FINAL REPORT
NAG2-653

ADAPTATION OF SKELETAL MUSCLE TO SPACEFLIGHT: COSMOS RHESUS PROJECT

COSMOS 2044 and 2229

Sue Bodine-Fowler, Ph.D.
UCSD School of Medicine

October 3, 1994

The proposed experiments were designed to determine the effects of the absence of weight support on hindlimb muscles of the monkey: an ankle flexor (tibialis anterior, TA), two ankle extensors (medial gastrocnemius, MG and soleus, SOL), and a knee extensor (vastus lateralis, VL). These effects were assessed by examining the biochemical and morphological properties of muscle fibers obtained from biopsies in young Rhesus monkeys (3-4 Kg). Biopsies taken from ground base experiments were analyzed to determine: 1) the effects of chair restraint at 1 G on muscle properties and 2) the growth rate of flexor and extensor muscles in the Rhesus. In addition, two sets of biopsies were taken from monkeys which were in the flight pool and the four monkeys that flew on the Cosmos 2044 and 2229 biosatellite missions. These studies were done in collaboration with Drs. V.R. Edgerton, R.R. Roy and J.A. Hodgson at UCLA.

HYPOTHESES

Muscle fibers will atrophy when subjected to a 0 gravity environment. Based on previous data obtained in rats after a 5-14 day spaceflight and after hindlimb suspension, we hypothesize that muscle fibers in a slow muscle will atrophy more rapidly than fibers in a fast muscle, and slow fibers in either a predominantly slow or mixed muscle will atrophy more than fast fibers.

The normal oxidative and glycolytic potential of muscle fibers will be sustained during the rapid phases of muscle atrophy while myosin isozymes of some fibers will change from slow toward fast types.

OBJECTIVE

Define the degree of muscle atrophy and metabolic changes that occur in muscle fibers of the VL, SOL, MG, and TA after 14 days of spaceflight.

METHODS

BIOPSY PROCEDURES for Cosmos 2044

Muscle biopsies were obtained pre- and postflight in two male monkeys flown on Cosmos 2044, a 14-day spaceflight, and in four control monkeys of approximately the same body weight, age and sex. The flight monkeys (782 and 2483) weighed 3.9 and 3.8 Kg, respectively, and were 3 years 6 months old at the time of the flight. Each of these monkeys gained approximately 300 grams between the time that the pre and postflight biopsies were taken. The four control monkeys (2471, 839, 2442, 2587) were part of the flight candidate pool and, consequently, received the same training and restraint as the two monkeys chosen for flight. The control monkeys weighed between 4.0 and 4.2 Kg at the time of the flight and gained approximately 500 grams between the time that the pre and postflight biopsies were taken. The control monkeys remained in their individual cages during the 14-day flight and were fed the same diet as the flight monkeys.

Biopsies were taken from two independent sites in the Sol, MG and TA muscles using a small (3-mm diameter) Bergstrom biopsy needle (Figure 1). The first (preflight) biopsies were taken 107 days prior to launch , and the second (postflight) biopsies were taken 24 hours after the recovery of the capsule. All biopsies were taken from the right leg since the left leg was implanted with EMG electrodes. The biopsies weighed between 8

and 14 mg and contained between 100-200 fibers of which an average of 40 ± 16 fibers were acceptable for metabolic and size analyses.

The preflight biopsies were taken while the animal was under a general anesthesia (Ketamine). Under sterile conditions, one incision was made on the medial side of the lower leg to give access to the Sol and MG muscles, and one incision was made on the anterior side of the leg to give access to the TA muscle. Using blunt dissection, the belly of each muscle was exposed and a small cut was made in the overlying fascia. The biopsy needle was inserted into the muscle parallel to the direction of the muscle fibers, and suction was applied to increase the size of the biopsy. The needle was withdrawn and the sample was removed from the needle and frozen in freon-12 cooled with liquid nitrogen. The fascia and skin were closed with absorbable sutures (Vicryl ™) and a topical antibiotic (Neosporin™) was placed on the wound. The preflight biopsies were taken from the superficial, medial regions of the distal Sol, the distal MG, and the proximal TA.

The postflight biopsies were taken while the animal was restrained in a primate chair (Primate Products Restraint System™) using local anesthesia applied to the biopsy site. A sterile field was made around the right leg and the leg was washed with betadine and alcohol. Local anesthesia was induced using a 2% lidocaine solution mixed with epinephrine (0.2 mls of 1:1000 epinephrine mixed with 4 mls of 2% lidocaine). The lidocaine solution was infiltrated along the incision line by inserting a needle through the skin and injecting lidocaine subcutaneously (~3-4 cc). Lidocaine was occasionally injected into the muscle (<1cc) proximal to the biopsy site. When the monkey showed no reaction to a skin prick, an incision was made to the skin, the muscle identified and the biopsy taken as described above. The postflight biopsies were taken from the superficial, medial regions of the proximal Sol,the proximal MG, and the distal TA.

The biopsy sites were selected to ensure that the same muscle fibers were not sampled during the pre and postflight biopsies and were determined based on detailed architectural analyses of each muscle. In addition, the feasibility of obtaining reproducible biopsies from the selected sites has been verified, i.e., the regions sampled within each muscle have been shown to have similar fiber type distributions.

BIOPSY PROCEDURES for Cosmos 2229

The biopsy procedures were modified in Cosmos 2229 in order to obtain larger and better quality muscle samples. All of the biopsies were shared with the Russian Science team.

Muscle biopsies were obtained pre- and postflight in two male monkeys flown on Cosmos 2229, a 12-day spaceflight, and in four control monkeys of approximately the same body weight, age and sex. The four control monkeys were part of the flight candidate pool and, consequently, received similar training and restraint as the two monkeys chosen for flight. The control monkeys remained in their individual cages during the 12-day flight and were fed the same diet as the flight monkeys.

Biopsies were taken from two independent sites in the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA) and vastus lateralis (VL) muscles using an open biopsy technique. The first (preflight) biopsies were taken approximately 91 days prior to launch, and the second (postflight) biopsies were taken 3 to 5 days after the recovery of the capsule. All biopsies were taken from the right leg. The Sol, MG, TA and VL on the left leg were implanted with EMG electrodes and a force transducer was placed on the tendon of the MG.

The biopsies were taken while the animal was under a general anesthesia. Under sterile conditions, small incisions were made on the medial side of the lower leg to expose the Sol and MG muscles, on the anterior side of the leg to expose the TA muscle, and on the lateral side of the thigh to expose the VL. 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 approximately 10 mm long x 5 mm wide x 5 mm deep (~100 to 150 mg wet weight). 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 a saline soaked guaze. The fascia and skin were closed with absorbable sutures (Vicryl ™). The tissue sample was weighed, stretched to approximately the in situ length, and mounted on cork using pins to insure a perpendicular orientation of the muscle fibers. The samples were frozen in isopentane cooled with liquid nitrogen and stored at -80° C. The preflight biopsies were taken from the medial regions of the proximal Sol, the distal MG, and the proximal TA. The postflight biopsies were taken from the medial regions of the distal Sol and the distal TA; and the middle region of the proximal MG.

TISSUE ANALYSIS

Fiber Cross-Sectional Area: The mean fiber cross-sectional area of each biopsy sample was determined from a population of 100 to 400 fibers measured from a serial cross-section immunohistochemically stained with a monoclonal antibody for laminin. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a diaminobenzidine (DAB) peroxidase reaction. Laminin stains the basal lamina just outside the plasma membrane (26). Using a color camera attached to a light microscope and image analysis system (Image I-AT, Universal Imaging Corporation), the region within the laminin boundaries was automatically filled in by the computer and the fiber cross-sectional area was calculated in μm^2.

