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Concurrent Exercise on a Gravity-Independent Device during Simulated Microgravity

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Abstract

Purpose—To examine the effect of a high-intensity concurrent training program utilizing a single gravity-independent device on maintaining skeletal muscle function and aerobic capacity during short-term unilateral lower limb suspension (ULLS).

Methods—Nineteen subjects (10 male; 9 female; 21.0 ± 2.5 yr, 65.4 ± 12.2 kg) were separated into 2 groups: 1) 10 day unilateral lower limb suspension only (ULLS; $n = 9$) and 2) 10 day ULLS plus aerobic and resistance training (ULLS+EX; $n = 10$). Exercise was performed on a single gravity-independent Multi-Mode Exercise Device (M-MED) with alternating days of high-intensity interval aerobic training and maximal exertion resistance training.

Results—Aerobic capacity increased by 7% in ULLS+EX ($P < 0.05$). Knee extensor and ankle plantar flexor three repetition max increased in the ULLS+EX group ($P < 0.05$) but this change was only different than ULLS in the plantar flexors ($P < 0.05$). Peak torque levels decreased with ULLS but were increased for the knee extensors and attenuated for the ankle plantar flexors with ULLS+EX ($P < 0.05$). A shift towards type IIx myosin heavy chain mRNA occurred with ULLS and was reversed with ULLS+EX in the vastus lateralis ($P < 0.05$) but not the soleus. Myostatin and atrogin increased with ULLS in both the vastus lateralis and soleus but this change was mitigated with ULLS+EX only in the vastus lateralis ($P = 0.0551$ for myostatin; $P < 0.05$ for atrogin). Citrate synthase was decreased in the soleus during ULLS but was increased with ULLS+EX ($P < 0.05$).

Conclusion—These results indicate that an M-MED class countermeasure device appears to be effective at mitigating the deconditioning effects of microgravity simulated during a modified-ULLS protocol.

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Keywords

flywheel; mRNA; skeletal muscle function; soleus; space flight; unilateral lower limb suspension

INTRODUCTION

Space exploration requires astronauts to endure an environment that results in rigorous deconditioning in a number of key physiological systems. It is well known that two of the primary systems negatively influenced by microgravity include the musculoskeletal and cardiovascular systems. In regards to skeletal muscle, reductions in muscle breakdown can be detected within days in a microgravity environment (46) which may lead to further decreases in muscle function (26) often observed in the antigravity muscles, or those muscles that play a postural role in standard gravity conditions (18). Specifically, 17 weeks of bed rest showed the greatest regional changes in muscle mass predominantly in the lower limb muscles (30). Additionally, maximal oxygen uptake may be reduced by as much as 25% with exposure to a microgravity environment impacting time intensive physical tasks (31). Considering these adaptations, concurrent exercise training programs consisting of both aerobic exercise (AE) and resistance exercise (RE) will need to be employed. With future plans for space missions in duration of 1 year or longer, such as extended missions to the International Space Station (ISS) and ultimately, a manned mission to Mars, designing optimal exercise countermeasures for mitigating physiological deconditioning will be essential for astronaut performance and health.

Although a considerable amount of research has been conducted in the last decade, there are still challenges that need to be resolved regarding the development of successful exercise countermeasures for space flight (4, 11, 39, 45, 48). This is true especially for missions of 6 months or longer where current exercise countermeasures have had some success in protecting against muscle mass and performance loss in the quadriceps but are lacking in other musculature (28, 29, 47, 52). Moreover, the complexity of designing optimal exercise countermeasures for space flight is clear when considering the need for the integration of both cardiorespiratory and musculoskeletal systems, time efficient exercise strategies, adequate muscle loading and constraints on hardware mass, volume and power consumption.

Vigorous AE is a necessary component for a countermeasure program, even in the short-term, with decreases in maximal exercise capacity being reported in as little as 17 days of space flight or bed rest (15). Cycle ergometry, which has been implemented in-flight, has generally been sufficient in the maintenance of aerobic capacity but is ineffective for preserving musculoskeletal health (13). Exercise utilizing a motorized treadmill has also been performed but impact loading may be limited and discomfort with the harness system used to secure the crewmember has been reported (19). Rowing may be a feasible alternative to cycling or treadmill exercise due to the usage of large postural muscles in both the upper and lower body. Recently, it has been reported that rowing exercise on a device antecedent to the one utilized in the current study (referred in the authors' manuscript as the Resistance and Aerobic Device; RAD) offers cardiovascular and metabolic responses equivalent to

indoor rowing on a commercial rowing ergometer (44). To date, much of the flight crew aerobic training program has focused on lower to moderate-intensity exercise for durations of up to 2 hours of daily exercise which may limit the time available for completing other required tasks and decrease exercise compliance.

The Advanced Resistive Exercise Device (ARED), currently being utilized on the ISS, has shown equivalent changes in muscular strength and volume to that of exercising with free weights in ambulatory subjects (32). This device uses pneumatic cylinders to provide a constant force throughout the range of motion and has resulted in musculoskeletal effects that were not significantly different than training with a flywheel device (32). Although showing potential as a countermeasure for losses in muscular strength and volume, the ARED is lacking an AE component and requires a considerable amount of space.

Integration of both AE and RE has been commonly referred to as concurrent training (12) and is a required characteristic for an exercise countermeasure protocol. Although the classic paper by Hickson (25) is often referenced in regards to an interference effect of AE on strength development with concurrent training, recent studies have reported otherwise. Lundberg and colleagues (34) observed increased in vivo muscular strength and power with concurrent training equivocal to that of resistance training only but was accompanied with greater amounts of hypertrophy. Additionally, the authors examined the basal expression of several genes related to angiogenesis, mitochondrial biogenesis, and protein turnover but showed no differences in gene expression following the 5 week training period (34). Further examination of gene expression during the initial periods of training may provide insight into the early cellular responses to training that lead to the resultant phenotypic adaptations. To date, no single exercise device has been employed to address both the cardiovascular and musculoskeletal components associated with spaceflight. Additionally, spacecraft constraints such as volume, weight and energy consumption as well as minimal time requirements on the device should be considered in this specialized area of interest.

Previously, an exercise device utilizing inertia produced by rotating flywheels has successfully maintained or increased muscle volume and strength during ambulation (43) and unilateral lower limb suspension (ULLS) (45) after a 5-week knee extensor resistance training regimen. The device also proved successful at maintaining or increasing muscular strength during 110 days of simulated space station confinement (2). Quadriceps muscle use during a squat on this device was equivalent to that of a squat using free weights in a gravity environment (36). The current study expands on this knowledge by utilizing a rotational flywheel device that allows for both high-intensity AE and RE eliminating the need for multiple exercise devices. Furthermore, the device is relatively light, low in volume, and requires no energy source. Given this background, we tested two hypotheses: 1) A concurrent training protocol consisting of high-intensity AE coupled with maximal effort RE would be able to maintain muscular function and aerobic capacity during ULLS and 2) that this exercise protocol would be effective at maintaining or improving molecular markers related to growth and atrophy.

