LOW-VOLUME RESISTANCE EXERCISE ATTENUATES THE DECLINE IN STRENGTH AND MUSCLE MASS ASSOCIATED WITH IMMOBILIZATION

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ABSTRACT: We determined the effectiveness of low-volume resistance exercise (EX) for the attenuation of loss of muscle mass and strength during leg immobilization. Men (N = 5) and women (N = 12, age 24 ± 5 years, body mass index 25.4 ± 3.6 kg/m²) were divided into two groups: exercise (EX; n = 12) and control (CON; n = 5). Subjects wore a knee brace on one leg that prevented weight bearing for 14 days. Resistance exercise (EX; 80% of maximal) was performed by the immobilized limb every other day. Immobilization induced a significant reduction (P < 0.05) in muscle fiber and thigh cross-sectional area (CSA), isometric knee extensor, and plantarflexor strength in the CON (P < 0.01) but not in the EX group. There were significant losses in triceps surae CSA in the CON and EX groups (P < 0.05), but the losses were greater in CON subjects (P < 0.05). A minimal volume (140 contractions in 14 days) of resistance exercise is an effective countermeasure against immobilization-induced atrophy of the quadriceps femoris but is only partially effective for the triceps surae.

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The most conspicuous physiological adaptations to muscle disuse are atrophy and strength loss1 (for reviews see Yasuda et al.2 and Phillips et al.3). Both have a rapid onset and are evident after as little as 10–14 days of unloading.1,4–7 Identifying countermeasures to atrophy to attenuate loss of muscle mass and function is important, because even partial maintenance of muscle mass during disuse situations is beneficial during, for example, rehabilitation or during prolonged exposure to microgravity.5,8 The alleviation of atrophy has focused on exercise4,9–18 and nutritional countermeasures.5,19–23 Resistance exercise training that employs workloads known to elicit hypertrophy is successful in preserving quadriceps femoris muscle mass and function during short-term (<21 days) unilateral lower limb suspension14 and bed rest.10,12 Despite the ability of resistance exercise to successfully maintain muscle mass and strength of the quadriceps femoris, the triceps surae muscle group is less responsive to exercise and often undergoes atrophy.10,11,15,16

Exercise-based atrophy countermeasures have typically employed resistance exercise protocols similar to those used to induce hypertrophy4,9–18, thus, it is perhaps not surprising that atrophy is offset or at least diminished. Recent evidence suggests that brief high-intensity contractions may be all that is needed to induce genetic changes that offset atrophy.24 Thus, the aim of this study was to use a lower exercise volume than has been employed previously during 14 days of immobilization-induced atrophy in humans. We hypothesized that low-volume resistance exercise performed every other day would be sufficient to attenuate, or possibly prevent, loss of muscle cross-sectional area (CSA) and strength normally observed during 14-day immobilization.

METHODS

Subjects. Seventeen recreationally active (i.e., exercise ≤2 days/week) men (N = 5) and women (N = 12) volunteered to participate in our study. Subjects were randomly assigned with the goal of showing a significant decline in fiber/muscle size with the minimal number of subjects in the control group and the number needed to see no decline in mid-thigh CSA in the intervention group. For sample size in the intervention, we assumed a population variance of 20% in the atrophic response1 and coefficients of variation of 10% in magnetic resonance imaging (MRI). Thus, to detect a cross-sectional difference of 50% between groups (i.e., an attenuation of the atrophy by at least half), with 80% confidence, we required at least 7 subjects in the active group (Table 1). To protect power we recruited and tested 12 subjects. All subjects underwent 14 days of unilateral knee brace–mediated immobilization. Subjects were divided into a control (CON) group or a resistance exercise countermeasure (EX) group. Subjects were screened to exclude smokers, any person with lower limb injury within 1 year prior to the start of the study, or family history of thrombosis. The study was approved by the McMaster University and the Hamilton Health Sciences Research Ethics Boards and conformed to the 1983 Helsinki

