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

T. Garma, C. Kobayashi, F. Haddad, G. R. Adams, P. W. Bodell, and K. M. Baldwin

Department of Physiology and Biophysics
University of California, Irvine
Irvine, CA 92697

Running Head: Acute resistance training in different contraction modes

Corresponding Author:

K.M. Baldwin
Department of Physiology and Biophysics
University of California, Irvine
Irvine, CA 92697

Phone: 949-824-7192
Fax: 949-824-8540
Email: kmbaldwi@uci.edu
Abstract:

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 insulin-like growth factor -I (IGF-I), pro-collagen III α1, 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.

Key Words: isometric; concentric; eccentric; resistance exercise; hypertrophy; anabolic signaling.
Introduction:

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 IRS-1 (insulin receptor substrate-1), Akt (protein kinase B) and mTOR (mammalian target of rapamycin) (19). This pathway appears to converge on processes which 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 effected 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 or 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 work is that of Hather et al. and Colliander & Tesch (14, 27). However, these papers did not directly compare pure lengthening and shortening actions, nor was isometric training included. For example,
Hather et al. reported that training using a combination of shortening and lengthening (CON/ECC) actions resulted in a significantly greater increase in fiber size when compared to 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, 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 which 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 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 current study is 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 prior to 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 current 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 an molecular outcome measures indicative of anabolic responses to acute increases in loading. The hypothesis of this study was that a given contraction mode would 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.
Experimental design and treatment protocol.

This study was conducted in conformity with APS’s *Guiding Principles in the Care and Use of Animals*, and the protocol was approved by the University of California, Irvine IACUC. Young adult female Sprague Dawley rats weighing $263 \pm 5$ grams were randomly assigned to four experimental groups (n=9 each), designated as Non-trained Control (SHAM), Isometric-Trained (ISO), Concentric-Trained (CON), and Eccentric-Trained (ECC). 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 five days with the first day of training being performed on designated day 1 followed by three additional training sessions (days 2, 4 & 5) with sacrifice occurring 24 hrs 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 40ga. insulated nickel chromium wire (Stablohm 800B, CA Fine Wire) were introduced into the subcutaneous region adjacent to the popliteal fossa via 27 ga. hypodermic needles. The needles were then withdrawn leaving the wire in place. Prior to 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) to induce 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± 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 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 actions contractions, tenotomy of the primary antagonistic muscle, the tibialis anterior (TA), resulted in less than 10% difference in force output measured pre- and post-tenotomy (data not shown). This result is similar to that originally reported by Wong & 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 isometric, concentric and eccentric modes. For the ISO mode exercise, the foot was positioned at an angle of approximately 44° relative to the tibia and no change in the foot-plate angle allowed. Because of the inherent drop in force output as the muscle shortens, the CON mode contractions were limited to 10° relative to the starting position (e.g., 44° to 54°) providing for a slower contraction relative to the eccentric group. For the ECC mode muscle actions, the foot was positioned at 64° relative to the tibia and allowed to move to 44°, the starting point of the concentric (and isometric contractions). All muscles
were stimulated for 2 seconds 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 isometric and concentric groups. For all exercise bouts the stimulation parameters were 2 sec. of stimulation with 19 seconds of rest between each contraction, 10 contractions per set. Five sets of contractions were applied with 5 minutes of recovery between sets. In each case this protocol resulted in less than 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 using LabTech Notebook (Laboratory Technologies Corp.). Data analysis was conducted using AcqKnowledge software (Biopac Systems). Force output was monitored in real time on the computer screen during each contraction. Rats were trained ~five hours after the beginning of their standard light cycle, during each training session.

**Tissue Collection.** Twenty-four hours after the last exercise bout (fourth session), the rats were killed via an injection of Pentosol euthanasia solution (Med-Pharmex) at a dose of 0.4 ml / kg (~160mg/kg Pentobarbitol), I.P. At the cessation of heart beat, a skin incision is made and the medial gastrocnemius muscles (MG) of both legs were dissected free of connective tissue, weighed, snap-frozen between blocks of dry ice, and stored at -80°C for later analysis.

**Biochemical & Molecular Analyses.**
A pre-weighed portion of each mixed MG 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 HCl, pH 7.0. Myofibrillar proteins were quantitatively extracted from 500 ul of the total homogenate by a modification of the original procedure described by Solaro et al (43).