METHODS

Subjects

A total of 19 subjects (10 male; 9 female; 21.0 ± 2.5 yr, 65.4 ± 12.2 kg, 167.0 ± 9.2 cm) were recruited to participate in the study. Subjects provided written consent prior to their participation and were block-randomized into two groups consisting of: 1) 10 day unilateral lower limb suspension only (ULLS; n = 5 male, 4 female; 21.0 ± 1.9 yr, 62.4 ± 9.2 kg, 166.8 ± 6.7 cm) and 2) 10 day ULLS plus AE and RE (ULLS+EX; n = 5 male, 5 female; 21.0 ± 3.0 yr, 68.0 ± 14.3 kg, 167.1 ± 11.4 cm). Subjects initially attended several preliminary sessions to be familiarized with the exercise and testing equipment and to be properly instructed on the ULLS protocol and safety precautions. Following familiarization, subjects attended six additional sessions for pre-testing (Figure 1). Day one consisted of the needle biopsy procedure for the vastus lateralis and soleus muscle samples. Days two and three included isokinetic and fatigue testing of the knee flexors, extensors, and ankle plantar flexors. Day four tested unilateral three repetition max (3RM) utilizing a leg press and calf raise movement. Three repetition max testing was repeated on day five to ensure that maximum strength levels were obtained. Peak oxygen consumption (VO_{2peak}) on a cycle ergometer was completed on day six. Ten consecutive days of ULLS were then carried out following the pre-testing phase with the ULLS+EX group participating in daily exercise training. Post-testing followed the same schedule as pre-testing with the exception that 3RM testing was performed only once. Subjects continued ULLS throughout the post-testing procedures. The study protocol was approved in advance by the University of California, Irvine Institutional Review Board.

Exercise Device Instrumentation and Configuration

The Multi-Mode Exercise Device (M-MED) design is based on the principle of rotational inertia and can be configured for both AE and RE modes. The M-MED is a variant of the RAD device described previously by Tesch et al (44). Three flywheels (diameter 44 cm, 2.5 kg each) with a total inertia of 0.1105 kg-m^2 are accelerated by shortening muscle contractions that unwind a strap connected to a shaft. At the end range of motion, the strap rewinds due to the rotating flywheels while the subject actively contracts during muscle lengthening providing an eccentric overload. The movement is then repeated until the desired repetitions are completed. Resistance training on the M-MED consisted of both horizontal squats and calf raises. Subjects were positioned supine with hips and knees flexed for the beginning position of the horizontal squat (Figure 2A). Calf raises required the subject to lie prone with the ankle in a dorsiflexed position (Figure 2B). After the initial position was obtained, subjects were instructed with strong verbal encouragement to maximally attempt a pushing action by extending at the knee or ankle joint. The M-MED device accommodates the loss in muscle force throughout the exercise set allowing for maximal exertion for each subsequent repetition given the fatigue state of the subject. Force, power, and electromyographic activity have previously been published for supine squats and calf raises with a previous iteration of the device (2–4, 7, 8, 36). In the aerobic training mode, the M-MED was configured for rowing exercise in which the flywheel was accelerated against varying magnetic resistance during the pulling motion (Figure 2C). The magnetic resistance was adjusted to achieve target heart rates (discussed below). Further

information on device configuration for AE including exercise biomechanics and cardiovascular responses has been previously reported (44).

Training Protocol

The ULLS+EX group completed both AE and RE during the ULLS period. Training alternated on a daily basis between AE and RE equaling five sessions for each mode of exercise. Aerobic exercise consisted of high intensity interval training with the MMED in the rowing configuration. Subjects warmed up for five minutes increasing intensity to reach a heart rate equivalent to 50% VO_{2peak}. Following the warm-up period, subjects were required to perform a four minute bout at a heart rate equivalent to 90% of VO_{2peak}. Immediately after, the subjects exercised for another four minutes at a lower intensity equivalent to 50% of VO_{2peak}. This sequence of high and low intensity intervals was repeated three more times such that each subject completed four total sets. Subjects were encouraged to reach their target heart rate as quickly as possible and to maintain that heart rate for the interval duration. Resistance exercise consisted of a five minute warm-up on a cycle ergometer. After warm-up, subjects completed four sets of seven repetitions of horizontal squats followed by six sets of fifteen repetitions of calf raises with two minute rest periods between sets. Subjects were given strong verbal encouragement to provide maximal exertion during both the shortening and lengthening phases of the exercises.

Unilateral Lower-Limb Suspension (ULLS)

Unilateral lower-limb suspension was implemented utilizing the modified strap-free model as previously described (37). In brief, subjects were required to perform all ambulatory activities on crutches while wearing shoes with a 5 cm modified sole on the right foot. This prevented the left foot from ground contact thereby unweighting the left leg (unloaded leg). The weight of the subject was supported by the crutches and the right leg (loaded leg). All ambulatory activities were performed on crutches 24 hours/day, 7 days/week, until the final day of post-testing (Figure 1). Familiarization sessions for techniques regarding the proper use of crutches were performed prior to the start of the ULLS period and included instructions about walking, stair ascent/descent, getting in and out of a seated position, and making transitions from surfaces with different levels of firmness. Subjects were interviewed daily by an investigator to monitor compliance. Over the entire period of the study, subjects had an average of 11.89 ± 7.54 instances where the suspended leg came into contact with the ground ranging from a light tap of the foot to partial body weight.

Aerobic Capacity

Aerobic capacity was assessed by measuring VO_{2peak} during an incremental exercise protocol on an electronically braked cycle ergometer (SensorMedics, Ergoline 800S Yorba Linda, CA). Work rate was continuously increased by 20 W per minute until the subjects reached volitional fatigue. Subjects were required to pedal between 60 and 80 rpm. Gas exchange was measured breath-by-breath by an Encore VMax metabolic cart (SensorMedics, Yorba Linda, CA). Heart rate and ECG were monitored and recorded throughout the test. Ratings of perceived exertion were assessed every three minutes.

Muscular Strength

Strength testing was conducted on a leg press machine (HF-4357 Leg Press, Hoist Fitness, San Diego, CA). This included the leg press for knee and hip extension strength and calf raises for plantar flexion strength. Subjects warmed up on the cycle ergometer for approximately five minutes. A moderate weight derived from the familiarization sessions was then used as a specific warm-up before 3RM attempts were made. A 3RM test was chosen due to previous recommendations for utilizing a 3RM versus a 1RM for reducing injury risk in lesser experienced individuals (5, 42). Valid repetitions were counted when subjects hit a pre-marked depth of approximately 1.57 rad of knee flexion for leg press and a pre-marked height determined by the end range of motion for the calf raise. Weight was then subsequently increased until subjects were unable to achieve three repetitions at a given weight. Rest periods of at least one minute were given between attempts consistent with literature for squat and bench press strength testing (35, 51). If three repetitions could not be performed, the previous successful attempt was considered the 3RM. Subjects obtained their 3RM after an average of 5.06 ±1.97 attempts across all testing sessions.

