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UPPER LIMB

High-intensity interval training improves fatty infiltration in the rotator cuff through the β3 adrenergic receptor in mice

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Aims

Rotator cuff muscle atrophy and fatty infiltration affect the clinical outcomes of rotator cuff tear patients. However, there is no effective treatment for fatty infiltration at this time. High-intensity interval training (HIIT) helps to activate beige adipose tissue. The goal of this study was to test the role of HIIT in improving muscle quality in a rotator cuff tear model via the β3 adrenergic receptor (β3AR).

Methods

Three-month-old C57BL/6 J mice underwent a unilateral rotator cuff injury procedure. Mice were forced to run on a treadmill with the HIIT programme during the first to sixth weeks or seventh to 12th weeks after tendon tear surgery. To study the role of β3AR, SR59230A, a selective β3AR antagonist, was administered to mice ten minutes before each exercise through intraperitoneal injection. Supraspinatus muscle, interscapular brown fat, and inguinal subcutaneous white fat were harvested at the end of the 12th week after tendon tear and analyzed biomechanically, histologically, and biochemically.

Results

Histological analysis of supraspinatus muscle showed that HIIT improved muscle atrophy, fatty infiltration, and contractile force compared to the no exercise group. In the HIIT groups, supraspinatus muscle, interscapular brown fat, and inguinal subcutaneous white fat showed increased expression of tyrosine hydroxylase and uncoupling protein 1, and upregulated the β3AR thermogenesis pathway. However, the effect of HIIT was not present in mice injected with SR59230A, suggesting that HIIT affected muscles via β3AR.

Conclusion

HIIT improved supraspinatus muscle quality and function after rotator cuff tears by activating systemic sympathetic nerve fibre near adipocytes and β3AR.

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Keywords: Rotator cuff, Fatty infiltration, Atrophy, High-intensity interval training, β3 adrenergic receptor

Article focus

- The current study was conducted to investigate the effect of high-intensity interval training (HIIT) on fatty infiltration in rotator cuffs, employing a rotator cuff tear mouse model to provide a new non-invasive treatment regimen for rotator cuff tears.

Key messages

- HIIT improved supraspinatus quality in a rotator cuff tear mouse model.
- The effect of HIIT on the supraspinatus is dependent on β3 adrenergic receptor (β3AR).
- The effect of HIIT on fatty infiltration may be a result of the systemic activation of sympathetic nerve fibres.

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Strengths and limitations

- HIIT could offer a new non-invasive and economical treatment regimen for rotator cuff tears.
- Human adipose tissue expresses low β3AR, but relatively high β2AR; the effect of HIIT on humans might be different.
- This article used an acute rotator cuff tear model, but a large portion of clinically observed rotator cuff tears are also chronic.

Introduction

For adults in the USA, rotator cuff tears are the most common tendon injury, affecting more than 10% of people older than 60 years.[1] In a descriptive analysis of 1,688 patients suffering from rotator cuff tears, intermediate and severe fatty infiltration (Goutallier stage 2 to 4) occurs in more than 30% of patients, most of which is in the supraspinatus.[2] Stage 2 and 3 fatty infiltration accounts for approximately 30% of these cases.[3] Many studies have demonstrated that fatty infiltration correlates with poor clinical outcomes after surgical repairs: Collin et al[4] reported that patients' Constant scores were associated with fatty infiltration of the supraspinatus, and Gladstone et al[5] found that the amount of supraspinatus fat correlated with external rotation and forward elevation strength. Historically, fatty infiltration has been considered irreversible.[6] However, some research has shown reversible FI in muscle, with FI decreasing two years after RC repair surgery.[7,8] If muscle FI is reversible, then many patients suffering from rotator cuff tears could rehabilitate well.

Recent laboratory studies in mice have shown the possibility of reversing FI.[9,10] This may be dependent on the activation of brown/beige-like adipose tissue, since it can clear lipids via thermogenesis with UCP1.[11] Beige adipose tissue was recently reported to be morphologically like white fat, but it becomes brown-like fat after certain stimuli.[12] In addition, batokines such as VEGF and IGF1, released by brown/beige adipose tissue, are beneficial to muscle regeneration. Bryniarski and Meyer[13] tried an intermuscular fat transplant model. The results showed that brown fat transplant promoted adjacent muscle regeneration, possibly achieved with paracrine cross-talk in mice. Different factors can activate brown/beige-like tissue, such as exercise,[12,14] cold exposure,[12,15] and pharmacological factors.[12] A common point of these stimuli is the activation of the sympathetic system.[12] Research shows that local sympathetic activation induces white fat browning and UCP1 expression through β1 adrenergic receptor (AR) and β3AR in mice.[16] As a typical protein highly expressed by brown fat, UCP1 plays a critical role when dissipating energy stored by lipids, and is regarded as a marker of white fat browning.[17] Other studies also imply a causal relationship between β3AR activation and UCP1 expression.[12,18,19] Based on these lab studies in mice, exercise might be a candidate to improve fatty infiltration through β3AR.

High-intensity interval training (HIIT) is a novel exercise model. It can be incorporated into running, cycling, swimming, and other activities.[20] Compared with moderate-intensity constant training (MICT), HIIT is believed to be more time-efficient for losing weight.[20,21] Some studies indicate that HIIT is more powerful than MICT in inducing white fat browning in diet-induced obese mice.[22,23] A meta-analysis also concluded that HIIT is more efficient in improving cardiorespiratory fitness.[24] Therefore, we planned to study the effect of HIIT on muscle fatty infiltration with a rotator cuff tear mouse model. We hypothesized that HIIT helps to improve muscle quality and decrease fatty infiltration by activating the sympathetic system.

