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

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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 messenger RNA and micro-RNA 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 after the 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. In addition, we identified that the ectopic fat accumulation that occurs in chronic cuff tears does not occur by activation of canonical intramyocellular lipid storage and synthesis pathways.

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

Keywords: Fatty degeneration; rotator cuff; sarcopenia; atrophy; lipid droplets; macrophages

Shoulder pain is a common complaint among the elderly population in the United States, with 25% of elderly individuals experiencing moderate to severe shoulder pain.[25] The most common cause of shoulder pain in elderly

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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] Because full-thickness rotator cuff tears do not spontaneously heal, nonoperative 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 time.[25] Even with rotator cuff tears that are successfully repaired arthroscopically, elderly patients are more likely to have reduced shoulder function than younger patients.[16] 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.

After a rotator cuff tear develops, 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 pathophysiologic changes is often referred to as "fatty degeneration" and is frequently exacerbated in elderly patients with rotator cuff tears.[25] 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 aging.[39] 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 sarcopenia.[18] We previously showed 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 intramyocellular and intermyocellular lipid content, and an accumulation of lipid-laden macrophages.[17] However, the effect of rotator cuff tear on muscle fiber force production and on 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 elderly male rats. We evaluated changes in muscle fiber contractility, fiber type distribution and size, and the expression of messenger RNAs (mRNAs) and micro-RNAs (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 after 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

The study used 24-month-old male Sprague-Dawley rats (n = 6) obtained from the National Institutes on Aging (NIA) Aged Rodent Colony (National Institutes of Health, Bethesda, MD, USA). This age was chosen because the maximum lifespan of rats is 32 to 36 months, 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 described.[17] 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. After induction of tear, rats were given postoperative buprenorphine (0.05 mg/kg) for analgesia.

After 30 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 midbelly for histologic analysis 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 one end was secured to a servomotor (Model 322C, Aurora Scientific, Aurora, ON, Canada) and the other end to a force transducer (Model 403A, Aurora Scientific). Fiber length was adjusted to obtain a sarcomere length of 2.5 μm. Fiber cross-sectional area (CSA) was calculated by analyzing 5 different diameters along the fiber from high-magnification images of the top and side views. Maximum isometric force (F_o) was elicited by immersing the fiber in a high calcium solution, and specific force (sF_o) was calculated by dividing F_o by CSA. Ten to 20 fast fibers were tested from each infraspinatus muscle.

Histologic analysis

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^{o}C until needed. Muscles were cryosectioned at a thickness of 10 μm and stained with Oil red O and hematoxylin or prepared for immunohistochemistry. Histologic analysis was conducted as previously described.[17]

The distribution of fiber types was determined by labeling sections with antibodies against type I, type IIA, and type IIB myosin heavy chain (all from Developmental Studies Hybridoma