate that the three modes of contraction produced nearly identical cell signaling, indicative of an anabolic response involving factors such as increased levels of mRNA for IGF-I, procollagen III  $\alpha$ 1, decreased myostatin mRNA, and increased total RNA concentration. The resulting profiles collectively provide evidence that pure mode of muscle action, in and of itself, does not appear to be a primary variable in determining the efficacy of increased loading paradigms with regard to the initiation of selected muscle anabolic responses.

concentric mode; eccentric mode; hypertrophy; anabolic signaling

IT IS WELL KNOWN that high force loading paradigms targeting key muscle groups can induce hypertrophic responses as manifested in net protein accumulation and increases in fiber size (10). Recent findings suggest that this adaptive response involves, in part, the activation of intracellular signaling pathways involving signaling intermediates such as insulin receptor substrate-1 (IRS-1), Akt (protein kinase B), and mammalian target of rapamycin (19). This pathway appears to converge on processes that create an anabolic response via enhanced protein translational events while restraining catabolism via the down-regulation of key enzymes in the ubiquitination of proteins for degradation (41, 46). We have recently shown that key elements of this pathway are also negatively affected at the onset of muscle atrophy due to hindlimb unloading (24). Observations of the regulatory steps leading to increased protein translation and decreased degradation have enhanced our understanding of key processes that govern protein balance in response to increased loading in skeletal muscle. However, this understanding has not been extensively applied as a scientific basis for selecting specific resistance exercise training parameters such as mode of action [e.g., static-isometric (Iso), shortening-concentric (Con), or lengthening-eccentric (Ecc) or combinations thereof] to elicit an anabolic state.

In general, the current dogma places a great deal of emphasis on the lengthening/Ecc mode of muscle actions for the optimal development of muscle hypertrophy [e.g., Kraemer et al. (29)]. With regard to the effectiveness of lengthening muscle actions during resistance training, the most commonly cited works are those of Hather et al. (27) and Colliander and Tesch (14). However, these studies did not directly compare pure lengthening and shortening actions, nor was isometric training included. For example, Hather et al. (27) reported that training using a combination of shortening and lengthening (Con/Ecc) actions resulted in a significantly greater increase in fiber size compared with paradigms using only the shortening mode training of equivalent volume (Con/Con) (25 vs. 20% increase). In actuality, few studies have made comparisons between pure lengthening, shortening, and isometric training modes in human subjects. In one such study, muscle hypertrophy was similar for all three modes (28).

Much of the confusion regarding the efficacy of the various training modes appears to result from the wide variation in outcome measures, e.g., isometric vs. dynamic strength testing. In general, changes in strength appear to follow the principle of specificity of training, i.e., dynamic training being most efficacious in increasing dynamic strength, and isometric training generally producing the greatest changes in isometric strength (38). However, there is often crossover, for example, with isometric training resulting in similar or superior performance relative to dynamic training (38).

In a recent study focused on muscle atrophy, we found that isometric exercise was ineffective at eliminating the initial muscle atrophy associated with 5 days of unloading (24). We were therefore interested in determining whether, during the initial stages of a resistance training program, there are differences in the responses of skeletal muscles based on the mode of muscle action (e.g., Iso vs. Con vs. Ecc). If this were the case, it might suggest that a particular muscle action could be more effective in preventing atrophy at the critical early time point at the initiation of muscle unloading. We had previously reported that there was little difference between isometric, shortening, and lengthening contraction modes in inducing hypertrophy of the medial gastrocnemius (MG) muscle of rats after 20 days of training (10 training sessions) (2). However, that study did not provide any data on the initial period of muscle adaptation that might be critical for effective countermeasures at the onset of unloading.

In a separate series of studies, we have identified a number of cellular and molecular level changes that are indicative of an

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anabolic response (1, 3, 4) and have demonstrated that these markers are sensitive to acute bouts of resistance exercise in both rodents and humans (8, 9, 22). The present study was designed to use these cellular and molecular level anabolic indicators to evaluate the response of skeletal muscle to increased loading at an early time point before the development of frank muscle hypertrophy but one that could be critical for the prevention of unloading-induced muscle atrophy.

In our previous training study, all variables, other than action mode, were equivalent (2). As a result, the three modes of muscle actions produced widely varying levels of force accumulation (Ecc > Iso > Con). For the purposes of the present study of acute resistance exercise, the Iso, Con, or Ecc mode of contraction was imposed with conditions of activation designed to result in equivalent volumes of force accumulation. Since this was an acute study, the analysis was focused on previously validated cellular and molecular outcome measures indicative of anabolic responses to acute increases in loading. The hypothesis of this study was that a given contraction mode would be more effective than another in initiating processes leading to an anabolic response at an early time point, for example, that an equal volume of Ecc mode contractile activity will result in a greater increase in total muscle RNA.

The primary results of this experiment clearly indicate that the three modes of contraction, which produced equivalent volumes of force accumulation, produced nearly identical cell signaling and adaptive responses. These observations provide further evidence that, in the case of pure muscle actions, the mode of action does not appear to be a primary variable directing the anabolic responses in limb skeletal muscle.

