Effect of High-Intensity Interval Training on Fatty Infiltration After Delayed Rotator Cuff Repair in a Mouse Model

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Background: Fatty infiltration (FI) of the rotator cuff muscles is correlated with shoulder function and retear rates after rotator cuff repair. High-intensity interval training (HIIT) induces beige adipose tissue to express more uncoupling protein 1 (UCP1) to consume lipids. The beta-3 adrenergic receptor (β3AR) is located on adipocyte membrane and induces thermogenesis.

Purpose: To test the role of HIIT in improving muscle quality and contractility in a delayed rotator cuff repair mouse model via β3AR.

Study Design: Controlled laboratory study.

Methods: Three-month-old C57BL/6J mice underwent a unilateral supraspinatus (SS) tendon transection with a 6-week delayed tendon repair. Mice ran on a treadmill with the HIIT program for 6 weeks after tendon transection or after delayed repair. To study the role of β3AR, SR59230A, a selective β3AR antagonist, was administered to mice 10 minutes before each exercise through intraperitoneal injection. The SS, interscapular brown adipose tissue (iBAT), and subcutaneous inguinal white adipose tissue (ingWAT) were harvested at the end of the 12th week after tendon transection and were analyzed by histology and Western blotting. Tests were performed to assess muscle contractility of the SS.

Results: Histologic analysis of SS showed that HIIT prevented and reversed muscle atrophy and FI. The contractile tests showed higher contractility of the SS in the HIIT groups than in the no-exercise group. In the HIIT groups, SS, iBAT, and ingWAT all showed increased expression of tyrosine hydroxylase, UCP1, and upregulated β3AR thermogenesis pathway. However, SR59230A inhibited HIIT, suggesting that the effect of HIIT depends on β3AR.

Conclusion: HIIT improved SS quality and function after delayed rotator cuff repair through a β3AR-dependent mechanism.

Clinical Relevance: HIIT may serve as a new rehabilitation method for patients with rotator cuff muscle atrophy and FI after rotator cuff repair to improve postoperative clinical outcomes.

Keywords: rotator cuff tear; delayed repair; high-intensity interval training; fatty infiltration

Muscle atrophy and fatty infiltration (FI) are common in patients who have sustained chronic rotator cuff (RC) tears. Intermediate and severe FI (Goutallier stages 2-4) occurs in >30% of patients.[38] Many studies have demonstrated that the amount of atrophy and FI correlates with high retear rates and poor clinical outcomes after surgical repairs.[7,12,19] It was historically believed that FI is irreversible, even after surgical repair.[21,27]

Recent studies have shown the possibility of reversing FI.[22,28,48] Laboratory research has shown that brown/beige-like adipose tissue might play an important role in this process since brown adipose tissue (BAT) (see Appendix Table A1 for expansions of specialized terms used in this article) can clear lipids by thermogenesis with uncoupling protein 1 (UCP1).[9] In addition, batokines released by BAT, such as VEGF and IGF1, are beneficial to muscle regeneration. Bryniarski and Meyer[5] tried an intermuscular fat transplant model, and their results showed that brown fat transplant promoted adjacent muscle regeneration, which is possibly achieved with paracrine cross-talk. Lee et al[30,31] found that transplantation of beige adipose fibro-adipogenic progenitors (FAPs) was beneficial to muscle regeneration after RC tears. Exosomes secreted by UCP1+ FAPs showed potent promyogenic activity in vitro and in vivo.[11] Meyer et al[39] found epimuscular adipose-derived stem cells from RC. These stem cells tended to increase the expression of beige-selective genes when RC was torn and promoted myogenesis in vitro.

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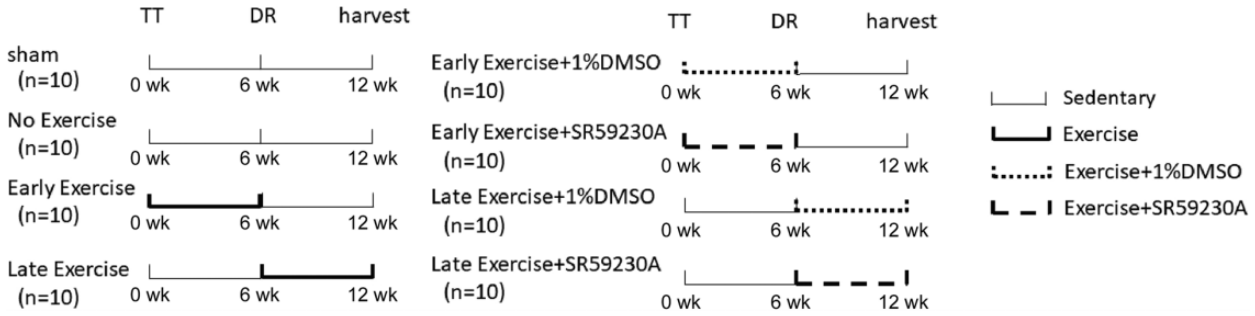

Figure 1. Flow diagram of experimental design. DMSO, dimethyl sulfoxide; DR, delayed repair; TT, tendon tear.

Cold exposure,[17,53] diet,[24] exercise,[46,53] and some other stimuli activate BAT through the sympathetic nervous system.[40] Noradrenaline released by sympathetic nerve fibers stimulates the beta-3 adrenergic receptor (β3AR) and activates the downstream cAMP-PKA axis.[42] As a result, HSL and UCP1 start hydrolyzing lipid droplets and thermogenesis.[42] High-intensity interval training (HIIT) is an effective method for activating the sympathetic system[45] and losing weight,[26,52] including various training models, such as cycling, running, swimming, and other activities.[26] According to some studies, HIIT promotes white fat browning more effectively than moderate-intensity continuous training,[37,47] but there is no research about HIIT and FI.

Herein, we aimed to study the effect of HIIT on muscle atrophy and FI with a mouse model of delayed RC tendon repair.[49] We hypothesized that HIIT helps to improve muscle atrophy and FI in RC muscle by activating BAT in muscle through the sympathetic system.

METHODS

Experiment Design

The protocol for this animal study received ethics committee approval. To study the effect of HIIT on RC tears, 40 mice were randomly divided into 4 groups of 10 (n = 5 for histological analysis and n = 5 for contractile tests and Western blot) as follows: (1) sham, (2) tendon tear (TT) + delayed repair (DR) + no exercise (NE group), (3) TT + DR + early exercise (EE group), and (4) TT + DR + late exercise (LE group) (Figure 1). All 30 mice outside of the sham group underwent unilateral (right side) supraspinatus (SS) tendon tear followed by delayed repair 6 weeks later. This TT + DR model has been described in our former study.[49] Briefly, after the shoulder was opened for repair, a 0.5-mm tunnel was created through the humerus with p-6 cutting needles (8648G; Ethicon) and the SS tendon was fixed to the anatomic position with No. 7-0 polypropylene sutures (8648G; Ethicon). The sham group and the NE group underwent no exercise during the 12 weeks. The EE group underwent exercise during the 6 weeks between TT and DR and then no exercise after DR. The LE group underwent exercise for 6 weeks from day 3 after DR. To study the role of β3AR, we administered SR59230A (SR), a selective β3AR antagonist, to mice before each HIIT session (Figure 1).

