Published in final edited form as:
J Shoulder Elbow Surg. 2011 September ; 20(6): 917–927. doi:10.1016/j.jse.2011.02.015.

Full-thickness supraspinatus tears are associated with more synovial inflammation and tissue degeneration than partial-thickness tears

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Abstract

Background—The objective of this study was to determine whether the tear size of a supraspinatus tendon correlated with synovial inflammation and tendon degeneration in patients that underwent shoulder arthroscopy for rotator cuff repair. We hypothesized that increased synovial inflammation would correlate with greater tear size of the supraspinatus tendon at the time of surgery.

Materials and Methods—Tissue from the synovium, bursa, torn supraspinatus tendon and subscapularis tendon were obtained from patients during shoulder arthroscopy in order to evaluate the mRNA expression of pro-inflammatory cytokines, tissue remodeling and angiogenesis factors in the tendon, bursa, and synovium. Additional tissue was fixed to determine histological changes including inflammation, vascular ingrowths, and collagen organization.

Results—Increased expression of IL-1β, IL-6, COX-2, MMP-9, and VEGF was found in the synovium of patients with full-thickness tears versus partial-thickness tears ($p<0.05$). In the supraspinatus tendon, increased expression of MMP-1, -9, and -13 and VEGF was found in the full-thickness group. The upregulation of these genes in the full-thickness group was consistent with enhanced synovium inflammation, greater vascular ingrowth and the loss of collagen organization in both supraspinatus and subscapularis tendons as determined by histology.

Conclusion—Increased synovium inflammation and tissue degeneration correlates with the tear size of the supraspinatus tendon. A better understanding of the relationship between synovial inflammation and the progression of tendon degeneration can help design novel and effective treatments to limit the advance of rotator cuff diseases and to improve their clinical outcomes.

Level of evidence—Basic Science, Molecular and Cell Biology Study

Keywords

Rotator cuff tear; synovial inflammation; pro-inflammatory cytokines; matrix metalloproteinase

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Financial Disclaimer: None

INTRODUCTION

Rotator cuff disease is a common cause of shoulder pain and dysfunction, especially in older and sedentary people. A spectrum of pathology exists with painful but low-grade partial thickness tears at one end and massive rotator cuff tears causing pseudo-paralysis at the other.[7–8,21–22,25,29]. Many extrinsic (impingement, demographic factors) and intrinsic (age-related degeneration, hypovascularity, inflammation, and oxidative stress, etc) factors have been proposed to explain the cause and the differences of why the disease advances rather rapidly in some patients but shows little or no progression in others.[7–9,21–22,29] Repetitive damage was considered one of the major extrinsic factors for the cause of a rotator cuff tear.[7,21,29] In a series of studies, Neer et al showed that impingement of the coracoacromial ligament and anterior acromion can lead to repetitive damage and tearing of the supraspinatus tendon.[21] This finding was supported by good clinical outcomes in patients where the offending subacromial spur was removed. Similarly, stronger anchors and suture materials have improved the biomechanical strength of repair constructs. And yet, failure rates of 20 to 80% after rotator cuff repair, depending on tear type, continue to be reported.[3,10,14,28–29,31] This advocates that certain intrinsic factors such as genetic discrepancy in synovial inflammation may play a role in rotator cuff remodeling and its healing rate. Since inflammation-associated leukocyte infiltration often gives rise to the elevation of catabolic cytokines and degradative enzymes, the presence of synovial inflammation is regarded as a pivotal intrinsic factor related to many joint diseases.[3,10,14,28–29,31]

Growing evidence shows that low- to mild-grade synovial inflammation is present in shoulders with a rotator cuff tear.[6,12,18,23,26] In an early study, Gotoh el al[12] found that along with synovial inflammation, the expression of interleukin 1β (IL-1β), was highly upregulated in the synovium in patients with rotator cuff transmural lesion. IL-1β is well known to initiate a cascade of catabolic responses by upregulating a group of degradative enzymes including matrix metalloproteinase 1 (MMP-1), MMP-9 and MMP-13.[32] This finding highlights the pivotal role of synovial inflammation in modulating rotator cuff degeneration. Indeed, two recent studies reported an overexpression of MMP-1 and MMP-13 genes in the torn supraspinatus tendon[18] and an elevation of MMP-1 and MMP-13 in synovial fluid of the shoulder with rotator cuff tears.[23] Since both MMP-1 and MMP-13 are major collagenases and can thus degrade intact collagen, their presence indicates cell-mediated tendon degeneration. In addition to tissue remodeling, IL-1β has also been associated with the spontaneous overexpression of vascular endothelial growth factor (VEGF) and von Willebrand factor (vWF), two important growth factors for neo-angiogenesis, in an overuse shoulder injury model.[6,26] Together, these findings suggest that the manifestation of chronic synovitis is associated with rotator cuff tears.

Several recent studies showed that the repair of Partial-thickness (PTh) tears is associated with better clinical outcomes compared to Full-thickness (FTh) tears, although the mechanism is not completely clear.[9–11,20] Given the importance of joint inflammation, one immediate question is whether or not synovial inflammation in the glenohumeral joint is associated with the severity or tear size of a rotator cuff tendon. One recent study of synovial fluid suggested there was a trend of increased degradative enzymes correlating to the increased tear size of the supraspinatus tendon (partial versus full-thickness),[23] but the difference between groups was not significant. This may be due in part to the small sample size and the detection limitations of the biochemical analysis used in this study. The aim of this study was to determine the gene expression of inflammation, tissue remodeling, and angiogenesis genes in the torn supraspinatus tendon, subscapularis (non-torn adjacent) tendon, local bursa, and synovium using quantitative real-time PCR. We also compared

these changes with gross tissue assessment changes using histological analysis. The hypothesis of this study is that full thickness supraspinatus tears would have greater synovial inflammation and tendon degeneration compared to partial thickness tears.

MATERIALS AND METHODS

Patients and Tissue Samples

Forty patients with rotator cuff tears were enrolled based on an institutional review board (IRB) approved protocol with the following inclusion/exclusion criteria: age between eighteen and seventy-five years with a partial or full thickness tear of the supraspinatus confirmed by MRI, symptomatic despite a minimum of 6 months of conservative treatment, no oral administration of non-steroidal anti-inflammatory drug within three days and/or no corticosteroid injection within three months, and no prior surgery. A pre-operative questionnaire was given to all patients that included demographic data, duration of symptoms, and other clinical variables. At arthroscopy, patients for the full thickness group were included only if the tear was isolated to the supraspinatus tendon, greater than 1 cm in size, and no further pathology was noted in the glenohumeral joint. At the time of surgery, tear size, tissue quality, and degree of bursitis were recorded by the operating surgeon.

