

Mechanics and Fatigability of the Rat Soleus Muscle During Early Reloading

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In order to elucidate muscle functional changes by acute reloading, contractile and fatigue properties of the rat soleus muscle were investigated at three weeks of hindlimb suspension and the following 1 hr, 5 hr, 1 d, and 2 weeks of reloading. Compared to age-matched controls, three weeks of unloading caused significant changes in myofibrillar alignments, muscle mass relative to body mass (-43%), normalized tension (-35%), shortening velocity (+143%), and response times. Further significant changes were not observed during early reloading, because the transitional reverse process was gradual rather than abrupt. Although most of the muscle properties returned to the control level after two weeks of reloading, full recovery of the tissue would require more than the two-week period. Delayed recovery due to factors such as myofibrillar arrangement and fatigue resistance was apparent, which should be considered for rehabilitation after a long-term spaceflight or bed-rest.

Key Words: Contractility, fatigability, hindlimb suspension, lactate, reloading

INTRODUCTION

Morphological and functional properties of postural or antigravity muscles change significantly depending on the weight-loading status.^{1,2} Muscle responses during hindlimb unloading have been well-studied. However, responses that

occur during early reloading have been less well studied, even though the morphological and functional problems during this process are equally serious.^{1,4} Previous studies have demonstrated that an unloaded muscle shows significant atrophy in mass and cross-sectional area, with loss of contractile proteins, decrease in protein synthesis, and disruption of myofibrillar alignment.⁵⁻⁸ Slow-twitch fibers are preferentially atrophied, while the number of fast type II fibers and the expression of fast-type myosin isoforms in slow fibers are increased.^{1,5,9} In concert with these alterations, muscle force and power are decreased, whereas shortening velocity and fatigability are increased.^{1,5,10}

When the atrophied muscle is reloaded, alterations in the muscle properties appear to be reversed, but the early recovery process is not well documented. Witzmann et al.¹¹ examined the time-course of contractile responses of rat hindlimb muscles after six weeks of immobilization. Although their investigation was extensive, they immobilized the animals with a cast that restricted free movement of the hindlimbs, and thus, it was difficult to compare their results of muscle contractile function with those of hindlimb suspension studies. Moreover, the recovery process during the first few hours after hindlimb suspension remains an open question.

Previous studies have demonstrated that muscle mass and cross-sectional area increase within 5 hrs of reloading.¹² This increase is accompanied by interstitial edema and sarcomere lesions due to eccentric contraction, which are deleterious to force generation and fatigue resistance.^{13,14} Because

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of the intrinsic problems, normalized tetanic tension (P_0) of the rat soleus muscle is decreased by 32% and 50% during day one and two of reloading, respectively, in comparison with that examined 7 days after unloading.¹⁵ Apart from the tension data, little is known about how other properties of antigravity muscles are affected by reloading. According to Mercier et al.,³ the rat soleus muscle exhibited shorter contraction times and half-relaxation times after three weeks of hindlimb unloading, and these properties returned to control levels after two weeks of reloading. This result suggests that the shortening velocity of the muscle may slow down during the recovery period (the reverse of the unloading process). In their report, P_0 declined 53-64% in the unloaded soleus but resumed its control level after two weeks of reloading.

In addition to muscle mechanics, fatigability of the reloaded muscle and its temporal recovery process from fatigue have yet to be thoroughly studied. Among many potential factors, lactate concentration and sarcomere disruption may be the most serious attributes affecting fatigue resistance during reloading. An increase in the lactate level results in muscle acidosis, and the sarcomere disruption causes a loss of myofibrillar integrity, which essentially hinders normal contractile function of the muscle.^{14,16,17} Thus, considering the qualitative and quantitative responses of the tissue, it needs to clarify whether the temporal recovery process by reloading would simply be the reversed process of unloading or not.

Based on these preceding reports, we hypothesized that during early reloading, the antigravity muscle shows a further decrease in tension and fatigue resistance and an increase in rate responses like shortening velocity, which add to prior alterations that have occurred by unloading. In order to test this hypothesis, the mechanics and fatigability of the rat soleus muscle were examined during the transitional period between unloading and reloading. A hindlimb suspension model was used, as in other studies. We defined the temporal profiles of the myofilament ultrastructure, muscle contractility (tension, shortening velocity, power, and time variables), and lactate and fatigue levels during unloading and reloading, with a particular emphasis on the early

reloading period.

MATERIALS AND METHODS

Subjects

Sprague-Dawley rats of five weeks of age (100-120g) were reared at 22-24°C in a 14:10 light:dark cycle. Standard Purina rat chow and water were provided *ad libitum*. On the last day of week 5, the rats were randomly divided into seven groups: one unloading group of 3 wk (U3w); 4 reloading groups, which experienced the 3 wk unloading followed by 1 hr, 5 hr, 1 d, or 2 wk reloading (R1h, R5h, R1d, and R2w, respectively); and two representative age-matched control groups kept for 3 and 5 weeks under standard conditions (C3w and C5w). The 3 wk unloading period and the 2 wk reloading period seemed long enough to observe sufficient alterations in muscle mechanical properties.³

Hindlimb suspension

The hindlimb suspension model was described by Nyhan et al.¹⁸ Each animal was weighed and anesthetized intraperitoneally with pentobarbital sodium (30 mg·kg⁻¹). Two pieces of flexible Tygon tubing (5.6 mm O.D.) were placed along the entire dorsum of the animal and were sutured with silk threads to provide a firm support. The animal was jacketed with a piece of nylon mesh that was sutured to the tubing for further support. The tail was taped gently to the Tygon tubing. On the following day (the first day of six wk) after the animal had completely recovered from the anesthetics, the tubing at the tip of the tail was connected to a tether so that the animal was suspended at an incline of 30-35° in a head-down orientation. The animal was free to move around the cage on its front feet. The mesh jacket was replaced in 7-10 days as the animal grew. Similar tubing and jackets were applied to the control groups with the exception that the tail was free from the taping to the tubing. At the time of reloading, the animals were freed from all tubing and jackets and were housed in individual cages. The general procedure for animal experiments

followed Guidelines of the University Animal Care and Use Committee.

