Spaceflight effects on single skeletal muscle fiber function in the rhesus monkey

ROBERT H. FITTS,1 DOMINIQUE DESPLANCHES,2 JANELL G. ROMATOWSKI,1 AND JEFFREY J. WIDRICK1
1Department of Biology, Marquette University, Milwaukee, Wisconsin 53201; and 2Laboratoire de Physiologie, Faculté de Medecine, 69373 Lyon, France

Received 28 October 1999; accepted in final form 29 June 2000

Fitts, Robert H., Dominique Desplanches, Janell G. Romatowski, and Jeffrey J. Widrick. Spaceflight effects on single skeletal muscle fiber function in the rhesus monkey. Am J Physiol Regulat Integrat Comp Physiol 279: R1546–R1557, 2000.—The purpose of this investigation was to understand how 14 days of weightlessness alters the cellular properties of individual slow- and fast-twitch muscle fibers in the rhesus monkey. The diameter of the soleus (Sol) type I, medial gastrocnemius (MG) type I, and MG type II fibers from the vivarium controls averaged 60 ± 1, 46 ± 2, and 59 ± 2 μm, respectively. Both a control 1-G capsule sit (CS) and spaceflight (SF) significantly reduced the Sol type I fiber diameter (20 and 13%, respectively) and peak force, with the latter declining from 0.48 ± 0.01 to 0.31 ± 0.02 (CS group) and 0.32 ± 0.01 mN (SF group). When the peak force was expressed as kiloNewtons per square meter (kN/m²), only the SF group showed a significant decline. This group also showed a significant 15% drop in peak fiber stiffness that suggests that fewer cross bridges were contracting in parallel. In the MG, SF but not CS depressed the type I fiber diameter and force. Additionally, SF significantly depressed absolute (mN) and relative (kN/m²) force in the fast-twitch MG fibers by 30% and 28%, respectively. The Ca²⁺ sensitivity of the type I fiber (Sol and MG) was significantly reduced by growth but unaltered by SF. Flight had no significant effect on the maximal fiber-shortening velocity in any fiber type or muscle. The post-SF Sol type I fibers showed a reduced peak power and, at peak power, an elevated velocity and decreased force. In conclusion, CS and SF caused atrophy and a reduced force and power in the Sol type I fiber. However, only SF elicited atrophy and reduced force (mN) in the MG type I fiber and a decline in relative force (kN/m²) in the Sol type I and MG type II fibers.

contractile properties; morphology; slow twitch; fast twitch

EXPERIMENTS BOTH FROM COSMOS and space shuttle missions have shown weightlessness to result in a rapid decline in the mass and force of rat hindlimb extensor muscles (2, 15). Additionally, despite an increased maximal fiber-shortening velocity (Vₒ), peak power was reduced in rat soleus (Sol) muscle after a 14-day spaceflight (2). Weightlessness has also been shown to induce muscle atrophy, increase fatigue, and reduce the physical work capacity in humans (3, 20). Declines in voluntary peak isometric ankle extensor torque ranging from 15 to 40% have been reported after long- and short-term spaceflight and prolonged bed rest in humans (7, 11). However, it is not clear to what extent these changes in neuromuscular performance are due to alterations in neural mechanisms of recruitment, to changes in muscle architecture and/or fiber-type composition, and/or to alterations in cellular processes of contraction. To assess the cellular basis of the functional changes associated with limb unloading in humans, we have used the single-skinned fiber preparation to study the effects of 17 days of bed rest and weightlessness (19–21). In addition to a reduced fiber size and force, both bed rest and spaceflight increased the Vₒ and reduced the power in the slow type I fiber of the Sol. The increased velocity was not caused by an increased expression of fast-type myosin, because the adapted fibers contained only slow myosin (19, 20).

When comparing the effects of weightlessness on human versus rodent skeletal muscle, it is clear that species differences exist. For example, spaceflights as short as 6–9 days have been shown to cause slow-to-fast-twitch fiber transitions of up to 10% in rat skeletal muscle, whereas in humans, no significant fiber-type transition occurred even after a 17-day flight (2, 8, 20). It has been hypothesized that larger animals such as nonhuman primates may more closely mimic the human in their musculoskeletal response to microgravity (1). Consequently, we recently characterized the contractile properties of single slow and fast fibers prepared from the medial gastocnemius (MG) and Sol muscles of adult rhesus monkeys and compared the results to those obtained in humans and rats (5, 23). In general, the contractile properties of the monkey fibers were closer in function to the human than the rat. For example, Vₒ of the slow type I fiber averaged 0.91, 0.73, and 0.51 fiber lengths/s in the rat, monkey, and human, respectively (23). Fiber diameter and peak power also showed distinct species differences with fibers from the monkey and human showing larger diameters but lower peak power than rat fibers (23). On the basis...
of these data, one could hypothesize that muscle and neuromuscular adaptations to microgravity would be similar in monkeys and humans.

