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Skeletal muscle adaptations following blood flow-restricted training during 30 days of muscular unloading

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Cook SB, Brown KA, DeRuisseau K, Kanaley JA, Ploutz-Snyder LL. Skeletal muscle adaptations following blood flow-restricted training during 30 days of muscular unloading. J Appl Physiol 109: 341–349, 2010. First published June 3, 2010; doi:10.1152/japplphysiol.01288.2009.—This study evaluated the effectiveness of low-load resistance training with a blood flow restriction (LLBFR) to attenuate muscle loss and weakness after 30 days of unilateral lower limb suspension (ULLS). Sixteen subjects (ages 18–50 yr) underwent 30 days of ULLS. Measurements of muscle strength, cross-sectional area, and endurance on the knee extensors and plantar flexors were collected before and after ULLS. Plasma concentrations of IGF-1 and IGFBP-3 were also assessed. During ULLS, eight subjects (5 males, 3 females) participated in LLBFR three times per week (ULLS + Exercise) while eight subjects (4 males, 4 females) did not exercise (ULLS). The blood flow-restricted exercise consisted of dynamic knee extension at 20% of the subject’s isometric maximum voluntary contraction coupled with a suprasystolic blood flow restriction. After 30 days of limb suspension, the ULLS + Exercise group experienced minimal and insignificant losses in knee extensor cross-sectional area and strength (1.2% and 2.0%, respectively; \( P \approx 0.05 \)), while the ULLS group demonstrated significant reductions in cross-sectional area and strength (7.4% and 21%, respectively). Decrements in plantar flexor strength (23.7%) and cross-sectional area (7.4%) were observed after ULLS (\( P < 0.05 \)) and were of similar magnitude between the experimental groups (\( P > 0.05 \)). Muscular endurance in the knee extensors improved 31% in the ULLS + Exercise group, while it decreased 24% in the ULLS group (\( P = 0.01 \)). No changes were seen in hormone concentrations throughout the study. In conclusion, LLBFR of the knee extensors is effective in maintaining muscle strength and size during 30 days of ULLS and results in improved knee extensor muscular endurance.

High-load resistance training during prolonged bed rest and limb suspension has been a widely used countermeasure to preserve muscle mass and strength (5, 33, 34). However, it may not be prudent for individuals to perform resistance training at near-maximal loads after injury or surgery. While resistance exercise is routinely performed on custom exercise devices during prolonged space travel, the large size of the devices and lack of portability make the use of high-load resistance exercise in the newly designed vehicles for lunar and Martian exploration very challenging. Recently, the concept of resistance training at low loads (\( \sim 20–40\% \) of maximum strength) coupled with a moderate blood flow restriction (LLBFR) has received considerable attention as an additional way to improve muscle size and strength. Takarada et al. (40) demonstrated significant improvements in knee extensor strength, cross-sectional area, and muscular endurance in athletes who completed LLBFR training twice per week for 8 wk. Moreover, it has been reported that the skeletal muscle adaptations following LLBFR training are analogous to adaptations observed following high-load training (24, 42).

The mechanisms whereby LLBFR results in hypertrophy and strength gains after training are currently unknown. These muscular adaptations could arise from increased motor unit recruitment, cellular signaling events from mechanical changes on the muscle fibers, and/or various endocrine responses. Currently, much of the research investigating the mechanisms of LLBFR has been endocrine related with a particular interest in growth hormone. Growth hormone concentrations increase after heavy resistance exercise (26) and LLBFR (30, 38, 39).

Potentially, muscle hypertrophy may be mediated through the growth hormone-insulin-like growth factor-1 (IGF-1) axis and the PI3K-Akt-mammalian target of rapamycin (mTor) signaling pathway (36). A study utilizing LLBFR resistance training has linked increases in serum IGF-1 levels to improvements in muscle mass and strength (1). While delineating the factors of hypertrophy through systemic measures may not be as specific as analyzing local growth factors and intracellular signaling pathways, it does not exclude the possibility that increases in growth hormone after LLBFR training can lead to the stimulation of hepatically derived IGF-1, which may elevate circulating IGF-1 and, in turn, activate muscle IGF-type receptors to begin protein synthesis (8). As LLBFR training may increase serum IGF-1 levels, the interplay between muscular unloading and the performance of an exercise countermeasure is ambiguous. Since LLBFR exercise leads to similar muscular adaptations as high-load resistance training (40, 42) and high-load resistance training attenuates muscle atrophy and weakness during prolonged unloading (5, 33, 34), it is possible that LLBFR exercise can be an effective and practical countermeasure to disuse-mediated muscle atrophy. Therefore, the purpose of this study was to evaluate the effectiveness of LLBFR