Succinate dehydrogenase Activity : Succinate dehydrogenase (SDH) activity and fiber cross-sectional area were determined for individual fibers (50-90 fibers) in 10 μm cross sections taken from each of the biopsy samples. Tissue sections were analyzed on a computer-assisted image analysis system (Image-I/AT, Universal Imaging Corporation). To measure SDH activity, repeated digitized images were taken of a single tissue section every 2 min over a period of 14 min while the tissue was incubated in a medium without the substrate, succinate. A medium with succinate then was added and repeated scans were taken every 2 min over the next 14 min. Reaction rates for each fiber were based on a linear regression line determined from the 8 points acquired with the medium containing substrate. Although the absolute optical density readings may vary slightly from day to day, the slope or reaction rate is not affected.

The medium for determining SDH activity contained: 100 mM phosphate buffer (pH=7.6), 1.5 mM sodium azide, 3 mM 1-methoxyphenazine methylsulfate, 1.5 mM nitro blue tetrazolium, 5.5 mM EDTA-disodium salt, and 58 mM succinate disodium salt. This incubation medium is a modification of the one used for the rat muscle and has been optimized for the monkey muscle. The reaction rates for fibers in the monkey are approximately 5 times slower than those in the rat.

Fiber Types: Fibers were classified as type I, IIa, IIb, IIx or hybrid (coexpression of slow and fast) using monoclonal antibodies which label the different myosin heavy chain (MHC) isoforms. Serial cross-sections were incubated with primary antibodies (BA-F8, BF-13, BF-35 and SC-71 generously donated by S. Schiaffino (Padova, Italy)) overnight at 25°C. Sections incubated without primary antibody were used as controls to visualize non-specific labeling. A Vectastain ABC™ kit (Vector Labs, Burlingame, CA, USA) was

used to amplify the antigen-antibody complex, which in turn, was visualized by treatment with a DAB peroxidase reaction.

The immunohistochemical labeling was compared to standard histochemical staining for myofibrillar ATPase after acid (pH, 4.35) and alkaline (pH, 10.0) preincubation. Serial sections also were stained for hemotoxylin and eosin (H&E) for routine histological examination.

Electrophoretic Separation of MHC: The MHC isoforms were separated by sodium docecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Frozen muscle samples were homogenized by hand in glass tissues grinders in 200-400 µl of ice-cold homogenization buffer [250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM Tris (hydroxymetyl) aminomethane (Tris), pH 6.8]. The myofibril homogenate was boiled in sample buffer (23) for 2 min at a final protein concentration of 0.125 µg/µl.

The separating gel was composed of 30% glycerol, 8% acrylamide-N,N'-methylene-bis -acrylamide (bis) (50:1), 0.2M Tris (pH 8.8), 0.1M glycine and 0.4% sodium dodecyl sulfate (SDS). The stacking gel was composed of 30% glycerol, 4% acrylamide-bis, 70 mM Tris (pH 6.7), 4 mM EDTA and 0.4% SDS. Polymerization was initiated with 0.05% N,N,N',N'-tetramethyletheylenediamine and 0.1% ammonium persulfate.

The upper running buffer consisted of 0.1M Tris (base), 150 mM glycine and 0.1% SDS. The lower running buffer consisted of 50 mM Tris (base), 75 mM glycine and 0.05% SDS. Both buffers were cooled to 4° C prior to use. The entire gel unit was placed in a stryrofoam box containing cooling packs to maintain the temperature below 10°C. The gel was run at 275V (constant voltage) for 22-24 hours. Quantification of the protein bands was accomplished by scanning the Coomasie Blue stained gels with a Pharmacia LKB Ultrascan XL laser scanning densitometer.

GROUND BASED EXPERIMENTS

To determine the effects of restraint at 1G, three male monkeys weighing between 3.5 to 4.1 Kg (mean 3.7 $\pm$ 0.23 Kg) were placed in a replication of the flight chair and restrained for 14-days to simulate the flight duration. All ground based experiments were performed at the NASA/Ames Research Center. Muscle biopsies were taken from the Sol, MG, TA, and VL approximately 2-weeks prior to the restraint and 3 days after the 14-day restraint period. The ground base experiments were performed prior to the actual flight and were designed to simulate the proposed 14-day flight. The actual flight was shortened for technical reasons and extended only 12-days.

RESULTS (COSMOS 2044)

FIBER CROSS-SECTIONAL AREA

The mean cross-sectional areas of fibers sampled from the pre and postflight biopsies of the two flight monkeys (782 and 2483) and the four control monkeys (2471, 839, 2442, and 2587) are given in Figure 2. In several instances, the biopsies were damaged (usually due to thawing) during the storage and transfer of the biopsies and, consequently, there are several missing data points.

The 14-day spaceflight appeared to have no effect on fiber size in the Sol and MG muscles. In each of the flight animals, the mean fiber size was significantly larger in the postflight compared to the preflight biopsies. In both the Sol and MG there appeared to be no differential effect on those fibers that stained light (presumably slow) or dark (presumably fast) for myosin ATPase, alkaline preincubation. In the Sol, the light and dark fibers were similar in size, whereas in the MG, the light fibers were significantly smaller than the dark fibers.

In the control monkeys, the mean fiber size was generally greater in the postflight (second) biopsies than in the preflight (first) biopsies. The first and second biopsies in the control monkeys were taken during the same time interval as the biopsies from the flight monkeys. Given that these animals had not reached maturity, the increase in size most probably reflects the growth of the muscles during this period. The absolute increase in size of fibers in the Sol and MG varied among the four control animals. Figure 3 compares the increase in body weight to the increase in fiber size in the Sol and MG. In general, there was a positive relationship between the absolute increase in body weight and the absolute increase in fiber size. Of interest is the interrelationship between the growth in the Sol and MG. In monkeys 2483, 782 and 2587, the fiber size increase was approximately the same in the Sol and MG. In monkeys 2442 and 839, however, there was a dissociation between the growth in these two muscles. In monkey 2442, the increase in the mean fiber size in the Sol was double that which occurred in the MG (1910 vs 914 μm, respectively). In contrast, in monkey 839 the MG fibers doubled in size relative to the fibers in the Sol.

In the TA, the results are less clear regarding the effects of spaceflight since no preflight data were obtained for flight monkey 2483. In general, the fibers in the TA of the control monkeys were larger in the second than the first biopsy, but the difference in size was not as large as that observed in the Sol and MG. In flight monkey 782, the postflight biopsy had significantly ($p<0.05$) smaller fibers than the preflight biopsy. Atrophy seemed to have occurred in both fiber types in the TA of monkey 782 . As in the MG, the light fibers in the TA tended to be smaller in size than the dark fibers.

SDH ACTIVITY

The mean SDH activities of the fibers sampled in the pre and postflight biopsies from the flight and control monkeys are shown in Figure 4. In the Sol and TA muscles, there was no apparent effect of spaceflight in that the flight monkeys showed the same trends as the control monkeys. In contrast, the MG seemed to be affected by the 14-day spaceflight. In the MG of control monkeys, the mean SDH activity was greater in the postflight than the preflight biopsies in those three monkeys with muscle samples at both time periods. However, in the MG of both flight monkeys, the mean SDH activity was less in the postflight than the preflight biopsies. Consequently, SDH activity in the fibers of the MG appeared to decrease in response to spaceflight, while no effect on the Sol and TA muscle fibers was evident.

