Velocity, force, power, and Ca\(^{2+}\) sensitivity of fast and slow monkey skeletal muscle fibers

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Fitts, Robert H., Sue C. Bodine, Janell G. Romatowski, and Jeffrey J. Widrick. Velocity, force, power, and Ca\(^{2+}\) sensitivity of fast and slow monkey skeletal muscle fibers. J. Appl. Physiol. 84(5): 1776–1787, 1998.—In this study, we determined the contractile properties of single chemically skinned fibers prepared from the medial gastrocnemius (MG) and soleus (Sol) muscles of adult male rhesus monkeys and assessed the effects of the spaceflight living facility known as the experiment support primate facility (ESOP). Muscle biopsies were obtained 4 wk before and immediately after an 18-day ESOP sit, and fiber type was determined by immunohistochemical techniques. The MG slow type I fiber was significantly smaller than the MG type II, Sol type I, and Sol type II fibers. The ESOP sit caused a significant reduction in the diameter of type I and type I/II (hybrid) fibers of Sol and MG type II and hybrid fibers but no shift in fiber type distribution. Single-fiber peak force (mN and KN/m\(^2\)) was similar between fiber types and was not significantly different from values previously reported for other species. The ESOP sit significantly reduced the force (mN) of Sol type I and MG type II fibers. This decline was entirely explained by the atrophy of these fiber types because the force per cross-sectional area (kN/m\(^2\)) was not altered. Peak power of Sol and MG fast type II fiber was 5 and 8.5 times that of slow type I fiber, respectively. The ESOP sit reduced peak power by 25 and 18% in Sol type I and MG type II fibers, respectively, and, for the former fiber type, shifted the force-pCa relationship to the right, increasing the Ca\(^{2+}\) activation threshold and the free Ca\(^{2+}\) concentration, eliciting half-maximal activation. The ESOP sit had no effect on the maximal shortening velocity (\(V_o\)) and ATPase and reduce peak power in the slow type I fibers of the Sol (19, 20). The percentage of fibers expressing fast myosin heavy chain (MHC) increased from 4 to 29% by 3 wk of HU; however, the majority of the fibers showed a significant increase in \(V_o\) while maintaining an exclusively slow type myosin isozyme profile (20).

Clearly, there is a need for additional information about the cellular adaptations induced by 0 G in humans and/or other large nonhuman primates before scientifically sound exercise countermeasures can be developed. Without the latter, it will be impossible to safely conduct the prolonged space missions required for exploration of Mars and other points of the universe. Recently, US and Russian scientists have studied the effects of spaceflight on the neuromuscular system in rhesus monkeys (2). The general hypothesis is that the rhesus monkey may be an excellent model for studying not only the mechanisms of 0-G-induced muscle atrophy but also the adaptive responses and strategies of the neuromuscular system. To date there is a scarcity of information concerning the functional properties of limb skeletal muscles in rhesus monkeys. Before alterations induced by 0 G can be studied and understood, it is important to understand the monkey's normal physiology. Consequently, the purpose of this work was to characterize the fiber type distribution and size characteristics of the slow Sol and fast gastrocnemius muscles of adult rhesus monkeys and determine the force, velocity, power, and Ca\(^{2+}\) sensitivity of individual fast- and slow-twitch fibers. A second purpose was to determine the effect of the flight facility itself on contractile function. Because the animals must fly in an experiment-support primate facility [Experimental System for the Orbiting Primate (ESOP)], it is important to

A major problem associated with space travel is the multifaceted deterioration of limb skeletal muscle (5, 26, 31). Skeletal muscles from rats flown in space have shown fiber atrophy, degeneration of motor innervation, muscle fiber segmental necrosis and central-core lesions, and disruption of the microvasculature, with the greatest change observed in antigravity muscles such as the soleus (Sol) (18, 26). For example, the relative atrophy observed with both models of weightlessness and 0 gravity (G; magnitude of the force of gravity on the surface of the Earth) was Sol > gastrocnemius = plantaris > extensor digitorum longus (8, 18, 28). Considerably less is known about the effects of weightlessness on skeletal muscle function in humans and nonhuman primates. During Skylab flights, the crew experienced a 12% decrease in leg volume and a 20% decrease in muscle strength (5). The greater decline in strength relative to muscle size suggests that factors in addition to cell atrophy contributed to the strength loss. Models of weightlessness employing rodents have clearly demonstrated that both peak force (N) and tension [N/fiber cross-sectional area (CSA)] decrease in response to unloading (11, 19–21). The reduced tension is thought to be caused by a selective loss in contractile proteins, such that the number of active cross bridges per CSA declines (29).

To assess the cellular basis of the functional changes induced by weightlessness, we have used the single skinned-fiber preparation and the hindlimb unloading (HU) rat model (11, 19–21). In addition to fiber atrophy and the decline in force, HU has been shown to increase maximal fiber shortening velocity (\(V_o\)) and ATPase and reduce peak power in the slow type I fibers of the Sol (19, 20). The percentage of fibers expressing fast myosin heavy chain (MHC) increased from 4 to 29% by 3 wk of HU; however, the majority of the fibers showed a significant increase in \(V_o\) while maintaining an exclusively slow type myosin isozyme profile (20).

rhesus monkey muscle; slow type I and fast type II fibers; spaceflight facility

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METHODS

Selection of animals and general care. The study described herein was conducted as part of a large joint effort between the US and French space agencies [National Aeronautics and Space Administration (NASA) and Centre d'Études Spatiales, respectively] designed to develop baseline ground-based data on the basic physiology and psychomotor performance of adult rhesus monkeys (Macaca mulatta). The project was approved by the animal care and use committee at NASA-Ames Research Center (Moffet Field, CA) and Marquette University (Milwaukee, WI).

