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Relationship between fatty infiltration and gene expression in patients with medium rotator cuff tear

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Background: Fatty infiltration (FI) is a key prognostic factor that affects outcomes after rotator cuff repair and is radiologically evaluated using the Goutallier classification. The purpose of this study was to assess alterations in gene and protein expression according to the Goutallier classification in the supraspinatus muscle and any relationships among various gene expression profiles.

Methods: Twenty-four samples of the supraspinatus muscle from 12 patients with a high FI grade (grade 3 or 4) and 12 patients with a low FI grade (grade 1 or 2) with medium-sized tears were acquired during arthroscopic surgery. Alterations in the expression of genes and proteins associated with adipogenesis, fibrosis, inflammation, and muscle atrophy were compared between the high- and low-FI groups using reverse-transcription quantitative polymerase chain reaction, Western blotting, and immunohistochemistry.

Results: mRNA expression of not only the adipogenic genes (peroxisome proliferator–activated receptor γ and CCAAT/enhancer-binding protein α; $P < .001$ and $P = .020$) but also the fibrosis-related gene (α-smooth muscle actin; $P < .001$), inflammation-related genes (interleukin [IL]-1β and tumor necrosis factor α; $P = .041$ and $P = .039$), and muscle atrophy–related genes (atrogin 1 and myostatin; $P = .006$ and $P < .001$) was higher in the high-FI group compared with that in the low-FI group. In addition, adipogenic gene expression was significantly correlated with the expression of other categories of genes (all $P < .05$, except atrogin 1). A correlation of gene and protein expression was observed for IL-1β ($P = .027$) and myostatin ($P = .029$).

Conclusions: The radiologic grading of FI was associated with the expression of various genes, including adipogenic, fibrotic, inflammatory, and atrophy-related genes, and these genes were closely correlated with each other in terms of expression. This information could be helpful in patient counseling.

Level of evidence: Basic Science Study; Molecular and Cell Biology

Keywords: Rotator cuff tear; fatty infiltration; Goutallier classification; radiologic grading; gene expression; protein expression

The degree of fatty infiltration (FI) of the rotator cuff muscle is usually assessed radiologically and, although there are some concerns regarding the reliability of the qualitative Goutallier classification,[19,24] it is the most widely used classification system for FI degree.[10] The original Goutallier staging system used computed tomography, but it was modified by Fuchs et al,[7] and, now, evaluation through magnetic resonance imaging (MRI) is

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more commonly used. Severe FI of rotator cuff muscles, resulting in muscle degeneration, as assessed using the Goutallier classification system, is closely related to poor structural and functional outcomes following rotator cuff repair.[9,11,16,21] Decreased postoperative strength, limited shoulder motion, and healing failure after tendon repair are the main clinical implications of FI and degeneration of rotator cuff muscles.[8,9,15,27,29]

Several authors have tried to identify the molecular mechanism underlying FI; adipogenic transcription factors, such as peroxisome proliferator–activated receptors gamma (PPAR-γ) and CCAAT/enhancer-binding proteins alpha (C/EBP-α), are involved in the process of FI of the rotator cuff muscle in rotator cuff tears.[6,30] In addition, rotator cuff tear–induced muscle degeneration is at least partially induced by an inflammatory process and fibrotic changes, which may be bigger problems than FI.[3,12,26] However, we cannot evaluate molecular changes in the rotator cuff muscle in every patient, and it is hard to measure fibrotic or inflammatory changes in the rotator cuff muscle radiologically, which may cause poor clinical outcomes.

To our knowledge, there has been no study that has evaluated differential gene and protein expression according to a radiologic grading system. Thus, the purpose of this study was to investigate the association between the Goutallier classification and the muscle degradation–related expression profiles of various genes, including fibrotic, inflammatory, and atrophy-related genes. We hypothesized that a higher grade of FI is associated with molecular changes in the rotator cuff muscle.