**DISUSE ATROPHY** is a consequence of muscular unloading and is commonly experienced during periods of joint immobilization, bed rest, limb suspension, and exposure to microgravity (7, 23). Following prolonged muscular disuse, alterations in muscle mass, strength, and neuromuscular function have been reported. For example, quadriceps strength has been shown to decrease \( \sim 20\% \) following 30 days of bed rest (15). The loss can also be augmented following an injury or surgical procedure as 60% reductions in strength in the month following total knee replacement surgery have been shown (37). Since strength is a predictor of physical functioning (29), disuse-mediated muscle dysfunction resulting in weakness can significantly impact one’s ability to perform daily tasks on resumption of weight-bearing activity. Therefore, preserving muscle strength through the use of exercise countermeasures during the disuse period is critical to prevent muscle atrophy and weakness.

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exercise to prevent muscle atrophy and weakness during 30 days of unloading and to gain insight on the IGF-1 axis during human limb suspension and LLBFR exercise. We hypothesized that subjects who performed LLBFR exercise during unilateral lower limb suspension (ULLS) would maintain muscle mass, strength, and endurance and their plasma IGF-1 levels would remain constant, while those who did not exercise would experience decrements in the aforementioned variables.

METHODS

General Overview of the Experimental Design

Skeletal muscle size, strength, endurance, and systemic endocrine function were assessed before and after a 30-day control period and again immediately following a 30-day ULLS experimental period on the knee extensors and plantar flexors (Fig. 1). Subjects were stratified by sex to one of two ULLS groups: 1) LLBFR of the knee extensors three times per week (ULLS + Exercise), or 2) received no intervention (ULLS) (n = 8/group).

Subjects

Sixteen subjects (age 18–50 yr) completed the study. Eight subjects were assigned to the ULLS group and eight subjects were assigned to the ULLS + Exercise group. Participants were excluded if they had any orthopedic limitations, neurological disorders, blood clotting disorders and/or familial histories of blood clotting disorders, or were currently using any type of hormone therapy (including birth control pills or patches). The experimental protocol was approved by the Syracuse University and SUNY Upstate Medical University Institutional Review Boards, and all subjects provided written informed consent before participation.

Limb Suspension Model

The ULLS protocol has been previously described and deemed a reasonable ground-based analog of spaceflight as well as an adequate model of general muscle disuse (3, 32). Subjects performed all physical activity for 30 days on axillary crutches while wearing a shoe with a 10-cm-thick sole on the right foot, thereby unloading the left lower limb. Accordingly, subjects were free to move through a relatively normal range of motion and perform most normal daily activities. To ensure compliance with the ULLS protocol, subjects continuously wore an accelerometer (AMP 331, Dynastream Innovations, Alberta, Canada) on the unloaded ankle during the 30 days of ULLS using methods previously described (10, 13). Subjects wore the AMP 331 for 3 days during the control period to quantify their daily physical activity levels and to obtain their individual acceleration patterns during walking. During the ULLS protocol, the acceleration patterns of the unloaded limb were compared with the acceleration patterns observed during walking for each subject. Steps were detected during ULLS if the subject’s acceleration rate was similar to normal ambulation. The sensitivity of this device in detecting steps during walking is 96%, and the specificity for not detecting steps during ULLS ambulation is 97% (13). Other measures of ULLS compliance (daily interviews with subjects and monitoring of skin temperature and circumference) were also obtained (32).

LLBFR

Eight subjects performed supervised bouts of LLBFR on the knee extensors 3 days/wk during ULLS. The plantar flexors were not exercised in either group, but they served as a within-subject control muscle for comparisons to be made. The LLBFR exercise sessions consisted of three sets of dynamic repetitions at 20% of their initial maximum voluntary contraction (MVC) with a blood flow restriction set to 1.3 times systolic blood pressure completed to volitional muscle failure (90 s of rest between sets) (12). It has been suggested that a pressure 30% higher than a subject’s resting systolic blood pressure impedes venous blood flow causing blood to pool in the capacitance vessels distal to the cuff while restricting some arterial blood flow (40). A metronome was used to pace the speed of the contractions and subjects completed each concentric and eccentric phase of the knee extension exercise in 2 s. The blood flow restriction was applied via a 6 × 83 cm tourniquet cuff (Hokanson, Bellevue, WA) placed at the most proximal position on the subject’s thigh and inflated (E20 Rapid Cuff Inflator, Hokanson) to the designated pressure. The cuff remained inflated throughout the entire exercise session, including rest periods. This averaged ~8.5 min throughout the training during ULLS.