An additional 40 mice were randomly divided into 4 groups of 10, as follows: (1) TT + DR + EE + dimethyl sulfoxide (DMSO) (EE DMSO group), (2) TT + DR + EE + SR (EE SR group), (3) TT + DR + LE + DMSO (LE DMSO group), and (4) TT + DR + LE + SR (LE SR group). All these mice underwent TT + DR, with SR (2 mg/kg) or 1% DMSO-PBS placebo administered by intraperitoneal injection 10 minutes before each exercise. SR (Sigma-Aldrich) was dissolved in 1% DMSO-PBS buffer.

The mice were housed in cages with a 12-hour dark-light cycle with free access to water and regular chow diet.

Exercise Protocol

HIIT exercise was performed on a motorized mouse treadmill 5 days per week for 6 weeks, according to a protocol slightly modified from that described by Wang et al[47] and Martinez-Huenchullan et al.[37] Before the first exercise, a maximal running capacity (MRC) test was performed on the mice that received surgery (the EE group after TT, the

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Ethical approval for this study was obtained from the Animal Welfare Committee of Central South University.

LE group after DR). The HIIT program included 7 rounds (2 minutes at 50% of the MRC followed by 4 minutes at 90% of the MRC). The MRC test was performed every 2 weeks, and the velocity was adjusted accordingly.

Muscle Harvesting and Wet Muscle Weight

All mice were sacrificed 12 weeks after the first surgery. For assessment of muscle atrophy, wet weights of the bilateral SS muscles were measured immediately after harvesting. The percentage change in wet muscle weight was determined with the following equation: $([SS_{right} - SS_{left}]/SS_{left}) \times 100\%$.[16]

Masson Trichrome Staining, Oil Red O Staining, and Cross-sectional Area

The treated-side SS (n = 5 per group) muscles were flash-frozen and then cryosectioned as described previously.[33] Masson trichrome (G1345; Solarbio) stain was used to assess fibrosis and atrophy in SS muscles. Oil red O (G1260; Solarbio) was used to assess FI in the SS. Fibrosis and FI were assessed as a percentage of the muscle section area. The cross-sectional area (CSA) of myocyte was assessed in the Masson trichrome–stained slides. Slides were covered with 10% glycerol in PBS (for oil red O) or 50% resinene in xylene (for Masson trichrome) and observed on an optical microscope. Cross sections were chosen randomly from the midbellies of the SS. Pictures were analyzed with ImageJ software (National Institutes of Health) as described previously.[33]

Immunofluorescence Staining

The treated-side SS (n = 5 per group) cryosection slides were fixed in 4% paraformaldehyde for 30 minutes, rinsed in PBS, placed in 0.1 M glycine (diluted in PBS; Fisher Scientific) for 30 minutes, and washed again in PBS. They were then covered with blocking solution (0.2% Triton X-100; 2% bovine serum albumin in PBS) for 1 hour at room temperature. Primary antibodies against UCP1 (an indicator of BAT activation or white fat browning) (diluted 1:100; NB100-2828; Novus Biologicals), tyrosine hydroxylase (TH) (an indicator of activation of sympathetic nerve fibers) (diluted 1:500; 66334-1-Ig; Proteintech), laminin (diluted 1:500; L9393; Sigma-Aldrich), and perilipin (diluted 1:500; abs137082; Absin) were diluted in a block mix and added to the sections for overnight incubation at 4°C. After a PBS rinse, the sections were incubated with a mixture containing FITC-conjugated (diluted 1:500; SA00003-8, SA00003-2; Proteintech) and Cy3-conjugated (diluted 1:500; SA00009-3, SA00009-1; Proteintech) secondary antibodies at room temperature for 120 minutes. After a PBS rinse for 2 × 10 minutes, the slides were covered with DAPI containing antifade mounting medium.

Western Blot

Total proteins were extracted using RIPA-lysis buffer containing proteinase inhibitors (No. 04693159001; Roche) and phosphatase inhibitors (No. 04906845001; Roche), separated by SDS-PAGE, transferred to PVDF membranes, and analyzed by immunoblotting. Primary antibodies against the following proteins were used: TH (66334-1-Ig; Proteintech), PKA (AF5450; Affinity), p-HSL (AF8026; Affinity), HSL (AF6403; Affinity), p-p38 MAPK (AF4001; Affinity), p38 MAPK (AF6456; Affinity), PGC1α (AF5395; Affinity), UCP1 (NB100-2828; Novus Biologicals), and GAPDH (60004-1-Ig; Proteintech). The antibodies were diluted 1:1000 with PBS. The membranes were then incubated with a peroxidase-conjugated secondary antibody (BS13278 [Bioworld], SA00001-1 [Proteintech], SA00001-3 [Proteintech]), and antibody-specific signals were detected by enhanced chemiluminescence and quantified using an automatic chemiluminescence imaging system (Complex 2000; Nanjing PuoAoXin Biotechnology).

SS Contractile Test

Preparation was performed while the mouse was anesthetized. We exposed the SS and scapular spine and removed the trapezius and deltoid. We sutured around the distal tendon part and proximal part of the SS. Then, we cut the scapula across the scapular spine and cut the humerus. After fixing the 2 sides of SS, we assessed the tetanic contraction force with a BL-420F acquisition system (Chengdu TME Technology). During the test, the muscle was stimulated with parallel wire electrodes (20 V; 100 Hz; 0.6 s), maintained in a bath of Ringer solution (137 mM NaCl, 5 mM KCl, 1 mM NaH_2PO_4, 24 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 11 mM glucose) bubbled with CO_2 to maintain pH.

Statistical Analysis

We applied the Dunnett multiple comparison test to determine the significant differences among the sham, NE, EE, and LE groups and applied the Sidak multiple comparison test to determine the significant differences among the EE DMSO, EE SR, LE DMSO, and LE SR groups. All data are shown as the mean ± SD. Statistical differences were determined when $P < .05$.