Materials and methods

Patient selection

This was a retrospective case-control study with a laboratory trial. Between February 2017 and January 2018, 187 patients were surgically treated for full-thickness rotator cuff tears at the authors' institution. Among these, we only included patients with medium-sized rotator cuff tears (n = 78: medium tears were defined as having a 1-3-cm tear size, according to the rating system introduced by DeOrio and Cofield[5]), and all patients underwent preoperative MRI evaluation within 4 months prior to surgery. Tear size was measured arthroscopically using a calibrated probe at the time of surgery. Exclusion criteria were as follows: steroid injection within 3 months before the surgery (n = 5), traumatic onset of rotator cuff tear (n = 1), preoperative stiff shoulder (n = 3), systemic inflammatory disorder (n = 1), previous surgery on the same shoulder (n = 2), and refusal to participate in the study (n = 2). Traumatic onset was defined as an obvious high-energy trauma that could cause acute rotator cuff tear, such as a falling accident or acute shoulder dislocation. For the remaining 64 patients, 2 orthopedic surgeons with specialization in the shoulder joint and 6 and 15 years of experience (S.Y.K. and S.W.C.), respectively, evaluated the FI of each rotator cuff muscle, according to the criteria established by Goutallier et al[10] and modified by Fuchs et al[7] using preoperative MRI (3.0-tesla system; Signa HDx; GE Healthcare, Pewaukee, WI, USA). The interobserver reliabilities were evaluated using the interclass correlation coefficient, a 2-way random model with absolute agreement, and were found to be excellent (supraspinatus, 0.845; infraspinatus, 0.771; and subscapularis, 0.710). The patients with a Goutallier grade of 0, 1, or 2 for the supraspinatus were classified into the low-FI group, and those with a Goutallier grade of 3 or 4 for the supraspinatus were classified into the high-FI group, as described in several previous studies.[4,28,33] For 5 cases, the grade was not clear, and these cases were excluded from this study. Among the remaining 59 patients, 12 patients belonged to the high-FI group and 47 to the low-FI group. Among the 47 low-FI group patients, we selected 12 patients who showed the most similar tear size as that of patients in the high-FI group for further biomolecular analyses. Demographic and clinical data of the groups are shown in Table I.

Tissue acquisition

All patients underwent arthroscopic surgery under general anesthesia in the beach chair position. For each patient, 3 supraspinatus muscle tissue samples (3 × 3 mm) were acquired from approximately 1 cm from the musculotendinous junction after repair using an arthroscopic punch through the lateral portal. Two samples were frozen immediately at –80°C for polymerase chain reaction (PCR) analysis and Western blot analysis, respectively. Another sample was fixed in fresh 10% buffered formalin for 16-24 hours at 4°C and then dehydrated and paraffin embedded for immunohistochemical analysis.

Quantitative reverse transcription PCR analysis

To assess the expression of various genes that may be related to the radiologic FI in the supraspinatus muscle, quantitative reverse transcription PCR was performed on adipogenic genes (PPAR-γ and C/EBP-α), a fibrogenic gene (alpha smooth muscle actin [α-SMA]), a protein degradation–related gene (atrogin 1), inflammation-related genes (interleukin 1beta [IL-1β] and tumor necrosis factor alpha [TNF-α]), and a negative regulator of skeletal muscle growth–related gene (myostatin). Total RNA was extracted from the isolated muscles using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, and cDNA was generated using the Maxime RT PreMix Kit (iNtRON Biotechnology, Korea). Quantitative PCR was conducted on a Light Cycler 480 System (Roche Diagnostics, Basel, Switzerland) with 2× qPCR BIO SyGreen Mix Lo-ROX (PCR Biosystems, London, UK). All gene expression data were normalized against glyceraldehyde 3-phosphate dehydrogenase expression. The sequences of the primers used are listed in Table II.

