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mTOR Regulates Fatty Infiltration through SREBP-1 and PPARg after a Combined Massive Rotator Cuff Tear and Suprascapular Nerve Injury in Rats

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Abstract

Rotator cuff tears (RCTs) are among the most common injuries seen in orthopedic patients. Chronic tears can result in the development of muscular atrophy and fatty infiltration. Despite the prevalence of RCTs, little is known about the underlying molecular pathways that produce these changes. Recently, we have shown that mammalian target of rapamycin (mTOR) signaling plays an important role in muscle atrophy that results from massive RCTs in a rat model. The purpose of this study was therefore to extend our understanding of mTOR signaling and evaluate its role in fatty infiltration after a combined tendon transection and suprascapular nerve denervation surgery. Akt/mTOR signaling was significantly increased and resulted in the up-regulation of two transcription factors: SREBP-1 and PPARγ. We also saw an increase in expression of adipogenic markers: C/EBP-α and FASN. Upon treatment with rapamycin, an inhibitor of mTOR, we observed a decrease in mTOR signaling, activity of transcription factors, and reduction in fatty infiltration. Therefore, our study suggests that mTOR signaling mediates rotator cuff fatty infiltration via SREBP-1 and PPARγ. Clinically, our finding may alter current treatment methods to address rotator cuff fatty infiltration. © 2012 Orthopaedic Research Society.

Keywords

rotator cuff tear; fatty infiltration; Akt/mTOR signaling; SREBP-1; PPARγ

Rotator cuff tears (RCTs) are a common musculoskeletal injury seen by orthopedic surgeons. The prevalence of RCTs is estimated between 15% and 51%, with higher rates above the age of 50.[1] While repair of small tears is successful in relieving pain and

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improving muscle biomechanics, repair of large and massive tears remains as a challenge.[2,3] Several prognostic factors have been identified that may affect the outcome of rotator cuff repairs. Of these, the development of fatty infiltration is one key factor that has been correlated with poor clinical outcomes even after successful surgical repair.[3-5] Molecular understanding of this phenomenon will leverage the development of therapeutics that may alter current treatment modalities.

Previous studies have suggested that the presence of adipocytes in atrophied rotator cuff muscles is due to the differentiation of pre-adipocytes into adipocytes, a process that is mediated by a transcription factor, peroxisome proliferator activated receptor gamma (PPARγ).[6,7] It is speculated that PPARγ operates synergistically with another transcription factor, CCAAT/enhancer-binding protein-a (C/EBPα) to trigger adipogenesis. An in vitro study has demonstrated that adipogenic differentiation induced by PPARγ up-regulation was under the control of transcription factor sterol regulatory element binding protein 1 (SREBP-1).[8] Despite current findings that indicate a possible role of PPARγ and SREBP-1 in promoting lipid biosynthesis, the regulation of these-molecules has yet to be studied following a massive RCT.

The Akt/mammalian target of rapamycin (mTOR) signaling pathway has a central role in regulating muscle size and also has been shown to regulate SREBP-1 activation.[9-11] Previously, we evaluated Akt/mTOR signaling to study muscle atrophy in rats that either underwent a simulated RCT or suprascapular nerve (SSN) denervation.[12] We found that mTOR signaling activity was up-regulated after SSN denervation. In this study, we evaluated the role of the mTOR signaling pathway in the development of fatty infiltration using our combined rotator cuff tendon and SSN injury model that we and others have confirmed is capable of reproducing fatty infiltration that is seen clinically.[12-14] We hypothesized that mTOR signaling induces fatty infiltration via SREBP-1 and PPARγ, and that inhibition of mTOR would decrease the development of fatty infiltration.

MATERIALS AND METHODS

Animal Surgery

Twelve adult female Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) that initially weighed 250 g were used for surgeries. A combined supraspinatus and infraspinatus tendon transection and SSN transection surgery (TT + DN) was performed on the right shoulder as previously described in order to simulate a massive RCT accompanied with nerve injury.[12] Sham surgery was performed on the contralateral side to serve as an internal control. All procedures were approved by San Francisco Veterans Affairs Medical Center (SFVAMC) Institutional Animal Care and Use Committee (IACUC). Based on our previous rat study,[15] four rats are needed to determine a significant difference in mTOR expression using the following assumptions $\alpha = 0.05$, $\beta = 0.80$.