Muscle preparation

On the day of the experiment, each animal was anesthetized with pentobarbital sodium (i.p., 50 mg·kg⁻¹). The soleus muscle tissues of both the right and left hindlimbs were quickly removed, and the left soleus muscle was either used for electron microscopic analysis (n=2) or was frozen immediately in liquid nitrogen for lactate concentration measurement. The right soleus muscle was placed horizontally in a 70 ml muscle bath containing cold oxygenated Ringer's solution (115 mM NaCl, 5 mM KCl, 4 mM CaCl₂, 1 mM MgCl₂, 1 mM NaH₂PO₄, 24 mM NaHCO₃, 11 mM glucose, pH 7.39) with 0.02 g·L⁻¹ tubocurarine chloride. One tendon was tied to the 300B-LR servomotor arm (Aurora Scientific, Canada), and the other tendon to a micrometer to adjust to the optimal muscle length (L₀).

Experimental apparatus

The servo system made both force and length steps in 1.3 ms and transition from the length control to the force control without any length transient. A custom software program (DMC, Aurora Scientific) was used to control both the servo system and a Grass S48 stimulator. The stimulator was connected to a pair of bright platinum electrodes and was supplied a 1.0-ms square wave pulse or pulse train. Data on force or length changes were stored in an IBM compatible PC and were analyzed by a custom software (DMA, Aurora Scientific).

Procedure for muscle contraction

All the experiments were conducted at 25°C tissue temperature. For each preparation, the optimum muscle length (L₀), supramaximal voltage and a stimulus frequency of 200 Hz that generated a fully fused, maximum tetanic force (F₀) were determined. The F₀ was usually stabilized after the first three or four trials. A rest period of 20 min was given between electric stimulations.¹⁹

The shortening velocity, power, and fatigue level of each soleus muscle were determined in a time course after F₀ was established. Because the muscle should not be fatigued before the fatigue experiment (see below), shortening velocity and power were examined in a minimum number of contraction trials before the fatigue experiment. Thus, as in a previous study,²⁰ two arbitrary values of loads (F) were set, and the shortening velocity (V) and power (F × V) at each F were determined. In an attempt to find the two F's, we first measured shortening velocity of the muscle from quick-release isotonic contractions, with 10-12 preset loads ranging from 0.1 F₀ to 0.8 F₀. Length changes were measured over 10-50 ms periods starting about 10 ms after the release. A hyperbola was fitted to the data using Hill's equation, and force-velocity and power-velocity relationships were established.¹⁹ From these experiments (n=3), two F's, 0.35 F₀ and 0.40 F₀ were determined, which best represented the forces generating near-maximum power (see Results). F₀ was rechecked before the muscle fatigue experiment, and the muscle and data were discarded if this last F₀ was less than 93% of the initially established F₀. Muscle fatigability was examined with a series of 2-sec stimulations and 2-sec rests repeated for about 80 sec. This protocol was made from the preliminary works stating that a complete plateau of the tetanic force curve required more than 1.8 sec of stimulation (see Results). Muscle fatigability was determined in two ways: relative decrement in force between the start and 60 sec of the stimulation protocol, and within a 2-sec force exertion generated at 60 sec in the stimulation protocol.

After completion of each experiment, L₀ was measured in place using a micrometer under a light microscope, and the muscle fiber length (FL) was approximated to be 2/3 L₀.²¹ The tissue was frozen in place with liquid nitrogen, and its mass (M_m) was measured with a chemical balance after the tendons and connective tissues were removed. The muscle tissue was then thin-sectioned (8 μm) in a Microm HM 505E cryostat and stained by routine haematoxylin and eosin procedure. The cross-sectional area (CSA) of the section was determined as in the previous study.²² Muscle force was normalized with CSA,

shortening velocity with FL , and power with M_m . From isometric tetanic force curves, rate of tension production (dP/dt), tetanic rise time (TRT), and half-relaxation time (HRT) were determined. The dP/dt was defined as the highest slope in the rising phase of the curve; TRT was defined as the time length between $0.1 F_0$ and $0.9 F_0$ in the rising phase; and HRT was defined as the time length between $0.9 F_0$ and $0.5 F_0$ in the falling phase of the curve.

Electron microscopy

The soleus muscles used in the electron microscopic (EM) study were collected from two rats per group. Each tissue was pre-fixed for 4 hrs in 2.5% glutaraldehyde, cut into $1 \times 1 \times 2$ m blocks, rinsed for 15 min in 0.1 M phosphate buffer (pH 7.2), and post-fixed for 2 hrs in 1% OsO_4 . The fixation and rinsing processes were conducted at $4^\circ C$ to minimize autolysis and extraction. For embedding, the fixed samples were dehydrated by rinsing each two times in a series of 50%, 60%, 70%, and 90% ethanol, and then two times in 100% ethanol. The samples were then infiltrated with propylene oxide, were affixed on dodecenyl succinic anhydride, epon 812, nadic methyl anhydride, and tridimethyl amino-methyl phenol (DMP-30) for 1 hr at room temperature, and polymerized for at least 1 d at $60^\circ C$. After trimming and semi-thin sectioning, muscle samples were dried on a hot plate ($80^\circ C$) and were prestained with 0.5% toluidine blue. Semi-thin sections were trimmed, ultra-thin sectioned with an ultramicrotome, and double-poststained with 1% uranyl acetate for 15 min and lead citrate for 5 min. Ultra-thin sections ($0.05 \mu m$) were supported on a grid. Longitudinal-sections were adjusted to $6,000 \times -7,000 \times$ magnification under an electron microscope (TEM, JEM-1200EX, Jeol, Japan), photographed by a digital TEM camera (Megaview III), and analyzed in an Image Analysis System (SIS, Munster, Germany).

Muscle lactate concentration

On the soleus muscle previously frozen in liquid nitrogen under resting conditions, assays were conducted with lactate assay kits (Sigma

Chemical, St. Louis, MO, USA) according to the manufacturer's instructions. Lactate concentration was calculated with an equation given within the instruction.

