Recovery of the Dog Quadriceps after 10 Weeks of Immobilization Followed by 4 Weeks of Remobilization

*Richard L. Lieber, Thalia McKee-Woodburn, and David H. Gershuni

*Division of Orthopaedics and Rehabilitation, Department of Surgery, Veterans Administration Medical Center and University of California, San Diego, California, U.S.A.

Summary: Skeletal muscle fiber areas were measured in three heads of the dog quadriceps after 10 weeks of immobilization followed by 4 weeks of remobilization. Two-way analysis of variance demonstrated a significant decrease in both type 1 (p < 0.005) and type 2 (p < 0.001) fiber area. However, there was no significant difference among the three heads of the quadriceps (p > 0.2). Although muscle fiber areas had not returned to control levels following remobilization, the area fraction of perimysial and epimysial connective tissue was not significantly different from control values (p > 0.15). These data suggest that although the degree of muscle atrophy following 10 weeks of immobilization is severe and muscle specific, following 4 weeks of remobilization, muscles uniformly recover to about 70% of control values. Key Words: Immobilization—Remobilization—Quadriceps—Dog—Muscle fiber areas.

Skeletal muscles atrophy when the muscle use level is decreased secondary to myopathy (17), suspension hypokinesia (4, 21), spinal cord transection (10, 11, 14, 16), and limb immobilization (2, 6, 7, 9, 13, 20). Under such conditions, investigators have demonstrated that muscles composed mainly of type 1 fibers (i.e., "slow" muscles) atrophy to a greater extent than muscles composed mainly of type 2 fibers (i.e., "fast" muscles) (2, 6, 7, 9, 13, 20). Additionally, antigravity muscles atrophy to a greater extent than their antagonists (2, 6, 13, 20).

In a recent study of dog quadriceps muscles, we demonstrated that three heads of the quadriceps; the vastus medialis (VM), vastus lateralis (VL), and rectus femoris (RF), did not atrophy to the same extent, but responded differentially to the immobilization procedure (12). These results indicated that a blanket concept of "slow fiber atrophy" did not apply to all muscles. Rather, it was a combination of factors that determined the muscular response to decreased use.

Although a plethora of immobilization studies exists, skeletal muscle recovery following immobilization, especially long-term immobilization, such as is often required clinically, has not been well studied. In a study of short-term immobilization (i.e., 5 weeks), Booth and Seider (3) measured decreased muscle force-generating capacity and biochemical properties following immobilization, which recovered to control values after only 14 days of remobilization. However, long-term immobilization (i.e., 10–12 weeks) resulted in muscle atrophy that required more time for recovery. Booth and Seider (3) documented that the soleus (SOL) muscle (composed of predominantly slow-contracting fibers) did not recover full contractile function until about 120 days following 90 days of immobilization. However, Fitts and Brimmer (8) reported that the SOL and extensor digitorum longus (EDL) both recov-
erected much more rapidly, within 60 and 90 days, respectively, after 90 days of immobilization. It is clear, therefore, that the response of fast and slow muscles is not well understood and that muscular recovery from long-term immobilization differs qualitatively in comparison with recovery from short-term immobilization. There have been no studies of muscle recovery that included analysis of muscle fiber areas. Such morphometric investigations have not been shown to be more sensitive to subtle changes occurring at the cellular level compared to whole-muscle physiological or biochemical studies, where the properties of the various fibers are averaged together (11). For example, muscle tetanic tension can decrease due to either slow (type 1) or fast (type 2) fiber atrophy, or both. Similarly, contractile speed can decrease due to type 1 fiber atrophy, type 2 fiber hypertrophy, or both. Clearly, such adaptations represent different muscular responses.

The dog model is well-suited to studies of immobilization and remobilization, as three of the quadriceps heads (VM, VL, and RF) contain nearly identical architectures and fiber lengths (15,23), but differ in fiber type percentage and number of joints crossed (1). The RF acts both as a knee extensor and hip flexor and is composed of about 50% type 1 fibers. The VM and VL both function as knee extensors only, but the VM contains about 50% type 1 fibers, whereas the VL contains only about 20% type 1 fibers (1,18). This model thus allows comparison between the VM and VL, which can be immobilized at precisely the same length but contain different percentages of type 1 and type 2 fibers. Similarly, comparisons between the RF and VM can be made, which have similar fiber type percentages but cross different joints. As dog muscles contain no type 2B fibers (1,18), unequivocal identification of fiber types can be made from a single histochemical stain for myofibrillar adenosine triphosphatase (ATPase) activity. Finally, the external skeletal fixation procedure used in this study permits more reproducible setting of joint angle between animals and a produces more rigid fixation than does cast immobilization.