Muscle Harvest

Rats were sacrificed at 6 weeks after surgery. Supraspinatus muscles from both surgical and sham sides were harvested and the remaining tendon and scar tissue were removed at the

muscle/tendon junction. For rats used for biochemical analysis (n = 6), supraspinatus muscles from both surgical and sham sides were isolated and a portion of the muscle was homogenized in 500 ml of T-PER solution (Pierce Biotechnology, Inc., Rockford, IL.) with a protease inhibitor cocktail (Sigma–Aldrich, Inc., St. Louis, MO) for total protein extraction and the other half was homogenized in 500 μl of Trizol® solution (Invitrogen, Inc., Carlsbad, CA) for total RNA extraction. For histological analysis (n = 6), muscle samples were mounted on cork disks to obtain frozen sections for histology as previously described.[14]

Western Blot Analysis

Sixty-five microgram of protein from muscle samples was loaded on 10% NUPAGE Bis-Tris gels and transferred to PVDF membranes (Invitrogen, Inc.). Membranes were blocked and incubated in primary and secondary antibodies as previously described.[12] Bands of developed blots were quantified using ImageJ Software (NIH). The following rabbit-anti-rat primary antibodies (Cell Signaling Technology, Inc., Danvers, MA) were used at a dilution of 1:200 to 1:500: anti-mTOR, anti-phospho-mTOR (Ser2448), anti-Akt, antiphospho-Akt (Ser473), and anti-GAPDH. Rabbit-anti-rat PPARγ and SREBP-1 primary antibodies were used at dilution of 1:1,000 (Santa Cruz Biotechnology, Santa Cruz, CA). HRP conjugated goat-anti-rabbit secondary antibody (Cell Signaling Technology, Inc.) was used at a dilution of 1:10,000.

Real-Time Reverse Transcript Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed to quantify the expression of the following adipogenic markers: PPARγ, SREBP-1, C/EBPα, and FASN in muscle samples using a SYBR Green I Master kit (Roche Applied Bioscience, Indianapolis, IN) with the following primers: PPARγ: (forward) 5′-TGGTGCCTTCGCTGATGCACTG-3’ and (reverse) 5′ -AGATCGCCCTCGCCTTGGCT-3’; SREBP-1: (forward) 5′-AGCCGTGGTGAGAAGCGCAC-3′ and (reverse) 5′ -ACTGCTGCTGCCTCTGCTGC-3′; C/EBPα: (forward) 5′-CCCGATGAGCAGCCACCTCCA-3′ and (reverse) 5′-TACCCCGCAGCGTGTCCAGT-3′ ; and FASN: (forward) 5′-TGCTGCATGCCAGTGGACGG-3′ and (reverse) 5′ -GCAGCGTGGGGGCAATTCCT-3′. Gene expression was normalized to the house keeping gene, GAPDH.[12] Fold change in mRNA expression was calculated by using ΔΔCT.

Histology

Muscle samples were sectioned at −20°C at a thickness of 10 μm. Only sections at the belly of the muscles were used for histological analysis. To localize p-mTOR, SREBP-1, and PPARγ activity within surgical and sham supraspinatus muscles, immunohistochemistry (IHC) was performed using phospho-mTOR, PPARγ, (Cell Signaling Technology, Inc.) and SREBP-1 (Novux Biologicals, Littleton, CO) antibodies at a dilution of 1:200. A DAB staining kit (Vector Laboratories, Inc., Burlingame, CA) was used for developing as previously described.[16]

Rapamycin Inhibition

In order to investigate the role of mTOR in fatty infiltration, we inhibited the activity of mTOR using rapamycin,[17,18] a potent immunosuppressive agent, in rats. Another set of 12 rats was used for this part of the study. These rats underwent TT + DN surgery as described above. They were then randomly assigned to one of two treatments (n = 6/group): rapamycin (Biotang, Inc., Waltham, MA) or vehicle (2% carboxymethylcellulose). Treatments began on the day of surgery and were delivered once daily via intraperitoneal injection at a dose of 1.5 mg/kg, dissolved in 2% carboxymethylcellulose (Sigma–Aldrich, Inc.).[18] This protocol was approved by SFVAMC IACUC. Animals were sacrificed at 6 weeks after surgery. Operated supraspinatus muscles from both treated and vehicle groups were harvested and homogenized in T-PER solution as described above. Western blot for anti-phospho-mTOR, SREBP-1, and PPARγ was performed comparing these treatment groups to the TT + DN group using the protocol described above. RT-PCR was performed to quantify the expression of PPARγ, SREBP-1, C/EBPα, and FASN in order to assess changes at the mRNA level following rapamycin administration. In order to evaluate the change in fatty infiltration upon rapamycin treatment, frozen sections of supraspinatus muscles from all three groups (rapamycin, vehicle, TT + DN) were also stained with oil red-O as previously described.[19]

Statistical Analysis

A paired t-test was used for data analysis between the surgical and sham sides. An ANOVA with a Tukey post hoc comparison was used for data analysis among the rapamycin, vehicle, and TT + DN groups. Significance was defined as a $p < 0.05$. Data are presented as the mean ± standard deviation. For RT-PCR, data are presented as fold change ± stanstandard error.

RESULTS

Significant Muscle Atrophy after 6 Weeks

Six weeks after TT + DN surgery, the supraspinatus muscles at the surgical side exhibited significant muscle weight loss compared to those at the sham side (n = 12). The wet weight of supraspinatus muscle was 155.83 ± 29.4 mg compared to the contralateral control shoulder 412.17 ± 26.3 mg ($p < 0.0001$).

