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Aging-associated exacerbation in fatty degeneration and infiltration following rotator cuff tear

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Abstract

Background—Rotator cuff tears are one of the most common musculoskeletal complaints and a substantial source of morbidity in elderly patients. Chronic cuff tears are associated with muscle atrophy and an infiltration of fat to the area, a condition known as “fatty degeneration.” To improve the treatment of cuff tears in elderly patients, a greater understanding of the changes in the contractile properties of muscle fibers and the molecular regulation of fatty degeneration is essential.

Methods—Using a full-thickness, massive supraspinatus and infraspinatus tear model in elderly rats, we measured fiber contractility and determined changes in fiber type distribution that develop 30 days after tear. We also measured the expression of mRNA and miRNA transcripts involved in muscle atrophy, lipid accumulation, and matrix synthesis. We hypothesized that a decrease in specific force of muscle fibers, an accumulation of type IIb fibers, and an upregulation in atrophic, fibrogenic, and inflammatory gene expression would occur in torn cuff muscles.

Results—Thirty days following tear, we observed a reduction in muscle fiber force and an induction of RNA molecules that regulate atrophy, fibrosis, lipid accumulation, inflammation and macrophage recruitment. A marked accumulation of advanced glycation end products, and a significant accretion of macrophages in areas of fat accumulation were observed.

Conclusions—The extent of degenerative changes in old rats was greater than that observed in adults. Additionally, we identified that the ectopic fat accumulation that occurs in chronic cuff

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Level of evidence: Basic Science Study, Molecular and Cell Biology, Animal Model

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tears does not occur by activation of canonical intramyocellular lipid storage and synthesis pathways.

Keywords

fatty degeneration; rotator cuff; sarcopenia; atrophy; lipid droplets; macrophages

Introduction

Shoulder pain is a common complaint among the elderly population in the US, with 25% of elderly individuals suffering from moderate to severe shoulder pain²⁵. The most common cause of shoulder pain in elderly patients is rotator cuff disease^{25; 56}. Elderly patients with rotator cuff disease often present with complaints of pain and a loss of strength and functional capacity that result in significant morbidity and disability^{25; 35}. As full-thickness rotator cuff tears do not spontaneously heal, non-operative treatments such as physical therapy, anti-inflammatory medication, and activity modification are directed at improving compensatory mechanisms to manage the functional deficits, but up to 74% of patients report persistent shoulder pain and dysfunction that continue to worsen with time²⁵. Even with rotator cuff tears that are successfully repaired arthroscopically, elderly patients are more likely to have reduced shoulder function than younger patients¹⁶. The reasons behind the aging-associated decrease in rotator cuff regeneration are not well understood, and further insights in this area are critical to improve the treatment of elderly patients with rotator cuff tears.

Following the development of a rotator cuff tear, there is often an atrophy of muscle fibers, fibrosis and an accumulation of fat within and around muscle fibers^{13; 15; 21; 42}. This set of pathophysiological changes is often referred to as “fatty degeneration” and is frequently exacerbated in elderly patients with rotator cuff tears²⁵. Compounding the decline in the regenerative capacity of muscles in elderly patients is sarcopenia, which is broadly defined as the loss of skeletal muscle mass and strength that occurs with aging³⁹. There is an elevation in the proteolysis of muscle fiber contractile proteins, a reduction in contractile protein synthesis, and an increase in connective tissue accumulation that impairs the normal function of skeletal muscle in individuals with sarcopenia¹⁸. We have previously shown that rotator cuff tears in adult rats result in reduced muscle fiber force production, conversion of type I and IIA fibers to type IIB, increased total intra- and intermyocellular lipid content and an accumulation of lipid laden macrophages¹⁷. However, the impact of rotator cuff tear on muscle fiber force production, and lipid and macrophage accumulation in elderly rats was not known.