Abbreviations: ANOVA, analysis of variance; CON, control; CSA, cross-sectional area; EMG, electromyography; EX, exercise; IEMG, integrated electromyography; ITT, interpolated twitch torque; MRI, magnetic resonance image; MUA, motor unit activation; MVC, maximal voluntary contraction; PTT, potentiated twitch torque; RE, resistance exercise; RM, repetition maximum

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Countermeasures for Disuse Atrophy

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Declaration on the use of human subjects in research. Informed written consent was obtained from each participant prior to commencement of the study.

**Experimental Protocol.** All subjects were familiarized with the techniques used for muscle strength and function testing at least 2 weeks prior to beginning the study. These included isometric maximal voluntary contraction (MVC) for knee extension and plantarflexion and maximally evoked twitch contraction using muscle stimulation electrodes. Subjects in the EX group participated in an additional familiarization session with the strength training equipment (Universal Gym Equipment, West Point, Mississippi). At this time, each subject’s voluntary single-repetition maximum (1RM) was determined for unilateral knee-extension, leg press (with plantarflexion at full extension), and seated calf-raise exercises.

All subjects completed a weighed 3-day food record (2 weekdays and 1 weekend day) before and at the end of the intervention to assess overall energy intake as well as a macronutrient intake. Food records were analyzed using the ESHA Food Processor SQL program (ESHA Research, Salem, Oregon).

The study consisted of 14 days of unilateral knee immobilization. The choice of the knee for immobilization was made in a randomized manner and balanced for dominance according to maximal isometric strength. Testing was performed before immobilization (PRE; 1 day prior to the start of the 14-day immobilization period) and after immobilization (POST; morning after 14-day immobilization). Measurements at each of these sessions included: thigh and leg CSA by magnetic resonance imaging (MRI); percent motor unit activation (%MUA); and isometric MVC for knee extensor and plantarflexor muscle groups using custom-built dynamometers, previously utilized by Hamada and colleagues. Both the MRI scan and the muscle biopsies were performed on the afternoon prior to the PRE testing session, because the subjects were immobilized on the morning of day 1. POST testing was conducted on the morning of day 15.

On the first day of immobilization, subjects arrived in the lab by 9:00 A.M. and were fitted with a knee-immobilization brace (IROM; DonJoy, Vista, California) and a set of crutches. The brace is actually designed to immobilize the knee joint only, and thus the subjects could freely move their ankle and hip joints. The brace was set to an angle of 60° of flexion so that subjects had complete toe clearance when ambulating using crutches, which they used for the entire immobilization period.

With this degree of knee flexion, subjects could not place weight on their immobilized leg, and the leg was effectively suspended. The Velcro strap of the brace was bound with plastic adhesive tape over which the investigators’ signatures were inscribed. Breaking the tape seal, so the brace could be adjusted or removed, would thus render the tape irreplaceable without damaging the signature, ensuring compliance with the immobilization procedures. Subjects returned to the laboratory daily, at which time they were permitted to remove the brace, under supervision, for approximately 10 minutes to allow for inspection by the investigators. Subjects had their legs and feet examined daily for signs of edema by observing leg color and looking for unusual vein patterns. Using palpation we checked for warmth, tenderness, and cords, and gently squeeze the calf muscle against the tibia to check for deep pain. We also had subjects dorsiflex the foot to look for Homán’s sign. Any signs of chafing or swelling were noted, and the brace was re-adjusted. Following visual inspection, the brace was reapplied and secured as described previously. All subjects found the brace to be tolerable. They did not report any adverse events, such as tightness or swelling, and only minor chafing and itching occurred, which could usually be corrected by adjusting some aspect of the brace.