Akt/mTOR Signaling was Significantly Up-Regulated following TT + DN Surgery

Total mTOR, phospho-mTOR, total Akt, phospho-Akt, PPARγ, and SREBP-1 protein activity was significantly increased in supraspinatus muscles from the surgical side compared to the sham side as evident from Western blotting and ImageJ quantification (n = 6; Figs. 1 and 2). Immunohistochemistry (n = 6) of phospho-mTOR, SREBP-1, and PPARγ supported our Western blot results as we found increased expression of these bio-molecules on the surgical side compared to the sham side (Fig. 3).

Up-Regulation of Adipogenic Genes following TT + DN Surgery

Real-time RT-PCR results demonstrated that expression of SREBP-1 and C/EBPα at the mRNA level was not significantly changed (2.5 ± 1.2-fold for SREBP-1 and 2.5 ± 1.9-fold for C/EBPα) in supraspinatus muscles of TT + DN rats 6 weeks after surgery. However, the mRNA levels of PPARγ and FASN genes were significantly increased (4.6 ± 1.7-fold for PPARγ and 4.4 ± 1.8-fold for FASN; $p < 0.05$) in supraspinatus muscles after surgery ($n = 6$; Fig. 4).

Administration of Rapamycin Significantly Reduced mTOR-SREBP-1-PPARγ Activity and Fatty Infiltration following a Massive RCT

The wet weight of supraspinatus muscle following rapamycin treatment was 200.17 ± 26.5 mg compared to 177.33 ± 35.0 mg following vehicle treatment ($p = 0.232$). Western-blot results ($n = 6$) showed that 6 weeks of rapamycin treatment significantly reduced activity of p-mTOR ($p = 0.04$) in operated supraspinatus muscles. Administration of rapamycin also significantly decreased SREBP-1 ($p = 0.001$) and PPARγ ($p = 0.002$) activity (Fig. 5). Vehicle treatment did not change the activity of p-mTOR, SREBP-1, or PPARγ compared to non-injected TT + DN rats. Real-time RT-PCR results demonstrated that expression of SREBP-1, PPARγ, C/EBPα, and FASN at the mRNA level was significantly ($p < 0.05$) reduced (−8.3 ± 3.3-fold for SREBP-1, −16.6 ± 13-fold for PPARγ, −21.3 ± 15.8-fold for C/EBPα, and −31.5 ± 14.5-fold for FASN) in operated supraspinatus muscles following rapamycin treatment (Fig. 6). Oil-red-O staining ($n = 6$) demonstrated that fatty infiltration was reduced in rapamycin treated supraspinatus muscles compared to the control non-injected muscle (Fig. 7).

DISCUSSION

Despite the importance of fatty infiltration in surgical outcomes of chronic rotator cuff repairs, little is known about the pathophysiology of this process. Our study aimed at defining the role of mTOR signaling in the development of fatty infiltration following rotator cuff tears in a rat model. Though its role in modulating muscle development and hypertrophy[17] has been well studied in other models, to our knowledge, this is the first study examining the role of mTOR signaling in rotator cuff fatty infiltration using a small animal model. Our results indicate that mTOR activity is upregulated following rotator cuff tendon transection and denervation (TT + DN). Increased mTOR activity upregulates SREBP-1 and PPARγ expression, two known master regulators of adipogenesis. Moreover, inhibiting mTOR with rapamycin significantly reduces the expression of SREBP-1 and PPARγ and fatty infiltration. Taken together, our findings provide a molecular basis that could guide the development of potential therapeutics to reverse fatty infiltration after RCTs and subsequent repair.

We utilized our TT + DN surgery model to simulate major pathological changes seen clinically following massive RCTs. While several animal models have surveyed fatty infiltration solely resulting from the tenotomy of healthy rotator cuffs,[3,14,15,20-22] other studies have demonstrated that fatty infiltration was more severe when tendon tears were coupled with nerve injury.[14,20,23-25] Moreover, electrophysiological studies conducted in

RCT patients have suggested that large tears change the course of the SSN through the suprascapular notch, increasing tension on the nerve and placing it at risk of injury. Mallon et al. studied eight patients with a known massive RCT and found that SSN denervation occurred concurrently. They concluded that if denervation were to accompany a RCT, it would create "a double-crush effect on shoulder biomechanics."[23] Studies performed in patients with massive RCTs have reported EMG findings that signify SSN neuropathy.[24] A reduction in nerve conduction has been hypothesized to explain the lipid accumulation and anatomical changes seen after denervation.[23,24] Through electrodiagnostic analysis Boykin et al.[26] found that 43% of patients with a RCT had a SSN injury. In an anatomic study, Albritton et al. showed that supraspinatus tendon retraction dramatically changed the course of the SSN as it passed through the suprascapular notch. The increased tautness in the nerve could place the nerve at risk of injury.[27] From these clinical studies, the contribution of SSN injury to rotator cuff fatty infiltration warrants investigation. Therefore, to understand the role of mTOR signaling in fatty infiltration, we conducted our study using a combined TT + DN model. Our previous study has shown that DN of SSN increases mTOR signaling while supraspinatus and infraspinatus TT decreases mTOR signaling. In the present study, we also observed a similar increase in mTOR signaling following a combined TT + DN surgery suggesting that denervation drives up-regulation of Akt/mTOR signaling following RCTs.

