Adaptations in human neuromuscular function following prolonged unweighting: I. Skeletal muscle contractile properties and applied ischemia efficacy

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Adaptations in human neuromuscular function following prolonged unweighting: I. Skeletal muscle contractile properties and applied ischemia efficacy. J Appl Physiol 101: 256–263, 2006. First published March 2, 2006; doi:10.1152/japplphysiol.01402.2005.—Strength loss following disuse may result from alterations in muscle and/or neurological properties. In this paper, we report our findings on human plantar flexor muscle properties following 4 wk of limb suspension (unilateral lower limb suspension), along with the effect of applied ischemia (Isc) on these properties. In the companion paper (Part II), we report our findings on the changes in neurological properties. Measurements of voluntary and evoked forces, the compound muscle fiber action potential (CMAP), and muscle cross-sectional area (CSA) were collected before and after 4 wk of unilateral lower limb suspension in adults (n = 18; 19–28 yr). A subset of subjects (n = 6) received applications of Isc 3 days/wk (3 sets; 5-min duration). In the subjects not receiving Isc, the loss in CSA and strength was as expected (~9 and 14%). We observed a 30% slowing in the duration of the CMAP, a 10% decrease in evoked doublet force, a 12% increase in the twitch-to-doublet force ratio, and an altered postactivation potentiation response (11% increase in the postactivation potentiation-to-doublet ratio). We also detected a 10% slowing in the ability of the plantar flexor to develop force during the initial phase of an evoked contraction, along with a 6% reduction in in vivo specific doublet force. In the Isc subjects, no preservation was observed in strength or the evoked muscle properties. However, the Isc group did maintain CSA of the lateral gastrocnemius, as the control subjects’ lateral gastrocnemius atrophied 10.2%, whereas the subjects receiving Isc abolished 4.7%. Additionally, Isc abolished the unweighting-induced slowing in the CMAP. These findings suggest that unweighting alters the contractile properties involved in the excitation-contraction coupling processes and that Isc impacts the sarcolemma. The mechanisms accounting for a reduction in strength could arise from a variety of factors, which can be broken into two broad categories, 1) neurological, and 2) skeletal muscle dysfunction, as it is well known that the output from these two sources controls force production (19). One of the most obvious explanations for decreased strength following periods of unloading is muscle atrophy; however, the observation of a greater decrease in strength relative to the loss of muscle mass following unweighting (4, 15, 41), along with the recent finding of no relationship between change in muscle size and strength following 20 days of bed rest (31), suggests that other alterations in the force-generating capacity of the neuromuscular system are compromised.

Within the neuromuscular system, there are several potential sites that could affect voluntary force output, such as excitatory drive to the lower motoneurons, motoneuron excitability, neuromuscular transmission, sarcolemma excitability, excitation-contraction (E-C) coupling, and contractile mechanisms (5). It was the purpose of the present work to comprehensively investigate adaptations in both neurological and skeletal muscle function following prolonged unweighting, along with assessing the efficacy of interventions designed to selectively target the nervous system (motor imagery) or skeletal muscle [applied ischemia (Isc)]. In this paper (Part I), we report our findings on the changes in skeletal muscle properties and the effectiveness of Isc (periodic bouts of blood flow occlusion) in preserving contractile function. In the companion paper following (Part II), we report our findings on the changes in neurological properties and the effectiveness of motor imagery (imagined maximal muscle contractions) in preserving neural function. Additionally, in Part II, we collectively analyze our findings to determine the relative contribution of neural and muscular factors in strength loss (11a). We chose to utilize “passive” interventions, in terms of the muscle and peripheral nervous systems, because an active intervention (i.e., resistance exercise) should result in a perturbation to both the nervous and muscular systems, making it difficult to determine the relative contribution of the respective systems. Additionally, from an applied perspective, investigations of these types of interventions are warranted, as they could prove clinically useful to many populations who are predisposed to unweighting-induced dysfunction but may not be able to effectively perform traditional interventions involving heavy loading of muscles and joints.

