Adaptations in human neuromuscular function following prolonged unweighting: II. Neurological properties and motor imagery efficacy

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Clark, B. C., T. M. Manini, S. J. Bolanowski, and L. L. Ploutz-Snyder. Adaptations in human neuromuscular function following prolonged unweighting: II. Neurological properties and motor imagery efficacy. J Appl Physiol 101: 264–272, 2006. 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 neurological properties following 4 wk of limb suspension [unilateral lower limb suspension (ULLS)], along with the effect of motor imagery (MI) training on these properties. In the companion paper (Part I), we report our findings on the changes in skeletal muscle properties. Additionally, in the present paper, we analyze our findings to determine the relative contribution of neural and muscular factors in strength loss. Measurements of central activation, the H-reflex, and nerve conduction were made before and after 4 wk of ULLS (n = 18; 19–28 yr). A subset of the subjects (n = 6) performed PF MI training 4 days/wk. Following ULLS, we observed a significant increase in the soleus H-reflex (45.4 ± 4.0 to 51.9 ± 3.7% expressed relative to the maximal muscle action potential). Additionally, there were longer intervals between the delivery of an electrical stimulus to the tibial nerve and the corresponding muscle action potential (M-wave latency; mean prolongation 0.49 ms) and H-reflex wave (H-wave latency; mean prolongation 0.46 ms). The efficacy of MI on strength was ambiguous, with no significant effect detected (although a modest effect size was observed; r² = 0.18). These findings suggest that unweighting induces plastic changes in neural function that appear to be spatially distributed throughout the nervous system. In terms of the relative contribution of neural and muscular factors regulating strength loss, we observed that neural factors (primarily deficits in central activation) explained 48% of the variability in strength loss, whereas muscular factors (primarily sarcolemma function) explained 39% of the variability.

Disuse atrophy; electromyography; bed rest; nerve conduction

In the preceding companion paper to this article, we made the assertion that the excessively large loss of muscle strength following prolonged periods of disuse may be due to alterations in neurological function (8a). In this paper (Part II), we investigate this postulate, as we report our findings on the changes in neural function following prolonged unweighting, and the effectiveness of motor imagery (MI) (imagined maximal muscle contractions) in preserving the neural function. Additionally, in this paper, we collectively analyze our findings to determine the relative contribution of the neural vs. muscular factors of strength loss.

Several authors have postulated that much of the disuse-induced loss of strength is related to “neural factors” (5, 10, 27); however, knowledge surrounding the precise neural mechanisms that are altered is limited. The majority of prior investigations examining this issue have evaluated the voluntary surface electromyogram (EMG) signal during maximal or submaximal voluntary contractions before and after disuse (5, 6, 10, 14, 45) and, based on their findings, made inferences about neural changes. Unfortunately, interpreting longitudinal changes in the voluntary interference EMG signal is difficult and may not rigorously reflect altered levels of neural drive to the muscle (19) and certainly does not provide accurate spatial information regarding at what level of neural control alterations may be occurring.

There are many sites in the nervous system in which the overall neural output to skeletal muscle can be modified. With respect to disuse, there is evidence that adjustments in both central and peripheral nerve function result in both humans and animals. For example, a reduced ability to centrally (voluntarily) activate the quadriceps femoris muscles following 20 days of bed rest in four subjects has recently been reported (27). At the level of the spinal cord, neural plasticity with disuse has been evaluated using the H (Hoffman) reflex with discrepant findings (2, 12, 49). In addition, a slowing in axonal nerve conduction velocity in cats following immobilization has been reported (41), along with a slowed conduction velocity in the branching axon terminals following bed rest in humans (43). Thus, although adaptations in the nervous system with disuse have not been as extensively studied as its skeletal muscle counterpart, there is still substantial evidence as to its plastic changes.

If indeed one of the limiting factors to strength following prolonged periods of disuse is of neural origin, it is possible, if not probable, that this deficit would arise from supraspinal factors. This postulate is supported by previous experiments that have observed changes in motor cortex organization and excitability following immobilization (29, 51). A recent study observed a reduction in the transcranial magnetic stimulation motor-evoked potential following immobilization while subjects performed imagined motor movements, which indicates that immobilization induced a functional reorganization or decreased excitability in the cerebral cortex area involved in executing movement (26). To our knowledge, no published reports exist on the effect of MI training in minimizing the disuse-induced loss in muscle strength; however, five studies have been conducted on its overall effectiveness in altering strength (24, 39, 46a, 50, 52), with the majority of these studies have shown favorable results (39, 46a, 50, 52). The theoretical conclusions made in the present paper are based on a limited number of subjects and should be viewed with caution.