The purpose of this study, therefore, was to compare muscle fiber morphometric properties among three heads of the dog quadriceps after long-term (i.e., 10 weeks) immobilization, followed by 4 weeks of remobilization. Specifically, we were interested in the muscle fiber area changes that would accompany this type of treatment. In muscle atrophy, the decrease in muscle size is, to a large extent, due to the decrease in the area of the individual fibers (2).

**MATERIALS AND METHODS**

Immobilization methodology and duration were as previously described (12). Briefly, the right knees from four mature mongrel dogs (mass range 20–25 kg) were immobilized at 90° of flexion for 10 weeks using an external skeletal fixator. The fixator was composed of two threaded Steinman pins placed transversely in the femur and two pins placed across the tibia. All four pins were then clamped to a medial and lateral side bar. On both legs, arthrotomies were performed to mimic the common clinical occurrence prior to postoperative immobilization. The left leg served as a nonimmobilized control. All procedures were performed in accordance with the National Research Council's guide for the care and use of laboratory animals. Following 10 weeks of immobilization, legs were remobilized for 4 weeks. During this 4-week period, normal cage activity was permitted, and daily 1-h walking/running outings were encouraged. Normal weight-bearing resumed spontaneously within about 1 week. Following remobilization, open biopsies (approximate size 1.5 cm³) were taken with the overlying fascia from a well-defined superficial portion of the VM, VL, and RF, 10 cm proximal to the knee and distant from the Steinman pin tracts, as previously described (12). The muscle biopsy was stretched to approximate rest length (fascia used as reference), frozen in isopentane cooled by liquid nitrogen (−159°C), and stored at −80°C for subsequent quantitative histological and enzyme histochemical analyses as previously described (11). Fibers were classified as either type 1 or type 2, depending on their optical density following myofibrillar ATPase staining at pH 9.4.

Muscle morphometric parameters were determined according to the point counting stereological methods developed by Weibel (22). The following parameters were quantified: type 1 fiber area (μm²); type 2 fiber area (μm²); percentage of each fiber type; and area fraction of endomysial and perimysial connective tissue (%).

Statistical analysis of the morphometric parameters was accomplished using the BMDP statistical package (5). A two-way analysis of variance (ANOVA; Program P7D) was used, with side (treated or control) and muscle (VM, VL, or RF) as the grouping variables. In this way, simultaneous
comparisons between the three muscles and two legs could be made, and the potential interaction between side and muscle could be investigated. Subsequent paired comparisons between all treated muscles (three paired comparisons) and between the treated and control side for each muscle (three paired comparisons) were made a posteriori using the Bonferroni approximation for multiple paired comparisons (19). All statistical results were considered significant for $p < 0.05$.

RESULTS

In general, specimens from the treated side displayed muscle fibers with a more variable distribution of size, shape, and staining intensities relative to control samples. Neither necrotic nor inflammatory cells were observed. The dramatic differences between muscles that were previously observed in response to immobilization (12) were not observed in the present study.

No difference in type 1 or type 2 fiber areas was observed among any of the control muscles (Table 1). Fiber type percentages of control muscles obtained agreed with those reported by Armstrong et al. (1) for the superficial portion of all muscles, implying that no sampling problems were encountered. Ten weeks of immobilization followed by 4 weeks of remobilization resulted in a significant decrease (or not full recovery) of both type 1 ($p < 0.005$) and type 2 ($p < 0.001$) fiber areas (Table 1; Figs. 1 and 2). Interestingly, no significant interaction terms were obtained in the two-way ANOVA, which was the most significant aspect of the previous study in that it documented the differential effect of immobilization on the three muscles. In the present study, recovery following immobilization was not a function of muscle or fiber type.

No significant difference in the area fraction (22) of endomysial and perimysial connective tissue percentage (percent extracellular space) was observed following treatment. Thus, the previously elevated area fraction of connective tissue (about 20%) (12), which was observed following immobilization, returned to control levels (about 10%, Table 1) following the remobilization period.

A significant difference in fiber type percentage (Table 1) was observed among muscles ($p < 0.05$), but not among treatments (immobilized/remobilized versus control). This reflected the basic differences among muscles rather than any effect of the treatment itself.

