Numerous investigations demonstrated that the unloading of skeletal muscle results in a significant loss of strength (1–5, 10, 14, 16, 18, 20, 23). Some of these studies associated unloading-induced strength decrements with atrophy at the whole muscle (1, 10, 14, 18, 20, 23) or myofiber (4, 14, 16) levels. These findings indicate that a loss of contractile proteins accounts for the diminished force production capacity of unloaded muscle. However, the authors reporting whole muscle and myofiber atrophy imposed extended periods of muscle unloading, generally 4–6 wk.

Unloading interventions of shorter duration, e.g., 9 or 10 days, have also been associated with decreased muscle function (5, 20). Mechanisms explaining this adaptation to short-term muscle unloading have yet to be identified. It is unlikely, however, that such immediate alterations in strength are due to muscle atrophy, because the turnover rate of contractile proteins is relatively slow (17). This suggests then that the early diminutions in muscle strength exhibited during muscle unloading are related to alterations in the neural activation of myofibers.

In contrast to muscle unloading, resistance training results in strength gains and muscle hypertrophy. In what has become a landmark investigation, Moritani and deVries (21) demonstrated that improvements in strength detected in the first 2 wk of a resistance training program were due to enhanced neural activation of muscle, rather than muscle hypertrophy.

It was our hypothesis that similar to exercise-induced strength gains, initial alterations in strength resulting from muscle unloading would be attributable to adaptations of the neural system rather than modifications of myofiber size. Thus the aim of the present study was to identify the mechanisms, neural vs. muscular, involved in the abatement of strength after a relatively short period of muscle unloading.

METHODS

Subjects. Six male and four female college students (21.0 ± 0.4 yr, 174.0 ± 2.3 cm, 78.7 ± 7.3 kg; means ± SE) volunteered for the study; all were healthy, but untrained. After receiving a verbal description of the study, the experimental procedures to be used, and the potential risks involved, the subjects provided written informed consent. Each subject completed a medical history form to ensure that no contraindications to participation existed. All experimental procedures were approved by the Committee for the Protection of Human Subjects at The College of William & Mary.

Experimental design. Subjects initially performed two familiarization trials on an isokinetic dynamometer (model 900–350, Biodex, Shirley, NY). After a 7- to 10-day recovery period, subjects returned to the laboratory to perform the...
preintervention muscle function test. Subjects were weighed and then, after a 5-min equilibration period while seated, a 3-ml blood sample was obtained from an antecubital vein. Whole blood was collected into a heparin-treated tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 3,000 g for 15 min at 4°C. The resultant plasma was stored at −75°C until blood-borne variables were analyzed.

Subjects then completed a 5-min nonspecific warm-up on an electrically braked cycle ergometer (Excaliber Unit, Lode, Groningen, The Netherlands) set at 50 W. After the warm-up, subjects were seated on the Biodex dynamometer with the right knee positioned at a 95° angle and aligned with the axis of the dynamometer. In preparation for the collection of electromyographic (EMG) data, a 1-in.2 area of skin over both the right vastus lateralis (VL) and vastus medialis (VM) was shaved, abraded, and cleansed with an alcohol wipe. Along the longitudinal contour of the muscle, 2-mm diameter electrodes filled with electrolyte gel were secured on the skin with adhesive collars at an interelectrode distance of 2 cm and traced with ink. These tracings enabled electrode placement at the same sites for the postintervention test 2 wk later.

EMG data were collected during a 5-s maximal isometric effort of the knee extensors. Subsequent to this, subjects were tested for maximal isokinetic muscle performance of the right knee extensors and flexors at the movement velocities of 0.53, 1.05, 2.09, and 3.14 rad/s. At all but the fastest velocity, sets of five repetitions were conducted, but at 3.14 rad/s, 30 maximal repetitions were completed to assess muscle endurance. Weight of the tested limb was assessed by the dynamometer to allow performance variables to be corrected for that resistance. Three-minute rest intervals interspersed sets, and verbal encouragement was provided throughout each set.

Subjects were asked to return to the laboratory 24 h later to obtain muscle samples and to begin the 2-wk intervention period of muscle unloading. Muscle specimens were collected 24 h after the muscle testing protocol to avoid any immediate postexercise edema that may have affected myofiber size. Muscle samples were obtained from the right vastus lateralis using the percutaneous needle biopsy procedure described by Bergstrom (6) and later modified by Evans et al. (13) to include suction. Specimens were properly oriented on cork with OCT embedding compound (Tissue-Tek, Miles, Elkhart, IN) using a stereo microscope (Stereomaster, Fisher Scientific, Pittsburgh, PA) at a magnification of ×45. Samples were then frozen in isopentane chilled with liquid nitrogen and stored at −75°C until analysis. After biopsy samples were collected, subjects immediately began the 2-wk intervention period of unloading of the right leg.