RESULTS (COSMOS 2229)

The body weights of the flight (151 and 906) and control (803, 907,775 and 1401) monkeys at the time of the first biopsy (pre) in Sept. 1992, at the time of the launch in Dec. 1992 and at the time of the second biopsy (post) in Jan. 1993 are shown in figure 5. Both flight monkeys gained weight between the first biopsy and the launch : 151 gained 700 gms and 906 gained 600 gms. During the flight, which lasted 12 days, monkey 151 lost 700 gms and 906 lost 200 gms. In comparison, the three monkeys which were restrained at 1G for a period of 14-days lost an average of 200 grams. The loss of body weight in monkey 906 was most likely related to fluid loss since his average daily food consumption during the flight was 1.2 times that on ground, whereas his total fluid intake per day was 76% that on ground. In monkey 151, his daily food and fluid intake during the flight were 33 and 28% of that on the ground.

No significant changes were observed in the whole body lean mass or the right leg mass during the flight period. There was a significant increase (12%, p=0.03) in the right leg lean mass during the recovery period. There was a decrease (22%, p=0.01) in right arm lean mass during the flight period which was primarily due to a large loss in flight monkey 151.

FIBER CROSS-SECTIONAL AREA

To determine whether atrophy occurred in response to microgravity, mean fiber areas were compared between pre and postflight biopsies from the same animal and between the postflight biopsies of each flight monkey and the mean of the postflight biopsies from the control monkeys (Table 1). There was no significant atrophy in the Sol or MG based on the differences between the mean fiber sizes of the pre and postflight biopsies (Table 1). However, the response of each of the flight monkeys was not identical. In monkey 906 there was an increase in the mean fiber areas of the postflight biopsies in both the Sol (112% of preflight) and MG (168% of preflight) and there was a shift in the distribution of fiber sizes. In both muscles the fast fibers showed the greatest increase in size. In the Sol, the type I fibers showed no change in size, while there was a 3% and 19% increase in size for the type IIa and hybrid fibers. In the MG, increases in fiber size of 14, 31,70 and 35% were found in the type I, IIa, IIx and hybrid fibers, respectively. The mean fiber sizes of the postflight Sol and MG biopsies were similar to the mean fiber sizes of the control monkeys (Table 1).

In contrast, in monkey 151, the mean fiber areas of both the Sol and MG after flight were 88% of the preflight values. Further, the fiber sizes of the postflight biopsies from the Sol and MG were 75% and 71% of the control post biopsy means. Fiber size histograms illustrated a a decrease in the percentage of large fibers following the flight in both muscles. In the Sol there was no change in fibers size among the type I fibers and a 21% decrease in the type IIa and hybrid fibers. All fiber types in the MG showed a decrease in fiber size: 26, 13 and 20% in the type I, IIa and IIx fibers, respectively..

The TA in both flight monkeys showed significant ($p<.001$) atrophy in response to the spaceflight (Table 1). The mean fiber size of the postflight biopsies from 151 and 906 were 69 and 54% of the overall mean of the post biopsies from the control monkeys (Table 1). In monkey 151, atrophy was observed only the type IIx fibers (30% decrease in size). In contrast, in monkey 906, all fibers atrophied (22,22, and 31% in the type I, IIa and IIx fibers, respectively).

As was observed in the Sol and MG, the response in the VL differed for the two flight monkeys. In monkey 151, the mean fiber size postflight was 57% of preflight (Table 1). Further the fiber size distribution changed from a bimodal to a unimodal distribution

and shifted to the left (i.e., smaller sizes). In monkey 906 there was no apparent atrophy in the VL as demonstrated by the mean fiber sizes (Table 1) and fiber size distributions. However, the fact that the VL of the control monkeys (Table 1) and the fibers in the Sol and MG of the same monkey showed significant growth suggests that there may have been some "atrophy" or slowing of growth during the flight.

To determine the effect of chair restraint, trained monkeys were placed in a chair which resembled that flown in the biosatellite for a period of 14-days at 1G. In general, the 14-day restraint had no effect on the fiber cross-sectional areas of the Sol, MG, TA or VL (Table 2). In these monkeys, growth was not a factor since the pre-restraint biopsy was taken approximately two-weeks prior to the experiment. The post-restraint biopsies were taken 3 days after the end of the restraint procedure to simulate what was to occur during the flight experiment. The three day delay was dictated by other neurophysiological tests that were apart of the overall flight experiment and had to occur immediately after the flight.

FIBER TYPES

Muscle fibers from the four hindlimb muscles studied could be classified into four types based on their immunohistochemical staining to monoclonal antibodies to the myosin heavy chain. In the Sol fibers were classified as either type I (slow), type IIa (fast) or hybrid (slow/fast). The type I fibers were positive for the F-8 and BF-35 antibodies. The type IIa fibers were positive for all of the antibodies except the F-8 antibody which is specific for the slow MHC. The hybrid fibers stained positively for all the antibodies and presumably expressed both type I and IIa MHCs. The hybrid fibers stained dark at an acid pH and intermediate to dark at an alkaline pH for myosin ATPase. Hybrid fibers composed between 20 to 40% of the soleus muscle in the young control monkeys, but, were rarely found in the MG, TA or VL.

Based on the immunohistochemical staining three fiber types were found in the MG, TA and VL: types I, IIa and IIx. The type I and IIA fibers stained the same as in the Sol. The classification of IIx was based on the negative staining of fibers for the BF-35 monoclonal antibody. These fibers were also negative for BA-F8, positive for BF-13 and intermediate for SC-71. In the rat, the SC-71 antibody is specific for type IIa fibers. In the monkey and humans (personnal communication, S. Schiaffino), however, the IIx fibers stain intermediate for SC-71. Based on these antibodies we could not detect any type IIb fibers in the MG, TA or VL muscles. Consequently, it appears that mixed hindlimb muscles of the monkey have few, if any, IIb fibers.

The lack of IIb MHC was confirmed by SDS-PAGE. In the Sol there were two bands which migrated to a location on the gel similar to that found for type I and IIa MHCs in other species. In the MG, TA and VL there were also only two bands which had migrations typical of type I and IIa MHCs. A band located where IIb MHC is typically found was not observed in any of the samples. The IIx MHC in other species migrates just below the IIa MHC band. Using the same procedure that is used to separate the four bands in rats, the IIa and IIx MHCs could not be separated and appear to migrate together.

In response to microgravity, there was a significant increase in the percentage of fast MHC in the Sol, and no change in fiber type in the MG and TA (Table 3). In the Sol of monkey 151 the percentage of type I, IIa and hybrid fibers was 54, 19 and 27 % in the preflight biopsies and 44, 30, and 26 % after the flight. In the Sol of monkey 906, the shift in fiber type was primarily from type I to hybrid. The percentage of type I, IIa and hybrid fibers was 51, 6 and 43% in the preflight biopsies and 29, 8 and 63% in the postflight biopsies. There was no difference in the percentage of slow and fast MHC between the pre and postflight biopsies taken from the the Sol, MG and TA of the control monkeys.

In the VL muscle there was an increase in the percentage of type IIx fibers after the flight. The percentage of type I, IIa and IIx fibers in the VL of monkey 151 was 11, 33 and 56% prior to the flight and 0, 21 and 79% after the flight. The percentage of type I, IIa

and IIx fibers in the VL of monkey 906 was 7, 37 and 56% prior to the flight and 4, 25 and 71% after the flight.

SDH ACTIVITY

The mean SDH activities of the pre and postflight biopsies from the Sol, MG, TA and VL of the flight and control monkeys are shown in Figure 6. Within an animal, the mean SDH activity was similar across muscles. There was no obvious effect of microgravity on the SDH activity of any of the muscles. For example, although the SDH activity decreased in the MG and TA of both flight muscles, it also decreased in some of the control animals. In the Sol, the mean SDH activity decreased in monkey 151 and increased in monkey 906 after the spaceflight. Interestingly, all of the control monkeys showed a decrease in SDH activity between the two biopsies, consequently, the increase in 906 was unusual.