Biopsies of the torn supraspinatus edge, subacromial bursa, synovium, and intact subscapularis tendon (used as an indication of the changes in the adjacent non-torn tendon) were harvested during arthroscopy using a 3mm biter. The samples were snap-frozen for mRNA analysis or fixed for histology. In order to minimize the variation of sampling, the bursal, synovial, and subscapularis tissues were taken at the same anatomical location as described in the following. The biopsy of the supraspinatus tendon was obtained from the most diseased portion of the tendon. For partial thickness tears, a biopsy of the diseased tendon was taken after the tendon was released from the tuberosity prior to repair. Synovial samples were obtained from the area of the rotator interval.

Histomorphological Analysis of Collagen Integrity, Vascular Ingrowth and Inflammation

Histomorphological analyses for synovial inflammation and collagen integrity were performed as follows. The tissue was fixed in 4% paraformaldehyde (Sigma, St. Louis, MO, USA), subjected to a routine dehydration process of a graded series of ethanol, embedded in paraffin, and cut in 6 μm serial sections. The sections were stained with hematoxylin and eosin (H&E, Sigma, St. Louis, MO, USA) or picrosirius red, and then viewed with an illumination of monochromatic or polarized light, respectively, using a microscope (Optishot-2, Nikon, Melville, NY, USA). The images were captured by a color CCD camera (DXM1200, Nikon, Melville, NY) and analyzed using commercially available software (Scan Pro; SPSS, Chicago, IL, USA).

The histomorphological changes for tendon were analyzed using the Khan/Bonar score.[16,19] Briefly, tendon degeneration was determined based on the changes in tenocytes, ground substance, collagen organization and vascularization (rated 0–3 for no, mild, moderate, or severe degeneration). Synovial inflammation was analyzed using an established synovium score system.[24] Synovial inflammation was graded as absent (0: only scattered peri-vascularization), very low grade (1: extensive peri-vascularization), low grade (2: multiple perivascular mononuclear and lymphocytic aggregates), or high grade (3: perivascular and diffuse lymphocytic or lymphoplasmacytic infiltrates). Collagen organization (or birefringence) was determined using picrosirius-red staining under polarized light microscopy with established protocol.[27] Briefly, the color image of picrosirius stained slides was taken under a polarized light and then converted into gray scale (level 1 to 255). The brightness (level 1 to 100) of 10 (50μm × 50 μm) boxes was analyzed and then averaged for

each specimen to determine the level of birefringence using commercially available software (Scan-Pro; or NIH Image J, NIH, Bethesda, MD, USA). The histomorphological analysis was performed by two independent observers and the variation in inter-observer and intra-observer were recorded.

Isolation and Analysis of mRNA Expression for Cytokines, MMPs and Angiogenesis

Samples used for mRNA analysis were first snap-frozen in liquid nitrogen and stored at −80°C. Before isolation, the samples were submerged in 10 volumes of RNA*later*-ICE (Ambion, Applied Biosystems, Carlsbad, CA, USA) to help stabilize the mRNA at −80°C for at least 24 hours. Briefly, samples were first minced at 4°C using a sterile surgical blade prior to homogenization. Total RNA in sample was extracted using Trizol (Sigma, St. Louis, USA) agent twice using the standard protocol provided by the vender. The remaining supernatant was purified using RNeasy Mini kit (Qiagen, Valencia, CA, USA) before suspension in RNase-free water. Total RNA content was quantified using a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, DE, USA). An equal amount of mRNA from each sample was reverse transcribed into cDNA using reverse transcriptase (iScript, BioRad, Hercules, CA, USA) and a PCR machine (ABI 7700, Applied Biosystems, Carlsbad, CA, USA) according to the vendor's standard protocol.

Real-time PCR was performed to determine expression of selected pro-inflammatory factors (IL-1, IL-6, TNF-α, COX-2), tissue-remodeling factors (MMP-1, MMP-9, MMP-13, TIMP-1), collagen matrix (type I collagen-COL1A1, type III collagen- COL3A1, smooth muscle actin (SMA), biglycan), and an angiogenesis factor (VEGF). Briefly, real time PCR reactions utilized the SYBR Green detection method. The reactions contained cDNA derived from the previously mentioned reverse transcription reactions for each sample, along with primer pairs for detecting the target transcript and the SYBR Green Master Mix (BioRad, Hercules, CA, USA) containing the polymerase and free deoxy-nucleotides. All primers were designed for use with real-time PCR (Invitrogen, Carlsbad, CA, USA) and verified based on human cell-lines stimulated by IL-1β (R&D, Minneapolis, MN, USA). The primer sets, product sizes and gene names from these genes are listed in Table 1. Control reactions were also performed containing cDNA samples with the reverse transcription buffer but lacking the reverse transcriptase enzyme. The reactions were displayed as amplification plots (fluorescence level vs. cycle number) and quantified based on the cycle threshold (C_T) method as described by the manufacturer (BioRad, IQ-Cycler, Hercules, CA, USA). For each gene, the final expression level was normalized to GAPDH, a reference housekeeping gene.

Statistical Analysis

Statistical analysis (Student's t-test or one-way ANOVA with Tukey post-hoc) was performed using Excel (Microsoft, Redmond, WA, USA) or Systat (10.2, Chicago, IL, USA) to determine the differences in mRNA expression between groups. Linear regression was performed using Systat to determine the relationship between pro-inflammatory cytokines, remodeling genes, and angiogenesis factors. Inter-observer agreement was calculated using Pairwise Spearman's test. To assess inter-observer variability for histologic parameters, Spearman's rho correlation for pairwise data and the Kappa statistic for categorical variables were derived. The significance level was set at 0.05.

RESULTS

Patient Population

Forty patients (24 with full-thickness (FTh) supraspinatus tears and 16 with partial-thickness (PTh) tears) were enrolled. The average age for all patients was 59.6±1.5 years. The average

age in the FTh group was 62.4±2.0 years, while average age the PTh group was 56.3±1.7 years at the time of surgery. The mean duration of symptoms was 13.8 months (2 months to 52 months), although four patients described their symptoms as lasting for “many years” and were therefore not included in this mean. patient reported recent anti-inflammatory use or previous shoulder surgeries.