In terms of alleviating the disuse-induced loss of muscle function, one novel approach may be through the periodic cessation of blood flow to the affected muscles. It has recently been shown that chronic blood flow restriction in rats results in muscle hypertrophy, along with increased levels of heat shock proteins and other adaptations (23). The mechanisms underlying these adaptations are not yet fully understood, but it appears that ischemic conditions can induce novel adaptations in muscle fiber composition and function, which may be therapeutically useful for a variety of conditions, including disuse atrophy.

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protein 72 and decreased myostatin (30), both of which are thought to be important regulators of atrophy (28, 39). Additionally, moderate vascular occlusion in combination with resistance exercise has been shown to exert beneficial effects on skeletal muscle and endocrine responses (8, 49, 51). It is uncertain how ISc is capable of altering parameters leading to favorable outcomes in human skeletal muscle, but these findings do suggest that alterations in blood flow may be useful in situations leading to atrophy. To our knowledge, only one report exists on this topic, which observed atrophy being reduced by ~50% following surgically induced bed rest (50).

The purpose of the present study was to determine the adaptations in plantar flexor (PF) muscle function following unilateral lower limb suspension (ULLS), and to evaluate the efficacy of applied ISc on these functional properties. We hypothesized that alterations in muscle cell membrane function and E-C coupling would exist following ULLS (assessed through the evaluation of the evoked electromyographic and force signals). Additionally, we hypothesized that the group receiving ISc would demonstrate a lesser degree of disuse-induced alterations.

METHODS

General Overview of the Experimental Design

Skeletal muscle contractile function of the unweighted PF was assessed before and after a 4-wk control period and immediately following 4 wk of ULLS. These tests measured a variety of contractile properties, including muscle size and electrophysiological and force characteristics, and are described in detail below. While undergoing the ULLS protocol, subjects were assigned to one of three groups: 1) ULLS with no intervention (ULLS), 2) motor imagery 4 days/wk (ULLS+MI), and 3) applied ISc 3 days/wk (ULLS+Isc). Because there was no statistically significant differences between the ULLS+MI and ULLS groups on the contractile variables, the data from this group were combined with those not receiving an intervention (collectively called ‘ULLS control groups”). However, because there is a theoretical basis for motor imagery altering strength, for this variable the groups were not pooled.

Subjects

A total of 18 volunteers completed the study. For one subject, we only obtained one evaluation session before the start of the ULLS protocol due to scheduling difficulties. Twelve of these subjects were assigned to the control groups (3 men and 9 women; 21.25 ± 0.96 yr, 164.05 ± 2.55 cm, and 67.36 ± 2.98 kg), whereas six were assigned to the ULLS+Isc group (3 men and 3 women; 20.00 ± 0.52 yr, 171.45 ± 4.89 cm, and 62.23 ± 4.64 kg). The subject’s physical activity levels were similar among groups (see RESULTS). Exclusion criteria included subjects known to have a family history of blood clotting disorders or who smoked. Additionally, women currently taking oral contraceptives and individuals who were a carrier for sickle-cell anemia were excluded from the applied Isc group. The Syracuse University and SUNY Upstate Medical University Institutional Review Board approved the experimental protocol, and all subjects provided written, informed consent before participation.

Limb Suspension Model

The ULLS protocol has been previously described (41). Briefly, it requires subjects to perform all ambulatory activity on crutches while wearing a shoe with a 10-cm-thick sole on the right foot, eliminating ground contact by the left foot, thereby unloading the left lower limb. To decrease the risk of venous thrombosis (6), subjects wore graduated compression stockings (37); took enteric coated Aspirin (1); and were advised to elevate their left leg whenever possible and avoid air travel, sitting for long periods of time, crossing their legs, and drinking alcoholic beverages. Additionally, we examined the subject’s height and weight, as well as various signs and symptoms (i.e., redness, tenderness, localized warmth, pitting edema) of venous thrombosis several times per week.