RESULTS

SS Muscle Atrophy and FI After HIIT

The repaired tendon was found to be intact in all specimens after HIIT in the LE group (Appendix Figure A1). Compared with the sham group, mice in the NE group showed an increased area of FI and collagen, but decreased SS weight and CSA (Figure 2). However, if mice ran with either the early or the late HIIT schedule, their SS quality turned out better than that in the NE groups. The area of FI was 1.05% ± 0.13% for EE (vs 10.7% ± 3.0% for NE; $P < .0001$) and 1.14% ± 0.44% for LE (vs NE; $P < .0001$) (Figure 2, A and D). The collagen area was 1.78% ± 0.19% (EE) (vs 3.52% ± 0.54% for NE; $P = .003$) and 1.79% ± 0.63%

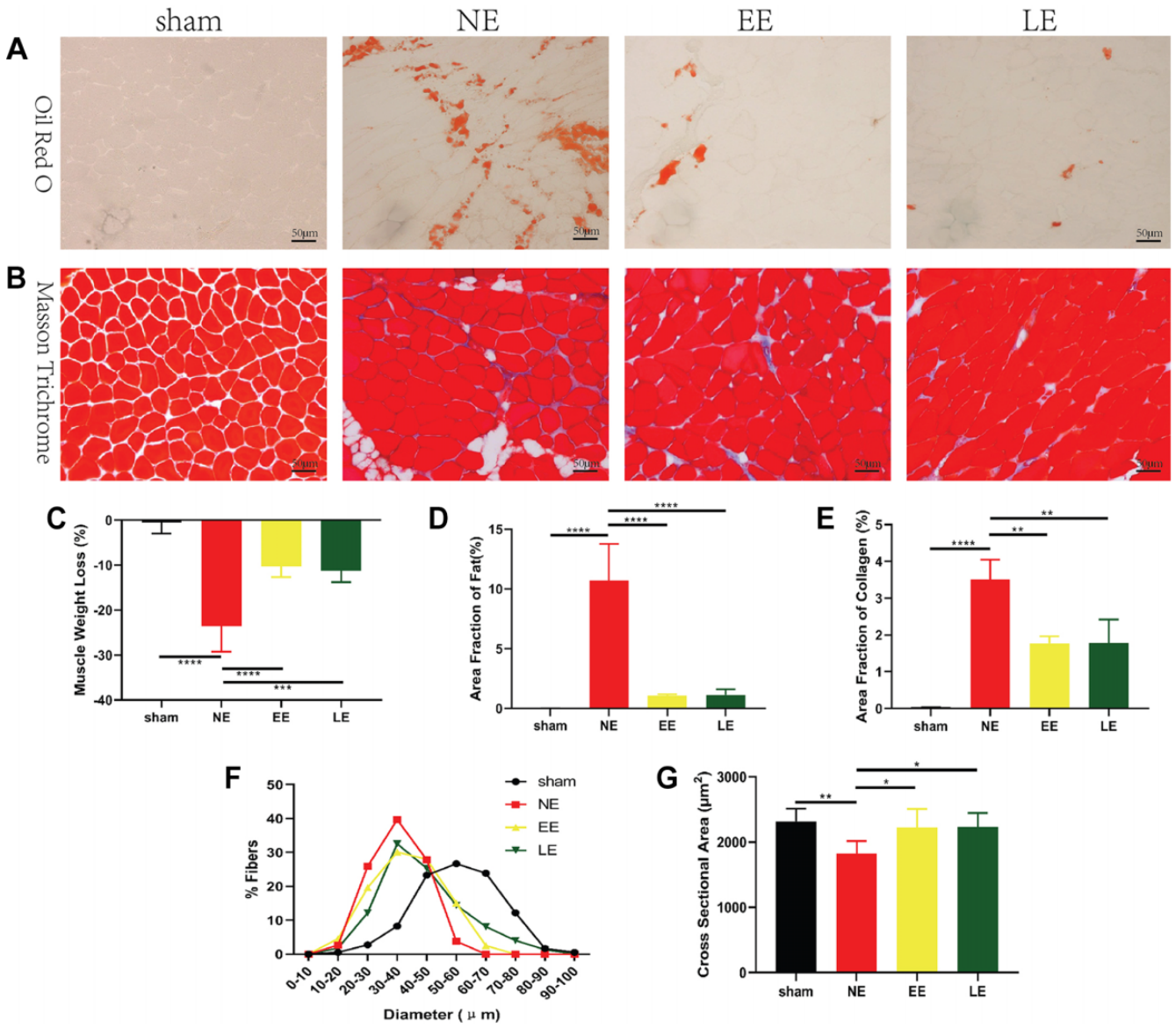

Figure 2. Histologic staining showing the effect of high-intensity interval training on the supraspinatus (SS). (A) Oil red O staining and (B) Masson trichrome staining of the SS. (C) SS weight loss was lower in the early exercise (EE) and late exercise (LE) groups compared with the no exercise (NE) group. (D) Fatty infiltration in the EE and LE groups was less than that in the NE group. (E) In the EE and LE groups, fibrosis was less than that in the NE group. (F and G) The muscle fiber cross section was larger in the EE and LE groups than in the NE group. Statistically significant difference: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

(LE) (vs NE; $P = .003$) (Figure 2, B and E). Muscle weight loss was –10.2% ± 2.4% (EE) (vs –23.5% ± 5.7% for NE; $P < .0001$) and –11.3% ± 2.5% (LE) (vs NE; $P < .001$) (Figure 2C). Compared with the NE group (median, 35.75 μm), more myofibers had a larger diameter in the EE group (median, 38.47 μm) and LE group (median, 40.71 μm) (Figure 2, B and F). The mean CSAs of myofibers were 2084 ± 320 μm^2 (EE) (vs 1738 ± 291 μm^2 for NE; $P = .031$) and 1903 ± 284 μm^2 (LE) (vs NE; $P = .026$) (Figure 2, B and G). There was no significant difference in FI, collagen area, muscle weight loss, and CSA between the EE and LE groups.

Expression of UCP1 and TH in the SS and Fat Depots After HIIT

Increased UCP1 expression in the EE (vs NE; $P < .003$) and LE (vs NE; $P < .0001$) groups indicated browning of adipocytes in SS (Figure 3A). Increased expression of TH in the EE (vs NE; $P = .049$) and LE (vs NE; $P < .003$) groups suggested activation of sympathetic fibers (Figure 3B). We also harvested interscapular brown adipose tissue (iBAT) and subcutaneous inguinal white adipose tissue (ingWAT). The results were similar to those in SS. UCP1 and TH expression increased in the EE groups (vs NE:

