FINAL SCIENCE REPORT SUMMARY OF RESEARCH FOR NAG 2-633

EXPERIMENT TITLE

Development of a Deltoid Shoulder Muscle Model for Rhesus Monkey Spaceflight Studies

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KEYWORD LIST

acromiodeltoid muscle, muscle fiber types, autoantibodies

ABSTRACT

The acromiodeltoid shoulder muscle was demonstrated to be a suitable model for spaceflight studies. The muscle contains a mixture of fast and slow fibers, permitting analysis of muscle fiber type specific changes. Two biopsy sites per muscle were identified that provided samples not degraded by the biopsy procedure. Both sites contained sufficient numbers fibers for determining changes in fiber type percentages and size. There was adequate bilateral symmetry regarding fiber type composition in the left and right muscles such that a total of four times points can be compared. The ESOP cage did not cause atrophy of deltoid muscle fibers; this means that microgravity-induced atrophy should be detectable. As expected, muscle excision stimulated muscle IgM and IgG muscle autoantibody production. Nonrestrained control animals suppressed this response whereas restrained monkeys showed an abnormally pronounced response indicative a compromised immune system. The presence of ESOP cage-induced changes in the immune response may mask spaceflight-induced effects. The ESOP cage modified the dominant hand operation of the PTS. These results demonstrate the importance of high fidelity ground based controls.

INTRODUCTION

The objective of this study was to demonstrate that the acromiodeltoid shoulder muscle in the rhesus monkey is a suitable model for examining the effects of spaceflight on the skeletal muscle of a subhuman primate subjected to simulated flight chair restraint. The deltoid abducts and flexes the arm against gravity. In microgravity, the muscle should atrophy because the limb becomes weightless. Following return to terrestrial gravity, the weight of the limb should cause eccentric contraction loading of the deltoid. Eccentric loading is known to produce muscle fiber damage, and astronauts experience delayed onset muscle soreness following return to Earth which indicates eccentric damage [Friden and Leiber, 1992; Riley et al., 1995]. On Earth, the deltoid muscles of chair restrained monkeys should not atrophy because the arms are not immobilized, although the range of motion in the abduction direction is limited. Two biopsies are needed from each muscle in order to characterize preflight properties and the postflight changes. Biopsy 1 must not degrade the structure of fibers in biopsy 2, and muscle fiber type properties in the two sites should be comparable to permit detection of changes. Furthermore, if the same sites have comparable properties in the left and right deltoid muscles, opportunity exists for monitoring postflight recovery as well.

Cosmonauts developed heart muscle autoantibodies following spaceflight [Tashpulatov et al., 1980]. Since functioning of the immune system is not normal during spaceflight, muscle autoantibodies may persist and exacerbate muscle damage [Taylor et al., 1986]. Muscle autoantibodies are known to be produced following surgery when skeletal muscles are cut. It is expected that the biopsy surgery 1 will release muscle proteins and stimulate an autoimmune response, involving IgM antibody production. Biopsy 2 reintroduces muscle proteins into the blood as a second immunogenic stimulus. A normally functioning system would suppress the autoantibodies. An abnormally functioning system may generate second

stage IgG autoantibodies [McCarty et al., 1984]. If restraint produces muscle atrophy in the less mobile lower limb muscles, the degenerating muscle may stimulate antibody production. An objective of this study is to assay for muscle antibodies 7 days following biopsy 1, after restraint, and 7 days following biopsy 2.

Monkeys skilled in the use of the Psychomotor Test System (PTS) show a hand dominance for operation of the joystick and collection of food pellet rewards [Hopkins et al., 1989]. It is expected that muscle fiber sizes would be larger in the acromiodeltoid of the dominant limb compared to the nondominant limb because of greater use. No differences are predicted for the percentages of slow, intermediate, and fast fibers in the two sides. The 18 days of chair restraint are anticipated to produce no atrophy of the dominant limb because of the continued use of the limb during PTS operation. Atrophy is expected in the nondominant limb because of reduced use during restraint.

METHODS

Two control (84-318, H-593) and three restraint (H-534, 85-309, H-602) adult male monkeys were used in this study. Chair restraint was performed for 18 days in the Experiment System for Orbiting Primates (ESOP). The left and right acromiodeltoid muscles were biopsied before (biopsy 1) and after restraint (biopsy 2). A portion of each biopsy was put on a labeled index card and quick frozen by quenching in liquid nitrogen-cooled Freon 22. The frozen muscles were shipped in a dry nitrogen shipper (-195°C) to the Medical College of Wisconsin (MCW) for histochemical analysis of muscle fiber type percentages and sizes as performed previously on rodent muscles [Riley et al., 1990]. Frozen cross sections were reacted for myofibrillar ATPase activity to resolve slow (lightly stained), fast (darkly reactive) and intermediate (moderately stained) fibers. Fiber cross sectional areas were measured by computer-assisted digitizing morphometry (R&M Biometrics System II). For each section, 100-200 fibers were sampled.