Applied ISc

Six subjects received the vascular occlusion intervention 3 days/wk. The occlusive stimulus was applied by compressing the proximal end of the unloaded lower leg using a pneumatic tourniquet cuff (Hokanson). The occlusive pressure was applied at 100 mmHg above systolic blood pressure, with a duty cycle consisting of 5 min of applied ISc, followed by 3 min of normal perfusion for a total of three sets (total ischemic time: 15 min). Using Doppler ultrasound technology, we have previously demonstrated our ability to successfully occlude blood flow using this procedure (8).

Measured Dependent Variables

Note, all of the methodologies utilized in this study have been previously described, along with the test-retest reliability of these parameters when separated by 4 wk with data obtained from the control period in these subjects (10). For greater details regarding the methodologies used, please see this paper by Clark et al. (10).

Muscle cross-sectional area. Serial axial plane MRI scans were acquired from the lower leg using a 1.5-T Philips Intera whole body scanner (Philips Medical Systems, Bothell, WA). Muscle anatomical cross-sectional area (CSA) was calculated for the soleus (Sol), medial gastrocnemius (MG), and lateral gastrocnemius (LG) muscles. This calculation was based on an average CSA over five slices obtained from the midbelly of the muscle. The same investigator, blinded to the time point of each image, performed all magnetic resonance analyses.

Muscle forces. PF muscle force was measured while subjects were seated in a custom-modified dynamometer (Parabody 826, LifeFitness, Schiller Park, IL). 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).

To assess muscle strength, subjects performed a minimum of four maximal voluntary isometric contractions (MVC) with a 1- to 2-min rest period between each contraction. During testing, strong verbal encouragement was provided by the investigators, and the highest value was considered the MVC force. Additionally, knee extension strength was assessed to aid in delineating the effect of ISc (which was specific to the PFs) on voluntary muscle strength. The setup for measuring knee extension strength has previously been described (8). The knee extension strength was assessed before the PFs at all time points during the study.

In addition to voluntary strength, measurements of electrically evoked contraction forces were obtained. Evoked responses were
elicited by transcutaneous stimulation of the tibial nerve by use of a cathode located in the popliteal fossa and an anode placed on the lower one-third of the posterior thigh. Supramaximal stimulation was obtained by increasing the stimulation intensity 15% beyond that required to elicit peak twitch force. Peak net forces were obtained under several stimulation frequencies and conditions, including twitch, doublet (100 Hz), and postactivation potentiation (PAP). The PAP doublet was administered ~2 s after the cessation of a 5-s MVC. This doublet was delivered following the third and fourth MVC trials, and an average of the two was calculated. The ratio of twitch to doublet force was calculated to investigate E-C coupling, as changes in the low-to-high-frequency ratio are indicative of alterations in E-C coupling (18, 29). Additionally, the ratio of PAP to resting doublet force was calculated to evaluate the differential response between the two evoked doublet characteristics, as a change in PAP force relative to resting doublet force is typically thought to be indicative of alterations in phosphorylation of myosin regulatory light chains (46).

Rates of evoked force development (+dF/dt) and relaxation (~dF/ dt) were calculated for the resting doublet force response. For the +dF/dt, force-time curves displayed a biphasic waveform with an initially faster +dF/dt through the first half compared with the second half. Therefore, we calculated the +dF/dt for the early phase (10–40% of peak force) and late phase (60–90% of peak force) of the contraction. Additionally, the ~dF/dt was calculated between 90 and 50% of peak force. Data were expressed in absolute terms (N/ms) as well as relative terms (%/ms) to account for the effect of peak force on the rate of force production (36, 45). The physiological mechanisms of force-time properties of evoked contractions are driven by a number of different factors and in general are thought to provide information on intracellular Ca^2+ transients, muscle fiber type, and cross-bridge function (24, 26, 40, 52, 53).

Additionally, in vivo specific doublet force (force per unit area) was estimated by dividing the evoked doublet force by the MRI-derived muscle CSA (N/cm^2). Because this assessment does not include pennation angle, it can only be considered an estimate of the force-generating capacity per CSA.

