Substrate and enzyme profile of fast and slow skeletal muscle fibers in rhesus monkeys

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Grichko, Varvara P., Gwen J. Gettelman, Jeffrey J. Widrick, and Robert H. Fitts. Substrate and enzyme profile of fast and slow skeletal muscle fibers in rhesus monkeys. J. Appl. Physiol. 86(1): 335–340, 1999.—Results from the Russian Cosmos program suggest that the rhesus monkey is an excellent model for studying weightlessness-induced changes in muscle function. Consequently, the purpose of this investigation was to establish the resting levels of selected substrate and enzymes in individual slow- and fast-twitch muscle fibers of the rhesus monkey. The second objective was to determine the effect of an 18-day sit in the Spacelab experiment-support primate facility [Experimental System for the Orbiting Primate (ESOP)]. Muscle biopsies of the soleus and medial gastrocnemius muscles were obtained 1 mo before and immediately after an 18-day ESOP sit. The biopsies were freeze-dried, and individual fibers were isolated and assayed for the substrates glycogen and lactate and for the high-energy phosphates ATP and phosphocreatine. Fiber enzyme activity was also determined for the glycolytic enzymes phosphofructokinase and lactate dehydrogenase (LDH) and for the oxidative markers 3-hydroxyacyl-CoA dehydrogenase (β-OAC) and citrate synthase. Consistent with other species, the fast type II fibers contained higher glycogen content than did the slow type I fibers. The ESOP sit had no significant effects on the metabolic profile of the slow fibers of either muscle or the fast fibers of the soleus. However, the fast gastrocnemius fibers showed a significant decline in phosphocreatine and an increase in lactate. Also, similar to other species, the fast fibers contained significantly higher LDH activities and lower 3-hydroxyacyl-CoA dehydrogenase activities. For the muscle enzymes, the quantitatively most important effect of the ESOP sit occurred with LDH where activities increased in all fiber types post sit except the slow type I fiber of the medial gastrocnemius.

monkey muscle; substrate content and enzyme activities; fast- and slow-twitch fibers

OBSERVATIONS FROM THE SKYLAB MISSIONS in the 1970s, and more recently the Russian space station Mir and US Spacelab missions, have shown weightlessness not only to induce muscle atrophy but also to reduce the physical work capacity and increase the fatigability of the crew (5). In prolonged space missions, the atrophy and increased fatigability could provide serious limitations to the crew’s ability to work in space and to rapidly egress in an emergency on return to Earth. To date, most of the information regarding the cellular mechanisms responsible for muscle atrophy, the altered contractile function, and the increased fatigability induced by weightlessness and models of weightlessness have come from rat studies (1, 3, 7, 8, 17, 21, 24, 25). Such studies have shown the increased fatigability associated with spaceflight and hindlimb unloading (HU) in the rat to be at least in part caused by an increased dependence on carbohydrate metabolism in the face of a reduced ability to oxidize fats (1, 11, 19). Baldwin et al. (1) found a 9-day spaceflight to produce a 37% inhibition in the ability of both the high- and low-oxidative regions of the vastus muscle to oxidize long-chain fatty acids. The observations that pyruvate oxidation and the activity of tricarboxylic acid (TCA) cycle marker enzymes were unaffected by weightlessness suggests that the reduced ability to oxidize fats was not caused by a reduced substrate flux through the TCA cycle (1). Additionally, the authors found weightlessness to have no effect on the β-oxidative enzyme 3-hydroxyacyl-CoA dehydrogenase (β-OAC). Consistent with the theory of an increased dependence on carbohydrate metabolism, both spaceflight and HU have been shown to increase insulin-stimulated glucose uptake and the activity of hexokinase in rat muscle (3, 26). Additionally, HU has been shown to increase the resting glycogen concentration in the rat soleus (Sol) (11).