## METHODS

### *Experimental Design and Treatment Protocol*

This study was conducted in conformity with the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings," and the protocol was approved by the University of California, Irvine Institutional Animal Care and Use Committee. Young adult female Sprague-Dawley rats weighing  $263 \pm 5$  g were randomly assigned to four experimental groups ( $n = 9$  each) designated as nontrained control (sham prepared), Iso trained, Con trained, and Ecc trained. Rats were grouped housed in standard vivarium cages on a 12:12-h light-dark cycle and were allowed access to food and water ad libitum. The experiment lasted a total of 5 days with the 1st day of training being performed on designated day 1 followed by three additional training sessions (days 2, 4, and 5), with animals being killed 24 h after the last resistance exercise session.

**Muscle activation and resistance exercise.** For each training bout, the rats were lightly anesthetized with ketamine-xylazine-acepromazine (30/4/1 mg/kg). Stimulation electrodes consisting of 40-gauge insulated nickel chromium wire (Stablohm 800B, CA Fine Wire) were introduced into the subcutaneous region adjacent to the popliteal fossa via 27-gauge hypodermic needles. The needles were then withdrawn, leaving the wire in place. Before electrode insertion a small section of insulation 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. The stimulation wires were then attached to the output poles of a Grass stimulus isolation unit interfaced with a Grass S8 stimulator. This allowed for the delivery of current to the sciatic nerve, resulting (without any direct contact of the nerve) in the induction of muscle contraction. The rats were then positioned in a specially built training platform described previously (11). The left leg was positioned in a foot plate attached to the shaft of a Cambridge model H ergometer. The voltage and stimulation

frequency ( $57 \pm 1$  Hz) were adjusted to produce maximal isometric tension. Previous studies indicated that this approach resulted in reproducible torque production within and between rats over multiple training sessions (2, 22). During each training session sham-prepared rats were anesthetized similarly to the contraction mode groups except that they were not mounted on the training platform.

The stimulation technique used in this study should activate both the anterior and posterior muscles of the leg. This being the case, the antagonistic actions of the anterior compartment might be expected to counter some of the force produced by the posterior muscle groups. In pilot studies we determined that, for isometric, lengthening, and shortening muscle action contractions, tenotomy of the primary antagonistic muscle, the tibialis anterior, resulted in <10% difference in force output measured pre- and posttenotomy (data not shown). This result is similar to that originally reported by Wong and Booth (47).

**Training protocol.** One of the key aims of this study was to compare outcome variables from muscles that had experienced a similar degree of loading regardless of the mode of action. To accomplish this aim, contractile parameters differed between the Iso, Con, and Ecc modes. For the Iso mode exercise, the foot was positioned at an angle of  $\sim 44^\circ$  relative to the tibia, and no change in the foot-plate angle was allowed. Because of the inherent drop in force output as the muscle shortens, the Con mode contractions were limited to  $10^\circ$  relative to the starting position (e.g.,  $44^\circ$  to  $54^\circ$ ), providing for a slower contraction relative to the Ecc group. For the Ecc mode muscle actions, the foot was positioned at  $64^\circ$  relative to the tibia and allowed to move to  $44^\circ$ , the starting point of the Con (and Iso) contractions. All muscles were stimulated for 2 s for each action. The combination of starting with the muscle in a shortened state and a relatively high rate of movement resulted in an Ecc force integral that was similar to that of the Iso and Con groups. For all exercise bouts the stimulation parameters were 2 s of stimulation with 19 s of rest between each contraction, 10 contractions per set. Five sets of contractions were applied with 5 min of recovery between sets. In each case this protocol resulted in <30% fatigue (first set vs. last set) during each training session. Following each training session, the electrodes were withdrawn. 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, control of stimulus triggering, and foot-plate excursion were programmed by using LabTech Notebook (Laboratory Technologies). Data analysis was conducted by using AcqKnowledge software (Biopac Systems). Force output was monitored in real time on the computer screen during each contraction. Rats were trained  $\sim 5$  h after the beginning of their standard light cycle, during each training session.

**Tissue collection.** Twenty-four hours after the last exercise bout (session 4), the rats were killed via an injection of Pentosol euthanasia solution (Med-Pharmex) at a dose of 0.4 ml/kg ( $\sim 160$  mg/kg pentobarbital sodium) ip. At the cessation of heart beat, a skin incision is made, and the MG muscles of both legs were dissected free of connective tissue, weighed, snap-frozen between blocks of dry ice, and stored at  $-80^\circ\text{C}$  for later analysis.

### *Biochemical and Molecular Analyses*

A preweighed portion of each mixed MG muscle sample was homogenized in 20 vol of a homogenization buffer, which contained 250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 10 mM Tris·HCl, pH 7.0. Myofibrillar proteins were quantitatively extracted from 500  $\mu\text{l}$  of the total homogenate by a modification of the original procedure described by Solaro et al. (43)

Briefly, muscle samples were homogenized in 20 vol of 0.25 M sucrose, 100 mM KCl, 5 mM EDTA, 10 mM Tris·HCl, pH 6.8. The homogenate was then centrifuged at 1,000 g for 10 min. The pellet obtained was homogenized in 20 vol of a buffer containing 175 mM

KCl, 2 mM EDTA, 0.5% Triton X-100, and 10 mM Tris·HCl, pH 6.8, at 4°C. This homogenate was then centrifuged at 1,000 g for 10 min. This Triton X-100 treatment was then repeated. The resultant pellet was suspended in 20 vol of wash buffer (150 mM KCl, 10 mM Tris·HCl, pH 7.0.) and then centrifuged at 1,000 g for 10 min. After the pellet was washed once, the final myofibrillar pellet was suspended in 150 mM KCl at a volume equivalent to one-half the total homogenate volume that was extracted. Protein concentration in the homogenate and myofibril suspension was determined by using the Bio-Rad protein assay with gamma globulin as a standard. Muscle total protein and myofibril content were calculated on the basis of the homogenized muscle piece weight and total muscle weight. An aliquot of myofibril suspension was then added to a solution containing 50% vol/vol glycerol, 100 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and 5 mM EDTA at a concentration of 1 mg/ml and stored at -20°C.