**Countermeasure–Resistance Exercise.** When participants reported to the laboratory, during the 15-min visual inspection period when the knee brace was removed, subjects in the EX group performed a bout of resistance exercise training. Subjects had their 1RM assessed at least 1 week prior to the immobilization period by a trained experienced investigator who tested all subjects. Briefly, each subject performed some light repetitions to warm-up before making between three and four attempts, with at least 4 min of rest in between, to lift (full extension) a load that could not be lifted more than once. Using this protocol, a subject’s 1RM was determined within three or four attempts. This value was verified 2 days later as being a true 1RM. The exercise consisted of one set of 10 ± 2 repetitions at 80% 1RM for knee extension, leg press with plantarflexion at full extension, and seated calf-raises, and was performed every other day. The total volume of resistance exercise during the training was 20 contractions for each of the quadriceps femoris and triceps surae muscle groups, and training sessions occurred every 2 days (i.e., 140 contractions total for the knee extensors and plantarflexors).

**Magnetic Resonance Imaging.** The PRE MRI scan was performed on the afternoon preceding day 1 of immobilization. The MRI was performed in a 3-
T HD scanner (Signa MRI System; GE Medical, Milwaukee, Wisconsin) at the Brain–Body Institute, Imaging Research Centre, St. Joseph’s Healthcare (Hamilton, Ontario). Image acquisition was carried out using T1 fluid attenuation inversion recovery (FLAIR) in the axial plane with the following parameters: repetition time/echo time = 2574 ms/6.7 ms; field of view = 25–30 cm; matrix size = range from 320/320 to 512/512 phase/frequency; inversion time = 958 ms; slice thickness = 5 mm. Thigh image acquisition utilized an eight-channel torso coil with two excitations, and the calf image was collected using a single-channel transmit/receive extremity coil with four excitations. During the pre-immobilization scan, bony landmarks for the thigh and leg were identified, and the distance from these landmarks to the first axial scan was recorded. These distances were used in the post-immobilization scan to ensure identical positioning. Nine slices were obtained from the mid-thigh and seven slices from the mid-leg. MRI image analysis was performed using Medical Image Processing, Analysis and Visualization (MIPAV) software (downloaded with permission from the National Institutes of Health; http://mipav.cit.nih.gov/). A bilateral scan was performed on a subset of 5 subjects to calculate changes in CSA for the contralateral (non-immobilized) leg.

Muscle Biopsy. The biopsy procedure was performed as described previously. Briefly, muscle was extracted using a 5-mm Bergstrom biopsy needle under local anesthesia (2% xylocaine). The biopsy taken both before and after the immobilization period was wiped free of blood and cleaned of any connective tissue prior to being embedded in OCT medium (Tissue-Tek, Sakura Finetechnical, Japan), with its fibers oriented perpendicular to horizontal, and frozen immediately in isopentane cooled with liquid nitrogen. Embedded tissue was sectioned and assayed for determination of muscle fiber types by histochemical analysis, as previously described.

Muscle Strength. For isometric knee-extension strength testing, subjects were seated on a bench with the immobilized leg secured in a custom-made dynamometer equipped with a strain gauge, as described previously. For knee-extension strength testing, the subject was seated upright, positioned with the trunk at 90° to horizontal, and the knee joint fixed at an angle of 110° (70° below horizontal), and securely fastened with Velcro straps. Maximal isometric strength was taken as the peak torque achieved during a 5-second MVC. The MVC was repeated three times with a minimum 2-min rest between trials. Similarly, for isometric plantarflexion strength testing, subjects were seated in a chair with the lower segment of the immobilized leg secured in a custom-made dynamometer. Subjects were seated upright with the trunk 90° to horizontal, the knee joint bent to 90°, and the ankle joint 10° in dorsiflexion (10° above horizontal). The same protocol was followed to determine the isometric strength of the plantarflexion muscle group as described for knee-extension strength. To determine the voluntary force-generating capacity of the muscle, specific strength was calculated as voluntary peak force per unit CSA of total quadriceps femoris [from the MRI slice as described earlier; i.e., N-m/(cm²-m)], as described elsewhere.  