Methods

Ethics. Animal experiments were performed based on the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health,[25] and approved by the Institutional Animal Care and Use Committee of our university. Extensive efforts were made to minimize both the number and suffering of the included animals. We have included an ARRIVE checklist to show that we have conformed to the ARRIVE guidelines.

Experiment design. The C57BL/6 J mouse is a common inbred strain of laboratory mice that is widely used as a model in simulating many human diseases, including rotator cuff tears.[26,27] In this study, tendon tear mice received unilateral (right side) supraspinatus and infraspinatus tendon transection.

To study the effect of HIIT on rotator cuff tears, 40 mice were randomly divided into four groups (n = 10/group: five for histological analysis, and five for contractile tests and Western blot): sham, tendon tear + no exercise, tendon tear + early exercise, and tendon tear + late exercise (Figure 1). The sham group and no exercise group underwent no exercise during the 12 weeks. The early exercise group underwent exercise for six weeks from day 3 post-transection. The late exercise group underwent exercise for six weeks from week 7 to week 12 post-transection (Figure 1).

To study the role of β3AR, we administered SR59230A, a selective β3AR antagonist into mice before HIIT ((Figure 1). A total of 40 mice were randomly divided into four groups (n = 10/group): tendon tear + early exercise + dimethyl sulfoxide (DMSO) (EE + DMSO), tendon tear + early exercise + SR59230 A (EE + SR), tendon tear + late exercise + DMSO (LE + DMSO), and tendon tear + late exercise + SR59230 A (LE + SR). SR59230A (2 mg/kg) or 1% DMSO–phosphate-buffered saline (PBS) placebo was administered by intraperitoneal injection ten minutes before each exercise. SR59230A (Sigma-Aldrich, USA) was dissolved in 1% DMSO-PBS buffer.

Mice were housed in cages with a 12-hour dark-light cycle with free access to water and a regular chow diet. The animal study protocols were reviewed and approved by the Animal Welfare Committee of Central South University.

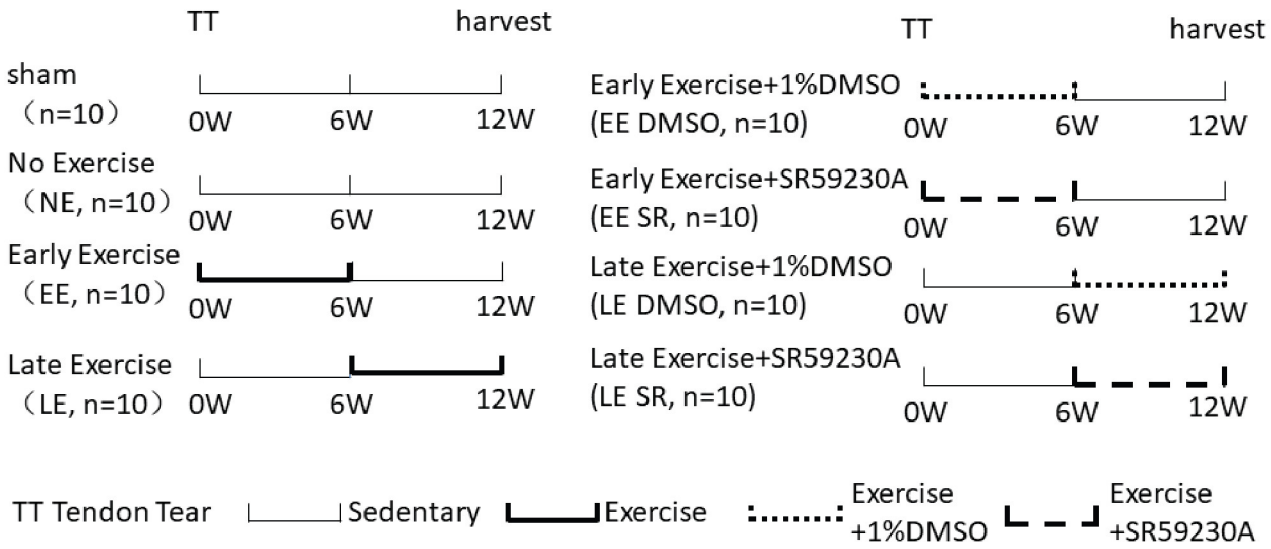

Fig. 1

Flow diagram exhibiting our experiment design. DMSO, dimethyl sulfoxide; TT, tendon tear; W, weeks.

Exercise protocol. HIIT exercise was performed on a motored mouse treadmill five days/week for six weeks, according to a protocol slightly modified from that described by Wang et al[23] and Martinez-Huenchullan et al.[22] Before the first exercise, a maximal running capacity (MRC) test was performed. The HIIT programme includes seven bouts (two minutes at 50% of the MRC followed by four minutes at 90% of the MRC). The MRC test was performed every two weeks, and the velocity was adjusted accordingly.