Muscle total DNA concentration was calculated on the basis of total DNA concentration in the total homogenate and was determined by using a fluorometric assay with the DNA-specific fluorescent Hoechst 33258 dye (30).

**Total RNA isolation.** Total RNA was extracted from preweighed frozen muscle samples of mixed fiber-type MG (comprising the belly of the MG) by using the TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the company's protocol. This procedure is based on the method described by Chomczynski and Sacchi (13). Extracted RNA was precipitated from the aqueous phase with isopropanol, and after washing with ethanol, the extract was dried and suspended in a known volume of nuclease-free water. The RNA concentration was determined by optical density at 260 nm (by using an OD<sub>260</sub> unit equivalent to 40 µg/ml). The muscle total RNA concentration was calculated on the basis of total RNA yield and the weight of the analyzed sample. The RNA samples were stored frozen at -80°C and were used subsequently in relative RT-PCR procedures.

**Reverse transcription.** One microgram of total RNA was reverse transcribed for each muscle sample by using the SuperScript II RT from Invitrogen (Carlsbad, CA) 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 RT reaction, the tubes were heated at 90°C for 5 min to stop the reaction, and then they 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, IGF-1 binding protein 4 (IGFBP-4), atrogen 1, procollagen III-α1, and suppressor of cytokine signaling (SOCS)-2 and -3. The sequences for the primers used for the specific target mRNAs are shown in Table 1. These primers were purchased from Operon Biotechnologies (Huntsville, AL). In each PCR reaction, 18S rRNA 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 the 18S amplification we used the Alternate 18S Internal Standards (Ambion, Austin, TX), which yields 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 be in 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 by using the same reagent premix for all the samples to be compared in the study. To validate the consistency of the analysis procedures, at least one representative sample from each group was included in each RT-PCR run.

One microliter of each RT reaction (0- to 10-fold dilution depending on target mRNA abundance) was used for the PCR amplification. The PCR reactions were carried out in the presence of 2 mM MgCl<sub>2</sub> by using standard PCR buffer (Bioline), 0.2 mM deoxynucleoside triphosphate, 1 µM specific primer set, 0.5 µM 18S primer/competitor mix, and 0.75 unit of Biolase DNA polymerase (Bioline, Genesee, San Diego, CA) 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°C (55–60°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 2.5% agarose gel by electrophoresis and stained with ethidium bromide. The ultraviolet light-induced fluorescence of stained DNA bands was captured by a digital camera, and the band intensities were quantified by densitometry with ImageQuant software (GE healthcare) on digitized images and were reported as arbitrary scan units. In this approach, each specific mRNA signal is normalized to its corresponding 18S. For each primer set, PCR conditions (cDNA dilutions, 18S competitor/

Table 1. PCR primer sequence

Target mRNA	PCR Primer Sequence 5'→3'	Product Size, bp	GenBank Accession No.
IGF-I	Fwd: GCATTGTGGATGAGTGTTC	202	M15480
	Rev: GGCTCCTCCTACATTCTGTA		
IGFBP-4	Fwd: CTGACAAGGATGAGAGTGAGCA	205	NM_001004274
	Rev: GAGGCGGCCAGTCGTTTC		
SOCS-3	Fwd: GGGGCCCTCCTTCTCTTTAC	238	AF075383
	Rev: GCAGCTGGGTCACTTCTCATAGG		
SOCS-2	Fwd: CGAAGGCCCTGCGTGAGC	207	AF075382
	Rev: ATCTGAATTTCCCGTCTGGTA		
Atrogen	Fwd: CAGAACAGCAAACAAAACCTC	218	NM_133521
	Rev: GCGATGCCACTCAGGGATGT		
MuRF-1	Fwd: TACCGAGAGCAGTTGGAAAAGT	215	AY059627
	Rev: CTCAAGCCCTCTGCTATGTGTT		
Myostatin	Fwd: CAAACAGCCTGAATCCAACCTAG	215	NM_019151
	Rev: CCCTGAGGGGTAGCGACAG		
IL-6	Fwd: CAGAAATACAAAGAAATGATGGATGCT	242	NM_012589
	Rev: ATTGGAAGTTGGGGTAGGAAGGAC		
Procollagen III α1	Fwd: CTCACCCTGCTTCAACCCTCTC	234	XM_343563
	Rev: TGGACATATTGCACAACATTTCTC		
Cyclin D1	Fwd: AAGTGGTGCAGAGGGAGAT	267	NM_171992
	Rev: GGGGGGATAGAGTTGTCAG		

Fwd, forward primer; Rev, reverse primer; IGFBP-4, IGF-1 binding protein-4; SOCS-2 and -3, suppressor of cytokine signaling-2 and -3, respectively; MuRF-1, muscle ring finger-1.

primer mix, MgCl<sub>2</sub> concentration, and annealing temperature) were optimized so that both the target mRNA and 18S product yields were in the linear range of the semilog plot when the yield is expressed as a function of the number of cycles (9).