After the unloading intervention, subjects returned to the laboratory to be weighed, provide a second blood sample, and to repeat the data collection protocol for EMG activity and muscle function. Again, biopsy samples were taken 24 h after completing the muscle function test protocol. To avoid potential spurious effects caused by circadian variation, each subject completed the postintervention data collection session at the same time of day (±10 min) that the preintervention session occurred. Testing of all subjects occurred between 1200 and 1500.

Muscle unloading. The model used to elicit unloading of the knee extensors and flexors of the right leg was a modification of the unilateral lower limb suspension (ULLS) technique first described by Berg et al. (3). The right leg was immobilized in a light weight orthopedic knee brace (Donjoy, DJOrthopedics, Chicago, IL) set at an angle of 70°, thus eliminating weight-bearing activity of the leg. Subjects were then fitted with crutches and instructed on the proper technique of crutches-assisted ambulation. Subjects were instructed to remove the knee brace and to perform range of motion activities of the right knee when they were retiring to bed for the evening. It should be noted that this version of ULLS resulted in both limb immobilization and muscle unloading. No subjects reported or displayed circulatory complications during the 2-wk period of ULLS.

Quantitation. Plasma concentrations of cortisol and ACTH were determined in duplicate using solid-phase 125I-radioimmunoassays (Diagnostic Systems Laboratories, Webster, TX). To eliminate interassay variance, all samples were analyzed in the same assay run; mean intra-assay variance for each hormone was <10%. Sensitivities for the detection of cortisol and ACTH were 13.8 nmol/l and 0.77 pmol/l, respectively.

During EMG recordings, signals were amplified by a factor of 1,000 and passed through a bandwidth filter set at 30 and 500 Hz, along with a 60-Hz notch filter. Signals were digitized at a sampling frequency of 1,000 Hz and were recorded by an online computer system during the 5-s maximal isometric contraction of the right quadriceps. The EMG signal was then full-wave rectified and integrated (iEMG).

Muscle performance variables of interest, peak torque, total torque (isometric), total work (isokinetic), and muscle endurance, were calculated by the Advantage software accompanying the Biodex dynamometer. Resistance to muscle fatigue was assessed as the difference in total work produced during the first ten repetitions compared with the last 10 repetitions during the 30-repetition set executed at 3.14 rad/s.

Neuromuscular efficiency, viewed as the responsiveness of muscle to neural excitation, was determined by dividing the total torque generated by the knee extensors during the 5-s isometric action by the average iEMG activity of the VL and VM muscles during that maximal effort.

To quantify myofiber profiles, 10-μm-thick transverse sections of muscle samples were obtained on a cryostat (Cryocut 1800, Reichert-Jung, Nümbrecht, Germany) set at −20°C. Sections were then stained for myofib AtPase activity using a preincubation pH of 4.55 in accordance with the procedure of Nemeth and Pette (22). A small subset of sections were simultaneously stained with a preincubation pH of 10.3 to confirm reversibility of staining. After being stained, muscle sections were visualized at ×400 with an Olympus BX60 microscope (Olympus America, Melville, NY). Fibers were identified as type I, IIA, or IIB according to staining intensity with the preincubation pH of 4.55, according to the classification system of Brooke and Kaiser (8). In humans, type IIB fibers predominantly express the IIX isoform of myosin heavy chain (24). Cross-sectional areas of myofibers were ascertained with the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Adequate pre- and postintervention muscle samples were obtained from eight subjects, and an average of 238 myofibers were assessed per sample.

Statistical analysis. Standard descriptive procedures were employed in determining subject characteristics. Nonparametric statistics (Mann-Whitney U-test) were employed to compare pre- with postintervention responses (%change) between men and women. None of the variables quantified displayed significant differences between genders. Male and female data were then collapsed together to provide a higher number, enabling pre- to postintervention differences to be analyzed with dependent t-tests. In all cases, an α-level of 0.05 was used to establish statistical significance.
Muscle function before (Pre) and after (Post) 2 wk of muscle unloading

