Rabbit supraspinatus tendon detachment

Effects of size and time after tenotomy on morphometric changes in the muscle

Jaroslaw Fabis[1], Marian Danilewicz[2] and Aleksandra Omulecka[2]

[1]Chairman, Clinic of Orthopaedics, Medical University of Lódz, [2]Department of Pathology, Morphometry Division, Medical University of Lódz. Correspondence: Dr. Fabis, 95-071 Rabien 78, Poland. E-mail: hobo@psk2.am.lodz.pl
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ABSTRACT – We studied the effects of size and time after rabbit supraspinatus tenotomy (group A – small tenotomy, group B – large tenotomy) on muscle morphometric changes in 48 rabbits. Animals were killed 6 (subgroups A1 and B1), 12 (subgroups A2 and B2) and 24 weeks (subgroups A3 and B3) after tendon detachment. Statistically significantly greater increases in interstitium volume were noted in subgroups A1-A3 and B1-B3 than in controls. Reductions in type I and II fiber diameters were mainly due to the length of observation. However, statistically significant differences in comparison with controls appeared earlier after large tenotomy. The size of the tenotomy primarily affected muscle fiber composition. Our results suggest that these changes were caused by fiber transformation from type I to type II and vice versa.

These findings indicate that the interdigitations between the supraspinatus and infraspinatus and between the supraspinatus and subscapularis tendons are important in dynamics and the degree of morphometric changes in the rabbit supraspinatus after tenotomy.

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Only a few studies have been done on the pathomorphological changes in the supraspinatus muscle after experimental detachment of its tendon (Björkenhaim 1989) or rotator cuff tear (Goutallier et al. 1994, Nakagaki et al. 1996). Such basic knowledge may be helpful in understanding the recovery potential of the muscle. We evaluated the effects of the size of the rabbit supraspinatus tendon detachment and the length of observation on morphometric changes in the muscle.

Animals and methods

We used 55 adult male rabbits of mixed race (weight 3.7–4.8 kg). They were anesthetized with an intravenous vetbutal injection (30 mg/kg) supplemented by skin infiltration with 1% lidocaine. The left shoulder was exposed and about 3 mm of the supraspinatus tendon was resected from the greater tubercle—group A (small tenotomy), and detached from the greater tubercle as well as from the subscapularis and infraspinatus tendons (cut interdigitation-length of split about 1 cm)—group B (large tenotomy). 7 rabbits were not operated on but were used as controls.

Tissue samples

The animals were killed 6 weeks (subgroups A1 and B1), 12 weeks (subgroups A2 and B2) and 24 weeks (subgroups A3 and B3) after the of tendon detachment. Full-thickness muscle samples taken from the middle of the muscle, were frozen in isopentane and cooled to about –150 °C with liquid nitrogen. Care was taken to ensure that the cuts were perpendicular to the long axis of the muscle fibers. The transverse 10 µm thick cryostat sections were stained with hematoxylin and eosin (HE) and Gomori's trichrome. Histochemical reaction for adenosine triphosphatase (ATPase) was done with preincubation at pH 9.4.

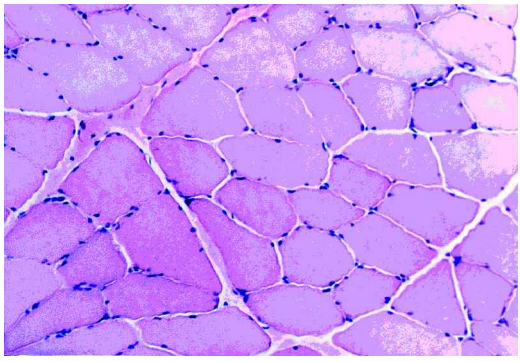

Figure 1. Normal (control) supraspinatus (HE, ×250).

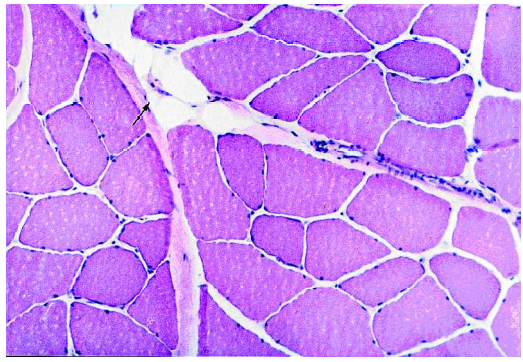

Figure 2. Supraspinatus 6 weeks after large tenotomy - increase in endomysial and perimysial connective tissue as well as fat tissue (arrow) (HE, ×250).