Muscle Function. Percent motor unit activation (%MUA) was determined for the knee extensor and plantarflexor muscle groups. The %MUA was calculated using an adaptation of the method described by Kawakami and colleagues. Briefly, a supramaximal electrical stimulus was externally applied to a muscle during a maximal voluntary isometric contraction. Prior to applying the stimulation electrodes, electrode gel was applied to the surface electrode and the underlying skin. The stimuli were rectangular voltage pulses, 200 µs in duration, derived from a stimulator (Device 3072; Medical Systems, Welwyn, Garden City, Herts, UK). The evoked maximal potentiated (i.e., following a 10-s maximal “conditioning” isometric contraction) peak twitch torque (PTT) was obtained by administering a series of paired 200-µs pulses of increasing voltage until an increase in voltage of ~20% elicited no further increase in PTT. The resulting interpolated twitch torque (ITT) is expressed relative to the evoked potentiated PTT and %MUA calculated as follows: %MUA = [1 – (ITT/PTT)] × 100%. The M-wave associated with the evoked twitches and the surface electromyography (EMG) associated with the MVC were recorded from the vastus medialis and soleus muscles for knee extension and plantarflexion, respectively. EMG, silver–silver chloride, disposable recording electrodes (3.8 mm in diameter) were applied to the surface of the skin above the vastus medialis and soleus muscles to record electrical data from the muscle. The EMG signals were amplified (1000×) and filtered (10 Hz to 2 kHz), as previously described. Integrated EMG (iEMG) was measured over a 1-s window (500 ms on either side of the MVC).

Statistical Analyses. Initial analysis of all data proceeded in an unblinded fashion; however, we confirmed, in a blinded manner, the same investigator’s ability to obtain the same results for the CSA of MRI scans and muscle fiber. The intraclass correlation coefficients for these analyses were,
RESULTS

Thigh and Leg Muscle CSA. There was a 6.2 ± 1.7% reduction in the muscle CSA of the thigh for the CON group following 14 days of knee immobilization, but there was no change over time in the EX group (P < 0.01; Fig. 1a). There was a significant reduction in CSA of the leg for the CON groups (P < 0.05), but losses were attenuated in the EX group (P < 0.05; Fig. 1b). The CON group showed a 7.6 ± 2.2% reduction for the quadriceps femoris (P < 0.05). Similar to total thigh CSA, there were no changes in quadriceps femoris CSA following the 14-day immobilization period for the EX group (P < 0.01).

With respect to the constituent muscles of the triceps surae muscle group, the gastrocnemius showed a significant reduction in CSA in CON (−9.4 ± 2.4%; P < 0.01), and there was an attenuated (P < 0.05), but still significant loss in the EX group (−6.6 ± 1.3%, P < 0.05 for time). The soleus showed a significant reduction (−6.8 ± 2.1%) in the CON group (P < 0.05), but there were no changes in CSA following immobilization in the EX group (−0.8 ± 1.2%, P = 0.7) group.

Changes in the CSA of quadriceps femoris, soleus, and gastrocnemius were also quantified from images obtained from the contralateral (non-immobilized) leg. There were no changes in CSA in the non-immobilized leg (N = 5) following 14 days of unilateral knee immobilization for quadriceps femoris (0.4 ± 1.3%, P = 0.83) and triceps surae (0.8 ± 1.8%, P = 0.58) muscle groups.

Dietary Intake. Subjects’ dietary energy intake remained unchanged during the intervention [CON: PRE = 9.32 ± 0.69 MJ/day, POST = 9.68 ± 0.81 MJ/day (P = 0.58); EX: PRE = 9.84 ± 0.59 MJ/day, POST = 9.76 ± 0.87 MJ/day (P = 0.69)]. In addition, the macronutrient ratio did not differ during the intervention for either group and was not different between groups (all P > 0.39; mean carbohydrate intake = 56 ± 3% of total energy; mean fat intake = 28 ± 3% of total energy intake; and mean protein intake = 16 ± 2%). All subjects reported a protein intake of >0.8 g/kg/day (mean across all groups = 1.26 ± 0.1 g/kg/day).