Muscle fiber action potentials. The amplitude and duration of the evoked Sol compound muscle fiber action potential (CMAP; also commonly referred to as M_max) was measured. The surface electromyographic signal was recorded from the resting Sol muscle, while the subject lay prone on an examination table. Electrodes were located on the belly of the muscle (Ag-AgCl, potential sensitive area of 22 mm, 40-mm center-to-center interelectrode distance; Kendall MediTrace 200, Chicopee, MA) (10). After placement during the first testing assessment, anatomical locations were noted by taking distance measurements from the midline of the popliteal fold, and these measurements were utilized for electrode placement during the latter sessions (10). A single, supramaximal electrical stimulation pulse was delivered to the tibial nerve, and the evoked Sol CMAP was recorded. The peak-to-peak amplitude of the CMAP was calculated, as well as the duration of the first negative peak (10). To control for the influence of temperature on these variables, Sol skin temperature was measured (SKT100C, MP150, BioPac Systems, Goleta, CA) and adjusted to within 30–32°C, if necessary.

Statistical Analysis

Mixed-model analysis of variance techniques were utilized to determine the effect of the independent variables (i.e., within-subjects factor: time; between-subjects factor: treatment group) on the dependent variables. Initially, the control period data were subjected to these analyses (precontrol vs. postcontrol). As expected, no significant differences were observed during the control period for any of the respective dependent variables; thus an average of the two control periods was calculated and used in separate analysis to assess ULLS-induced changes (average control vs. post-ULLS). For all analyses, a preset α-level of significance equal to 0.05 was required for statistical significance, and significant main effects and/or interaction terms were followed up with Sidak post hoc tests. The SPSS statistical package (version 10.0, Chicago, IL) was used for data analysis. Data are presented as means ± SE, unless otherwise stated.

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 a P ≤ 0.05 (41). However, with relatively small sample sizes, as in the present study, rather large differences may not reach statistical significance and result in Type II error. Therefore, effect sizes (here, referring to partial η^2, which represents the proportion of total variation attributable to the factor, partialing out other factors from the total nonerror variation) are reported as an additional statistical parameter to aid in interpretation of the findings.

RESULTS

Subject Compliance with ULLS

As expected, significant reductions were observed in the number of steps per day during the ULLS protocol compared with the control period (P < 0.00, η^2 = 0.80). No differences were observed in physical activity levels between the experimental groups during the control period (ULLS control groups: 5,237 ± 642 steps/day vs. ULLS+Isc group: 4,394 ± 302 steps/day; P = 0.61, η^2 = 0.06). Additionally, no differences were observed during the ULLS protocol, as reductions in weight-bearing steps were 99.64 ± 0.13 and 99.86 ± 0.10% for the ULLS control groups and ULLS+Isc groups, respectively (P = 0.99, η^2 < 0.00).

Reliability of Dependent Variables

The test-retest reliability of the control period data has been published elsewhere (10). In brief, the overall reliability of all of the reported variables had coefficients of variation (CV) between 1.9 and 13.2% and intraclass correlation coefficients (ICC) between 0.70 and 0.99. Strength displayed a CV of 4.2% and ICC = 0.97. CSA displayed CVs between 1.9 and 3.8% and ICCs between 0.93 and 0.99. The evoked peak force properties displayed a CV between 9.2 and 11.8% and ICC between 0.70 and 0.80. The +dF/dt and ~dF/dt displayed CVs between 10.2 and 13.2% and ICCs between 0.78 and 0.84. The CMAP parameters displayed CVs between 7.5 and 8.3% and ICCs between 0.81 and 0.86. Specific doublet force displayed a CV = 8.4% and an ICC = 0.71.