To gain greater insight into aging-associated changes in fatty degeneration, we used a well-established chronic experimental model of full-thickness rotator cuff tear^{17; 27; 45} in a group of elderly male rats. We evaluated changes in muscle fiber contractility, fiber type distribution and size, and the expression of mRNAs and miRNAs involved in lipid synthesis, lipid storage, extracellular matrix production, inflammation, and autophagy in old rats that underwent a full thickness supraspinatus and infraspinatus tear. We hypothesized that, 30 days following a rotator cuff tear in old rats, there would be a reduction in muscle specific fiber force production and an induction in the expression of mRNA and miRNA transcripts that regulate atrophy, inflammation, fibrosis, lipid accumulation and autophagy.

Materials and Methods

Animals

This study was approved by the University of Michigan IACUC. Twenty four month-old male Sprague Dawley rats (N=6) obtained from the NIA Aged Rodent Colony were used in this study. This age was chosen as the maximum lifespan of rats is 32-36 months of age, and the aging-related decline in force generating and regenerative capacities of rats of this age closely reflect what is often observed in elderly humans^{5; 6}. Full-thickness tears of the supraspinatus and infraspinatus tendons were created as previously described¹⁷. Briefly, a full thickness tenectomy of the right supraspinatus and infraspinatus was performed using a deltoid splitting transacromial approach to simulate a massive rotator cuff tear and to prevent scarring and healing of the detached tendons in the rodent model. The left shoulder served as a sham operated control in which a deltoid splitting procedure was performed, but the supraspinatus and infraspinatus tendons were left intact. Following induction of tear, rats were given post-operative buprenorphine (0.05 mg/kg) for analgesia. After a period of thirty days, rats were anesthetized with sodium pentobarbital (50 mg/kg), and the supraspinatus and infraspinatus muscles were harvested. The distal end of the muscle was free and showed no signs of lateral adhesion formation. Rats were then humanely euthanized with pentobarbital overdose. Supraspinatus muscles were weighed, finely minced, and prepared for biochemical measures. Infraspinatus muscles were weighed, and separated at the mid-belly for histology and single fiber contractility.

Muscle Fiber Contractility

Contractile measurements of permeabilized muscle fibers of the proximal infraspinatus were performed as previously described^{17; 32}. Fibers were placed in a temperature controlled chamber containing relaxing solution and secured at one end to a servomotor (Aurora Scientific, Model 322C, Aurora, ON, Canada) and the other end to a force transducer (Aurora Scientific, Model 403A, Aurora, ON, Canada). Fiber length was adjusted to obtain a sarcomere length of 2.5µm. Fiber cross sectional area (CSA) was calculated by analyzing five different diameters along the fiber from high-magnification images of the top and side views. Maximum isometric force (F_0) was elicited by immersing the fiber in a high calcium solution, and specific force (sF_0) was calculated by dividing F_0 by CSA. Ten to 20 fast fibers were tested from each infraspinatus muscle.

Histology

Distal infraspinatus segments were frozen in Tissue-Tek (Sakura, AJ Alphen aan den Rijn, The Netherlands) using isopentane cooled liquid nitrogen, and stored at -80°C until needed. Muscles were cryosectioned at a thickness of 10µm, and stained with Oil red O and hematoxylin or prepared for immunohistochemistry (IHC). Histology was conducted as previously described¹⁷. The distribution of fiber types was determined by labeling sections with antibodies against type I myosin heavy chain (DSHB), type IIA myosin heavy chain (DSHB) and type IIB myosin heavy chain (DSHB). Extracellular matrix (ECM) was identified using wheat germ agglutinin (WGA) lectin conjugated to AlexaFluor 488 (Invitrogen, Grand Island, NY, USA). Type IIX fibers were detected by the absence of signal. To identify macrophages and areas of lipid accumulation, slides were incubated with antibodies against the macrophage-specific antigen F4/80 (AbCam) and the lipid stain BODIPY 493/503 (Invitrogen). Primary antibodies were detected using highly cross-adsorbed secondary antibodies conjugated to AlexaFluor probes (Invitrogen). Images were obtained using a Zeiss Axioplan 2 microscope with AxioCam cameras. Quantitative measurements were performed using ImageJ software (NIH).