The relationship between SDH activity, fiber cross-sectional area and myosin type is illustrated for the Sol (Fig 7), and MG (Fig. 8) of the two flight monkeys. In the Sol, all three fiber types (I, IIa, and hybrid) had similar SDH activites and fiber areas. In the MG and TA, the type I and IIa fibers had overlapping SDH activites and fiber areas and the type IIx fibers had a lower SDH activity and significantly larger fiber areas than the type I and IIa fibers. The type I and IIa fibers in the MG and TA were generally smaller in size than the type I and IIa fibers in the Sol. Interestingly, the relationship between type I, IIa and IIx fibers in the MG and TA was very similar to the relationship seen between type I, IIa and IIb fibers in other species. The decrease seen in the overall mean SDH activities in all of the muscles appears to have been due to a decrease in the activities of the type I and IIa fibers.

SUMMARY

Based on data collected in rats it is generally assumed that extensors atrophy to a greater extent than flexors in response to spaceflight or hindlimb suspension. Consequently, the finding that fibers in the TA (a fast flexor) of the flight monkeys atrophied, whereas fibers in the Sol (a predominantly slow extensor) and MG (a fast extensor) grew after a 14-day spaceflight (Cosmos 2044) and 12-day spaceflight (Cosmos 2229) was unexpected. In Cosmos 2044, the TA in both flight monkeys had a 21% decrease in fiber size, whereas the Sol and MG both had a 79% increase in fiber size. In Cosmos 2229, the TA in both flight monkeys showed significant atrophy, whereas the Sol and MG showed slight growth in one monkey (906) and slight atrophy in the other monkey (151).

The reason for the differential response in the two flight monkeys in Cosmos 2229 is unclear, but may relate to differences in the activity levels of the two flight monkeys (151 and 906) while in space. During the 12-day flight (Cosmos 2229) monkey 906 was more active than monkey 151 based on the number of times it performed its motor task. The motor task that each animals was trained to perform was a sinusoidal lever movement (60 degrees of movement) performed at a specific rate and amplitude against a torque which was adjustable depending on the abilities of the monkey. The monkey was cued to perform the task at least once every day of the flight for a 20 min period. Edgeerton and colleagues (see technical report of Edgerton) found that the integrated EMG activity of the Sol and MG during the 20 minutes periods of the motor task was as high or higher during the flight as during pre and postflight testing. Further, the amount of activity recorded in the Sol and

MG during the 20 min sessions during the flight generally exceeded the total activity that occurred during 24 hours of continuous recordings during normal cage activity.

Although the monkeys were not trained to perform a task with their right leg they often placed their foot on a fixed bar that was located on the right side of the chair next to the movable lever. Consequently, it was possible that the monkeys performed periodic loaded contractions with the extensors of the right ankle. One possible explanation for the differences in the fiber size responses of the extensors of the two flight monkeys was that monkey 906 "exercised" more than monkey 151, and was able to counteract the atrophic response of unloading due to spaceflight. Several studies have shown that daily periods of load-bearing can attenuate the atrophic response in the Sol, and to some degree the MG, associated with hindlimb suspension. Unfortunately, we do not have any data on the movements of the un-trained (right) leg.

In Cosmos 2229, the interpretation of the muscle morphological and biochemical data has been greatly assisted by the data provided by the whole body and limb densitometry, metabolic, and hormone status and neuromuscular activity levels. In this respect, the monkey has provided a useful and very unique model which enabled us to assess the response of microgravity from a highly integrated perspective. The apparent differences in the response of extensors and flexors in the rat and monkey to microgravity are more likely related to the ability of the monkey to counteract the effects of unloading with some form of "load-bearing" contractions during the flight rather than a species difference. In future flights this hypothesis can be tested by monitoring the activity and forces generated by the "non-trained" right limb.

PUBLICATIONS

Roy, R.R., Bodine-Fowler, S.C., Kim, J., Rudolph, W., Haque, N., deLeon, D., and Edgerton, V.R. Architectural and fiber type distribution properties of selected Rhesus leg muscles: feasibility of multiple independent biopsies. *Acta Anat.* 140: 350-356, 1991.

Hodgson, J.A., Bodine-Fowler, S.C., Roy, R.R., deLeon, R.D., deGuzma, C.P., Koslovskaya, I., Sirota, M. and Edgerton, V.R. Changes in recruitment of Rhesus soleus and gastrocnemius muscles following a 14-day spaceflight. *Physiologist* 34 (Suppl) S102-S103, 1991.

Bodine-Fowler, S.C., Roy, R.R., Rudolph, W., Haque, N., Kozlovskaya, I.B., and Edgerton, V.R. Spaceflight and growth effects on muscle fibers in the rhesus monkey. *J. Appl. Physiol.* 73: 82S-89S, 1992.

Bodine-Fowler, S.C., Pierotti, D.J. and Talmadge, R.J. Preferential atrophy of flexor muscles of the monkey after spaceflight. *J. Appl. Physiol.* (submitted August 1994)

TABLE 1: *FIBER CROSS-SECTIONAL AREA* (μm^2)

MONKEY		SOL		MG		TA		VL	
		PRE	POST	PRE	POST	PRE	POST	PRE	POST
151	MEAN	1868	1652	1539	1361	2587	1952	2934	1662
	SD	394	442	609	455	927	646	787	489
	n	195	167	436	387	286	310	315	408
906		2018	2271	1175	1976	2128	1507	2327	2391
		407	565	298	690	830	566	826	672
		215	234	294	453	322	331	379	337
907		1749	1754	1743	1688	2241	2470	1818	2450
		322	492	547	747	967	910	567	714
		394	308	369	201	214	96	406	367
803		2711	2466	1771	1837	1849	3149	3290	2906
		481	564	358	679	708	815	833	838
		254	255	277	244	364	308	394	325
775		2452	2359	1778	2201	2699	2798	2929	3431
		407	793	402	655	1022	1228	695	833
		262	190	352	222	329	253	331	364
CONTROLS	Mean	2304	2193	1764	1909	2263	2806	2679	2929
	SD	498	384	18	264	425	340	767	491
	n	3	3	3	3	3	3	3	3

Data are means ± standard deviation for a sample of muscle fibers (n) measured from a single biopsy. Fiber cross-sectional area was measured from a cross-section immunohistochemically stained for laminin. Monkeys 151 and 906 were the flight monkeys. Monkeys 907, 803 and 775 were control monkeys.

TABLE 2: *FIBER CROSS-SECTIONAL AREA* (μm^2) *AFTER GROUND-BASE RESTRAINT*

MONKEY		SOL		MG		TA		VL	
		PRE	POST	PRE	POST	PRE	POST	PRE	POST
148	MEAN	1167	1332	1138	1480	1686	1707	1458	1521
	SD	325	342	369	491	606	407	419	403
	n	217	247	345	367	380	318	364	333
149		1581	1414	2033	1487	2816	2806	1318	1091
		318	397	463	424	841	1196	408	295
		413	495	331	334	310	203	383	390
150		1599	1447	1304	1103	1567	1551	1838	1653
		349	342	442	491	575	453	524	495
		217	247	277	294	206	214	352	330
OVERALL	MEAN	1449	1398	1492	1357	2023	2021	2023	2021
	SD	244	59	476	218	689	684	689	684
	n	3	3	3	3	3	3	3	3

Data are means ± standard deviation for a sample of muscle fibers (n) measured from a single biopsy. Fiber cross-sectional area was measured from a cross-section immunohistochemically stained for laminin. Biopsies were taken 2 weeks prior to restraint (pre) and 3 days after the restraint (post). Monkeys were restrained for a 14 day period at 1G in a chair similar to the chair in the biosatellite (Cosmos 2229)..

