■ SHOULDER AND ELBOW

Muscle regeneration following repair of the rotator cuff

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doi:10.1302/0301-620X.98B10.37231 $2.00

Bone Joint J 2016;98-B:1389–94.
Received 8 September 2015; Accepted after revision 29 June 2016

Aims

The aim of this study was to analyse human muscle tissue before and after rotator cuff repair to look for evidence of regeneration, and to characterise the changes seen in the type of muscle fibre.

Patients and Methods

Patients were assessed pre-operatively and one year post-operatively using the Oxford Shoulder Score (OSS) and MRI. The cross-sectional area and distribution of the type of muscle fibre were assessed on biopsies, which were taken at surgery and one year post-operatively. Paired samples from eight patients were analysed. There were three men and five women with a mean age of 63 years (50 to 73).

Results

All but one patient showed improvement in OSS ($p = 0.004$). The mean increase in the cross-sectional area of the muscle was 1220 μm^2 (-801 to 3712; $p = 0.03$). There was a reduction of type 2a fibres ($p = 0.02$). A clear relationship could not be seen between the MRI findings and the histological appearances.

Conclusion

This is the first study to provide evidence that atrophy of the supraspinatus muscle is reversible. Changes in the types of fibre are discussed. MRI assessment of muscle atrophy may not be fully representative of myofibre atrophy.

Cite this article: *Bone Joint J* 2016;98-B:1389–94.

The clinical results after rotator cuff repair are better than both the natural history of the condition and those achieved in symptomatic patients treated non-operatively.[1-3] Rates of re-rupture of between 7% and 31% have been reported after repair of small to intermediate tears and up to 94% for massive tears.[2,4-10] Although the outcome is better than the pre-operative state after a re-rupture,[11,12] the degenerative process continues and function is poorer than that with a completely healed repair.[12]

Traditionally, rotator cuff tears have been characterised by their size and the number of tendons involved, and suitability for surgery has been determined on this basis. The size of the tear has a significant effect on the outcome of surgery.[9] Large and chronic tears tend to be associated with more severe muscle degeneration or atrophy and with fatty deposition or infiltration.[13,14]

Thomazeau et al[15] originally described the occupation ratio as the ratio of the cross-sectional area of the supraspinatus to that of the supraspinatus fossa on an oblique-sagittal MRI image (the “Y” view), as a method of determining the degree of muscle atrophy in the rotator cuff. Zanetti, Gerber and Hodler[16] described the “tangent sign” whereby an atrophic supraspinatus is identified as one which does not cross a line drawn from the superior border of the coracoid process to the superior border of the scapular spine as seen on the “Y” view. Warner et al[17] proposed a similar scale for assessing atrophy. A line is drawn from the tip of the coracoid to the scapular spine and also to the inferior tip of the scapula. Both supraspinatus and subscapularis can be assessed by relating the muscle bulk to these lines.

Only a few authors have described the histology of cuff tears.[18-21] In a study of 13 biopsies of supraspinatus in patients with a chronic tear, Mendias et al[21] showed disruption of the myofibril architecture, the presence of multinuclear complexes of macrophages, and the accumulation of lipid droplets in and around muscle fibres. Similarly, Steinbacher et al[18]

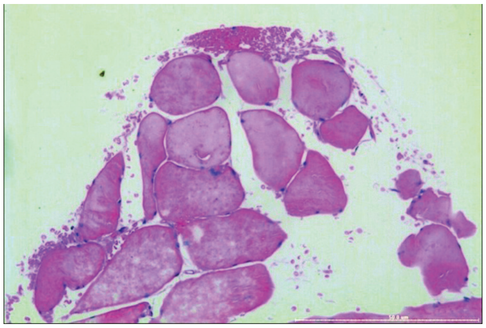

Fig. 1a

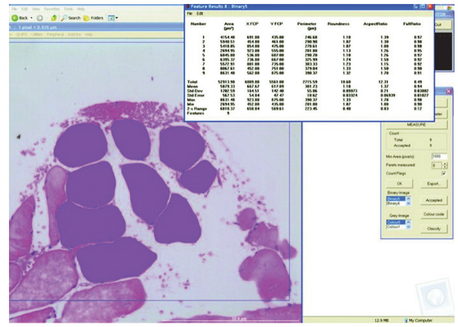

Fig. 1b

Images depicting how myofibre cross-sectional areas were measured. The values were then calculated to give a form factor and included if this was greater than 0.75.