<table>
<thead>
<tr>
<th></th>
<th>Peak Torque</th>
<th>Total Torque (isometric)</th>
<th>Total Work (isokinetic)</th>
<th>Muscle Endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Knee extensors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 rads/s</td>
<td>216.5 ± 19.3</td>
<td>169.4 ± 21.1*</td>
<td>649.0 ± 57.7</td>
<td>508.3 ± 63.3*</td>
</tr>
<tr>
<td>0.53 rads/s</td>
<td>210.4 ± 14.7</td>
<td>170.3 ± 14.9*</td>
<td>852.9 ± 59.4</td>
<td>652.3 ± 54.7*</td>
</tr>
<tr>
<td>1.05 rads/s</td>
<td>194.8 ± 13.0</td>
<td>159.7 ± 13.3*</td>
<td>800.9 ± 52.0</td>
<td>620.9 ± 47.3*</td>
</tr>
<tr>
<td>2.09 rads/s</td>
<td>166.5 ± 11.7</td>
<td>142.8 ± 10.5*</td>
<td>668.6 ± 44.1</td>
<td>544.0 ± 37.5*</td>
</tr>
<tr>
<td>3.14 rads/s</td>
<td>133.6 ± 8.4</td>
<td>116.4 ± 8.0*</td>
<td>2,735.3 ± 207.6</td>
<td>2,339.0 ± 163.6*</td>
</tr>
<tr>
<td>Knee flexors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.53 rads/s</td>
<td>110.8 ± 9.4</td>
<td>95.6 ± 8.8*</td>
<td>436.8 ± 35.7</td>
<td>358.6 ± 31.8*</td>
</tr>
<tr>
<td>1.05 rads/s</td>
<td>101.9 ± 8.6</td>
<td>89.2 ± 8.0*</td>
<td>414.0 ± 32.2</td>
<td>343.0 ± 23.2*</td>
</tr>
<tr>
<td>2.09 rads/s</td>
<td>89.2 ± 7.2</td>
<td>79.5 ± 7.3*</td>
<td>358.6 ± 27.0</td>
<td>303.6 ± 20.9*</td>
</tr>
<tr>
<td>3.14 rads/s</td>
<td>75.0 ± 5.1</td>
<td>72.9 ± 6.0</td>
<td>1,452.2 ± 120.2</td>
<td>1,308.2 ± 87.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10. Units of measurement for peak torque at 0 rads/s = N; peak torque at all other velocities = Nm; total work = J; muscle endurance = % decrease in work performed during first 10 repetitions vs. last 10 repetitions. *Statistically significant (P < 0.05) decrease in Post vs. Pre performance of variable at same speed of movement; †statistically significant (P < 0.05) improvement in Post vs. Pre muscle endurance. Note: at 3.14 rads/s 30 repetitions were conducted.

RESULTS

Body and limb mass. The 2-wk period of ULLS affected neither body mass nor mass of the unloaded limb (data not shown).

Muscle function. Virtually every aspect of muscle performance was affected by the 2-wk period of muscle unloading. In the quadriceps, ULLS resulted in a significant decrease in peak torque produced during the isometric contraction and at each of the velocities used during isokinetic testing. In the knee flexors, ULLS elicited significant decrements in peak torque at each speed of movement tested, except for the fastest speed (3.14 rads/s). Averaged across velocities of movement, including 0 rads/s, muscle unloading evoked a 17.2% decrease in peak torque, or strength, of the knee extensors and a 10% loss of strength in the knee flexors.

Total torque produced by the quadriceps during the 5-s isometric contraction was significantly lower during the postintervention testing, as was total work generated by the quadriceps and hamstrings at each of the velocities measured during isokinetic contractions. Including the different speeds of movement tested, the average decrement in total work performed by the quadriceps incorporating total torque during the isometric effort, was 20%, whereas the hamstrings’ total work output declined an average of 15% as a result of unloading.

For both peak torque and total work, ULLS-induced decrements were progressively less pronounced as the speed of movement increased. For example, at 0 rads/s, peak torque of the quadriceps decreased by 22%, whereas a 13% reduction occurred at 3.14 rads/s.

In contrast to strength and total force produced, resistance to muscle fatigue was enhanced after the ULLS intervention. This was true of both the knee extensors and flexors. All muscle function data are presented in Table 1.

EMG and neuromuscular efficiency. The ULLS protocol was also found to significantly alter iEMG activity during maximal isometric effort of the quadriceps. When data from the VL and VM muscles were averaged together, less neural excitation was evident in postintervention recordings compared with preintervention values. In contrast, neuromuscular efficiency, muscle work performed relative to iEMG activity during the 5-s isometric contraction, remained unaffected by the unloading intervention. Indeed, values for neuromuscular efficiency were almost identical during pre- and postintervention testing, 0.883 and 0.888, respectively. Results of iEMG and neuromuscular efficiency testing are shown in Table 2.

Myofiber profile. In assessing histochemically stained muscle fibers, it was determined that 14 days of ULLS failed to elicit any significant changes in fiber size or fiber type distribution. The results from our analysis of muscle samples are presented in Table 3.