Measured Dependent Variables

Muscle cross-sectional area (CSA), strength, and endurance were assessed three times during the study (before and after a 30-day control period and immediately following 30 days of ULLS) on both the knee extensors and plantar flexors of the unloaded limb. Each time period of testing took place over two visits. The first visit included a magnetic resonance image (MRI) scan to measure CSA of the lower limbs and then skeletal muscle function was assessed on the second visit approximately 1–2 days later. The testing of the knee extensors and plantar flexors was counterbalanced between the subjects but at post-ULLS testing of the knee extensors was always performed before the plantar flexors because we could not be certain the knee extensors were not activated during the plantar flexion movement.

CSA. Serial axial MRI scans were acquired from the lower and upper leg using a 1.5-T Philips Intera whole body scanner with software Release 11 (Phillips Medical Systems, Bothell, WA), Ten-nm-thick transaxial images (2,122-ms repetition time, 10.12-mm

Fig. 1. Experimental design of the study. At each time when endocrine function is assessed (except basal endocrine function) the blood is sampled 5 times: before the acute bout of exercise, immediately after the bout of exercise, and then 12, 24, and 36 h after the exercise. The control group was tested exactly the same, but they do not perform any exercise. ↓ denote the days that the subject performed exercise. ULLS, unilateral lower limb suspension.

NOTE: Each ↓ denotes one bout of exercise for individuals assigned to the countermeasures.
slice-to-slice interval) were obtained after a 30-min supine rest period to allow for fluid equilibration. The images were transferred to a computer for calculation of muscle CSA using the NIH ImageJ software (2). For each axial slice, the CSA (cm²) of the individual muscles of the knee extensors and plantar flexors were identified in the images and manually traced with a computer mouse and then automatically computed. To obtain CSA of the knee extensors all the images were traced from the appearance of the distal portion of the rectus femoris to the appearance of the femoral neck. For the plantar flexors, the anatomic landmarks were determined from the distal appearance of the lateral gastrocnemius toward the proximal portion of the tibia in which the individual plantar flexors were no longer distinguishable. The same number of slices was measured for each particular subject at each of the testing time points, and care was taken to ensure within-subject measurement replication. The same investigator performed all the analyses and was blinded to the time point of the images. The CSA of each muscle group was calculated as the sum of the rectus femoris and vasti CSA for the knee extensors, and the sum of the medial gastrocnemius, lateral gastrocnemius, and the soleus for the plantar flexors. The test-retest reliability of CSA of the plantar flexors was previously determined to have an intraclass correlation coefficient (ICC) of 0.96 (9) and in the present study the ICC for the CSA of the knee extensors before and after the control period was 0.99.

Muscle force. Isometric muscle force was obtained on a knee extension dynamometer (MedX, Ocala, FL). The back rest was adjusted to create a hip joint angle of 100° from flexion, and a seat belt was secured across the subject’s hips to reduce any movements of the hip joint and to minimize assistance from other muscle groups. For isometric testing, the knee joint angle was set at 60° from extension and the right limb was extended and rested on a pad. To obtain an MVC, subjects were instructed to push as hard as possible against an immovable arm attached to a force transducer (model 1U1, HBM, Marlborough, MA; sensitivity of 0.002 V/N), and output was amplified and recorded at 1,000 Hz using a 16-bit data-acquisition card (MP150, BioPac Systems, CA). Plantar flexion force was measured while subjects were seated in a custom-modified dynamometer (Parabody 826, LifeFitness, Schiller Park, IL) (9). The subjects’ left leg was positioned in the dynamometer with the hip, knee, and ankle joint angles all secured at 90°, and force was measured by a force transducer (MLP-300-T, Transducer Techniques, Temecula, CA).

Subjects performed a minimum of three MVCs with a 1- to 2-min rest period between each contraction. The MVCs were conducted until two consecutive trials were within 5% of each other and the highest value was considered the MVC force. The force exerted by the subjects was displayed on a 43-cm computer monitor located directly in front of the subject. During testing, strong verbal encouragement was provided by the investigators. Using the ICC, the test-retest reliability of the MVC for knee extension and plantar flexion MVC is 0.99 and 0.97, respectively (9). Determination of one-repetition maximum (1-RM) of the knee extensors has been previously described (31). Subjects performed a warm-up of 10 dynamic repetitions at a light to moderate weight, and then single attempts at progressively heavier weights were performed until 1-RM (the heaviest weight that could be lifted for one repetition) was determined. This test was only performed for knee extension as our custom-modified plantar flexion dynamometer was designed for isometric contractions only. In the present study the ICC for 1-RM before and after the control period was 0.98.