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basis for MI increasing muscle strength is based on recent neuroimaging studies demonstrating that MI and actual movements share, at least in part, common neural pathways. For example, a host of brain functional imaging studies indicate that MI activates several cortical areas, including the primary motor cortex, supplementary and premotor areas, and cingulated gyrus (30, 38, 40, 42, 47), all of which are known to contain corticospinal neurons in monkeys (16). Additionally, MI has been shown to acutely alter corticospinal excitability (7, 17, 20, 22, 23, 48). Although it is difficult to determine whether the acute effects of MI will translate into long-term changes following MI training, nonetheless, there is a conceptual basis for it when performed concomitantly with unweighting to counteract the loss of strength and supraspinal function.

The purposes of the present study were to determine the adaptations in plantar flexor (PF) neural function following unilateral lower limb suspension (ULLS), and evaluate the efficacy of MI on these functional properties. We hypothesized that alterations in neural dysfunction would result following ULLS. Additionally, we hypothesized that the group performing MI would demonstrate a reduction in disuse-induced strength loss and that, overall, the degree of strength loss would be due to maintenance of central activation. While the feasibility of using MI training to lessen disuse-induced strength loss may not be overly practical, studies of this nature are needed as they will help to establish a therapeutic theory by which to approach brain motor function and aid in elucidating the relative contribution of the nervous system in voluntary muscle force generation.

Another overall goal of this project was to determine the relative contributions of neural and muscular changes in regulating muscle strength loss following unweighting. Thus in this paper we combine data sets presented in Part I and Part II to predict the relative influence of the respective variables in mediating the loss of strength.

METHODS

General Overview of the Experimental Design

Neurological function was assessed before and after a 4-wk control period and immediately following 4 wk of ULLS. These tests measured a variety of neural properties, including central (voluntary) muscle activation, H-reflex excitability, and nerve conduction velocity, and are described in detail below. This limb suspension model involves subjects performing all ambulatory activity on crutches while wearing a shoe with a 10-cm-thick sole on the right foot, thus eliminating ground contact by the left foot, thereby unweighting the left lower limb. While undergoing the ULLS protocol, subjects were assigned to one of three groups: 1) ULLS with no intervention (ULLS), 2) MI 4 days/wk (ULLS+MI), and 3) applied ischemia 3 days/wk (ULLS+Isc). Because there was no statistically significant differences between the ULLS+Isc and ULLS with no intervention groups on the neurological 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 ischemia altering strength, for this variable the groups were not pooled.

Subjects and the Limb Suspension Model

Full details on subjects and the ULLS model are provided in Part I (8a). In brief, a total of 18 volunteers completed 4 wk of ULLS. Twelve of these subjects were assigned to the control groups (5 men and 7 women; 20.75 ± 0.76 yr, 166.77 ± 3.26 cm, and 66.30 ± 3.50 kg), whereas six were assigned to the MI group (2 men and 4 women; 21.00 ± 1.41 yr, 166.01 ± 3.61 cm, and 64.35 ± 3.12 kg). During the control period and the ULLS period, subjects wore a planar accelerometer (Dynamstream Innovations, Alberta, CA) on their left leg, which allowed for quantification of steps taken during the respective time periods. The Syracuse University and SUNY Upstate Medical University Institutional Review Board approved the experimental protocol, and all subjects provided written, informed consent before participation.

MI

MI training sessions were held four times per week. Subjects were brought to the laboratory alone and instructed to perform 10 imagined maximal contractions of the left PF muscles. The duration of each imagined contraction was ~15 s and was followed by ~1 min of rest. To teach subjects how to perform MI, we initially had them sit in the PF dynamometer and told them to relax all of the leg muscles, especially the PFs, as much as possible. Next, they were instructed to maximally activate the brain, but not the muscles. Soleus EMG was qualitatively measured during the first week of MI training to ensure that muscle activation did not occur. The verbal directions were based on those previously used in MI studies (24, 39, 50). On a verbal signal to begin, the subject was instructed to “concentrate on the left calf muscles, and imagine the foot pushing maximally against the footpad. Feel yourself pushing up as hard as you can with your left calf muscles. Keep pushing up as hard you can with your left calf muscles. Keep imagining you are pushing as hard as you can, push harder, push harder...now stop.” It should be noted that this mental exercise was not simply a visualization of oneself performing the task; rather, the performers were instructed to adopt a kinesthetic imagery approach, in which they urged the muscles to contract maximally (39).