Ten adult male rhesus monkeys were selected from the colony at the NASA-Ames Research Center. During the course of this study, the animals were housed at the NASA primate test research facility, Ames Research Center. Animal care was in accordance with the guidelines established by the National Institutes of Health and NASA. The animals had free access to water, but the total intake was limited to 2,000 ml/day.

The ESOP facility contained space for two monkeys, and each compartment (rhesus experiment compartment) contained a psychomotor test system (PTS). The PTS was developed by Washburn and Rumbaugh (30) and consisted of a package of software tasks, together with the computer hardware required to administer each task. The monkeys manipulated a joystick with their right hand to control the movements of a cursor on a computer screen. In this way, they responded to computer-graphic stimuli in accordance with the demands of 1 of 18 psychological tasks (30). Before initiation of the study, the animals were fully trained in the operation of the PTS, and they were required to work PTS tasks to obtain food. One purpose of the study was to evaluate the effect of the ESOP facility on skeletal muscle function. Because the ESOP was built as a Spacelab payload, and the maximal flight time of the space shuttle is 18 days, 8 of the 10 animals were housed in the ESOP in a sitting position for 18 days (ESOP sit). The remaining two animals served as vivarium controls. During the ESOP sit procedure, the monkeys were presented with menus that contained icons representing each PTS task. When the monkey selected one of the icons, it received five trials of the corresponding task, after which the menu of options was again presented. Animals that completed trials successfully were reinforced with fruit-flavored chow pellets. Thus the monkeys could choose both when to work and also on what task to work during the 16 h per day that the lights were on and the PTS was continuously available. The number of pellets was limited only by each animal's performance. Animals that did not work a sufficient number of PTS tasks were supplemented with wafers made from the same ingredients as the food pellets. Food intake averaged 287 g/day. The monkeys' average age was 8.3 yr, and the pre- and post-ESOP weights were 9.4 ± 0.4 kg and 10.0 ± 0.4 kg, respectively.

The ESOP facility did not restrain the legs. The monkeys were able to move their ankles through a full range of movement and touch the sides of the capsule and a bar positioned below their feet. Video of the animals and electromyographic (EMG) recordings from the Sol and MG were obtained at selected times throughout the 18-day sit. Although the monkeys could push off the sides of the capsule, the video indicated that they generally did not. Additionally, the EMG data demonstrated that the activity of both the Sol and medial gastrocnemius (MG) was reduced compared with pre-ESOP recordings (J. Hodgson, personal communication).

Generally, the animals curled their feet slightly inward so that the lateral surface of the feet rested on the foot bar.

Biopsy procedure. Muscle biopsies were obtained 4 wk before (pre) and immediately after (post) the 18-day ESOP sit. The pre- and postbiopsies were taken from two independent sites in the Sol and the MG muscles by using an open-biopsy technique. All biopsies were taken from the right leg, and the specific location of each biopsy site is shown in Fig. 1. The sites were selected to ensure that the same muscle fibers were not sampled during the pre- and postflight biopsies, as determined from detailed architectural analyses of each muscle (27). Additionally, the regions sampled within each muscle have been shown to have similar fiber type distributions (27).

After general anesthesia (isoflurane), a small incision (3–4 cm) was made on the medial side of the lower leg to expose the Sol and MG muscles. By using blunt dissection, the belly of each muscle was exposed and a small cut was made in the overlying fascia. To obtain the biopsies, the tip of a scalpel blade was used to isolate a piece of tissue ~10 mm long × 5 mm deep (~150 mg wet wt). All samples were taken from the superficial muscle belly, and the cut was made parallel to the direction of the muscle fibers. The muscle sample was removed and immediately placed on saline-soaked gauze and processed as described below. The fascia and skin were covered with absorbable sutures (Vicryl t). The tissue sample was weighed and then divided in half with a longitudinal cut parallel to the fiber orientation. One of the sections was divided again and used for the skinned and freeze-dried fiber preparations, respectively. The skinned-fiber preparation is described below, whereas the studies conducted with the freeze-dried fibers will be presented elsewhere (V. Grichko, G. J. Gettelman, J. J. Widrick, and R. H. Fitts, unpublished).

The pre- and postbiopsies were taken from two independent sites in the Sol and the MG muscles by using an open-biopsy technique. All biopsies were taken from the right leg, and the specific location of each biopsy site is shown in Fig. 1. The sites were selected to ensure that the same muscle fibers were not sampled during the pre- and postflight biopsies, as determined from detailed architectural analyses of each muscle (27). Additionally, the regions sampled within each muscle have been shown to have similar fiber type distributions (27).
observations). The other one-half was stretched to approximately the in situ length and mounted on cork by using pins to ensure a perpendicular orientation of the muscle fibers. The samples were then frozen in isopentane cooled with liquid nitrogen and stored at −80°C until processed for immunohistochemical analysis of fiber type. The muscle section to be used for skinned-fiber analysis was placed in skinning solution (4°C) and stored at −20°C for up to 4 wk. The skinning solution contained (in mM) 125 K-propionate, 2 EGTA, 4 ATP, 1 MgCl₂, and 20 imidazole and 50% (vol/vol) glycerol. The section to be used for single-fiber biochemical analysis was aligned longitudinal on a small index card, frozen in liquid nitrogen, freeze-dried under vacuum at −40°C, and then stored under vacuum at −80°C.

Histochiometric identification of fiber type. Fbers were classified as type I, IIa, IIx, or type I/II (hybrid; coexpression of slow and fast myosin) by using monoclonal antibodies that label the different MHC isoforms. Serial cross sections were incubated with primary antibodies [BA-F8, BF-13, BF-35, and SC-71 generously donated by S. Schiaffino (Padua, Italy)] overnight at 25°C. Sections incubated without primary antibody were used as a control to visualize nonspecific labeling. A Vectastain ABCt kit (Vector Labs, Burlingame, CA) was used to amplify the antigen-antibody complex, which, in turn, was visualized by treatment with a diaminobenzidine peroxidase reaction.