Data analysis

All data are presented as mean \pm SE, unless otherwise noted. Differences in the means of each morphological and functional variable among C3w, U3w, R1h, R5h, and R1d were examined with a oneway analysis of variance (ANOVA) and Scheffé's multiple comparison tests, assuming that individuals of these groups were within a comparable age range. Differences in the means between C3w and C5w, and between C5w and R2w were examined by independent pairs *t*-tests. The statistical procedure was performed using SPSS/PC+ (SPSS Inc. Chicago, IL, USA)

RESULTS

Body and muscle mass. Body mass (M_b) decreased significantly after three weeks of unloading and during the early stages of reloading (R1h - R1d), but it recovered to the control level after two weeks of reloading (Table 1). Compared with C3w, muscle mass relative to body mass (M_m/M_b) decreased significantly by 43% in U3w and 32-36% in R1h - R1d (oneway ANOVA, $F_{4,39}=10.2$, $p<0.01$) and recovered fully (97.5%) in 2 weeks of reloading (*t*-test, $p>0.05$). The cross-sectional area (CSA) of the muscle decreased by 46% in U3w, 39-44% in R1h - R1d ($p<0.01$), and returned to 87.2% of the C5w level after two weeks of reloading ($p<0.05$). There was no significant difference in muscle fiber length (FL) between the UR groups and each corresponding control.

Myofibrillar arrangement

Representative electron micrographs were shown for four groups in Fig. 1. In C3w, the regular pattern of the I-band and A-band was clear, and mitochondria that appeared cross-sectioned were aligned along the straight Z-lines in the inter-myofibrillar space (Fig. 1A). However,

Table 1. Morphological Traits of the Unloading-reloading Groups Compared with the Age-matched Controls

Group (n)	Mb (g)	Mm (mg)	Mm/Mb	FL (mm)	CSA (mm ²)
C3w (6)	271.63 ± 2.35 ^{a,b}	106.6 ± 8.7 ^{a,b}	0.39 ± 0.03 ^b	16.80 ± 0.73	4.21 ± 0.24 ^{a,b}
U3w (10)	231.31 ± 7.41 ^b	51.0 ± 3.66 ^b	0.22 ± 0.02 ^b	15.12 ± 0.50	2.28 ± 0.18 ^b
R1h (9)	228.08 ± 5.31 ^b	56.8 ± 3.71 ^b	0.25 ± 0.02 ^b	16.10 ± 0.46	2.35 ± 0.31 ^b
R5h (7)	228.33 ± 7.29 ^b	59.9 ± 2.08 ^b	0.25 ± 0.02 ^b	15.68 ± 0.57	2.60 ± 0.15 ^b
R1d (10)	229.29 ± 9.45 ^b	60.6 ± 2.04 ^b	0.27 ± 0.01 ^b	15.74 ± 0.29	2.57 ± 0.11 ^b
C5w (7)	381.34 ± 4.40 ^a	155.4 ± 2.40 ^a	0.41 ± 0.02	17.60 ± 0.20	5.89 ± 0.10 ^{a,b}
R2w (7)	362.89 ± 24.83	140.5 ± 8.28	0.40 ± 0.03	18.20 ± 0.43	5.14 ± 0.23 ^b

Values are presented as mean ± 1SE. Numbers in parentheses are sample sizes in each group. Mb, body mass; Mm, muscle mass; FL, muscle fiber length; CSA, cross-sectional area.

Statistical significance ($p < 0.05$): ^abetween C3w and C5w; ^bbetween the UR groups and the corresponding controls.

the myofibrillar arrangement was disrupted considerably in U3w as the Z-lines were often crumbled or misaligned, and a great proportion of mitochondria were longitudinally situated in the A-band region (Fig. 1B). In R1d, the width of the myofibrils was narrower than that in C3w, Z-lines remained crumbled, and mitochondria resided in the A-band (Fig. 1C). The general features of myofibrillar arrangement resumed in R2w, but the Z-lines were still interrupted by mitochondria that were longitudinally located over the Z-lines into the A-band (Fig. 1D).

Muscle contraction

We obtained the typical isometric twitch and tetanic force curves, and the load-length records as a function of time, and the relative force-velocity and power-velocity curves were illustrated in Fig. 2A-1C. The F/F_0 ratio at which the maximum power occurred was mostly in a range of 0.35-0.40 for both the UR and control groups (Fig. 2C; also see Adams et al.¹⁰). Thus, 0.35 F_0 and 0.4 F_0 were used for preset isotonic loads (F), shortening velocities were determined at both loads, and then a value of shortening velocity was chosen at either loads, whichever generated a greater power for that muscle.

Muscle force and tension

Variations in maximum tetanic force (F_0) and normalized tension (P_0) of the UR groups were compared to those of the control groups (Fig. 3). The F_0 was 70.9 ± 5.2 g in C3w and 94.3 ± 4.2 g in C5w, and they differed significantly between the two groups (independent paired t-test, $p < 0.05$). Compared to C3w, the force decreased by 65% in U3w and 71-77% in R1h-R1d (oneway ANOVA, $F_{4,39}=38.8$, $p < 0.01$). After 2 weeks of reloading, the force recovered to 88% of C5w (t-test, $p > 0.05$). The P_0 was 165.1 ± 7.8 kN·m⁻² in C3w and 157.2 ± 7.4 kN·m⁻² in C5w and did not differ between the two. Compared to C3w, the P_0 significantly declined by 35% in U3w and 49-55% in R1h-R1d ($F_{4,39}=10.7$, $p < 0.01$). P_0 recovered to nearly 100% of the control level after two weeks of reloading (160.3 ± 15.8 kN·m⁻² in R2w). Although there were further decrements in force (6-12%) and tension (14-20%) from U3w to R1h-R1d, the differences were not significant (Scheffe's test, $p > 0.05$). The ratio of twitch to tetanic tension ranged between 0.16 and 0.18 in both the control and the UR groups.