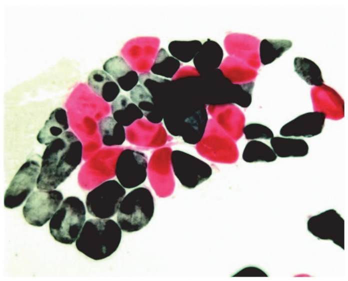

Fig. 2

Image showing the immune stained sample to detect type 1 fibres - dark grey/ black; type 2a fibres - pink/red; and type 2b fibres – pale grey.

showed that fat accumulates not only in the interstitium, but also intracellularly with a corresponding reduction in contractile apparatus and volume of the muscle fibres. They also confirmed previous findings that the atrophy is caused by shrinkage (not death) of the muscle fibres. In a study of 37 biopsies of supraspinatus muscle, Irlenbusch and Gansen[19] showed that with a torn supraspinatus tendon, there is a disturbance in the distribution of the muscle fibres, especially the fast twitch fibres (type 2 fibres). With increasing severity from a partial to a complete tear, there is a reduction in the diameter of the muscle fibres particularly again the fast twitch fibres. A greater variation in the diameter of the fibres was also seen.[19] There have been no human studies investigating the effect of rotator cuff repair on the size of the muscle fibres or the distribution of different types of fibre.

We aimed to investigate whether the muscle atrophy associated with rotator cuff tears is reversible. The distribution of the types of muscle fibre and the relative cross-sectional areas were also studied.

Patients and Methods

Patients placed on the waiting list for repair of the rotator cuff were approached for enrolment. Final inclusion was decided at the time of surgery based on whether the tear was amenable to repair or not. The tears were repaired using a mini-open technique with either a single or double row repair. The operations were undertaken by or under the supervision of one of two senior surgeons (IAT, JH). An independent physiotherapist (AB) assessed the patients and an Oxford Shoulder Score (OSS)[22] was documented pre-operatively and one year post-operatively.

The intra-operative muscle biopsies were taken from the bursal side of the muscle belly (the most atrophic portion) under direct vision using a Tru-Cut biopsy needle (CareFusion UK 306 Ltd., Basingstoke, United Kingdom). A further biopsy was taken one year post-operatively by the senior author (IAT) using a Tru-Cut needle under ultrasound guidance performed by a senior musculoskeletal radiologist (DT) again from the bursal side of the muscle belly of supraspinatus. Biopsies were fixed in paraffin wax and cut at 5 µm using a microtome. The sections were dewaxed in xylene and rehydrated through ethanol. Staining was performed using haematoxylin and eosin (H&E). Double-labelled immunohistochemical staining was also performed using a validated technique to differentiate the types of muscle fibre.[23]

Samples were photographed at 10× magnification under light microscopy and analysed using Leica QWin software (Leica Microsystems, Bensheim, Germany). Only biopsies with a minimum yield of 60 muscle fibres, with a form factor of > 0.75, were included for analysis (Figs 1a and 1b). This applied to both the pre- and post-operative biopsies. The immune staining enabled further analysis of the type of muscle fibre (Fig. 2) and relative cross-sectional areas of type 1 and type 2 fibres.

MRI was undertaken pre-operatively and one year post-operatively. Scans were analysed by an independent senior musculoskeletal radiologist (DT). Atrophy was evaluated on the oblique sagittal images using the occupation ratio.[15] Post-operative scans were also assessed for the integrity of the repair as identified by a discontinuity of the tendon or its insertion. The study had ethical approval and all patients gave informed consent.