In contrast to rats, there is little information concerning the effects of weightlessness (or models of weightlessness) on muscle substrate and/or enzyme profile in humans or nonhuman primates. In humans, bed rest is frequently employed to model 0 gravity (G; magnitude of the force of gravity on the surface of the Earth). Recently, we observed 17 days of bed rest to significantly increase muscle glycogen and the enzymes phosphorylase and hexokinase in human Sol slow type I fibers (10). To totally understand how weightlessness alters substrate metabolism and increases fatigue, additional information is needed on humans and/or other large nonhuman primates. As a result of the Russian Cosmos program, it has become apparent that the rhesus monkey is an excellent model for studying the deleterious effects of 0 G on muscle structure and function and on whole body performance (2). Consequently, the objective of this work was to characterize the concentration and activity of selected muscle substrates and enzymes in individual slow- and fast-twitch fibers of the rhesus monkey. These data will provide a basis for comparison with similar data on humans and provide a database for subsequent studies on the effects of spaceflight. Because any future study of weightlessness would require that the animals be housed in an orbiting facility, we also assessed whether the reduced mobility imposed by the experiment-support primate facility [Experimental System for the Orbiting Primate...
ESOP) designed to be used aboard Spacelab had any effect on the measured variables.

**METHODS**

Selection of animals and general care. The studies described here were conducted as part of a large joint effort between the US and French space agencies (National Aeronautics and Space Administration (NASA) and Centre d'Etudes Spatiales) designed to develop baseline ground-based data on the basic physiology and psychomotor performance of adult male rhesus monkeys (Macaca mulatta). The project was approved by the animal care and use committee at NASA-Ames and Marquette University, and the animals were housed at the NASA primate test research facility, Ames Research Center (Moffet Field, CA). Animal care was in accordance with the guidelines established by the National Institutes of Health and NASA. The age and weight of the animals (pre- and post-ESOP), their food and water intake, and the ESOP were exactly as described previously (6).

Muscle biopsy procedure and fiber isolation and identification. Muscle biopsies were obtained from eight animals 4 wk before (pre) and immediately after (post) an 18-day ESOP sit. The pre- and postbiopsies were taken from two independent sites in the Sol and medial gastrocnemius (MG) muscles by using a general anesthesia (isoflurane) and an open-biopsy procedure exactly as described previously (6, 22). Once obtained, the biopsy was divided longitudinally, and one section was used for physiological and morphological studies described elsewhere (6) while the other was aligned longitudinally on a small piece of index card, stretched to approximately in situ length, and quick frozen in liquid nitrogen. The samples were then shipped in a dry-liquid-nitrogen shipper to Marquette University, where the they were dried under vacuum at −35°C and stored under vacuum at −80°C (7).

Individual fibers (~2-mm-long segments) were dissected free at room temperature, weighed on a quartz-fiber balance, and stored separately under vacuum at −80°C (7, 16). On average, 40 fibers were dissected from each of the biopsied muscles. For the slow Sol, the average number of slow and fast fibers studied per animal for each assay was 11 and 8, respectively. For the fast MG, the distribution averaged 14 fast and 4 slow fibers per animal per assay. At the time of an assay, fibers were brought to room temperature and divided into three equal sections. Two of the sections were used for the enzyme or substrate assays described in Fiber substrates and Enzyme assays, while the third was run on 5 and 12% SDS-PAGE for fiber type determination. A given section was used for either two substrate [ATP and phosphocreatine (PCr) or glycogen and lactate] or two enzyme ([β-OAC and lactate dehydrogenase (LDH) or citrate synthase (CS) and phosphofructokinase (PFK)]) assays. The fiber segment used for fiber typing was solubilized in 10 µl of 1% SDS sulfate sample buffer and stored at −80°C. Approximately 0.5 nl of fiber volume was run on a Hoefer SE 600 gel system consisting of a 3% (wt/vol) acrylamide stacking gel and a 5% (wt/vol) separating gel, and fiber type was determined from the myosin heavy chain (MHC) expression (Fig. 1A). To determine the myosin light chain composition and confirm the fiber typing, ~1 nl of fiber volume was loaded on a gel consisting of a 3.5% acrylamide stacking gel and a 12% acrylamide separating gel (Fig. 1B). All gels were silver stained as described by Giulian et al. (9).