Briefly, muscle samples were homogenized in 20 volumes of 0.25M sucrose, 100mM KCl, 5mM EDTA, 10mM Tris HCl, pH 6.8. The homogenate was then centrifuged at 1000 g for 10 minutes. The pellet obtained was homogenized in 20 volumes 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 1000g for 10 minutes. This triton X treatment was then repeated. The resultant pellet was suspended in 20 volume of wash buffer (150 mM KCl, 10 mM Tris-HCl; pH 7.0.) then centrifuged at 1000g for 10 minutes. After washing the pellet once, the final myofibrillar pellet was suspended in 150 mM KCl at a volume equivalent to half the total homogenate volume that was extracted. Protein concentration in the homogenate and myofibril suspension was determined using the Biorad Protein assay with gamma globulin as a standard. Muscle total protein and myofibril content were calculated based on the homogenized muscle piece weight and total muscle weight. An aliquot of myofibril suspension was then added to a solution containing 50% v/v glycerol, 100mM Na4P2O7, 5mM EDTA at a concentration of 1 mg per 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 using a fluorometric assay using the DNA-specific fluorescent Hoechst 33258 dye (30).
**Total RNA Isolation.** Total RNA was extracted from pre-weighed frozen muscle samples of mixed fiber-type MG (comprising the belly of the MG) 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 (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 (using an OD260 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 used subsequently in relative RT-PCR procedures.

**Reverse Transcription (RT).** One µg of total RNA was reverse transcribed for each muscle sample 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 minutes, according to the provided protocol. At the end of the RT reaction, the tubes were heated at 90°C for 5 minutes to stop the reaction and then they were stored at -80°C until used in the PCR reactions for specific mRNA analyses (see below).

**Polymerase Chain Reaction (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 Insulin-like growth factor –I (IGF-I), IGF-1 binding protein 4 (IGF1BP4), atrogin 1, procollagen III-alpha-1 and SOCS-2 & -3). The sequence for the primers used for the specific target mRNAs is shown in Table 1. These primers were purchased from Operon Biotechnologies Inc., (Huntsville, AL). In each PCR reaction, 18S ribosomal RNA was
co-amplified 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 competimers at an optimized ratio that could range from 1:4 to 1:10, depending on the abundance of the target mRNA. Inclusion of 18S competimers was necessary in order to bring down the 18S signal, which allows its linear amplification to be in the same range as the co-amplified 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 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 µl 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 2mM MgCl2 using standard PCR buffer (Bioline), 0.2 mM dNTP, 1µM specific primer set, 0.5µM 18S primer/competimer 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 minutes at 96°C, followed by 25 cycles of 1 minute at 96°C, 1 minute at 55°C (55-60°C depending on primers), 1 minute at 72°C, and a final step of 3 minutes 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 competimer/primer mix, MgCl₂ concentration, and annealing temperature) were optimized so that both the target mRNA and 18S product yields were in the linear range of the semi log 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 (Figure 1A). These analyses included contractions obtained at the beginning and at the end of each set and they included all four training sessions in order to provide a synopsis of the resistance exercise program. These areas were analyzed via AcqKnowledge software (Biopac Systems) (See Figure 1B).

**Data Presentation and Statistical Analysis.**

All values are reported as mean and standard error of the mean (SEM). Treatment effects were determined by one way ANOVA with post-hoc testing (Neuman-Keuls multiple comparison tests) using the Prism software package (Graphpad). The post-hoc testing provided comparisons between all data sets, 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-second contraction recording in the three modes of action depicting constant force output in the isometric mode, and the fall and increase in force output for the concentric and eccentric actions, respectively. The mean integrated force that was accumulated across the four training sessions was equivalent among the three contraction mode groups (Figure 1B).

Body and Muscle Weight, and Protein Concentrations.

Body weights were essentially identical across the four experimental groups (Table 2). Medial gastrocnemius (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).

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 (Figure 2).

Messenger RNA

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 figure 3, the pattern of increases in mRNA for IGF-I, IGF-BP4 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 ProCol3α1 mRNA, a marker of increased collagen synthesis (Figure 4).

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

The mRNA for interleukin-6 (IL-6) was essentially undetectable in non-exercised muscles (Figure 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 (Figure 6B).