Determination of the torque-velocity relationship and assessment of a fatigue profile was performed using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY). Seated knee extension (KE) and knee flexion (KF) were executed with the hips at 1.57 rad, hands holding on to anchored hand holds and straps used to fix the upper torso, thigh, and ankle. Maximal KE and KF torques were measured at angular velocities of 0.52, 1.05, 1.57, 2.09, 3.14, 4.19 and 5.24 rad·s^{-1}. Isometric KE and KF were assessed with the knee fixed at 1.05 rad for five second trials with five second rest periods. Ankle plantar flexion (PF) was performed in the supine position with the knees and hips flexed at 2.09 rad. Maximal ankle PF torques were measured at angular velocities of 0.52, 1.05, 1.57, 2.09, 2.62, 3.14, 3.67, and 4.19 rad·s^{-1}. All assessments were performed through the entire range of motion. Five trials were conducted for shortening contractions and three trials for isometric contractions. Peak torque was measured at each velocity.

The fatigueability of the KE and KF was determined by having the subjects perform three sets of thirty maximal repetitions at 3.14 rad·s^{-1} and 2.09 rad·s^{-1} for KE and KF, respectively. Each set was separated by a 1 min rest interval. Verbal encouragement and visual feedback from the Biodex software (Rev. 3.44, 2009) was provided. Peak torque values were averaged for every five repetitions to give six time points per set.

Muscle Biopsies

Percutaneous muscle biopsies were obtained from the left lower limb before and after the ULLS period using a suction-modified needle biopsy technique (14). In brief, following the injection of local anesthetic (2% Lidocaine), a 5–6 mm incision was made before muscle samples were collected from the vastus lateralis and soleus using a 6G × 4¾" U.C.H. biopsy needle (Cadence Science, Lake Success, NY). Tissue for histology was mounted on cork and quickly frozen in isopentane pre-cooled with liquid nitrogen. The remaining tissue was immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

RNA Extraction and RT-PCR

Total RNA was extracted from pre-weighed frozen muscle samples using the Tri Reagent according to the manufacturer's protocol (Molecular Research Center, Cincinnati, OH). The extracted RNA pellet was suspended in a known volume of nuclease-free water (1 μl/mg tissue). The RNA concentration was determined by OD 260 in a Nanodrop spectrophotometer (using the conversion factor of 40 μg/ml per 1 unit OD 260). The RNA samples were stored frozen at −80°C for subsequent analyses for specific gene expression using an end point RT-PCR approach. Prior to cDNA synthesis, RNA integrity was checked by electrophoresis of 400 ng total RNA on a 1% agarose gel stained with Gelgreen stain (Biotium, Hayward, CA) and was found to be good quality RNA based on intact 28S and 18S bands.

One microgram of total RNA was reverse transcribed into cDNA for each sample using the SuperScript II RT (Invitrogen, Grand Island, NY) and a mix of oligo dT (100 ng/reaction) and random primers (200 ng/reaction) in a 20-μl total reaction volume at 44°C for 50 min according to the provided protocol. At the end of the reverse transcription reaction, the tubes were heated at 90°C for 5 min to stop the reaction and then were stored at – 80°C until used in the PCR reactions for specific mRNA analyses.

Specific PCR primers were designed using PrimerSelect software (Lasergene, DNASTar, Madison, WI) and the reference mRNA sequence from NCBI GenBank (see Table, Supplemental Digital Content 1, PCR primer sequence and information). The forward and reverse primers were designed on different exons separated by large introns so that GDNA product will separate from the cDNA PCR product. Primers were purchased from Operon Biotechnologies (Huntsville, AL). For each target, the PCR reactions were carried out using 0.1 to 1 μl cDNA/reaction (corresponding to 5 to 50 ng of total RNA) in the presence of 2 mM $MgCl_2$ by using standard PCR buffer (Bioline, Tuanton, MA), 0.2 mM dNTP, 1 μM specific primer set, and 0.75 unit of Biolase DNA polymerase (Bioline, Taunton, MA) in 25 μl total volume. For each primer set, PCR conditions (cDNA dilution and PCR cycle number) were set to optimal conditions so that the target mRNA product yields were in the linear range of the semilog plot when the yield is expressed as a function of the number of PCR cycles and for a given condition target mRNA PCR yields were tightly correlated to input cDNA (9).

Amplifications were carried out in a Stratagene Robocycler with an initial denaturing step of 3 min at 96°C, followed by 24–27 cycles of denaturing 1 min at 96°C, annealing 1 min at 59°C, extending 1 min at 72°C and a final extension 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, Fairfield, CT) on digitized images and were reported as arbitrary scan units as reported previously (11). In this approach, each specific mRNA signal is expressed in arbitrary units (AU) per ng of total RNA. The PCR signal representing a specific mRNA expression was normalized to tissue weight by using total RNA concentration to generate the calculation. Reporting RNA expression per unit muscle weight is preferred since the total RNA concentration may vary in muscle tissue in response to training or other activity

paradigms, whereas the traditional internal controls such as GAPDH and large ribosomal proteins were shown to vary in muscle subjected to different activity paradigms (24).

MHC mRNA Isoform Distribution

Myosin heavy chain mRNA composition was determined by competitive PCR on the cDNA using a synthetic DNA fragment as control, common forward primers for all the MHC mRNAs and an isoform-specific reverse primer. In this method, the MHC mRNA is expressed as a % of the total MHC mRNA pool. The synthetic DNA fragment was built using the same base DNA backbone as used previously (1, 16). An overlapping PCR approach was used to extend the DNA backbone with the specific primers for the human MHC mRNA. At the 5' end of the control fragment the common forward primer for the rat was replaced with a common primer for the human MHC mRNA. Similarly, at the 3'end of the control fragment, the DNA was extended stepwise with primers that are complementary to 3'UTR of specific MHC mRNA in the order of type I, IIa, IIx, and IIb MHC respectively. The common primer corresponds to a highly conserved sequence from exon 37 and is 100% identical in all MHC mRNA isoforms including developmental and extraocular. The PCR was performed on a 1 μl of 1 to 80 dilute cDNA in the presence of 2 attomoles of the control fragment for 25 cycles in the presence of 2 mM $MgCl_2$, 15 pmoles of PCR primers and using Biolase DNA polymerase (Bioline, Taunton, MA).

At the end of PCR, PCR products were separated by electrophoresis on a 2.5% agarose gel stained with SYBR green. At the end of the run, the UV exposed gel image was taken using a digital camera and the digitized image was used in quantification of the density of the bands using ImageQuant software. For each sample, the cDNA band was normalized to the corresponding control fragment and this ratio was used to determine the relative percent of the total MHC mRNA expression (I+IIa+IIx). Note that under the PCR conditions used, the IIb MHC mRNA was not detected in any of the samples.

Statistical Analyses

Two-way ANOVAs (group and time as main factors) with repeated measures were used to analyze the VO_{2peak}, 3RM and fatigue data. For the torque-velocity relationship, post-ULLS values were expressed as a percentage change from pre-ULLS values and then analyzed using a two-way ANOVA (group and angular velocity as main factors) with repeated measures. Post-ULLS values obtained from PCR were expressed as a percentage change from pre-ULLS values and then analyzed using a student's paired t-test. Data points and variance are reported throughout as mean ±SD. Statistical significance was defined as P 0.05 and all analyses were performed with GraphPad Prism 6.01 (GraphPad Software Inc, La Jolla, CA).

RESULTS

Aerobic Capacity

Exercise training (ULLS+EX) for 10 days incorporating 5 days of high intensity interval training showed a trend ($P = 0.0533$) for an increase in VO_{2peak} by 7% (data not shown). No change was seen in the ULLS group.