This investigation was part of a multinational (Russian, French, and American) integrated project designed to study how the physiological and behavioral functions of the rhesus monkey respond to weightlessness. The rhesus biosatellite program was initiated by the Russian Institute for Biomedical Problems (IBMP) and the Soviet Space Agency in 1983. The rhesus monkeys studied in this investigation flew aboard the Bion 11 biosatellite that was the sixth in the series of biosatellite flights. The specific objectives of our study were to 1) establish the effects of microgravity of the contractile properties of individual slow- and fast-twitch fibers isolated from the Sol and MG muscles of the rhesus monkey; 2) integrate our results with those of others to sort out the relative importance of changes in motor recruitment versus the reduced loading in leading to the atrophy and reduced performance of skeletal muscle; and 3) determine the extent to which the microgravity-induced changes in skeletal muscle fiber function mirror those observed in humans. The latter objective will allow researchers to determine the extent to which rhesus monkey biosatellite studies relate to adaptations in humans.

MATERIALS AND METHODS

Animal care, and training. The Bion 11 mission was a 14-day flight (12/24/96 to 1/6/97) that carried two 4- to 5-kg male rhesus monkeys. The animals were selected from a flight pool of 12, and the flight selection was based on the quality of the preflight data, viable implants, and success in completing motor and behavioral tasks. A description of the animal selection procedures, the transportation to the launch site, recovery procedures from the landing site, and the electromyogram (EMG) and tendon force sensor implants was published elsewhere (14). The animal care and experiments described here followed the Principles of Laboratory Animal Care (National Institutes of Health Publication 85–23, revised 1985) and were approved by the animal care and use committees at National Aeronautics and Space Administration (NASA)-Ames Research Center (Moffett Field, CA), the IBMP, and Marquette University (Milwaukee, WI). Except for 3 days preflight and during the flight, the animals were housed individually in standard 4.3-ft² stainless steel cages at the IBMP in Moscow, Russia. The module within the Bion satellite contained a capsule with two contoured chairs, the life support systems, and test equipment including physiological data recording systems. Three days prelaunch, the two flight monkeys were placed in the flight chairs and lightly secured with a chest harness attached to the back of the chair to limit leaning forward at the waist (14). The chair also limited movement of the legs at the hip in the sagittal plane, whereas the lower legs were unsecured (14). The flight capsule and living conditions in flight were the same as published for the Bion 10, Cosmos 2229 mission (10).

Experimental groups and flight capsule. In addition to the two flight animals, we studied five vivarium control and two capsule control animals. All nine monkeys were juvenile male rhesus monkeys with an average age of 40.3 ± 0.4 mo. The capsule control group was studied postflight, and the animals were housed in a capsule identical to the flight capsule for 17 days (1/24/97–2/9/97) under environmental conditions (temperature, light-dark cycle, food intake) that mimicked those experienced by the flight animals. The duration of the 17-day capsule sit was identical to the flight capsule exposure (3-day prelaunch and 14-day flight). To be consistent with other publications, this group is referred to as the 14-day capsule-sit (R+17) control group.

Due to the time needed to train the animals and implant biosensors, the preflight muscle biopsies were obtained −4.5 mo before the flight. Any functional changes in the cellular properties of individual slow- and fast-twitch fibers due to growth during the 4-mo period were controlled for by the vivarium control group. During flight, the two flight animals were restrained in contoured chairs within the capsule. The 1-G, R+17 capsule control animals were studied to evaluate the effects of the capsule environment independent of spaceflight.

Biopsy procedure. After general anesthesia (isoflurane), the pre- and postbiopsies were taken from two independent sites in the right Sol and MG muscles using an open-biopsy technique. Both the pre- and postbiopsies were taken from the right leg muscle. The left leg contained EMG and tendon force-transducer implants. The procedures used were exactly as described previously (5). The sites were selected to ensure that the same muscle fibers were not sampled during the pre- and postbiopsies and that the fiber-type distribution within each sampled region was similar (16). The postflight biopsies were obtained according to the following schedule: the two flight animals were biopsied at ~24 h after reentry (R+1), which was as soon as possible after return of the animals from the landing site to Moscow; two of the vivarium control animals were biopsied on flight days 3 and 4, respectively, and two were biopsied 1 day after the flight animals. The fifth vivarium control animal was biopsied the same day as the two R+17 capsule control animals that were biopsied 24 h after the chair-restraint period.

The biopsied sample was weighed and then divided longitudinally into three sections. Two of the three sections were used by other investigators. A small piece (~1 mm thick) was cut from the bottom of the third section and used for the morphological studies described below. The remaining tissue was then divided longitudinally, and one section was used for the skinned-fiber studies described here. The other section was aligned longitudinally on a small index card and frozen in liquid nitrogen and used for enzyme and metabolic studies (R. H. Fitts, V. Grichko, and L. B. De La Cruz, unpublished observations). The sections to be used for the skinned-fiber experiments were placed in 4°C skinning solution [composition in mM: 125 K propionate, 20.0 imidazole (pH 7.0), 2.0 EGTA, 4.0 ATP, and 1.0 MgCl₂, and 50% glycerol (vol/vol)] and shipped back to Marquette University (4°C shipper). On arrival at Marquette, the bundles were placed in fresh skinning solution and stored at −20°C for up to 4 wk (21, 22). The single-fiber experiments described in this manuscript were carried out within this 4-wk period.