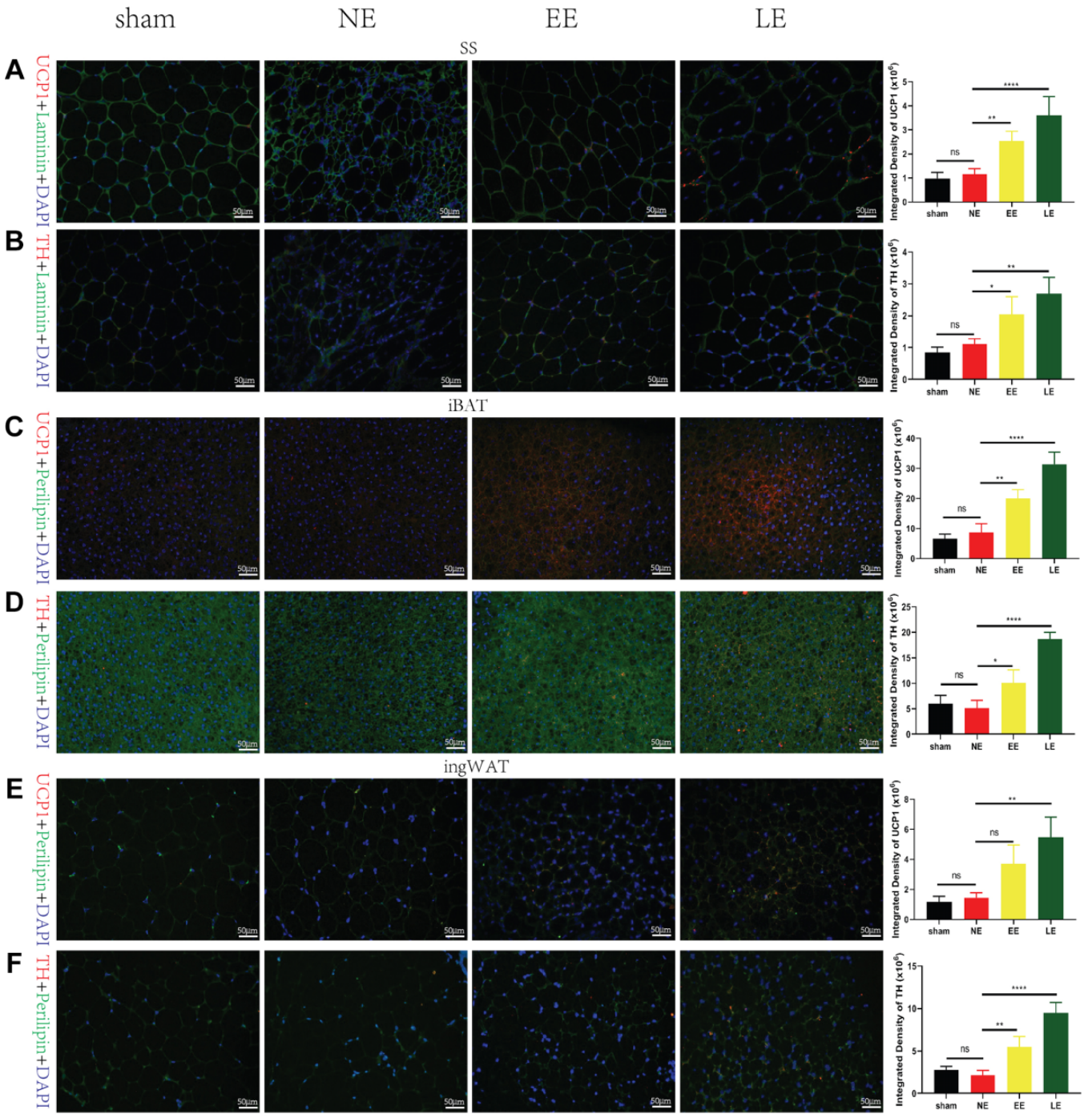

Figure 3. Immunofluorescence staining showing higher uncoupling protein 1 (UCP1) (red) and tyrosine hydroxylase (TH) (red) expression in the early exercise (EE) and late exercise (LE) groups. In the (A and B) supraspinatus (SS), (C and D) interscapular brown adipose tissue (iBAT), and (E and F) inguinal white adipose tissue (ingWAT), immunofluorescence staining showed higher UCP1 (red) and TH (red) expression in the EE and LE groups compared with the no exercise (NE) group. Statistically significant difference: *$P < .05$, **$P < .01$, ****$P < .0001$. DAPI, 4′,6-diamidino-2-phenylindole; ns, not significant.

$P = .005$ for iBAT UCP1; $P = .025$ for iBAT TH; $P = .007$ for ingWAT TH) and the LE groups (vs NE: $P < .0001$ for iBAT UCP1; $P < .0001$ for iBAT TH; $P = .001$ for ingWAT UCP1; $P < .0001$ for ingWAT TH) (Figure 3, C-Fs).

Effect of HIIT on FI and β3AR

To study whether β3AR mediated the effect of HIIT, SR and DMSO were administered to mice before exercise. We found