Morphometry

Histological morphometry was performed with an image analysis system consisting of a computer equipped with an Aver 2000 card (frame grabber, true color, real time), color TV camera, linked to a microscope. This system was programmed (program MultiScan, CSS-Poland) to calculate the distance between 2 points and a regulated area of a structure using a stereological net (with a regulated number of points). The cross-sectional areas of type I and type II fibers (samples stained with ATPase, pH 9.4) as well as the interstitial volumes (samples stained with Gomori's trichrome) were determined using a point counting method that is an adaptation of the principles of Weibl (1979) using a stereological net. The point spacing was 16 µm. The total number of points in a net was 169, and the total area 0.37 mm^2. We studied 10 fields of the muscle under the above-described net. We measured the smallest diameter of each fiber type (I and II, samples stained with ATPase, pH 9.4) in 150 fibers of each specimen (magnification ×400).

Statistics

Differences between groups were tested using analysis of variance (one-way ANOVA) and the post-hoc LSD test, preceded by evaluation of normality and Levene's test. The Mann-Whitney U-test was used, when appropriate. Results were considered statistically significant if $p < 0.05$.

Table 1. Interstitium volume of the rabbit supraspinatus muscle

		Interstitium volume (%)		
Group	n	Mean	Range	SD
Control	7	2.9	2.2–4.0	0.6
A1	10	9.1 [a]	5.3–12	2.1
A2	8	9.1 [b]	6.6–12	1.5
A3	6	9.5 [c]	6.6–12	0.9
B1	7	13 [d]	9.7–17	3.0
B2	9	21 [e]	9.7–31	6.1
B3	8	22 [f]	15–34	6.6

[a] $p < 0.0007$ v. (versus) control; $p < 0.01$ v. B1
[b] $p < 0.002$ v. control; $p < 0.002$ v. B2
[c] $p < 0.003$ v. control; $p < 0.002$ v. B3
[d] $p < 0.002$ v. control; $p < 0.01$ v. B2; $p < 0.008$ v. B3
[e] $p < 0.001$ v. control
[f] $p < 0.002$ v. control

Results

The results of the light microscopy examinations at 6, 12 and 24 weeks after small and large tenotomies were very similar. We found reductions in the diameters of the muscle fibers. Focal necrosis of single muscle fibers with inflammatory infiltration, mostly by phagocytes, was seen. The endomysial and perimysial connective tissue and fat tissue between the muscle fibers were increased.

The interstitium volume was significantly greater in all subgroups A1–A3 and B1–B3 than in the controls (Figures 1 and 2) (Table 1).

The fibers of type I diameter gradually decreased in groups A and B over time (Table 2) and the

Table 2. Diameters of type I and type II fibers in the rabbit supraspinatus muscle

Group	n	Fiber type and diameter (μm)					
		Mean		Range		SD	
		I	II	I	II	I	II
Control	7	49	50	37–55	41–61	6	7
A1	10	43	45	29–52	31–77	9	14
A2	8	40 [a]	42	32–50	33–55	6	7
A3	6	39 [b]	38 [f]	29–55	31–46	11	6
B1	7	39 [c]	42	28–52	24–55	8	11
B2	9	40 [d]	42 [g]	32–48	28–55	6	8
B3	8	38 [e]	36 [h]	29–46	21–51	6	10

[a] $p < 0.04$ v. control
[b] $p < 0.04$ v. control
[c] $p < 0.02$ v. control
[d] $p < 0.02$ v. control
[e] $p < 0.01$ v. control
[f] $p < 0.02$ v. control
[g] $p < 0.04$ v. control
[h] $p < 0.003$ v. control

Table 3. The percentages of type I and type II fibers in the rabbit supraspinatus muscle of the control group and subgroups A1-A3 and B1-B3

Group	n	Fiber type distribution (%)					
		Mean		Range		SD	
		I	II	I	II	I	II
Control	7	20	80	12–32	67–88	7.0	7.0
A1	10	10 [a]	90 [f]	6.0–18	82–94	4.0	4.0
A2	8	10 [b]	90 [g]	6.4–14	86–94	3.0	3.0
A3	6	11 [c]	89 [h]	8.0–17	83–93	3.0	3.0
B1	7	12 [d]	82 [i]	7.4–20	80–93	4.0	4.2
B2	9	15 [e]	85 [j]	8.0–23	77–92	5.0	5.0
B3	8	29	71	15–48	52–85	10	10

Fiber type I:
[a] $p < 0.004$ v. control
[b] $p < 0.003$ v. control
[c] $p < 0.02$ v. control; $p < 0.003$ v. B3
[d] $p < 0.02$ v. control; $p < 0.002$ v. B3
[e] $p < 0.0009$ v. B3
Fiber type II:
[f] $p < 0.004$ v. (versus) control
[g] $p < 0.003$ v. control
[h] $p < 0.02$ v. control; $p < 0.003$ v. B3
[i] $p < 0.02$ v. control; $p < 0.002$ v. B3
[j] $p < 0.0009$ v. B3

difference was significant between subgroups A2, A3 and B1-B3 versus the controls (Table 2). As regards type II fibers statistically significant differences were noted between subgroups A3, B2 and B3 versus the controls (Table 2).