Gene Expression

Total RNA was isolated from supraspinatus muscles using a miRNeasy kit (Qiagen), treated with DNase I (Qiagen), and the concentration of isolated RNA was measured on a NanoDrop (Thermo). RNA was reverse transcribed to cDNA using the RT² First strand kit (Qiagen) and amplified in a CFX96 real time thermal cycler (Bio-Rad) using a custom array of primers for specific mRNA and miRNA species, and an RT² SYBR Green qPCR mix (Qiagen). Expression of mRNA transcripts were normalized to the stable housekeeping gene β -actin, and miRNA transcripts to the stable housekeeping gene Rnu6⁴¹. A list of transcripts and RefSeq and miRBase information is presented in Supplementary Tables 1 and 2.

Measurement of Advanced Glycation End Product and N^ε-(carboxymethyl) Lysine Adducts

Twenty five milligrams of finely minced supraspinatus muscle was digested into peptides by placing samples in a 20mg/mL solution of proteinase K (Invitrogen) for 24 hours at 55°C. Following digestion, the proteinase was inactivated by heating samples to 95°C for 10 minutes. ELISAs (Cell Biolabs) for advanced glycation end product (AGEs) and N^ε-(carboxymethyl) lysine (CML) protein adducts were performed following manufacturer's directions. ELISA plates were read in a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Measured AGE and CML adduct content was normalized to input wet muscle mass.

Statistical analysis

Data are presented as mean±SE. Differences between the sham and tear side were tested using t-tests ($\alpha=0.05$) in GraphPad Prism 5.0b.

Results

Thirty days after creation of the rotator cuff tear, there was a 40% decrease in the mass of supraspinatus muscles (sham 386±37.6mg, tear 230±22.7mg, $P<0.05$) and a 50% decrease in the mass of infraspinatus muscles (sham 394±27.1mg, tear 198±12.7mg, $P<0.05$). For single muscle fibers, rotator cuff tear had no effect on CSA (Figure 1A), but there was a 40% reduction in F_o , and a 30% decrease in sF_o (Figure 1B-C). Infraspinatus muscles were then analyzed for myosin heavy chain isoforms to determine fiber type size and distribution. The size of type IIB and hybrid type I/IIA muscle fibers (Figure 2A), and the amount of type IIA fibers (Figure 2B), was reduced in torn rotator cuff muscles. A representative image of muscle fiber typing is shown in Figure 2C. We next measured the levels of the oxidative stress markers AGE and CML, and observed an increase in both AGE and CML adducts in torn rotator cuff muscles (Figure 3).

A marked increase in lipid accumulation within and around muscle fibers was observed (Figure 4A-B), and fatty macrophage deposition was also frequently noted in torn rotator cuff muscles (Figures 4C-D). Gene expression of torn muscles showed increases in the expression of PPAR γ and C/EBP α , and a decrease in the expression of glycerol-3-phosphate acyltransferase (GPAT4, Figure 5A). There was no change in DGAT1, the fatty acid transporter, FAT/CD36, or adipose triglyceride lipase (ATGL), a lipolytic enzyme (Figure 5A). For lipid storage transcripts, there was a dramatic increase in the expression of perilipin-1 (PLIN1), fat specific protein of 27kDa (FSP27), and phospholipase D1 (PLD1, Figure 5B), with no change in PLIN2 (ADRP), PLIN5 (OXPAT), the perilipin binding partner, CGI58, or the ER associated lipid droplet binding proteins, fat storage-inducing transmembrane proteins 1 and 2 (FIT1 and FIT2, Figure 5B).

For transcripts involved in fibrosis and ECM maintenance, there were increases in Col1 α 2, FSP-1, MMP14, MMP2, PDGFR α , TIMP1, TIMP2, and tenomodulin (Figure 5C). There

was no change in MMP9 and a decrease in scleraxis expression (Figure 5C). Transcripts involved in autophagy, Atg16L1, Atg5, Beclin-1, and Vps34 were unchanged following rotator cuff tear (Figure 5D). For atrophy and inflammation gene expression, there was a decrease in the E3 ubiquitin ligase, MuRF-1, but no change in Atrogin-1 (Figure 5E). Additionally, there were increases in 5-LOX, COX-2, IL-10, and IL-1 β , but no change in COX-1 or IL-6 (Figure 5E).