Histomorphologic Analysis of Tendon Integrity and Synovium Inflammation

The results from H&E staining demonstrate that there was a decrease of the fibroblast population and less organized collagen fibrils in the supraspinatus tendon in the FTh group as compared to the PTh group (Figs. 1B and 1A, respectively). The results from the picrosirius-red staining for collagen alignment suggested that there was a 35% decrease of birefringence (58.6±9.3 versus 35.8±3.1 for PTh and FTh groups, respectively; p=0.04) in the supraspinatus tendon. However, there was no difference in overall histological scores between PTh and FTh groups (1.84±0.18 and 1.61±0.14 PTh and FTh groups, respectively; p=0.33). In general, there was a good agreement in Khan/Bonar score between the observers (kappa = 0.705). A slight decrease of collagen organization in the subscapularis was also seen in the in the FTh group as compared to the PTh group (Figs. 1D and 1C, respectively). This reflected the decrease of birefringence (9%) of collagen alignment also seen (65.3±8.8 versus 59.6±12.5 for PTh and FTh groups, respectively) in the subscapularis tendon. These findings paralleled the findings in histological scores where a trend of increased tendon degeneration in the FTh group was found (Figs. 2; p=0.06). This suggests that subscapularis tendon, which was non-torn and adjacent to torn tendon, was being affected by the joint inflammation and possibly other (kinematics, mechanics, etc.) alterations due to the injury.

In the synovium, increased pro-inflammatory cells, vascularity, surface irregularity and synovial thickening were found in the FTh group as compared to the PTh group (Figs. 1F and 1E, respectively). A significant increase of histological score was found in the FTh group (1.18±0.10) in comparison with the PTh group (0.72±0.14) as shown in Fig 2 (p=0.001). There was an excellent agreement in synovial scores between the observers (kappa=0.931). In the bursal tissue, a greater occurrence of pro-inflammatory cells and enhanced vascularity were also seen in the FTh group versus the PTh group (Fig. 1H and 1G, respectively), which indicated that there was a greater degree of synovial inflammation in the FTh group. Since no validated histological scoring system is currently available for bursa, no scoring was performed for these tissues.

Gene Expression of Supraspinatus (torn) and Subscapularis (adjacent) Tendons

Results from the supraspinatus tendon showed that there was a 34-fold increase of the COX-2 gene in the FTh group as compared to the PTh group (Table 2, Fig. 3A). There were no other major differences in pro-inflammatory expression between FTh and PTh groups (Table 2) except a 67% decrease of the iNOS gene (Fig. 3A). On the other hand, there was a 7.2 and 2.5-fold increase of MMP-13 and MMP-9, respectively, found the FTh group as compared to the PTh group (Table 2; Fig. 4A). There was also a trend of increased COL1A1 expression (6.2 fold) in the FTh group (Table 2, Fig. 4A). Together, these changes indicated greater activities of tissue remodeling and also neo-vascularization in the FTh group, which was consistent with the histological as described above. This increased vascularity can be attributed to the attempt of tissue repair in the FTh group. Of particular interest, we found a decreased expression of VEGF (72.7%), COL3A1 (80.4%), and Biglycan (63.9%) in the seemingly uninvolved subscapularis tendon in the FTh group as compared to the PTh group (Table 2, Fig. 3A, 4A). These changes at the mRNA level for neo-vascularization and fibril regeneration were consistent with the upregulation of COX-2 gene (2.2 folds) in the supraspinatus tendon of the FTh group (Fig. 3A).

Gene Expression of Synovium and Bursa

In the synovium, IL-1β (5.2 fold), IL-6 (13.8 fold), TNF-α (3.4 fold), iNOS (9.5 fold), COX-2 (26.2 fold) and VEGF (25.2 fold) were upregulated in the FTh group as compared to the PTh group (Table 3 and Fig. 3B). These increases in pro-inflammatory activities were consistent with the heightened number of pro-inflammatory cells and vascularity seen in the histology (Figs. 1E and 1F). In the synovium, significant upregulation of MMP-1 (16.5 fold) in the FTh group was also found, which would contribute to the overall collagenase level in the synovial fluid as well as to the collagen degradation of the tendons surrounding the synovial joint (Fig. 4B). There was no significant difference in the gene expression of pro-inflammatory cytokines in the bursa of those patients with FTh rotator cuff tears as compared to those in the PTh group except slightly increased expression of iNOS expression ($p=0.088$, Fig. 3B). This indicated that bursal tissues played a lesser role in the differential tissue remodeling between PTh and FTh groups.

Relationship between Pro-inflammation and Tissue Remodeling Factors

By analyzing the mRNA expression in the supraspinatus tendon, we found some correlations between pro-inflammatory and tissue remodeling factors. A correlation was seen between angiogenesis genes (VEGF) and tendon remodeling genes (COL3A1, COL1A1, Table 3). There was a good correlation between COL3A1 and IL-1β as well as between COL3A1 and IL-6 (Table 3). A strong correlation was also evidenced between tissue remodeling genes: MMP-9 versus MMP-1 ($R=0.74$), COL1A1 versus COL3A1 $R=0.68$), COL3A1 versus SMA ($R=0.63$), and COL3A1 versus Biglycan ($R=0.90$, Fig. 5A). The low correlation between some genes was due in part to the mixture of two sets data which depended on the size of the tear. This was well-demonstrated in the graph MMP-9 versus IL-1β (Fig. 5B) where a greater increase (correlation coefficient) of the MMP-9 versus IL-1β expression was found in the FTh group as compared to that of PTh group. This suggested that the upregulation of MMP-9 in the FTh group was more sensitive to the presence of IL-1β. Similar changes were also found in other tissue remodeling genes (MMP-1 and MMP-13, not shown). Together, these findings highlighted the important role of pro-inflammatory cytokines in modulating matrix remodeling in rotator cuff. Still, the differential effects on tendon degradation might require a larger sample size to determine.