During pilot testing, we assessed our ability to successfully coach individuals on performing MI. To do this, we measured the H-reflex at rest and during MI in 10 subjects, as increases in the H-reflex have been observed in individuals performing MI (17, 20, 22). In this experiment, we had subjects lie prone on an examination table while soleus EMG was recorded and periodic electrical stimulation was delivered to the tibial nerve while at rest or during MI (complete details of the H-reflex are provided below). Our results were similar to those reported by Hale et al. (22), as we observed a slight facilitation in the H-reflex acutely during MI [H-reflex excitability ~48.5 ± 3.6% of compound muscle fiber action potential (CMAP) at rest and increasing to 50.6 ± 3.7% during MI; P < 0.01; n² = 0.40] (9). Therefore, based on this experiment revealing an altered physiological response to MI, which has previously been shown to be elevated during MI by other laboratories, we feel that our MI cuing was successful in activating the corticospinal pathways.

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 (8). For greater details regarding the methodologies used, please see this paper by Clark et al. (8).

Muscle strength and mass. PF muscle cross-sectional area (CSA) was assessed using magnetic resonance imaging, and muscle strength was assessed using a customized dynamometer. For complete details on these procedures, please see Part I (8a).

Central activation. To determine what percentage of the total force-generating capacity of the unweighted PFs can be produced voluntarily, a combination of voluntary and electrically stimulated contractions were performed following the commonly utilized interpolated twitch technique (4, 8, 27). Briefly, a supramaximal doublet (100 Hz) was delivered to the tibial nerve while the subject performed a maximal voluntary contraction (MVC). The increase in force following the doublet was expressed relative to a potentiated doublet...
The excitability of spinal fusiform fibers. As such, it is a commonly utilized tool to assess the influence of muscle spindle sensitivity and γ-activation of intrafusal fibers. As such, it is a commonly utilized tool to assess the excitability of spinal α-motoneurons, while also reflecting transmission efficiency in Ia afferent synapses (1). Because the nature of this disuse paradigm (ULLS) results in one limb being unweighted and the other one maintaining/increasing the weight-bearing load, we felt it would be particularly interesting to evaluate neural plasticity as the level of the spinal reflexes in the two different sides. Thus we assessed the H-reflex excitability from both the left (unweighted) and right (weight-bearing) limbs.

The excitability of the H-reflex response in the resting soleus muscle was assessed while the subject lied prone on an examination table with the ankle joint maintained at 45° of plantar flexion. To compare and interpret changes in evoked H-reflex responses following periods of unweighting, it is vital that the effective stimulus strength remain invariant between recording sessions, because the size of the H-reflex is heavily modulated by even small changes in stimulus intensity. Therefore, it is suggested that, when repeated H-reflex measurements are performed, it is an advantage to use a stimulation intensity that produces M-wave responses corresponding to a constant percentage of the maximal M-wave to ensure that the same number of motor axons are recruited in each trial, which indicates that stimulus intensity to the efferent nerve is also kept constant (1). Thus, in the present study, the electrical stimulation was first increased to obtain a maximal CMAP (also known as M\text{max}), and the stimulation intensity was adjusted and continuously monitored to evoke a muscle action potential (M-wave) with a peak-to-peak amplitude equal to 20 ± 2.5% of the CMAP. The corresponding peak-to-peak amplitude of the H-wave was then calculated and expressed as a percentage relative to the CMAP (H\text{2}/CMAP) (Fig. 1).

Averages of eight trials were used in this calculation. For this measurement, EMG sampling rate was 2.500 Hz.

In addition to providing an index of spinal reflex excitability, the H-reflex recordings can be used to derive indexes of peripheral nerve conduction (44). Here, the latency responses in the soleus EMG H-reflex recordings were evaluated (Fig. 1). Specifically, two measures of nerve conduction were made. First, the time difference between stimulus onset and the upshot of the M-wave was calculated (8) and represents the time required for conduction down the larger diameter (higher threshold Type II) motor units to the terminal branches and propagation across the neuromuscular junction (Fig. 1). Second, the time interval between stimulus onset and the upshot of the H-wave was calculated (8) and represents the time required for signal propagation through the reflex arc [including the Ia afferent, synaptic delay at the motorneuron, and the smaller diameter (lower threshold Type I) efferent motor units back across the neuromuscular junction] (Fig. 1). In addition to this parameter, the time difference between the upshot of the two respective waves (H- minus M-wave latency) was calculated. To increase the resolution of these temporal computations, EMG sampling rate was increased to 200 kHz.