Endurance. Muscular endurance of the knee extensors was assessed using repeated dynamic contractions at 40% MVC (obtained before the control period and the absolute load was held constant throughout the remaining testing periods) at 15 contractions/min. The concentric and eccentric portions of the knee extension exercise each lasted for 2 s. The test was terminated when a subject could no longer maintain maximum extension or repetition frequency for two consecutive repetitions. The number of repetitions was recorded. The ICC for the knee extension endurance protocol from pre- to post-control period was 0.91.

Blood Sampling

Blood samples were obtained at four time points over the course of the study: at the beginning of the control period, the morning before subjects began ULLS, after 1 day of ULLS, and on approximately day 26 of ULLS (Fig. 1). At each time point (except for the morning before the subjects began ULLS which consisted of just one blood draw in the fasted state), 20-ml blood samples were obtained at rest in the fasted state, immediately after a bout of LLBFR exercise and 12, 24, and 36 h later. Subjects in the ULLS only group were studied in the same manner but did not perform the exercise. This resulted in a total of 16 blood samples per subject over the course of the study.

Dietary Controls

Before their first day of blood draws, subjects completed a 3-day dietary intake diary and were asked to replicate this diet before each of their subsequent blood draw sessions. On the exercise blood drawing days, the subject’s meals were designed to conform to a constant diet, which had the following criteria: no caffeine, aspartame, or snacks; macronutrient distribution of approximately 55% carbohydrate, 20% protein, and 25% fat. Caloric intake was based on the Harris Benedict standard formula plus an appropriate activity factor based on the subject’s age, sex, and physical activity. Subjects were instructed to consume meals immediately after the morning blood draw (at ~0700) and at 1200 and 1800 (after the evening blood draw). The subjects kept food logs throughout the duration of the blood draws and there were no differences in caloric intake or meal composition during the various testing time points. Dietary intake was assessed using Diet Analysis Plus 7.0 (Thomson Learning, Belmont, CA).

Analytical Methods

Blood was collected in glass vacutainers and centrifuged for 20 min at 800 g at 4°C. Plasma was then aliquotted and stored at −80°C until later analysis. IGF-1 and IGFBP-3 concentrations were measured by enzyme-linked immunosorbent assays (ELISA) (Immunodiagnostic Systems, Fountain Hills, AZ) and had a sensitivity of 3.1 µg/l and 137 ng/ml, respectively. The intra- and interassay coefficients of variation for IGF-1 were 5.6% and 5.5%, respectively. The intra- and interassay coefficients of variation for IGFBP-3 were 10.4% and 5.0%, respectively. Samples were analyzed in duplicate and all samples for a given subject were run in the same assay to eliminate interassay variance.

Statistical Analysis

All data are presented as means ± SD. Box plots were used as a nonparametric method to display the data. Sample size for the present study was based on previous data from our group and was powered (≥0.80) to detect significant decreases in muscle CSA and strength at P < 0.05 (10, 32).

A mixed-model ANOVA with repeated measures was used to determine the effect of the independent variables [i.e., between-subjects factors: intervention group (ULLS or ULLS + Exercise)] and within-subjects factor of time on the dependent variables of muscle CSA, strength, and endurance for the knee extensors and plantar flexors. These analyses were initially performed on the control period data, and no significant differences were observed among the dependent variables during the control period. Therefore an average of the control period measurements was calculated and used in a separate analysis to assess changes as a result of ULLS (pre-ULLS vs. post-ULLS). The ANOVA assumption of homogeneity of variances was tested using Box’s test and Levene’s test, and all variables met the assumption. If the assumption of sphericity was not met, the Geisser- Greenhouse correction was applied to the analysis. The statistics were computed using SPSS version 16.0 (Chicago, IL).
Additionally, three-level multilevel modeling using SAS Proc Mixed (version 9.1, Cary, NC) was used to analyze the acute and chronic responses of IGF-1 and IGFBP-3 during ULLS and LLBFR. Specifically, we explored whether there was a relationship between the experimental groups (ULLS and ULLS + Exercise) for IGF-1 and IGFBP-3 levels at the five blood draws (preexercise, postexercise, 12 h, 24 h, and 36 h) during the control period, 1 day into ULLS and 26 days into ULLS. At level 1, each subject’s variability was determined as a function of their own mean and the deviation of their mean from the sample mean. At level 2, the within-subject variable, time period, was introduced to examine changes in IGF-1 and IGFBP-3 over the three time periods. The level 3 within-subject variable, blood draw, was then added into the model to examine whether there was significant change across the five blood draws at each of the three time periods. Between-subject differences were tested using group as a predictor of level (intercept) as well as change across time period and blood draw. Because age was a significant predictor in the IGF-1 analysis, all models were controlled for age. Before the analysis, an unconditional model was run to estimate the intraclass correlation (ICC), or the ratio of between- to within-person variability in IGF-1 and IGFBP-3 levels, to justify the use of multilevel modeling (35). All models were tested with and without random effects (i.e., allowing subjects to have their own slope as well as their own intercept) for the within-subjects variables and the comparative fits were examined using the Akaike information criterion (AIC). For the AIC, smaller values indicated better model fit and would justify including the random effect in the model (35).