DISCUSSION

In this study, we found an increased number of pro-inflammatory cytokines and angiogenesis factors in the synovium of the FTh group as compared to the PTh group. We also found an increased expression of tissue remodeling factors, including MMP-1 and MMP-13 (collagenases 1 and 3, respectively) in the torn supraspinatus tendon in the FTh group. The upregulation of mRNA was supported by histomorphological findings where more advanced synovial inflammation and a more degraded matrix of the supraspinatus tendon in the FTh group were seen. Together, these findings supported our hypothesis that increased tear severity of a rotator cuff was correlated with greater synovium inflammation and a more degraded matrix in the torn supraspinatus tendon. In addition, we also found certain degeneration in the subscapularis tendons of the FTh group even though they appeared normal based on the pre-surgical MRI evaluation and at the time of arthroscopy. This suggested that joint inflammation affected the properties and normal activities of non-injured tendons which were submerged in the synovial fluid with elevated levels of pro-inflammatory cytokines. However, we did not find that the size of a tear enhanced the inflammation and degradation in the bursal tissue.

Apart from these limitations, our findings in synovial inflammation and tissue remodeling in rotator cuff tears were consistent with the current literature. As noted earlier, the level of

IL-1β in synovial fluid was increased in diseased shoulders.[12,23] Even though the cell type that produced IL-1β was not reported in these studies,[12,23] synovium was considered the main source of joint inflammation. In our study, we found the expression of pro-inflammatory cytokines, including IL-1, IL-6, and COX-2, was indeed increased in the synovium of the FTh group compared to the PTh group.[1] The increase in pro-inflammatory molecules was also supported by the histological results which demonstrated an increase in angiogenesis and inflammation. Together, these findings support the concept that the synovium contributes to the elevation of pro-inflammatory cytokines in an inflamed joint. In addition, our study also suggests that the severity of the tear correlated with synovial inflammation. This finding was supported by several recent studies of collagen and fibronectin fragments.[13,15,31] In these studies, either synovial fibroblasts or chondrocytes were co-cultured with degraded matrix protein, including collagen and fibronectin. IL-1 production and other pro-inflammatory responses were elevated in the group co-cultured with collagen fragments or fibronectin fragments.[13,15] Although we did not directly study the production of fibronectin, previous studies have indicated that there is increased fibronectin present in a torn tendon.[31] Taken together, these findings support the concept that the synovium responded to the insult of a tear with a hypertrophied and inflamed synovitis which in turn increased the severity of the injury.

Our findings also showed that the increase in joint inflammation was closely correlated with tendon remodeling. It is well known that the elevation and activation of IL-1β can induce many other pro-inflammatory cytokines and initiate a cascade of catabolic events.[5,17,30] In this study, we found strong correlations between IL-1 related cytokines (IL-1β, IL-6 and TNF-α) and tissue remodeling genes (MMP-1, MMP-9, MMP-13) in the synovium and torn tendon. This was consistent with increased IL-1β and MMP-13 found in the synovial fluid of diseased shoulders.[12,23] A correlation between inflammation and tissue remodeling factors was also found in patients with subacromial bursitis. Two recent studies by Blaine et al[3,33] showed that the expression of cytokines TNF-α, IL-1β, and IL-6) along with matrix metalloproteases (MMP-1 and MMP-9) and cylcooxygenases (COX-1 and COX-2) were significantly upregulated in the bursal tissue as compared to the normal specimens, although the effects on tendons and synovium were not reported. In our study, we did not find any difference in the bursal tissue between groups. This might be due to the fact that the bursal inflammatory response was independent of tear size, but related to other factors, such as the presence of impingement. It is also possible that alterations in gene expression occurred early in the course of rotator cuff disease and thus were not significantly related to the size of tear.

Of particular interest, we found that MMP-1 upregulation in the synovium and MMP-13 upregulation in the torn supraspinatus tendon were increased with the severity of tear. The increase of MMP-13 has been found in the torn supraspinatus tendon and synovial fluid.[18,23] In this study, we found the upregulation of MMP-13 was significantly lower in the PTh group. A possible explanation for this is that the mechanical stimuli in the torn tendon of partial-thickness tears mediated the overexpression of MMP-13. Arnoczky et al[1,2] have demonstrated using a rat tail tendon model that MMP-13 expression is elevated in response to stress deprivation (loss of tension) and may be reversed with the administration of MMP inhibitors (TIMPs). Since both MMP-1 and MMP-13 are collagenases which can cleave intact type I and Type III collagen, their elevation could predispose the tendon to weakening and tear progression or indicate an attempted healing response.[4] Hence, our findings of MMP-1 and MMP-13 upregulation in the FTh group may also reflect a loss of biomechanical stability imparted by the rotator cuff or failure of the barrier formed by the cuff tendons which usually isolate the glenohumeral joint. Also notable was the elevation of MMP-9 in the tendon, synovium, and bursa in the FTh group. MMP-9 or gelatinase B can degrade type I collagen once cleaved by collagenases; MMP-9 can also cleave type IV and

V collagen. The upregulation MMP-9 is also required for neo-vascularization and the infiltration of pro-inflammatory cells. Together, our findings of MMP-1, -9, and -13 in conjunction with other pro-inflammatory cytokines suggested that MMP-1 and MMP-13 tissue remodeling genes are important for the progression of rotator cuff tears and that synovial inflammation may play a role on regulating tissue remodeling.

Some limitations of this study include that there could have been sampling variations among patients and among different operating surgeons, even though standardized protocols were utilized. Second, like most clinical studies using biological tissues, there was only a single time point (at the time of arthroscopic surgery) used in this study. This limited us from determining the time-course responses of each individual determine how synovial inflammation affected the progression of a tear and corresponding symptoms. Finally, there was no normal tendon included in this study. Some previous studies have used cadaveric tissue or samples taken from patients with other shoulder disorders, such as instability or trauma.[33–34] We could not include these samples in this study in consideration of the highly degradable and/or sensitive nature of mRNA.

CONCLUSION

Overall, we found an increase in the expression of tissue remodeling factors in the torn rotator cuff and inflammation in the glenohumeral synovium in full versus partial thickness tears. A better understanding of the role of joint inflammation in rotator cuff disease is important for designing novel and effective treatments to limit the progression of the tear and consequently improve clinical outcomes. Our findings suggest that synovial inflammation and the progression of a rotator cuff tear are closely related to each other. However, it is not certain at this point whether the matrix debris of a massive torn rotator cuff induces greater synovial inflammation or whether the highly inflamed synovium encourages active tissue remodeling that results in a massive rotator cuff tear. It is very likely that synovial inflammation and tendon degradation affect each other mutually as shown in Figure 6. Treatments that reduce synovial inflammation or that inhibit tissue degeneration in the torn tendon may break the cycle and therefore mitigate the progression of tears and/or improve clinical outcomes. In conclusion, our study shows that the severity of a tear is closely related to chronic synovial inflammation and tissue remodeling in the torn supraspinatus tendon, both of which are important for the advancement of rotator cuff treatment.