Statistical analysis. This was performed using Microsoft Excel (Microsoft, Redmond, Washington) for Mac 2011 and SPSS version 21 (IBM, Armonk, New York). Paired

Table I. Showing the radiological, histological and clinical findings of patients before and after cuff repair

n	Age at surgery (yrs)	Tendon involved	Pre-operative tear size on MRI (cm^2)	Re-tear	Goutallier grade		Occupation ratio		Occupation ratio grade		Mean myofibrecross-sectional area (μm^2)			Oxford Shoulder score	
					Pre	Post	Pre	Post	Pre	Post	Pre	Post	Change	Pre	Post
1	62	SS	4.1	1	1	1	0.46	0.52	2	2	3649	4600	951	15	45
2	69	SS	NA*	No	NA*	3	NA	0.22	NA	3	1947	3005	1058	17	48
3	73	SS	5.5	2	2	2	0.33	0.35	3	3	1098	1769	672	12	37
4	65	SS/SB	2.2	No	1	1	0.51	0.42	2	2	1556	5268	3712	28	47
5	50	SS/SB	6.6	No	0	1	0.5	0.46	2	2	2609	3241	632	31	41
6	67	SS	NA†	No	1	1	0.57	0.69	2	1	4378	3577	-801	29	44
7	65	SS	2.2	2	1	3	0.38	0.38	3	3	2588	4253	1665	36	46
8	61	SS/IS	6.8	2	1	1	0.48	0.6	2	1	2854	4726	1872	43	44

* Pre-operative MRI scan undertaken by 3rd party with images unavailable for review
† Tear reported as partial tear found to be full thickness at surgery
SS, supraspinatus; SB, subscapularis; IS, Infraspinatus; Pre, prior to cuff repair; Post, following cuff repair
Re-tear: 1 – Footprint tear, 2 – Medial Failure

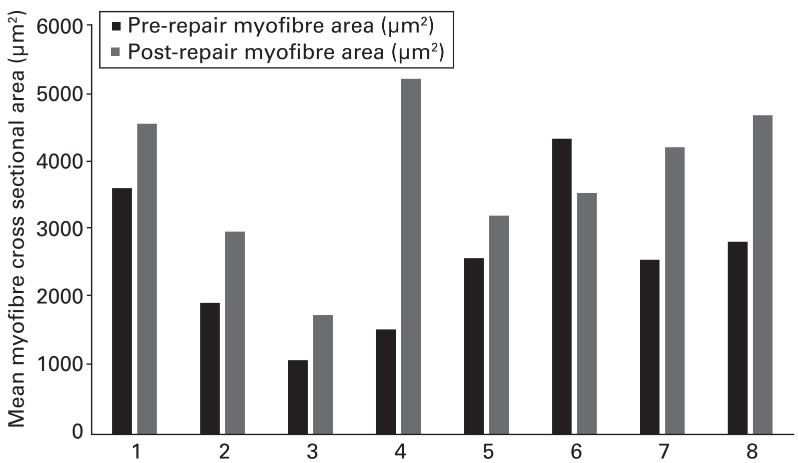

Fig. 3

Chart showing the mean myofibre cross-sectional area in pre- and post-cuff repair samples.

sample *t*-tests were used to compare the pre- and post-operative cross-sectional areas of the muscle fibres and the clinical outcome scores. Wilcoxon Signed rank test for related samples was used for comparison of the proportions of the different types of fibre. The alpha value was set at 0.05.

Results

In all, eight patients with adequate tissue samples were studied. There were three men and five women with a mean age at the time of repair of 63 years (50 to 73). All but one had an improvement in OSS at a mean follow-up of 1.5 years (1.3 to 2.3; $p = 0.004$).