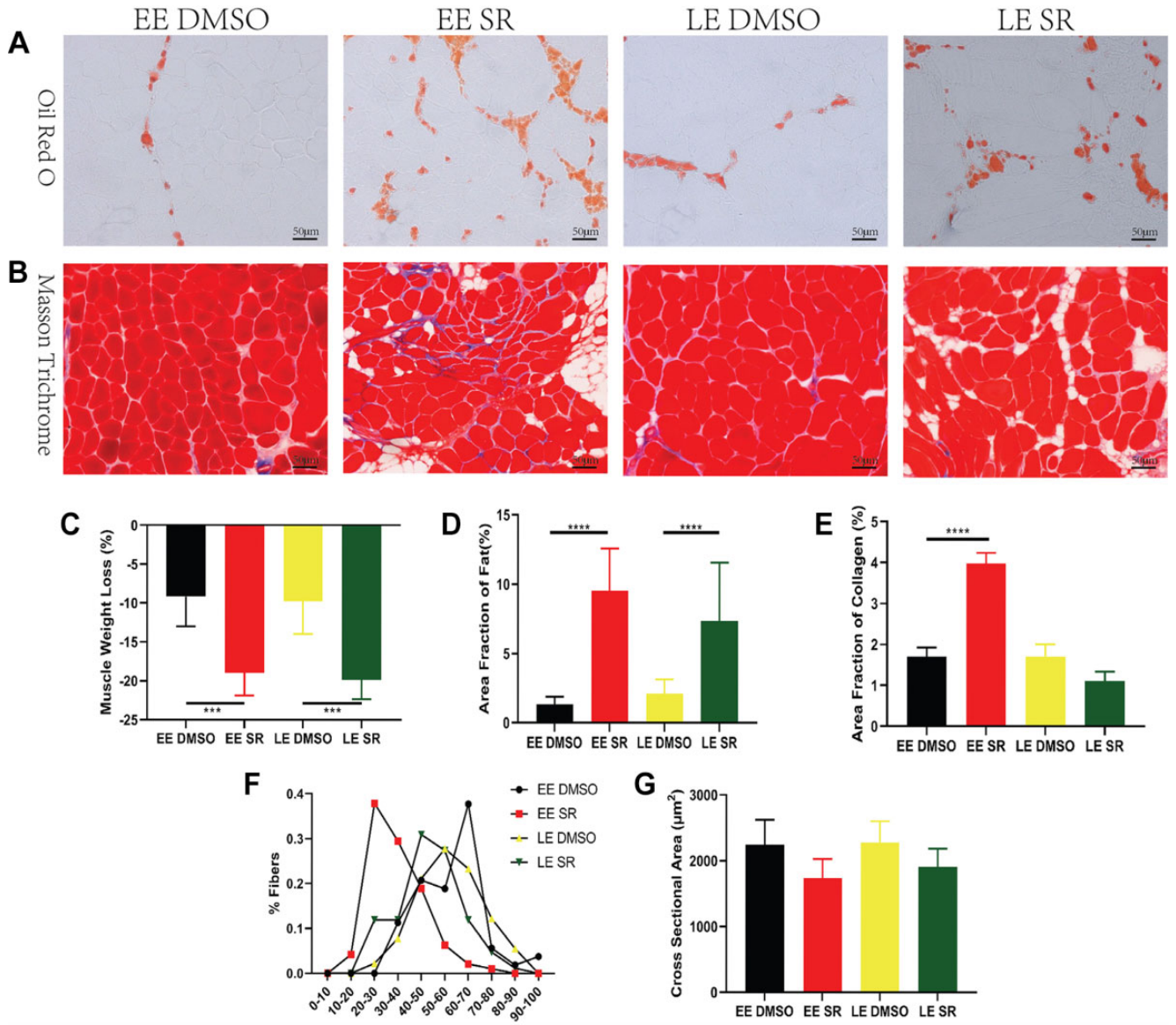

Figure 4. SR59230A (SR) inhibited fatty infiltration and fibrosis decrease. (A) Oil red O staining and (B) Masson trichrome staining of the supraspinatus. (C) Muscle weight loss was greater in the early exercise (EE) SR and late exercise (LE) SR groups. (D) Oil red O staining showed increased fatty infiltration in the EE SR and LE SR groups. (E) Masson trichrome staining showed increased fibrosis in the EE SR group compared with the EE dimethyl sulfoxide (DMSO) group. (F and G) Myofiber showed a decreasing trend in the EE SR and LE SR groups. Statistically significant difference: ***$P < .001$, ****$P < .0001$.

that the SS quality of mice injected with SR was worse than that of mice injected with DMSO. Similar to the NE group, SS in the SR groups had more FI ($P < .0001$ for EE SR vs EE DMSO; $P < .0001$ for LE SR vs LE DMSO) (Figure 4, A and D). Masson trichrome staining showed more collagen area in the EE SR group (vs EE DMSO; $P < .0001$) (Figure 4, B and E). SS in the SR groups had less weight ($P < .001$ for EE SR vs EE DMSO; $P < .001$ for LE SR vs LE DMSO) (Figure 4C). SR did not induce FI or fibrosis in SS from mice in the sham surgery group (Appendix Figure A2).

UCP1 expression also decreased in the SR groups ($P < .0001$ for EE SR vs EE DMSO; $P < .0001$ for LE SR vs LE DMSO) (Figure 5A). However, TH expression was similar whether the mice were injected with SR or DMSO (Figure 5B). In other words, HIIT still activated local sympathetic fibers in the SR groups. In iBAT and ingWAT, just like SS, UCP1 expression decreased if the mice were injected with SR (in iBAT: $P < .0001$ for EE SR vs EE DMSO, $P < .0001$ for LE SR vs LE DMSO; in ingWAT: $P = .003$ for EE SR vs EE DMSO, $P < .001$ for LE SR vs LE DMSO) (Figure 5, C and E), whereas TH expression had no change (Figure 5, D and F).

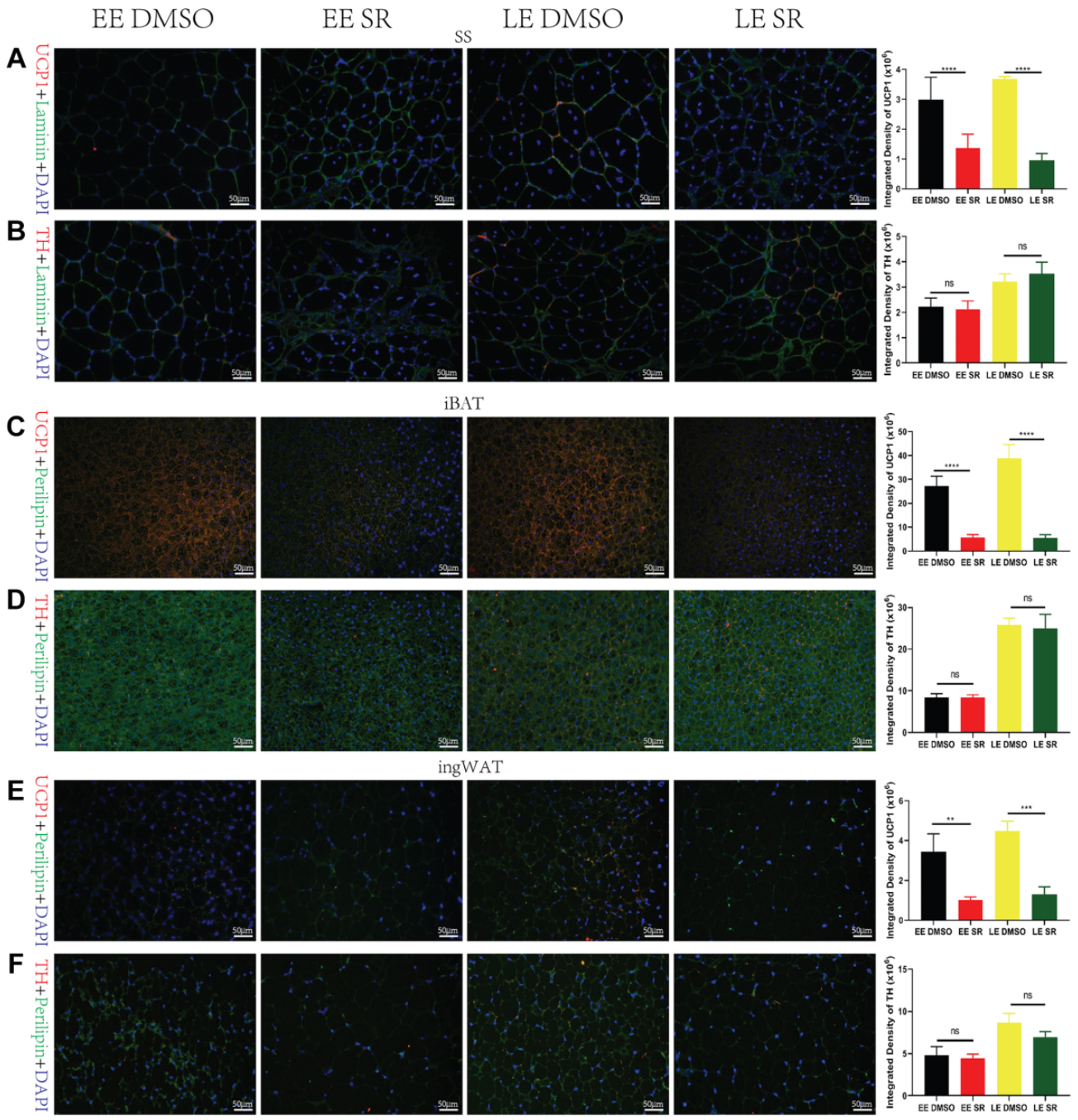

Figure 5. SR59230A (SR) inhibited the expression of uncoupling protein 1 (UCP1) in the (A and B) supraspinatus (SS), (C and D) interscapular brown adipose tissue (iBAT), and (E and F) inguinal white adipose tissue (ingWAT). Immunofluorescence showed lower UCP1 (red) expression in the early exercise (EE) SR and late exercise (LE) SR groups and no change in tyrosine hydroxylase (TH) (red) expression. Statistically significant difference: $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$. DAPI, 4′,6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; ns, not significant.