For transcripts associated with macrophage accumulation, an increase in the pan macrophage marker F4/80 (Figure 5F) was observed. No change was detected for the expression of the pro-inflammatory M1 macrophage marker, CCR7, but an increase in two other M1 macrophage markers, CD11b and CD68 (Figure 5F) were observed. The anti-inflammatory M2 macrophage marker, CD168, was unchanged following tear (Figure 5F). ApoE, a transcript related to fatty macrophage formation, was increased following tear, but there was no change in the primary fatty macrophage cholesterol storage transcript ACAT1 (Figure 5F).

We also evaluated the expression of several miRNAs. For miRNAs associated with muscle adaptation, miR-499, 133a, -29b, and -143 were downregulated, while miR-23a was upregulated in torn muscles (Figure 6A). There was no change in miR-133b, -126, -23b, -1, or -206 (Figure 6A). Lipid metabolism and adipogenesis related transcript miR-130a was upregulated following tear, but no changes in miR-27a, -138, -103, -196a, and -107 were detected (Figure 6B). miRNAs associated with ECM synthesis, miR-21, -381, -221, -214, and -31 were upregulated following tear, but miR-338 and -222 (Figure 6C) did not change. All transcripts measured from the let-7 cluster were upregulated (Figure 6D).

Discussion

Understanding the impact of full-thickness, massive rotator cuff tear on muscle fiber force production, and lipid and macrophage accumulation is of critical importance to improve outcomes after rotator cuff repair surgery in elderly patients. This was the first study, to our knowledge, that measured changes in contractility, fiber type distribution, gene expression of various mRNAs and miRNAs, and markers of oxidative stress following a rotator cuff tear in an elderly rodent model. Rotator cuff tear reduced F_0 and sF_0 , indicating there was a functional decrease in the number of force generating myofibrils within muscle fibers. The relative decrease in force production was more pronounced in elderly rats than in adult rats from our previous study¹⁷. Adult rats showed decreases in the CSA of type I, IIX, and IIA/IIX fibers following rotator cuff tear, but in the current study old rats had decreases in the size of type IIB and I/IIA fibers. We also expected an increase in the number of type IIB fibers, but did not see this in elderly rats. Combined, these results suggest that elderly rats experience greater decreases in force production after tear, and the aging-related impact of rotator cuff tear is fiber type specific.

Interleukins, prostaglandins, and leukotrienes have diverse roles in promoting inflammation, fibrosis and atrophy after muscle injury^{23; 33; 43; 46}. IL-1 β generally is a pro-inflammatory cytokine that promotes muscle atrophy⁴⁴ and was upregulated following tear. COX-2 catalyzes the formation of pro-inflammatory prostaglandins that inhibit muscle stem cell proliferation and muscle regeneration^{33; 43}, and 5-LOX which converts arachidonic acid into pro-inflammatory leukotrienes⁴⁶, were also upregulated. AGEs and CML are markers of chronic oxidative stress in muscle that promote local inflammation^{1; 8}, and elevations in AGE and CML protein adducts were observed in torn rotator cuff muscles of old rats. For miRNAs associated with myogenesis and muscle regeneration¹¹, miR-1, -133b, and -206 were unchanged following tear. However, miR-133a and miR-499, which promote differentiation of myoblasts¹¹, were downregulated following tear. The let-7 cluster of

miRNAs play an important role in regulating myogenesis⁹, and all let-7 transcripts were elevated in torn rotator cuff muscles. While induction in autophagy related transcripts was observed in torn rotator cuff muscles from adult rats, no changes in the autophagy related transcripts Atg16L1, Atg5, Beclin-1 or Vps34 were observed in the current study. MuRF-1 and atrogin-1 are the major E3 ubiquitin ligases in skeletal muscle and serve as important rate limiting enzymes in protein degradation⁴⁰. miR-23a is a miRNA that binds to the 3' UTR of MuRF-1 and atrogin-1 transcripts, and plays an important role in inhibiting MuRF-1 and atrogin-1 translation⁴⁹. Unlike adult rats which showed no change in MuRF-1, atrogin-1 or miR-23a after rotator cuff tear¹⁷, old rats had an upregulation in miR-23a expression and a corresponding downregulation of MuRF-1 expression. While MuRF-1 and atrogin-1 have a well described role in acute muscle atrophy following injury or immobilization, there is conflicting data regarding the role of ubiquitin mediated proteolysis in chronic muscle atrophy^{18; 42}. The results from the current study suggest that although several proinflammatory markers are upregulated after prolonged rotator cuff tear, activation of autophagy pathways and sustained induction of MuRF-1 and atrogin-1 are not required for chronic atrophy in torn rotator cuff muscles of old rats.