Blood was collected in heparinized tubes before the first biopsy, 7 days after biopsy 1, before restraint, immediately following restraint, and 7 days after biopsy 2. The tubes were spun, and the plasma was removed, placed in 1.5 ml Microfuge tubes and frozen at -20°C. The frozen samples were shipped overnight on dry ice to MCW. During screening for antimuscle antibodies, the plasma was thawed and applied at dilutions of 1/10, 1/20, 1/40, and 1/80 on 8 μm cryostat sections of rat plantaris and rhesus monkey deltoid muscle. Following phosphate buffered saline (PBS) washes, bound monkey IgM was detected by indirect immunofluorescence staining with 1/50 dilutions of goat anti-monkey IgM FITC, and monkey IgG was localized with goat anti-monkey IgG FITC. Plasma was omitted in control sections to determine the background levels of labeling of the fluorescence secondary antibodies alone. Immunostaining intensities were quantitated by recording the exposure times of the photomicroscope light meter. Photomicrographs were taken to document the patterns of immunostaining.

Continuous (1 frame/sec) video of the upper body and head was collected throughout the 18

day period of restraint. Upper limb movements were quantified during hours 4-12 of the light cycle for each restrained monkey on restraint days 1, 5, 10, 13 and 15 by counting the number of left and right arm reaches/min during the first 15 minutes of each hour of the 9 hour periods sampled per day.

RESULTS

Muscle fiber types

The mean percentages of slow, intermediate and fast fibers were similar in the deltoid muscles of nonrestrained controls and restrained test animals (Table 1). Fiber type percentages were also similar in the left and right muscles (Table 2). Muscle fiber sizes were not different in control and restrained monkeys (Table 3). There was no indication that biopsy 1 caused denervation, devascularization or physical damage to fibers in the biopsy 2 site.

TABLE 1

Muscle Fiber Type Percentages As Defined by Myofibrillar ATPase Activity In Sections Of Deltoid Muscles From ARRT Control and Restrained Monkeys

GROUPS	SLOW	INTERMED.	FAST
Controls (n=5)	27±2	27±6	46±6
Restrained (n=3)	27±4	32±8	41±4

SLOW - fibers lightly stained for alkaline myofibrillar ATPase activity
INTERMED. - moderately stained fibers
FAST - darkly stained fibers

TABLE 2

Comparison of Muscle Fiber Type Percentages In The Left and Right Deltoid Muscles of ARRT Control and Restrained Monkeys

GROUPS	SLOW	INTERMED.	FAST
Controls (n=4)			
Left	27±4	22±9	53±11
Right	28±1	31±6	41±4
Post restraint (n=3)			
Left	28±7	34±12	39±5
Right	27±2	30±5	43±4

TABLE 3

Muscle Fiber Type Areas (μm^2) As Measured In Alkaline Myofibrillar ATPase Reacted-Sections of Deltoid Muscles From ARRT Control and Restrained Monkeys

GROUPS	SLOW	INTERMED.	FAST
Controls (n=5)	2878±634	3171±1025	4731±586
Restrained (n=3)	2790±675	3639±720	4689±138

Muscle autoantibody immunoreactivity

Increased immunoreactivity in rat plantaris sections presented as elevated cytoplasmic staining of all muscle fibers with and without indications of myofibril cross striation localization. Extracellular immunostaining was also increased, indicating immunoreactivity in capillaries. Immunoreactivity was similar within monkey deltoid fibers, except that extracellular staining was always high because immunoglobulins normally were present. No consistent differences in staining patterns were recognized for IgG and IgM distributions.

Seven days after biopsy 1, three of five monkeys showed mildly elevated IgM and mild to high IgG immunoreactivity (Tables 4,5). This immunoreactivity decreased with the passage

of time in the control non-restrained monkeys and in all but one of the three restrained monkeys. Seven days following biopsy 2, control monkeys had the expected decreased immunoreactivity, except for mild elevation of IgM in one animal. In contrast, restrained monkeys showed marked elevation of one or both immunoreactive immunoglobulin species.