DISCUSSION

This study demonstrated that (a) the 4 weeks of remobilization that followed 10 weeks of immobi-

### TABLE 1. Morphometric parameters of dog quadriceps muscles

<table>
<thead>
<tr>
<th>Parameter (two-way ANOVA values leg/muscle)</th>
<th>Vastus medialis</th>
<th>Vastus lateralis</th>
<th>Rectus femoris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left (n = 4)</td>
<td>Right (n = 4)</td>
<td>Left (n = 4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right (n = 4)</td>
</tr>
<tr>
<td>Type 1 area ($\mu m^2$) (0.004/0.239)</td>
<td>2,104</td>
<td>1,521</td>
<td>1,990</td>
</tr>
<tr>
<td></td>
<td>$\rightarrow$</td>
<td></td>
<td>$\rightarrow$</td>
</tr>
<tr>
<td></td>
<td>1,157</td>
<td></td>
<td>2,103</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,183</td>
</tr>
<tr>
<td>Type 2 area ($\mu m^2$) (0.000/0.791)</td>
<td>2,233</td>
<td>1,477</td>
<td>2,255</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\rightarrow$</td>
</tr>
<tr>
<td></td>
<td>1,183</td>
<td></td>
<td>2,103</td>
</tr>
<tr>
<td>Percent extracellular space (% of total)</td>
<td>10.5</td>
<td>14.0</td>
<td>12.3</td>
</tr>
<tr>
<td>(0.157/0.605)</td>
<td></td>
<td></td>
<td>$\rightarrow$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.5</td>
</tr>
<tr>
<td>Percent type 1 fibers (0.535/0.040)</td>
<td>19.0</td>
<td>26.4</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\rightarrow$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.0</td>
</tr>
<tr>
<td>Percent type 2 fibers (0.535/0.040)</td>
<td>81.0</td>
<td>73.6</td>
<td>85.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\rightarrow$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72.0</td>
</tr>
</tbody>
</table>

* Two-way ANOVA values in the parameter column represent the $p$-value for the main leg and muscle effects, respectively. There were no significant interaction terms. Values in the table are connected by lines ending in "$\rightarrow$" if the values are not significantly different or in "$\times$" if means are significantly different ($p < 0.05$).

RESPONSE OF DOG QUADRICEPS TO REMOBILIZATION

FIG. 1. Type 1 (slow) muscle fiber area from three heads of the quadriceps muscle. Data from immobilized legs are from the previous study (12) for comparison. Note that upon remobilization, the type 1 fiber area is still below control values.

FIG. 2. Type 2 (fast) muscle fiber area from three heads of the quadriceps muscle. Data from immobilized legs are from the previous study (12) for comparison. Note that upon remobilization, the type 2 fiber area is still below control values.

smaller recovery because the immobilization effect is relatively small to begin with. Subsequent remobilization does not represent a large change in use level, and the muscle fibers remain about the same size.

Muscle fiber areas are relatively equivalent among muscles prior to immobilization, in spite of the fact that different muscles are used to different extents. By changing the level of use to the same absolute level, the differential change in use results in a differential atrophic response. Following remobilization, the differential increase in use again causes a differential response. However, the end result is to bring all of these fiber sizes back to parity. This concept is consistent with the familiar Wolf's law, which applies to other connective tissues such as tendon and ligament.

In response to immobilization, the VM demonstrated a significant increase in fast fiber percentage (11). This was attributed to the prolonged level of decreased use, which has been shown to result in a slow to fast fiber type transformation in other models of decreased use, such as spinal cord transection (11) and immobilization (12). Interestingly, following remobilization, the VM fiber type distribution returned to control values, in spite of the fact that the VM remained in an atrophic state. This implies that on increased use, fiber type transformation (fast to slow) precedes hypertrophy. Conversely, we would hypothesize that following prolonged immobilization, muscle fibers would atrophy before transformation. Thus, the cellular control mechanism for regulating the amount and type of myosin synthesized may be dependent on the state of the tissue itself.

Clinically, the knee is frequently immobilized for periods in the range of 10 weeks, as in this experimental study. After such immobilization and significant quadriceps atrophy, it is clear that only moderate recovery occurs after 4 weeks of active exercise. A longer time period and more aggressive therapy is presumably required to achieve a more complete restitution of muscle morphology.

Acknowledgment: This work was supported by the Veterans Administration (D.H.G., R.L.L.) and USPHS/NIH Grant AR35192 (R.L.L.). The authors thank Sonny Schacher for her excellent technical assistance.

REFERENCES