Contraction rate and time variables

The maximum rate of tetanic tension production (dP/dt), the tetanic rise time (TRT), and the

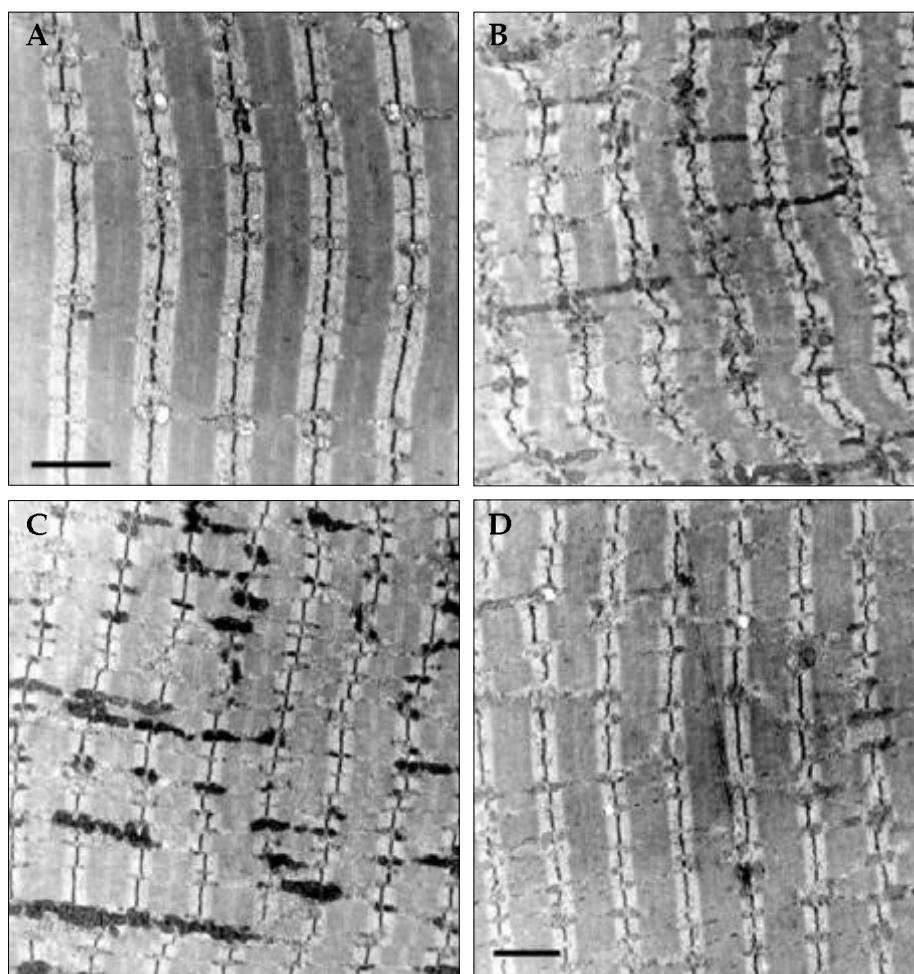


Fig. 1. Electron micrographs showing the longitudinal sections of the soleus muscle in C3w (A), U3w (B), and R1d (C) and R2w (D). Two soleus tissues from two subjects were used in each group. The general feature of arrangements in myofibrils and mitochondria of C5w was much alike that of C3w, and the arrangements in myofibrils and mitochondria of R1h and R5h was like that of R1d. Magnification: $\times 7,000$ for A and B; $\times 6,000$ for C-D. Horizontal bar= $2\mu\text{m}$.

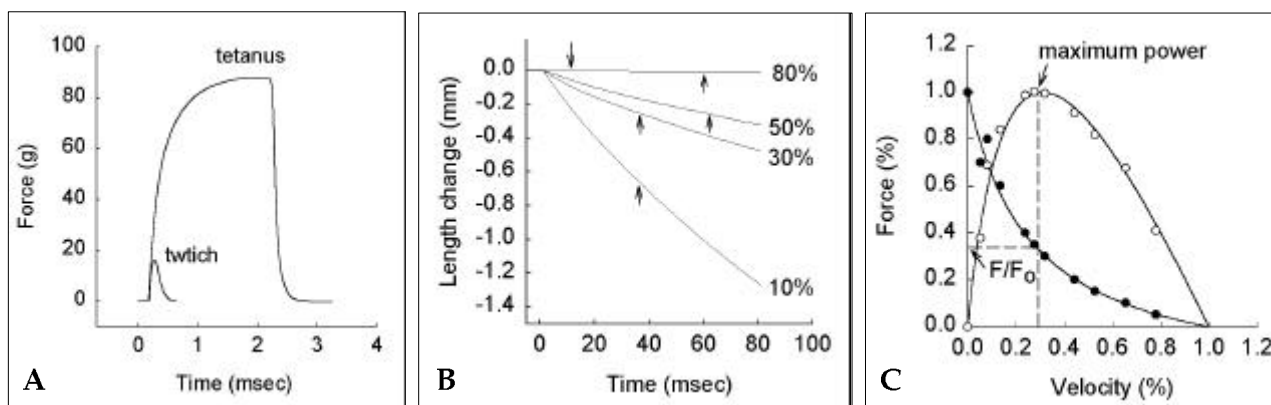


Fig. 2. (A) Representative isometric twitch and tetanic curves for the R2w soleus muscle stimulated at 200 Hz. Twitch to tetanus ratio was 0.178, $M_m=0.157\text{g}$, and $FL=17.7\text{mm}$. (B) Typical load-length records as a function of time from the soleus muscle of a C3w animal. Four out of 10 length records are shown. The time interval over which shortening velocity was determined for each indicated load level was between the downward arrow and each upward arrow. (C) Relative force-velocity (black dots) and power-velocity (open dots) curves were obtained from the load-length relationships in B. For B and C, $M_m=0.080\text{g}$; $FL=13.7\text{mm}$; $F_o=67.63\text{g}$; $F/F_o=0.351$; maximum shortening velocity= $2.80\text{FL}\cdot\text{s}^{-1}$, maximum power= $30.86\text{W}\cdot\text{kg}^{-1}$, and Hill's constant (a/P_o)= 0.257 .