HIIT and the β3AR Thermogenesis Pathway

In total, we obtained proteins from SS, iBAT, and ingWAT in the 8 groups. We found that in the HIIT groups (EE and LE), PKA, p-HSL, p-p38 MAPK, and PGC1α in the β3AR thermogenesis pathway[2,32,43] were elevated compared with those in the NE group. In the SR groups (EE SR and LE SR), the expression of these proteins was depressed compared with that in the DMSO groups (EE DMSO and LE DMSO) (Figure 6). A diagram of the β3AR thermogenesis pathway is shown in Figure 7.

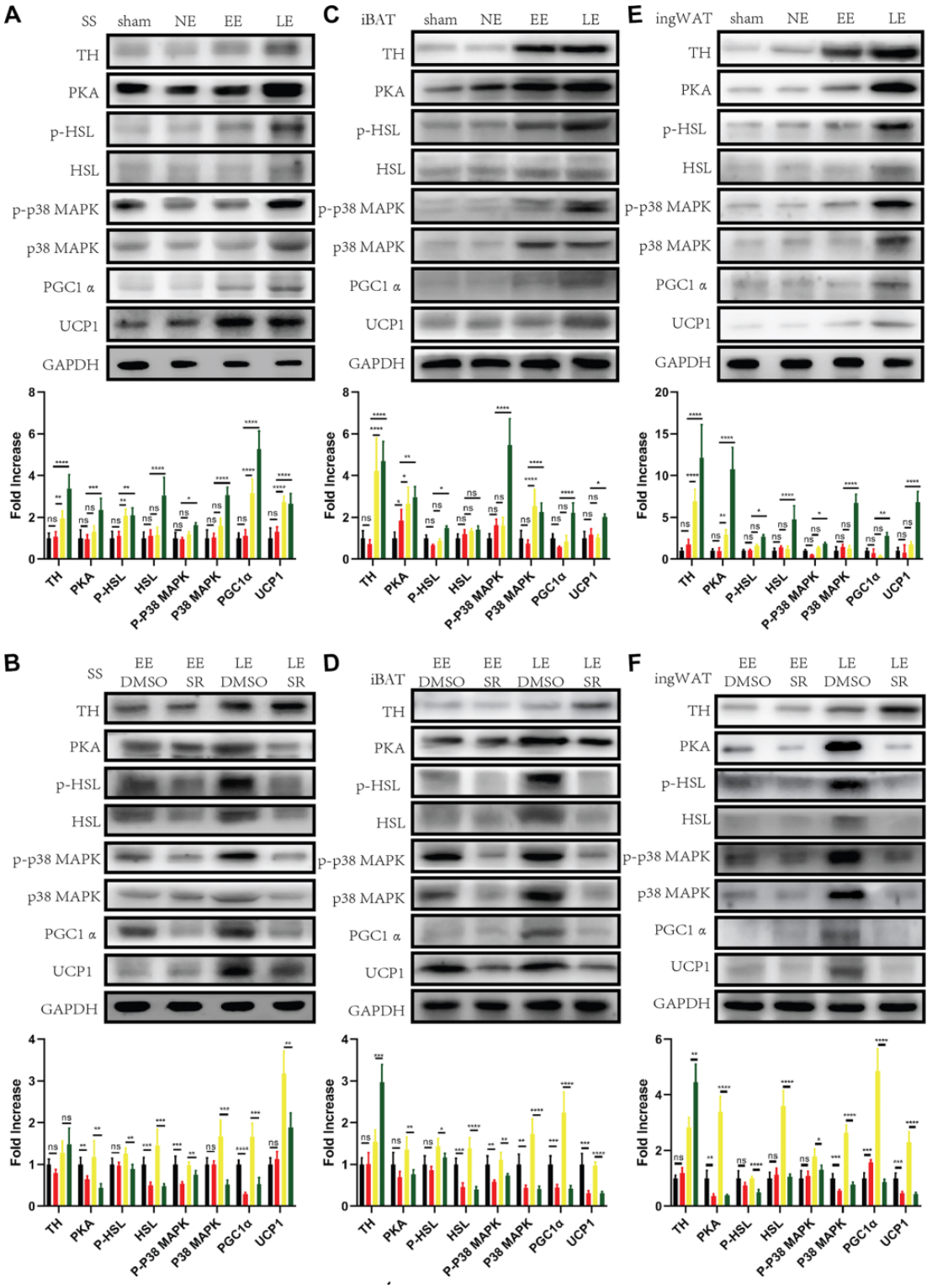

Figure 6. Western blot of critical proteins in the β3AR thermogenesis pathway for the sham (black bars), NE (red bars), EE (yellow bars), and LE (green bars) groups and for the EE DMSO (black bars), EE SR (red bars), LE DMSO (yellow bars), and LE SR (green bars) groups. In the (A and B) supraspinatus (SS), (C and D) interscapular brown adipose tissue (iBAT), and (E and F) inguinal white adipose tissue (ingWAT), for TH, PKA, HSL, p38 MAPK, PGC1α, and UCP1, fold change was calculated as target protein divided by GAPDH. For p-HSL and p-p38 MAPK, fold change was calculated as phosphorylated target protein divided by total target protein. Statistically significant difference: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$. EE, early exercise; LE, late exercise; NE, no exercise; ns, not significant; SR, SR59230A. See Appendix Table A1 for remaining abbreviation expansions.

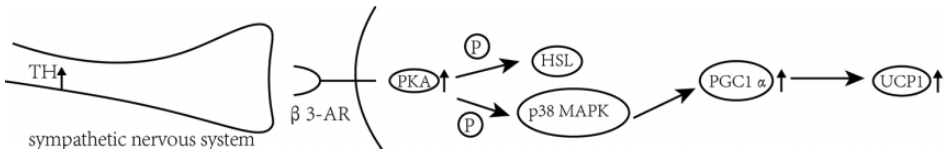

Figure 7. Graphic abstract of the β3AR thermogenesis pathway. See Appendix Table A1 for abbreviation expansions.