#### Assessment of Integrated Force-Time Areas

During each training session, traces of the stored contractions were analyzed for the integrated force area (Fig. 1A). These analyses included contractions obtained at the beginning and at the end of each set, and they included all four training sessions to provide a synopsis of the resistance exercise program. These areas were analyzed via AcqKnowledge software (Biopac Systems) (see Fig. 1B).

#### Data Presentation and Statistical Analysis

All values are reported as means and SE. Treatment effects were determined by one-way ANOVA with post hoc testing (Newman-Keuls multiple comparison tests) using the Prism software package (Graphpad). The post hoc testing provided comparisons between all datasets, allowing for the identification of differential responses due to exercise mode. For all statistical tests the 0.05 level of confidence was accepted for statistical significance.

## RESULTS

### Average Integrated Force

Figure 1A presents a typical 2-s contraction recording in the three modes of action depicting constant force output in the Iso mode, and the fall and increase in force output for the Con and Ecc actions, respectively. The mean integrated force that was accumulated across the four training sessions was equivalent among the three contraction mode groups (Fig. 1B).

### Body and Muscle Weight and Protein Concentrations

Body weights were essentially identical across the four experimental groups (Table 2). MG wet muscle weight was not different among the four experimental groups (Table 2). There were no significant differences in total protein or myofibrillar protein between any of the groups (Table 3).

Table 2. Body and muscle mass measurements

Group	Body Mass, g	Right MG, mg	Left MG,* mg
Sham	261 ± 3	698 ± 13	711 ± 15
Iso	261 ± 5	688 ± 17	724 ± 16
Con	259 ± 3	659 ± 12	693 ± 18
Ecc	264 ± 4	656 ± 10	664 ± 17

Values are means ± SE. Exercise training modes of action were static-isometric (Iso), shortening-concentric (Con), or lengthening-eccentric (Ecc). Sham, nontrained control; MG, medial gastrocnemius. \*Resistance exercise.

### RNA and DNA Concentration

At this early time point, there were no significant changes in DNA concentration across the three trained MG muscle groups relative to their untrained counterparts (data not shown). However, there were statistically significant increases in RNA concentration in the stimulated muscle (Fig. 2).

### mRNA

There were a number of changes in the production and/or accumulation of mRNA as a result of the resistance exercise protocol used in this study.

As presented in Fig. 3, the pattern of increases in mRNA for IGF-I, IGFBP-4, and cyclin D1 mRNA levels was similar in the stimulated MG of all three contraction mode groups. However, cyclin D1 mRNA did not change significantly in the Con group. Resistance exercise also stimulated an increase in the levels of procollagen III α1 mRNA, a marker of increased collagen synthesis (Fig. 4).

The mRNA for myostatin was significantly decreased in the exercised muscles from all groups (Fig. 5A). A similar pattern of response was seen in the mRNA for SOCS-2; however, the Con group did not demonstrate a significant change (Fig. 5B). The resistance exercise protocol used in this study did not appear to have any effect on the mRNA levels on atrogenin-1 (data not shown).