TABLE 3: *MYOSIN HEAVY CHAIN COMPOSITION*

MONKEY ID	BIOPSY	SOL % FAST MHC	MG % FAST MHC	TA % FAST MHC
151	PRE	23.7	93.5	85.8
	POST	39.0	92.6	79.5
906	PRE	13.9	75.5	93.2
	POST	34.4	89.4	93.0
FLIGHT	**PRE**	**18.6**	**84.5**	**89.5**
	POST	**36.7 ***	**91.0**	**86.3**
803	PRE	11.0	77.5	94.5
	POST	11.8	77.4	97.0
907	PRE	4.1	77.8	97.3
	POST	4.5	75.8	82.9
1401	PRE	12.5	75.6	93.4
	POST	12.0	70.6	93.0
CONTROL	**PRE**	**9.2**	**76.9**	**95.1**
	POST	**9.4**	**75.6**	**90.9**

The percentage of slow and fast MHC were determined by SDS-PAGE. Data presented in the table are percentage of fast MHC (type IIa and IIx) for biopsies taken prior to the flight (PRE) and 3 to 5 days after the flight (POST) from the two flight monkeys (151 and 906) and age-matched control monkeys (803, 907 and 1401). The means for the FLIGHT (n=2) and CONTROL (n=3) groups are shown in bold. Significant differences at $p < 0.10$ are indicated by an asterisk.

Fig 1

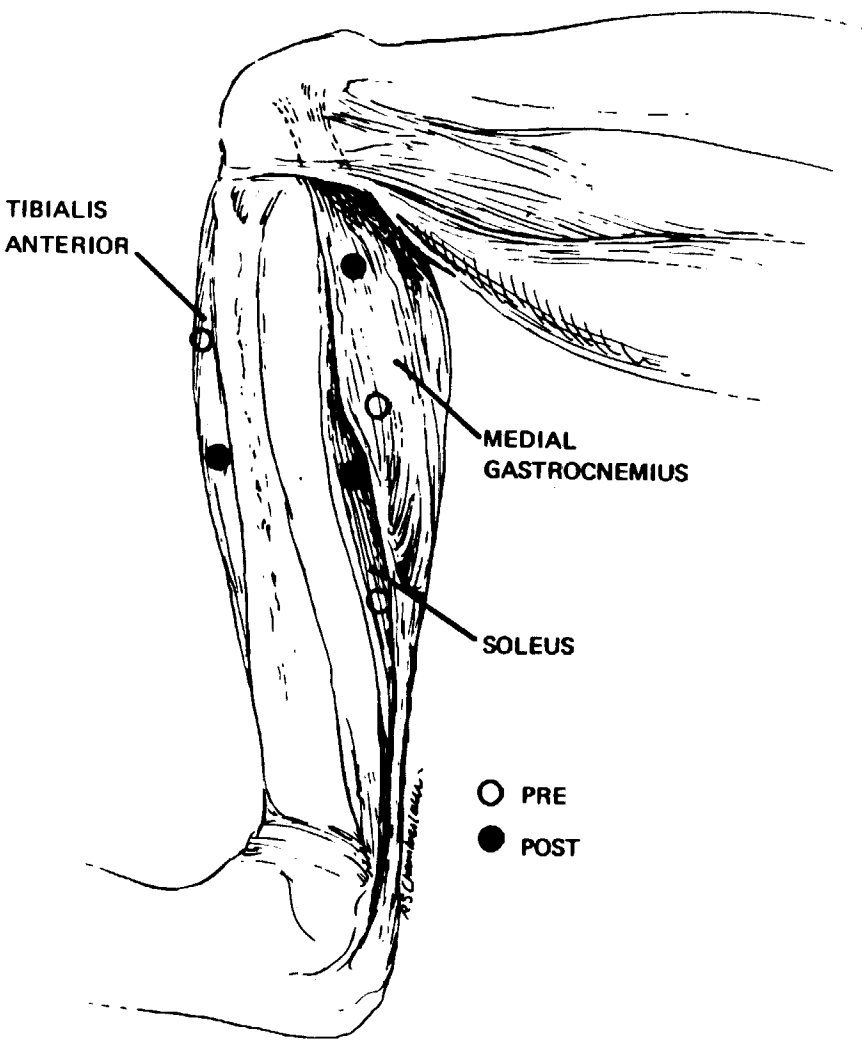

TIBIALIS
ANTERIOR
MEDIAL
GASTROCNEMIUS
SOLEUS
PRE
POST

Fig 2

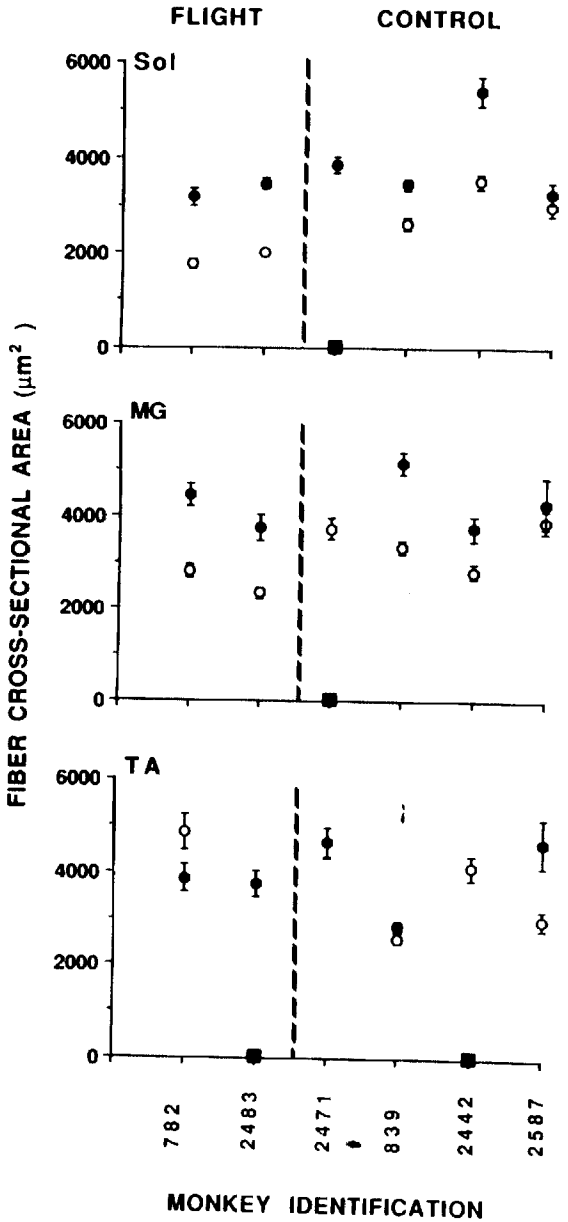

FLIGHT
CONTROL
Sol
MG
TA
6000
4000
2000
0
FIBER CROSS-SECTIONAL AREA (μm²)
782
2483
2471
839
2442
2587
MONKEY IDENTIFICATION