In each case an additional statistical analysis was conducted on the values of the change in a given parameter (e.g., EX - Contra) in order to further evaluate the possibility that one or more training modes may have resulted in a greater change. These analyses failed to detect and mode specific differences in the change in total RNA, or the mRNAs for IGF-I, IGF-BP4, cyclin D1, ProCol3α1 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 onset of training. This experiment was driven by our previous observations that isometric exercise, of a type proven to induce muscle atrophy, was found to be ineffective in preventing the early atrophy response to muscle unloading (2, 24). In the current study, all three contraction paradigms that were examined, e.g., concentric, eccentric, and isometric, consisted of similar durations, activation patterns and the 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 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).

Muscle Output

The mean force integral measured following activation in the concentric, eccentric and isometric exercise groups was essentially the same (Figure 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 sub-set 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, 4, 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 cellular RNA pool consists of ribosomal RNA 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). In order to evaluate the strength of this previously observed tendency, the current study included a total of four exercise bouts. The increase in total RNA seen in the current 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 (Figure 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 which enable the muscle to enter a anabolic state thereby resulting in eventual net protein accumulation as the training program continues beyond the acute stage (3, 5, 41). In the current 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 (Figure 3A&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 (Figure 3C).

Pro-collagen Type III  Recently we have reported that procollagen III α1 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 current study induced a marked up regulation of this gene in the MG muscle (Figure 4). In a pilot study we have observed significant up regulation of the procollagen III gene in response to just one bout of exercise (unpublished observation) providing strong evidence that 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  Myostain 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 current study, all three resistance exercise protocols resulted in a similar decrease in myostain mRNA (Figure 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 current 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 (Figure 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 current study would be appropriate for muscles preparing to enter a hypertrophic phase in order 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 current 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 over expression 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 feed back 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 SOCS3 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 up regulation of SOCS-3 may participate in negative feedback, impacting both the amount and activity of IRS-1 in skeletal muscle.

In the current 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 animals (Figure 6A). Similarly, SOCS-3 mRNA increased in the exercised muscles of all groups (Figure 6B).

Recently, Spangenburg reported that over expression of SOCS-3 in myoblasts induces differentiation in a IGF-I independent manner (44). 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. have recently reported that endurance mode training increases skeletal muscle SOCS-3 mRNA levels (45). These authors suggested that the exercise induced increase in SOCS-3 resulted in increased IL-6 mRNA levels via the recruitment of NF-κB (nuclear factor-κB) to the IL-6 promoter. In the context of the current study, it is not possible to rule out a similar metabolically driven role for IL-6 in resistance exercise based on the results in hand.

**General Considerations and Summary**

There are a number of factors which 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 & 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 current 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 standout via the induction of more robust changes in indicators of the initiation of anabolic responses was not supported.
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Table 1: PCR primer sequence

| Target mRNA | PCR Primer sequence 5'→3' | Product Size (bp) | GenBank Acc. # |
|-------------|---------------------------|-------------------|----------------|
| IGF-I       | Fwd: GCATTGTGGATGAGTGTTGC Rev: GGCTCCTCCTACATTCTGTA | 202               | M15480         |
| IGF-BP4     | Fwd: CTGACAAGGATGAGTGAGCA Rev: GAGGCGGCCAGTCTGTC | 205               | NM_001004274   |
| SOCS 3      | FWD: GGGGCCCTCCTCTTCTTAC REV:               | 238               | AF075383       |
| SOCS 2      | FWD: CGAAGGCCCTGCCTGAGC REV: ATCTGAATTTCCTGCTTGTA | 207               | AF075382       |
| Atrogin     | Fwd: CAGAACAGCATAAAACAAACTC Rev: GCGATGCCACTCAGGGATGT | 218               | NM_133521      |
| Myostatin   | Fwd: CAAACAGCTGAATCCAACTTAG Rev: CCGTGAGGGGGTAGCGACAG | 215               | NM_019151      |
| IL6         | Fwd: CAGAAAATCAAAAGAAATGATGGATGCT              | 242               | NM_012589      |
| Procollagen III α1 | Fwd: CTCACCCCTGCTTTCAACCCCTTC | 234               | XM_343563      |
| Cyclin D1   | Fwd: AAGTGGCTGCGAGGGGAGAT Rev: GGGGCGGATAGGTGTCAG | 267               | NM_171992      |

Fwd: Forward primer  
Rev: Reverse primer
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        |

*resistance exercise

Table 3. Total Protein and Myofibrillar Protein Concentration

| Group | Total Protein (mg/g) | Myofibrillar Protein (mg/g) |
|-------|----------------------|-----------------------------|
|       | Right MG (mg) | Left MG (mg)* | Right MG (mg) | Left MG (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         |

*resistance exercise
Figure Legends

Figure 1. Force production in a rodent model of resistance exercise.
(A.) Representative force tracings. (B.) The integral value calculated from the first contraction of the first bout of each of 4 trainings session was calculated for all animals in each group. The mean ± standard error 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.