Western blot analysis

To analyze protein expression according to the radiologic FI grade, we performed Western blot analysis using supraspinatus muscle proteins. Whole cell extracts from isolated muscle tissue were prepared using radioimmunoprecipitation assay buffer (Elpis-Biotech, Daejeon, Republic of Korea). Proteins from whole cell lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to

Table I Demographic and clinical data

	High-FI group (n = 12)	Low-FI group (n = 12)	*P* value
Age, yr (range)	65.8 ± 10.1 (41-81)	60.0 ± 7.8 (48-68)	.13
Sex, M:F, n	3:9	7:5	.11
Duration of symptoms, mo	21.9 ± 33.7	30.9 ± 35.2	.53
Side of involvement, D:ND, n	6:6	5:7	.68
Tear size of AP dimension, cm*	20.1 ±4.4	19.1 ± 2.9	.52
Tear size of medial retraction, cm*	15.2 ± 6.0	13.3 ± 5.8	.43
FI of the supraspinatus†	3.2 ± 0.5	1.6 ± 0.8	<.001
FI of the infraspinatus†	2.6 ± 1.2	1.8 ± 0.5	.031
FI of the subscapularis†	1.3 ± 1.0	0.8 ± 1.0	.20
Initial pain VAS	5.7 ± 1.9	6.3 ± 2.1	.43

M, male; *F*, female; *D*, dominant; *ND*, nondominant; *AP*, anteroposterior; *FI*, fatty infiltration; *VAS*, visual analog scale.
High-FI group was defined as the Goutallier grade 3 or 4 for the supraspinatus and low-FI group as the Goutallier grade 0, 1 or 2 for the supraspinatus
* Tear size was measured intraoperatively by using a calibrated probe after debridement of degenerated tendon edges. AP dimension was measured at the lateral edge of the footprint, and medial retraction was estimated based on the distance from the apex of the tear to the lateral footprint.
† FI of each rotator cuff muscle (supraspinatus, infraspinatus, and subscapularis) was graded according to the criteria established by Goutallier et al.

Table II Primers used for quantitative RT-PCR

Gene full name	Gene symbol (human)	Main role	Sequences; forward (F) / reverse (R)
Peroxisome proliferator–activated receptor gamma	PPAR-γ	Adipogenic transcription factor	(F) 5′-CGGTTTCAGAAATGCCTTGC-3′ (R) 5′-ATCTCCGCCAACAGCTTCTC-3′
CCAAT/enhancer-binding protein alpha	C/EBP-α	Adipogenic transcription factor	(F) 5′-AAGAAGTCGGTGGACAAGAAC-3′ (R) 5′-GTCATTGTCACTGGTCAGCTC-3′
Actin, alpha 2, smooth muscle	α-SMA	Myofibroblast formation	(F) 5′-CTGTTCCAGCCATCCTTCAT-3′ (R) 5′-CCGTGATCTCCTTCTGCATT-3′
F-box protein 32 (Atrogin1)	FBXO32	E3 ligase, ubiquitination of muscle protein	(F) 5′-GGCTGCTGTGGAAGAAACTC-3′ (R) 5′-CCTTCCAGGAAAGGATGTGA -3
Interleukin 1 beta	IL-1β	Inflammatory cytokine	(F) 5′-GGACAAGCTGAGGAAGATGC-3′ (R) 5′-TCGTTATCCCATGTGTCGAA-3′
Tumor necrosis factor	TNF-α	Inflammatory cytokine	(F) 5′-GAGGCCAAGCCCTGGTATG-3′ (R) 5′-CGGGCCGATTGATCTCAGC-3′
Myostatin	MSTN	A negative regulator for skeletal muscle cell proliferation and differentiation	(F) 5′-GCAGTGATGGCTCTTTGGAA-3′ (R) 5′-GTCAGACTCTGTAGGCATGGTAA-3′
Actin, beta	ACTB	Cytoskeletal actins (used as a loading control)	(F) 5′-TCTGGCACCACACCTTCTAC-3′ (R) 5′-TCGTAGATGGGCACAGTGTGG-3′

RT-PCR, reverse transcription polymerase chain reaction.

nitrocellulose membranes. The membranes were probed with anti-TNF-α (sc-133192; Santa Cruz Biotechnology, Dallas, TX, USA), anti-IL-1β (3A6; Cell Signaling Technology, Danvers, MA, USA), anti-C/EBP-α (sc-365318; Santa Cruz Biotechnology), anti-PPAR-γ (C26H12; Cell Signaling Technology), and anti-α-SMA (ab7817, Abcam, Cambridge, MA, USA) primary antibodies. Immunoreactive proteins were visualized using an Amersham Enhanced Chemiluminescence kit (GE Healthcare, NJ, USA), according to the manufacturer's instructions. Immunoreactive proteins were assessed using a LAS-3000 Image Analyzer (Fuji Film, Tokyo, Japan). Protein amounts were determined by densitometric analysis using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Immunohistochemistry