TABLE 4

Percentage Change From A Previous Screening Point in Monkey IgM Anti-Muscle Antibody Immunostaining

Animals	7 d Post Biopsy 1	Post Restraint	7 d Post Biopsy 2
Controls			
84-318	9.7	4.7	-10.9
H-593	-6.7	-10.9	4.7
ESOP Group			
H-534	0.0	-10.9	28.7
85-309	9.7	-2.3	12.2
H-602	12.2	-8.8	0.0

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7 d Post Biopsy 1 - plasma sample drawn 7 days after biopsy 1
Post Restraint - sample taken after 18 days in ESOP
7 d Post Biopsy 2 - sample taken 7 days after biopsy 2

TABLE 5

Percentage Change From A Previous Time Point in Monkey IgG Anti-Muscle Antibody Immunostaining

Animals	7 d Post Biopsy 1	Post Restraint	7 d Post Biopsy 2
Controls			
84-318	20.3	4.7	-25.9
H-593	0.0	4.7	-10.9
ESOP Group			
H-534	-18.7	7.6	-9.2
85-309	7.2	-10.9	28.8
H-602	12.2	-3.5	28.8

Frequency of left and right arm movements

The restrained monkeys operated the PTS and received food pellet rewards in a dispenser located in a recess in the left wall of the cage. They retrieved pellets mostly using the right arm (Table 6). Interestingly, monkey 85-309 used the left arm much more extensively than the other two animals during the first week. This was a more awkward and difficult reach than reaching across with the right arm. Gradually, this monkey shifted to dominant use of the right arm.

TABLE 6

Use of the Right and Left Arms During ESOP Cage Restraint
(Mean number of reaches per minute ± SD)

Monkey		Day 1	Day 5	Day 10	Day 13	Day 15
H602	Right	1.4±1.8	1.4±2.2	2.2±2.5	5.0±5.1	3.4±4.5
	Left	0.2±0.2	0.2±0.3	0.1±0.1	0.4±0.6	0.1±0.1
H534	Right	3.2±4.5	3.3±3.7	4.4±6.5	3.1±4.1	no video
	Left	0.4±0.4	0.9±1.0	0.5±0.6	0.4±0.4	no video
85-309	Right	1.7±1.7	4.5±1.7	4.2±3.9	3.9±3.7	3.8±3.6
	Left	0.7±0.5	2.5±1.0	0.9±0.7	0.3±0.3	0.6±0.3

DISCUSSION & CONCLUSIONS

The acromiodeltoid (middle deltoid) muscle is well suited for spaceflight studies. It contains a mixture of fast and slow fiber types. Spaceflight is expected to produce preferential atrophy of slow fibers, and these atrophic fibers are expected to be susceptible to reloading damage. Spaceflight-induced atrophy should be detectable because the fibers do not atrophy in the ground base ESOP restraint model. Two biopsies per muscle are obtainable without interference of one biopsy with the integrity of the fibers in the second biopsy site. Tissue removal produced no observable problems with normal limb movements. Biopsy 1 is taken in the anterior portion of acromiodeltoid and biopsy 2 from the posterior region. Reversal of this pattern in earlier tests resulted in unwanted denervation atrophy and muscle fiber damage. The fiber type composition in the two sites is comparable which means that preflight and postflight comparisions can be made regarding fiber sizes and fiber type percentages. The same sites in the contralateral muscle also have comparable properties which means that recovery can be studied by taking biopsies 3 and 4 from this muscle. Concern was raised that the deltoid is vulnerable to physical injury as the animal moves through its environment. Bruising injuries would elevate the proportion of aberrant muscle

fibers, making the muscle less suitable for detecting spaceflight induced degeneration. This is not a problem because only $0.9 \pm 0.3\%$ aberrant fibers were present in 4 control animals. Our previous studies showed that 4-7% aberrant fibers are expected following spaceflight.

While chair restraint does not alter the parameters of the muscle examined in this study, there is a significant effect on muscle antibody production. Restraint does not elicit muscle antibodies, but restrained monkeys exhibit an abnormal elevation of muscle antibodies following post restraint biopsy 2. Spaceflight is also expected to compromise suppression of muscle autoantibodies. This change may not be detectable because restraint alone produces the expected effect.

Chair restraint modified arm use behavior [Hopkins et al., 1989]. The preferred use of the left arm to retrieve food pellet rewards was changed to right hand use after one week in the restraint cage. It appeared that the location of the pellet cup caused a more awkward use of the left arm and retrained the monkeys to favor the right arm. The deltoid fibers in the dominant arm were not larger than those in the nondominant arm. This indicates that muscle fiber sizes are not influenced by lightly loaded voluntary movements. This conclusion was further supported by the absence of atrophy in the little used nondominant limb during restraint.

ACKNOWLEDGMENTS

Special thanks are given to Shawn Bengston, Denice Helwig and Samatha Edmonds for supporting this study. Funded by NAG 2-633.

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