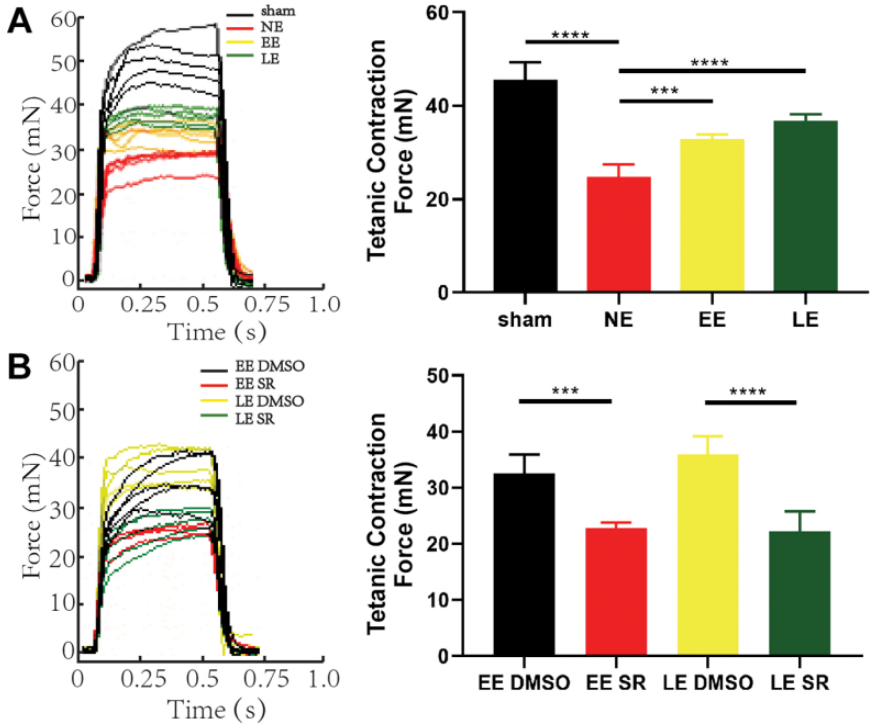

Figure 8. Contractile tests of supraspinatus. (A) High-intensity interval training improved the tetanic contraction force in the early exercise (EE) and late exercise (LE) groups. (B) SR59230A (SR) inhibited the improvement of tetanic contraction force. Statistically significant difference: ***$P < .001$, ****$P < .0001$. DMSO, dimethyl sulfoxide; NE, no exercise.

SS Contractility Caused by HIIT

The tetanic contraction force of SS in the EE (32.9 ± 0.9 mN; $P < .001$) and LE (36.8 ± 1.5 mN; $P < .0001$) groups was larger than that in the NE group (24.8 ± 2.6 mN) (Figure 8A). In the SR groups, the tetanic contraction force was depressed to 22.8 ± 1.0 mN (EE SR: $P < .001$ vs 32.6 ± 3.4 mN for EE DMSO) and 22.2 ± 3.6 mN (LE SR: $P < .0001$ vs 35.9 ± 3.2 mN for LE DMSO), similar to the values in the NE group (Figure 8B). There was no significant difference in tetanic contraction force between the EE and LE groups.

DISCUSSION

As expected, we observed significant FI in SS in the delayed RC repair mouse model, and the muscle contraction was weaker than that in the sham group. In this delayed RC repair model, SS degenerates and has significant FI before the RC repair surgery.[49] However, HIIT, a model of exercise, largely reversed FI, atrophy, and the decrease in contractile force of SS after delayed RC repair in mice. If proven to do the same in humans, HIIT could serve as a new rehabilitation treatment for patients with chronic RC tears after surgery.

We also found that HIIT stimulated systemic beige/brown fat dependent on β3AR. Previous studies showed that a group of muscle residential interstitial progenitor cells, named FAPs, are the main source of FI.[34] When mice received β3AR agonist, FAPs and FI tended to adopt a BAT phenotype.[3] HIIT is a novel exercise style to help one lose weight. Some studies show that HIIT activates the sympathetic nervous system and improves metabolic diseases, especially fat metabolism.[14,18,36] Previous studies have found that HIIT increased the expression of UCP1 and other metabolism-related genes in subcutaneous adipose tissue, especially in obese mice fed a high-fat diet.[37,47] HIIT can be employed in different exercises, such as running, cycling, and swimming. It is controversial whether early active shoulder exercise increases retear rates,[25] but strengthening exercises are usually recommended starting

in the 12th week after surgery.[29,35] To decrease the retear risk from HIIT, exercise with a low shoulder burden starting in the 12th week after repair may be a safer rehabilitation method.

In this study, we showed that HIIT significantly improved muscle quality and contractile force by reducing muscle atrophy and FI before RC repair and after RC repair. SS in the EE and LE groups had less FI, less fibrosis, wider myofiber, and better contractile force. Clinical research showed that RC muscle quality before surgery is a predictor of clinical outcomes after RC repair.[44] The results in the EE group indicated that HIIT is an option for "pretreatment" to prepare patients for surgical intervention of their RC tears. Interestingly, the RC was torn when the EE group underwent HIIT. This indicates that HIIT prevents FI through something else instead of local muscle exercise. Because of the increased TH and UCP1 expression in the SS and other adipose depots, we attribute the effect to the systemic activation of sympathetic nerve fibers. The results in the LE group challenge the current concept that RC muscle FI is irreversible.

Previous studies have found that exercise affected the metabolism of local muscle. Consitt et al[8] reviewed the impact of endurance and resistance training on skeletal muscle energy metabolism in older adults. An interesting point is that endurance training increases intramuscular triglycerides in older adults just like the athlete's paradox put forward by Goodpaster et al,[20] while resistance training does not. Intramuscular triglycerides do not equal FI in muscle. Intramuscular triglycerides may come from the adipocytes in muscle or molecules in myocytes. They are explained as energy storage for those who experience endurance training. However, muscles have more glycolytic demand than lipid oxidation demand in resistance training. According to our research, another reason for low intramuscular triglycerides might be consumption by activated FAP in muscle. Effting et al[15] applied a ladder-climbing model in mice in which obesity was induced by a high-fat diet. They found that 8-week exercise increased the phosphorylation of RAC-alpha serine/threonine-protein kinase (Akt) and AMPK to help take up glucose and oxidize fatty acid in the quadriceps. Ladder climbing also reduced the expression of some inflammation genes, such as TNFα and IL1β in the quadriceps muscles of obese mice. A meta-analysis including 12 studies found that exercise improved muscle quality and FI in adults experiencing myosteatosis.[41]

The effects of HIIT on the SS were largely attributed to activated UCP1 expression. UCP1 is a marker of BAT. Different from WAT with unilocular morphology, BAT is characterized by multiple lipid droplets and high expression of UCP1.[1] Recent studies have found that a group of unilocular adipose tissue changed to BAT-like morphology after certain stimuli.[53] This adipose tissue was named beige or brite fat tissue, located in ingWAT typically.[53] In activated BAT and beige fat tissue, UCP1 expression increases and consumes free fatty acid quickly.[9] Previous studies confirmed that FI is a kind of beige fat and that UCP1 played a key role in decreasing FI.[50,51] Our results showed that HIIT significantly increased the expression of UCP1 and TH. TH is the rate-limiting enzyme in the synthesis of norepinephrine, a sympathetic neurotransmitter.[54] Increased expression of TH indicates activation of local sympathetic nerve fibers,[13,54] and increased expression of UCP1 indicates activation of BAT or beige fat.[23] Previous research found some interesting interactions between sympathetic nerves and adipose tissues. Cui et al[10] found that a fat-derived "adipokine," neurotrophic factor neurotrophin 3, acted on tropomyosin receptor kinase C as a key regulator of sympathetic nerve growth and innervation in adipose tissue. In turn, it helps to activate brown or beige adipocytes. Cao et al[6] found that partial chemical denervation of iBAT sympathetic nerves with 6-OHDA, a selective neurotoxin to sympathetic nerves, activated sympathetic nerve fibers and beige fat browning in ingWAT. In contrast, if ingWAT was also treated with 6-OHDA, no beige fat browning was detected in ingWAT.