Statistical Analysis

Mixed-model analysis of variance techniques were utilized to determine the effect of the independent variables (i.e., within-subjects factor: time, side; between-subjects factor: treatment group) on the dependent variables. Initially, the control period data were subjected to these analyses (precontrol vs. postcontrol), and no significant differences were observed during the control period for any of the respective dependent variables. Therefore, to assess ULLS-induced changes, an average of the two control periods was calculated and used in a separate analysis (average control vs. post-ULLS). Our laboratory has previously reported the reliability and variability in these dependent variables when separated by 4 wk in these same subjects (8).

Sample size for the present study was based on previous data from Kawakami et al. (27) investigating bed rest-induced changes in central activation failure following 20 days of bed rest, and on data from Ploutz-Snyder et al. (37) on loss of strength following ULLS. These calculations were based on power ≥0.80 and a P value ≤0.05. 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 (partial η²) are reported as an additional statistical parameter to aid in interpretation of the findings. Additionally, to gain further insight into the effect of MI on strength, the degree of muscle atrophy (percent decrease in PF CSA) was used as a covariate.

Neural vs. Muscular Factors in Strength Loss

One of the overall goals of this project was to delineate the relative contribution of neurologic changes and muscle changes in mediating the strength loss. By design, subsets of the subjects received interventions designed to perturbate primarily the nervous or muscular system in an attempt to gain a substantial amount of between-subject variability in the various measured physiological variables, and therefore multiple regression analytical techniques may elucidate the predictive relationships between and among select factors in strength loss. We identified nine variables that we felt may theoretically be predictive of strength loss (neural property variables: central activation, H-reflex excitability, H- minus M-wave latency, M-wave lat...
tency; muscle property variables: CMAP amplitude, CMAP duration, rate of evoked force development and relaxation, and soleus muscle CSA). From the average control data and the post-ULLS data, the natural logarithm for each of these variables as well as muscle strength were calculated, and the percent change was determined [the analysis of the log-transformed variable provides the most accurate estimate of the percent difference (25)]. Next, the aforementioned variables were entered into a blocked multiple-regression analysis where percent decrease in strength served as the dependent variable, and the neural variables were entered into the first block (model 1) and the muscle variables into the second (model 2). From this analysis, the coefficient of determination ($R^2$) for model 1 was determined and represented the percentage of explained variance in strength loss due to neural factors. The increment to $R^2$ in model 2 was also determined and represented the variance explained due to muscle factors. To evaluate the independent contribution of each predictor, the semipartial $r^2$ (sp-$r^2$) values from model 2 were calculated. The sp-$r^2$ value is interpreted as the percentage of variance in strength loss (the dependent variable) uniquely attributable to the given independent variable (by factoring out shared variance contributions with other predictors).

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 ($P < 0.00, \eta^2 = 0.80$). No differences were observed in physical activity levels between the experimental groups during the control period (ULLS control groups: 4,672 ± 360 steps/day, vs. ULLS + MI group: 5,524 ± 1,150 steps/day; $P = 0.61, \eta^2 = 0.06$). Additionally, no differences were observed during the ULLS protocol, as reductions in weight-bearing steps were 99.68 ± 0.13 and 99.72 ± 0.12% for the ULLS control and MI groups, respectively ($P = 0.99, \eta^2 < 0.00$).

Reliability of Dependent Variables

The test-retest reliability of the control period data has been published elsewhere (8). In brief, the overall reliability of all of the reported variables had coefficients of variation (CV) between 1.3 and 4.3% and intraclass correlation coefficients (ICC) between 0.75 and 0.97. Strength displayed a CV = 4.2% and ICC = 0.97. Central activation displayed a CV = 3.91%. H-reflex excitability displayed a CV = 11.0% and an ICC = 0.93, while the spinal reflex latencies displayed CVs between 1.3 and 4.3% and ICCs between 0.75 and 0.97.