Morphometric analysis of myofibrils. The 1-mm cross-sectional piece was processed for electron microscopy by fixing in a 6.25% solution of glutaraldehyde. After a 1-h fixation, samples were cut into small blocks and put into a solution of 1% osmic acid in 0.06 M veronal-acetate buffer for 2 h. After dehydration in increasing concentrations of ethanol, the samples were embedded in Epon (9). The morphometric analysis of the samples was carried out on ultrathin sections (60–90 nm) from two tissue blocks randomly chosen from each biopsy. Twenty micrographs per block and hence 40 micrographs per biopsy were taken with a systematic sampling procedure in consecutive frames of 200-square mesh grids. A final magnification of ×24,000 was used to estimate the...
myofibrillar density. Contact prints of the 35-mm films were projected on a screen fitted with a grid C 16 (144 test points). The myofibrillar density was determined by standard stereological procedures (17). To determine Z band and sarcomere lengths, a magnification of ×20,000 was used, and measurements were made on 10 fibers per animal.

**Experimental solutions and single fiber preparation.** The composition of the relaxing and activating solutions were exactly as described previously (21, 22). The relaxing solution had a free Ca$^{2+}$ concentration of pCa 9.0 (where pCa = − log Ca$^{2+}$ concentration) that contained the following (in mM): 20 imidazole, 7 EGTA, 14.5 creatine phosphate, 4.74 ATP, 5.40 MgCl$_2$, and 0.016 CaCl$_2$. The maximal activating solution, pCa 4.5, contained (in mM) 20.0 imidazole, 7.0 EGTA, 14.5 creatine phosphate, 4.81 ATP, 5.26 MgCl$_2$, and 7.0 CaCl$_2$. The pH of the relaxing and activating solutions was adjusted to 7.0 with KOH, and total ionic strength was adjusted to 180 mM with KCl. For the force-pCa experiments, the fibers were activated with a series of solutions ranging from pCa 6.8 to 5.0. These solutions were made by mixing appropriate volumes of the activating (pCa 4.5) and relaxing (pCa 9.0) solutions (21).

On the day of an experiment, a muscle bundle (Sol or MG) was transferred to a dissecting chamber containing relaxing solution. A single fiber was isolated from the bundle and transferred to an experimental chamber containing relaxing solution. This stainless steel chamber contained three troughs that allowed the fiber to be moved from a low-Ca$^{2+}$ relaxing solution to activating solutions of various pCa values. A fiber segment (~2 mm long) was mounted between a force transducer (model 400, Cambridge Technology, Cambridge, MA; sensitivity 2 mV/mg) and an isotonic direct-current torque motor (model 300H, Cambridge Technology), as described previously in detail (18). The experimental chamber was mounted on the stage of an inverted microscope, and temperature was maintained at 15°C. Sarcomere length was adjusted to 2.5 μm by using an eyepiece micrometer (×800), and segment length was determined by moving the microscope stage with a micrometer so that the fiber segment moved across the visual field of the eyepiece. The segment length was determined directly from the micrometer displacement. A Polaroid photograph was taken of the fiber while it was briefly suspended in air. Fiber diameter was determined at three points along the length of the photograph, and fiber cross-sectional area was calculated from the mean width, assuming the fiber forms a circular cross section when suspended in air. Any fiber showing a high degree of striation nonuniformity or a damaged region was discarded.

Peak fiber force (N and kN/m$^2$), stiffness, $V_{0}$, and the force-velocity, force-power, and force-pCa relationships were determined exactly as described previously (5). During the determination of the force-pCa relationship, fiber stiffness was also determined at all pCa values tested. Immediately after the determination of $V_{0}$, the rate of tension redevelopment ($k_{tr}$) was measured by activating the fiber in pCa 4.5. Once peak force was obtained, a 400 μm slack for 60 and 20 ms, respectively, for slow and fast fibers was introduced, after which the fiber was rapidly restretched to mechanically break the actin-myosin cross bridges (12). The rate constant for the tension redevelopment was defined as $k_{tr}$. A computer program employing a least-squares fit was used to solve for $k_{tr}$ (12). Figure 1 shows representative slack-unslack tests for two slow fibers for which $k_{tr} = 3.04$ and 3.56/s. Due to the need to conserve time such that multiple fibers could be studied per day, a laser clamp to maintain a constant sarcomere length during tension redevelopment was not employed. However, in preliminary experiments, we showed that slow fiber $k_{tr}$ was the same with and without a laser clamp. In contrast, fast fibers showed lower values when a clamp was not used. Apparently, the likelihood of sarcomere nonuniformity developing is higher in fast fibers. Consequently, fast fiber $k_{tr}$ values are not reported.

After the contractile measurements, the myosin heavy chain (MHC) and light chain (MLC) composition of each fiber was determined by SDS-PAGE exactly as described previously (22). On the basis of their MHC profile, each fiber was identified as slow type I, fast type II, or a hybrid containing both slow and fast myosin isozymes (22). Fast type II fibers were considered as a single group and not subdivided into type IIa and IIx, because in monkey fibers, it is not possible to consistently subdivide these isozymes by SDS-PAGE analysis (5).