Strength

The group that received no intervention (n = 6; as the motor imagery subjects were not pooled for this variable due to the theoretical basis of it potentially altering strength) lost 14.2% of their PF muscle strength (231.9 ± 32.4 vs. 198.9 ± 24.8 N), whereas the Isc group lost 15.2% (Fig. 1) (274.9 ± 39.7 vs. 233.1 ± 33.4 N). Both of these decreases were significant (P < 0.00, η^2 = 0.78), and there were no differences between the two groups (P = 0.51, η^2 = 0.05). When these changes were considered in light of the changes in the knee extensors (entering muscle group into the ANOVA model), again no differences were observed between the groups (time × muscle group × treatment group, P = 0.92, η^2 < 0.00) (ULLS control group: 540.8 ± 45.8 vs. 449.1 ± 46.9 N; ULLS+Isc group: 567.9 ± 59.8 vs. 471.6 ± 36.8 N); however, the knee extensors did display a greater loss in muscle strength compared with the PFs (Fig. 1, P = 0.02, η^2 = 0.45).
Interestingly, the loss of SOL and MG muscle CSA was significantly less than in the control period (pre-unilateral lower limb suspension [ULLS]), *P < 0.05. **KE strength loss greater than PF loss, P < 0.05.

Muscle Atrophy

Following ULLS, both groups displayed significant decreases in muscle CSA; however, the ULLS+Isc group displayed a preferential maintenance of the LG CSA (Fig. 2; SOL: ULLS control groups: 40.92 ± 6.28 vs. 38.03 ± 7.43 cm²; ULLS+Isc: 45.03 ± 8.39 vs. 42.05 ± 8.63 cm²; MG: ULLS control groups: 22.65 ± 5.50 vs. 20.56 ± 5.49 cm²; ULLS+Isc group: 26.32 ± 8.69 vs. 24.73 ± 9.34 cm²). Interestingly, the loss of SOL and MG muscle CSA was not different between the two groups (Fig. 2) (SOL: ULLS control groups: 40.92 ± 6.28 vs. 38.03 ± 7.43 cm²; ULLS+Isc group: 45.03 ± 8.39 vs. 42.05 ± 8.63 cm²; MG: ULLS control groups: 22.65 ± 5.50 vs. 20.56 ± 5.49 cm²; ULLS+Isc group: 26.32 ± 8.69 vs. 24.73 ± 9.34 cm²). When the CSA of the respective muscles is summed, no group difference was observed, as the control groups demonstrated an 8.6% decrease in PF muscle size, whereas the applied ischemia group lost 6.6% (*P = 0.69, \( \eta^2 = 0.03 \)). This nonsignificant difference at the level of the entire PF muscle appears to be due to large individual variability in muscle atrophy (ranges between 2.3 and 19.3% decreases in CSA for the ULLS control group and 4.92 vs. 14.63 cm²; ULLS+Isc group: 26.32 ± 8.69 vs. 24.73 ± 9.34 cm²).

Evoked Peak Forces

Following ULLS, the ULLS+Isc group was not different from the ULLS control groups for any of the peak forces or their respective ratios (\( P \geq 0.37 \) and \( \eta^2 \leq 0.06 \)). Thus for this analysis, data from all of the groups were pooled for presentation. No changes in twitch force were observed (Fig. 4A; 18.45 ± 1.39 vs. 18.83 ± 1.49 N; \( P = 0.84, \eta^2 < 0.00 \)), nor was there a change in PAP force (Fig. 4A; 55.02 ± 3.31 vs. 53.74 ± 4.34 N; \( P = 0.76, \eta^2 = 0.01 \). However, doublet force decreased ~10% (Fig. 4A; 40.586 ± 2.88 vs. 36.62 ± 3.45 N; \( P < 0.02, \eta^2 = 0.32 \)). Accordingly, the ratios of twitch to doublet force and PAP to doublet force increased (Fig. 4B; \( P = 0.03 \) and 0.05, \( \eta^2 = 0.31 \) and 0.21, respectively).