RESULTS

Subject Descriptive Statistics and Compliance

There were no significant differences in age, height, or body weight between the ULLS and ULLS + Exercise group (Table 1). As expected, reductions were observed in the number of steps per day from pre-ULLS to post-ULLS (P < 0.001). There were no differences in physical activity levels between the experimental groups at pre-ULLS, and no differences were detected during the ULLS protocol as the percentage of weight-bearing steps declined in both groups (Table 1).

The subjects in the ULLS + Exercise group demonstrated 100% compliance with the exercise intervention as all subjects completed three LLBFR exercise sessions per week during the unloading phase. The load (11.8 ± 4.2 kg) and blood flow restriction pressure (150 ± 10 mmHg) was not altered during the intervention, but the number of repetitions completed increased significantly with exercise training (61 ± 25 repetitions to 99 ± 35 repetitions, P < 0.001), resulting in increases of 12–185% in the number of repetitions performed among the subjects.

Table 1. Descriptive statistics of the sample

<table>
<thead>
<tr>
<th></th>
<th>ULLS</th>
<th>ULLS + Exercise</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4 M, 4 F</td>
<td>5 M, 3 F</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>18.8 (1.0)</td>
<td>26.1 (10.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.3 (12.2)</td>
<td>175.9 (11.7)</td>
<td>0.22</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.9 (14.2)</td>
<td>78.0 (14.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Physical activity, steps/day</td>
<td>6,027 (2,689)</td>
<td>8,147 (4,499)</td>
<td>0.23</td>
</tr>
<tr>
<td>ULLS exercise compliance, % step reduction</td>
<td>99.4 (0.6)</td>
<td>99.7 (0.3)</td>
<td>0.39</td>
</tr>
<tr>
<td>Pre-ULLS measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extensors 1-RM, kg</td>
<td>38.7 (19.7)</td>
<td>50.5 (19.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>Knee extensors CSA, cm²</td>
<td>69.3 (33.9)</td>
<td>75.3 (26.2)</td>
<td>0.70</td>
</tr>
<tr>
<td>Plantar flexors strength, N</td>
<td>279.6 (124.6)</td>
<td>343.3 (93.2)</td>
<td>0.27</td>
</tr>
<tr>
<td>Plantar flexors CSA, cm²</td>
<td>36.6 (11.5)</td>
<td>40.9 (6.6)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are means (SD). ULLS, unilateral lower limb suspension; 1-RM, one repetition maximum; CSA, cross-sectional area; M, males; F, females.

Control Period

Figure 2A depicts the box plots of the dependent variables before and after the 30-day control period, and the differences from control period varied by <15%. There were no differences between the pre- and post-control period data for knee extensor strength and CSA for both experimental groups (P = 0.77 and 0.47, respectively). There were also no differences between the pre- and post-control period data for plantar flexor strength and CSA for both experimental groups (P = 0.56 and 0.33, respectively). When the data for the control period were averaged, there were no significant differences between the ULLS and ULLS + Exercise groups on strength and CSA measures of the knee extensors and plantar flexors (Table 1). There were also no differences between the number of repetitions completed on the knee extension endurance task in the pre- to post-control period (P = 0.23).