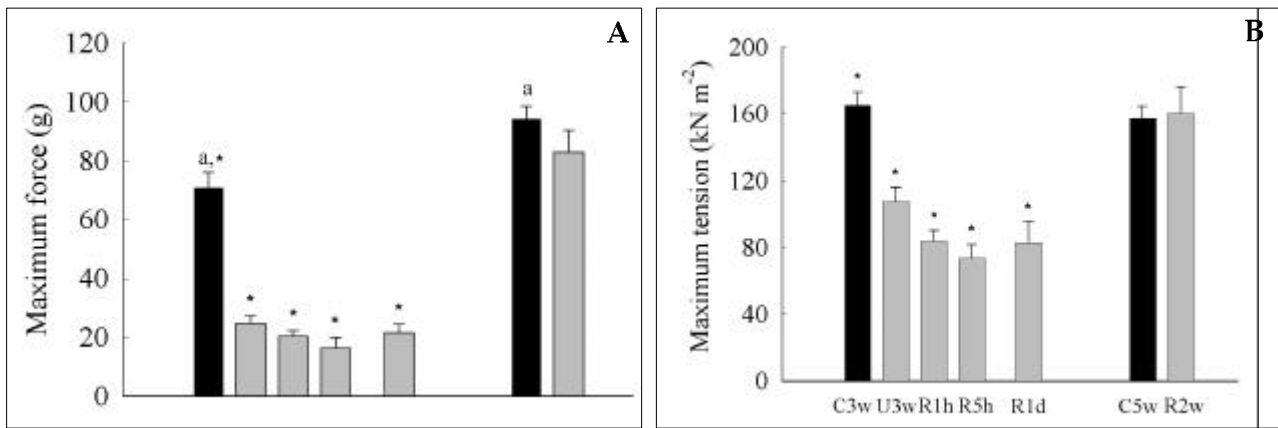


Fig. 3. Maximum tetanic force (A) and tension (B) for control and UR groups. Sample sizes are given in Table 1, and bars represent SE. Statistical significance: a, between C3w and C5w (independent *t*-test); *, among C3w, U3w, R1h, R5h, and R1d (oneway ANOVA and Scheffe's tests).

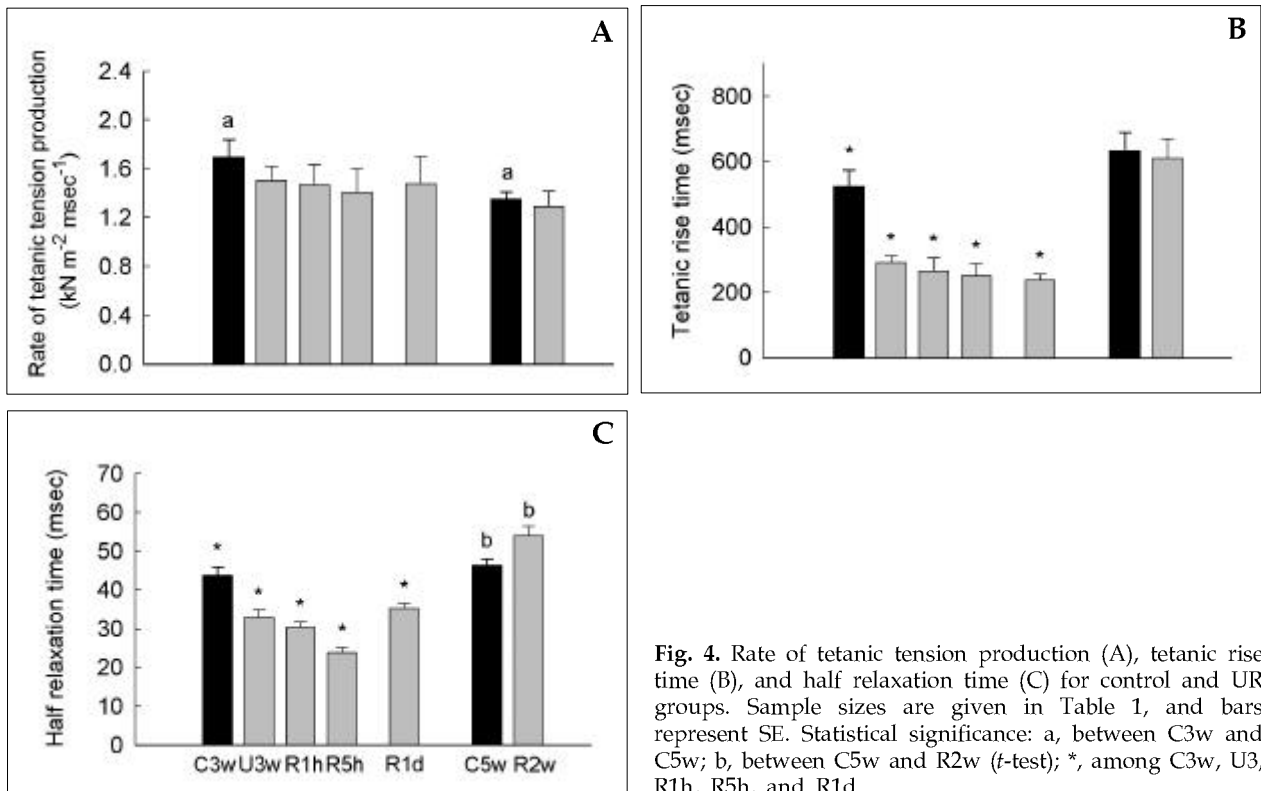


Fig. 4. Rate of tetanic tension production (A), tetanic rise time (B), and half relaxation time (C) for control and UR groups. Sample sizes are given in Table 1, and bars represent SE. Statistical significance: a, between C3w and C5w; b, between C5w and R2w (*t*-test); *, among C3w, U3w, R1h, R5h, and R1d.

half relaxation time (HRT) during maximum tetanic exertion were measured (Fig. 4). Between C3w and C5w, there was a significant difference in dP/dt ($p < 0.01$) but not in TRT and HRT. In comparison with the age-matched controls, the dP/dt changed little in the UR group (Fig. 4A). TRT, however, decreased significantly by 49–58%

in U3w, R1h, R5h, and R1d ($p < 0.05$), but it returned to the control level within two weeks of reloading (Fig. 4B). HRT also decreased significantly by 41–57% in U3w to R1d (Fig. 4C). HRT then increased to 117% of C5w after two weeks of reloading ($p < 0.05$).