**Statistical analysis.** Data are presented as means ± SE. Statistical differences between groups were assessed using a

| Table 1. Diameter (μm) of slow type I and fast type II fibers |
|-----------------|-----------------|-----------------|-----------------|
| Group           | Sol Type I      | MG Type I       | MG Type II      |
| Preflight       | 58 ± 1 (69)     | 50 ± 1 (46)     | 59 ± 2 (39)     |
| Postflight      | 56 ± 1 (73)     | 46 ± 2 (30)     | 59 ± 2 (31)     |
| Vivarium        | 60 ± 1 (73)     | 46 ± 2 (20)     | 59 ± 2 (31)     |
| R+17            | 48 ± 1 (41)*    | 43 ± 1 (24)     | 61 ± 2 (52)     |
| Flight          | 52 ± 1 (65)†    | 38 ± 1 (21)*†   | 58 ± 3 (17)     |

Values are means ± SE for number of fibers shown in parentheses. R+17, 14-day capuole-sit group; Flight, 14-day flight group; Sol, soleus; MG, medial gastrocnemius. *Value significantly different from R+17 value (P < 0.001). †Value significantly different from postvivarium group (P < 0.001).
Table 3. Hill plot analysis of the force-pCa relationships of slow type I and fast type II fibers

<table>
<thead>
<tr>
<th>Group</th>
<th>Activation Threshold</th>
<th>n1</th>
<th>n2</th>
<th>pC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sol type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preflight (57)</td>
<td>6.98 ± 0.03*</td>
<td>1.60 ± 0.08</td>
<td>3.03 ± 0.14</td>
<td>6.12 ± 0.01</td>
</tr>
<tr>
<td>Postflight</td>
<td>7.04 ± 0.06</td>
<td>1.36 ± 0.18</td>
<td>2.44 ± 0.23</td>
<td>5.97 ± 0.07</td>
</tr>
</tbody>
</table>

| MG type I |                      |    |    |      |
| Vivarium (15) | 7.00 ± 0.08 | 1.16 ± 0.07 | 2.43 ± 0.20 | 5.86 ± 0.05* |
| R+17 (12) | 6.90 ± 0.07 | 1.33 ± 0.11 | 2.67 ± 0.23 | 5.91 ± 0.05 |
| Flight (10) | 7.04 ± 0.06 | 1.36 ± 0.18 | 2.44 ± 0.23 | 5.97 ± 0.07 |

| MG type II |                      |    |    |      |
| Preflight (10) | 6.73 ± 0.08* | 2.21 ± 0.20* | 5.44 ± 0.74* | 6.16 ± 0.03 |
| Postflight | 6.71 ± 0.11 | 1.65 ± 0.31 | 3.96 ± 0.51 | 5.99 ± 0.05 |

Values are means ± SE for number of fibers shown in parentheses. *Value significantly different from preflight type I group (P ≤ 0.01).
unaffected by either the capsule sit (R)
controls (Table 1). The fast type II fiber diameters were
with the precondition, but only flight produced signif-
showed a reduced MG type I fiber diameter compared
flight groups (Table 1). Both of these groups also
maximal fiber-shortening velocity; $k$

Fiber force and stiffness. The peak force ($F$ and
kN/m$^2$) and stiffness for the slow type I and fast type II
fibers are shown in Table 2. Type I Sol fibers from both
the capsule control (R+17) and the flight animals
showed a significant decline in peak force (N) compared
with the vivarium controls. However, when these data
were expressed as kiloNewtons per square meter, only
the flight group showed a significant decline. Peak
force of the types I and II fibers of the MG were
unaffected by the capsule sit, whereas microgravity
significantly reduced the absolute peak force of both
fast and slow fibers and the force per cross-sectional
area of the fast type II MG fibers (Table 2). Hybrid
fibers showed a tendency to respond in a manner similar
to the slow type I fiber type. In the Sol, the hybrid fiber peak isometric force ($P_o$) was 0.39 ± 0.04, 0.30 ±
0.02, and 0.27 ± 0.03 mN in the vivarium, capsule, and
flight groups, respectively, whereas in the MG, the
hybrid fiber $P_o$ values were 0.38 ± 0.06 (vivarium),
0.41 ± 0.10 (R+17 capsule), and 0.21 ± 0.04 (flight) mN. For all groups except the capsule control, hybrid
fibers represented 11–16% of the fiber population for
both the Sol and MG. In the Sol capsule control group,
the hybrid fibers represented 51% of the total popula-

Fiber stiffness ($E_o$) is thought to partially reflect the
type and number of attached cross bridges. The Sol type I
and MG type II fibers showed a reduced $E_o$, postflight, but

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_o$, FL/s</th>
<th>$k_{tr}$, per second</th>
<th>$V_o$, FL/s</th>
<th>$k_{tr}$, per second</th>
<th>$V_o$, FL/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preflight</td>
<td></td>
<td></td>
<td>MG Type I</td>
<td></td>
<td>MG Type II</td>
</tr>
<tr>
<td>(R+17)</td>
<td>0.94 ± 0.04</td>
<td>2.48 ± 0.05</td>
<td>0.84 ± 0.03</td>
<td>2.90 ± 0.08</td>
<td>5.82 ± 0.43</td>
</tr>
<tr>
<td>Vivarium</td>
<td>0.85 ± 0.03</td>
<td>2.07 ± 0.05</td>
<td>0.55 ± 0.03</td>
<td>1.64 ± 0.09</td>
<td>4.37 ± 0.36</td>
</tr>
<tr>
<td>Flight</td>
<td>0.96 ± 0.04</td>
<td>1.89 ± 0.05</td>
<td>0.52 ± 0.04</td>
<td>1.57 ± 0.11</td>
<td>4.13 ± 0.47</td>
</tr>
</tbody>
</table>