Post-ULLS

CSA. Following unloading, the ULLS group showed an average decrease in CSA of the knee extensors and plantar flexors (7.5% and 8.5%, respectively). The loss in CSA in this group ranged from 0 to 16% from pre- to post-ULLS in the plantar flexors and 4 to 15% in the knee extensors (Fig. 2B). Figure 3 is a cross-sectional MRI image of the knee extensors in a ULLS subject who had an overall reduction in CSA of 7%. The ULLS + Exercise group demonstrated an insignificant reduction (~1%) in CSA of the knee extensors. This loss ranged from 0 to 6% of pre-ULLS CSA with two subjects experiencing a 3% gain in CSA. The ULLS + Exercise subjects had a significant reduction (5.4%) in CSA of the plantar flexors, and this loss ranged from 2 to 9% (Fig. 2B). When the individual muscles of the knee extensors were assessed, there was no muscle × time × group interaction (P = 0.18), but there was a muscle × time interaction (P = 0.02) indicating that muscle atrophy was greater in the vasti muscle than the rectus femoris, regardless of experimental group. A significant time × group interaction (P = 0.04) for knee extensor CSA revealed the ULLS group experienced a greater reduction than the ULLS + Exercise group. The individual muscles (lateral gastrocnemius, medial gastrocnemius, and soleus) of the plantar flexors atrophied similarly with no group differences (P > 0.05). When the MRI slices were assessed along the length of the knee extensors, CSA of the vasti muscles was significantly reduced only in the mid thigh region (slices 5–14) (P = 0.02), and this did not differ between
group was highly variable as subjects experienced up to a 21% increase in MVC strength while others had decrements close to 30% of pre-ULLS MVC strength. The ULLS and ULLS + Exercise groups experienced a 26.7% and 20.7% decrease in PF MVC, respectively \((P < 0.05)\) (Fig. 3). There was a significant difference in 1-RM strength of the knee extensors between the groups after ULLS \((P = 0.02)\) (Fig. 2B). The ULLS group decreased 21% in 1-RM strength while the ULLS + Exercise group exhibited an insignificant loss of 1.5%.

**Endurance.** Following unloading, the ULLS group had a 24% reduction in the number of repetitions performed and the ULLS + Exercise group showed a 28% increase \((group \times time interaction P = 0.003)\) (Fig. 5).

**IGF-1 and IGFBP-3.** Results from the MLM analysis produced ICCs of 0.76 and 0.81 for IGF-1 and IGFBP-3, respectively. The ICC for IGF-1 indicated that 76% of the variability was between subjects and 24% was within subjects. The ICC for IGFBP-3 partitioned 81% of the variability between subjects and 19% within subjects. The variability for both dependent variables was significant within and between the subjects \((P < 0.05)\). Overall, IGF-1 levels during the five blood draws at each of the three time points did not change and were not different within or between the groups \((P > 0.05)\). However, there was an overall difference in IGF-1 levels between the experimental groups \((ULLS: 196.3 \pm 47.8 \mu g/l; ULLS + Exercise: 139.4 \pm 46.7 \mu g/l, P = 0.004)\), and subject age was determined to be a significant predictor of IGF-1 levels \((P = 0.012)\). IGFBP-3 levels did not change during the study, and there were no significant group differences \((ULLS: 3,243.57 \pm 151.0; ULLS + Exercise: 2,477.9 \pm 229.2 \mu g/ml; P = 0.12)\). Age was not a predictor of IGFBP-3 levels \((P > 0.05)\).

**DISCUSSION**

The main objective of this study was to evaluate the effectiveness of LLBFR exercise as a countermeasure against disuse-mediated muscle atrophy following prolonged unloading. Our major findings were that muscle mass, strength, and endurance following LLBFR resistance exercise performed on the knee extensors during 30 days of unloading was preserved in the knee extensors compared with no intervention. Thus LLBFR resistance training may be a suitable countermeasure to prevent disuse atrophy and even improve muscular endurance.

Previous studies have reported an average loss of 11% and 21% in knee extensor CSA and strength, respectively, following unloading with no intervention \((14, 32, 33, 44, 45)\). The loss of strength \((-8\%)\) and CSA \((-16\%)\) in the knee extensors in the ULLS group of the present study is slightly lower but reasonably comparable to those prior studies. Plantar flexor strength and CSA losses were also similar to our previous study \((10)\) and to those of Schulze et al. \((33)\). There was no discernable difference in the atrophic response among the knee extensors \((-8\%)\) and plantar flexors \((-9\%)\) suggesting that both muscles are susceptible to the effects of muscular unloading. The disproportionate loss of muscle strength compared with muscle size is not a novel finding as it has been consistently demonstrated in many ULLS studies \((7, 10, 14, 32, 33, 45)\). In addition to reduced skeletal muscle protein synthesis and increased protein degradation \((as demonstrated by diminished CSA)\), there must be other alterations in force-generating capacity in the neuromuscular system that compromise muscle

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**Fig. 2.** Box plots of percent changes in muscle cross-sectional area (CSA) and muscle strength in the knee extensors (KE) and plantar flexors (PF) during a 30-day control period (A) and after 30 days of ULLS (B); \(n = 16\). The top and bottom lines and the line through the middle of the box represent the 75th percentile (top quartile), 25th percentile (bottom quartile), and 50th percentile (median), respectively. The whiskers on the bottom extend from the 10th percentile (bottom decile) and top 90th percentile (top decile). The dots outside of the box and whiskers represent “far-out” outliers.
function. Neuromuscular impairments following disuse-mediated atrophy have been seen in the ability to voluntarily recruit motor units, the transmission of neural impulses, and the contractile properties of skeletal muscle (11).