Shortening velocity and power

Next, the shortening velocity (V , determined at either 35% or 40% maximum tetanic force, F_0) and the mechanical power for the UR and control groups (Fig. 5) were examined. The average V was $0.70 \pm 0.11 \text{ FL} \cdot \text{s}^{-1}$ in C3w and $0.58 \pm 0.05 \text{ FL} \cdot \text{s}^{-1}$ in C5w, and was not statistically different between the two (Scheffe's test, $p > 0.05$). The V increased by 43% in U3w, by 61-70% in R1h-R1d ($p < 0.01$), and returned to the C5w level after two weeks of reloading. Although V increased 19-25% more in R1h-R1d than U3w, the difference was not significant (Scheffe's tests, $p > 0.05$).

Muscle power was statistically similar between C3w and C5w (44.1 and $33.4 \text{ W} \cdot \text{kg}^{-1}$, respectively) (Fig. 5B). The muscle power of the UR groups ranged between 35.7 - $47.1 \text{ W} \cdot \text{kg}^{-1}$ and did not significantly deviate from that of the age-matched controls ($p > 0.05$ for all the pairs).

Muscle fatigability

Forces generated with the stimulation protocol (a series of 2-sec stimulation and 2-sec rest) were recorded for about 80 sec (Fig. 6A and B). The 2-sec force traces that were attained at 60 sec of the stimulation protocol were used to measure the force decrement (Fig. 6C and D). To gauge muscle fatigability, relative decrement in force over the 60-sec trial (% maximum) or during the 2-sec exertion at 60 sec (% initial) was summarized in

Figure 7A and 7B. Other than the case of R1h (oneway ANOVA and Scheffe's test, $p = 0.047$), the force decrement over the 60-sec trial was statistically indistinguishable between the UR groups and the age-matched controls (Fig. 7A). However, the force decrement seen during the 2-sec exertion at 60 sec (Fig. 7B) was significantly greater in the UR groups than in each of the age-matched controls.

Muscle lactate concentration

Lactate concentration in the soleus muscle was within a similar range for C3w and C5w (0.15 and $0.17 \text{ mg} \cdot \text{dL}^{-1} \cdot \mu\text{g protein}^{-1}$, respectively; $p > 0.05$) (Fig. 8). Compared to C3w, the lactate concentration increased by 140% in U3w and 89% in R1h (Scheffe's tests, $p < 0.05$). The lactate level of R5h and R1d, and R2w did not differ significantly from the levels of each corresponding control.

DISCUSSION

Our data show that developmental changes in the muscle function were minimal between C3w and C5w and were only seen in the force and rate of tetanic tension production (dP/dt). The increase in force (33%), for instance, seemed merely due to the size effect of the soleus muscle (40% in CSA) for the two-week period (Table 1). It is known that differentiation of skeletal muscle in

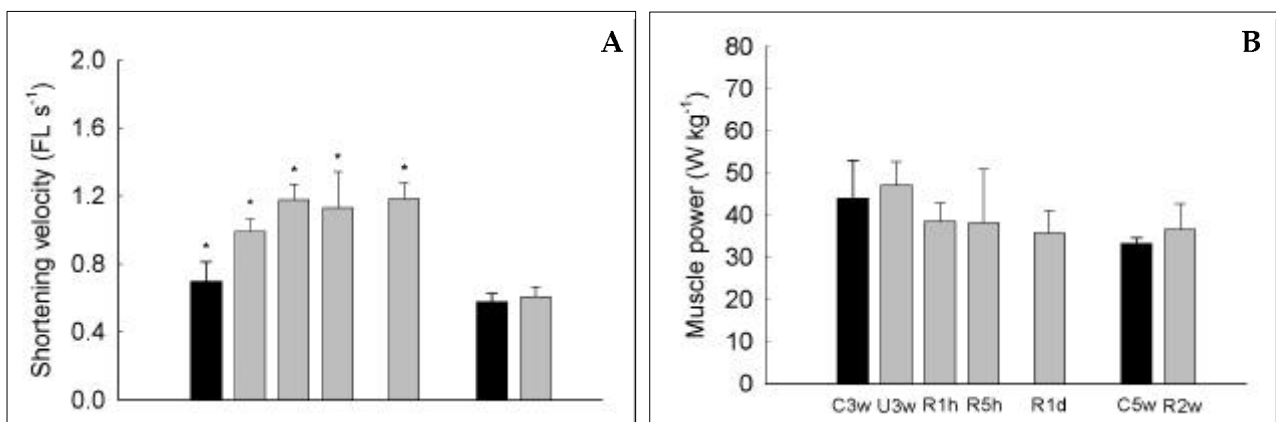


Fig. 5. Shortening velocity (A) and power (B) of the soleus muscle for the control and UR groups. Sample sizes are given in Table 1, and bars represent SE. * denotes statistical significance among C3w, U3, R1h, R5h, and R1d.