Muscular Strength and Fatigue

Muscular strength data obtained from 3RM testing is shown in Figure 3. Leg press 3RM increased in the ULLS+EX group ($P = 0.0327$). However, 10 days of ULLS alone did not affect leg press 3RM. A significant interaction effect ($P = 0.0002$, F ratio = 21.86) for the calf raise 3RM demonstrated that exercise training was effective at preventing the 9.5% loss in strength from unloading with a 9.8% increase in 3RM.

Knee flexor, knee extensor, and ankle plantar flexor torque-velocity relationships are presented in Figure 4 (A–C). ULLS decreased peak torque levels for the knee flexors, extensors and ankle plantar flexors. Exercise increased peak torque for the knee extensors ($P = 0.0105$, F ratio = 6.734) while attenuating the loss in the ankle plantar flexors ($P = 0.0145$, F ratio = 6.119). It should be noted that although exercise attenuated the decrease in overall torque for the plantar flexors, a decrease of 3.5% was still experienced. Exercise training elicited a 7% increase in knee extension muscular endurance, shown by an interaction effect ($P = 0.0030$, F ratio = 12.095), whereas there was no change with unloading (Figure 4D and 4E).

Muscle mRNA and Morphological Analysis

Changes in MHC mRNA expression are shown in Figure 5 for the vastus lateralis and soleus muscles. Exercise training reversed the MHC mRNA expression patterns with unloading by increasing type I ($P = 0.0333$) and type IIa ($P = 0.0092$) MHC mRNA while decreasing type IIx ($P = 0.0012$) in the vastus lateralis. Alternatively, exercise training did not provide a sufficient stimulus to impact MHC mRNA expression in the soleus.

Sarcomeric and Growth/Atrophy Molecular Markers

A summary of the mRNA analysis for sarcomeric and molecular markers affecting growth and atrophy is shown in Figure 6. Total RNA concentration, a marker of translational capacity, increased in the ULLS+EX group only for both the vastus lateralis ($P = 0.0096$) and soleus ($P = 0.0055$). Myostatin and atrogin are both considered negative regulators of muscle mass. A trend towards a decrease in myostatin mRNA expression was seen with ULLS+EX in the vastus lateralis ($P = 0.0551$) with no difference in the soleus. It should be noted that myostatin expression appears to show an exaggerated increase in the soleus compared to the vastus lateralis in response to unloading ($P = 0.0064$). Similarly, unloading increased the expression of atrogin in both muscles yet exercise training blunted this response in the vastus lateralis ($P = 0.0265$) with a similar trend in the soleus ($P = 0.0847$). No group differences were seen in the expression of mRNA for the structural proteins actin, titin, and nebulin in both the vastus lateralis and soleus. Unloading decreased citrate synthase mRNA expression, an essential protein in the citric acid cycle, in the soleus ($P = 0.0059$) but not the vastus lateralis. Exercise training was able to increase citrate synthase mRNA expression levels but was only significantly different than unloading in the soleus ($P = 0.0235$).

DISCUSSION

Numerous studies have established that microgravity has profound deleterious effects across a broad range of physiological parameters, in particular, those associated with both the cardiovascular and musculoskeletal systems. A number of countermeasures have been developed to mitigate the cardiovascular and musculoskeletal deconditioning that occurs in microgravity, but virtually every countermeasure system / modality suffers from one or more of the following limitations: *i)* benefits only one physiological system; *ii)* physiological benefits do not fully mitigate effects of microgravity; *iii)* large upload mass; *iv)* large volume requirement; and/or *v)* requires power.

M-MED class devices appear to represent an overall superior design compared to other countermeasure devices studied to date (see Table, Supplemental Digital Content 2, Key physiological and engineering considerations for exercise countermeasure devices to be utilized in spaceflight). From an overall engineering perspective (i.e., volume, mass, and power requirements), M-MED class devices appear to be approximately equivalent to that of treadmills and cycle ergometers (two devices with the smallest volume and mass requirements). From a physiological perspective, M-MED and artificial gravity (AG) class countermeasure devices clearly have greater potential (as compared to treadmills, cycle ergometers, or lower body negative pressure) to effectively act as a countermeasure to microgravity related declines in structure and function. AG class devices such as the human powered Space Cycle (27) can be used to impose unique stresses on the vestibular, cardiovascular, and musculoskeletal systems, with the potential to produce increases in aerobic capacity, strength across a broad spectrum of muscles, and bone mineral density. M-MED class devices also have a similar potential. Importantly, it should be stressed that M-MED class devices do not have the large volume requirements needed for AG class devices. Therefore, from a practical perspective, it seems like M-MED class devices may represent a superior countermeasure platform when engineering requirements and physiological impact are considered in total.

Given this background, we performed a proof-of-principle study that examined whether a low volume concurrent training program (i.e., resistance + aerobic training) using an M-MED class device would be effective in mitigating the loss of muscle function as induced by simulated microgravity (i.e., ULLS). There are several key findings to this study. First, concurrent exercise training (i.e., resistance + aerobic training) on the M-MED device positively impacted muscle function while improving aerobic capacity. Second, we observed that the effects of the concurrent exercise training program collectively (muscle function + cellular/molecular changes) were more effective in mitigating the deconditioning effects of ULLS for the knee extensors as compared to the plantar flexors. Third, key markers of muscle atrophy were positively influenced by the resistance training program. Fourth, the concomitant aerobic interval aspect of the training program produced a 7% increase in $VO2_{peak}$ and a significant increase in citrate synthase mRNA levels. Collectively, these findings support the concept that M-MED class devices can be used to benefit multiple physiological systems.

Current scientific evidence suggests that the plantar flexor muscle group is both more sensitive to unloading and more refractory to countermeasures as compared to the knee extensor muscle group. Consistent with this perspective, we observed that the functional effects of ULLS were more pronounced for the plantar flexors as compared to the knee extensors. For instance, we observed that the torque velocity curve of the knee extensors was reduced by approximately 4%, while that of the plantar flexors was reduced by approximately 10%. A similar trend was also evident for the 3RM data. It should be noted that the decrease in 3RM for the plantar flexors that resulted from ULLS was reversed with exercise training whereas the decrease in torque with isokinetic testing was only mitigated. These differences may be a result of several factors. The knee angle utilized for each mode of testing was different with the knee angle being 0 rad for the 3RM and 2.09 rad for isokinetic testing. It is known that selective recruitment of the triceps surae muscles can occur through the manipulation of knee angles (23, 40). Additionally, isolation of the plantar flexors during isokinetic testing has previously been known to be difficult. Even with a specially designed torque velocity device to isolate the plantar flexors a 1.9 to 7.4% day to day variation was reported (49). In our lab, we attempted to isolate the calf muscles by having the subject lay supine with the knee flexed to 2.09 rad while strapping the upper torso, thigh, and foot securely to the machine. Surface electrodes for EMG were applied to the vastus lateralis and lateral head of the gastrocnemius to provide verbal feedback on limiting quadriceps involvement during testing. Finally, by exercise training the plantar flexors, previously untrained individuals may better isolate the plantar flexors post-training thereby reducing overall torque production. Regardless of the differences between modes of testing, we demonstrate similar positive responses to exercise training with the plantar flexors as the knee extensors.