Performing LL_BFR resistance exercise during ULLS was effective at mitigating muscle atrophy suggesting that LL_BFR training may have an impact on protein synthesis and/or degradation rates as well as neuromuscular function. An intriguing element was that the LL_BFR exercise was performed three times per week on only the knee extensors and was always completed in <10 min; thus muscle quadriceps size and strength were preserved and endurance increased with only 30 min of exercise per week using loads of only 20% of maximum. Prior LL_BFR studies have recommended a higher frequency of exercise bouts because it is believed that LL_BFR exercise does not require a long recovery time between training sessions and minimal muscle damage occurs when such a low load is utilized (1, 39). Potentially greater effects would be seen if the LL_BFR exercise was performed more than three times per week.

Exercise training-induced muscle hypertrophy is most likely not uniform along the length of a limb. The greatest training-induced muscle hypertrophy has been found in the midthigh region where CSA is the largest and that changes in CSA became progressively smaller toward the proximal and distal regions of the knee extensors (46). In another study (19), CSA of the knee extensors increased throughout the length of the thigh, but the individual muscles of the quadriceps femoris hypertrophied differently along the thigh, suggesting there is selective hypertrophy. In the present study, when the knee extensors were examined separately the loss of CSA was more evident in the vasti muscles than that of the rectus femoris, and this atrophy was most apparent in the midthigh. This is similar to previous ULLS and bed rest studies (4, 20, 45). The most obvious difference between the vasti and rectus femoris is that the rectus femoris is biarticular crossing both the knee and hip joints whereas the vasti muscles cross only the knee joint. It seems likely the hip movements in bed rest or ULLS may induce sufficient muscle activity to alleviate atrophy, or possibly the baseline activity of the rectus femoris is lower than the vasti muscles such that unloading is a less dramatic atrophy stimulus.

Perhaps the most intriguing finding in our study was that muscular endurance increased as a result of LL_BFR exercise during ULLS. Berg et al. (6) reported a decline in work capacity (i.e., decline in average peak torque during exercise) accompanied by significant decreases in citrate synthase following 4 wk of ULLS. Interestingly, Kaijer et al. (22) demonstrated that ischemic cycling training increased work capacity and endurance in conjunction with higher citrate synthase levels. To our knowledge, this adaptation as a result of LL_BFR resistance training has not been investigated and would be particularly interesting since LL_BFR resistance exercise has been promoted as an alternative to high-load exercise, rather than aerobic exercise. Regardless, after a period of muscle unloading, the maintenance of muscle mass and strength accompanied by an improved metabolic profile of skeletal muscle would be ideal on resumption of daily physical activity.

While our study demonstrated that LL_BFR resistance training maintained muscle mass and strength of the knee extensor during ULLS, we did not observe any changes in plasma IGF-1 and IGFBP-3 concentrations during unloading or during acute and chronic bouts of LL_BFR. While no studies have assessed these endocrine variables during ULLS, this finding is in accordance with a previous bed rest study (16). A rise in growth hormone following LL_BFR exercise is thought to be mediated by the accumulation of metabolites, particularly lactic acid, H+ and adenosine (28, 39) resulting in the stimulation of chemosensitive afferents (group III and IV afferents) that may subsequently elicit a neurohumoral response to the hypothalamus to increase circulating growth hormone levels. Theoretically, elevated growth hormone levels should increase circulating IGF-1 levels (26), but frequently it has been shown that IGF-1 levels do not respond after acute bouts of high-load resistance exercise (25) and chronic training (21). However, an elevation in IGF-1 activity following LL_BFR training was reported to be a mediator of muscle hypertrophy by Abe et al. (1) who demonstrated a 24% increase in IGF-1 following 2 wk of twice daily LL_BFR exercise. Since growth hormone levels have been shown to rise immediately after LL_BFR exercise (30, 38, 39) there is typically a delayed secretion of IGF-1 that may be seen 16–28 h postexercise (26). Therefore, it is possible that in the previous study (1) the training effect of LL_BFR exercise on IGF-1 levels could be confounded by the timing of when the