Strength and Atrophy

Overall, the ULLS control group ($n = 6$; as the applied ischemia subjects were not pooled for this variable due to the theoretical basis of it potentially altering strength) lost 14.2% of their PF strength (231.9 ± 32.4 vs. 198.9 ± 24.8 N), while the MI group lost 9.9% (Fig. 2) (236.9 ± 17.9 vs. 213.3 ± 28.2 N). Both of these decreases were significant ($P < 0.00, \eta^2 = 0.56$), and there were no differences between the two groups ($P = 0.56, \eta^2 = 0.04$).

In addition to assessing PF strength, we also measured knee extensor (KE) strength to aid in delineating the effect of MI, which was specific to the PFs, on voluntary strength. Thus, when the PF strength loss was considered in light of the changes in the KE (entering muscle group into the ANOVA model), again no differences were observed between the groups (time × muscle group × treatment group, $P = 0.17$) (ULLS control group: 540.8 ± 45.8 vs. 449.1 ± 46.9 N; MI group: 550.8 ± 62.7 vs. 400.1 ± 40.2 N), although a modest effect size was observed (0.18) (Fig. 2).

We also analyzed the PF strength loss between these two groups using atrophy (percent decrease in CSA) as a covariate. Again we observed no significant difference, but a small effect size, as the estimated marginal means revealed the MI subjects lost 8.5% of their strength compared with 15.7% in the nonintervention control subjects (Fig. 2, inset; $P = 0.32, \eta^2 = 0.11$).

Central Activation

No significant changes were observed in the ability to voluntarily activate the PF following ULLS (Fig. 3) ($P = 0.31, \eta^2 = 0.07$). Additionally, there was no significant difference between the ULLS control and MI groups in this response ($P = 0.55, \eta^2 = 0.03$) (ULLS control groups: 93.06 ± 2.20 vs. 89.22 ± 3.38%; MI group: 93.84 ± 1.08 vs. 92.83 ± 4.12%). It appears that this analysis is being skewed by one outlier in the MI group who experienced a large decrement in central activation following ULLS. To illustrate this, it should be noted that 83% (5 of 6) of the subjects in the MI group demonstrated either minimal changes or increases in the ability to voluntarily activate their PF maximally, whereas 55% of the subjects in the ULLS control group experienced a decrease in this ability (defined as a decrease ≥ 2.0% (mean decrease −10%), while 9% experienced no change, and 36% increased (mean increase −4%).

H-Reflex Excitability

Following ULLS, no changes were observed in the CMAP amplitude (see Ref. 8a), and the MI countermeasure group was not different from the ULLS control groups in the ULLS-induced H-reflex excitability response ($P = 0.70, \eta^2 = 0.05$). Therefore, for this analysis, data from all of the groups were
pooled for presentation. Following ULLS, plastic changes in the spinal reflex excitability were observed, which was dependent on the weight-bearing side (Fig. 4) \((P < 0.01, \eta^2 = 0.38)\). Further analysis revealed a differential response, with the unweighted side demonstrating an increase in the H-reflex \((45.44 \pm 3.6 \text{ vs. } 51.88 \pm 4.2 \, \text{H}_20/\text{CMAP})\), while the weight-bearing side was similar to that during the control period \((46.05 \pm 4.0 \text{ vs. } 41.99 \pm 3.7 \, \text{H}_20/\text{CMAP})\).

**Evoked Response Latencies**

Following ULLS, the MI countermeasure group was not different from the ULLS control groups for any of the nerve conduction measures \((P \geq 0.31, \eta^2 \leq 0.10)\). Therefore, for this analysis, data from all of the groups were pooled for presentation. Overall, the M-wave latency increased \(-8.5\% \) (Fig. 5; \(P < 0.00, \eta^2 = 0.51\)) \((6.18 \pm 0.62 \text{ to } 6.67 \pm 0.36 \text{ ms})\). Additionally, the H-wave latency increased a similar absolute amount as the M-wave latency (Fig. 5; \(P < 0.00, \eta^2 = 0.47\)) \((29.20 \pm 1.43 \text{ to } 29.66 \pm 1.41 \text{ ms})\), whereas no changes were observed in conduction through the spinal reflex loop (H-minus M-wave latencies) (Fig. 5; \(P = 0.67, \eta^2 = 0.05\)) \((23.02 \pm 1.38 \text{ vs. } 22.99 \pm 1.51 \text{ ms})\).