To validate the local expression of target proteins, immunohistochemistry was additionally performed for molecules that showed significant differences in Western blot results. For immunohistochemistry analysis, 5-μm paraffin-embedded tissue sections were prepared, deparaffinized in xylene, and rehydrated in an ethanol-water series. Antigen retrieval was conducted using citrate buffer (pH 6.0). The slides were incubated with primary antibodies for 1 hour at room temperature, washed 3 times with phosphate-buffered saline, and incubated with the corresponding secondary antibody conjugated to horseradish peroxidase for 30 minutes at room temperature; this was followed by washing with phosphate-

buffered saline thrice. Expression levels determined through immunohistochemistry staining for molecules that showed significant differences in Western blot results were graded as absent or minimally present, mildly present, moderately present, and severe or markedly present, corresponding to grades 0, 1, 2, and 3, respectively. To eliminate observer bias, all examinations were performed in a randomized and blinded fashion by a pathologist with more than 10 years of training (J.Y.K.). All slides were analyzed under an Eclipse Ni-U microscope (Nikon, Tokyo, Japan), and images were acquired with a Nikon DS-Ri1 and analyzed using NIS Elements F4.00.00, version 4.0.

Statistical analysis

Data are expressed as the mean ± standard error. Mean values were compared using the Mann-Whitney *U* test for continuous variables and the chi-square or Fisher exact test for the categorical variables to determine differences between groups. Intraclass correlation coefficients were used to evaluate the interobserver reliability of the Goutallier-grade evaluation. The Pearson correlation coefficient was used to examine the association between mRNA expression values of each gene. Differences were considered significant at $P < .05$. Statistical analyses were performed using SPSS for Windows (version 18.0; IBM, Armonk, NY, USA).

Results

Gene expression pattern according to radiologic FI severity

As shown in Fig. 1, mRNA expression of the adipogenic genes, PPAR-γ and C/EBP-α, varied significantly based on the severity of the radiologic FI ($P < .001$ for PPAR-γ and $P = .020$ for C/EBP-α). In addition to the adipogenic genes, the mRNA expression of α-SMA, a gene related to fibrogenesis, varied significantly depending on the degree of radiologic FI ($P < .001$). Similarly, the inflammation-related genes, IL-1β and TNF-α, exhibited a significantly higher expression in the high-FI group ($P = .041$ and $P = .039$, respectively). Furthermore, the muscle atrophy–related genes, atrogin 1 and myostatin, also showed higher mRNA expression in the high-FI group ($P = .006$ and $P < .001$, respectively).

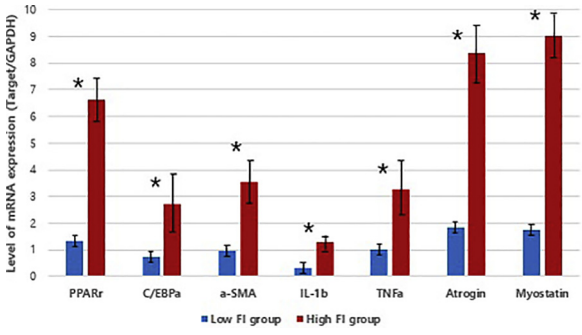

Figure 1 Comparison of the relative mRNA expression levels (target/glyceraldehyde 3-phosphate dehydrogenase) in supraspinatus muscles between the low– and high–fatty infiltration (FI) groups by real-time quantitative reverse transcription polymerase chain reaction analysis. *Significantly different, $P < .05$. *PPAR-γ*, peroxisome proliferator–activated receptors gamma; *C/EBP-α*, CCAAT/enhancer-binding protein alpha; *α-SMA*, alpha-smooth muscle actin; *IL*, interleukin; *TNF-α*, tumor necrosis factor alpha.)