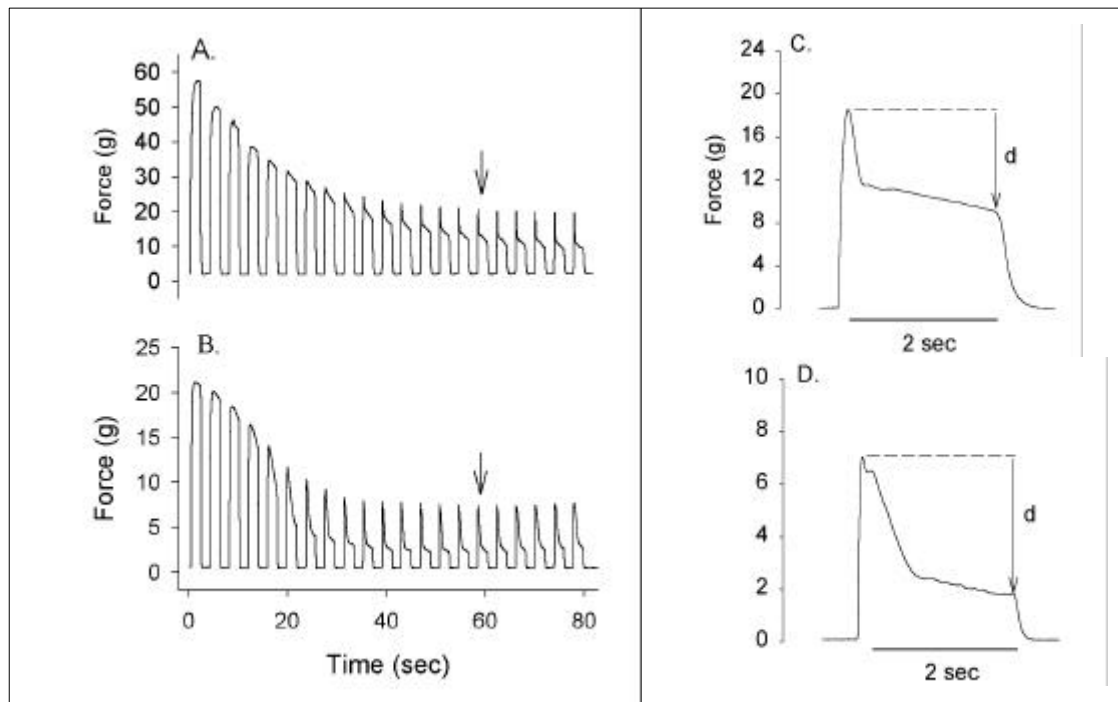


Fig. 6. A - B. Typical traces of forces for C3w (A) and U3w (B) generated by a series of 2-sec stimulations and 2-sec rest periods repeated for about 80 sec. The two arrows indicate forces produced at 60 sec, which were used to gauge muscle fatigability. C - D. The force curves at 60 sec in A and B are enlarged to show the magnitude of force decrement (d) in C3w (C) and U3w (D).

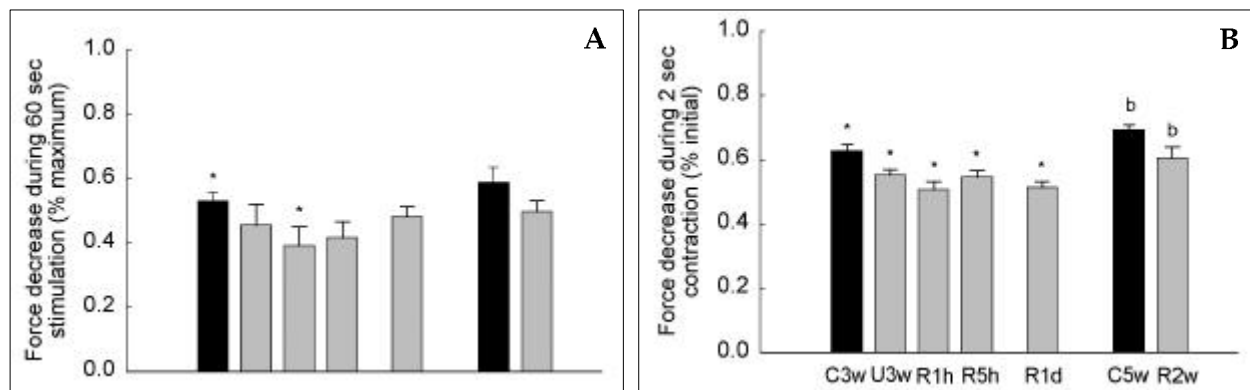


Fig. 7. (A) Force decrease during 60 sec of the stimulation (% maximum). (B) Force decrease during 2 sec contraction (% initial) generated at 60 sec. Sample sizes are given in Table 1, and bars represent SE. Statistical significance: b, between C5w and R2w; *, among C3w, U3w, R1h, R5h, and R1d.

rats completes at around four weeks of postnatal age.²²

Major changes in morphological and contractile properties of the tissue occurred during the three weeks of unloading, while additional changes by acute reloading were marginal. This observation

made it difficult to accept our hypothesis that further significant changes would occur in the muscle function through acute reloading due to the potential effects of edema or sarcomere lesion on muscle contractility.

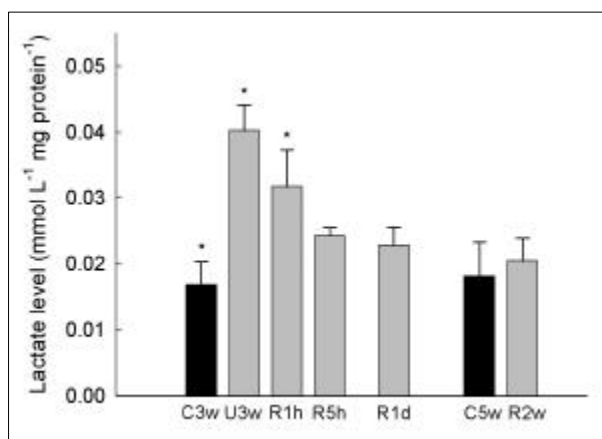


Fig. 8. Lactate levels in the soleus muscle for the UR and control groups. $n=4$ for C3w, C5w, and R2w; $n=5$ for U3w, R1h, R5h, and R1d. Error bars represent SE. * denotes statistical significance among C3w, U3w and R1h (ANOVA and Scheffe's tests).

Transitional period

More specifically, three weeks of unloading caused significant atrophy of the soleus muscle, as seen in the relative mass (43%) and misalignment of myofilaments (Fig. 1). The magnitude of the muscle atrophy was similar to that observed in other studies.^{3,5,23} The decreasing trend in muscle size was apparently reversed to a gradual increasing trend immediately after reloading, which would have probably involved muscle edema as well as an increment of myofibrillar constituents (e.g., contractile proteins).¹³⁻¹⁵ The arrangement of myofilaments was considerably disrupted in U3w, and this misalignment continued until day one of reloading (Fig. 1), suggesting its impact on muscle contractile function.