Fig 3

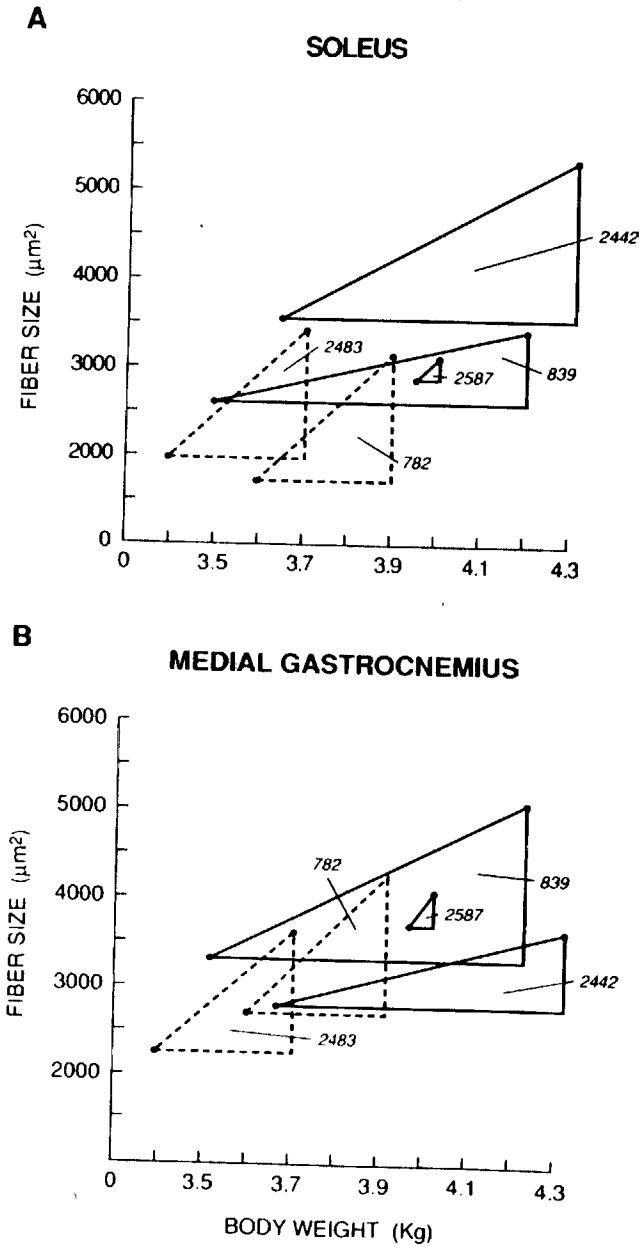

A
SOLEUS
FIBER SIZE (μm²)
6000
5000
4000
3000
2000
0
0
3.5
3.7
3.9
4.1
4.3
2442
2483
2587
839
782
B
MEDIAL GASTROCNEMIUS
FIBER SIZE (μm²)
6000
5000
4000
3000
2000
0
3.5
3.7
3.9
4.1
4.3
BODY WEIGHT (Kg)
782
839
2587
2442
2483

Fig 4

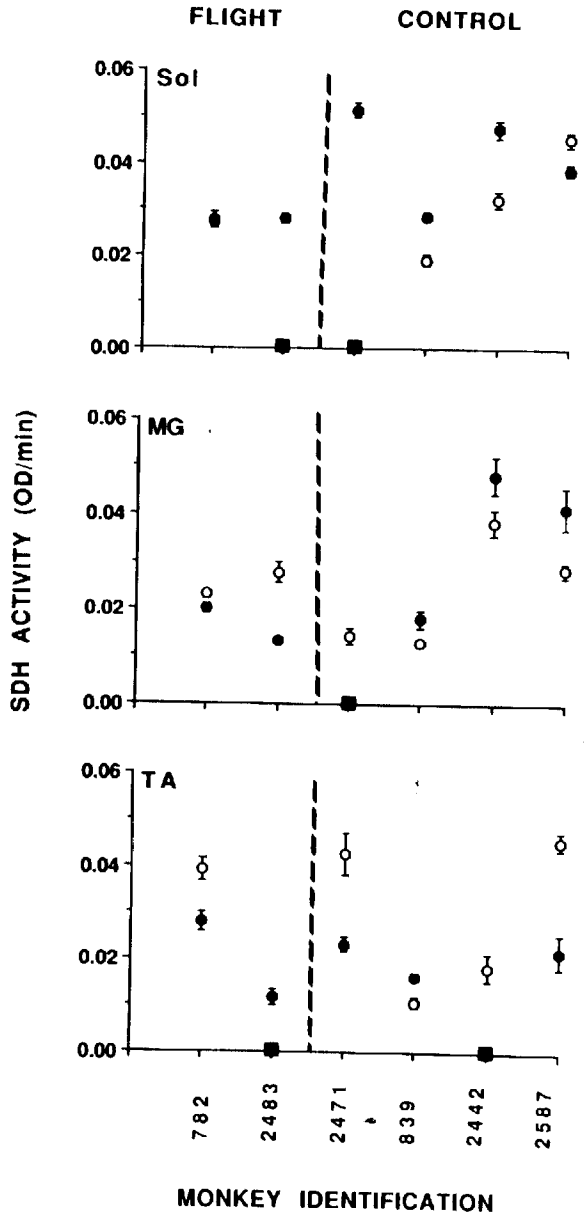
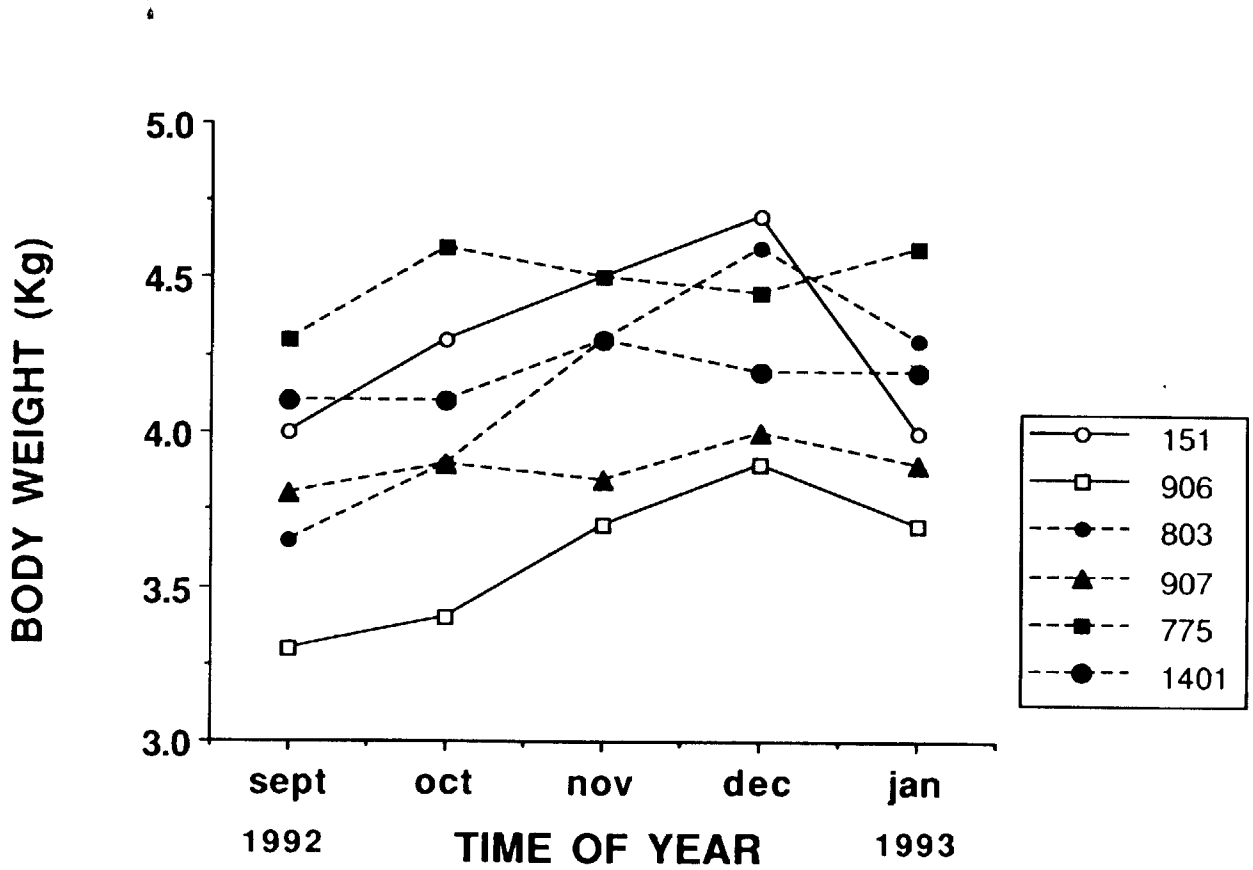