Adipogenesis is a tightly regulated process that includes two key stages: commitment of mesenchymal stem cells (MSCs) to produce pre-adipocytes and differentiation into adipocytes.[28] Each of these processes is regulated by different molecular cascades. While the former is mediated by BMP, Wnt, and hedgehog signaling, the differentiation of MSCs into mature adipocytes can involve different pathways including C/EBPs, PPARγ, GLP-1, TIP-3, and RhoA.[28-31] Thus, it is important to determine which regulatory pathway is regulating adipogenesis in different injury and disease states. In our study, we focused on PPARγ as previous studies have explored its role in rotator cuff fatty infiltration. Frey et al.[6] reported the up-regulation of PPARγ in a sheep RCT model. Likewise, Kim et al. and Gumucio et al. demonstrated an increase of PPARγ and C/EBPα expression in rats that underwent a combined RCT and nerve injury.[13,20] Consistent with previous studies, we observed increased expression of PPARγ at both the mRNA and protein level in supraspinatus muscles that demonstrated fatty infiltration, which was confirmed with histologic analysis. The increase of PPARγ expression seen on the surgical side was independent of sham surgery.[12] Therefore, our result confirms that PPARγ is involved in rotator cuff fatty infiltration. However, our study did not distinguish which lineage commits MSCs to adipogenesis. Activity of other cascades (i.e., Wnt, RhoA) may also have a role.

SREBP-1 is a well-known regulator of lipid biosynthesis, which transcriptionally regulates the expression of adipogenic genes.[10] Once SREBP-1 is activated, it can directly mediate PPARγ induction via E-Box motifs located in the PPARγ promoter region.[8] In addition to direct signaling, a study has shown that SREBP-1 indirectly triggers PPARγ expression by activating endogenous ligands in the cytoplasm that translocate into the nucleus.[32] This dual signaling activity of SREBP-1 could explain the significant increase of PPARγ that we saw at both the protein and mRNA levels. Interestingly, we found SREBP-1 to have significant protein activity but baseline gene expression, suggesting that SREBP-1 activity is governed by increased activation (rather than increased expression) in rotator cuff fatty infiltration.

Significant increase of SREBP-1's downstream effector, FASN, verifies that SREBP-1 was up-regulated in our study, as studies in other models have also shown a correlation between SREBP-1 and FASN activity.[9,33] However, we are the first to show that SREBP-1 activity is increased following massive RCTs. Likewise, this increase in SREBP-1 activity is independent of sham surgery.[12]

Our study demonstrated that pharmacological inhibition of mTOR using rapamycin had no significant effect on muscle mass. This finding is in line with previously reported literature.[17,18] However, rapamycin treatment significantly reduced the expression of PPARγ at the mRNA and protein levels, thus confirming our hypothesized role of mTOR signaling in regulating PPARγ and rotator cuff fatty infiltration. Kim et al. also demonstrated that attenuation of mTOR signaling impairs PPARγ's ability to trigger pre-adipocyte differentiation.[34] Key studies analyzing rat adipose tissue have also shown that rapamycin reduces the expression of several PPARγ genes[35] and fat accretion.[36] Collectively, our study and the aforementioned studies indicate cross-talk between PPARγ and mTOR. This information may lead to the development of innovative treatments targeting rotator cuff fatty infiltration.

Rapamycin treatment also was able to significantly reduce SREBP-1 activity in our study at both the mRNA and protein levels. Similar to PPARγ, this data indicate cross-talk between mTOR signaling and SREBP-1 which is supported by models of fatty infiltration.[9-11,37,38] Using epithelial cells, Porstmann et al. were able to show that mTOR complex 1 regulated lipogenesis via SREBP-1. They blocked mTOR signaling using rapamycin. Reduced mTOR activity prevented SREBP-1 nuclear translocation and decreased the expression of lipogenic genes.[9] Peterson et al.[37] supported this claim and identified Lipin 1 as a regulator of SREBP-1 transcriptional activity. Future work is needed to further define the detailed mTOR-SREBP-1-PPARγ pathway in rotator cuff fatty infiltration. It is unclear whether mTOR activates SREBP-1, which in turn induces PPARγ activity or whether mTOR interacts with each of these transcription factors independently following RCTs.