Importantly, it should be noted that the M-MED training program used in the current study produced beneficial functional effects for both the knee extensors and plantar flexors. Both muscles groups had improved 3RM and torque-velocity performance. Interestingly however, at the cellular/molecular level, there was a differential response to the M-MED training program with the knee extensors appearing to be the most responsive relative to the plantar flexors with regard to putative regulators of muscle mass. A slow-to-fast transition in MHC occurs with unloading and has consistently been shown to occur with both bed rest and spaceflight (6). These changes are also consistent with those seen in rats during 10- and 14-days of microgravity with the slightly longer duration displaying fast-to-faster transitions (MHC IIa → MHC IIx → MHC IIb) (41). These fiber type shifts may contribute to changes in performance capacity. Following the classic slow-to-fast fiber type transition associated with unloading/disuse, ULLS caused a similar increase in type IIx MHC mRNA expression with both the vastus lateralis and soleus muscles. In contrast, exercise training generally induces a fast-to-slow fiber type transition with an increase in type IIa fibers and a maintenance or increase in type I fibers (6). In the current study, concurrent exercise training was able to induce this change with the vastus lateralis but not the soleus. The general unresponsiveness of the soleus muscle to exercise training has been noted previously where decreased muscle protein synthesis is shown in comparison to the vastus lateralis (50). This response may be due to the high percentage of type I fibers that comprise the soleus (~65% type I MHC mRNA) versus the vastus lateralis (~30% type I MHC mRNA) although single-

fiber differences have been found when comparing fibers of the same MHC isoform (33). MHC I and MHC IIa fibers of the soleus were reported to have larger fiber diameters but lower specific tension than those found in the vastus lateralis (33). Regardless of the apparent mRNA differences in expression, no change in muscle CSA as measured by histological staining was seen with the vastus lateralis or soleus with 10 days of ULLS or ULLS+EX (data not shown). Previously, decreased muscle size as measured by MRI have reported varied outcomes ranging between 6 and 20% and may be due to the longer duration of unloading which was between 16 and 42 days of ULLS (22). The reader should be cautioned when making comparisons between morphometric analyses, as was done in the current study, and whole muscle CSA measured by MRI due to methodological differences (43). Given the phenotypic changes associated with longer durations of unloading, mRNA analysis may be useful in detecting the early responses to unloading. It should be noted that while we are reporting changes in mRNA expression, these could be the result of either transcriptional or posttranscriptional-pretranslational changes. Furthermore, these mRNA changes may or may not translate to changes at the protein level.

Further differences between the vastus lateralis and the soleus were evident with respect to markers of muscle atrophy and muscle growth inhibition. Atrogin is an E3 ubiquitin ligase selectively located in striated muscle that exhibits increased expression with conditions of disuse (10) whereas myostatin acts as a negative regulator of muscle mass through its effects on satellite cell proliferation/differentiation and the IGF-Akt and ubiquitinproteasomal pathways (17). Although both muscles experienced increases in myostatin mRNA expression during ULLS, it appears that exercise was only able to reverse this increase in the vastus lateralis (shown by a trend of $P = 0.0551$). Atrogin, on the other hand, was increased by ULLS and this increase was mitigated with exercise training in the vastus lateralis and to a lesser extent, the soleus ($P = 0.0847$). These follow similar patterns to data reported on exercise training utilizing artificial gravity created by a short-arm centrifuge during 21 days of bedrest (statistically nonsignificant) (11). Both the vastus lateralis and soleus may go through different time-dependent changes following unloading. Gustafsson et al. showed that it was the vastus lateralis, and not the soleus, incurring early increases in factors relating to protein degradation with the exception of myostatin (21). Taken together, these results suggest that the regulation of muscle atrophy during the early stages of unloading may encounter muscle-specific time-dependent changes. This warrants further investigation as early changes seen at the molecular level may be indicators for the longer term changes observed in muscle function.

One of the unique aspects of this study was examining the effects of ULLS and resistance training on the mRNA levels of key sarcomeric genes. In particular, we looked at the mRNA levels of actin, nebulin, and titin. As a first approximation, it seems reasonable to postulate that a down regulation of the transcription of sarcomeric genes mediates, in part, the atrophic response typically seen with mechanical unloading. However, we observed that mechanical unloading via ULLS did not affect the mRNA levels of any of these key sarcomeric genes nor did M-MED training. This suggests that at early time-points the mRNA levels of key sarcomeric genes are unaffected by mechanical unloading. Indeed, Reich et al. (38) examined the effects of 48 hours of ULLS and found no changes in any of

the key sarcomeric genes. In contrast to short periods of mechanical unloading, we observed that 21 days of bedrest study produced substantial losses in MHC and actin mRNA levels (11). Clearly, a comparison between early and intermediate periods of unloading has the potential to provide rich information about the control of key sarcomeric genes to both unloading and reloading of human skeletal muscle.

The aerobic component of the exercise training program utilized a high-intensity interval training approach. This type of training has been shown to be an effective alternative to traditional steady-state exercise showing similar or even superior physiological adaptations with lower time commitments (20). During the 10 day training period, subjects exercised aerobically for 5 days which amounted to a total of 160 minutes of aerobic training of which half was done at high intensity. This resulted in a trend for a 7% increase ($P = 0.0533$) in VO_{2peak} assessed by a graded cycle ergometer test. Although testing was not conducted unilaterally, the results obtained in a relatively short duration of time nevertheless exhibit the aerobic potential of the M-MED during the initial unloading period. This change was supported at the mRNA level through a mitigation of the unloading-induced decreases in citrate synthase found in the soleus.