Correlation between mRNA expression levels of various genes

There were significant correlations between the mRNA expression levels of most of the genes. Specifically, the expression of adipogenic genes (PPAR-γ and C/EBP-α) was strongly correlated with the expression of the fibrosis, inflammation, and muscle atrophy–related genes (Table III).

Protein expression and immunohistochemical analysis

As shown in Fig. 2, the IL-1β and myostatin protein expression levels significantly increased in the high-FI group compared to those in the low-FI group ($P = .047$ and $P = .046$, respectively). In addition, even though they were not significantly different, the protein expression levels of C/EBP-α and α-SMA were almost 2-fold higher in the high-FI group compared with those in the low-FI group ($P = .110$ and $P = .19$, respectively).

In accordance with Western blot analysis, immunohistochemical staining revealed the increased expression of IL-1β and myostatin in the high-FI group (Fig. 3, *B* and *D*, respectively) compared with that in the low-FI group (Fig. 3, *A* and *C*, respectively). Semiquantitative grading of immunohistochemical staining also showed significant differences in the protein expression levels between the low- and high-FI groups ($P = .008$ for IL-1β and $P = .012$ for myostatin) (Table IV).

Discussion

FI, resulting in muscle degeneration, is one of the most important prognostic factors that affects outcomes after rotator cuff repair and is radiologically evaluated using the Goutallier classification.[10,14] The Goutallier grades 0, 1, and 2, forming the low-FI group, and Goutallier grades 3 and 4, forming the high-FI group, are based on several previous studies.[4,28,33] Williams et al[33] reported that Goutallier grade 3 is an objective radiologic marker for muscle FI and atrophy. In addition, Sheean et al[28] reported that Goutallier grade 3 is the criteria for irreparability; similarly, Collin et al[4] suggested that Goutallier grades 3 and 4 represent nonfunctional muscles. Thus, we did not consider Goutallier grade 2 as indicating high FI and divided the groups accordingly.

Table III Pearson correlation coefficient between the mRNA expressions of various genes

Parameter	PPAR-γ	C/EBP-α	α-SMA	Atrogin	IL-1β	TNF-α	Myostatin
PPAR-γ	1.000	0.800*	0.790*	0.201	0.482†	0.705*	0.791*
C/EBP-α		1.000	0.535*	0.097	0.076	0.449†	0.583*
α-SMA			1.000	0.090	0.552*	0.533*	0.803*
Atrogin				1.000	-	0.127	0.106
IL-1β					1.000	0.708*	0.467†
TNF-α						1.000	0.510†
Myostatin							1.000

PPAR-γ, peroxisome proliferator–activated receptors gamma; *C/EBP*, CCAAT/enhancer-binding proteins; *α-SMA*, alpha-smooth muscle actin; *IL-1β*, interleukin 1beta; *TNF-α*, tumor necrosis factor alpha.

* Correlation is significant at the .01 level.

† Correlation is significant at the .05 level.