Histological analysis. There was an increase in the mean cross-sectional area of the fibres in all but one sample of 1220 μm^2 (-801 to 3712; $p = 0.03$), at a mean of 1.5 years (1.3 to 1.7) post-operatively. The mean cross-sectional areas for each paired sample can be seen in Table I and Figure 3. The cross-sectional areas of all the fibres are further represented histographically for both pre- and post-operative samples in Figures 4a and b. A right shift towards a larger area can be seen post-operatively. On differential assessment of the types of fibre, there was an increase in mean cross-sectional area in both type 1 (1262μm^2, -1176 to 5977; $p = 0.07$) and type 2 fibres (963μm^2, -1148 to 3060; $p = 0.05$).

The proportions of type 1, 2a and 2b fibres were assessed in seven samples. There was a statistically significant trend in the reduction of type 2a fibres ($p = 0.02$). No pattern could be discerned for type 2b fibres. There was a trend towards the preservation of type 1 fibres though this was not statistically significant ($p = 0.2$).

Radiological analysis. There were four re-tears on the MRI scans one year post-operatively. One was at the footprint; the other three were medial failures. The radiological parameters of the cuffs can be seen in Table I. The grade of fatty infiltration had progressed in two patients (one of whom had a re-tear) and remained static in the others. The occupation ratio was seen to improve one grade in two patients (one of whom had a re-tear) and remained static in the others. The mean change in cross-sectional area of those without a re-tear was 1290 μm^2 (-801 to 3712) and 1150 μm^2 (672 to 1872) in those with a re-tear ($p = 0.4$).

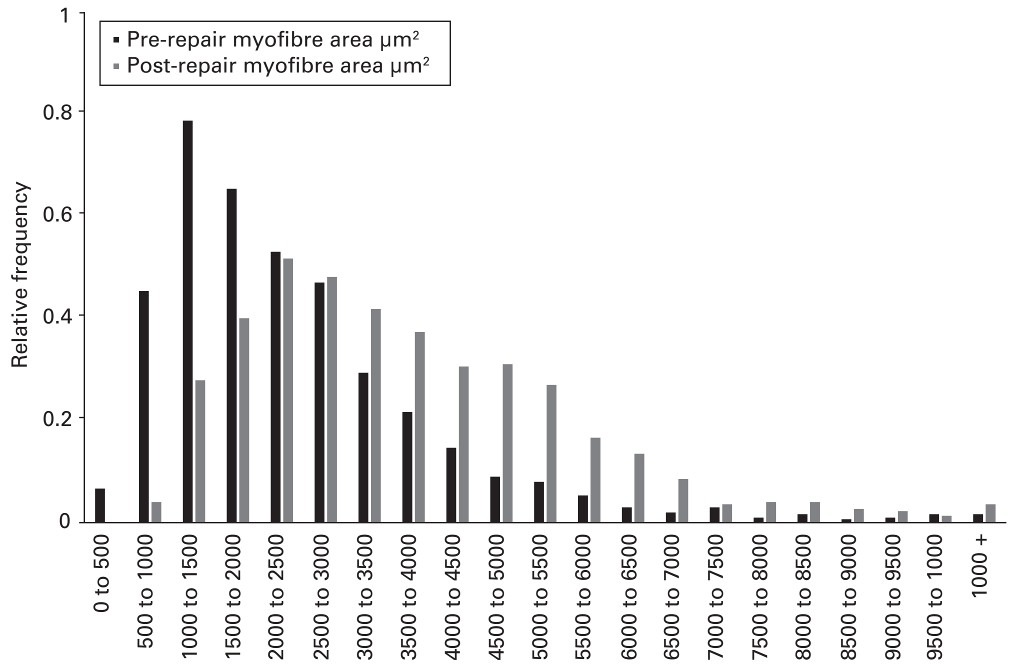

Fig. 4

Histogram showing a right shift towards a larger cross-sectional area of the muscle fibres comparing the pre- and post-operative samples.

All re-tears occurred in those who underwent a single-row rather than a double-row repair. The change in occupation ratio from before to after surgery showed a non-linear, non-monotomic relationship when compared with the changes in mean cross-sectional area.