Fig. 3. Representative example of a magnetic resonance image (MRI) of the knee extensors in the unloaded leg before (A) and after (B) ULLS in a nonexercising subject. The dotted lines traced around the vastus lateralis demonstrate ~7% reduction in muscle mass in this particular subject.
last exercise sessions occurred. Despite this, we did not observe any acute or chronic changes in plasma IGF-1 or IGFBP-3 after LLBFR exercise and unloading. This finding does not imply that protein synthesis did not occur in the ULLS + Exercise group as Wilkinson et al. (50) and West et al. (49) reported the upregulation of local muscle growth factors independent of elevated systemic anabolic hormone concentrations. Moreover, Fujita et al. (18) compared a bout of blood flow-restricted resistance exercise to a volume-matched exercise at the same intensity. They assessed serum concentrations of growth hormone and IGF-1 and used muscle biopsies to investigate the cellular mechanisms of the mTor signaling pathway. While the levels of serum growth hormone increased significantly, IGF-1 levels remained fairly constant. There was evidence of upregulation of the mTor pathway and an increase in muscle protein synthesis following the blood flow-restricted exercise (18) providing evidence that serum IGF-1 concentrations may not be associated with muscle protein synthesis. Currently it cannot be determined that the growth hormone response was related directly to the anabolic effect on the skeletal muscle either, but its consistent elevation after LLBFR exercise certainly deserves further investigation. Since plasma IGF-1 levels during LLBFR exercise do not seem to change, its importance in determining the effects on skeletal muscle mass is questionable, and more local factors should be evaluated in future studies.

As in many human studies, there can be considerable subject variability. Previous ULLS studies have reported decrements in the plantar flexors and knee extensor muscle CSA to range from 2 to 19% (10) and 7 to 26% (32), respectively, which is similar to observations of muscle atrophy after prolonged spaceflight (17). Therefore, in response to unloading, it is difficult to tease out the effectiveness of the countermeasure from intersubject variability. Additionally, the group variability could have been impacted by sex and age as the ULLS + Exercise group had more males and tended to be older. In light of this, the within-subject experimental design, as well as the control period, allowed for greater statistical power and offset some of this variability that occurred.

Although we have shown that LLBFR resistance exercise can be beneficial during reduced physical activity, a limitation to the present study is that it did not have an intervention group that received only a blood flow restriction or only engaged in
low-load resistance exercise. Muscle atrophy has been shown to be slightly attenuated following repetitive blood flow restriction bouts during muscular unloading (27, 41). While we did not specifically have a group that received only a blood flow restriction, we did evaluate strength and CSA of the plantar flexor muscle group in all of our subjects. We found no evidence that a blood flow restriction at the thigh would prevent muscle atrophy downstream in the nonexercised plantar flexor muscles. It is important to note that the blood flow restriction pressures and the total time under occlusion (1.3 times systolic blood pressure for <10 min) was much less than the previously mentioned studies that utilized pressures close to 300 mmHg for at least 15 min (11, 29, 43). Prior LL-BFR training studies during normal ambulation have included intervention groups that also engaged in low-load resistance exercise without a blood flow restriction (42, 43). In those cases, the exercise volume in the low-load group was matched to the LL-BFR exercise group with the results demonstrating greater strength gains with LL-BFR. Exercise. When low-load resistance exercise with and without a blood flow restriction are each performed to muscular failure, the blood flow-restricted exercise has a greater effect on the rate of fatigue (47), but both regimens result in high levels of muscular activity (measured by EMG) (48). This implies that in either mode of exercise there is substantial recruitment of type II muscle fibers that could result in hypertrophy and strength gains. If that is the case, then an advantage of LL-BFR resistance exercise would be that the muscles could be fatigued more quickly. Evaluating both LL-BFR resistance training and load-low exercise regimens performed to muscular failure may provide further insight into the effectiveness of LL-BFR resistance training.

Overall, it was observed that almost all subjects in the nonexercising group experienced substantial strength and CSA losses in the knee extensors and plantar flexors. The two subjects in which we did not observe decrements experienced <2% changes in these variables which were within the expected coefficient of variation of these measurements. Conversely, three subjects that performed LL-BFR on the knee extensors during ULLS actually experienced improvements in muscle strength and CSA while the others showed decrements of <10%. While the exercise was specific to the knee extensors, it did not preserve muscle mass of the plantar flexors as all subjects experienced decrements of similar magnitude to the nonexercising group. The overall tendency to attenuate muscle loss and weakness after performing LL-BFR resistance exercise during ULLS certainly warrants more investigation.

In summary, we have found that LL-BFR exercise on the knee extensors during 30 days of ULLS maintained muscle mass and strength and improved endurance. Despite the need for more invasive techniques to understand the endocrine responses, this training has been shown to be an effective countermeasure against the effects of disuse on use atrophy.

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