Muscle Fiber CSA. The CSAs of both type I and II fibers were reduced in the CON group by −8.8 ± 2.3% and −9.2 ± 3.2%, respectively (both P < 0.01; Figure 4). A non-paired t-test also revealed no significant difference between the reduction in CSA of type I and type II fibers (P = 0.78). The CSA of both type I and II fibers in the EX group was, in accordance with the muscle CSA, unchanged (type I fibers: −1.4 ± 2.6%, P = 0.43; type II fibers: −0.8 ± 2.1%, P = 0.59).

The reduction in CSA of type I and II fibers in subjects (n = 5) who had their non-immobilized limb biopsies before and after immobilization also showed no changes in type I or II CSA (both P > 0.83).
Isometric Maximal Torque. With respect to knee-extension isometric MVC, there was a 22.3 ± 4.0% reduction in isometric torque production for the CON group following the immobilization period ($P < 0.05$; Fig. 2a). There were no changes in knee-extension MVC for the EX group. Plantarflexion strength exhibited similar losses of approximately 25.3 ± 2.5% in the CON group ($P < 0.05$; Fig. 2b), but there was no reduction in the EX group.

Voluntary Specific Isometric Maximal Torque. There was a significant reduction in knee-extension and plantarflexion specific strength (voluntary isometric 1RM per CSA) post-immobilization in the CON group only ($P < 0.05$; Fig. 3a and b).

Motor Unit Activation and iEMG Activity. The $\%$MUA and iEMG, for both knee extension and plantarflexion, were unaltered following 14 days of unilateral knee immobilization (Table 2).

DISCUSSION

The novel finding of this study is that a relatively low-volume exercise program consisting of 140 intense contractions per 14 days as 20 contractions every other day per muscle group was an effective countermeasure against muscle mass and strength loss during 14 days of knee immobilization. Phrased alternatively, only ∼30 s of high muscle tension on alternate days for the quadriceps femoris and triceps surae muscle groups prevented losses in thigh and soleus and attenuated loss in the gastrocnemius muscle CSA as well as knee-extension and plantarflexion isometric torque. The relevance of our results is underscored by the fact that the majority of atrophy for both the quadriceps femoris and triceps surae muscle groups is thought to manifest itself during the first 28 days of unloading.3,21

Several studies have reported successful amelioration of unloading-induced muscle mass and strength losses with the use of resistance exercise countermeasures4,10,12,15,16; however, these studies4,10,12,15,16 used protocols that would have been
sufficient to induce hypertrophy under normal circumstances, at least based on a comparison of the intensities and volumes employed; hence, it is not surprising that these same programs would offset atrophy. Previously, the lowest volume of high-intensity resistance exercise reported to protect against losses in muscle size and function was that undertaken by Schulze et al.,14 who employed three sets of high-intensity resistance exercises in addition to maximal isometric contractions for knee extension and plantarflexion, with exercises performed every third day. In our study, we used ~65% of the number of repetitions used by Schulze et al.,14; thus, based on our data, and those of others,14 a relatively low volume of resistive contractions appears to be sufficient to maintain mass and function of skeletal muscle. However, the exercise volume we used did not completely preserve lower leg gastrocnemius/triceps surae muscle CSA. In agreement with previous studies,10,11,15,16,30 we observed that the triceps surae was somewhat refractory to disuse countermeasures. With a combination of resistance and endurance work, Trappe et al.20,21,23 were able to offset atrophy of the triceps surae; hence, it may be that this muscle group requires a larger and different type (i.e., aerobic exercise) of stimulus than the thigh to maintain mass during inactivity. We acknowledge that, as a method for rehabilitation of immobilized patients or patients with fractures, our intervention is impractical; however, as a general demonstration of how powerful an anti-atrophic-stimulus, low-volume loading can be, we view our data to be of practical significance for the design of countermeasures for disuse atrophy.