Following ULLS, the ULLS+Isc intervention group was not different from the ULLS control groups on either of these dependent variables (\( P \geq 0.71, \eta^2 \leq 0.01 \)). Thus for this analysis, data from all of the groups were pooled for presentation. Overall, ULLS resulted in a significant slowing in the action potential (CMAP) duration following 4 wk of limb suspension. ULLS resulted in a significant prolongation of the action potential, whereas applied ischemia alleviated this alteration. *Significantly different from control period (pre-ULLS), \( P < 0.05 \). **Significantly different from ULLS control groups, \( P < 0.05 \).
observed in the late-phase +dF/dt in either absolute (Fig. 5; 0.222 ± 0.016 vs. 0.206 ± 0.023 N/ms; \( P = 0.28, \eta^2 = 0.08 \)) or relative terms (Fig. 5; 0.542 ± 0.028 vs. 0.541 ± 0.033 \%/ms; \( P = 0.97, \eta^2 = 0.01 \)).

\[ -dF/dt \]

Following ULLS, the ULLS + ISc intervention group was not different compared with the ULLS control groups in either of these dependent variables (absolute \(-dF/dt\); \( P = 0.46, \eta^2 = 0.04 \); relative \(-dF/dt\); \( P = 0.82, \eta^2 = 0.01 \)). Therefore, for this analysis, data from all of the groups were pooled for presentation. Overall, ULLS resulted in an 11% slowing of \(-dF/dt\) when the data were expressed in absolute terms (\(-0.2302 \pm 0.029\) vs. \(-0.2044 \pm 0.024\) N/ms; \( P = 0.05, \eta^2 = 0.24 \)); however, this difference disappeared when data were expressed relative to peak force (\(-0.5380 \pm 0.032\) vs. \(-0.5168 \pm 0.021\) \%/ms; \( P = 0.39, \eta^2 = 0.05 \)).

**Specific Doublet Force**

Following ULLS, specific doublet force decreased 6.2% when data from the ULLS control and ULLS + ISc groups are considered collectively (\( P = 0.05, \eta^2 = 0.22 \)). The applied ISc did not exhibit any effect on the change in specific doublet force following ULLS (ULLS control groups: 5.37 ± 0.35 vs. 4.99 ± 0.30 N/cm²; ULLS + ISc group: 5.48 ± 0.53 vs. 5.25 ± 0.77 N/cm²; \( P = 0.62, \eta^2 = 0.02 \)).

**DISCUSSION**

The primary purposes of this study were to more fully elucidate adaptations in the electrophysiological and E-C coupling mechanisms of human skeletal muscle following prolonged unweighting and to determine the effectiveness of periodic bouts of ISc on preventing disuse-induced changes in muscle performance. To this end, our major findings were that 4 wk of ULLS slowed the muscle fiber action potential and altered E-C coupling (as assessed from the peak evoked forces and force-speed characteristics), with an end result being a decreased evoked doublet force per unit area. Interestingly, the ISc prevented the slowing in the CMAP and minimized LG atrophy, but did not affect the voluntary or evoked force properties.

**Adaptations in Muscle Function Following Prolonged Unweighting**

The present study demonstrated a dramatic 30% slowing in the duration of the Sol CMAP (Fig. 3), similar to that reported by Duchateau and Hainaut (14) in the adductor pollicis after 6-wk fracture-induced immobilization, but contrary to the observation of no change in the first dorsal interosseous following immobilization (21). The CMAP represents the summated electrical activity (arising from motor unit action potentials) resultant of the synchronous depolarization of the muscle fibers innervated by the depolarized nerve. Thus the CMAP waveform is determined by the effectiveness of temporal and spatial summation, which is affected by various factors. Specifically, the CMAP duration is most notably affected by temporal summation (33). Therefore, the physiological underpinnings of our observed slowing in the CMAP could be attributed to several factors, most notably changes in muscle fiber conduction velocity (MFCV), increased temporal dispersion between the responses of different motor units, and/or alterations in the muscle cell membrane properties (32, 33). Because MFCV could also impact temporal summation (7), it is possible that the slowing in CMAP is due to a slowing in the MFCV, which has previously been observed following disuse (12, 44). Because MFCV is directly proportional to fiber circumference in isolated frog muscle (25), the potential exists that muscle fiber atrophy is driving the slowed action potential response. How-
ever, because we observed a much greater relative change in the Sol CMAP duration than in the Sol muscle atrophy, along with a weak, nonsignificant relationship between the change in CMAP duration and the change in Sol CSA ($R^2 = 0.05, P = 0.39$), it seems unlikely that fiber atrophy alone is responsible for our observed prolongation.