Summary

In conclusion, the current study is a proof-of-principle study that examined the effectiveness of a countermeasure device allowing for both AE and RE training in mitigating the deconditioning effects of microgravity as simulated by a 10 day lower limb unloading protocol. This study produced four key findings. First, we found that a high-intensity, low-volume concurrent exercise program was able to show favorable changes in musculoskeletal and cardiovascular conditioning. Second, unloading provided typical slow-to-fast shifts in MHC mRNA expression that was mitigated by training in the vastus lateralis but not the soleus. Third, both atrogin and myostatin, molecular markers for the regulation of muscle atrophy, were positively manipulated with exercise training in the vastus lateralis but not the soleus. These results help explain the final key point that the soleus and the ankle plantar flexors are more sensitive to ULLS than the vastus lateralis and the knee extensors. The favorable results presented here show that a high intensity concurrent exercise training program can have a positive impact on musculoskeletal and cardiovascular conditioning. Nevertheless, the heightened sensitivity of the soleus to unloading interferes with the effects of exercise training and highlights the need for further examination for optimal exercise prescription to impact the plantar flexor muscle group. These results show that a concurrent high intensity resistance and aerobic interval training program using a single exercise device can positively affect the musculoskeletal and cardiovascular systems with short term ULLS. This provides the groundwork necessary to pursue future research examining the effectiveness of this device with longer duration unloading as might occur during extended space missions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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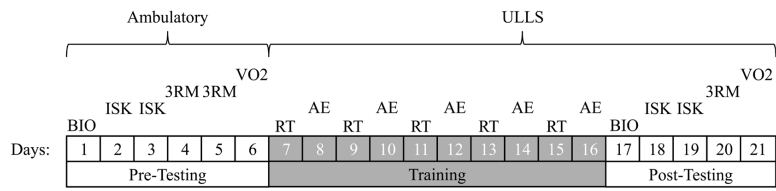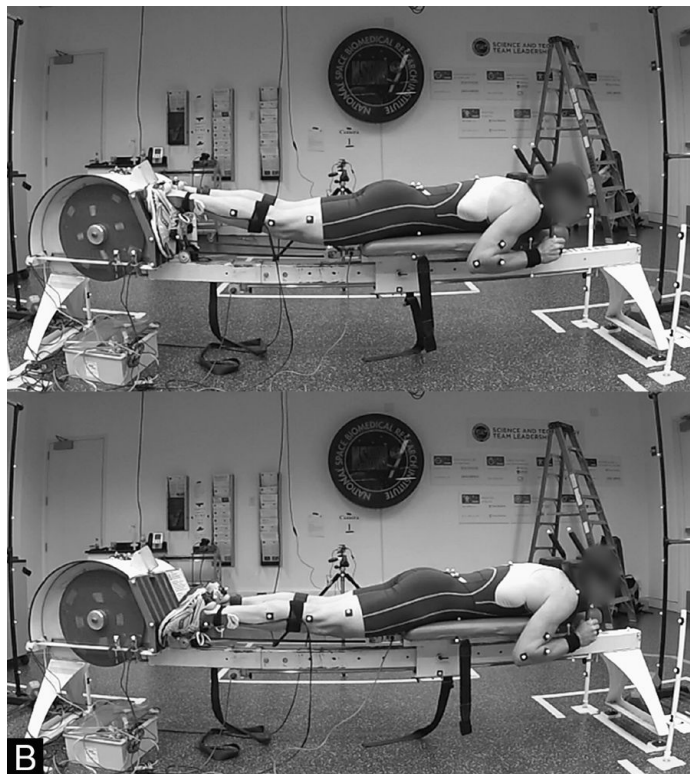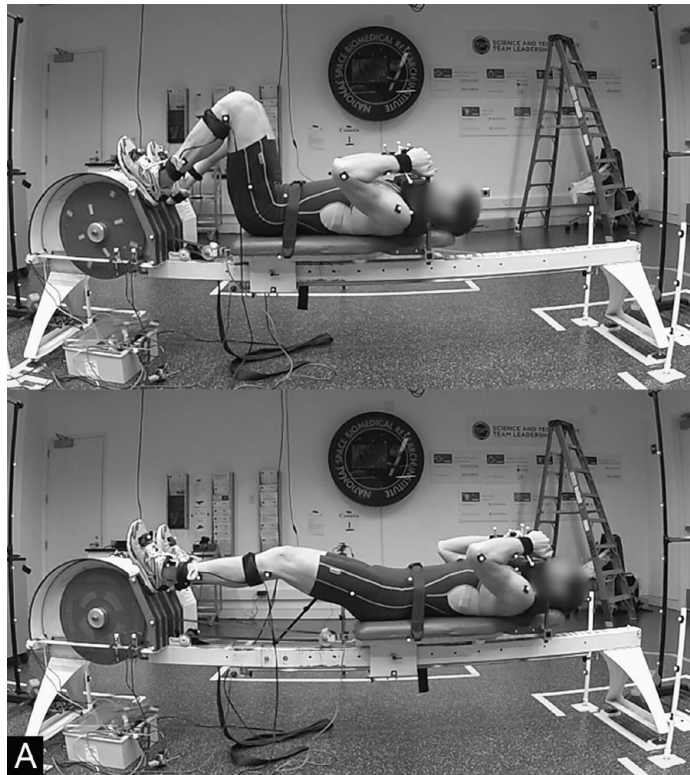
Figure 1.
Timeline of the study. BIO, biopsy; ISK, isokinetic testing; 3RM, three repetition max; VO_2, peak oxygen consumption; RT, resistance training; AE, aerobic training; ULLS, unilateral lower limb suspension.
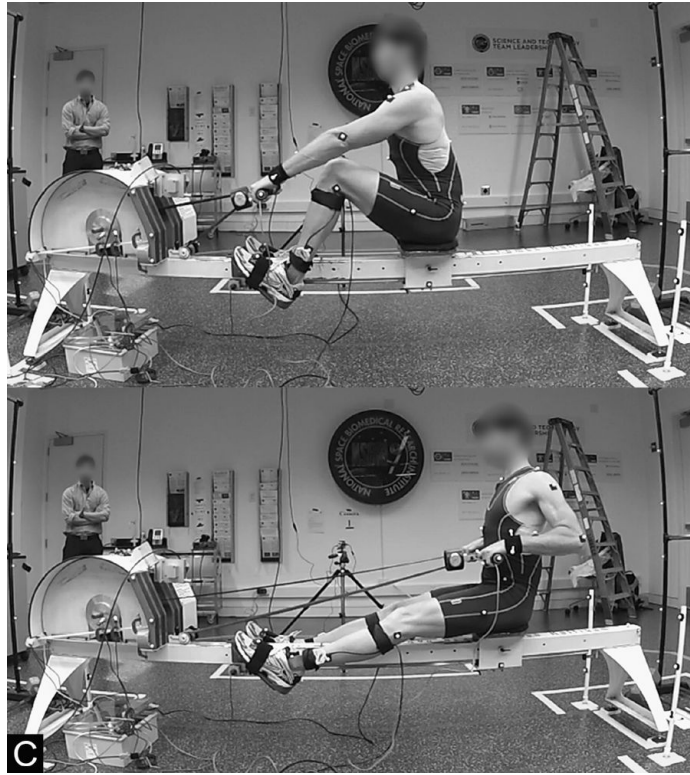
Figure 2.
M-Med Device configured for squats (A), calf raises (B), and aerobic rowing (C) displaying the start and end position for each movement. Photos courtesy of Lealem Mulugeta, Lead Scientist for NASA's Digital Astronaut Project.
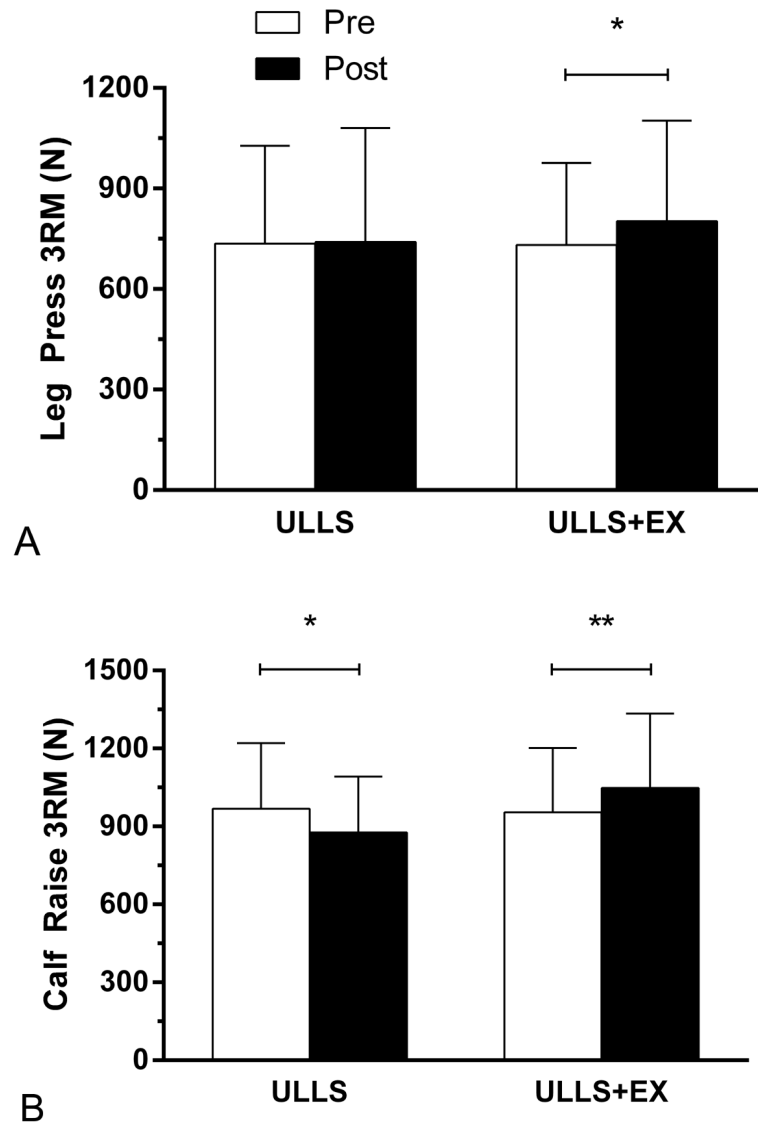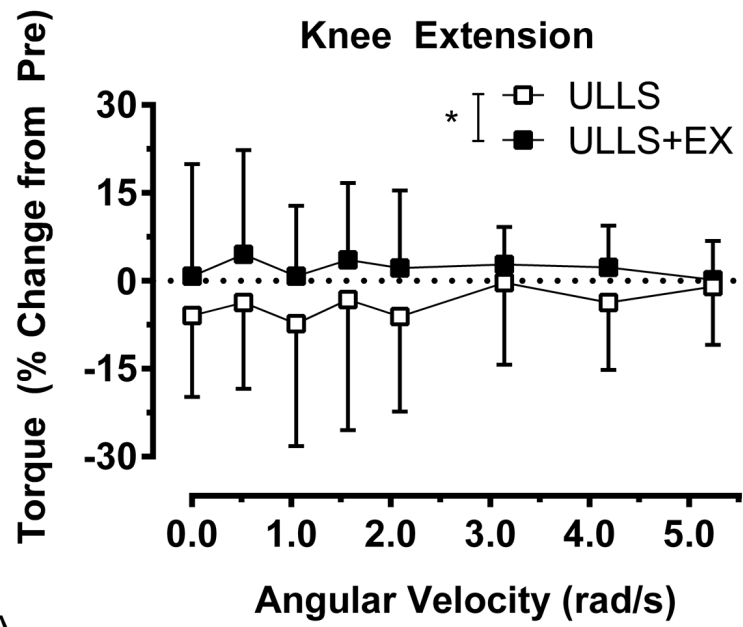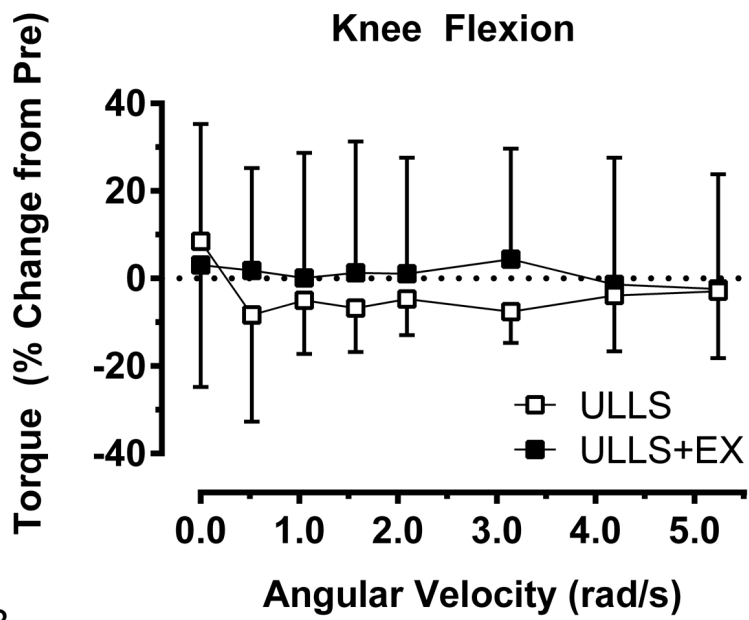
Figure 3.
Three repetition max values for the leg press (A) and calf raise (B) during ULLS or ULLS +EX. * Significant difference ($P < 0.05$) or ** ($P < 0.01$) between PRE and POST conditions.
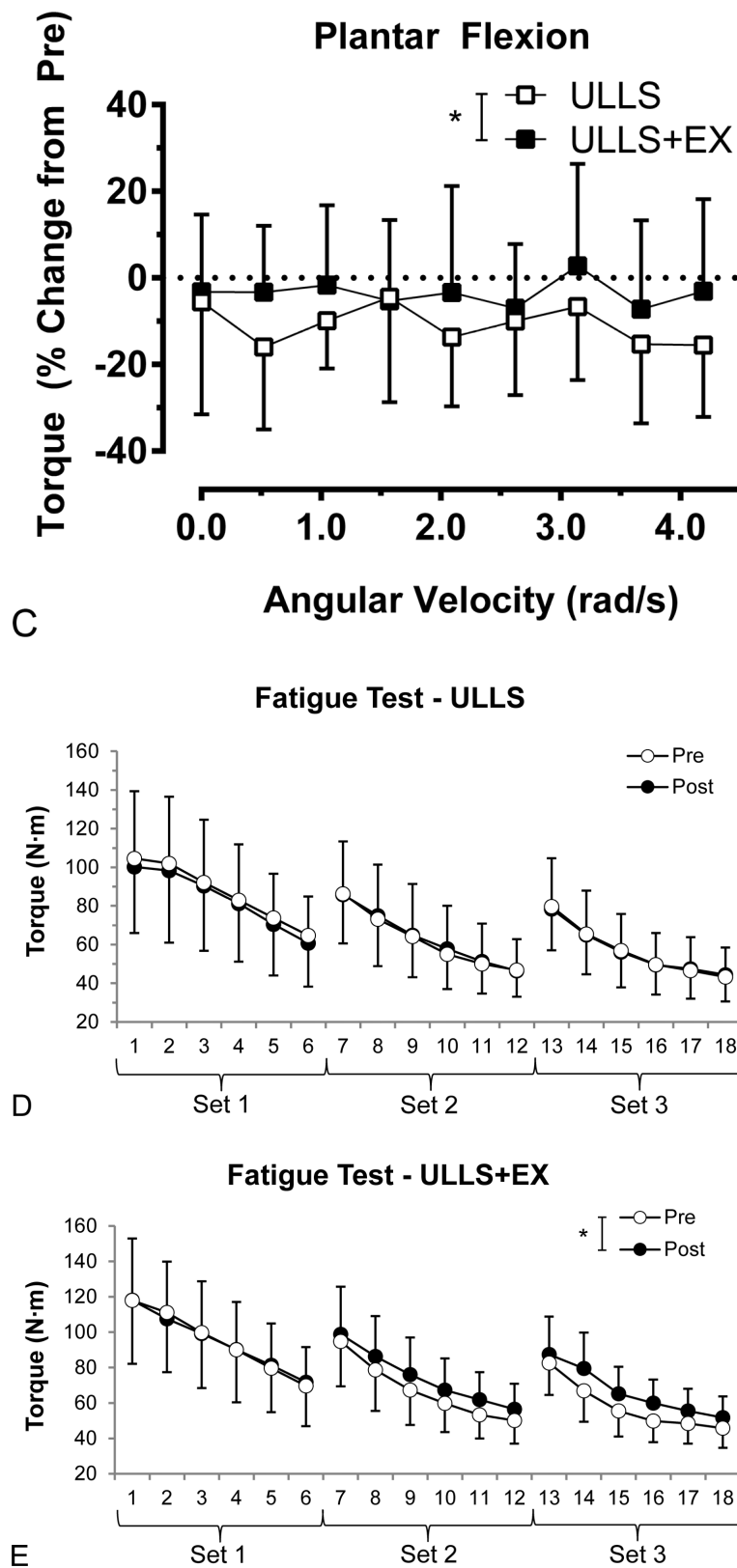
Figure 4.