The gastrocnemius muscle showed a reduction in CSA across both groups, although it was attenuated in the EX group, whereas soleus muscle CSA was fully maintained following the immobilization period in the EX group. The lack of complete maintenance of CSA in the triceps surae muscle group in the EX group is thus a consequence of inadequate preservation of the gastrocnemius muscle, which was of a magnitude large enough to obscure the effective maintenance of CSA in the soleus muscle. This result is perhaps not surprising when the muscle activity during the resistance exercise training sessions would have been effective for the soleus (i.e., seated, bent knee, calf-raises), but perhaps not as effective for the gastrocnemius (calf press during the last phase of the leg press). Although this work implies that additional stimulation may be required for full maintenance of muscle mass in the lower leg, it also provides evidence of responsiveness of the postural soleus muscle to a low volume of high-intensity resistance exercise. Our data also highlight the importance of examining the atrophic response of individual muscles when countermeasure interventions are utilized and not simply the aggregate measure of the triceps surae or lower leg.

The extent of atrophy observed in the CON group was comparable with data from other short-term (2–3-week duration) disuse studies for the quadriceps femoris muscle group (~7%)1,5,6,10,11,14,31 and the triceps surae muscle group (~8%),14 suggesting that our model of disuse atrophy was appropriate for study of disuse-induced wasting in humans. A secondary and yet

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<th>Table 1. Subject anthropometric characteristics.</th>
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Values are presented as mean ± SEM. BMI, body mass index. CON group: knee immobilization control group (3 women and 2 men); EX group: knee immobilization with resistance exercise (9 women and 3 men).

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<th>Table 2. Percent motor unit activation (%MUA) and integrated surface EMG (iEMG) from the vastus medialis (knee extension) and soleus (plantarflexion) during maximal knee extension and plantarflexion before (PRE) and immediately following (POST) 14 days of unilateral knee immobilization.</th>
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Values are expressed as mean ± SEM.
important observation was that the contralateral limb showed no change in muscle CSA (quadriceps femoris or triceps surae) or muscle fiber CSA during the immobilization period. This indicates that a contralateral, non-immobilized limb could serve, at least insofar as morphology is concerned, as a valid control for an immobilized limb, as we have observed previously. 32

We observed a disproportionate decline in isometric peak torque (20%) versus the decline in muscle CSA. We do not believe that this finding was due in any way to a reduction in central neural drive but was likely due to changes in other neural factors. A number of previous studies looked at isometric strength loss and changes in muscle CSA with immobilization. For example, Deschenes et al. 33 observed a 21% decline in isometric torque and no change in muscle fiber CSA. Miles et al. 34 immobilized the forearm for a period of 9 days and found a significant decrease in strength, yet no changes in muscle size were seen. Similar results were seen by Adams et al. 35 who noted disproportionately greater declines in strength versus muscle CSA during a 16-day unloading period. Similarly, Parcell et al. 36 noted that isometric extension torque declined by 12%, whereas cross-sectional muscle area was reduced by only 4% following 4 weeks of unloading.

In conclusion, this study provides evidence that unloading-induced muscle mass and strength losses can be offset with a relatively low volume of high-intensity resistive exercise. The successful implementation of such resistive work would have implications for persons exposed to reduction in muscle activity due to non–disease-mediated mechanisms (e.g., immobilization, bed rest, microgravity). Our results are in agreement with other investigators who have suggested that the triceps surae is less responsive to resistance exercise countermeasures and is more susceptible to wasting than the thigh during immobilization; however, the resistive contraction paradigm we utilized was effective in preserving soleus muscle CSA.

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