Figure 2. Resistance exercise effects on total RNA.
~80-85% of the total RNA pool consists of ribosomal RNA (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 this and subsequent plots, * indicates a significant change relative to the contralateral (CONTRA) muscles within groups. The ANOVA with post-doc testing used in this study compared all pairs of data. Differences between the CONTRA muscles and the RIGHT and LFET muscles of the SHAM group were never found. An * also indicates a significant difference from the SHAM muscles. No post-hoc differences were seen between the exercised muscles (EX).

Figure 3. Effects of resistance exercise on mRNA levels for (A.) IGF-I, (B.) IGFBP4 and (C.) cyclin D1. Exercise increased both IGF-I and IGFBP4 mRNA significantly relative to the contralateral muscles and the muscles of SHAM 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.

Figure 4. Effects of resistance exercise on mRNA levels of pro-collagen III α1. Exercise increased pro-collagen III α1 mRNA significantly relative to the contralateral muscles and the muscles of SHAM animals. There were no differences between the EX values.

Figure 5. Resistance exercise effects on Myostatin and SOCS-2 mRNA levels. (A.) Exercise decreased myostatin mRNA significantly relative to the contralateral muscles and the muscles of SHAM animals. There were no differences between the EX values across action modes. (B.) Exercise decreased SOCS-2 mRNA significantly relative to the contralateral muscles and the muscles of SHAM animals in both the ISO and ECC but not the CON groups. There were no differences between the EX values across action modes.

Figure 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 non-exercised 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). (B.) Exercise increased SOCS-3 mRNA significantly relative to the contralateral muscles and the muscles of SHAM animals. There were no differences between the EX values across action modes.
Literature Cited

1. Adams, G.R., V.J. Caiozzo, F. Haddad and K.M. Baldwin. Cellular and molecular responses to increased skeletal muscle loading after irradiation. *Am J Physiol* 283:C1182-C1195, 2002.

2. Adams, G.R., D.C. Cheng, F. Haddad and K.M. Baldwin. Skeletal muscle hypertrophy in response to isometric, lengthening and shortening training bouts of equivalent duration. *J Appl Physiol* 96:1613-1618, 2004.

3. Adams, G.R. and F. Haddad. The relationships between IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J. Appl. Physiol.* 81:2509-2516, 1996.

4. Adams, G.R., F. Haddad and K.M. Baldwin. Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. *J. Appl. Physiol.* 87:1705-1712, 1999.

5. Adams, G.R. and S.A. McCue. Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats. *J. Appl. Physiol.* 84:1716-1722, 1998.

6. Alexander, W.S. Suppressors of cytokine signaling (SOCS) in the immune system. *Nat Rev Immunol* 2(6):410-416, 2002.

7. Allen, D.L., R.R. Roy and V.R. Edgerton. Myonuclear domains in muscle adaptation and disease. *Muscle & Nerve* 22:1350-1360, 1999.

8. Bickel, C.S., J. Slade, E. Mahoney, F. Haddad, G.A. Dudley and G.R. Adams. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *J Appl Physiol.* 98:482-488, 2005.

9. Bickel, C.S., J.M. Slade, F. Haddad, G.R. Adams and G.A. Dudley. Acute molecular responses of skeletal muscle to resistance exercise in able-bodied and spinal cord-injured subjects. *J Appl Physiol* 94:2255-2262, 2003.
10. Booth, F.W. and K.M. Baldwin. Muscle plasticity: energy demand and supply processes. In, Handbook of Physiology Section 12. *L.B. Rowell, J.T. Shepard Ed. Oxford Univ. Press, 1966. pa. 1074-1123.*

11. Caiozzo, V.J., E. Ma, S.A. McCue, E. Smith, R.E. Herrick and K.M. Baldwin. A new animal model for modulating myosin expression by altered mechanical activity. *J. Appl. Physiol.* 73:1432-1440, 1992.

12. Carbia-Nagashima, A. and E. Arzt. Intracellular proteins and mechanisms involved in the control of gp130/JAK/STAT cytokine signaling. *IUBMB Life.* 56(2):83-88, 2004.