FLIGHT
CONTROL
Sol
MG
TA
SDH ACTIVITY (OD/min)
0.06
0.04
0.02
0.00
782
2483
2471
839
2442
2587
MONKEY IDENTIFICATION

Fig 5


BODY WEIGHT (Kg)
5.0
4.5
4.0
3.5
3.0
sept
1992
oct
nov
dec
jan
1993
TIME OF YEAR
151
906
803
907
775
1401

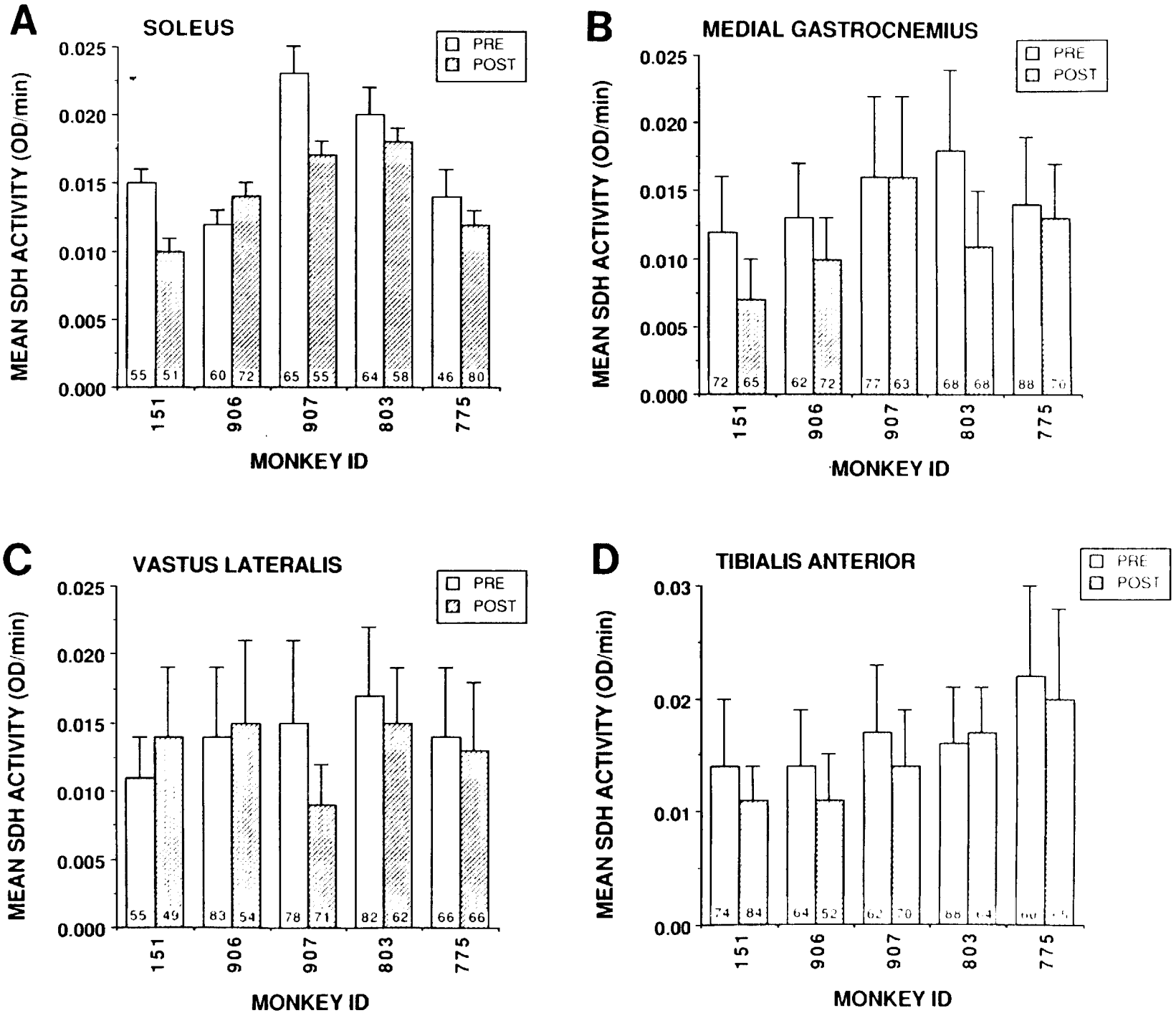

A
SOLEUS
PRE
POST
MEAN SDH ACTIVITY (OD/min)
0.025
0.020
0.015
0.010
0.005
0.000
55 51
60 72
65 55
64 58
46 80
151
906
907
803
775
MONKEY ID
B
MEDIAL GASTROCNEMIUS
PRE
POST
MEAN SDH ACTIVITY (OD/min)
0.025
0.020
0.015
0.010
0.005
0.000
72 65
62 72
77 63
68 68
88
151
906
907
803
775
MONKEY ID
C
VASTUS LATERALIS
PRE
POST
MEAN SDH ACTIVITY (OD/min)
0.025
0.020
0.015
0.010
0.005
0.000
55 49
83 54
78 71
82 62
66 66
151
906
907
803
775
MONKEY ID
D
TIBIALIS ANTERIOR
PRE
POST
MEAN SDH ACTIVITY (OD/min)
0.03
0.02
0.01
0.00
74 84
64 52
151
906
907
803
775
MONKEY ID