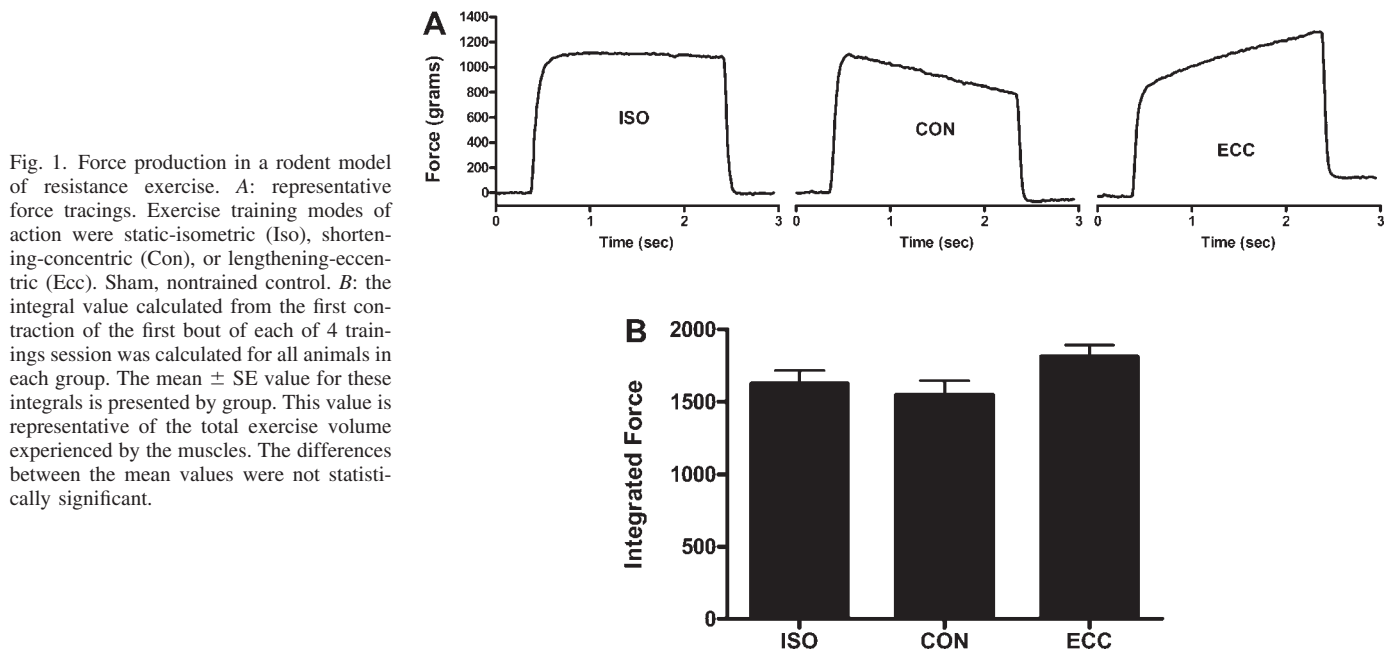


Fig. 1. Force production in a rodent model of resistance exercise. A: representative force tracings. Exercise training modes of action were static-isometric (Iso), shortening-concentric (Con), or lengthening-eccentric (Ecc). Sham, nontrained control. B: the integral value calculated from the first contraction of the first bout of each of 4 training sessions was calculated for all animals in each group. The mean ± SE value for these integrals is presented by group. This value is representative of the total exercise volume experienced by the muscles. The differences between the mean values were not statistically significant.

Table 3. Total protein and myofibrillar protein concentration

Group	Total Protein, mg/g		Myofibrillar Protein, mg/g	
	Right MG	Left MG*	Right MG	Left MG*
Sham	244±8	257±4	121±6	127±6
Iso	253±5	237±5	120±6	112±5
Con	260±6	239±8	127±7	107±5
Ecc	267±5	249±7	130±4	119±6

Values are means ± SE. \*Resistance exercise.

The mRNA for IL-6 was essentially undetectable in nonexercised muscles (Fig. 6A). However, resistance exercise resulted in accumulation of IL-6 mRNA in all three modes of action. In accord with this response, the mRNA for SOCS-3 was also significantly increased in the exercised muscles (Fig. 6B).

In each case an additional statistical analysis was conducted on the values of the change in a given parameter (e.g., exercised muscle vs. contralateral muscle within group) to further evaluate the possibility that one or more training modes may have resulted in a greater change. These analyses failed to detect any mode-specific differences in the change in total RNA or the mRNAs for IGF-I, IGFBP-4, cyclin D1, procollagen III- $\alpha$ 1 mRNA, SOCS-2, IL-6, or SOCS-3 (data not shown).

## DISCUSSION

The primary goal of this study was to test the hypothesis that a particular muscle action mode (i.e., shortening, lengthening, or isometric) is more effective at initiating muscle anabolic responses at the onset of training. This experiment was driven by our previous observations that isometric exercise, of a type proven to induce muscle hypertrophy, was found to be ineffective in preventing the early atrophy response to muscle

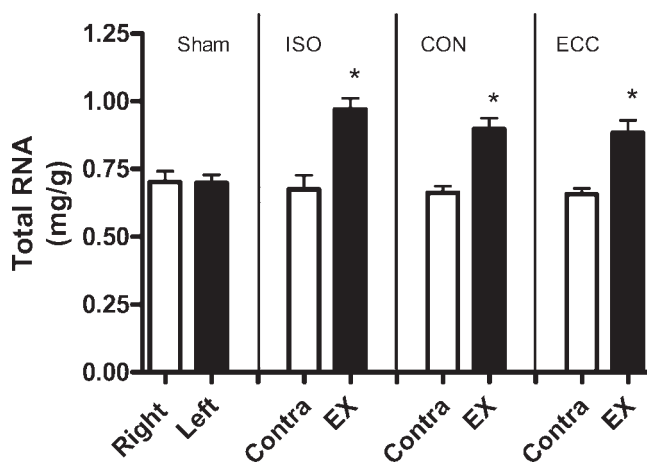


Fig. 2. Resistance exercise effects on total RNA. Approximately 80–85% of the total RNA pool consists of rRNA. Therefore changes in this value are dominated by rRNA. Increased total RNA most likely represents an increase in the translational capacity of the muscles. For Figs. 2–6, the asterisk (\*) indicates both a significant change relative to the contralateral (Contra) muscle within groups and a significant difference from the sham-prepared muscles. Differences between the Contra muscles and the right and left muscles of the sham-prepared group were never found. No post hoc differences were seen between the exercised (Ex) muscles.