Muscle fibers from the Sol and MG can be classified into four types on the basis of their immunohistochemical staining to monoclonal antibodies to the myosin heavy chain. The type I fibers were positive for the BA-F8 (specific for slow MHC) and BF-35 (positive for all MHCs except IIx) antibodies and negative for the BF-13 (positive for all type II MHCs) and SC-71 (specific for fast type IIa MHC) antibodies. The type IIa fibers were positive for all of the antibodies except BA-F8. The classification IIx was based on the negative staining of fibers for the BF-35 antibody. These fibers were also negative for BA-F8, positive for BF-13, and intermediate for SC-71. In rats, the SC-71 antibody is specific for type IIa fibers. In monkeys and humans, however, IIx fibers stain intermediate for SC-71 (S. Schiaffino, personal communication). Hybrid fibers stained positively for all the antibodies and presumably express both type I and IIa MHCs.

Single-fiber preparation. On the day of an experiment, a muscle bundle (Sol or MG) was transferred to a dissecting chamber containing relaxing solution pCa 9.0 (4°C, where pCa = −log Ca²⁺ concentration) that contained the following (in mM): 20 imidazole, 7 EGTA, 10.0 caffeine, 14.5 creatine phosphate, 4.81 ATP, 5.26 MgCl₂, and 7.0 CaCl₂·2H₂O. Both relaxing and activating solutions contained sufficient KOH and KCl to bring pH to 7.0 and total ionic strength to 180 mmol/l. The composition of the relaxing and activating solutions was determined by using the computer program of Fabiato and Fabiato (7) that uses the apparent stability constants reported by Godt and Lindley (13). Peak force (N) was determined in each fiber by computer subtraction of baseline force from peak force attained during maximal Ca²⁺ activation, and force (kN/m²) was calculated. Peak fiber stiffness was measured by applying small-amplitude sinusoidal changes in fiber length (FL; ΔL) at a frequency of 1.5 kHz and measuring the magnitude of the resultant change in force (ΔP). The elastic modulus (E₀) of each fiber was calculated as follows: E₀ = ΔP/ΔL × FL/fiber CSA. Peak stiffness was determined by subtraction of the stiffness recorded in relaxing solution from that measured during full activation (pCa 4.5), exactly as described previously (21).

Determination of fiber V₀, Vₘ was determined by the slack test, as previously described in detail (32). Briefly, the fiber was activated, allowed to attain peak force, and then rapidly shortened by a predetermined length step so that force dropped to zero. There was a rapid redevelopment of force once the fiber had shortened to take up the imposed slack. The fiber was then returned to relaxing solution and reextended to its original FL. This entire procedure was repeated so that each fiber was subjected to a minimum of five different slack distances, each ≈20% of FL. The computer plotted the slack step distances against the corresponding times required for the redevelopment of force and fit the data with a first-order least-squares regression line. The slope of this line was Vₛ (in mm/s), which was normalized to FL at a sarcomere spacing of 2.5 μm (FL/S).

Force-velocity-power relationships. In a subset of fibers, the segment was subjected to a series of isotonic contractions as follows. The mounted fiber was transferred into a chamber filled with activating solution maintained at 15°C and allowed to attain peak isometric force (Pᵢₘ). The position motor then rapidly stepped the fiber to three submaximal isotonic loads. The rate of fiber shortening was controlled by a servomechanism similar to that described by Julian and Moss (17). The duration of each isotonic step varied from 80 to 120 ms and was adjusted so that the total shortening that occurred over all three steps did not exceed 20% of FL. After the third isotonic step, the fiber was returned to relaxing solution and reextended to its original FL. The entire procedure was repeated until each fiber had been subjected to a total of 15–18 different isotonic loads.
Outputs from the force transducer and position motor were monitored on a digital-storage oscilloscope before being amplified and interfaced to a personal computer. Custom software determined $P_0$ (defined as the difference between resting force in relaxing solution and the force immediately before the initiation of the isotonic steps) and the force and velocity attained over the last half of each isotonic step. Force-velocity data pairs were fit with the hyperbolic Hill equation (16). Peak power was calculated from the force-velocity parameters $V_{\text{max}}$ (defined as the extrapolated intercept of the force-velocity relationship with the velocity axis), $a/P_0$, where $a$ is the constant with the dimension of force, and the maximal $P_0$ attained by the fiber during the experiment.

**Force-pCa relationships.** Force-pCa relationships were determined by measuring $P_0$ attained in activating solutions having free Ca$^{2+}$ concentrations ranging from pCa 6.8 to 5.0. These solutions were made by mixing appropriate volumes of the maximal activating (pCa 4.5) and relaxing (pCa 9.0) solutions described above. The submaximal forces attained during these contractions, $P_r$, were expressed as a fraction of the peak isometric force determined in pCa 4.5, i.e., $P_r = (\text{submaximal } P_0)/P_0$ at pCa 4.5. Every fourth or fifth contraction was performed at pCa 4.5. Separate Hill plots [where $\log (P_r/(1 - P_r))$ is plotted against pCa] were fitted to data points $<0.5P_0$ and $>0.5P_0$. The free-Ca$^{2+}$ concentration that elicited half-maximal activation was defined as the mean abscissa intercept of each plot. The Ca$^{2+}$ activation threshold was defined as the free-Ca$^{2+}$ concentration obtained when the plot of points $<0.5P_0$ was extrapolated to a value of $-2.5$.