Muscular harvesting and wet muscle weight. All mice were killed 12 weeks after tendon transection. For assessment of muscle atrophy, wet weights of bilateral supraspinatus muscles were measured immediately after harvesting. The percentage change of wet muscle weight was determined with the following equation: $[(SS_{Right} - SS_{Left})/SS_{Left}] * 100\%$.[28]

Masson trichrome staining, oil red O staining, and cross-sectional area. The supraspinatus (n = 5 per group) muscles were flash-frozen and cryosectioned as described previously.[29] Masson trichrome (G1345, Solarbio, China) and Oil Red O (ORO; G1260, Solarbio) stains were used to assess fibrosis, atrophy, and fatty infiltration in supraspinatus muscles. Slides were covered with 10% glycerol in PBS (for ORO) or 50% resinene in xylene (for Masson trichrome) and observed on an optical microscope. Cross-sections were chosen randomly from midbellies of supraspinatus. Pictures were analyzed with ImageJ software (National Institutes of Health, USA) as described previously.[29,30]

Immunofluorescence staining. Samples were fixed in 4% paraformaldehyde for 30 minutes, rinsed in PBS, placed in 0.1 M glycine (Thermo Fisher Scientific, diluted in PBS) 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 one hour at room temperature. Primary antibodies against UCP1 (indicator of activation of beige adipose tissue or white fat browning) (NB100-2828, Novus Biologicals, USA, diluted 1:100), tyrosine hydroxylase (indicator of activation of sympathetic nerve fibres) (66334-1-Ig, Proteintech, USA, diluted 1:500), laminin (L9393, Sigma-Aldrich, diluted 1:500), and perilipin (abs137082, Absin (China), diluted 1:500) were diluted in a blockmix and added to the sections for overnight incubation at 4°C. After a PBS rinse, the sections were incubated with a mixture containing fluorescein isothiocyanate-conjugated (SA00003-8, SA00003-2, Proteintech, diluted 1:500) and Cy3-conjugated (SA00009-3, SA00009-1, Proteintech, diluted 1:500) secondary antibodies at room temperature for 120 minutes. After a PBS rinse for ten minutes (done twice), the slides were covered with DAPI containing antifade mounting medium.

Western blot. Total proteins were extracted using radioimmunoprecipitation assay lysis buffer containing proteinase inhibitors (No. 04693159001, Roche Diagnostics, Switzerland), and phosphatase inhibitors (No. 04906845001, Roche), separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF) membranes, and analyzed by immunoblotting. Primary antibodies against the following proteins were used: TH (66334-1-Ig, Proteintech), protein kinase A (PKA) (AF5450, Affinity (China)), p-HSL (AF8026, Affinity), hormone-sensitive lipase (HSL) (AF6403, Affinity), p-p38 mitogen-activated protein kinase (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 1:1,000 with PBS. The membranes were then incubated with a peroxidase-conjugated secondary antibody (BS13278, Bioworld (China); SA00001-1, Proteintech; SA00001-3,

Proteintech), and antibody-specific signals were detected by enhanced chemiluminescence and quantified using the Automatic Chemiluminescence Imaging System (Complex 2000; Nanjing PuoAoXin Biotechnology, China).

Supraspinatus contractile test. Preparation was performed while the mouse was anaesthetized. We exposed the supraspinatus and scapular spine, and removed the trapezius and deltoid. We sutured around the distal tendon part and proximal part of the supraspinatus. Then, we cut the scapula across the scapular spine and cut the humerus. After fixing the two sides of the supraspinatus, we assessed the tetany contraction force with the BL-420F acquisition system (Chengdu TME Technology, China). During the test, the muscle was maintained in a bath of Ringer solution (137 mM NaCl, 5 mM KCl, 1 mM NaH2PO4, 24 mM NaHCO3, 2 mM CaCl2, 1 mM MgSO4, 11 mM glucose) at 37°C.

Statistical analysis. We applied Dunnett's multiple comparison test to determine the significant difference among the sham, no exercise, early exercise, and late exercise groups and applied Sidak's multiple comparison test to determine the significant difference among the early exercise DMSO, early exercise SR, late exercise DMSO, and late exercise SR groups. We applied two-way analysis of variance (ANOVA) and post-hoc test (Sidak's multiple comparison test) to determine the difference of fatty area among the early exercise DMSO, early exercise SR, late exercise DMSO, and late exercise SR groups. All data are shown as the mean and standard deviation (SD) with the exception of myofibre diameters, which are captured with medians, 25th percentiles, and 75th percentiles. Statistical differences were determined when $p < 0.05$.

Results

HIIT improved SS quality in the TT model and increased UCP1 and TH expression in SS. We found significant muscle loss (vs sham, $p < 0.001$) and fatty infiltration (vs sham, $p < 0.001$) in the no exercise group (Figure 2a). Masson trichrome staining showed increased fibrosis (vs sham, $p < 0.001$) and decreased cross-sectional area (vs sham, $p < 0.001$) in the no exercise group (Figure 2b). However, for mice with HIIT, their muscle quality improved compared to the no exercise group. Mean muscle weight loss was -13.0% (SD 1.6%) (early exercise) (vs -26.8% (SD 2.6%) for no exercise, $p < 0.001$) and -13.8% (SD 3.7%) (late exercise) (vs no exercise, $p < 0.001$) (Figure 2e). The area of fatty infiltration was 1.32% (SD 0.68%) (early exercise) (vs 15.6% (SD 4.0%) for no exercise, $p < 0.001$) and 1.08% (SD 0.67%) (late exercise) (vs no exercise, $p < 0.001$) (Figure 2f). More myofibres had a larger diameter in the early exercise group (median 34.49 μm (IQR 28.21 to 41.46) and the LE groups (median 35.81 μm (IQR 29.87 to 41.94) than in the NE group (median 28.85 μm (IQR 23.05 to 34.88) (Figure 2g). The mean CSA of myofibres was 1,815 μm^2 (SD 32) (early exercise) (vs 1,144 μm^2 (SD 136) for no exercise, $p < 0.001$) and 1,722 μm^2 (SD 60) (late exercise) ($p < 0.001$) (Figure 2h). The collagen area measured 2.31% (SD 0.43%) (early exercise) (vs 5.60% (SD 1.80%) for no exercise, $p = 0.007$) and 2.74% (SD 0.36%) (late exercise) (vs no exercise, $p = 0.015$) (Figure 2i). Increased UCP1 expression in the early exercise (vs no exercise, $p < 0.001$) and late exercise groups (vs no exercise, $p < 0.001$) indicated browning of adipocytes in supraspinatus (Figures 2c and 2j). Increased expression of tyrosine hydroxylase (TH) in the early exercise (vs no exercise, $p < 0.001$) and late exercise groups (vs no exercise, $p < 0.001$, Dunnett's multiple comparison test for all p-values) suggested activation of sympathetic nerve fibres (Figures 2d and 2k).