Consistent with our finding of a slowed CMAP duration is the observation of a 12% increase in the twitch-to-doublet ratio (Fig. 4), indicating a preferential loss of evoked force at higher stimulation intensities. Within the human skeletal muscle fatigue literature, the evaluation of low-to-high-frequency force generation is common, as it provides insight into changes in E-C coupling failure, with our observation of greater force decrements during higher frequency stimulation typically thought to be resultant from altered muscle action potential properties, such as the slowing in the waveform that we observed (29). It has been speculated that high-frequency fatigue is due in part to ion disturbances (i.e., increased extracellular K$^+$ concentration), which prevents action potential propagation along the surface membrane and blocks conduction along the t-tubules (29, 54). However, in addition to the slowing in the CMAP duration, high-frequency fatigue is generally associated with decreased CMAP amplitude (29), which we did not observe; thus it is possible that the mechanisms driving shifts in the force-frequency relationship during fatigue are dissimilar to those observed with disuse.

Interpreting the maintenance of potentiated doublet force, despite a loss in the resting doublet force following ULLS (11% increase in the PAP-to-doublet ratio) also provides insight into the adaptations in the contractile properties (Fig. 4). The principal mechanism of PAP is generally regarded to be phosphorylation of myosin regulatory light chains, which renders them more sensitive to Ca$^{2+}$ release from the sarcoplasmic reticulum (35, 46). Based on this common interpretation of PAP, our findings may be indicative of increased myofiber Ca$^{2+}$ sensitivity following ULLS. However, this explanation is in stark contrast to single-fiber studies observing a decreased Ca$^{2+}$ sensitivity following bed rest in humans (56) and hindlimb unloading in rats (3). Another potential explanation could be a shift toward a type II muscle fiber-type composition, as fast fibers are known to undergo a greater phosphorylation of myosin regulatory light chains in response to conditioning activity (48). However, our observation of a slowing in the $+dF/dt$ may not support this possibility. A slow-to-fast fiber-type transition is known to occur in lower mammals with disuse, but is not consistently observed in humans (16, 20); hence it is difficult to determine whether fiber-type shifts are an influential factor. Further research is needed to elucidate the mechanism(s) responsible for the increased PAP-to-doublet ratio. In any event, this observation is consistent with our observation of an increased twitch-to-doublet ratio, as PAP results in a disproportionate increase in twitch and low-frequency contraction force (46).

Another interesting finding of the present study was the observation of a slowing in the relative $+dF/dt$ during the initial phase, but not the latter phase (Fig. 5). Studies examining contractile force development properties following prolonged disuse have reported both a slowing in force development and/or prolonged time to peak tension (13, 34), as well as no change (14, 47). The most commonly cited factors that can result in a slowing of the $+dF/dt$ are a shift toward a slower (type I) fiber-type composition (26) and the amount and rate of sarcoplasmic reticulum Ca$^{2+}$ release (17, 40). It seems unlikely that a fiber-type shift toward a slower profile can explain the decreased $+dF/dt$, since fiber-type changes in human muscle are either nonexistent or change toward a faster profile (16, 20). Another potential explanation of this finding relates to musculoskeletal stiffness. Because these changes were during the initial phase of the contraction only, it seems plausible that ULLS-induced changes in stiffness of the series elastic component may be involved. For example, if a decrease in tendon stiffness occurred as a result of ULLS (as previously reported following bed rest) (43), the evoked force curve should exhibit a slowing in the $+dF/dt$, which may be most pronounced during the early phase of contraction, as it might initially provide a greater transfer of force. In terms of the $-dF/dt$, which is generally thought to be dependent on the rate of cross-bridge detachment and/or calcium reuptake (24, 52), we observed an 11% slowing when expressed in absolute terms, but a nonsignificant slowing of 4% when expressed in relative terms. Thus it appears that the absolute slowing was primarily due to the change in peak force vs. a slowing in the contractile properties per se.