**MHC composition.** After the contractile measurements, the MHC composition of each fiber was determined by SDS-PAGE. The segment was removed from the experimental apparatus and solubilized in 10 µl of 1% SDS sample buffer containing 6 mg/ml EDTA, 0.06 M Tris, 1% SDS, 2 mg/ml bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol. The sample buffer (2.5 µl) was loaded on a Hoefer SE 600 gel system that consisted of a 3% (wt/vol) acrylamide stacking gel and a 5% (wt/vol) separating gel (25). Gels were silver stained as described by Giulian et al. (12). The relative content of each MHC in hybrid fibers was determined by densitometric scanning (CliniScan 2, Helena Laboratories, Beaumont, TX).

Statistical analysis. Data are presented as means ± SE. A t-test was used to determine intergroup differences in contractile properties. Statistical significance was accepted at $P ≤ 0.05$.

**RESULTS**

**Fiber diameter and percent distribution.** The fiber type distribution was determined by immunohistochemical staining to monoclonal antibodies as described in Methods. A representative micrograph is shown in Fig. 2 and the percent fiber type distribution in Table 1. Consistent with other species, the monkey Sol contained primarily the slow type I fiber; however, the fast fiber population (IIa and hybrid) showed a higher percentage than we had previously observed in rats and humans (9, 10, 20). The MG contained primarily fast fibers, with the greatest percentage being type IIx. Interestingly, the monkey Sol and MG, similar to human muscle, contained no fast type IIb fibers. The slow type I fibers of the MG were significantly smaller than the Sol type I and Sol and MG type II fibers. This difference was apparent when fiber size was determined from a CSA analysis of the histochemically stained sections (Fig. 3) or from the diameter of the individual skinned fibers (Table 2). The latter technique is more sensitive and reliable because the diameter is measured at a constant sarcomere length of 2.5 μm. The ESOP sit caused a significant reduction in the diameter of the type I and hybrid fibers of the Sol and the type II and hybrid fibers of the MG, whereas the Sol type II and MG type I fibers were unaffected (Table 2; see Table 4; Fig. 3). The ESOL sit caused no major shifts in the fiber type distribution; however, we did observe an increased number of hybrid fibers postsit (Table 1; see Table 4).

**Fiber force (N and kN/m²) and stiffness.** Peak force (mN and kN/m²) for each fiber type is shown in Table 2. There were no major differences between the fiber types in their ability to generate force (kN/m²), although the Sol slow type I and fast type II fibers showed the lowest and highest force, respectively. The ESOP sit...
by the ESOP sit. The $V_o$ for the hybrid fiber was generally distributed toward the high end of the type I group (Figs. 5 and 6). The mean ratio of MHC II to the total MHC for the hybrid fibers is shown in Table 4. The ratio was unaffected by the ESOP sit, but when individual hybrid fibers of both muscles were evaluated a significant positive correlation was observed between the percentage of type II MHC and $V_o$ (Fig. 7). This relationship can be best described by a second-order polynomial equation

$$
V_o = 5.56 \frac{(MHC \text{ II}/\text{total MHC})^2}{-1.66 \frac{(MHC \text{ II}/\text{total MHC})}{+0.72}}
$$

Although the relationship was significant, the scatter about the fit was great ($r^2 = 0.61$).

Isotonic contractile properties of rhesus monkey muscle fibers. The force-velocity relationship of a representative type I fiber, obtained from the Sol of an adult rhesus monkey maintained under normal living conditions, is presented in Fig. 8A. Three parameters are necessary to describe this relationship: 1) the intercept

Table 1. Immunohistochemical determination of fiber type distribution in Sol and MG muscle biopsies obtained from control monkeys and from monkeys before and after 18 days of restraint in the ESOP

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Type I, %</th>
<th>Type IIa, %</th>
<th>Type IIx, %</th>
<th>Hybrid, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol</td>
<td>Precontrol</td>
<td>70 ± 10</td>
<td>13 ± 5</td>
<td>9 ± 4</td>
</tr>
<tr>
<td></td>
<td>Postcontrol</td>
<td>62 ± 14</td>
<td>21 ± 9</td>
<td>17 ± 5</td>
</tr>
<tr>
<td></td>
<td>Pre-ESOP</td>
<td>62 ± 6</td>
<td>22 ± 5</td>
<td>16 ± 3</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP</td>
<td>51 ± 6</td>
<td>25 ± 6</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>MG</td>
<td>Precontrol</td>
<td>23 ± 2</td>
<td>24 ± 6</td>
<td>49 ± 3</td>
</tr>
<tr>
<td></td>
<td>Postcontrol</td>
<td>19 ± 15</td>
<td>26 ± 4</td>
<td>51 ± 5</td>
</tr>
<tr>
<td></td>
<td>Pre-ESOP</td>
<td>20 ± 6</td>
<td>27 ± 4</td>
<td>49 ± 7</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP</td>
<td>17 ± 3</td>
<td>28 ± 5</td>
<td>51 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 2 control monkeys and 7 experimental monkeys. Sol, soleus; MG, medial gastrocnemius; hybrid, type I/II fibers; pre, before; post, after; ESOP, Experimental System for the Orbiting Primate; control, ordinary ambulatory living conditions. Precontrol biopsies were obtained at the same time as pre-ESOP biopsies; postcontrol biopsies were obtained at the same time as post-ESOP biopsies. An average of 403 ± 130 (SD) fibers were sampled from each biopsy.

significantly reduced the force of the Sol type I and MG type II fiber types. This decline was entirely explained by the significant atrophy of these fiber types because the force per CSA (kN/m²) was not altered by the ESOP sit. Fiber stiffness averaged 2.39 ± 0.24 and 2.07 ± 0.37 kN/m² × 10⁶ in the slow fibers and 0.87 ± 0.11 and 1.09 ± 0.23 kN/m² × 10⁶ in the fast fibers of the Sol and MG, respectively. The ESOP sit had no effect on fiber stiffness.