HIIT increased UCP1 and TH expression in fat depots. In addition to SS, we harvested interscapular brown fat and inguinal subcutaneous white fat and examined UCP1 and TH expression by immunofluorescence. The results were similar to those in supraspinatus muscle. UCP1 (Figures 3a and 3c) expression increased in the HIIT groups (early exercise vs no exercise, $p < 0.001$ for brown/beige-like adipose tissue, $p < 0.001$ for inguinal subcutaneous white fat; late exercise vs no exercise, $p < 0.001$ for brown/beige-like adipose tissue, $p < 0.001$ for inguinal subcutaneous white fat). There was also greater expression of TH (Figures 3b and 3d) in the HIIT groups (early exercise vs no exercise, $p = 0.037$ for brown/beige-like adipose tissue, $p = 0.039$ for inguinal subcutaneous white fat; late exercise vs no exercise, $p < 0.001$ for brown/beige-like adipose tissue, $p < 0.001$ for inguinal subcutaneous white fat; Dunnett's multiple comparison test for all p-values).

The HIIT effects were dependent on the β3-adrenergic receptor. To study the role of β3AR in this process, SR59230A and DMSO were administered to mice before exercise. We found that the quality of supraspinatus muscle in mice injected with SR59230A was worse than that of mice injected with DMSO. Similar to the no exercise group, supraspinatus muscle in the SR59230A groups had more fatty infiltration ($p < 0.001$ for early exercise SR vs early exercise DMSO, $p < 0.001$ for late exercise SR vs late exercise DMSO) than the HIIT groups (only injected with DMSO) (Figures 4a and 4f) but fewer and smaller myofibres ($p < 0.001$ for early exercise SR vs early exercise DMSO, $p = 0.024$ for late exercise SR vs late exercise DMSO) (Figures 4b and 4h). We applied two-way ANOVA and post-hoc test (Sidak's multiple comparison test) to determine the difference in fatty area between the groups. The HIIT period factor contributed 3.764% to the total variation ($p = 0.009$), the SR59230A factor contributed 88.34% to the total variation ($p < 0.001$), and the interaction contributed 5.883% ($p = 0.002$) (Supplementry Table i). Supraspinatus muscle lost more weight in the SR59230A groups ($p < 0.001$ for early exercise SR vs early exercise DMSO, $p < 0.001$ for late exercise SR vs late exercise DMSO) (Figure 4e). UCP1 expression also decreased in the SR59230A groups ($p < 0.001$ for early exercise SR vs early exercise DMSO, $p < 0.001$ for late exercise SR vs late exercise DMSO) (Figures 4c and 4j). However, TH expression was similar whether the mice were injected