Chronic rotator cuff tears often have substantial fibrosis^{27; 29}, and for torn muscles in the current study there was a gross accumulation of connective tissue apparent from immunohistochemistry. For gene expression, torn rotator cuff muscles had an elevation in the expression of type 1 collagen. Elevations of miR-214, -21, -221 and -222 are associated with fibrosis in other tissues^{3; 19; 36}, and while miR-222 expression was not different there was an increase in miR-214, -21 and -221 in torn rotator cuff muscles. The ECM degradation transcripts MMP2 and MMP14 were elevated, but increases in the MMP inhibitors TIMP1 and TIMP2 were also observed. The fibroblast proliferation markers FSP-1, PDGFR- α and tenomodulin were also elevated. Scleraxis, which is a bHLH transcription factor that promotes tendon fibroblast proliferation²⁸ was downregulated, while miR-381 which is predicted to inhibit scleraxis expression³¹ was upregulated. The downregulation in scleraxis is likely due to the lack of passive tension in torn muscles, as loss of tensile load reduces scleraxis expression²⁸. Overall the general pattern of changes in mRNA and miRNA molecules that regulate ECM production was similar to what was observed in our previous study in adult rats¹⁷, although the tear-induced changes were generally of greater magnitude for old muscles than adult muscles.

Macrophages play an important role in regulating muscle degeneration and regeneration following injury, and there are at least two well studied populations of macrophages that are thought to contribute to each of these processes. The classically activated M1 macrophages promote atrophy and inflammation, while the alternately activated M2 macrophages help to resolve inflammation and promote regeneration^{37; 47}. Previously, we demonstrated a marked accumulation of macrophages in torn rotator cuff muscles of adult rats¹⁷, although we did not evaluate markers of specific M1 and M2 subpopulations of macrophages. For old rats, increases in the expression of the M1 transcripts CD11b and CD68 were observed, but no change in the M2 marker CD168 was observed. Following most muscle injuries, CD11b and CD68 levels increase immediately following muscle injury and return to baseline levels 4-6 days after injury⁴⁷. The persistent elevation of M1 markers in a chronic rotator cuff tear in old rats suggests a prolonged activation of inflammatory and degenerative processes. In atherosclerosis, a population of fatty macrophages, often referred to as "foam cells", uptake high levels of lipid and promote local tissue inflammation⁷. Activation of the transcription factor PPAR- γ plays an important role in the differentiation of fatty macrophages, which upon differentiation express high levels of Apolipoprotein E (ApoE) and FSP27 at high levels^{24; 48}. In the current study, substantial elevations in PPAR- γ , ApoE and FSP27 were observed. In atherosclerosis, foam cells can exacerbate fatty lesions that worsen the severity

of the disorder⁷, and these cells may also contribute to chronic inflammation and degeneration in rotator cuff tears.

While the deposition of large amounts of fat in and around muscle fibers is commonly observed after tear, and there is a negative correlation between fat accumulation and clinical outcomes following rotator cuff tear¹⁴, the molecular etiology of fatty infiltration following tear is not well understood. PPAR- γ and C/EBP α have diverse roles in lipid synthesis and storage and are commonly used as general markers of adipogenesis following rotator cuff tear. In agreement with previous studies^{13; 17; 20; 22}, both transcripts are upregulated in old rats following rotator cuff tear. The ontogeny of lipid accumulation, and the specific cells and signaling pathways which drive this process in rotator cuff tears, remain largely unknown. A slight aging-associated increase in adipocytes has been reported in a rat model of full thickness rotator cuff tears¹². Marked increases in lipid accumulation have been observed in response to chronic endurance training, and in patients with type 2 diabetes mellitus – a phenomenon referred to as the “athlete’s paradox”⁵¹. The accumulation of fat in these states can have both adaptive and maladaptive effects on muscle function⁵¹. In order to gain a greater understanding of the mechanisms behind the development of fatty degeneration in the rotator cuff, we evaluated the expression of several genes with well known roles in canonical lipid synthesis, storage and metabolism.