$V_o$, A representative polyacrylamide gel illustrating MHC isoform expression in isolated single monkey fibers is shown in Fig. 4. The legend of Fig. 4 indicates the muscle source, and $V_o$ is given below each lane. Lane 1 represents a hybrid fiber containing both slow and fast MHC and a $V_o$ intermediate between the slow (lanes 2, 3, and 5) and fast (lane 4) fibers. Unlike with fibers in rats and humans, the various fast fiber types did not separate but rather migrated as a single band on the gel (Fig. 4). For Sol, this did not present a problem because the immunohistochemical results demonstrated that the type II fiber population to be entirely type IIa (Table 1). Consequently, the data for Sol type II fibers shown in Table 2 can be considered type IIa. The mean $V_o$ of 4.19 ± 0.39 FL/s was significantly higher than that of the slow type I fiber from either Sol or MG but was lower than that of the MG type II population (Table 2). However, this latter group contained both IIa and IIx fibers, and, at least in humans, the IIx fiber is known to have higher $V_o$ values than does the IIa fiber. If one assumes that the MG type II fibers with $V_o$ values >6.0 (see Fig. 6) were type IIx fibers, then the type IIa and IIx fibers showed a mean $V_o$ of 3.26 and 7.70 FL/s, respectively.

The ESOP sit had no significant effect on the mean $V_o$ of any fiber type in either Sol or MG (Table 2). Additionally, $V_o$ histograms (Figs. 5 and 6) demonstrate that the $V_o$ distribution within a fiber type was also unaffected

Fig. 3. Mean fiber cross-sectional area for (A) soleus and (B) MG pre- and post-18-day ESOP occupancy by aniamls in sitting position (ESOP sit). Solid, open, horizontally striped, and cross-hatched bars: type I, Ia, IIx, and I/II (hybrid) fibers, respectively; error bars, SE. *P < 0.05 vs. corresponding pre-ESOP mean.
of the curve with the force axis, or $P_0$; 2) the velocity axis intercept ($V_{\text{max}}$); and 3) the dimensionless parameter $a/P_0$, which describes the shape or curvature of the relationship. In this particular example, $P_0$, $V_{\text{max}}$, and $a/P_0$ values were 0.77 mN, 0.70 FL/s, and 0.029, respectively. Overlaid on the force-velocity curve is the force-absolute power relationship of the fiber. Peak power is attained at a unique point along the force-velocity relationship where the product of force ($P$) and shortening velocity ($V$) is maximal. In the present example, these conditions were attained at 14% of $P_0$, or 0.112 mN. At this external load, the fiber shortened at a velocity of 0.100 FL/s, producing a peak power output of 11.2 mN·FL·s$^{-1}$.

Figure 8B illustrates the force-velocity and force-power relationships of a Sol type II fiber obtained under the same control conditions. The force-velocity relationship of this fiber differs from that of the type I fiber in Fig. 8A in several ways. Although the type II fiber produced only slightly more $P_0$ (0.80 mN), its $V_{\text{max}}$ was 63% greater (1.14 FL/s) than that of the type I fiber. In addition, the greater $a/P_0$ (0.109) of the type II fiber indicates that the force-velocity relationship was less curved compared with that of the type I fiber. Because of this lower degree of curvature, the peak power output of the type II fiber occurred at 24% of $P_0$ vs. 14% of $P_0$ for the type I fiber. The type II fiber therefore produced almost five times more peak absolute power (51.9 mN·FL·s$^{-1}$) than the type I fiber because it was capable of shortening at a 2.7-fold greater velocity.

### Table 2. Fiber diameter, peak force, and maximal shortening velocity of Sol and MG type I and type II muscle fibers before and after 18 days of restraint in the ESOP

<table>
<thead>
<tr>
<th>Muscle</th>
<th>$n$</th>
<th>Diameter, $\mu$m</th>
<th>$P_0$, mN</th>
<th>$V_{\text{max}}$, FL/s</th>
<th>$a/P_0$, kN/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-I</td>
<td>Pre-ESOP 27</td>
<td>84 ± 2</td>
<td>0.83 ± 0.04</td>
<td>148 ± 5</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP 33</td>
<td>75 ± 2*</td>
<td>0.68 ± 0.03*</td>
<td>158 ± 5</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Sol-II</td>
<td>Pre-ESOP 14</td>
<td>77 ± 3</td>
<td>0.77 ± 0.06</td>
<td>164 ± 5</td>
<td>4.32 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP 21</td>
<td>77 ± 4*</td>
<td>0.67 ± 0.05*</td>
<td>146 ± 8</td>
<td>3.81 ± 0.35</td>
</tr>
<tr>
<td>MG-I</td>
<td>Pre-ESOP 16</td>
<td>67 ± 3</td>
<td>0.53 ± 0.04</td>
<td>153 ± 7</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP 12</td>
<td>60 ± 4</td>
<td>0.49 ± 0.06</td>
<td>171 ± 9</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td>MG-II</td>
<td>Pre-ESOP 37</td>
<td>89 ± 3</td>
<td>0.92 ± 0.05</td>
<td>148 ± 5</td>
<td>5.13 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Post-ESOP 29</td>
<td>79 ± 3*</td>
<td>0.75 ± 0.05*</td>
<td>148 ± 5</td>
<td>4.92 ± 0.46</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of fibers per mean. $P_0$, peak isometric force; $V_{\text{max}}$, maximal shortening velocity; Sol-I, Sol-II, MG-I, and MG-II: Sol and MG type I and type II fibers, respectively. *$P < 0.05$ vs. corresponding pre-ESOP mean.

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**Fig. 5.** Frequency distributions for $V_0$ of Sol fibers studied pre- (A) and (B) post-ESOP sit.

**Fig. 6.** Frequency distributions for $V_0$ of MG fibers studied pre- (A) and (B) post-ESOP sit.
while producing 1.7 times more absolute force (0.191 mN).