There are some limitations to our study. First, we did not distinguish which mTOR complex (1 or 2) was most active in our model.[10,11] However, other models have indicated a connection between lipid biosynthesis and mTORC1. We speculate that mTORC1 is also instrumental in rotator cuff fatty infiltration. In our previous muscle atrophy study, we observed increased mTOR signaling following SSN DN. Importantly, we saw significant activation of S6K1, a known downstream effector of mTORC1.[12] Since our TT + DN surgery Western blot results parallel the mTOR signaling trend we observed in DN previously, we infer that mTORC1 is also involved in fatty infiltration. Moreover, rapamycin is a known inhibitor for mTORC1 at the dose we used.[18] Previous studies also have implicated that SREBP-1 and PPARγ are regulated by mTORC1.[10,35] Another potential limitation was that we used a single dose of rapamycin at 1.5 mg/kg. Although recent studies have used a higher dose, other studies including ours have accomplished satisfactory in vivo inhibition of mTOR at this dose.[17,35,36] Our Western blot, PCR, and histologic results also support that rapamycin at this dose can significantly reduce mTOR activity in rat supraspinatus muscle after TT + DN surgery. Follow-up studies are aimed at quantifying the amount of fatty infiltration after rapamycin treatment with high resolution

MRI and biochemical quantification, and to evaluate novel tissue-specific inhibitors of mTOR.

Finally, like other animal studies, the anatomy and mechanics of a rat shoulder are different from that of a human shoulder. Thus, the results from this animal study may not be directly applicable to patients. However, we do believe that our model provides the best proxy to what is seen following RCTs in humans as evident by our previous data[14] and of others who utilized our model.[13] Though other rodent models have been reported to study rotator cuff fatty infiltration, they are limited. Gimbel et al.[39] have developed a rat model but this model has not clearly demonstrated fatty infiltration. Gupta et al. have reported a rabbit model that can develop fatty infiltration. However, the anatomy of the rabbit rotator cuff is considerably different than that of the human.[40] Thus, we believe our TT + DN model is suitable for the current study as it is capable of reproducing fatty infiltration that is seen clinically.

In summary, we demonstrated that mTOR signaling modulates rat rotator cuff fatty infiltration through SREBP-1 and PPARγ. Based on this study and our previous muscle atrophy study, mTOR signaling may be of interest in the development of future pharmacological strategies to address rotator cuff fatty infiltration and muscle atrophy. Likewise, our study may guide other studies that evaluate the molecular mechanisms behind fatty infiltration following other injury states.

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REFERENCES

1. Chaudhury S, Dines JS, Delos D, et al. Role of fatty infiltration in the pathophysiology and outcomes of rotator cuff tears. Arthritis Care Res (Hoboken). 2012; 64:76–82. [PubMed: 21770040]
2. Coffeld RH, Parvizi J, Hoffmeyer PJ, et al. Surgical repair of chronic rotator cuff tears. A prospective long-term study. J Bone Joint Surg Am. 2001; 83-A:71–77.
3. Gerber C, Meyer DC, Schneeberger AG, et al. Effect of tendon release and delayed repair on the structure of the muscles of the rotator cuff: an experimental study in sheep. J Bone Joint Surg Am. 2004; 86-A:1973–1982. [PubMed: 15342760]
4. Goutallier D, Postel JM, Bernageau J, et al. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. Clin Orthop Relat Res. 1994; 304:78–83.
5. Yamaguchi K, Ball CM, Galatz LM. Arthroscopic rotator cuff repair: transition from mini-open to all-arthroscopic. Clin Orthop Relat Res. 2001; 390:83–94. [PubMed: 11550880]
6. Frey E, Regenfelder F, Sussmann P, et al. Adipogenic and myogenic gene expression in rotator cuff muscle of the sheep after tendon tear. J Orthop Res. 2009; 27:504–509. [PubMed: 18932240]
7. Itoigawa Y, Kishimoto KN, Sano H, et al. Molecular mechanism of fatty degeneration in rotator cuff muscle with tendon rupture. J Orthop Res. 2011; 29:861–866. [PubMed: 21246616]
8. Fajas L, Schoonjans K, Gelman L, et al. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. Mol Cell Biol. 1999; 19:5495–5503. [PubMed: 10409739]
9. Porstmann T, Santos CR, Griffiths B, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 2008; 8:224–236. [PubMed: 18762023]
10. Laplante M, Sabatini DM. An emerging role of mTOR in lipid biosynthesis. Curr Biol. 2009; 19:R1046–R1052. [PubMed: 19948145]

11. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012; 149:274–293. [PubMed: 22500797]
12. Liu X, Joshi SK, Samagh SP, et al. Evaluation of Akt/mTOR activity in muscle atrophy after rotator cuff tears in a rat model. J Orthop Res. 2012; 30:1440–1446. [PubMed: 22378614]
13. Gumucio JP, Davis ME, Bradley JR, et al. Rotator cuff tear reduces muscle fiber specific force production and induces macrophage accumulation and autophagy. J Orthop Res. 2012 [Epub ahead of print].
14. Liu X, Laron D, Natsuhara K, et al. A mouse model of massive rotator cuff tears. J Bone Joint Surg Am. 2012; 94:e41. [PubMed: 22488625]
15. Liu X, Manzano G, Kim HT, et al. A rat model of massive rotator cuff tears. J Orthop Res. 2011; 29:588–595. [PubMed: 20949443]
16. Skittone LK, Liu X, Tseng A, et al. Matrix metalloproteinase-2 expression and promoter/enhancer activity in skeletal muscle atrophy. J Orthop Res. 2008; 26:357–363. [PubMed: 17960656]
17. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 2001; 3:1014–1019. [PubMed: 11715023]
18. Kline WO, Panaro FJ, Yang H, et al. Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. J Appl Physiol. 2007; 102:740–747. [PubMed: 17068216]
19. Koopman R, Schaart G, Hesselink MK. Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem Cell Biol. 2001; 116:63–68. [PubMed: 11479724]
20. Kim HM, Galatz LM, Lim C, et al. The effect of tear size and nerve injury on rotator cuff muscle fatty degeneration in a rodent animal model. J Shoulder Elbow Surg. 2011; 21:847–858. [PubMed: 21831663]
21. Rubino LJ, Stills HF Jr, Sprott DC, et al. Fatty infiltration of the torn rotator cuff worsens over time in a rabbit model. Arthroscopy. 2007; 23:717–722. [PubMed: 17637406]
22. Safran O, Derwin KA, Powell K, et al. Changes in rotator cuff muscle volume, fat content, and passive mechanics after chronic detachment in a canine model. J Bone Joint Surg Am. 2005; 87:2662–2670. [PubMed: 16322616]
23. Mallon WJ, Wilson RJ, Basamania CJ. The association of suprascapular neuropathy with massive rotator cuff tears: a preliminary report. J Shoulder Elbow Surg. 2006; 15:395–398. [PubMed: 16831639]
24. Costouros JG, Porramatikul M, Lie DT, et al. Reversal of suprascapular neuropathy following arthroscopic repair of massive supraspinatus and infraspinatus rotator cuff tears. Arthroscopy. 2007; 23:1152–1161. [PubMed: 17986401]
25. Rowshan K, Hadley S, Pham K, et al. Development of fatty atrophy after neurologic and rotator cuff injuries in an animal model of rotator cuff pathology. J Bone Joint Surg Am. 2010; 92:2270–2278. [PubMed: 20926720]
26. Boykin RE, Friedman DJ, Zimmer ZR, et al. Suprascapular neuropathy in a shoulder referral practice. J Shoulder Elbow Surg. 2011; 20:983–988. [PubMed: 21277806]
27. Albritton MJ, Graham RD, Richards RS II, et al. An anatomic study of the effects on the suprascapular nerve due to retraction of the supraspinatus muscle after a rotator cuff tear. J Shoulder Elbow Surg. 2003; 12:497–500. [PubMed: 14564276]
28. Tang QQ, Lane MD. Adipogenesis: from stem cell to adipocyte. Annu Rev Biochem. 2012; 81:715–736. [PubMed: 22463691]
29. Challa TD, Beaton N, Arnold M, et al. Regulation of adipocyte formation by GLP-1/GLP-1R signaling. J Biol Chem. 2012; 287:6421–6430. [PubMed: 22207759]
30. Jakkaraju S, Zhe X, Pan D, et al. TIPs are tension-responsive proteins involved in myogenic versus adipogenic differentiation. Dev Cell. 2005; 9:39–49. [PubMed: 15992539]
31. McBeath R, Pirone DM, Nelson CM, et al. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell. 2004; 6:483–495. [PubMed: 15068789]
32. Kim JB, Wright HM, Wright M, et al. ADD1/SREBP1 activates PPARγamma through the production of endogenous ligand. Proc Natl Acad Sci USA. 1998; 95:4333–4337. [PubMed: 9539737]

33. Jeon BN, Kim YS, Choi WI, et al. Kr-pok increases FASN expression by modulating the DNA binding of SREBP-1c and Sp1 at the proximal promoter. J Lipid Res. 2012; 53:755–766. [PubMed: 22331133]
34. Kim JE, Chen J. regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes. 2004; 53:2748–2756. [PubMed: 15504954]
35. Houde VP, Brule S, Festuccia WT, et al. Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes. 2010; 59:1338–1348. [PubMed: 20299475]
36. Blanchard PG, Festuccia WT, Houde VP, et al. Major involvement of mTOR in the PPARγamma-induced stimulation of adipose tissue lipid uptake and fat accretion. J Lipid Res. 2012; 53:1117–1125. [PubMed: 22467681]
37. Peterson TR, Sengupta SS, Harris TE, et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell. 2011; 146:408–420. [PubMed: 21816276]
38. Bakan I, Laplante M. Connecting mTORC1 signaling to SREBP-1 activation. Curr Opin Lipidol. 2012; 23:226–234. [PubMed: 22449814]
39. Gimbel JA, Van Kleunen JP, Mehta S, et al. Supraspinatus tendon organizational and mechanical properties in a chronic rotator cuff tear animal model. J Biomech. 2004; 37:739–749. [PubMed: 15047003]
40. Gupta R, Lee TQ. Contributions of the different rabbit models to our understanding of rotator cuff pathology. J Shoulder Elbow Surg. 2007; 16:S149–S157. [PubMed: 17903710]