Acknowledgments

The authors thank Drs. Edward Craig and Struan Coleman for their contribution as well as Ms. Lily Ying and Dr. David Kovacevic for their kind assistance. This project was support by Institutes of Sports Medicine, an AOSSM Young Investigator's Grant and a NIH grant (AR50549).

IRB: The Hospital for Special Surgery Institutional Review Board approved this study: 26088

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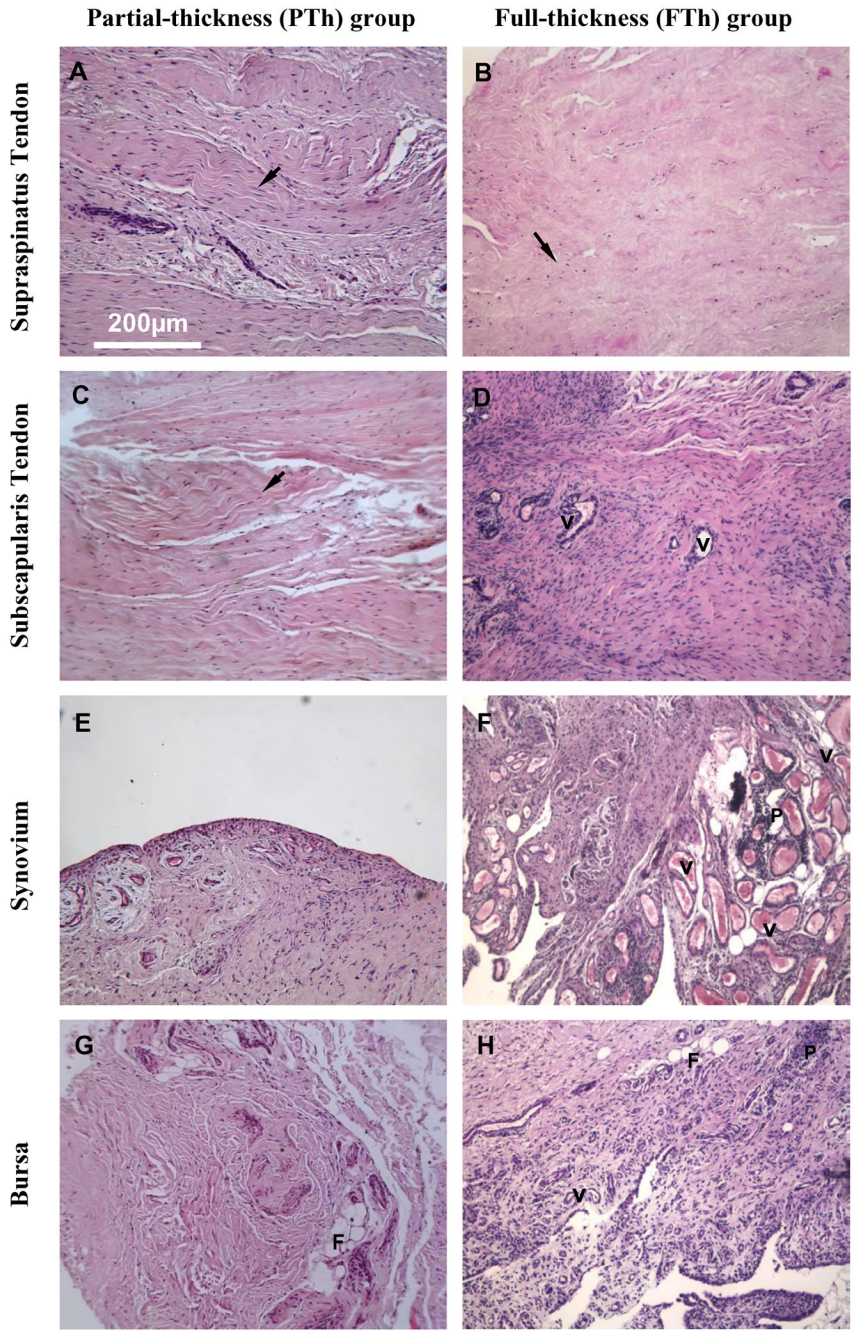

Figure 1.

Histomorphological changes (H&E staining) of supraspinatus tendon, subscapularis tendon, synovium and bursa with PTh and FTh tears. Bar indicates 200μm in (A). The H&E staining of supraspinatus tendon showed a decreased fibroblast population and less organized collagen fibrils (indicated by arrows) in the FTh group (B) as compared to the PTh group (A). An increase of neovascularization (indicated by V) and less organized fibroblasts/ collagen in the subscapularis were seen in the FTh group (D) as compared to the PTh group (C). Increased pro-inflammatory cells (indicated by P) and vascularity (indicated by V) were found in the FTh group (F) as compared to the PTh group (E). Increased pro-inflammatory

cells (indicated by P), fatty cells (F) and vascularity (V) were also seen the bursal tissue in the PTh (G) and FTh (H) groups.