These fundamental differences between the individual slow and fast fibers illustrated in Fig. 8 are representative of those obtained when statistical comparisons were conducted among groups of fibers expressing type I or type II MHC. Table 3 contains the average force-velocity-power parameters of single type I and II fibers obtained from Sol and MG of rhesus monkeys before (pre-ESOP) and immediately after the 18-day ESOP sit (post-ESOP). The \( V_{\text{max}} \) and \( a/P_0 \) were both significantly greater for the type II fibers, and, as a result, peak power output, whether expressed in absolute or in relative terms, was significantly greater for these fibers. On average, Sol fibers expressing both type I and type II MHC (type I/II) displayed isotonic contractile properties that were similar to fibers expressing type I MHC exclusively (Table 4).

Similar relationships between type I and type II fiber \( V_{\text{max}} \) and \( a/P_0 \) were observed for the control MG fibers (Table 3). However, type II fibers from this muscle were significantly larger in diameter and produced 50% more absolute peak force than the type I fibers (Table 2). As a result, average absolute peak power was 8.5 times greater for the type II vs. type I fibers from this muscle vs. the approximately fivefold difference noted between the type I and type II fibers of Sol (Table 3).

Effect of ESOP occupancy on isotonic contractile properties. Force-velocity and force-power parameters of single fibers obtained immediately after 18 days of ESOP occupancy are compiled in Tables 3 and 4. The force-velocity parameters (\( V_{\text{max}} \) and \( a/P_0 \)) were not affected by the ESOP sit in either muscle or fiber type. Consequently, the relative submaximal force and velocity that elicited peak power and the absolute shortening velocity at peak power output were also unchanged. However, the absolute force associated with peak power declined in parallel to the loss of fiber \( P_0 \), i.e., by 19% for Sol type I fibers and by 26% for MG type II fibers (Table 3). These alterations in force-velocity parameters are illustrated by post-ESOP Sol type I fibers plotted in Fig. 8A. Although \( V_{\text{max}} \) (0.77 FL/s) and \( a/P_0 \) (0.026) of this post-ESOP fiber were similar to the values noted for the control fiber, its \( P_0 \) was 23% lower (0.59 mN) and its peak power was reduced by 25% (8.4 \( \mu \)N·FL·s\(^{-1} \)). Overall, peak power was reduced by an average of 25 and 18% for Sol type I and MG type II fibers, respectively (Table 3). Because of the greater variability in the average type II fiber peak power, only the change for Sol fibers was statistically significant.

Additional information concerning ESOP-induced changes in power can be gained from examining the peak power frequency distributions compiled in Fig. 9. Before ESOP occupancy, the peak power produced by Sol type I fibers ranged from 5.5 to 20.3 \( \mu \)N·FL·s\(^{-1} \), with the largest number of fibers having values between 10 and 15 \( \mu \)N·FL·s\(^{-1} \). After the ESOP sit, the peak power frequency distribution was shifted to the left so that the peak power of individual fibers ranged from 4.5 to 17.6 \( \mu \)N·FL·s\(^{-1} \), with the greatest number
of fibers producing peak power in the 5–10 µN·FL·s⁻¹ range.

Our results imply that the reduced ability of Sol type I and MG type I fibers to produce peak power was due entirely to the atrophy they experienced during the ESOP sit procedure. The impact of fiber atrophy on peak power after the ESOP sit is evident when one examines peak power values normalized to fiber CSA (kN·m⁻²·FL·s⁻¹). Normalized peak power (Table 3) was unaffected by the ESOP sit, supporting the idea that a reduction in fiber diameter was the primary cause of the decreased peak power output of the post-ESOP fibers.

Fiber Ca²⁺ sensitivity before and after ESOP occupancy. Force-pCa relationships for Sol type I and MG type I and II fibers (there was an insufficient number of Sol type II fibers and hybrid fibers for analysis) are presented in Fig. 10. For all groups, relative force rose approximately to develop effective countermeasures. Rhesus monkeys offer a number of advantages over other smaller animals such as rats in that comprehensive studies of sensory-motor control and cellular responses to 0 G are possible. However, to date there are very few data concerning the functional properties of monkey limb skeletal muscle (6). The results of this study

### Table 3. Force-velocity and force-power properties of individual slow- and fast-twitch Sol and MG fibers

<table>
<thead>
<tr>
<th>Muscle</th>
<th>n</th>
<th>V_max, FL/s</th>
<th>a/P_o</th>
<th>P at Peak Power</th>
<th>V at Peak Power</th>
<th>Peak Power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mN</td>
<td>kN/m²</td>
<td>µN·FL·s⁻¹</td>
</tr>
<tr>
<td>Sol-I</td>
<td>27</td>
<td>0.66 ± 0.07</td>
<td>0.041 ± 0.003</td>
<td>0.129 ± 0.008</td>
<td>24.0 ± 1.1</td>
<td>11.8 ± 0.7</td>
</tr>
<tr>
<td>Pre-ESOP</td>
<td>23</td>
<td>0.68 ± 0.10</td>
<td>0.042 ± 0.026</td>
<td>0.105 ± 0.008*</td>
<td>26.2 ± 1.4</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>Post-ESOP</td>
<td>7</td>
<td>1.63 ± 0.28</td>
<td>0.093 ± 0.020</td>
<td>0.161 ± 0.032</td>
<td>36.2 ± 4.3</td>
<td>54.9 ± 8.1</td>
</tr>
<tr>
<td>MG-I</td>
<td>14</td>
<td>0.67 ± 0.13</td>
<td>0.034 ± 0.007</td>
<td>0.093 ± 0.111</td>
<td>26.5 ± 1.1</td>
<td>8.1 ± 0.8</td>
</tr>
<tr>
<td>Pre-ESOP</td>
<td>9</td>
<td>0.57 ± 0.09</td>
<td>0.025 ± 0.011</td>
<td>0.081 ± 0.007</td>
<td>30.8 ± 2.8</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>MG-II</td>
<td>15</td>
<td>1.38 ± 0.14</td>
<td>0.140 ± 0.020</td>
<td>0.207 ± 0.016</td>
<td>36.6 ± 2.1</td>
<td>68.5 ± 7.1</td>
</tr>
<tr>
<td>Pre-ESOP</td>
<td>14</td>
<td>1.42 ± 0.15</td>
<td>0.126 ± 0.011</td>
<td>0.154 ± 0.133*</td>
<td>36.9 ± 2.3</td>
<td>55.9 ± 8.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers. V_max, maximal shortening velocity determined from force-velocity relationship; a/P_o, dimensionless parameter; FL, fiber length; P, force; V, shortening velocity. *P < 0.05 vs. control mean of corresponding fiber type. †P < 0.05 vs. control type I mean.