13. Chomczynski, P. and N. Sacchi. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159, 1987.

14. Colliander, E.B. and P.A. Tesch. Effects of eccentric and concentric muscle actions in resistance training. *Acta Physiol. Scand.* 140:31-39, 1990.

15. Croker, B.A., D.L. Krebs, J.G. Zhang, S. Wormald, T.A. Willson, E.G. Stanley, L. Robb, C.J. Greenhalgh, I. Forster, B.E. Clausen, N.A. Nicola, D. Metcalf, D.J. Hilton, A.W. Roberts and W.S. Alexander. SOCS3 negatively regulates IL-6 signaling in vivo. *Nat Immunol* 4:540-545, 2003.

16. DeVol, D.L., P. Rotwein, J.L. Sadow, J. Novakofski and P.J. Bechtel. Activation of insulin like growth factor gene expression during work induced skeletal muscle growth. *Am. J. Physiol.* 259:E89-E95, 1990.

17. Febbraio, M.A., N. Hiscock, M. Sacchetti, C.P. Fischer and B.K. Pedersen. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes.* 53:1643-1648, 2004.
18. Flores-Morales, A., C.J. Greenhalgh, G. Norstedt and E. Rico-Bautista. Negative regulation of GH receptor signaling. *Mol Endocrinol.* 2005 ;, 2005.

19. Glass, D.J. Molecular mechanisms modulating muscle mass. *Trends Mol Med.* 9 :344-350, 2003.

20. Goldspink, G. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *J. Anat.* 194 :323-334, 1999.

21. Greenhalgh, C.J., P. Bertolino, S.L. Asa, D. Metcalf, J.E. Corbin, T.E. Adams, H.W. Davey, N.A. Nicola, D.J. Hilton and W.S. Alexander. Growth enhancement in suppressor of cytokine signaling 2 (SOCS-2)-deficient mice is dependent on signal transducer and activator of transcription 5b (STAT5b). *Mol Endocrinol* 16(6) :1394-1406, 2002.

22. Haddad, F. and G.R. Adams. Acute cellular and molecular responses to resistance exercise. *J Appl Physiol* 93(1) :394-403, 2002.

23. Haddad, F. and G.R. Adams. Aging Sensitive Cellular and Molecular Mechanisms Associated with Skeletal Muscle Hypertrophy. *J Appl Physiol* :100(4):1188-1203, 2006.

24. Haddad, F., G.R. Adams, P.W. Bodell and K.M. Baldwin. Isometric Resistance Exercise Fails to Counteract Skeletal Muscle Atrophy Processes During the Initial Stages of Unloading. *J. Appl. Physiol.* 100 :433-441, 2006.

25. Haddad, F., F.P. Zaldivar, D.M. Cooper and G.R. Adams. IL-6 Induced Skeletal Muscle Atrophy. *J Appl Physiol* 98(3) :911-917, 2005.

26. Hannan, K.M., R.D. Hannan and L.I. Rothblum. Transcription by RNA polymerase I. *Front Biosci.* 3 :376-398, 1998.
27. Hather, B.M., P.A. Tesch, P. Buchanan and G.A. Dudley. Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol. Scand. 143* :177-185, 1991.

28. Jones, D.A. and O.M. Rutherford. Human muscle strength training: The effects of three different regimes and the nature of the resultant changes. *J. Physiol. 391* :1-11, 1987.

29. Kraemer, W.J., K. Adams, E. Cafarelli, G.A. Dudley, C. Dooly, M.S. Feigenbaum, S.J. Fleck, B. Franklin, A.C. Fry, J.R. Hoffman, R.U. Newton, J. Potteiger, M.H. Stone, N.A. Ratamess and T. Triplett-McBride. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc. 34(2)* :364-380, 2002.

30. Labarca, C. and K. Paigen. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem. 102* :344-352, 1980.

31. Lee, S.J. Regulation of muscle mass by myostatin. *Annu Rev Cell Dev Biol. 20* :61-86, 2004.

32. Lieskovska, J., D. Guo and E. Derman. Growth impairment in IL-6-overexpressing transgenic mice is associated with induction of SOCS3 mRNA. *Growth Horm IGF Res. 13* :26-35, 2003.

33. Liu, W., S.G. Thomas, S.L. Asa, N. Gonzalez-Cadavid, S. Bhasin and S. Ezzat. Myostatin is a skeletal muscle target of growth hormone anabolic action. *J Clin Endocrinol Metab. 88(11)* :5490-5496, 2003.