**Neural vs. Muscular Factors in Strength Loss**

Collectively, the predictor variables (central activation, H-reflex excitability, H-minus M-wave latency, M-wave latency, CMAP amplitude, CMAP duration, rate of evoked force development and relaxation, and soleus muscle CSA) in our multiple-regression analysis explained \(-87\%\) of the variability of strength loss \((R^2 = 0.872, \text{ adjusted } R^2 = 0.727, P < 0.01)\) (Fig. 6A). Within this analysis, the neurological variables explained \(-48\%\) of the variation in strength loss \((R^2 = 0.479, P = 0.05)\), whereas the muscular variables explained \(-39\%\) \((R^2 = 0.392, P = 0.02)\) (Fig. 6A). To evaluate the contribution of each individual predictor variable, the sp-$r^2$ values (a measure of the independent variance contribution that each predictor contributes to the model after factoring out shared covariance contributions with other predictors) indicated that central activation was the most influential variable in mediating the loss of strength \((\text{sp-}r^2 = 0.593, \beta = 1.246, P < 0.01)\) followed by CMAP duration \((\text{sp-}r^2 = 0.308, \beta = -0.171, P < 0.01)\) and CMAP amplitude \((\text{sp-}r^2 = 0.211, \beta = -0.265, P < 0.01)\) (Fig. 6B). After these three variables, five others significantly predicted strength loss, explaining between \(-8\%\) and \(-10\%\) of the variability (Fig. 6B; M-wave latency, \(\text{sp-}r^2 = 0.108, \beta = -0.282, P = 0.03\); rate of force relaxation, \(\text{sp-}r^2 = 0.106, \beta = -0.125, P = 0.03\); nerve conduction velocity, \(\text{sp-}r^2 = 0.103, \beta = -0.066, P = 0.03\); soleus CSA, \(\text{sp-}r^2 = 0.0868, \beta = 1.047, P = 0.05\); and rate of initial phase force development, \(\text{sp-}r^2 = 0.084, \beta = -0.020, P = 0.05\) ). The H-reflex excitability measure did not significantly explain the loss in strength \((\text{sp-}r^2 = 0.012, \beta = -0.068, P = 0.42)\).

**DISCUSSION**

The primary purposes of this study were to more fully elucidate adaptations in neurological function following pro-
longed unweighting and determine the effectiveness of MI training on reducing the changes in neural properties and strength. To this end, our major findings are that 4 wk of ULLS appear to create plastic changes at the level of the spinal cord, with an increase in the H-reflex excitability in the unweighted limb and a slight decrease in the weight-bearing limb, and prolong the latency response of the muscle fiber action potential. Contrary to our hypothesis, we did not observe a significant decrease in central activation. MI training did not exert profound effects large enough to result in statistically significant differences in the maintenance of strength. Perhaps the most interesting finding from the present study relates to the analyses evaluating the relative contribution of the nervous system and the muscular system in regulating strength loss, with neural factors explaining 48% of the variation in strength loss and skeletal muscle factors explaining 39%. In addressing these findings, we will first discuss the novel findings regarding the underlying adaptations that we observed in neural properties, followed by an examination of the influence of MI training, and the relative contribution of neural vs. muscular factors in mediating unweighting-induced strength loss.

Adaptations in Neurological Function Following Prolonged Unweighting

The present study observed a differential H-reflex excitability response by weight-bearing limb, with the unweighted limb displaying hyperexcitability and the weighted limb displaying hypoxicitability (Fig. 4). To our knowledge, this is the largest study (in terms of sample size) to evaluate changes in the H-reflex following a period of disuse. The finding of an increased H-reflex excitability is in agreement with that of Duchateau (12), who observed a slight facilitation in the H-reflex after 5 wk of bed rest in one subject, and with those of Anderson et al. (2), who observed an increased response following 3-wk hindlimb unloading in juvenile rats. However, they are contrary to the report from Yamanaka and colleagues (49), who observed a drastic reduction in the H-reflex following 20 days of bed rest in five subjects (63% of $M_{\text{max}}$ before bed rest to 27% after). One possible explanation for the discrepant findings is the conditions under which the H-reflexes were obtained. In the present study, they were taken at rest while the subject was prone on an examination table, whereas Yamanaka et al. obtained their measurements during standing. Thus the disuse-induced response could vary with the level of activation in the test muscle, as has been described with exercise training inducing an adaptation in the H-reflex during contraction but not at rest (1). However, other possibilities explaining the discrepant findings could be differences in the disuse model utilized (ULLS vs. bed rest) or the potential confound of Yamanka et al. (49) employing a countermeasure, which included daily resistance exercise training (leg press), which has previously been shown to alter the H-reflex excitability (1).