Discussion

This study, which involved histological analysis of human muscle tissue before and after repair of the rotator cuff, is the first of its kind. Our findings, that the mean cross-sectional area of the muscle fibres of supraspinatus increases after repair, provides evidence that atrophy is reversible following this procedure. Even with re-tears, improvements were seen, which are most likely to be the result of muscle loading and hypertrophy that occur prior to subsequent failure. Tears of repaired rotator cuffs are frequently smaller than the original tears and tend not to increase in size over time as untreated tears frequently do.[12] Some re-ruptures may heal also spontaneously.[12]

Firm conclusions have been difficult to draw from previous human studies investigating the effect of rotator cuff repair on muscle atrophy, given that they have been image-based with disparate results. Some have reported that pre-operative atrophy of supraspinatus is irreversible and that progressive atrophy is halted or slowed by successful repair, and advances significantly after a failed repair.[2,24,25] Others have suggested that atrophy is reversible after repair.[4,5,8,26] Our MRI findings show that the occupation ratio grade can improve in keeping with these findings. When we compared the variables of change in mean cross-sectional area of the muscle fibres before and after repair with the change in occupation ratio, a non-linear, non-monotomal relationship was observed where one might have expected some correlation or indeed a linear relationship. It is difficult to be certain regarding the effect of a re-tear which has been confirmed on MRI, on the measured parameters given the small numbers, although there was no clear difference in histologically measured cross-sectional areas between those without (1290 µm^2) and those with a re-tear (1150 µm^2).

It is clearly easier to measure muscle changes associated with repair of the rotator cuff using radiological methods. However, one of the key drawbacks to this was highlighted in an important study that compared pre- and immediate post-operative MRI.[27] This showed an immediate improvement in both fatty infiltration and muscle atrophy after surgery, most probably representing a mechanical effect of re-tensioning the muscle rather than muscle regeneration. This raises a significant question mark over the applicability of imaging to characterise and determine the true reversibility of structural muscle changes and may partly explain why we did not see a linear relationship between the imaging and histological findings.

There are no human studies available in the literature which compare the size of muscle fibres before and after repair of the cuff. Animal studies with histological analysis which compare the findings before and after experimental simulation of cuff tears are available. In two similar studies which assessed the degree of muscle atrophy in rabbits after early and delayed cuff repair, the same authors found no reversal although they sacrificed the animals for analysis at only 12 and 24 weeks, respectively, after repair.[26,28] In an experimental study involving sheep, Gerber et al[29] found muscle atrophy to be partially reversible at 35 weeks following repair. The results of these studies are hard to translate to humans given the variability in animal models used and the inherent differences between humans and animals which bear weight on their shoulders. Furthermore, the rotator cuffs in animals are often analysed a few weeks after repair rather than at one-year post-operatively as in our study.

On analysis of the relative cross-sectional areas of type 1 (slow twitch) and type 2 (fast twitch) fibres, we did not find any statistically significant differences in the relative atrophy of the two types. Irlenbusch and Gansen[19] reported that atrophy preferentially affected the fast twitch fibres, interpreting this finding as a disturbance of muscular coordination and proposing that these fibres are most responsible for fine control.[19] It is not possible to quantify this finding on review of the histograms presented in that paper. In a more recent study involving muscle biopsies of rotator cuff tears, Lundgreen et al[20] found that the atrophy did not preferentially involve any particular type of fibre, in keeping with our findings.

With regards to the analysis of the types of fibre, the most notable finding was the statistically significant reduction in the proportion of type 2a fibres and a trend towards a slight increase or preservation in the proportion of type 1 fibres, albeit not statistically significant. Lundgreen et al[20] reported that the number of type 1 fibres was reduced in patients with a full thickness tear, suggesting the possibility of a shift in fibre phenotype in which endurance-type fibre characteristics are lost. The finding that, following repair of the cuff, this shift may be halted or reversed to some degree, may therefore be important. It would be interesting to know whether the number of type 1 fibres would decline and the type 2a fibres would gradually increase in number in an untreated cuff tear. Certainly there are no human data specifically looking at this, but there is experimental evidence of a decline in type 1 fibres and preservation of type 2a fibres in other diseased states of muscle such as that reported by Kawada and Ishii[30] in their assessment of muscle biopsies of rat hindlimbs which have been subjected to chronic venous occlusion. Indeed, changes in the type of muscle fibres have been studied widely in the animal population under varying conditions including loading, stretch, neuromuscular activity, hormonal influences and aging. Specifically, stretch and mechanical loading have been shown to induce a transition from fast to slow types.[31,32]