Plasma hormones. The 2-wk period of muscle unloading resulted in a significant increase (55%) in resting levels of blood-borne cortisol, a catabolic, or muscle wasting, steroid. No significant change in resting values of circulating ACTH was evident. To examine the sensitivity of the adrenal cortex to stimulation from ACTH, the ratios of circulating levels of cortisol to ACTH were calculated before and after the ULLS intervention. A statistical trend (P = 0.09) toward an increase in the ratio of cortisol to ACTH was documented after the intervention period. This suggests that unloading induced an enhanced responsiveness of the adrenal cortex to the pituitary gland, which resulted in a significant (P < 0.05) elevation in resting levels of circulating ACTH. The ratios of circulating levels of cortisol to ACTH were calculated before and after the ULLS intervention. A statistical trend (P = 0.09) toward an increase in the ratio of cortisol to ACTH was documented after the intervention period.
leaves ACTH. Plasma hormone data can be found in Table 4.

DISCUSSION
Various methods of unloading, including bed rest (2, 11, 16, 18), limb immobilization (20, 23), exposure to 0 gravity, i.e., space flight (12), and lower limb suspension (1, 3, 5, 10, 11, 14), have all been found to evoke reductions in muscle performance. Typically these studies have employed intervention periods of at least 4 wk and have documented atrophic responses of either the whole muscle (1, 10, 14, 18, 20, 23) or the myofibers within the involved muscle (4, 14, 16).

Only a few studies have investigated the effects of short-term unloading on muscle function. Miles et al. (20) immobilized the forearm for a period of 9 days and found a significant decrease in strength, yet no alterations in muscle size were detected. In view of this, the authors presumed that an impairment in the neural activation of muscle explained their results. Adams et al. (1) exposed subjects to a 16-day unloading intervention using the ULLS technique. They, too, documented a significant abatement of strength that could not be accounted for by muscle atrophy. These authors, similar to Miles et al. (20), concluded that impairments in the neural excitation of the unloaded muscle were primarily responsible for the observed strength decrements. However, neither of those studies directly assessed the neural activation of unloaded muscle.

In 1979, Moritani and deVries (21) published what has become a seminal report in the field of exercise physiology. In that study, individuals performed resistance exercise, weight training, for a period of 8 wk to improve muscle strength. The authors’ objective was to identify whether these strength gains were related to muscle hypertrophy or an enhanced capacity of the nervous system to recruit muscle during maximal efforts. After 2 wk of training, 80% of the strength increments noted were attributable to “neural factors” as evidenced by greater iEMG activity. By 4 wk of training, the majority of the strength gains were due to muscle hypertrophy. Beyond 4 wk of resistance exercise, strength improvements became increasingly dependent on muscle hypertrophy.

Given that neural mechanisms are responsible for early strength gains, and mindful of the role of neural alterations in early strength losses that were postulated by Adams et al. (1) and Miles et al. (20), we sought to determine directly whether neural modifications were accountable for the decreased strength associated with short-term muscle unloading. Our findings confirm that the loss of strength displayed by the quadriceps after 2 wk of unloading can primarily be attributed to a decreased capacity of the nervous system to excite the muscle. Indeed, the data show a strong \( r = 0.93 \) and significant \( P \leq 0.05 \) correlation between ULLS-induced declines in strength and iEMG activity. Our data also revealed that neuromuscular efficiency, or the responsiveness of muscle to neural excitation, was not affected by 2 wk of unloading. These results also support the notion that losses in strength accompanying short-term muscle unloading are more closely related to neural adaptations than disturbances in the contractile function of muscle. However, not all strength decrements are divorced from impaired neuromuscular efficiency. For example, the loss in strength noted after exercise-induced muscle damage is coupled with attenuated neuromuscular efficiency (9).

The results presented here are at odds with an earlier report by Berg and Tesch (5). In that investigation it was determined that although 10 days of ULLS elicited a significant reduction in strength, no alteration in the iEMG activity of the affected muscle was evident; muscle size was not measured. As is common, Berg and Tesch analyzed the EMG activity of the three surface muscles of the quadriceps, i.e., the vastus lateralis, vastus medialis, and rectus femoris. But in our study, EMG recordings of the rectus femoris were not collected. This muscle, unlike the vasti muscles, crosses two joints and serves as both a hip flexor and knee extensor (19). In its function as a hip flexor, the rectus femoris would continue to be regularly recruited during the forward swing phase of crutches-assisted ambulation. As a result, when iEMG data from the muscles were averaged together to determine the effects of unloading, readings from the rectus femoris would provide spurious results. Thus we elected to examine only the VL and VM muscles to assess the effects of ULLS on the neural activation of quadriceps.