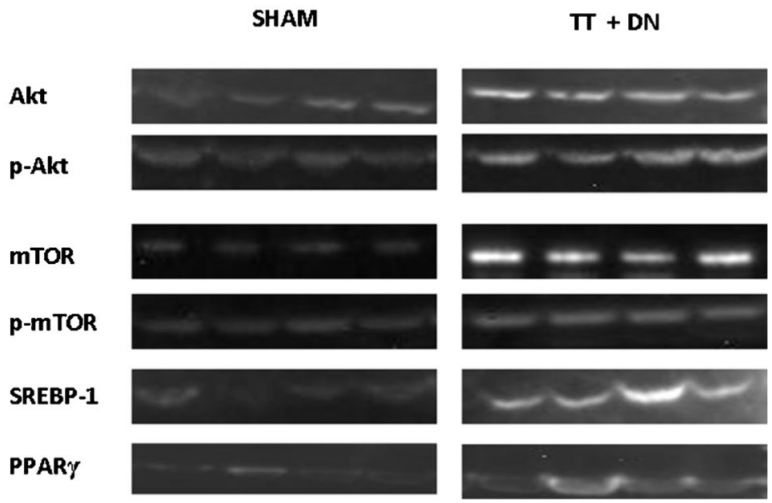

Figure 1.

Six weeks after TT + DN surgery, activity of Akt, p-Akt, mTOR, p-mTOR, SREBP-1, and PPARγ was up-regulated in supraspinatus muscles of the operated side compared to sham control as evident by Western blot analysis.

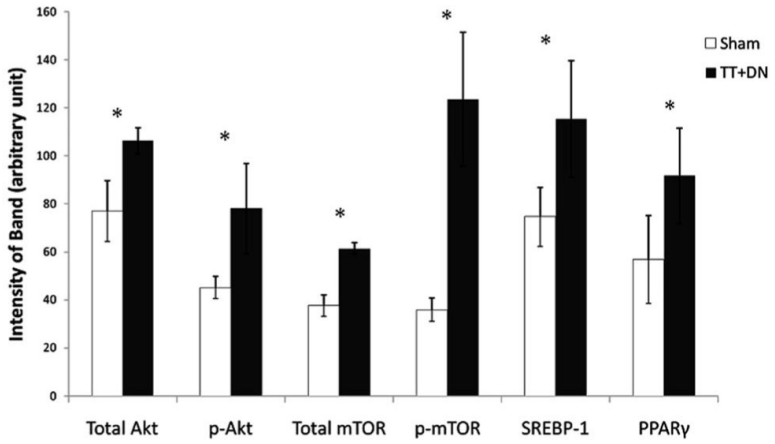

Figure 2.

ImageJ quantitative analysis of Western blot bands of Akt, p-Akt, mTOR, p-mTOR, SREBP-1, and PPARγ in supra-spinatus muscles 6 weeks after TT + DN surgery (* indicates $p < 0.05$).

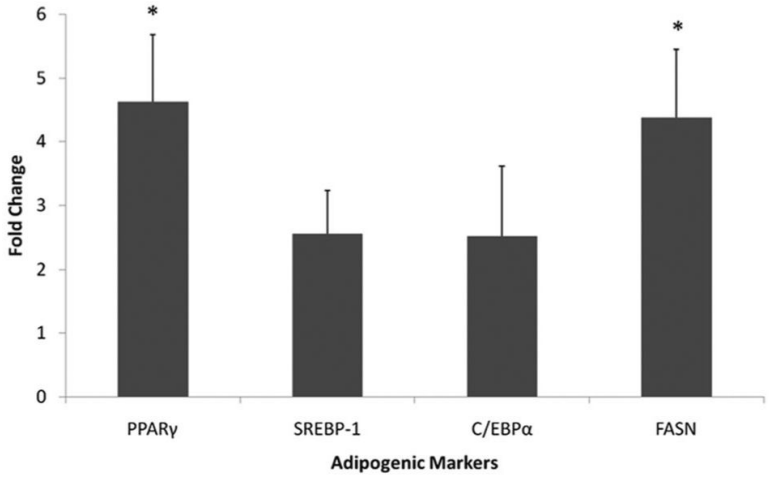

Figure 3.

Fold change of PPARγ, SREBP-1, C/EBPα, and FASN in supraspinatus muscles 6 weeks after TT + DN surgery (* indicates $p < 0.05$).

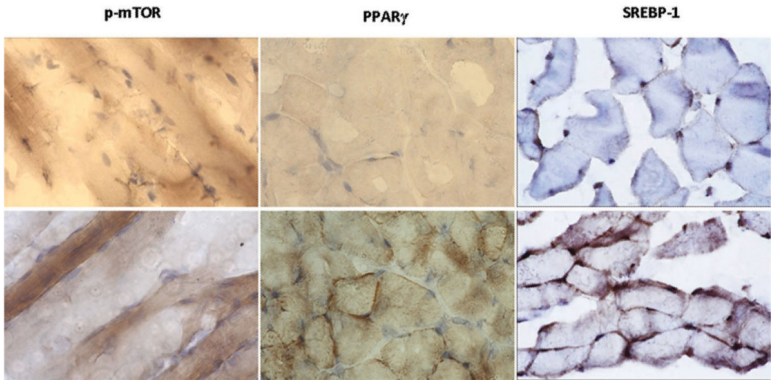

Figure 4.