One particularly interesting finding from the present study is the observation of exceptionally high levels of individual variability in muscle atrophy (range $\sim$2–19%). Individual variation in the disuse-atrophy response has received little attention, although it has been previously observed in astronauts following spaceflight, where it has proven difficult to determine how much of the variability reflects true individual differences as opposed to the use of countermeasures (20). Similar variability was observed in our laboratory’s prior ULLS studies of the quadriceps femoris muscle with individual atrophy ranging between $\sim$7 and 26% after 5-wk ULLS (41). It is possible that the individual variability is due to either compliance or biological differences. We did not observe a relationship between accelerometer-detected steps during ULLS and atrophy ($R^2 = 0.02, P = 0.67$); however, it is possible that the sensitivity and specificity of our measure of compliance are not high enough to discriminate this. Future endeavors should attempt to determine whether individual variation in atrophy may be attributed to biological parameters and, if so, what genetic factors are associated with the response.

Efficacy of Applied Isc

Although Isc did not significantly reduce strength loss or atrophy at the whole level of the PFs, it did result in atrophy attenuation of the LG muscles (Fig. 2). The finding of no attenuation at the whole muscle level is in disagreement with results from Takarada and colleagues (50), who observed that Isc reduces the knee extensor atrophy rate by one-half following 14 days of bed rest in postsurgical patients. However, it should be noted that the frequency and duration of the applied Isc in the present study was substantially less than that of Takarada et al., who twice daily administered five 5-min bouts of Isc (compared with our three 5-min bouts three times per week). Thus it is very likely that our Isc protocol was not as potent a stimulus as the aforementioned study.

Another interesting finding was the differential effect on the individual triceps surae muscles, with the ULLS+Isc group
displaying a preferential maintenance of the gastrocnemius CSA, but not Sol. This finding is particularly intriguing when considered in light of the Sol having a much greater type I fiber composition compared with the gastrocnemius (~80 vs. 50%) (23). These findings are in agreement with those recently reported by Kawada and Ishii (30), who observed skeletal muscle hypertrophy (7.8% increase in wet weight) in the type II muscles of the rat following 2 wk of chronic blood flow restriction, without a concomitant change in type I muscles (30). Thus the effect of blood flow restriction on muscle atrophy may depend on the muscle fiber composition. Additionally, because the Sol muscle has been shown to primarily contribute to the contractile characteristics of the PFs when the knee is in the flexed position (as in the present study) (55), care should be taken when interpreting our findings of ISc having no effect on the voluntary or evoked forces, as it is possible that a biomechanical arrangement allowing for a greater contribution of force from the gastrocnemius muscle would have yielded different findings.

The most dramatic effect of ISc on muscle function was the abolishment of the prolongation in the muscle fiber action potential observed in the control subjects (Fig. 3). It is well known that acute ISc exerts profound effects on the electrical properties of muscle (9, 22, 27, 45), but little is known on the adaptations associated with transient or chronic ISc. The limited data on this topic suggest that chronically ischemic tissue adapts with a change in the properties or regulation of K+ channels and a higher resting membrane potential (38).

Further research is needed to more fully identify the impact of ISc on muscle function. Additionally, the mechanisms underling the alterations in membrane performance need to be identified.

In summary, we have identified numerous adaptations in the electrical, mechanical, and morphological properties of human muscle following prolonged unweighting, and the influence of periodic bouts of muscle ISc on these functional characteristics. In addition to the expected loss in muscle mass and strength, we observed a slowing in the duration of the evoked muscle action potential, an increased twitch-to-doublet ratio, and the PAP force response (increased PAP-to-doublet ratio). We also detected a slowing in the ability of the muscle to develop force during the initial, but not the latter, phase of an evoked contraction, along with a reduction in in vivo doublet force per unit area. Although the transient application of ISc did not dramatically influence muscle strength or mass, it did result in a preferential maintenance of LG muscle size and prevent the unweighting-induced slowing in the muscle action potential. Overall, these findings suggest that unweighting alters the contractile factors involved in the E-C coupling processes, and that ISc impacts the action potential conduction properties of the sarcolemma without substantially altering the force generation properties.

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