Table 4. Plasma hormone concentrations before (Pre) and after (Post) 2 wk of muscle unloading

<table>
<thead>
<tr>
<th>Cortisol, pmol/l</th>
<th>ACTH, pmol/l</th>
<th>Cortisol/ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>230.8 ± 33.6</td>
<td>358.8 ± 42.7</td>
<td>9.9 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 10 \). *Statistically significant \( P \leq 0.05 \) difference from Pre value of same variable; †statistical trend \( P = 0.09 \) toward difference from Pre value of same variable.
This probably accounts for the contrast between our results, where significant neural impairment was detected, and those of Berg and Tesch, where iEMG activity was not significantly affected by ULLS. It is also noteworthy that we imposed a 14-day intervention period, whereas the study by Berg and Tesch featured a 10-day ULLS protocol.

Our analysis of muscle biopsy specimens revealed that 2-wk of unloading failed to remodel significantly the size or distribution of myofibers taken from the VL. However, with all fiber types pooled together, a slight degree (<5%, P = 0.29) of fiber atrophy was exhibited post-ULLS. Previously, Booth (7) demonstrated that the atrophic responses caused by unloading were predictable and progressive as the duration of limb immobilization increased. This would suggest that with longer intervention periods, significant muscle atrophy would be manifested. And, in fact, unloading durations of at least 4 wk are accompanied by significant myofiber atrophy (4, 14, 16).

The hormonal data presented here indicate that even within a 2-wk interval, muscle unloading affects the hormonal milieu in a manner that promotes muscle atrophy. ULLS resulted in a significant increase in the plasma concentration of cortisol, which is a potent catabolic, or muscle wasting, hormone synthesized by the adrenal cortex (15). This increase occurred in the absence of a concomitant elevation of ACTH, which is released by the pituitary gland into the bloodstream to stimulate the release of cortisol by the adrenal gland (15). After the unloading intervention, there was a 74% increase in the ratio of cortisol to ACTH in the circulation. In effect, 14 days of ULLS appears to have increased the sensitivity of the adrenal cortex to ACTH and modified the pituitary-adrenal axis. This is a novel finding and is interesting in light of the fact that increased activity in the form of endurance training has the opposite effect and blunts the response of cortisol to ACTH stimulation (25). Further research is needed to confirm these initial findings, however.

An unexpected result from our study was that muscle fatigability was decreased after 2 wk of unloading. Previous research had concluded that muscle unloading led to increased fatigability (4, 20). The contrast between those results and ours may be due to differences in muscles affected (20) and/or the duration of the unloading intervention (4) or in the method of testing fatigability (4, 20). In the testing procedure used here, resistance to fatigue was quantified as the difference in total work performed during the first and last 10 repetitions of a 30-repetition set. Subsequent to the unloading intervention, the decline in work performed from the beginning to the end of the set was tempered. On closer examination of the data, it was apparent that the work executed during the initial 10 repetitions had been reduced after unloading, whereas no appreciable change had occurred in the performance of the final 10 repetitions. In short, muscle fatigability had been diminished because total work during the first few repetitions had been compromised by ULLS. The fact that others (4, 16) reported that unloading did not increase the activity of either glycolytic or oxidative enzymes in the involved muscle suggests that the improved resistance to fatigue exhibited by our subjects is not due to alterations in bioenergetic pathways.

In conclusion, we established that the loss of strength observed after a short, i.e., 14-day, period of muscle unloading imparted with ULLS can be ascribed to a decreased capacity of the nervous system to activate the affected muscle tissue. The impairment of neural factors leading to decreased muscle force production after 2 wk of unloading is consistent with previous literature showing that strength gains made with 2 wk of resistance training are accounted for by enhanced neural recruitment of muscle. That is, initial variations in muscular strength, whether positive or negative in nature, are related to similar positive or negative adaptations of the nervous system. This study also demonstrates that although myofibers do not significantly atrophy during 14 days of ULLS, endocrine adaptations occur that result in a more catabolic environment that is conducive to muscle atrophy should a longer period of unloading occur.

Perspectives

The data presented here may have important clinical implications. Many individuals are relegated to conditions of unloading after injury or during recovery from illness. Our findings indicate that, at least in young adults, the loss of strength caused by up to 2 wk of muscle unloading occurring with casting or bed rest are mainly related to a decreased ability of the nervous system to stimulate muscle, not muscle atrophy. Consequently, recovery of normal muscle strength should be fairly rapid because retraining the nervous system would be required rather than the more time consuming resynthesis of lost contractile proteins.

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REFERENCES