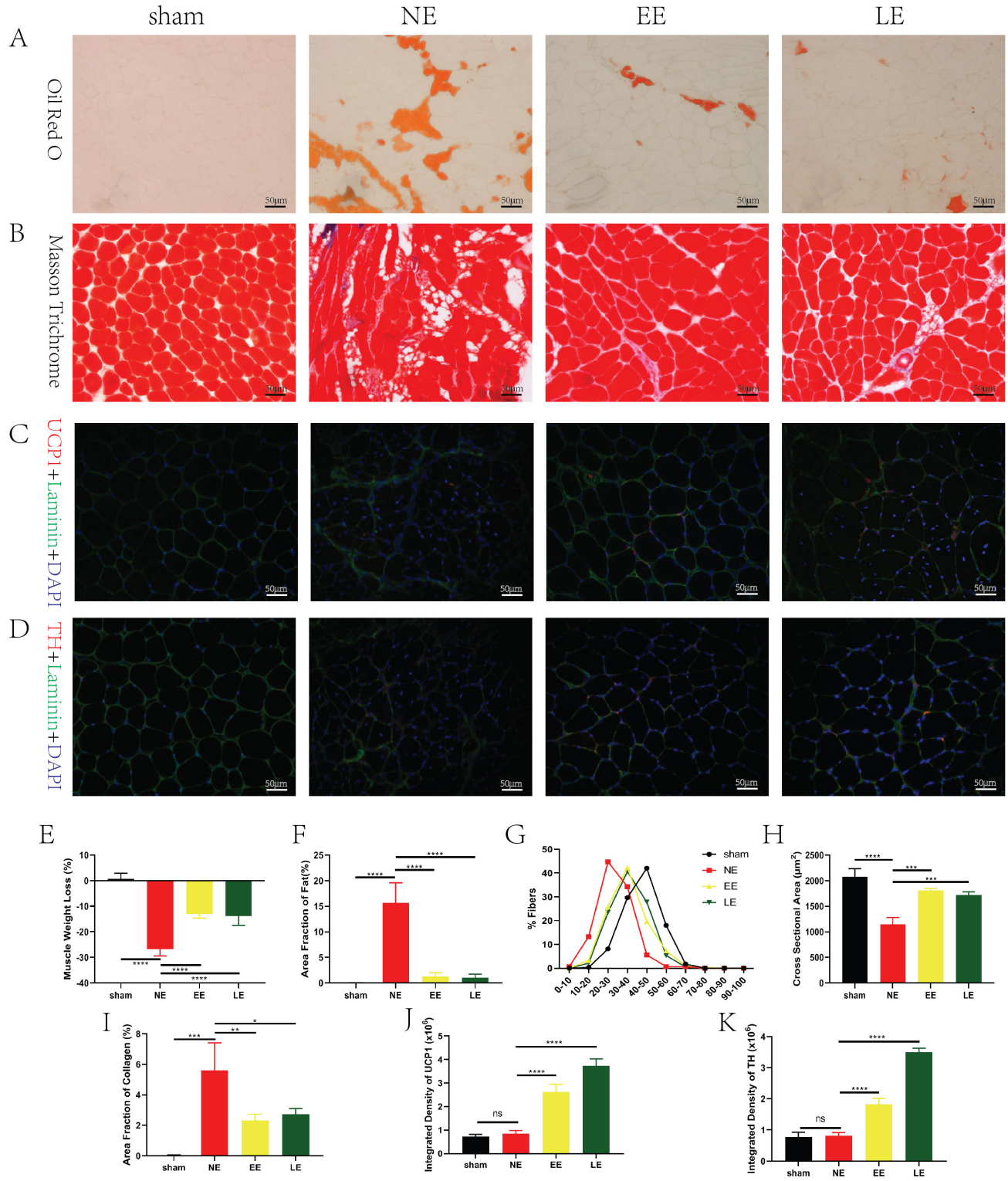

Fig. 2

Histological staining showed the effect of high-intensity interval training on supraspinatus muscle (n = 5 per group). a) Oil Red O staining showed less fatty infiltration in the early exercise (EE) and late exercise (LE) groups. b) Masson trichrome staining showed increased fibre diameter and cross-sectional area (CSA), and decreased fibrosis in the EE and LE groups. c) Immunofluorescence of laminin (green) and UCP1 (red) in supraspinatus. d) Immunofluorescence of laminin (green) and tyrosine hydroxylase (TH) (red) in supraspinatus muscle. e) Muscle weight lost less in the EE and LE groups. f) The fat area fraction (as a percentage) was calculated as the area of Oil Red O staining divided by the entire sample area. g) and h) The cross-section of muscle fibres was larger in the EE and LE groups than in the no exercise (NE) group. i) The collagen area fraction (as a percentage) was calculated as the area of aniline blue staining divided by the entire sample area. j) and k) Immunofluorescence showed higher UCP1 (red) and TH (red) expression in the EE and LE groups. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