Values are means ± SE for number of fibers shown in parentheses. *Value significantly different from Preflight value ($P ≤ 0.001$). $V_o$, maximal fiber-shortening velocity; $k_{tr}$, rate of tension development; FL, fiber length.
only in the former was the decline significant. The $P/E_o$ ratio was not altered by the capsule sit or spaceflight in either fiber type or muscle (Table 2). This finding suggests that there was no change in the force per cross bridge, and thus the decline in the peak force per cross-sectional area (kN/m²) of the Sol type I fiber would seem to be attributable to a selective loss in the number of myofilaments per myofibril and/or to a reduced myofibril density per fiber. The electron micrograph (EM) analysis of myofilament density did not support the former possibility. The myofilament density of the two preflight animals was 71.8 and 69.8% compared with 74.0 and 69.7% postflight. The vivarium control and R+17 capsule-sit groups showed myofilament densities that were not significantly different from the flight animals. Although we could not document a flight-induced decline in myofilament density, the EM analyses supported the fiber diameter measurements in that the postflight samples from the Sol type I fibers showed significantly shorter Z-band lengths (Fig. 2). Flight monkey 357 showed considerably more type I fiber atrophy and loss of force than flight monkey 484. Flight induced a 39% decline in the
peak force of the Sol slow type I fibers of monkey 357 compared with 22% for monkey 484. This variability in response is perhaps best observed in Fig. 3, in which peak force is plotted against fiber diameter for both the pre- and postflight data. Postflight, the 9 smallest and 11 weakest fibers all belonged to monkey 357.

pCa-force relationship. The growth period between the pre- and postflight biopsies resulted in a right shift in the pCa-force relationship for the slow type I fibers of the Sol and MG. This shift resulted in a significantly higher free Ca\(^{2+}\) (pCa\(_{50}\)) for half-maximal activation in the postvivarium controls for the slow type I fibers relative to the preflight group (Table 3). However, neither the capsule sit nor spaceflight had any effect on the pCa-force relationship in slow or fast fibers. Thus the activation threshold, \(n_1\) and \(n_2\), and the pCa\(_{50}\) values were not different between these groups and the postvivarium control group (Table 3). Figure 4 compares the pCa-force relationships (pre- vs. postflight) for Sol type I fibers from the vivarium control, R+17 capsule sit, and flight monkeys. The right shift in the relationship was observed in all three groups documenting that the change was related to growth and not the capsule sit or spaceflight.

\(V_o\) and \(k_{tr}\). In the slow type I fibers of the Sol, there was no significant effect of growth (post- vs. previvarium), the capsule sit (R+17 vs. postvivarium), or flight (flight vs. postvivarium) on the \(V_o\) or the peak \(k_{tr}\) after a slack-unslack (Table 4). In contrast, both the slow type I and fast type II fibers of the MG showed a significant decline in fiber \(V_o\) with growth, whereas this variable was not affected by the capsule sit or spaceflight (Table 4). The \(k_{tr}\) of the MG type I fiber also showed no change with the capsule sit or flight but declined with growth (Table 4).

The individual fibers studied were all identified as slow type I, hybrid, or fast type II based on their myosin isozyme pattern on 12% and 5% SDS gels. Figures 5 and 6 are representative 12% and 5% gels showing individual fibers and their \(V_o\)s. Figure 7 shows a histogram of Sol slow type I fiber \(V_o\) for each group (pre vs. post). The growth controls showed a large number of fibers with \(V_o\)s between 0.5 and 1.0 fiber length (FL)/s, whereas for the postflight group, there was a trend toward more fibers with \(V_o\) values >1.1 FL/s. However, the ANOVA analysis demonstrated that the number of fibers with \(V_o\) values >1.1 FL/s was not significantly altered by flight. Spaceflight had no effect on the Sol type I fiber MLC\(_3\)/MLC\(_{2s}\) ratio. On the basis of densitometric scanning of the 12% SDS gels of the Sol type I fibers shown in Table 3, the postflight MLC\(_3\)/MLC\(_{2s}\) ratio of 0.109 ± 0.013 was not significantly altered from the preflight value of 0.138 ± 0.029. Additionally, the \(V_o\)s of the fast slow fibers (\(V_o\)s >1.1 FL/s) could not be explained by an increased expression of fast troponin T, because there was no difference between the slow troponin T-to-total troponin T ratio between postflight type I fibers with relatively high \(V_o\)s compared with those with relatively low \(V_o\)s (0.96 ± 0.03 vs. 0.97 ± 0.02, respectively). Hybrid fibers from both the Sol and MG (pre-mean value, 1.39 ± 0.11 FL/s) were significantly faster than the slow type I fiber (\(P < 0.001\)) and, similar to the type I fibers from the Sol, were unaffected by growth (1.33 ± 0.22 FL/s), the capsule sit (1.48 ± 0.16 FL/s), or flight (1.23 ± 0.23 FL/s).