Similarly, neural activity has a profound effect. Denervation results in a transition from slow to fast types. Models of re-innervation can result in specific and orchestrated changes of myofibre phenotype depending on the pattern of neural impulse applied.[31,32] Cross re-innervation studies have shown that fast muscles become slow when re-innervated by a slow nerve, and slow muscles become fast when re-innervated by a fast nerve. Chronic low-frequency stimulation mimics the tonic low-frequency impulse pattern normally delivered to a slow-twitch muscle and can induce major changes in myosin with a replacement of fast by slow isoforms allowing for inter- and intra-species differences.[31,33] The possibility that cuff repair may result in less tension on the suprascapular nerve at the base of the scapular spine has previously been discussed.[34,35] The suggested point at which tension in the nerve is released would indicate that innervation to the infraspinatus would be predominantly affected. Nevertheless, Costouros et al[36] found improvements in electromyography and scores in both infraspinatus and supraspinatus after cuff repair. The implications, as applied to our study, are that repair of the rotator cuff may stimulate a transition of fast to slow types of fibre and lead to a return of more normal patterns through a combination of mechanical loading and also possibly through improvements in innervation.

This study has limitations. The sample size is small. However, we feel that the size is in keeping with other biopsy-based studies, which commonly involve animal rather than human tissue. A limitation and source of error inherent to all studies in this area, is that the radiological grading systems for muscle atrophy are based on a single cross-sectional image, which may be subject to variation between scans. Meyer et al[37] also showed that atrophy of the supraspinatus does not occur homogeneously following a tear of the tendon. In their MRI study, they found that the superficial (fascia-sided) portion of the muscle undergoes more atrophy than the deep (bone-sided) portion, and it is the deep portion of the muscle that tends to undergo fatty infiltration preferentially. It is clear that the location of biopsy samples may also be affected by the heterogeneity of atrophy. Our biopsies were taken from the bursal side of the surface of the muscle, which, as suggested, tends to represent its more atrophic area.

The potential reversibility of muscle atrophy has been extensively investigated in the literature with image-based studies which give disparate results and from which firm conclusions are difficult to draw. Our study provides evidence that atrophy of the supraspinatus after rotator cuff tear is reversible. The analysis of the types of muscle fibre suggests a reduction in type 2a fast twitch fibres and preservation of slow twitch fibres. This is most likely to reflect an effect of loading and possibly improved innervation that may result from repair of the rotator cuff. Together, these are important findings that may be a focus for future research into the biological augmentation of cuff repairs to further enhance the regeneration of muscle.

We would advise caution in the use of MRI assessment of muscle atrophy in guiding surgical decisions, as it may not fully indicate the atrophy or its reversibility following cuff repair.

Take home message:

Muscle regeneration is possible following the repair of a torn rotator cuff tendon.

Supplementary material

Figures showing how the occupation ratio was calculated can be fiound alongside the paper at http://www.bjj.boneandjoint.org.uk/

Author contributions:

U. Butt: Data collection/analysis, Writing the paper.
M. S. Rashid: Data collection/analysis.
D. Temperley: Radiology review/data collection, Performed scans/biopsies.
S. Crank: Tissue collection and preparation.
A. Birch: Data collection, Patient review.
A. J. Freemont: Tissue preparation/analysis, Data collection and supervision, Paper editing.
I. A. Trail: Performed surgery and biopsies, Data collection, Paper editing.

The authors would like to acknowledge J. Haines who supervised and performed some of the surgery, and P. Baird for her help with handling and preparing the tissue samples.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

This article was primary edited by J Scott and first proof edited by A. C. Ross.

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