The contractile variables most responsive to unloading were muscle force, tension, shortening velocity, tetanic rise time, and half relaxation time. Then, the changes in these variables slowed down during early reloading. The reduction in force (65%) outran loss of cross-sectional area (46%) during unloading, which resulted in a significant decrease in normalized tension (35%). This may indicate substantial intracellular alternations such as myofilament misalignment (Fig. 1) and substantial loss of myofilaments during unloading.^{3,6} In this study, the tension decreased 14-20% more in R1h - R1d than that in U3w, but the difference

was not statistically significant. However, Pottle and Gosselin¹⁵ showed that the rat soleus muscle exhibited an even greater decrement in P_0 after one day of reloading (32%) and two days of reloading (50%) following one week of unloading. The discrepancy between the two studies may arise from differences in the unloading time length and in magnitudes of gain in muscle mass and loss in force for the given reloading period. In the study of Pottle and Gosselin, unloading was undertaken for one week, and there was a 43% increase in muscle mass and a 21% decrease in force for 1 d of reloading compared to this study (3 weeks for unloading; 19% for mass; 15% for force).

The three weeks of unloading caused a significant increase in the shortening velocity (143%) of the tissue while an additional increase in the velocity during R1h - R1d was not significant (Fig. 5A). The increase in shortening velocity is accounted for, in part, by the selective reduction in type I fibers as well as by the expression of fast-type myosin isoforms.^{7,10} Therefore, relatively more abundant fast-type fibers or isoforms may increase the cross-bridge turnover rate, which results in curtailed time responses (TRT, HRT) and shortening velocities of the tissue.¹ In addition to the alteration in fiber types, the selective loss of actin filaments can be another factor in increasing the shortening velocity, because such a loss can increase the spacing between myosin and actin filaments and hence can make the cross-bridge cycle faster.⁶

Previous studies have demonstrated that fatigability is more or less independent of the amount of activity or loading status.^{24,25} In this study, a similar result was discovered stating that the relative decrement in tension (41-53%) over 60 sec of continuous force exertion did not differ between the unloading-reloading (UR) groups and the controls (Fig. 7A). However, another measure of fatigability illustrated that the magnitude of tension depression during a 2-sec maximum contraction was greater in the UR groups (Figs. 6C versus 6D, and 7B), indicating lower fatigue resistance than the control. This lower resistance to fatigue suggests a relatively poor quality of performance in the UR groups for a given strenuous task. The greater decrement in tension (Fig.

6D) would imply a lower capacity of cross-bridge maintenance, probably due to insufficient energy supply.⁵

Significant increases in the lactate level in U3w and R1h (Fig. 8) seemed to reflect the increased proportion of fast-type fibers, as well as reduced circulation in the soleus muscle during unloading.^{5,26} Blood flow to the hindlimbs decreased in the head-down posture, or microcirculation in the antigravity muscle is impaired during spaceflight and returns to the original level after normal standing resumes.^{6,17,26} Therefore, the lactate level would accumulate in the hindlimb muscle during the unloading period. This return of the lactate level to the control level soon after reloading (i.e., in R5h) indicated that the recovered circulation reduced lactate production and/or effectively removed lactate from the muscle. The lactate level that peaked in U3w and remained high in R1h correlated only modestly with muscle fatigability. Several other factors that affect muscle fatigability, such as myofibrillar disruption (Fig. 1) and ATP production capacity,^{5,12,27} should be considered in conjunction with the lactate level.

Recovery period

After the transitional period, morphological and functional changes seemed to take on the reversed processes of unloading. The reloading group showed an almost full recovery of muscle morphology during two weeks of reloading. While muscle mass increased 132% between R1d and R2w, myofiber length changed only 16% during this period (Table 1). The major recovery of the soleus tissue may, therefore, occur in the thickness of individual fibers, which suggest an increase in the number of sarcomeres across the fibers but only marginally along the fibers.

The increment in muscle tension (93%) between R1d and R2w (Fig. 3B) is likely to be associated with the recovery of intracellular constituents such as myofilament arrangements (Fig. 1) and contractile proteins.^{7,28} Between R1h and R2w, the shortening velocity (*V*) decreased 49%, and the contraction time (TRT) increased 179%. Both approached the levels of the C5w, but HRT increased to a greater value than the control value

(Fig. 4C). The slower *V*, longer TRT, and longer HRT would reflect a shift of muscle fibers from fast to slow types as well as preferential recovery of actin filaments.^{1,5} The longer HRT would result from the slower detachment of the cross-bridges during relaxation, which may reflect an insufficient rate of ATP supply.

In contrast to the contractile properties, fatigue resistance did not reach the control level even after two weeks of reloading (Fig. 7B). Because the lactate level first returned to the control level after 5 hrs of reloading (Fig. 8), the delayed recovery of the fatigue resistance might be due to the myofibrillar properties and/or ATP production capacity, which can possibly take a longer time to recover.^{5,27} Our micrographs of the soleus muscle showed that the myofibrillar alignment was not completely normal even after two weeks of reloading (Fig. 1).

The contraction rate (*dP/dt*) and power did not change significantly over the entire UR period (Figs. 3A and 4B). Both the decreased *P*₀ and TRT kept *dP/dt* constant, while the decreased *P*₀ and the increased *V* counteracted each other, resulting in relatively constant power. According to Widrick et al.,²⁹ 17 days of spaceflight caused about a 20% decrease in power to two crew members. However, in the other two astronauts, the spaceflight did not cause any effect on the power capacity because of the compensatory effect between muscle force and shortening velocity. Such maintenance of power was also seen in the rat soleus muscle after 6 d of spaceflight.^{1,10}

In conclusion, major changes in morphology and contractile function of the rat soleus muscle occurred during the three weeks of unloading, while further significant changes by acute reloading were marginal. The transitional reverse process during early reloading was gradual rather than abrupt. Most of the muscle properties returned to the control level after two weeks of reloading. Full recovery of the tissue, however, would require more than two weeks of reloading, as the myofibrillar arrangement and fatigue resistance were not completely resumed by the two-week period, which would be considered for rehabilitation after a long-term spaceflight or bed-rest. Some factors that were not

included in our discussion but would seriously affect the tissue atrophy were possibly the time length of unloading and age of animals.³ These factors may interactively determine the degree and rate of the degeneration process of cellular components and would thus be the future subject to be addressed.

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