Isotonic contractile properties. The isotonic force-velocity and force-power relationships for the Sol and MG type I fibers for each group are shown in Figs. 8...
and 9. From these data, $V_{\text{max}}$, dimensionless parameter ($a/P_o$), peak power, and the force and velocity obtained at peak power were determined. The mean values for each group and fiber type are shown in Table 5. For the Sol type I fiber, these parameters were unaffected by the growth period from the pre- to the postflight biopsy (Fig. 8). Similar to fiber $V_o$, the Sol type I fiber $V_{\text{max}}$ was not significantly altered by the capsule sit or spaceflight. In contrast, Sol type I fiber peak power significantly declined after the capsule sit and flight compared with the vivarium control (Table 5 and Fig. 8). The decreased peak power was primarily the result of the reduced $P_o$, but a greater curvature (lower $a/I_o$ ratio) of the force-velocity relationship also contributed to the decline (Fig. 8). As with fiber diameter and force, monkey 357 showed a greater decline in Sol type I fiber power than monkey 484 (Fig. 10). For monkey 357, Sol type I fiber peak power declined from $7.42 \pm 1.13$ to $4.92 \pm 0.44 \mu \text{N} \cdot \text{FL} \cdot \text{s}^{-1}$, whereas for monkey 484, in the same fiber type, peak power declined from $6.93 \pm 1.1$ to $5.73 \pm 0.39 \mu \text{N} \cdot \text{FL} \cdot \text{s}^{-1}$. Due to the reduced $P_o$ and $a/P_o$ ratio, the Sol type I fibers of

Fig. 5. Representative 12% SDS-polyacrylamide gels illustrating myosin light chain (LC) expression in single rhesus Sol (lanes 3–5) and gastrocnemius (lanes 6–14) fibers. Lanes 1 and 2 are myosin standards from rhesus Sol and gastrocnemius muscle, respectively. The fiber type and maximal fiber-shortening velocity ($V_o$) for each lane were: (3) type I, 0.64 fiber length (Fl)/s; (4) hybrid type I and II, 1.73 Fl/s; (5) type I, 0.88 Fl/s; (6) type II, 2.04 Fl/s; (7) type II, 2.18 Fl/s; (8) type II, 5.08 Fl/s; (9) type I, 0.63 Fl/s; (10) type II, 1.86 Fl/s; (11) type I, 0.37 Fl/s; (12) type I, 0.40 Fl/s; (13) type II, 1.70 Fl/s; (14) type II, 4.79 Fl/s. Nos. in parentheses represent lanes 3–14.

Fig. 6. Representative 5% gel demonstrating separation of type I, IIa, and IIX MHC’s in single rhesus Sol (lanes 3–11) and gastrocnemius (lanes 12–14) fibers. Lanes 1 and 2 are myosin standards from rhesus Sol and gastrocnemius muscle, respectively. The fiber type and $V_o$ for each lane were: (3) type IIa, 6.74 Fl/s; (4) type I, 1.35 Fl/s; (5) type I, 1.09 Fl/s; (6) type I, 0.76 Fl/s; (7) type I, 1.07 Fl/s; (8) type I, 0.99 Fl/s; (9) type I, 1.37 Fl/s; (10) type I, 0.59 Fl/s; (11) type I, 0.89 Fl/s; (12) type IIX, 8.73 Fl/s; (13) type IIX, 6.04 Fl/s; (14) type I, 0.91 Fl/s.

Fig. 7. $V_o$ histograms of Sol type I fibers from growth control, R+17 capsule, and flight group.
the R+17 (capsule sit) and postflight groups shortened at a slower velocity at any given load. At peak power, the postflight fibers showed a significant increase in velocity and decrease in force (both in mN and kN/m²) compared with the vivarium control group (Table 5).

In the MG type I fiber population, growth caused a significant decline in peak power as well as the force and velocity obtained at peak power (Fig. 9 and Table 5). However, spaceflight had no significant effect on the isotonic contractile properties of the MG except for an increase in the MG type I fiber a/Po ratio.

DISCUSSION

A major objective of NASA’s space program is to conduct a human exploration of Mars by 2014. For this to become a reality, a number of important biological problems need to be solved. Primary among these is the development of effective countermeasures to prevent the loss of skeletal muscle mass, force, and power (20). To date, the major limitations have been the high costs of human spaceflight missions, which necessarily limited their number, and the difficulty of studying the effects of weightlessness in humans independent of crew countermeasure programs. One solution to these problems is to fly animals without countermeasures in considerably less costly unmanned biosatellites. Results from earlier collaborative experiments between the United States and Russia, flown as part of the Cosmos biosatellite program, suggested that the nonhuman primate might adapt to microgravity in a manner similar to humans (1). Additionally, we have recently observed that the single-fiber contractile function of the nonhuman primate is similar to that observed in humans (23). Thus a primary purpose of this investigation was to determine the effects of 14 days of microgravity on the contractile function of individual slow type I and fast type II fibers in the rhesus monkey and compare the functional alterations with those observed in humans. If adaptations were the same or similar, future biosatellite experiments could be designed to explore the complex interactions of neural recruitment, altered endocrine function, and cell and molecular change within individual fibers and their relative importance to the microgravity-induced decline in skeletal muscle performance.

Fiber diameter and peak force. Spaceflight induced a 13% and 17% decline in the diameters of the slow type

---

Fig. 8. Composite force-velocity and force-power relationships for type I Sol fibers obtained from pre (solid lines)- and postbiopsies (dashed lines) for growth controls, R+17 capsule, and space animals.