FAT/CD36 is the primary protein used to transport fatty acids into cells⁵¹. Once fatty acids have been taken up into the cytosol, there are stored in lipid droplets as triglycerides, a process catalyzed by the enzymes GPAT4 and DGAT1⁵⁰. Small lipid droplets, such as those typically found in endurance trained or diabetic muscles, are coated by perilipin 2⁵⁰. FSP27 promotes the formation of large lipid droplets³⁸, and perilipin 1 preferentially coats droplets of this size¹⁰, like those observed in adipocytes and foam cells. When fatty acids are needed for energy metabolism, perilipin-5 coats lipid droplets and targets them to mitochondria⁵⁴. On the lipid droplet surface, CGI-58 activates ATGL to directly liberate fatty acids from stored triglycerides⁵⁷, which can then be put through the β -oxidation pathway for energy production. While there was a notable increase of ectopic fat, there was an upregulation of miR-130a, which is downregulated during adipogenesis⁵⁵, and no change in FAT/CD36 expression following tear. There was a downregulation of GPAT4 and unchanged DGAT1 expression following tear. Consistent with the large intramyocellular lipid droplets observed in torn rotator cuff muscles, there was a marked increase of perilipin 1 expression after rotator cuff tear, but no change in either perilipin 2 or perilipin 5. The expression of ATGL and its activator, CGI58, was not different following tear. There were also no changes in miR-103 and -107, which increase lipid metabolism and mitochondrial function⁵². In Oil red O images, the size of intramyocellular lipid droplets is substantially larger than what is typically observed for endurance athletes or type 2 diabetics^{2; 34}, and for intermyocellular lipid, many of the fat deposits contained high levels of macrophages. Together, these results suggest the dramatic increase in fat that occurs with fatty degeneration is not being actively used for metabolic purposes, and may be a pathological response to rotator cuff tear.

This study has several limitations. Although the rat is an accepted model of rotator cuff tear because of its morphological similarities to humans⁴⁵, rats do not develop the severity of fatty degeneration seen in human patients. We chose a single time-point of thirty days to study an early chronic tear condition to allow for comparisons to our previous study, but did not evaluate changes over multiple time points. A tenectomy model was also required to prevent spontaneously healing which can occur in rodents but does not occur in humans. While we make comparisons between observations in the current manuscript to our previous work¹⁷, we did not directly perform direct statistical analyses to evaluate the effect of age and tear on rotator cuff function and morphology. For single fiber measurements, we used

type II fibers only, and did not measure by specific myosin isoform. We did this due to the greater amount of type II fibers in the cuff, and sF_o does not change between fibers with different type II myosin isoforms⁴. We used the contralateral side as the control muscle, and although we did not notice any overt signs of muscle injury, it is possible that the control muscle experienced a small amount of muscle hypertrophy. We only measured changes in gene expression as opposed to protein level changes, and did not measure changes specific to myosin isoform or cell type. Despite these limitations, this study provided insight into the cellular and molecular changes in rotator cuff muscles of old rats and identified many transcripts and cytokines that may be important for causing many of these changes.

An estimated 50% or greater of elderly patients have a full thickness rotator cuff tear, and many of these patients are symptomatic with pain, weakness, and functional limitations with activities of daily living²⁵. The prevalence and size of tears increase with age²⁵, and treatment is limited. NSAIDs are used to decrease pain and inflammation, however use of these drugs can inhibit muscle regeneration^{33; 43} and the use of NSAIDs may not be appropriate for elderly individuals with certain cardiovascular conditions³⁰. Physical rehabilitation for elderly patients with rotator cuff tears follows a three phase system with coordinated pain control, and exercises to improve strength and proprioception⁵³, but despite these strategies, the incidence of fatty degeneration does not decrease. The ectopic fat seen in rotator cuff tears is an attractive therapeutic target, as pathological lipid that accumulates in muscle has been shown to increase inflammation and inhibit activation of the mTOR/Akt pathway³⁷. While lipid-mediated inhibition of mTOR/Akt signaling has been studied in the context of glucose transport and insulin sensitivity, many of the mTOR/Akt signaling pathway components also regulate protein synthesis in muscle⁴⁰. Liu and colleagues observed a decreased activation of Akt and mTOR in torn rotator cuff muscles²⁶, and the accumulation of lipid in torn rotator cuff muscle might inhibit protein synthesis pathways and reduce the recovery of muscle fiber force production. Further studies exploring the specific nature and regulation of lipid deposition in rotator cuff tears will be critical in development of treatment strategies to combat fatty degeneration and return patients to full function.