**Fiber substrates.** The fiber segments (~700 ng) were assayed for ATP and PCr or equally divided and assayed for glycogen and lactate exactly as described previously (7).

**Enzyme assays.** The assays were conducted by modification of the procedures of Lowry and Passonneau (16) as described previously (7), by using methods based on pyridine nucleotide enzyme reactions. The one exception is the CS assay, which was changed as described here. The fiber segment (300–400 ng) was added to 10 µl of preincubation solution and incubated at 20°C for 30 min. The preincubation solution contained 50 mM 2-amino-2-methyl-1,3-propanediol (pH 8.8), 0.25% citrate-free BSA, and 0.6 M KCl. After the preincubation, 50 µl of specific reagent were added and the reaction was incubated for 60 min at 20°C. The specific reaction was exactly as described previously (7). The reaction was stopped by adding 50 µl of 0.075 N NaOH, and the assay mixture was heated at 95°C for 5 min. After cooling to 20°C, 500 µl of citrate reagent were added and the reaction was incubated for 20 min. The citrate reagent contained 100 mM tetraethylammonium buffer (pH 7.9), 60 µM NADH, 100 µM ZnCl2, 0.02% BSA, 0.006 U/ml citrate lyase, and 0.6 U/ml malic dehydrogenase. After 20 min, the excess NAD was destroyed by adding 50 µl of 1 N HCl, and the reaction was incubated for 10 min at 20°C. One milliliter of 6 N NaOH containing 10 mM imidazole was added, and the reaction heated at 60°C for 20 min to destroy the NAD and to produce a fluorescent product (7). The reaction was cooled to 20°C, and the fluorescence was measured.

**Statistical analysis** Data are presented as means ± SE. A t-test was used to determine fiber type differences within a muscle (prevalues only), to compare a particular fiber type between muscles, and to assess the effects of the ESOP sit on the substrate and enzyme profiles of each fiber type.

**RESULTS**

**Fiber type.** As described in METHODS, the fiber type was determined by the MHC isozyme profile on 5 and 12% SDS-PAGE gels (Fig. 1). With use of immunohistochemical techniques, we previously showed that the...
fast fiber population of the monkey Sol was restricted to the type IIa fiber type, whereas the MG contained both IIa and IIx fibers (6). Although hybrid fibers containing both slow and fast isozymes of myosin were observed in both muscles, neither muscle contained the type IIb MHC (6). Similar to humans, the type IIx fibers displayed the least and most mobility on the 5% gels, respectively, while the IIa migrated between the IIx and I isozymes (Fig. 1). Compared with human and rat (18, 28), the monkey myosin isozyme separation was less distinct, and thus for some fast fibers it was impossible to determine whether the fiber was a type IIa or IIx. For this reason, the fast fiber enzyme data presented in Tables 3 and 4 are shown both as specific fiber types (IIa and IIx) and as a combined type II group. The latter represents all of the type II fibers studied, which in the case of the Sol (see Table 3) should be primarily if not entirely type IIa fibers, whereas for the MG (see Table 4) this group contains both IIa and IIx fibers (6).

Fiber substrates. The substrate data for slow type I and fast type II fibers pre- and post-ESOP sit are shown in Tables 1 and 2. The pre ESOP type II fibers showed higher glycogen concentration than the slow type I fibers. The ATP, PCr, glycogen, and lactate contents of a particular fiber type (type I or II) were not significantly different when fibers from the Sol and MG were compared. The ESOP sit had no significant effect on glycogen in any fiber type in either the Sol or the MG muscles. The only significant substrate changes induced by the ESOP sit was in the type II fibers of the MG, where PCr decreased and lactate increased (Table 2). Although six of the eight monkeys showed reduced PCr in the fast MG fibers, except for one animal (H-602) the decline was modest (Fig. 2).