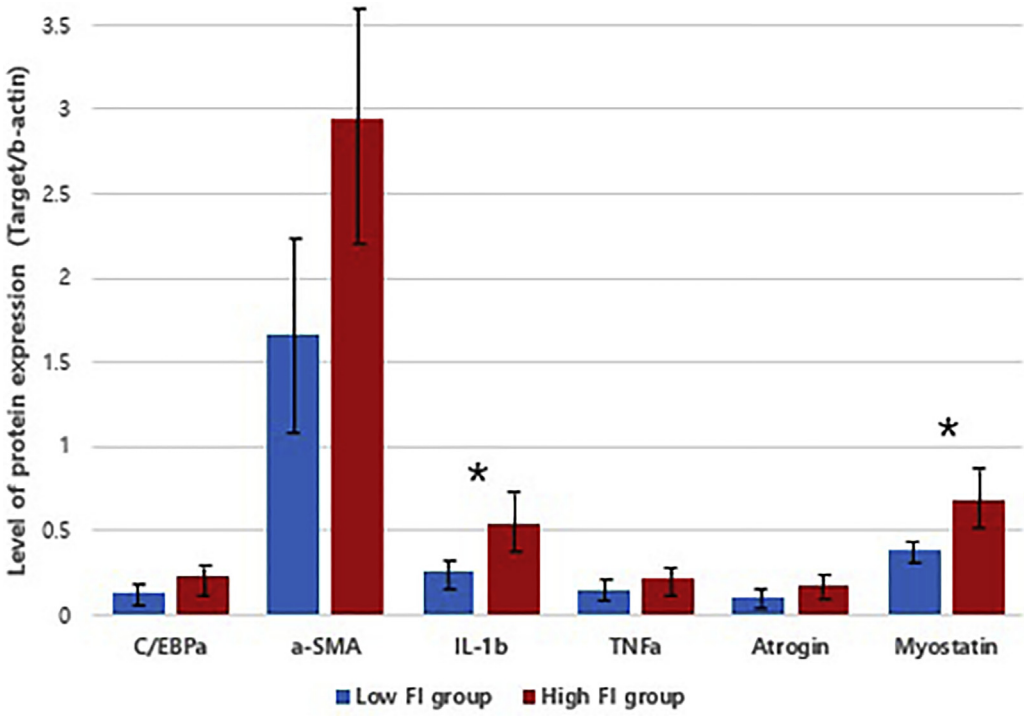

Figure 2 Relative protein expression levels (target/β-actin) in the supraspinatus muscle between the radiologic high– and low–fatty infiltration (FI) groups by Western blot analyses. Interleukin (IL)-1β and myostatin showed significantly higher expression levels in the high-FI group than in the low-FI group. *Significantly different, $P < .05$. *C/EBP-α*, CCAAT/enhancer-binding protein alpha; *α-SMA*, alpha-smooth muscle actin; *TNF-α*, tumor necrosis factor alpha.

In this study, we first demonstrated that the radiologic assessment of FI by the Goutallier classification was directly related to the expression levels of various genes and proteins in human muscle tissue. Based on this finding, we expected higher expression of adipogenic, proinflammatory, and atrophy-related genes in patients with a higher grade of radiologic FI. IL-1β, which is involved in inflammation, and myostatin, which is involved in the inhibition of myogenesis, show significantly higher protein expression in the high-FI group. In addition, even though we failed to detect significant differences, the adipogenic C/EBP-α and fibrogenic α-SMA proteins also showed an almost 2-fold higher expression (target/β-actin) in the high-FI group compared to that in the low-FI group. The statistical insignificance of the differential protein expression of C/EBP-α and α-SMA may be attributed to the relatively small number of samples or the discordance between mRNA and protein expression because of an unknown post-transcriptional modification that inhibits final protein synthesis or another unknown mechanism.