Fig 6

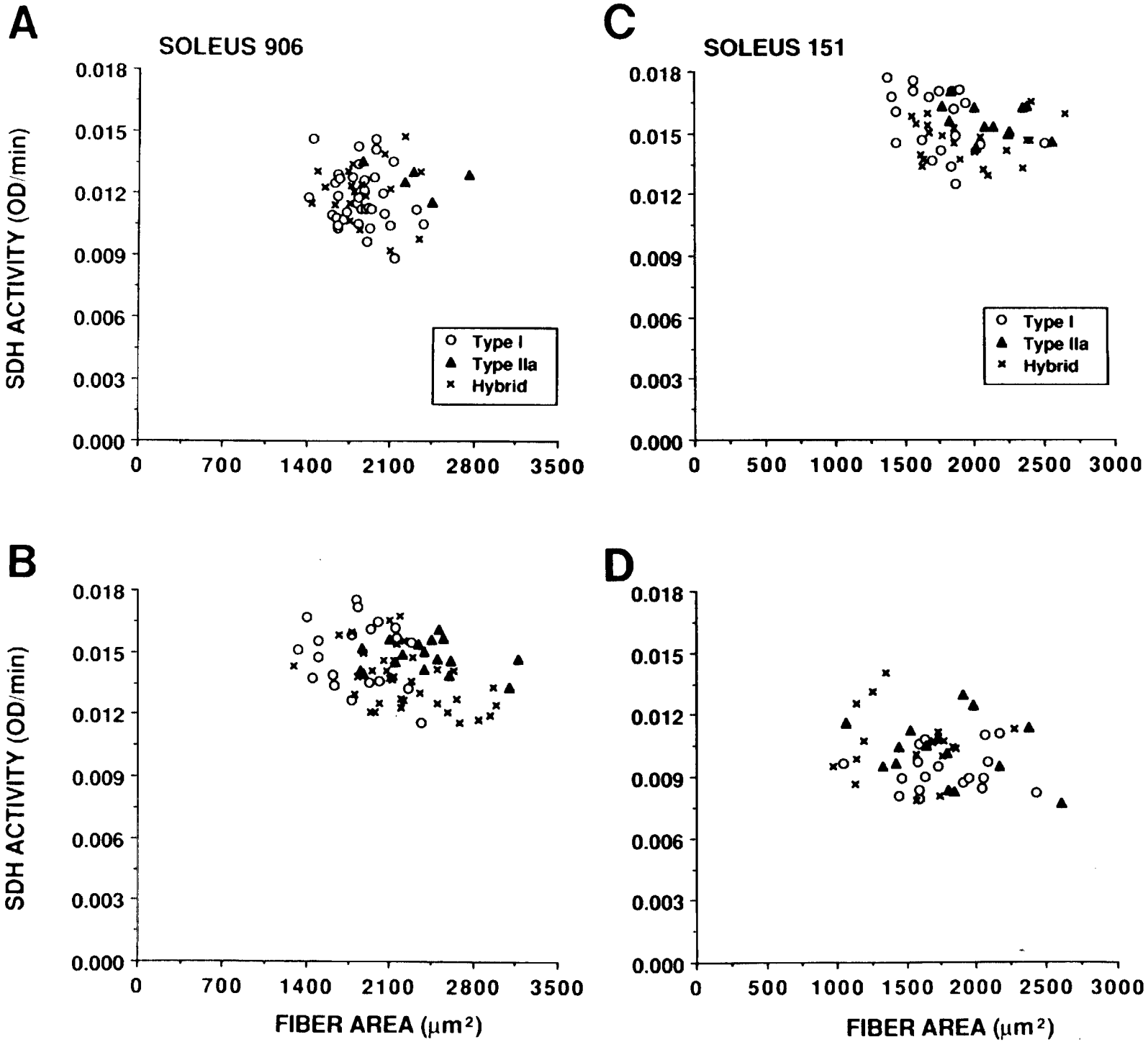

A
SOLEUS 906
SDH ACTIVITY (OD/min)
0.018
0.015
0.012
0.009
0.006
0.003
0.000
0
700
1400
2100
2800
3500
Type I
Type IIa
Hybrid
C
SOLEUS 151
0.018
0.015
0.012
0.009
0.006
0.003
0.000
0
500
1000
1500
2000
2500
3000
Type I
Type IIa
Hybrid
B
SDH ACTIVITY (OD/min)
0.018
0.015
0.012
0.009
0.006
0.003
0.000
0
700
1400
2100
2800
3500
FIBER AREA (μm²)
D
0.018
0.015
0.012
0.009
0.006
0.003
0.000
0
500
1000
1500
2000
2500
3000
FIBER AREA (μm²)

Fig 7

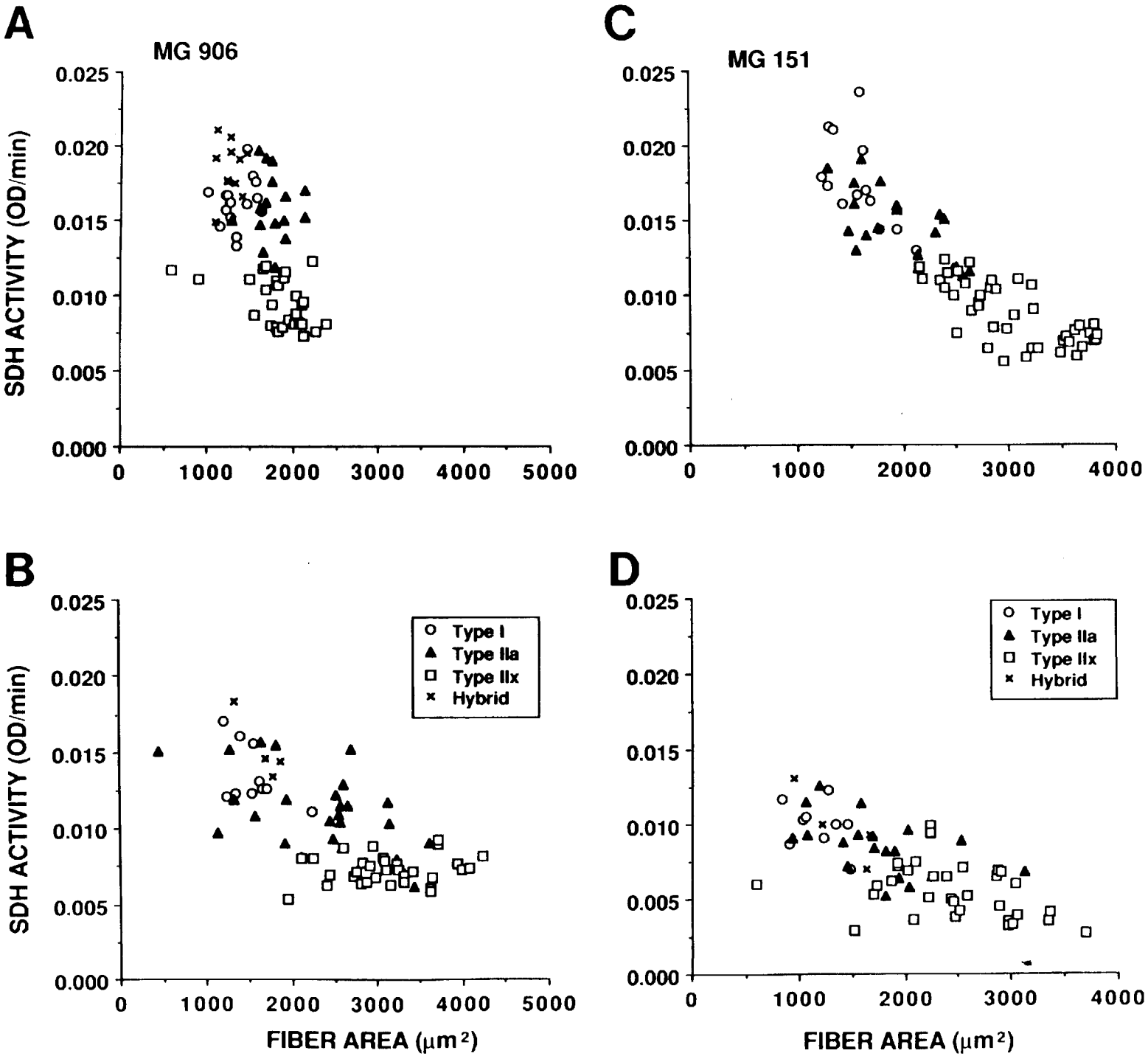

A
MG 906
SDH ACTIVITY (OD/min)
0.025
0.020
0.015
0.010
0.005
0.000
0
1000
2000
3000
4000
5000
C
MG 151
B
D
Type I
Type IIa
Type IIx
Hybrid
FIBER AREA (μm²)

Fig 8