Fiber enzyme activities. The pre- and post-ESOP values for the glycolytic enzymes LDH and PFK and oxidative enzymes β-OAC and CS for the slow type I and fast type II fibers are shown in Tables 3 and 4. Consistent with other species, the fast fibers contained significantly higher LDH activities and for the most part lower oxidative enzyme activities. The exception to the latter was the type IIa fiber of the Sol where CS activity was higher, not lower, than that in the Sol type I fiber. The oxidative enzymes β-OAC and CS were significantly higher in the Sol type II compared with the MG type II fibers, whereas the reverse was true for the glycolytic enzyme LDH. The ESOP sit had no effect on the oxidative enzyme activities of the fast and slow fibers of the MG (Table 4), but the β-OAC was slightly increased in the fast type IIa Sol fiber population (Table 3). The quantitatively most important effect occurred with LDH, where activities increased in all fiber types post sit except the slow type I fibers of the MG. In regard to LDH activity, the slow Sol type I fiber became more like the fast type IIa fiber type (Fig. 3). The ESOP induced increase in LDH activity was less apparent in the fast type IIa fiber type (Fig. 3). Regarding PFK, the post-ESOP increase was only significant in the fast type IIx MG fibers (Table 4). Thus, when the activity of PFK is plotted against CS for fibers in which both of these enzymes were measured, the slow type I Sol fibers showed a similar distribution pre- and post-ESOP sit (Fig. 4).

DISCUSSION

Substrate profile of slow and fast fibers. To our knowledge, there are no published reports on the substrate profile of either whole muscles or individual slow- and fast-twitch fibers in the rhesus monkey. Consistent with other species, the fast-twitch fibers contained significantly higher glycogen concentrations than the slow-twitch fibers (27). In rats, one generally observes higher high-energy phosphate stores (ATP and PCr) in fast type II fibers compared with slow type I fibers (12), but for the rhesus monkey we observed no...
significant fiber type differences in either of these substrates. This result agrees with the finding of Ivy et al. (14) in human muscle. Additionally, we have recently conducted a substrate analysis of human fast- and slow-twitch fibers by using the same assay conditions employed in this study, and we found no fiber type differences in the ATP and PCR content (unpublished observations).

Fig. 2. Mean phosphocreatine (PCr) concentration of fast type II medial gastrocnemius fibers for each monkey is shown pre- and post-Experimental System for the Orbiting Primate (ESOP) sit. Although the sit caused a significant decline in fast-fiber PCr, this figure demonstrates that, except for monkey H-602, decline induced by the sit was modest. In the case of monkey H-534, PCr content increased post sit.

![Graph showing mean phosphocreatine (PCr) concentration](image)

Table 3. Soleus muscle enzymes in rhesus monkey: effect of ESOP sit

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH</th>
<th>PFK</th>
<th>β-OAC</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>18.79 ± 1.69 (72)</td>
<td>1.08 ± 0.06 (87)</td>
<td>3.57 ± 0.22 (91)</td>
<td>2.57 ± 0.24 (71)</td>
</tr>
<tr>
<td>Post</td>
<td>31.41 ± 2.36 (86)†</td>
<td>1.15 ± 0.05 (80)</td>
<td>3.13 ± 0.16 (79)</td>
<td>2.66 ± 0.19 (76)</td>
</tr>
<tr>
<td>Type IIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>44.59 ± 4.25 (32)†</td>
<td>1.32 ± 0.13 (35)</td>
<td>1.35 ± 0.17 (28)†</td>
<td>3.85 ± 0.50 (27)†</td>
</tr>
<tr>
<td>Post</td>
<td>51.57 ± 3.89 (46)</td>
<td>1.62 ± 0.09 (37)</td>
<td>1.88 ± 0.14 (50)†</td>
<td>3.12 ± 0.31 (30)</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>47.99 ± 2.90 (71)</td>
<td>1.34 ± 0.08 (71)</td>
<td>1.37 ± 0.14 (60)</td>
<td>2.87 ± 0.29 (58)</td>
</tr>
<tr>
<td>Post</td>
<td>60.98 ± 3.77 (79)†</td>
<td>1.47 ± 0.09 (70)</td>
<td>1.71 ± 0.11 (88)</td>
<td>2.36 ± 0.25 (52)</td>
</tr>
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</table>

Values are means ± SE given in mol·h⁻¹·kg dry wt⁻¹ for no. of fibers in parentheses. LDH, lactate dehydrogenase; PFK, phosphofructokinase; β-OAC, β-hydroxyacyl-CoA dehydrogenase; CS, citrate synthase. *Significantly different from Pre, P ≤ 0.05. †Significantly different from type I fiber, P ≤ 0.05.