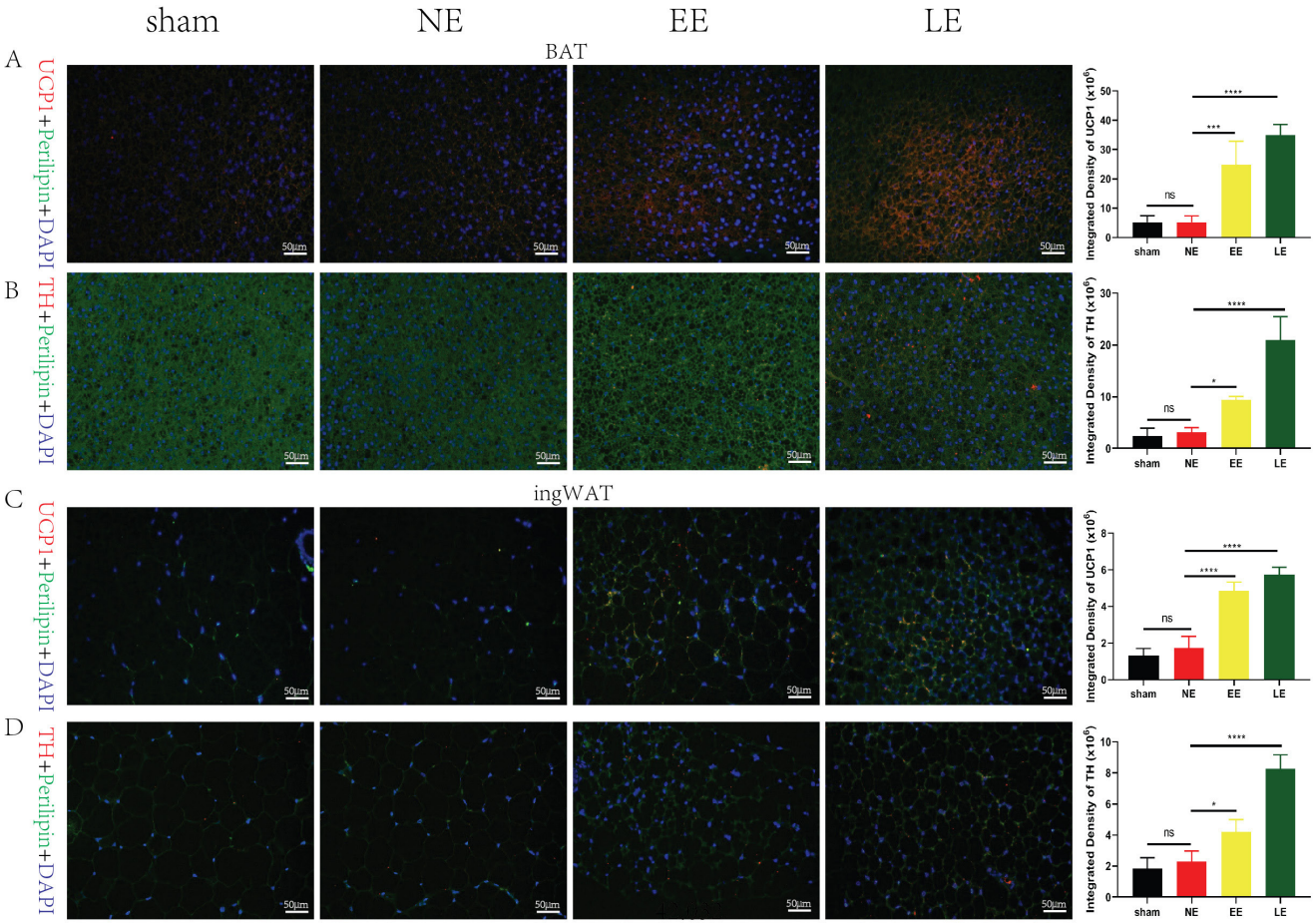

Fig. 3

Immunofluorescence showed the effect of high-intensity brown/beige-like adipose tissue (BAT) and inguinal subcutaneous white fat (ingWAT) (n = 5 per group). In a) and b) interscapular brown fat and c) and d) inguinal subcutaneous white fat. Immunofluorescence showed higher UCP1 (red) and tyrosine hydroxylase (TH) (red) expression in the early exercise (EE) and late exercise (LE) groups. *p < 0.05; ***p < 0.001; ****p < 0.0001.

with SR59230A or DMSO (Figures 4d and 4k). In other words, HIIT still activated local sympathetic fibres in the SR59230A groups.

In brown/beige-like adipose tissue and inguinal subcutaneous white fat, similar to supraspinatus muscle, UCP1 expression decreased if the mice were injected with SR59230A (Figures 5a and 5c), whereas TH expression showed no change (Figures 5b and 5d).

HIIT promoted the β3AR thermogenesis pathway. We obtained proteins from supraspinatus muscle, brown/beige-like adipose tissue, and inguinal subcutaneous white fat in the eight groups. We found that in the HIIT groups (early exercise and late exercise), PKA, p-HSL, p-p38 MAPK, and PGC1α in the β3AR thermogenesis pathway were elevated compared with those in the no exercise group.[31–33] In supraspinatus muscle and inguinal subcutaneous white fat, the expression of P-P38 MAPK did not increase significantly, but showed a trend to increase. In the SR59230A groups (early exercise SR and late exercise SR), the expression of these proteins was depressed compared to that in the DMSO groups (early exercise DMSO and late exercise DMSO). (Figure 6). Quantitative real-time polymerase chain reaction results in supraspinatus muscle corresponded with the protein expression (Supplementary Table ii).

SR59230A inhibited the improvement of supraspinatus function caused by HIIT. The mean tetany force of supraspinatus muscle in the early exercise (2.91 g (SD 0.16), vs 1.78 g (SD 0.08) for no exercise, $p < 0.001$, Dunnett's multiple comparison test) and late exercise (2.63 g (SD 0.15), vs no exercise group, $p < 0.001$, Dunnett's multiple comparison test) groups was larger than that in the no exercise group (1.77 g (SD 0.78)). In the SR59230A groups, tetany force was depressed to 1.66 g (SD 0.15) (early exercise SR, vs 2.83 g (SD 0.27) for early exercise DMSO, $p < 0.001$, Sidak's multiple comparison test) and 1.63 g (SD 0.92) (late exercise SR, vs 2.54 g (SD 0.07) for late exercise DMSO, $p < 0.001$, Sidak's multiple comparison test), similar to those in the no exercise group (Figure 7).

Discussion

Consistent with clinical observations and previous studies, we observed significant rotator cuff muscle atrophy and fatty infiltration in the rotator cuff tear