Torque-velocity relationship for the knee extensors (A), knee flexors (B), and ankle plantar flexors (C). Significant group differences were found for the knee extensors and ankle plantar flexors only. Fatigue testing for the knee extensors is presented for ULLS (D) and ULLS+EX (E). * Significant difference ($P < 0.05$).
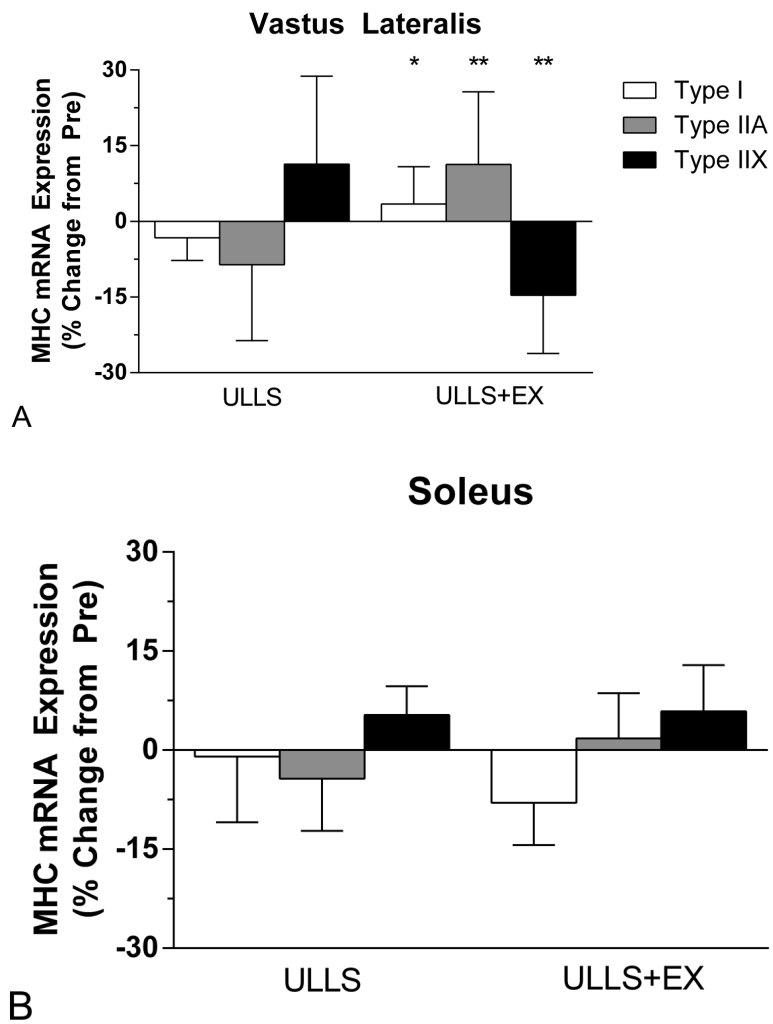
Figure 5.
Myosin heavy chain mRNA expression as a % change from PRE values for the vastus lateralis (A) and soleus (B). * Significant difference ($P < 0.05$) or ** ($P < 0.01$) between ULLS and ULLS+EX.
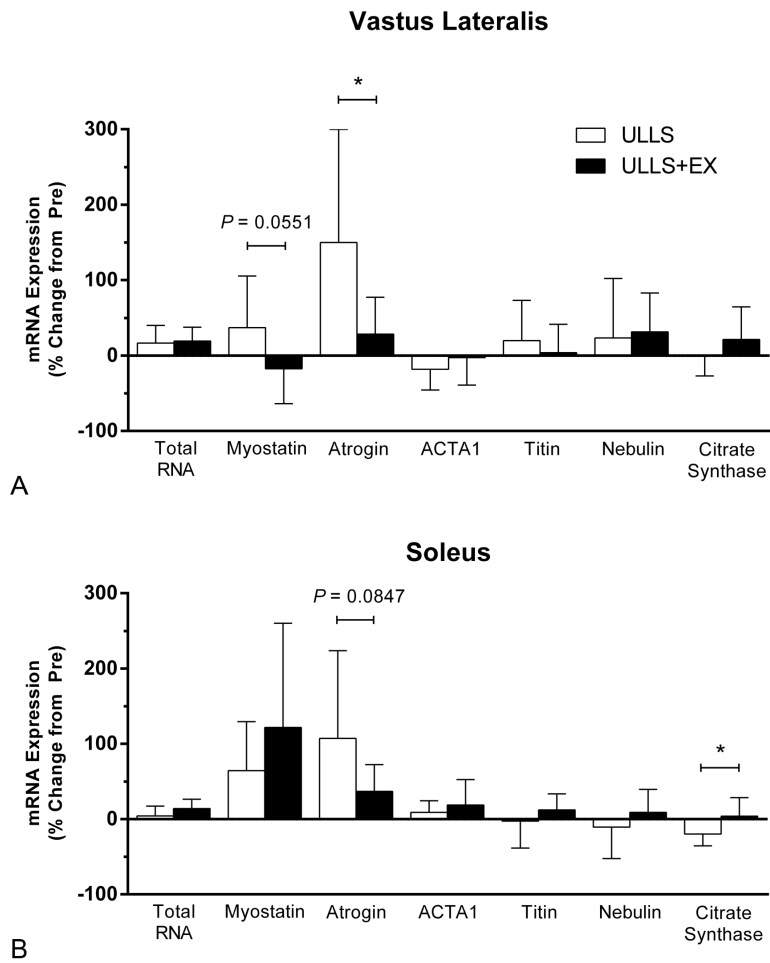
Vastus Lateralis

A

Soleus

B

Figure 6.
mRNA expression as a % change from PRE values for the vastus lateralis (A) and soleus (B) for selected markers of growth/atrophy and sarcomeric structural proteins. * Significant difference ($P < 0.05$) between ULLS and ULLS+EX.