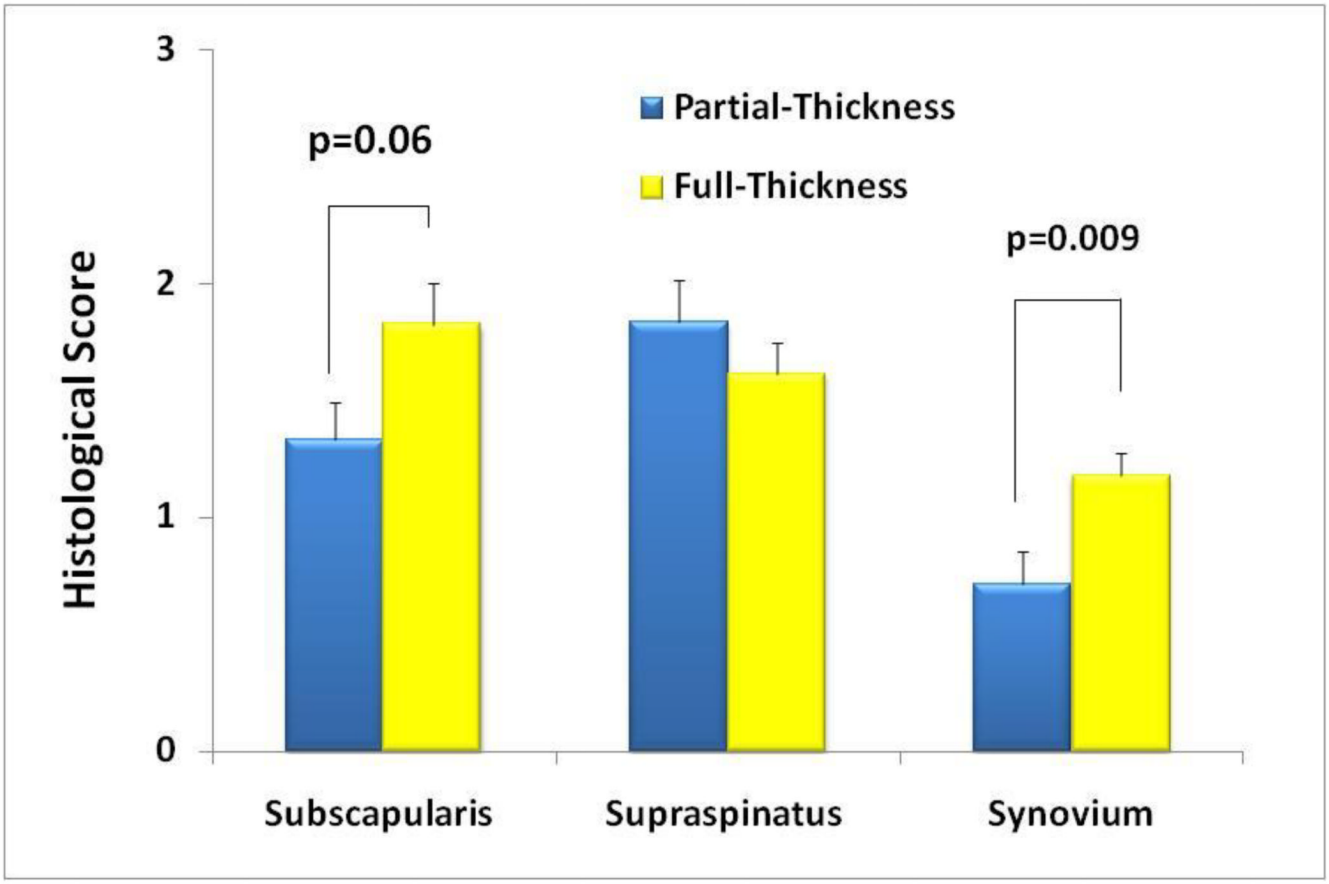

Figure 2.
A good interobserver's agreement was found in the Khan/Bonar scores for supraspinatus and subscapularis tendons (kappa=0.705) and in the synovium scores (kappa=0.931) for synovium. An significant increase of inflammation score was found in the FTh group ($p<0.01$).

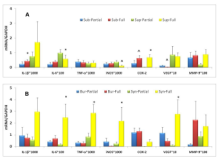

Figure 3.
mRNA expression of pro-inflammatory gene and angiogenesis factors using real-time PCR. *indicates $p<0.05$; ^ indicates $0.05 < p < 0.1$. A slight increase of pro-inflammatory gene expression (IL-1β, COX-2) and decrease of angiogenesis factors (iNOS, VEGF) in the supraspinatus and subscapularis tendons were found in the FTh group as compared to the PTh group. In synovium, a high upregulation of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, iNOS) and VEGF was found in the FTh group as compared to the PTh group. No changes in the bursal tissue were found.

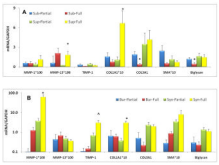

Figure 4.
mRNA expression of tissue remodeling genes expression. *indicates $p<0.05$; ^ indicates $0.05 < p < 0.1$. The high upregulation of MMP-13 and COL1A1 in the supraspinatus tendon was found in the FTh group as compared to the PTh group. There was also a downregulation of COL3A1 and Biglycan in subscapularis tendon found in the FTh group. In synovium, an upregulation of MMP-1 and COLA1 was found in the FTh group as compared to the PTh group. No changes in the bursal tissue were found.

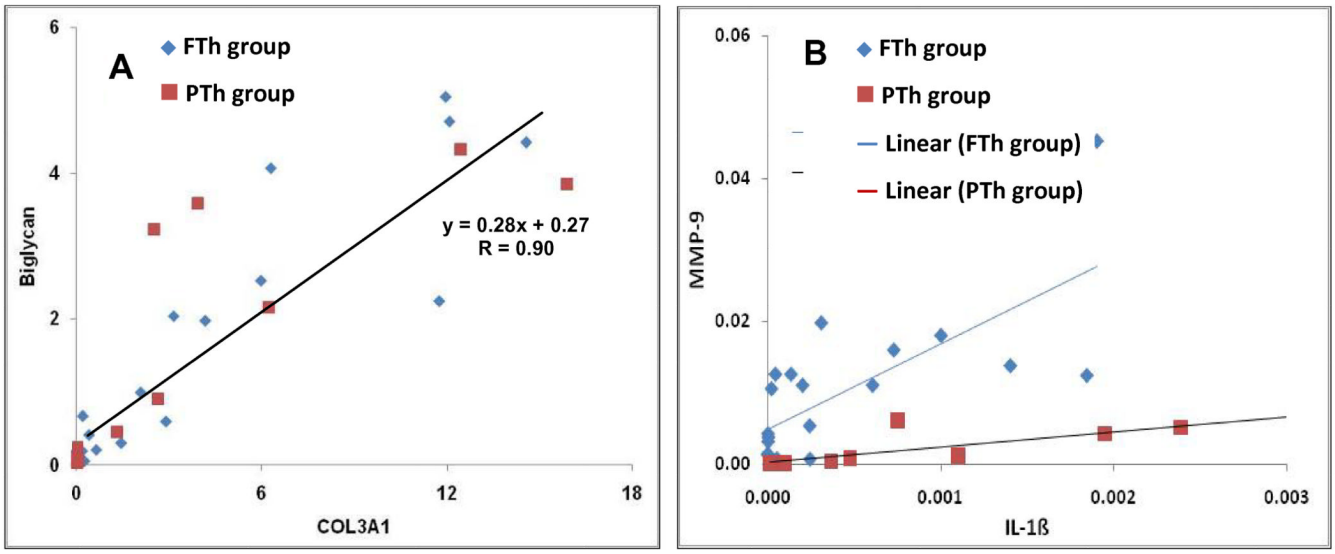

Figure 5.