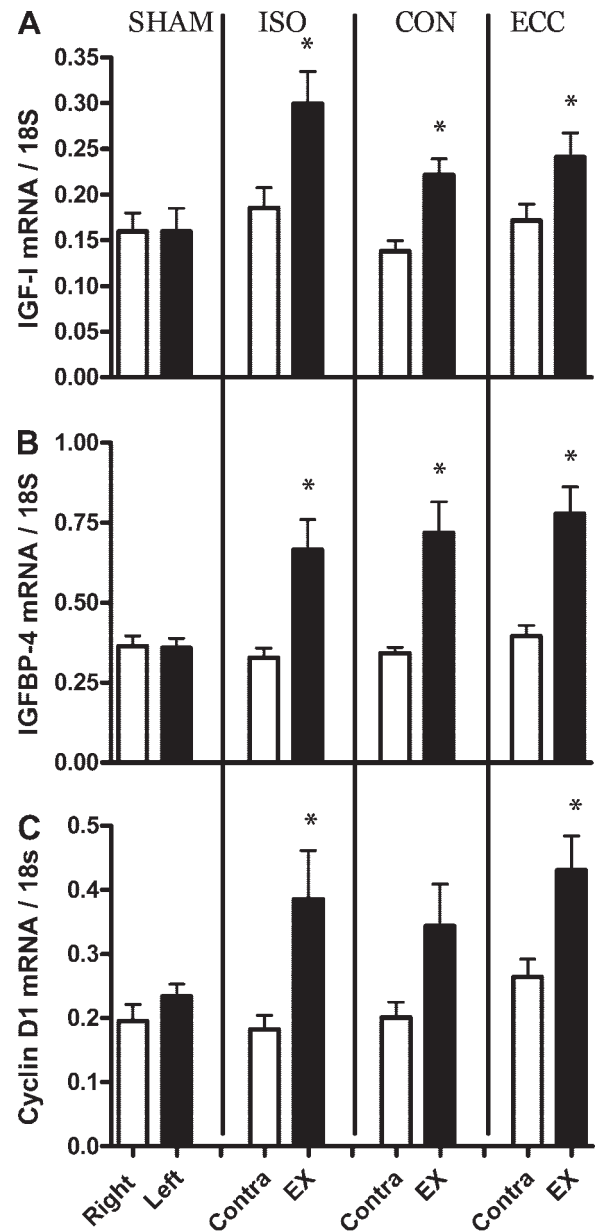


Fig. 3. Effects of resistance exercise on mRNA levels for IGF-I (A), IGF-1 binding protein-4 (IGFBP-4; B), and cyclin D1 (C). Exercise increased both IGF-I and IGFBP-4 mRNA significantly (\*) relative to the Contra muscles and the muscles of sham-prepared animals. There were no differences between the Ex values across action modes. In the case of cyclin D1 mRNA, the value for the Con group was not statistically different from the Contra muscles.

unloading (2, 24). In the present study, all three contraction paradigms that were examined, e.g., Con, Ecc, and Iso, consisted of similar durations, activation patterns, and number of contractions performed in each mode during each training session. However, in contrast to our previous study, muscle action parameters such as the starting relative length and degrees of movement were designed to provide an equivalent volume of force production (mean force integral) across the modes of action. We reasoned that, by holding muscle output equivalent, any observed differences in the signaling markers indicating a more potent initiation of an anabolic state would

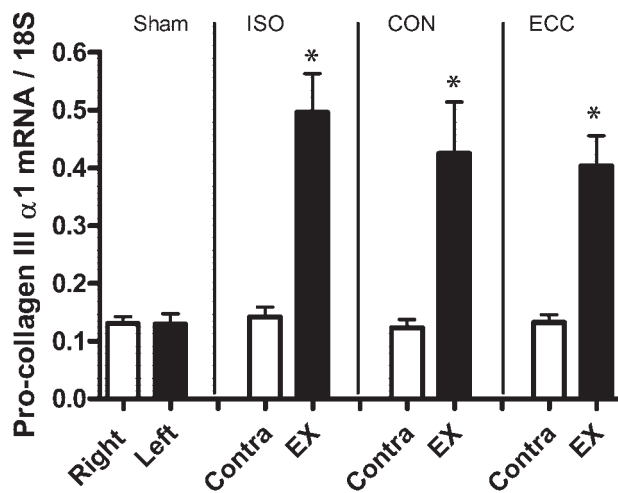


Fig. 4. Effects of resistance exercise on mRNA levels of procollagen III  $\alpha$ 1. Exercise increased procollagen III  $\alpha$ 1 mRNA significantly (\*) relative to the Contra muscles and the muscles of sham-prepared animals. There were no differences between the Ex values.

be a function of the muscle action mode. It was reasoned that this information may be particularly germane to instances of muscle unloading wherein there is a relative rapid initial decrease in muscle mass (24).

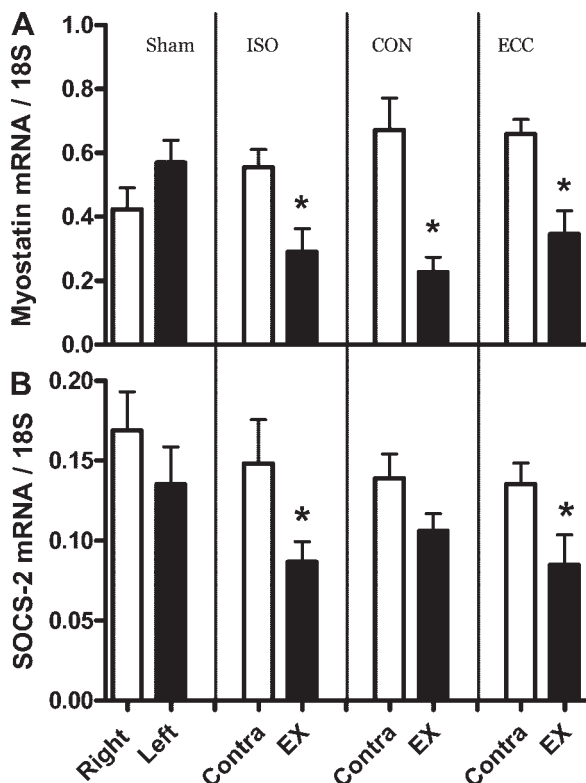


Fig. 5. Resistance exercise effects on myostatin and suppressor of cytokine signaling (SOCS)-2 mRNA levels. *A*: exercise decreased myostatin mRNA significantly (\*) relative to the contralateral muscles and the muscles of sham-prepared animals. There were no differences between the Ex values across action modes. *B*: exercise decreased SOCS-2 mRNA significantly (\*) relative to the Contra muscles and the muscles of sham-prepared animals in both the Iso and Ecc but not the Con groups. There were no differences between the Ex values across action modes.