Conclusion

Rotator cuff tears are common upper extremity injuries in the elderly and treatment options to reverse fatty degeneration are limited. While the elderly have high rates of rotator cuff tears, the majority of animal studies that have evaluated the biology of rotator cuff tears have used juvenile or adult animals. Using an elderly rat model, the present study evaluated the changes in muscle fiber contractility, fiber type distribution, gene expression and AGE accumulation that occurred after inducing a rotator cuff tear. We observed a reduction in muscle fiber force production, an induction of fibrogenic, mRNA and miRNA molecules, and a significant accumulation of macrophages in areas of fat accumulation. In general the magnitude of degenerative changes in old rats was greater than that observed in adult animals. We also identified that the ectopic fat accumulation that occurs in chronic rotator cuff tears in elderly rats does not occur by activation of canonical intramyocellular lipid storage and synthesis pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Disclaimer

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Figure 1. Permeabilized fiber contractility size and force. (A) Permeabilized fiber cross sectional area (CSA), (B) maximum isometric force (F_0) and (C) specific force (s F_0) of sham and torn rotator cuff muscles. Values are mean $\pm$ SE. N=6 muscles from each group. *, significantly different from sham group (P<0.05).

Figure 2.

Fiber type size and percent composition. (A) Cross sectional area (CSA) and (B) percent distribution of myosin heavy chain isoform of muscle fibers from sham and torn rotator cuff muscles. (C) Representative immunohistochemistry demonstrating different fiber types. White, extracellular matrix (WGA-lectin); blue, MHC I; red, MHC IIA; green, MHC IIB; black, MHC IIX; blue-red, hybrid MHC I/IIA; red-black, hybrid MHC IIA/IIX; green-black, hybrid MHC IIB/IIX. Values are mean±SE. N=6 muscles from each group. *, significantly different from sham group ($P<0.05$).

Figure 3. Oxidative stress markers. (A) Advanced glycation end product (AGE) protein adducts and (B) N^ε-(carboxymethyl) lysine (CML) protein adducts in sham and torn rotator cuff muscles, normalized to wet muscle mass. Values are mean±SE. N=6 muscles from each group. *, significantly different from sham group (P<0.05).

Figure 4. Lipid and macrophage immunohistochemistry. Representative Oil red O sections from (A) sham and (B) torn rotator cuff muscles. Blue, hematoxylin; red, Oil red O. Representative immunohistochemistry from (C) sham and (D) torn rotator cuff muscles. Blue, nuclei (DAPI); red, extracellular matrix (WGA lectin); green, lipid (BODIPY); white, macrophages (F4/80). Arrowheads indicate areas of macrophage accumulation concomitant with lipid deposition.

Figure 5. Gene expression. Expression of genes associated with (A) adipogenesis, (B) lipid storage, (C) extracellular matrix synthesis and fibrosis, (D) autophagy, (E) inflammation and atrophy and (F) macrophage and fatty macrophage accumulation. Target gene expression was normalized to β -actin. Values are mean $\pm$ SE. N=6 muscles from each group. *, significantly different from sham group ($P<0.05$).

Figure 6. miRNA expression. Expression of miRNAs associated with (A) muscle adaptation, (B) lipid storage and synthesis, (C) extracellular matrix synthesis and fibrosis, (D) the let7 cluster. Target miRNA expression was normalized to Rnu6. Values are mean $\pm$ SE. N=6 muscles from each group. *, significantly different from sham group ($P<0.05$).