(A) A plot of correlation between COL3A1 versus Biglycan in torn supraspinatus showed a good correlation (R=0.90) between COL3A1 and Biglycan. (B) There was a good correlation between IL-1β versus MMP-9 in both FTh and PTh groups, even though the correlation of pooled data was not significant. There was also an increased expression of MMP-9 as in the FTh as compared to the PTh group.

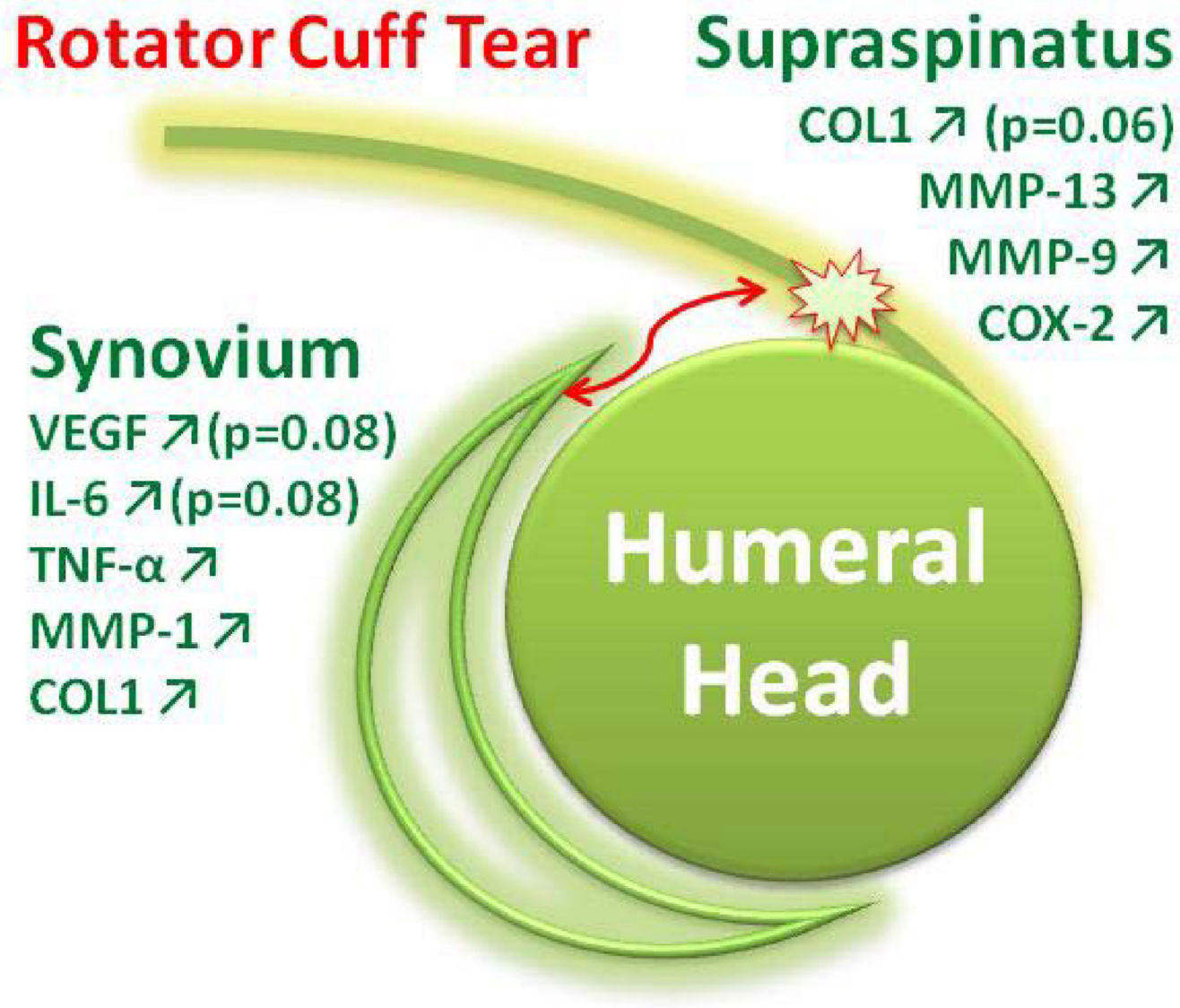

Figure 6.
An illustration showed the interactions between synovial inflammation and torn rotator cuff tendon. The increase of tear size was correlated with greater pro-inflammatory response in the synovium.

Table 1

Primer sets, product sizes and gene names used for real-time PCR experiments

Gene	Forward primer (5'→3')	Reverse primer (3'→5')	cDNA size (bp)	NCBI #
GAPDH	GAGTCAACGGATTTGGTCGTATT	GACAAGCTTCCCGTTCTCAGCCT	185(190–374)	NM_002046
IL-1 β	GGCTTATTACAGTGGCAATGAGGA	TCCATGGCCACAACAACTGA	180(123–302)	NM_000576
IL-6	TGCAGAAAAAGGCAAAGAATC	TAAAGCTGCGCAGAATGAGAT	135(577–711)	NM_000600
TNF-α	CCCAGGCAGTCAGATCATCTT	TCTCAGCTCCACGCCATTGGC	140(390–529)	NM_000594
COX2	TGGCTAGACAGCGTAAACTGC	AACCGTAGATGCTCAGGGACT	142(808–949)	NM_000963
iNOS	TCACCAGGAGATGCTGAACTAC	GAGCACAGCTTTGACCAAGAC	142(1692–1833)	NM_000625
VEGF	ATCAGTTCGAGGAAAGGGAAA	CTGCGGATCTTGTACAAACAAA	124(1464–1587)	NM_003376
MMP-1	CATGCCATTGAGAAAGCCTTCC	AGAGTTGTCCCGATGATCTCC	123(537–659)	NM_002421
MMP-9	TGCCCGGACCAAGGATACAG	TCAGGGCGAGGACCATAGAG	182(1181–1362)	NM_004994
MMP-13	CTTTTGGAATTAAGGAGCATGG	TTGTCCAGGTTTCATCATCATC	129(526–654)	NM_002427
TIMP-1	CCAAGATGTATAAAGGGTTCCAA	TTTCCAGCAATGAGAAACTCCT	136(389–524)	NM_003254
COL1A1	AGGAATTCGGCTTCGACGTT	GGTTCAGTTTGGGTTGCTTG	125(4481–4605)	NM_000088
COL3A1	GGGAACAACTTGATGGTGCT	CCTCCTTCAACAGCTTCCTG	173(37–209)	NM_000090
SMA	ACTGAGCGTGGCTATTCCTTC	TTCTCAAGGGAGGATGAGGAT	134(702–835)	NM_001613
Biglycan	ACGCCCTCGTCCTGGTGAACA	GGATGCGGTTGTCGTGGATGC	165(586–750)	NM_001711

Table 2

Levels of mRNA expression in subscapularis tendon (Subscp), supraspinatus tendon (Supras), bursa tissue (Bursa) and synovium (Synov). All expression was normalized to house-keeping gene GAPDH. Statistics comparison between Partial-thickness and Full-thickness groups was performed using Student's t-Test.