IHC of supraspinatus muscles 6 weeks after TT + DN surgery. There appears to be an up-regulation of p-mTOR (active), SREBP-1, and PPARγ in the surgical side (bottom row) compared to the sham control (top row).

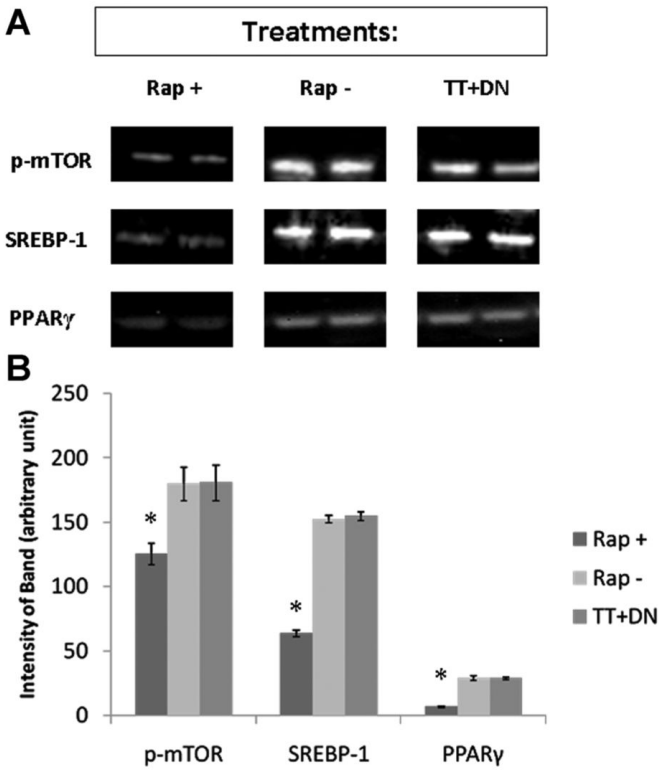

Figure 5.

(A) Activity of p-mTOR, SREBP-1, and PPARγ was decreased in supraspinatus muscles upon Rapamycin (Rap+) treatment as evident by Western blot analysis. No change was seen between vehicle (Rap-) and TT + DN groups. This data suggests there is a signaling relationship between mTOR, SREBP-1, and PPARγ in the setting of a RCT. (B) ImageJ quantitative analysis of Western blot bands (* indicates $p < 0.05$).

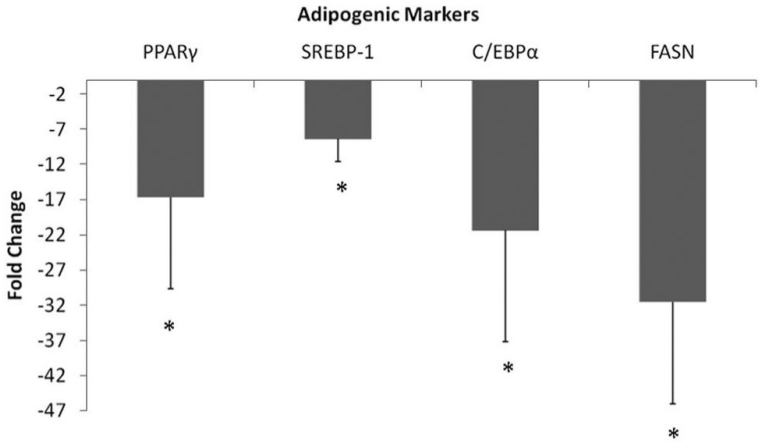

Figure 6.

Fold change of PPARγ, SREBP-1, C/EBPα, and FASN in supraspinatus muscles 6 weeks after TT + DN surgery and rapamycin treatment (* indicates $p < 0.05$).

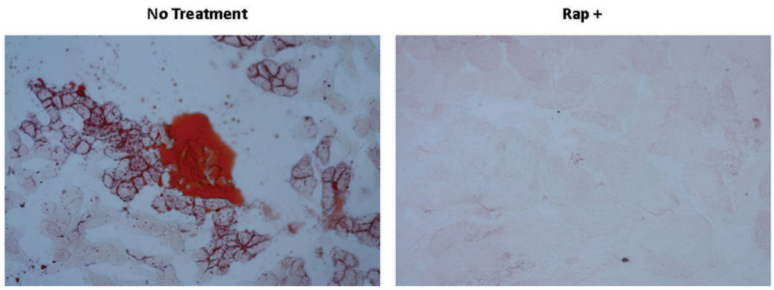

Figure 7.

Oil Red-O staining evaluating fatty infiltration between operated side supraspinatus muscles between TT + DN and Rapamycin groups. Although there was fat present in both groups, the Rapamycin group showed a reduction suggesting that mTOR signaling does influence rotator cuff fatty infiltration.