Fig. 9. Composite force-velocity and force-power relationships for type I Sol fibers obtained from pre (solid lines)- and postbiopsies (dashed lines) for growth controls, R+17 capsule, and space animals.
I fibers of the Sol and MG muscles, respectively. In contrast, the fast type II fibers of the MG showed no significant change. The extent of Sol type I fiber atrophy observed here for the rhesus monkey was similar to that found in humans after 11- and 17-day flights (4, 20). We observed considerable subject variability as the Sol type I fibers from monkey 357 showed a 16% decline in diameter, whereas the diameter of this fiber type in monkey 484 was not significantly altered. This subject variability has also been observed in humans (Fig. 10). In a recent 17-day spaceflight, only three of the four crew members studied experienced Sol type I fiber atrophy, and considerable intersubject variability was also observed after an 11-day spaceflight and a 17-day bed rest (4, 20, 22). In the human studies, a portion of the variability might have resulted from varying amounts (number and intensity) of exercise countermeasures employed by each subject. Alternatively, there may be intersubject differences in how the neuromuscular system responds to microgravity. One advantage of this study is that no countermeasures were performed, and thus the observed changes can be attributed to space travel. The recent publication of Recktenwald et al. (14) demonstrated that the two flight monkeys studied here showed a decreased cycle period and EMG burst duration of the primary extensors (Sol and MG) during locomotion postflight, whereas the burst amplitude of the tibialis anterior (flexor muscle) increased. Because this bias toward flexor activity was not observed in the R+17 monkeys, the authors concluded that the alterations in the neural regulation of movement could not be attributed merely to restriction of movement, but were caused by factors unique to the microgravity environment (14). Recktenwald et al. (14) also reported that the MG tendon forces were considerably greater post- compared with preflight during locomotion. Collectively, the authors concluded that microgravity induced a reorganization of the recruitment to favor fast versus slow motor units and flexor versus extensor muscles. This interpretation is consistent with our findings of selective atrophy of the slow type I fibers in the same two flight monkeys and with our recent observations in humans in which we observed greater atrophy of the slow Sol type I fibers compared with fast MG fibers (Ref. 20 and unpublished observations). Of particular importance to our results was the finding that in-flight monkey 357 showed a progressive change from predominantly Sol activity to mainly MG activity over the duration of the flight, whereas this recruitment ratio remained unchanged in monkey 484 (Hodgson, personal communication). This suggests that the Sol type I fiber atrophy observed in monkey 357, but not 484, was a direct result of the reduced activation of the Sol motor units in monkey 357. During treadmill walking postflight, both flight and R+17 capsule monkeys showed a reduced Sol muscle activation compared with preflight, whereas MG activity increased in the R+17 but not the flight animals (14). These recruitment pattern differences are consistent with our observation of significant atrophy of the MG type I fibers in the postflight but not the R+17 capsule group.

Monkey 357 showed a considerably greater decline in Sol type I fiber force compared with monkey 484, and in the MG, only flight reduced peak force (mN). This latter observation plus the finding that flight, but not the capsule sit, reduced relative force (kN/m²) in both slow and fast fibers represents an important difference between simply reducing activity (R+17 capsule group) and spaceflight. In the latter condition, the reduced activity was associated with muscle unloading. Our results do not allow us to make a definitive conclusion about why weightlessness reduced relative force. The observation that fiber stiffness declined whereas the $P_o/E_o$ ratio remained unaltered suggests that the decline in peak force (kN/m²) was not caused by a reduced force per cross bridge. These data are consistent with the hypothesis that the microgravity-induced decline in relative force was caused by myofibril atrophy producing a reduced myofibril density per fiber and/or a decline in the density of myofilaments within a myofibril. The morphometrical data do not
support the latter possibility, because flight had no significant effect on the density of myofilaments per myofibril. The possibility exists that a small, but functionally significant, decline in myofilament density did occur but that it went undetected by EM analysis. An additional possibility is that the myofibril and myofilament densities were unaltered but that the number of functional cross bridges per myofilament decreased. Whatever the mechanism, it is clear that the decline in relative force required not only inactivity, but also a zero-load condition.

**Fiber shortening velocity.** After a 17-day spaceflight in humans, we observed an ~25% increase in the Sol type I fiber $V_o$ (20). This increase was not associated with any change in the myosin isozyme pattern. We hypothesized that the increased $V_o$ was caused by a selective loss in the thin filament actin producing an increased distance between thin and thick filaments. This geometric change would cause the cross bridge to dissociate sooner, thus reducing the internal drag in the last phase of the cross-bridge power stroke (13). In this study, the increase in Sol type I fiber $V_o$ was less and not significant at the $P<0.05$ level (postflight vs. vivarium group). Clearly, fiber and myofibril atrophy occurred as a result of microgravity, but we have no evidence on whether or not there was a selective loss in actin relative to myosin. The reason for the reduced effect on fiber $V_o$ in rhesus compared with humans is unknown but may be related to the shorter duration of this flight. Importantly, spaceflight showed a tendency to increase the number of slow type I fibers with $V_o > 1.1$ FL/s (Fig. 7). Besides a shift to higher velocity type I fibers, whole muscle velocity would be increased post-flight if the percentage of hybrid or fast type II fibers increased. However, in this study, spaceflight had no significant effect on the distribution of fiber types isolated for study in either the Sol or MG. In contrast, a single-fiber analysis showed a significant decrease in the percentage of fibers expressing type I MHC and a corresponding increase in fibers containing type IIa MHC after a 17-day spaceflight in humans. However, when fiber type was determined by histochemical myosin ATPase analysis of the entire biopsy cross section, no significant change in fiber-type distribution was observed (20). Collectively these data suggest that if significant fiber-type shifting from slow type I to fast type II fibers occurs with weightlessness in nonhuman primates or humans, spaceflight durations longer than 17 days are required.