34. Marcell, T.J., S.M. Harman, R.J. Urban, D.D. Metz, B.D. Rodgers and M.R. Blackman. Comparison of GH, IGF-I, and testosterone with mRNA of receptors and myostatin in skeletal muscle in older men. *Am J Physiol 281(6)* :E1159-E1164, 2001.
35. McPherron, A.C., A.M. Lawler and S.J. Lee. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*. 387(6628):83-90, 1997.

36. Michaylira, C.Z., J.G. Simmons, N.M. Ramocki, B.P. Scull, K.K. McNaughton, C.R. Fuller and P.K. Lund. Suppressor of cytokine signaling-2 limits intestinal growth and enterotrophic actions of IGF-I in vivo. *Am J Physiol Gastrointest Liver Physiol* ; 2006.

37. Mooney, R.A., J. Senn, S. Cameron, N. Inamdar, L.M. Boivin, Y. Shang and R.W. Furlanetto. Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *J Biol Chem.* 276:25889-25893, 2001.

38. Morisse, M.C., E.A. Harman and M.J. Johnson. Resistance training modes: specificity and effectiveness. *Med. Sci. Sport and Exerc.* 27:648-660, 1995.

39. Nader, G.A., T.J. McLoughlin and K.A. Esser. mTOR function in skeletal muscle hypertrophy: Increased ribosomal RNA via cell cycle regulators. *Am J Physiol.* 289: C1457-C1465, 2005.

40. Olesen, J.L., K.M. Heinemeier, F. Haddad, H. Langberg, A. Flyvbjerg, M. Kjaer and K.M. Baldwin. Expression of insulin-like growth factor I, insulin-like growth factor binding proteins, and collagen mRNA in mechanically loaded plantaris tendon. *J Appl Physiol.* 101(1):183-188, 2006.

41. Rommel, C., S.C. Bodine, B.A. Clarke, R. Rossman, L. Nunez, T.N. Stitt, G.D. Yancopoulos and D.J. Glass. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nature Cell Biol* 3(11):1009-1013, 2001.
42. Siehl, D., B.H. Chua, N. Lautensack-Belser and H.E. Morgan. Faster protein and ribosome synthesis in thyroxine-induced hypertrophy of rat heart. *Am J Physiol* 248(3 Pt 1):C309-C319, 1985.

43. Solaro, J., D.C. Pang and F.N. Briggs. The purification of cardiac myofibrils with Triton X-100. *Biochim. Biophys. Acta* 245:259-262, 1971.

44. Spangenburg, E.E. SOCS-3 induces myoblast differentiation. *J Biol Chem.* 280(11):10749-10758, 2005.

45. Spangenburg, E.E., D.A. Brown, M.S. Johnson and R.L. Moore. Exercise increases SOCS-3 expression in skeletal muscle: potential relationship to IL-6 expression. *J Physiol* 2006. DOI: 10.1113/jphysiol.2005.104315.

46. Stitt, T.N., D. Drujan, B.A. Clarke, F. Panaro, Y. Timofeyva, W.O. Kline, M. Gonzalez, G.D. Yancopoulos and D.J. Glass. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell.* 14:395-403, 2004.

47. Wong, T.S. and F.W. Booth. Skeletal muscle enlargement with weight-lifting exercise by rats. *J Appl Physiol.* 65(2):950-954, 1988.
Figure 1

A.

B.
Figure 2

Bar chart showing Total RNA (mg/g) for different groups: Sham, ISO, CON, ECC. The chart indicates significant differences marked with asterisks (*) between the groups.
Figure 3

A. SHAM  ISO  CON  ECC

IGF-I mRNA / 18S

B. IGFBP-4 mRNA / 18S

C. Cyclin D1 mRNA / 18S

Right  Left  Contra  EX  Contra  EX  Contra  EX
Figure 4

![Graph showing Pro-collagen III α1 mRNA / 18S for different conditions: Sham, ISO, CON, ECC, Right, Left, Contra, EX. The graph includes error bars indicating variability.](image)
Figure 5

A.

B.
Figure 6

A. | Sham | ISO | CON | ECC |
--- | --- | --- | --- | ---
IL-6 mRNA / 18S

B. | Right | Left | Contra | EX | Contra | EX | Contra | EX |
--- | --- | --- | --- | --- | --- | --- | ---
Socs-3 mRNA / 18S