Table 4. Medial gastrocnemius muscle enzymes in rhesus monkey: effect of ESOP sit

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH</th>
<th>PFK</th>
<th>β-OAC</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>24.91 ± 5.29 (18)</td>
<td>1.25 ± 0.24 (19)</td>
<td>2.83 ± 0.43 (18)</td>
<td>3.74 ± 0.57 (23)</td>
</tr>
<tr>
<td>Post</td>
<td>27.25 ± 3.56 (15)</td>
<td>1.25 ± 0.13 (14)</td>
<td>5.29 ± 1.55 (28)</td>
<td>2.76 ± 0.43 (19)</td>
</tr>
<tr>
<td>Type IIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>51.24 ± 4.95 (25)†</td>
<td>1.50 ± 0.09 (26)</td>
<td>1.51 ± 0.22 (19)†</td>
<td>2.88 ± 0.40 (28)</td>
</tr>
<tr>
<td>Post</td>
<td>64.72 ± 4.09 (31)*</td>
<td>1.40 ± 0.10 (28)</td>
<td>1.04 ± 0.12 (24)</td>
<td>2.66 ± 0.22 (31)</td>
</tr>
<tr>
<td>Type IIx</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre</td>
<td>60.67 ± 2.90 (65)†</td>
<td>1.23 ± 0.07 (47)</td>
<td>0.86 ± 0.08 (67)†</td>
<td>1.69 ± 0.23 (39)†</td>
</tr>
<tr>
<td>Post</td>
<td>78.00 ± 3.87 (60)*</td>
<td>1.46 ± 0.09 (33)*</td>
<td>0.87 ± 0.06 (61)</td>
<td>1.55 ± 0.15 (43)</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>60.35 ± 2.21 (116)</td>
<td>1.30 ± 0.05 (110)</td>
<td>1.06 ± 0.07 (118)</td>
<td>1.82 ± 0.18 (91)</td>
</tr>
<tr>
<td>Post</td>
<td>71.83 ± 2.53 (135)*</td>
<td>1.54 ± 0.07 (92)*</td>
<td>1.08 ± 0.06 (114)</td>
<td>1.78 ± 0.12 (95)</td>
</tr>
</tbody>
</table>

Values are means ± SE given in mol·h⁻¹·kg dry wt⁻¹ for no. of fibers in parentheses. Type II group represents mean ± SE for all type II fibers (IIa and IIx) studied. *Significantly different from Pre, P ≤ 0.05. †Significantly different from type I value, P ≤ 0.05. ‡Significantly different from type IIa value, P ≤ 0.05.
Enzymatic activity of slow and fast fiber types. Our results document that in the rhesus monkey, like in other species, significant fiber type differences exist for both glycolytic and oxidative enzyme capacity. Although considerable variation in enzyme activity was observed within a fiber type (Figs. 3 and 4), the slow type I fibers were characterized by low LDH and PFK activity. The differences between fiber types was small compared with rat muscle but was similar to the range observed in humans (4, 13, 15).

Effect of ESOP sit. The ESOP reduces animal mobility, and thus it could in itself induce functional changes that mirror those observed with weightlessness. Thus it is important to understand exactly how the ESOP alters cell function. We previously showed that the 18-day ESOP sit produced a 10–11% decline in the slow type I fiber (4). In contrast to β-OAC, the TCA cycle enzyme CS did not show consistent differences between the slow type I and fast type IIa fiber type. The fast type IIx fiber had the lowest oxidative capacity and highest LDH activity. The differences between fiber types was small compared with rat muscle but was similar to the range observed in humans (4, 13, 15).

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