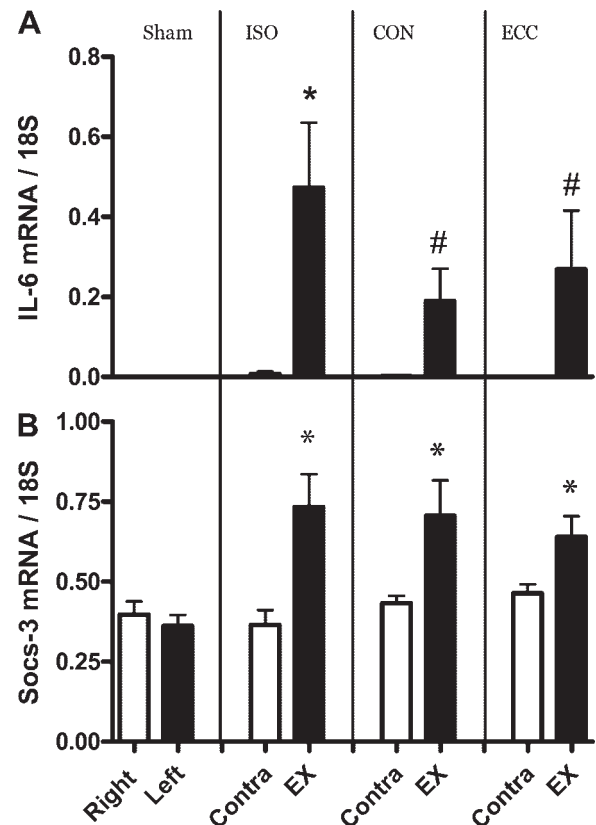


Fig. 6. Effects of resistance exercise on IL-6 and SOCS-3 mRNA levels. *A*: IL-6 mRNA was detected in just 4 of the 40 nonexercised muscles examined. Resistance exercise resulted in detectable levels of IL-6 mRNA in all of the Ex muscles. The increase in IL-6 seen in the Iso group was greater than that seen in the Con and Ecc muscles (#  $P < 0.05$  vs. Iso). \*Significant change relative to the Contra muscle within group and relative to the sham-prepared muscles. *B*: exercise increased SOCS-3 mRNA significantly (\*) relative to the Contra muscles and the muscles of sham-prepared animals. There were no differences between the Ex values across action modes.

### Muscle Output

The mean force integral measured following activation in the Con, Ecc, and Iso exercise groups was essentially the same (Fig. 1). This contrasts markedly with our previous training study in which this parameter was not controlled (2).

### Muscle Anabolic Responses

For these experiments we chose a subset of signaling markers that we and others have shown to be sensitive to increased loading in both human and animal muscles and that are clearly linked to hypertrophy responses under diverse experimental conditions (e.g., models of resistance exercise and chronic functional overload), including stimuli independent of muscle loading (1, 3–5, 8, 9, 16, 20).

**Total RNA.** Increases in translational efficiency and capacity are an important initiating step in an anabolic response. The majority of the cellular RNA pool consists of rRNA, and therefore total RNA measurements can be taken to roughly reflect the translational capacity of the muscle (26, 42). In a previous study, two consecutive bouts of resistance exercise, using this rat model, resulted in a tendency for an increase in total RNA (22). To evaluate the strength of this previously

observed tendency, the present study included a total of four exercise bouts. The increase in total RNA seen in the present study provides an important indication that anabolic adaptation was occurring well before any significant accumulation of contractile protein would be expected. Interestingly, there were no mode-specific differences in the increase in total RNA concentration in this study (Fig. 2).

**IGF-I.** It is apparent that the loading-sensitive muscle IGF-I system provides an early response autocrine/paracrine pathway that could be linked to a variety of signaling cascades that enable the muscle to enter an anabolic state, thereby resulting in eventual net protein accumulation as the training program continues beyond the acute stage (3, 5, 41). In the present study, the two components of this system that were measured, IGF-I and IGFBP-4 mRNA, both increased in a similar fashion across muscle action modes (Fig. 3, A and B).

**Cyclin D1.** Cyclin D1 has several potential roles in the initial response of muscle to a hypertrophic stimulus. In its traditional role as a cell cycle regulatory protein, increased cyclin D1 may indicate that some muscle satellite cells are leaving their quiescent state and preparing to proliferate. A second pro-anabolic role for increased cyclin D has been proposed in which this protein may promote translation (39). Similar to that of IGF-I, cyclin D1 mRNA demonstrated a similar pattern following all three muscle actions (Fig. 3C).

**Procollagen type III.** Recently we have reported that procollagen III  $\alpha 1$  is an early gene marker for the adaptation of connective tissue, at the myotendinous region in skeletal muscle, in response to functional overload (40). Consistent with these observations it is apparent that the resistance exercise stimulus in the present study induced a marked upregulation of this gene in the MG muscle (Fig. 4). In a pilot study we have observed significant upregulation of the procollagen III gene in response to just one bout of exercise (unpublished observation), providing strong evidence that the collagen remodeling and deposition process appears to play a pivotal role in translating anabolic stimuli into a stronger and larger infrastructure in the muscle/tendon architecture.