### DISCUSSION

A major challenge facing the field of space biology is to identify the cellular and molecular alterations in limb skeletal muscle with weightlessness and ultimately to develop effective countermeasures. Rhesus monkeys offer a number of advantages over other smaller animals such as rats in that comprehensive studies of sensory-motor control and cellular responses to 0 G are possible. However, to date there are very few data concerning the functional properties of monkey limb skeletal muscle (6). The results of this study

### Table 4. Contractile properties and MHC content of Sol and MG hybrid muscle fibers before and after 18 days of restraint in the ESOP

<table>
<thead>
<tr>
<th>Slack Test Results</th>
<th>Force-Velocity Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>P_o</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----</td>
</tr>
<tr>
<td>SOL-I/I1</td>
<td></td>
</tr>
<tr>
<td>Pre-ESOP</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Post-ESOP</td>
<td>66 ± 3*</td>
</tr>
<tr>
<td>MG-I/I1</td>
<td></td>
</tr>
<tr>
<td>Pre-ESOP</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>Post-ESOP</td>
<td>60 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers. *P < 0.05 vs. pre-ESOP mean.
Fig. 9. Peak absolute power-frequency distributions of Sol and MG fibers from adult rhesus monkeys. A: Sol fibers obtained during control conditions. B: Sol fibers obtained post-ESOP sit. C: MG fibers obtained during control conditions. D: MG fibers obtained post-ESOP sit.

address this void and provide important information on how the flight-chair environment in itself alters cell function.

Fiber type distribution, size, and peak force. In agreement with previous observations in monkeys (1, 2, 6, 27) and other species (10, 24), we found that Sol and MG muscles contain primarily slow type I and fast type II fibers, respectively. Although the rhesus monkey Sol has been reported to be 90% slow-twitch type I (27), three studies including ours have found the percentage of slow type I fibers to range from values in the low 60s to the high 70s (2, 6). Thus it appears that rhesus monkey Sol contains a higher percentage of fast fibers (all of which are fast type IIa fibers) than generally observed in rodents or humans (9, 10, 24). Nevertheless, we have recently shown that the fiber size and contractile properties of the type I fibers from rhesus monkey Sol more closely resemble those in humans than do those in rat Sol (33). When results from humans, rhesus monkeys, and rats were compared, the fiber force per CSA was found to be independent of species size and relatively constant across species (9, 33). In contrast, \( V_o \) and peak power were inversely related to species body mass (33). These data support the hypothesis that the rhesus monkey would be a good model for assessing the effects of weightlessness on human muscle function. Consistent with a previous report (2), we observed by two independent methods that MG type I fibers were significantly smaller than was any other fiber type. This difference has also been observed in humans but not in rats (9, 33). However, the extent of the effect was greater in monkeys, such that the mean diameter of the MG type I fiber observed herein was smaller than that of a similar fiber population in rats (33). This is an exception to the general observation of a small increase in fiber diameter with species size (33). The small fiber size in MG type I fibers caused the peak force to be low compared with that of the other fibers studied (Table 2). However, the force per CSA was similar to that of the other fiber types, which suggests

Fig. 10. Force-pCa relationships of single fibers obtained from adult rhesus monkeys. A: Sol fibers expressing type I MHC. B: MG fibers expressing type I MHC. C: MG fibers expressing type II MHC. • and solid lines, Fibers obtained during normal ambulatory living conditions; ○ and dotted lines, fibers obtained post-ESOP sit; error bars, ±1 SE. P, force; \( P_o \), peak isometric force.
that the myofilament content per CSA was identical between fiber types. Further support for this comes from the observation that fiber stiffness in Sol and MG type I fibers was similar. Because fiber stiffness, at least in part, reflects the number of attached cross bridges, it is apparent that no major difference in cross-bridge content existed between these fiber populations. It is not clear why monkey MG type I fiber diameter fell below that expected for the species size, but one possibility relates to the normal weight-bearing activity of this species. Monkeys spend a considerable amount of time in a squatting posture. Consequently, the triceps surae is placed in a shortened position, with the biarticular MG likely to be more affected than the Sol. Placing the muscle in a chronically shortened position would be expected to reduce reflex activation of the muscle, and the resulting inactivity could induce fiber atrophy relative to the type I population of the Sol. The fact that the muscle was still weight bearing likely protected against a loss of contractile protein, and thus peak force (kN/m²) was maintained.