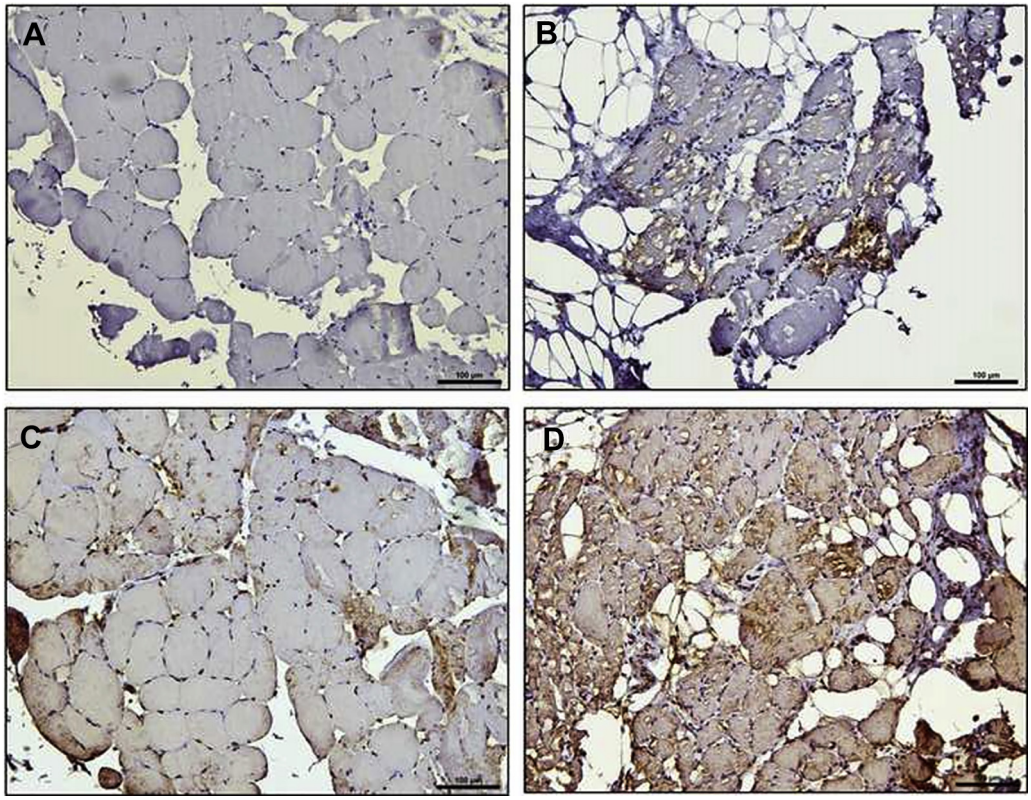

Figure 3 Protein expression in the supraspinatus muscle. Interleukin (IL)-1β: (**A**) low–fatty infiltration [FI] group and (**B**) high-FI group. Myostatin: (**C**) low-FI group and (**D**) high-FI group. in the supraspinatus muscle. Higher IL-1β and myostatin expression levels were observed in the high-FI group compared with those in the low-FI group.

Table IV Semiquantitative grading for the immunohistochemical staining

	Low-FI group				High-FI group			
	G0	G1	G2	G3	G0	G1	G2	G3
IL-1β	4	5	2	1	0	1	4	7
Myostatin	0	6	5	1	0	2	2	8

IL-1β, interleukin 1beta; *FI*, fatty infiltration; *G*, grade; *G0*, absent or minimally present; *G1*, mildly present; *G2*, moderately present; *G3*, severe or markedly present.

PPAR-γ and C/EBP-α are well-known transcription factors for adipogenesis in a rotator cuff tear.[13,15] We found similar results of increased PPAR-γ and C/EBP-α mRNA expression in rotator cuff tear patients, and this expression was correlated with the radiologic FI grade. In addition, α-SMA levels are also increased in patients with rotator cuff tears and have a detrimental effect on outcomes after rotator cuff repair.[25] In this study, α-SMA expression was higher in the high-FI group; thus, we expected more prominent fibrotic changes of the rotator cuff muscle in patients with severe FI. Such results are supported by previous studies that demonstrate collagen deposition and local fibrotic muscle change in patients exhibiting rotator cuff FI.[20,23] Furthermore, it could be presumed that α-SMA would have been upregulated if the FI, as assessed by the radiologic Goutallier classification, was severe. Atrogin 1 is a well-known factor related to the protein degradation of skeletal muscle,[12] and myostatin is also a potent negative regulator of skeletal muscle growth.[26] The increased expression of atrogin 1 and myostatin in the high-FI group suggested that FI might be associated with muscular atrophy. This is supported by previous studies that show the interrelationship between the FI process and muscular atrophy.[1,34] Although several studies have shown that proinflammatory cytokines are associated with rotator cuff tears,[2,22] studies showing a relationship with FI have not yet been clearly documented, especially using human tissue. In this study, we demonstrated that proinflammatory cytokine expression was associated with FI, using both radiologic and biomolecular assessments, indicating that severe FI might be accompanied by severe inflammation.