The morphometric study showed changes in the proportion of type I and type II fibers in groups A and B (Figures 3 and 4) (Table 3).

Discussion

We found no morphometric studies about the effects of time and size of tenotomy on muscle morphometric changes in an animal model of rotator cuff tear. An increase in the volume of intramuscular connective tissue in an animal model of muscle

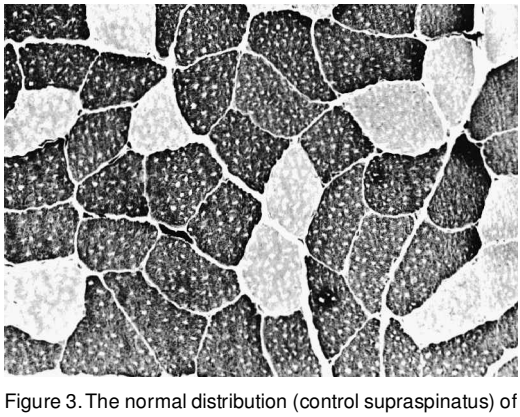

Figure 3. The normal distribution (control supraspinatus) of histochemical fiber types shown with the ATPase reaction at pH 9.4 (type I fibers stain light and type II fibers dark, ×250).

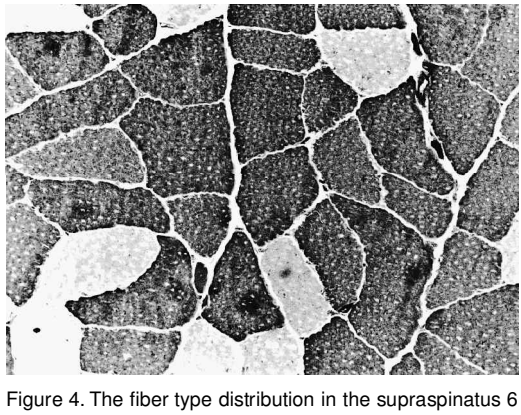

Figure 4. The fiber type distribution in the supraspinatus 6 weeks after large tenotomy, shown with the ATPase reaction at pH 9.4 (type I fibers stain light and type II fibers stain dark, ×250). Note the differences between the diameter of type II fibers.

disuse was reported by Cooper (1972), Williams and Goldspink (1984), Michelsson et al. (1989), Józsa et al. (1990). We found that the size of the rabbit supraspinatus tenotomy was the primary factor affecting the increase in interstitium volume. This process reached its maximum 6 weeks after a small tenotomy while it increased more after a large tenotomy during 3 months of observation. Our data agree with those of Björkenhaim (1989), who also noted a maximal reduction in the percentage of the muscle-fiber area in the rabbit supraspinatus muscle 6 weeks after tenotomy from the greater tubercle during 12 weeks of observation.

Our findings suggest that the changes in muscle fiber composition were caused by transformation from type I to type II fibers and vice versa. This view is supported by three findings. First, conversion from a type I fiber to type II and type II fiber to type I has been reported in several animal models (Romanul et al. 1967, Booth 1982, Hauschka et al. 1987, Templeton et al. 1988, Maxwell et al. 1992, Jarvis et al. 1996, Brunetti et al. 1997, Sutherland et al. 1998). Furthermore, Meier et al. (1997) found transformation of fiber type I to type II in multifidus muscle of young patients with idiopathic scoliosis. Secondly, segmental necrosis of single muscle fibers noted by us was not important and there were no statistically significant differences as regards the interstitium volume after large tenotomy between 12 and 24 weeks. Thirdly, Fabis et al. (1999) noted that 6 months after reattachment of the rabbit supraspinatus tendon in subgroup B2 (reattachment 3 months after large tenotomy), the fiber distribution was similar to the values in subgroup B1. In Burke et al.'s (1973) study, type I fibers are more resistant to fatigue while type II have less fatigue resistance, and are more specific for force creation. An in vivo study of electrophysiological properties of the rabbit supraspinatus muscle showed a stable decrease of twitch tension and fatigue index after its small tenotomy (Fabis et al. 1998) and a gradual reduction in these parameters after a large tenotomy during 6 months of observation (Fabis et al. 2000). These data, together with our findings of changes in fiber type distribution, suggest that the increase in percentage of type II fibers may reflect a process of compensation for the reduction in muscle twitch tension while the increase in percentage of type I fibers may reflect compensation for an increase in muscle fatigability.

We found that the interdigitations between the supraspinatus and infraspinatus tendons and between the supraspinatus and subscapularis tendons are important in dynamics and the degree of severity of the morphometric changes in the rabbit supraspinatus muscle. A comparison between electrophysiological data of rabbit supraspinatus muscle after small (Fabis at al. 1998) and large tenotomies (Fabis at al. 2000) further supports this observation. These interdigitations are present in the human rotator cuff (Clarc and Harryman 1992). It seems that more attention should be paid to careful reconstruction of the intertendinous connection during repair of tears in the human rotator cuff.

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