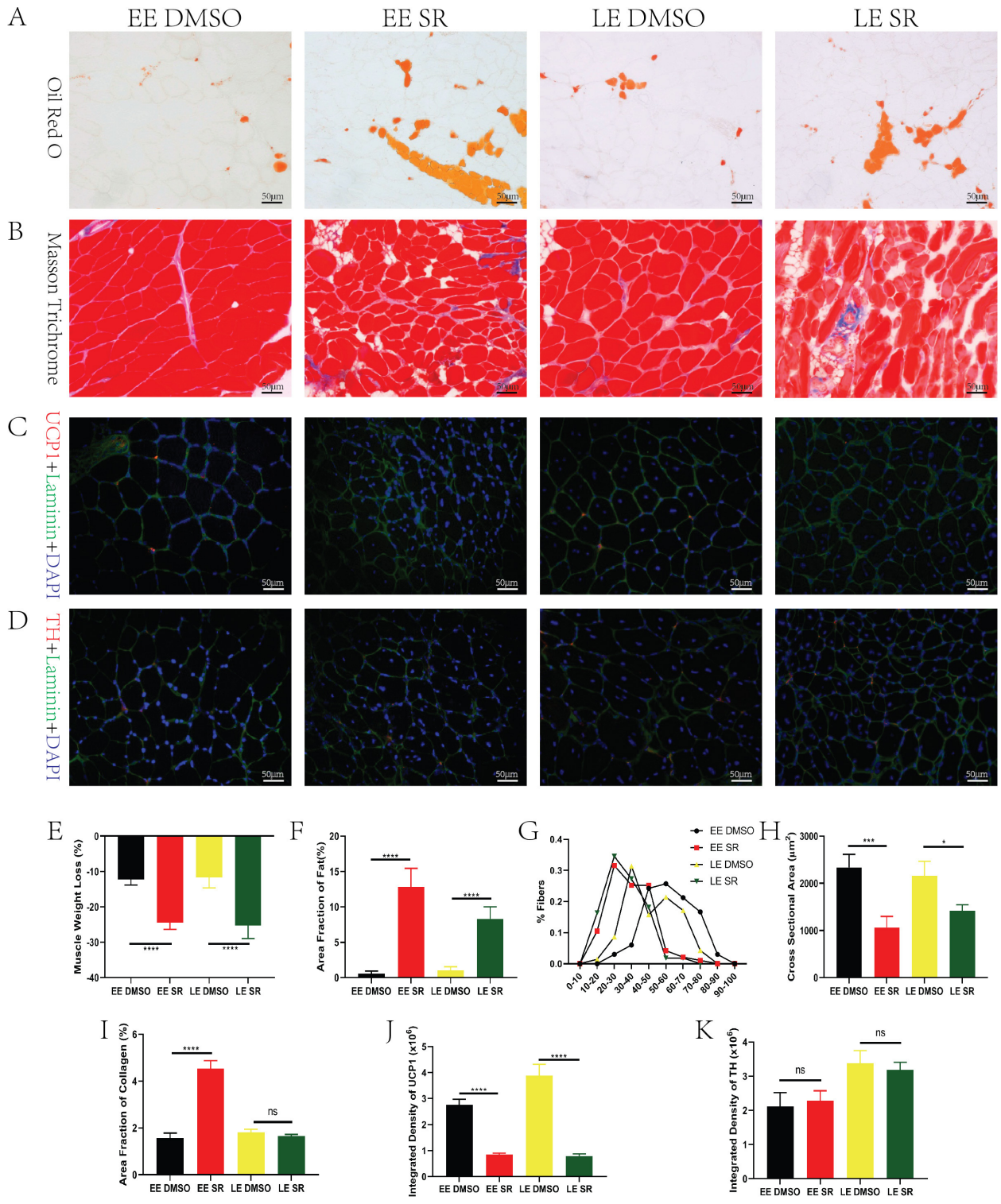

Fig. 4

Histological staining showing the effect of β3 adrenegic receptor (AR)-mediated high-intensity interval training on supraspinatus muscle (n = 5 per group). a) In supraspinatus muscle, Oil Red O staining showed increased fatty infiltration in the early exercise (EE) SR and late exercise (LE) SR groups. b) Masson trichrome staining showed decreased fibre diameter in the EE SR and LE SR groups, and increased fibrosis in the EE SR group. c) Immunofluorescence of laminin (green) and UCP1 (red) expression in supraspinatus muscle. d) Immunofluorescence of laminin (green) and tyrosine hydroxylase (TH) (red) expression in supraspinatus muscle. e)There was greater muscle weight loss in EE SR and LE SR groups. f) The fat area fraction (as a percentage) was calculated as the area of Oil Red O staining divided by the entire sample area. g) and h) The cross-section of muscle fibres was smaller in the EE SR and LE SR groups than in the EE dimethyl sulfoxide (DMSO) and LE DMSO groups. i) The collagen area fraction (as a percentage) was calculated as the area of aniline blue staining divided by the entire sample area. j) Immunofluorescence showed lower UCP1 (red) expression in the EE SR and LE SR groups. k) Immunofluorescence showed no change in TH (red) expression in the EE SR and LE SR groups. *p < 0.05; ***p < 0.001; ****p < 0.0001. ns, not significant.