The significantly higher stiffness in the type I compared with the type II fiber is similar to that observed in both rats and humans (22, 34). The increased type I fiber stiffness does not appear to be caused by an increased cross-bridge density because peak force (kN/m²) was the same in all fiber types (Table 2). The peak force observed in this study (140–170 kN/m²) was similar to values previously published by us and others (kN/m²) was the same in all fiber types (Table 2). The increased type I fiber stiffness, at least in part, reflects the number of attached cross bridges, it is apparent that no major difference in cross-bridge content existed between these fiber populations. It is not clear why monkey MG type I fiber diameter fell below that expected for the species size, but one possibility relates to the normal weight-bearing activity of this species. Monkeys spend a considerable amount of time in a squatting posture. Consequently, the triceps surae is placed in a shortened position, with the biarticular MG likely to be more affected than the Sol. Placing the muscle in a chronically shortened position would be expected to reduce reflex activation of the muscle, and the resulting inactivity could induce fiber atrophy relative to the type I population of the Sol. The fact that the muscle was still weight bearing likely protected against a loss of contractile protein, and thus peak force (kN/m²) was maintained.

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\( V_0 \), the activation threshold, mM Ca\(^{2+} \)

\( V_o \) for the monkey slow type I and fast type II fibers were intermediate compared with that in fiber types in rats and humans. For example, the monkey Sol fiber type I fibers were intermediate compared with that in fiber types in rats and humans. For example, the monkey Sol type I fiber \( V_o \) reported here (Table 2) was between the 1.34 and 0.52 FL/s published previously for rats and humans, respectively (33). MG type II fibers were faster than Sol type II fibers, undoubtedly due to the latter being entirely type II a whereas the former contained a mixture of IIa and IIx fibers. We know from data in humans that the type IIx fiber contains a significantly higher \( V_o \) than the type IIa fiber (34). The hybrid fibers showed a \( V_o \) higher than that for type I but considerably lower than that for type II fibers. Fibers with a relatively high type II-to-total MHC ratio (0.6–0.8) had velocities only slightly higher than pure type I fibers. This observation is consistent with the hypothesis that in hybrid fibers the slow MHC would be expected to have a disproportionately greater influence on \( V_o \) (25). Because the myosin heads composed of slow myosin would detach considerably more slowly than fast myosin heads, they would provide a significant internal drag to fiber shortening speed. Cordonnier et al. (6) suggested that the \( V_o \) of hybrid fibers would be determined by the dominant species; thus fibers with a predominant fast MHC content would resemble pure fast fibers. Our results (Fig. 7) and those of Reiser et al. (25) suggest that this is not the case, that, in fact, even a small amount of type I MHC significantly reduces fiber \( V_o \) compared with that of a pure fast fiber (Fig. 7).

**Force-velocity relationship and peak power.** The greater peak power of the control fast fibers was due to the following characteristics of their force-velocity relationships: 1) type II fibers displayed a lower degree of curvature so that peak power was attained at a greater percentage of both \( V_{\text{max}} \) and \( P_o \). 2) \( V_{\text{max}} \) was greater for the type II fibers; and 3) in the case of the MG only, type II fibers were capable of producing greater \( P_o \) (mN). These differences between the force-velocity parameters of type I and type II fibers in monkeys are qualitatively similar to those observed for single slow and fast fibers from other species, such as rats and humans (3, 35).

**P<sup>C</sup>A-force relationship.** In addition to differences in force-velocity parameters, type II fibers had a lower Ca\(^{2+} \) sensitivity than did type I fibers. This finding is in agreement with what others have reported for the rhesus monkey (6) and other species (14, 34). High-velocity fibers express fast isoforms of the regulatory protein troponin C (14). This isoform has two Ca\(^{2+} \)-binding sites, vs. a single binding site on the slow
tropinin C isoform, and this may be responsible in part for the reduced Ca$^{2+}$ sensitivity of fast fibers (23). It has also been proposed that the faster cross-bridge cycling rates of high-velocity fibers produce less cooperative activation of the thin filament compared with the slower cycling cross bridges of slow-velocity fibers (4). This would also tend to reduce the Ca$^{2+}$ sensitivity of fast fibers.

Effects of the ESOP sit. The primary effect of the ESOP sit was to produce a small but significant fiber atrophy. Sol type I and MG type II fibers showed an ~10% atrophy, whereas the diameter of the hybrid fibers of Sol and MG declined by 15 and 28%, respectively. The immunohistochemical results (Fig. 3) suggest that MG type II fiber atrophy was confined to type Ila fibers. The apparent increase in the number of hybrid fibers is difficult to interpret because the immunohistochemical results showed an increased hybrid number in both the control and experimental group (Table 1). The most logical explanation is that the ESOP sit induced a small number of type I fibers in both Sol and MG to express type II as well as type I myosin. The small size of hybrid fibers (Table 4) and their velocity distribution (Figs. 5 and 6) suggest that the hybrids developed from type I rather than type II fibers. The increase in hybrid fibers or the expression of fast myosin in slow fibers is consistent with what has been observed in rats after periods of HU (20). Presumably, the reduced activity and/or loading of the muscle triggers the fast-myosin expression.

The force and power reduction in Sol type I and MG type II fibers were all explained by the atrophy because the force and peak power per CSA and fiber stiffness were unaltered by the ESOP sit. This suggests that the myofibrillar protein concentration and number of cross bridges per CSA remained unchanged.

Summary. The peak force-generating capacity between the slow- and fast-twitch fibers in rhesus monkeys was not significantly different, nor was it different from values reported for other species. In contrast, $V_o$, $V_{\max}$, peak power, and the pCa-force relationship were dependent on fiber type, and the values were closer to those in humans than to those in rats. This observation is consistent with the known inverse relationships of $V_o$, $V_{\max}$ and peak power to body size. The major effect of the 18-day ESOP sit was to induce a small but significant decline in fiber diameter that, in turn, reduced the absolute magnitude of both peak force (mN) and power (µN·FL·s$^{-1}$). This suggests that future O-G studies evaluating the effects of weightlessness on rhesus monkey muscle function can be conducted with the confidence that any alterations in peak force and power per CSA or $V_o$ are induced by 0 G and not the ESOP facility.

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