A recent study demonstrated that supraspinatus muscle atrophy and FI are strongly correlated with loss in contractile force after rotator cuff tear.[32] With respect to the molecular mechanism, most reports have suggested that pluripotent stem cells or progenitor cells in muscles differentiate into adipocytes via adipogenic transcription factors, such as C/EBP-α and PPAR-γ.[6,30,31] In addition to this, we previously elucidated a new mechanism underlying muscle FI via fatty acid–binding protein 4, which does not involve adipogenic differentiation of muscle stem cells.[18] Previously, we also analyzed the expression of several genes associated with inflammation, adipogenesis, and myogenesis.[17] The mRNA expression of the inflammation-associated genes IL-1β and IL-6 was remarkably upregulated 1 week after rotator cuff tear, then decreased after 2 weeks, indicating an acute inflammatory response at the early stage following rotator cuff tear.[17] The adipogenic genes C/EBP-α and PPAR-γ were upregulated at 2 weeks and even more so at 4 weeks and onward after rotator cuff tear, whereas the expression of the myogenic regulators Myod1 and Myf5 was also significantly increased at 2 weeks after rotator cuff tear, as compared with the control.[17] However, the previous study was performed using animal tissue, and there is likely a difference in the biologic response to the injury between humans and animals. From the results of this study, we do not know the cause-effect relationship of the expression of the various genes. However, based on previous studies,[17,18] we speculate that the pathologic inflammatory process mediated by various inflammatory cytokines (IL-1β and TNF-α in this study) may lead to later FI or muscle degeneration, which is mediated through the upregulation of adipogenic (C/EBP-α and PPAR-γ), fibrogenic (α-SMA), and atrophy-related (atrogin and myostatin) genes.

In this study, we demonstrated that proinflammatory cytokine expression is associated with FI using both radiologic and biomolecular assessments, indicating that severe FI might be accompanied by severe inflammation.

Through radiologic assessment by the Goutallier classification, we can only distinguish the nonmuscle portion from the muscle portion using the difference in the MRI signal intensities. However, the nonmuscle area would not be just the fat area. This nonmuscle area may include fibrotic and inflammatory tissues, as well as fat tissue; however, these are hard to distinguish by radiologic assessment. Nevertheless, the results of this study demonstrated that radiologic assessment of FI was directly correlated with biomolecular changes in the expression of adipogenic, fibrogenic, inflammatory, and muscle atrophy-related genes. Thus, it may be possible that radiologic assessment reflects actual biomolecular and histologic changes. If a patient shows high FI by radiologic assessment, he or she may have not only severe FI but also fibrosis, inflammation, and atrophy of the rotator cuff muscle.

This is the first study to analyze the association and changes in biomolecular levels in human muscle tissue based on radiologic classification of FI in patients with rotator cuff tears. Nevertheless, there were several limitations to this study that require consideration. First, the number of patients was relatively small, which may have weakened the findings of the study. The post hoc power analysis indicated that we had only 58% power to obtain a significant result. This small to moderate power due to the small sample size in this study might lead to false negative results for several proteins. Second, we could not include all the cytokines, transcription factors, and enzymes relevant to rotator cuff tears. Instead, we selected the most representative factors as candidate molecules that are known to be important and were of our interest. Including more cytokines, transcription factors, and enzymes may allow us to detect other factors that may have a relationship with FI after rotator cuff tear. Third, even though it was not statistically different, and we confined the tear size to medium, there were differences in the demographic data of age, gender, and duration of symptoms. In addition, the histopathologic status of muscle specimens collected from each group may have differed, and there could have been interindividual variations that might have affected the outcomes. Fourth, because the Goutallier classification system is a qualitative system, there might have been a measurement error. However, the results of the interobserver reliability analysis showed excellent agreement. Grade 1 and 2 or grade 3 and 4 may not be clearly distinguishable, but it is relatively straightforward to distinguish grades 2 and 3. Furthermore, we excluded 5 patients from the study for whom the grade was not clear. Finally, even though we showed the radiologic and biomolecular associations of the expression of various genes in this study, we did not identify their cause-effect relationship. Thus, we cannot suggest potential molecular pathways by which genes in different categories are related; this is the next step of our research.

Conclusions

Severe radiologic FI was associated with upregulation of various genes, including adipogenic, fibrotic, inflammatory, and atrophy-related genes, and their expression levels were closely correlated with each other. This information could be helpful in patient counseling.

Disclaimer

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