**Myostatin.** Myostatin is thought to negatively regulate skeletal muscle mass (31). For example, a lack of myostatin expression results in large increases in body and muscle mass during development (35). We have previously reported that acute resistance exercise results in a decrease in muscle myostatin expression in young but not old rats (23). In the present study, all three resistance exercise protocols resulted in a similar decrease in myostatin mRNA (Fig. 5A).

**SOCS-2.** Similar to myostatin, SOCS-2 appears to exert a restraining influence on muscle size (21), apparently via regulation of growth hormone (GH)-associated intracellular signaling (18). Interestingly, there are reports that myostatin may also interact with the GH axis (33, 34). In addition to its impact on GH signaling, in some tissues SOCS-2 has been found to bind to the IGF-I receptor, reducing its signaling activity in vivo (36). In the present study, the pattern of changes in SOCS-2 mRNA levels seen following resistance exercise indicates that this parameter is most likely not differentially sensitive to the mode of action (Fig. 5B). However, as with cyclin D1, the apparent trend toward increased SOCS-2 mRNA was not significant in the muscles from the Con group. It is possible that this is a function of a lesser adaptive stimulus generated in this mode. However, examination of the data

suggests that it is equally likely that this result is a function of randomly increased variability.

In light of the negative relationship between myostatin and SOCS-2 and muscle mass, the responses seen in the exercised muscles from the present study would be appropriate for muscles preparing to enter a hypertrophic phase to compensate for sustained increases in loading.

**Muscle IL-6.** In addition to the suite of previously identified markers of potential anabolic responses, we included analysis related to IL-6 in the present study. This was prompted by our recent observation that SOCS-3 mRNA increased following acute resistance exercise in the muscles of young and old rats (23). SOCS-3 is most commonly associated with negative-feedback regulation of IL-6 signaling (15). In addition, the negative impact of IL-6 overexpression on growth appears to be mediated via an increase in the expression of SOCS-3 (32). We have previously reported that direct, local, infusion of IL-6 into a single targeted skeletal muscle resulted in a significant increase in muscle SOCS-3 mRNA as a result of increased Jak/STAT3 signaling (25). However, SOCS-3 can function as a general negative-feedback agent in response to ligands that signal via the Jak/STAT pathway (12). In addition to IL-6, both the insulin and IGF-I receptors have been reported to be sensitive to negative regulation by SOCS-3 (6, 37). One possible mechanism for the effects of SOCS-3 on insulin and IGF-I signaling appears to be the potential for modulation of IRS-1. We recently reported that there is a significant negative correlation between SOCS-3 mRNA levels and the tyrosine phosphorylation of IRS-1 (23). Interestingly, there was also a significant negative correlation between the mRNA levels of SOCS-3 and the amount of IRS-1 protein present in muscles. This suggests that upregulation of SOCS-3 may participate in negative feedback, impacting both the amount and activity of IRS-1 in skeletal muscle.

In the present study, resistance exercise resulted in the detection of IL-6 mRNA in the muscles of rats exposed to each of the three training modes, while this transcript was undetectable in the muscles of sham-prepared animals (Fig. 6A). Similarly, SOCS-3 mRNA increased in the exercised muscles of all groups (Fig. 6B).

Recently, Spangenburg (44) reported that overexpression of SOCS-3 in myoblasts induces differentiation in an IGF-I-independent manner. In this context, a resistance exercise-induced increase in SOCS-3 may promote the differentiation of satellite cells in preparation for their fusion with myofibers in order to support the maintenance of the myonuclear domain as muscle hypertrophy progresses (7).

While IL-6 is commonly associated with immune responses, recent results have demonstrated that, in skeletal muscle, IL-6 production is sensitive to, and plays an important role in, the regulation of energy substrate selection (17). The studies that have elucidated this concept have more or less uniformly involved relatively extended, endurance-type exercise. In rodents, Spangenburg et al. (45) have recently reported that endurance mode training increases skeletal muscle SOCS-3 mRNA levels. These authors suggested that the exercise-induced increase in SOCS-3 resulted in increased IL-6 mRNA levels via the recruitment of NF- $\kappa$ B to the IL-6 promoter. In the context of the present study, it is not possible to rule out a similar metabolically driven role for IL-6 in resistance exercise on the basis of the results in hand.



### General Considerations and Summary

There are a number of factors that must restrict the interpretation of the results presented in this basic study. For example, the present study did not examine whether different contraction modes interacting with one another (e.g., concentric and eccentric) may produce synergistic effects that could create end results greater than those attending a single pure muscle action protocol. Moreover, a limited range of potential markers of anabolic responses was chosen in the present study. It is clear that there are many other processes that are also essential for inducing an increase in net protein balance.

In the present study, in the presence of equivalent amounts of accumulated force, the various indicators of muscle anabolic responses, such as increased RNA levels, were very similar regardless of muscle action mode. As a result, the hypothesis that one particular muscle action mode would stand out via the induction of more robust changes in indicators of the initiation of anabolic responses was not supported.

### ACKNOWLEDGMENTS

We thank Ming Zeng, Li-Ying Zhang, Phuc Tran, Alvin Yu, Julianne Lynn, and Rudy Senstad for skillful technical assistance.

### GRANTS

This research was supported by National Space Biomedical Research Grant NCC-58 to K. M. Baldwin.

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