	Subscapularis Tendon		**Supraspinatus Tendon**		**t-Test (p-value)**	
	PTh	**FTh**	**PTh**	**FTh**	**Subscp**	**Supras**
IL-1β*1000	0.23±0.17	0.43±0.11	0.75±0.31	1.71±1.42	0.169	0.609
IL-6*100	0.21±0.13	0.38±0.09	0.98±0.23	0.59±0.23	0.185	0.284
TNF-α*1000	0.38±0.15	0.33±0.15	0.27±0.1	0.31±0.13	0.833	0.836
iNOS*1000	0.23±0.11	0.26±0.09	0.37±0.11	0.12±0.06	0.808	**0.038***
COX-2	0.27±0.20	0.62±0.18	0.02±0.01	0.68±0.20	**0.068^**	**0.017***
VEGF*10	0.11±0.05	0.03±0.01	0.86±0.59	0.75±0.24	**0.087^**	0.838
MMP-9*100	0.65±0.37	0.81±0.30	0.39±0.09	0.98±0.32	0.610	**0.045***
MMP-1*100	0.54±0.21	0.53±0.31	0.40±0.20	1.14±0.60	0.974	0.807
MMP-13*100	0.59±1.07	2.07±0.83	0.25±0.02	1.82±0.59	0.166	**0.046***
TIMP-1	0.30±0.15	0.08±0.02	0.42±0.14	0.23±0.07	0.264	0.450
COL1A1*10	1.54±0.60	0.76±0.34	1.08±0.47	6.66±1.93	0.732	**0.058^**
COL3A1	1.89±0.48	0.37±0.19	3.45±1.70	4.17±1.40	**0.003***	0.793
SMA*10	2.41±0.87	0.5±0.18	1.13±0.55	0.71±0.24	0.763	0.290
Biglycan	1.19±0.25	0.43±0.22	1.59±0.54	1.46±0.43	**0.030***	0.827
	Bursa		**Synovium**		**t-Test (p-value)**	
	PTh	**FTh**	**PTh**	**FTh**	**Bursa**	**Synov**
IL-1β*1000	0.94±0.70	0.74±0.32	0.57±0.25	2.96±1.18	0.772	0.110
IL-6*100	0.41±0.12	0.67±0.26	0.18±0.07	2.48±1.16	0.508	**0.077^**
TNF*1000	0.32±0.15	0.28±0.14	0.83±0.31	2.86±0.74	0.875	**0.036***
iNOS*1000	0.41±0.29	0.04±0.02	0.23±0.14	2.19±1.16	**0.088^**	0.139
COX2*10	1.18±0.40	1.29±0.28	0.02±0.01	0.42±0.20	0.829	0.163
VEGF*10	1.15±0.48	0.46±0.21	0.11±0.06	2.77±1.21	0.141	**0.084^**

	Subscapularis Tendon		Supraspinatus Tendon		t-Test (p-value)	
	PTh	FTh	PTh	FTh	Subscp	Supras
MMP-9*100	0.16±0.10	2.25±1.61	0.87±0.37	1.74±0.98	0.374	0.565
MMP-1*100	0.09±0.06	1.23±0.55	3.73±1.39	61.7±18.2	0.217	**0.037***
MMP-13*100	0.43±0.34	0.67±0.34	0.46±0.17	0.36±0.08	0.677	0.300
TIMP-1	0.11±0.05	0.14±0.05	0.69±0.26	3.03±0.89	0.621	**0.098**^
COL1*10	0.64±0.58	0.60±0.20	0.37±0.14	3.05±0.67	0.942	**0.013***
COL3	0.50±0.48	0.21±0.10	2.34±0.85	2.01±0.45	0.446	0.535
SMA*10	0.28±0.13	0.85±0.27	3.43±0.86	7.94±4.90	0.166	0.533
Biglycan	0.29±0.27	0.23±0.10	1.34±0.44	1.11±0.34	0.782	0.567

* indicates $p<0.05$ and

^ indicates $0.05<p<0.10$.

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Table 3

Correlation coefficients between genes in supraspinatus tendon with data pooled from both FTh and PTh groups

Correlation coefficients (R)													
	IL-1β	**IL6**	**TNF**	**iNOS**	**COX2**	**VEGF**	**MMP9**	**MMP1**	**MMP13**	**TIMP1**	**COL1**	**COL3**	**SMA**
IL-6	0.47												
TNF-α	0.25	0.08											
iNOS	0.07	0.10	0.12										
COX2	0.38	0.19	0.02	0.08									
VEGF	0.26	0.27	0.10	0.07	0.18								
MMP-9	0.21	0.17	0.40	0.03	0.31	0.06							
MMP-1	0.35	0.10	0.42	0.03	0.31	0.01	**0.74***						
MMP-13	0.05	0.19	0.40	0.09	**0.60***	0.18	0.41	0.44					
TIMP-1	0.20	0.07	0.33	0.15	0.47	0.13	0.17	0.11	0.29				
COL1	0.43	0.38	0.01	0.23	0.03	**0.57***	0.26	0.04	0.19	0.24			
COL3	**0.51***	**0.51***	0.13	0.23	0.38	**0.50***	0.25	0.34	0.14	0.43	**0.68***		
SMA	0.22	0.22	0.36	0.08	0.31	0.38	0.11	0.01	0.08	0.46	**0.55***	**0.63***	
Biglycan	0.48	0.38	0.18	0.00	0.46	0.39	0.47	0.51	0.31	0.54	0.48	**0.90***	**0.52***

*(indicates R>0.5)