To ensure the effect of HIIT is systemic, we also detected TH and UCP1 expression in other beige/brown fat depots by immunofluorescence. Similar results between FI and other beige/brown fat depots indicate that the effect of HIIT on FI is a localized reflection of the systemic effect rather than a result of shoulder exercise. To explore whether the effect is dependent on β3AR, we administered SR, a selective β3AR antagonist, into mice, just 10 minutes before HIIT and investigated the phenotypes of SS atrophy and FI. SR groups showed worse muscle quality and function, similar to those who did not exercise. However, TH expression in SS and other beige/brown depots was high, so HIIT still activated local sympathetic nerve fibers successfully. By comparison, UCP1 expression decreased in the SR groups. Therefore, the effect of HIIT on muscle quality depends on β3AR. We further examined related proteins by Western blotting of the pathway from TH to UCP1. The expression was as expected.

Limitations

Some limitations exist to this study. First, we should consider the difference between mice and humans. Some studies have indicated a low expression of β3AR in human muscle, with it expressing relatively high β2AR instead.[4] It is uncertain whether FI in human SS would reverse in a similar mechanism. Second, we used acute tendon transection in this RC tear model, but chronic RC tears are also present in a large portion of clinical cases. Despite the difference between our model and the clinical situation, the muscle degeneration seen is similar. Third, electrodes were used to stimulate the muscles during HIIT, and such forced intervention may have affected the nervous system. However, in the clinic, patients performing HIIT also need supervision. This supervision may have similar effects on the nervous system. Finally, the mice used here were very young relative to the age of patients in the clinic with common RC degeneration, and the amount of fat in this model was minuscule compared with these patients' conditions. The young age of the mice might limit the translational implications of the research, but HIIT may serve as an early rehabilitation method for young patients.

CONCLUSION

In the current study, we discovered that, no matter before repair or immediately after repair, HIIT could improve SS atrophy, FI, and function in a delayed RC repair mice model. The effects might be related to fat browning through the activation of β3AR by excited sympathetic nerve fibers.

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APPENDIX

TABLE A1
Abbreviations and Expansions of Specialized Terms Used in the Article

Abbreviation	Expansion
6-OHDA	6-Hydroxydopamine
AMPK	AMP-activated protein kinase
β3AR	Beta-3 adrenergic receptor
BAT	Brown adipose tissue
cAMP	Cyclic adenosine 3′,5′-monophosphate
DAPI	4′,6-Diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
FAP	Fibro-adipogenic progenitors
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HIIT	High-intensity interval training
HSL	Hormone-sensitive lipase
IGF1	Insulin-like growth factor 1
IL1β	Interleukin 1 beta
iBAT	Interscapular brown adipose tissue
ingWAT	Inguinal white adipose tissue
MAPK	Mitogen-activated protein kinase
PBS	Phosphate-buffered saline
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
p-HSL	Phosphorylated hormone-sensitive lipase
PKA	Protein kinase A hormone-sensitive lipase
PVDF	Polyvinylidene difluoride
RIPA	Radioimmunoprecipitation assay

(continued)

Table A1 (continued)

Abbreviation	Expansion
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TH	Tyrosine hydroxylase
TNFα	Tumor necrosis factor alpha
UCP1	Uncoupling protein 1
VEGF	Vascular endothelial growth factor

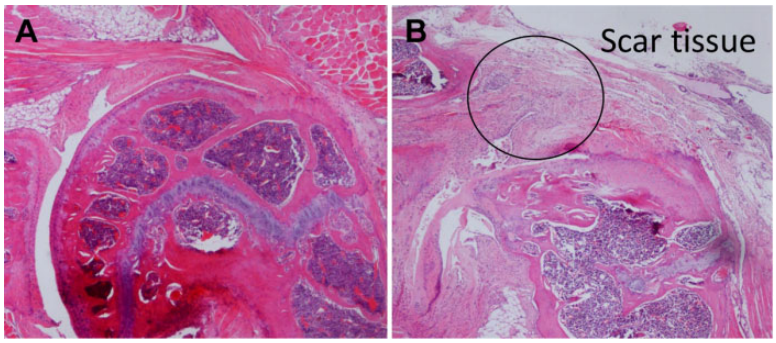

Figure A1. Repaired tendon kept intact after high-intensity interval training. Hematoxylin-eosin staining of mouse shoulder showed normal structure in the (A) sham group and that scar tissue linked tendon and humerus in the (B) late exercise group. Scar tissue is shown in the circle.

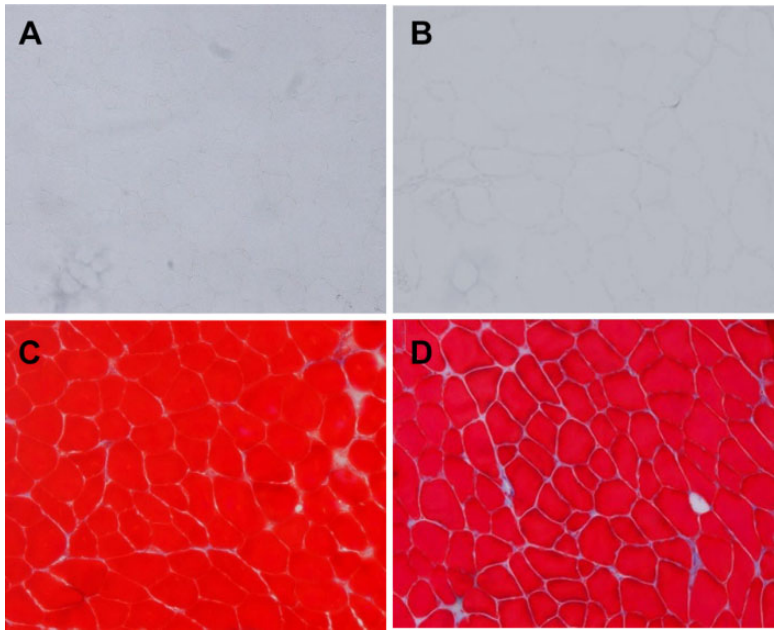

Figure A2. Oil red O staining showed no fatty infiltration in the supraspinatus of mice that received sham surgery followed by intraperitoneal injection of (A) dimethyl sulfoxide or (B) SR59230A 5 days per week for 6 weeks. Masson trichrome staining showed no obvious fibrosis in the supraspinatus of mice that received sham surgery followed by intraperitoneal injection of (C) dimethyl sulfoxide or (D) SR59230A 5 days per week for 6 weeks.