**Fiber peak power.** The ability of a muscle to perform work is related to the peak power that can be generated. In all species, due to their considerably higher shortening velocities, fast type II fibers generate significantly more peak power than slow type I fibers (6, 24). The results presented here agree with previously published data on rhesus monkeys and demonstrate that the peak power of slow and fast fibers in the rhesus monkeys are similar to the corresponding fiber types in humans and considerably lower than rat fibers (6, 23, 24).

Both spaceflight and the R+17 capsule sit significantly reduced the peak power of the Sol slow type I fibers. This decline can be entirely attributed to fiber atrophy and the reduced force-generating capacity of the fiber. Thus, when peak power was normalized to fiber volume, power was not affected by the capsule sit or spaceflight. In the postflight fibers, the velocity obtained at peak power was significantly higher than the postvivarium control group, and this served to partially compensate for the reduced fiber force at peak power in the postflight animals (Table 5).

**Conclusions.** The 14-day spaceflight induced muscle fiber atrophy and a decline in peak force in the slow type I fibers of the Sol and MG. In the former muscle, the decline in force could not be totally explained by fiber atrophy because relative force ($kN/m^2$) also declined. Flight monkey 357 showed the greatest fiber atrophy and force loss, and these changes were associated with an in-flight shift in EMG activity from predominantly Sol to mainly MG over the duration of the flight. In comparison, the R+17 capsule-sit group showed type I fiber atrophy and force loss only in the Sol and no change in relative force in either muscle or fiber type. This comparison suggests that the loss in relative force requires not only inactivity, but also a zero-load condition. The decline in type I fiber peak power after both the capsule sit and flight was entirely explained by the fiber atrophy. Spaceflight caused a tendency for more high-velocity slow fibers ($V_o > 1.1$ FL/s), and, during the development of peak power, the postflight Sol type I fibers shortened at significantly higher velocities. This adaptation in velocity compensated in part for the decline in force during the development of peak power.

These results demonstrate that the contractile properties of single rhesus monkey fibers and their adaptation to microgravity are similar to what we have previously observed in humans. As pointed out by Recktenwald et al. (14), an advantage to studying rhesus monkeys is that one can maintain chronically implanted intramuscular electrodes that allow accurate EMG recordings and direct comparisons between changes in recruitment and cellular alterations within the individual muscle fibers. With this model, it should be possible to determine whether microgravity induces an altered recruitment strategy from predominantly Sol to predominantly gastrocnemius, thus causing Sol type I fiber atrophy or the Sol type I fiber atrophy occurs first, which, in turn, initiates an increased gastrocnemius activity.

**Perspectives**

NASA has planned human exploration of Mars by the year 2014. It is expected that the trip would require ~6 mo and that the crew would live on Mars for 18 mo before returning to Earth. Thus the entire mission would require ~3 yr of exposure to either the microgravity of space or the gravitational forces of Mars, a planet with approximately one-third the Earth’s gravity. The data presented here demonstrate that, without
countermeasures, significant muscle atrophy and loss in force and power occur within 14 day of weightlessness with the antigravity slow type I fiber more susceptible than the fast type II fiber. Clearly, exercise countermeasures (and perhaps hormone supplementation) will have to be incorporated to prevent unacceptable loss of bone and muscle. Even if artificial gravity during flight proves possible and effective, it is unlikely to alleviate the need for exercise programs. The current results are in good agreement with previous work on humans exposed to 17 days of weightlessness (Fig. 10). In the latter study, the crew participated in an aerobic exercise countermeasure program consisting of bicycling ergometry and treadmill running. Despite this, the type I fiber atrophy and the decline in force were similar to that observed in the current study in which the monkeys performed no exercise countermeasures. This suggests that the aerobic exercise countermeasures employed by NASA are ineffective in preventing muscle wasting and the loss in force and power. Our results demonstrate that inactivity was enough to elicit fiber atrophy and reduced absolute force (mN), whereas the decline in relative force (kN/m²) appeared to require inactivity and unloading. Undoubtedly, heavy resistance exercise (rather than aerobic or endurance exercise) of the key antigravity muscles of the legs and back will be needed to prevent microgravity-induced muscle wasting.

The authors thank the NASA support team of Mike Skidmore (Project Manager), Dr. Richard Grindeland (Project Scientist), and Shawn Bengston (Science Operation Manager), whose extraordinary efforts made this work possible. We also thank the members of the Russian Space Agency and the Institute of Biomedical Problems and, in particular, Dr. Inessa Kozlovskaya (Russian Project Scientist) for support throughout this project. Finally, we thank the Centre d'Etudes Spatiales (CNES) Project Manager Dr. Michel Viso for coordinating the French-United States collaboration, Dr. Sue Bodine for conducting the muscle biopsies, and Dr. Hans Hoppeler for assistance in the analysis of the EMs.

This study was supported by NASA Grant NAG2-636 to R. H. Fitts and CNES Grant 071 979/CNES 6896 to D. Desplanches.

REFERENCES