Although many scientist interpret a facilitation of the H20/CMAP as an increase in the excitability of α-motorneurons, it is also reflective of properties of the Ia afferent. Modulation of the H-reflex amplitude can result from a number of factors, including presynaptic inhibition of the Ia fibers, variation in the amount of Ia neurotransmitter release, and changes in the excitability of the motorneurons due to changes in either the membrane potential arising from excitatory or inhibitory inputs or intrinsic properties of the neurons. We feel our findings are driven primarily by the Ia afferents and that this finding does not necessarily indicate that the motorneurons are more excitable or can be more easily activated from descending supraspinal drive. It is possible that our findings were due to increased Ia transmission efficacy (reduced presynaptic and/or postsynaptic inhibition), as chronically blocked peripheral nerve conduction (with both tetrodotoxin and nerve section) results in enhanced Ia synaptic transmission in rat spinal motoneurons, which is accounted for by the deprivation of sensory impulse activity (31). Although the present study certainly did not result in complete blockage of Ia afferents, it is plausible that the observed increase in the H-reflex response may be due to changes in afferent supersensitivity vs. motoneuron excitability. Further research is needed using other techniques to determine the neurophysiological explanation of our observed response.

Fig. 6. Relative contribution of neural vs. muscular factors in regulating the disuse-induced loss of muscle strength. $A$: overall, the multiple regression model explained 87% of the variance in strength loss, with 48% of the variance being attributed to alterations in neural function and 39% by muscular factors. $B$: percentage of variance in strength loss uniquely attributable to the various physiological factors after factoring out shared contributions with other predictors (solid bars, $P \leq 0.01$; hatched bars, $P \leq 0.05$).
Our observation of an increased latency time in the M- and H-waves indicates a slowing in nerve conduction following ULLS (Fig. 5). Because a slowed conduction was observed for both the H-wave and M-wave responses, without a slowing through the spinal reflex loop (as indicated by no change in H-minus M-wave latency time, Fig. 5), these findings suggest that the slowed responses are primarily related to the conduction velocity in the supply nerves, the branching axon terminals, and/or transmission across the neuromuscular junction (28). This assertion is based on the assumption that, if a slowing in axonal nerve conduction velocity occurred following ULLS, then a longer transmission time through the spinal reflex loop would be observed. Therefore, because this time was not altered, but a slowed response in both the M-wave and H-wave responses was observed, it seems likely that this prolonged response was due to altered function of neuromuscular transmission. This interpretation is consistent with similar suggestions from other authors (21, 43) and congruent with animal studies, indicating that the decreases in physical activity levels result in morphological adaptations of the neuromuscular junction (11, 18).

Contrary to our hypothesis, we did not find a significant reduction in the ability of subjects to centrally activate their PFs following ULLS. This outcome appears to exhibit a high degree of variability between subjects, as we did observe a mean decrease (from 93 to 89%), but that failed to attain statistical significance, likely due to the inconsistency of the mean decrease (from 93 to 89%), but that failed to attain degree of variability between subjects, as we did observe a PF following ULLS. This outcome appears to exhibit a high explanation (11, 18).

Factors Regulating Strength Loss

Relatively few studies have attempted to evaluate the relationship between neural and muscular properties in regulating disuse-induced losses in strength, despite it being suggested in a Research Roundtable article a decade ago (3). Thus, although our findings on this topic are certainly preliminary, they are novel and informative as they attempt to address this question. From the variables that we measured and entered into our regression model, we were able to explain all but 13% of the variability in strength loss, with the alterations in the nervous system being a higher contributor than the skeletal muscle counterpart (Fig. 6A). Further investigation into the physiological variables influencing the large deficits in strength following unweighting indicated the decrement in central activation was the highest predictor of strength loss. Although this finding may appear to contradict our finding of central activation not showing a significant decrease following ULLS, it can be explained when one considers what the two analyses are evaluating, with the ANOVA testing for mean differences at two time points (pre- vs. post-ULLS), while the regression analysis is evaluating whether variables (i.e., strength and central activation) go up or down together.

The finding that changes in CA were associated with changes in voluntary force generation following disuse is in agreement with a report from Kawakami and colleagues (27), who reported that ~55% of the variation in strength following 20 days of bed rest were explained by central activation. Their calculation was based solely on a correlation analysis, thus not accounting for shared variances with other variables (i.e., muscle size). The other variables that were highly predictive of strength loss were those associated with the muscle cell membrane, as both the duration and amplitude of the CMAP were highly significant and explanatory.

It has long been observed that there is a relationship between muscle size and strength when individuals are compared in cross-sectional studies (32–34). Thus we had hypothesized that the decrease in muscle CSA would be

Efficacy of MI

We did not observe any effect of MI on the H-reflex or nerve conduction responses, although we did not expect MI training to have any influence on peripheral and spinal nerve properties (latency times, H-reflex excitability) but rather expected it to influence supraspinal neural functional, as the primary mechanism underlying the strength increase following mental training-induced enhancement (in the absence of disuse) is the supraspinal command to muscle, probably mostly localized to the cerebral cortex (39). Interpreting the findings on the effect of MI on strength and central activation is less straightforward. Statistically speaking, we did not observe any effect of MI on strength or central activation (Figs. 2 and 3). However, we did observe small to modest effect sizes with respect to strength, indicating that the MI may have had some impact. This was most apparent when the loss in PF strength was considered in parallel with the loss in KE strength, as MI training was targeted only for the PF. Here, the ULLS control group displayed only slightly more KE strength loss compared with PF (~14 and 17%), while the MI group exhibited a much larger loss in KE than PF strength (27 vs. 10%). This observation indicates that the MI subjects may have had a larger predisposition for disuse-induced effects than the ULLS control subjects, and we must acknowledge that these MI findings may be susceptible to Type II error. It should be noted that it is possible results may vary with different muscles (39), as distal and proximal muscles differ in their size of cortical representation (36). Further research on the impact of mental imagery training concomitantly with disuse is warranted; specifically it is suggested that the effect on different muscle groups be investigated, along with higher frequencies and duration of MI training.
responsible for a large portion of the loss in strength following ULLS. However, contrary to our expectations, we did not observe a strong relationship, as atrophy alone explained <10% of the loss in strength. Although this finding is counter to the cross-sectional study findings, it is in agreement with Kawakami and colleagues (27), who reported a nonsignificant relationship between atrophy and strength following 20 days of bed rest.

We should caution our findings regarding the relative contribution of neural vs. muscular factors in several regards. First, the sample size in the study is rather small to definitively determine the exact contribution of these variables in predicting strength loss. Therefore, these findings should be interpreted as preliminary. However, the primary problem associated with regression and small sample sizes relates to the potential for an outlier to drive a relationship. We did investigate this (using casewise diagnostics), and it does not appear that single outliers are responsible for our findings. Second, despite our extremely large number of variables measured to assess function of the neuromuscular system, we have not accounted for all potentially influential variables upon strength loss. For example, there could be a number of other explanatory properties, such as changes in muscle architecture, muscle fiber type, or decreases in motor unit discharge rate. Decreases in fiber length and pennation angle (35), along with maximal motor unit discharge rate, have been observed following periods of disuse and could further aid in explaining the loss of strength (13, 46). Third, the time frame of our study should be considered when inferring our results to other circumstances. It is probable that protocols of different duration would alter the relative contribution of neural and muscular factors on strength loss, presumably with the neural factors being more important with shorter term protocols and contractile factors with longer durations.

In summary, we have identified numerous alterations in neurological properties that appear to be spatially distributed throughout the nervous system following prolonged unweighting and examined the influence of MI training on these functional characteristics. In addition to the expected loss in muscle mass and strength, we observed a heightened H-reflex excitability, along with prolonged M- and H-wave latencies. Although we did not find a significant decrement in the ability to centrally activate the PFs, we did observe an association between the change in this ability and strength loss. MI training did not exert significant effects on the maintenance of central activation function or strength, although a modest effect size was observed for strength preservation. These findings suggest that unweighting induces plastic changes in neural function at the level of the spinal cord and terminal branches of the motor axons and/or neuromuscular junction. Additionally, when these findings are considered in conjunction with adaptations in skeletal muscle, it appears that much of the loss in strength is associated with neural deficits in central activation, along with alterations in the CMAP amplitude and duration.

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