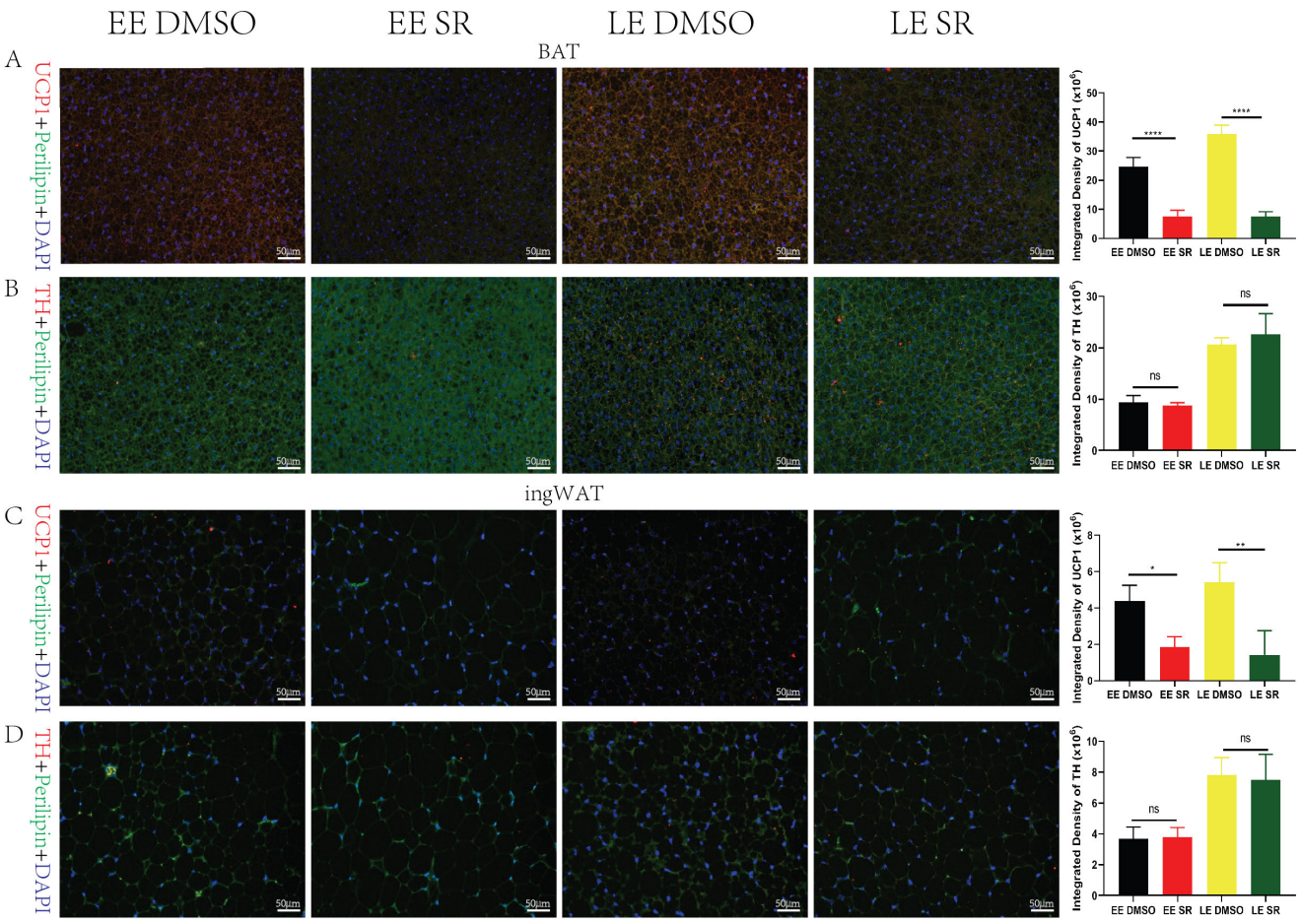

Fig. 5

Immunofluorescence showed the effect of β3 adrenegic receptor (AR)-mediated high-intensity interval training on brown/beige-like adipose tissue (BAT) and inguinal subcutaneous white fat (ingWAT) (n = 5 per group). a) and b) In interscapular brown fat, immunofluorescence showed lower UCP1 (red) expression in the early exercise (EE) SR and late exercise (LE) SR groups, but no change in tyrosine hydroxylase (TH) (red) expression. c) and d) In inguinal subcutaneous white fat, immunofluorescence showed lower UCP1 (red) expression in the EE SR and LE SR groups, but no change of TH (red) expression in the EE SR and LE SR groups. 208 × 146 mm (300 × 300 DPI). DMSO, dimethyl sulfoxide. *p < 0.05; **p < 0.01; ****p < 0.0001.

model. However, HIIT largely prevented fatty infiltration and atrophy, and also improved the contractile force of the supraspinatus in mice. HIIT protocols vary, and include running, cycling, swimming, and other activities. Some, like cycling, are friendly to patients with rotator cuff injury. HIIT could serve as a prevention method for fatty infiltration in patients with mild rotator cuff injury, if proven in humans. Some researchers listed severe fatty infiltration in rotator cuff muscle as a contraindication of the rotator cuff repair operation.[34] Thus, HIIT may serve as a preoperative preparation for these patients. In some situations, patients refuse surgery due to economic, religious, or other personal reasons. HIIT is suitable for them as a noninvasive, inexpensive therapy. In addition, we found that its effect is mediated by stimulating systemic beige/brown fat through β3AR.

Brown/beige-like adipose tissue is identified with UCP1 and is characterized by multiple small lipid droplets, while white fat is unilocular.[35,36] Brown/beige-like adipose tissue was beneficial to muscle regeneration when transplanted near the impaired muscle, which depended on local crosstalk between this tissue and the muscle.[13,37] Beige adipose tissue, such as inguinal subcutaneous white fat, was recently reported to be similar to white fat morphologically, but it becomes brown-like fat after being exposed to certain stimuli.[12] In activated beige adipocytes, with the increased expression of UCP1, proton potential energy produced by mitochondria is released to heat without producing adenosine triphosphate, which accelerates β-oxidation of free fatty acids and consumption of lipids in turn.[11]

In previous studies, researchers found significant FI, atrophy, and fibrosis in supraspinatus mouse muscles at six weeks after tendon tear surgery.[38] They found that a group of residential interstitial progenitor cells in muscles, named fibroadipogenic progenitors, are the main source of fatty infiltration.[10] Both fibroadipogenic progenitors and fatty infiltration could be induced to adopt a brown/beige-like adipose tissue phenotype by stimulating β3AR in mice.[39] HIIT activates the sympathetic nervous system and helps with weight loss.[40,41] Some studies believe that HIIT induces postexercise lipid oxidation by β-adrenergic

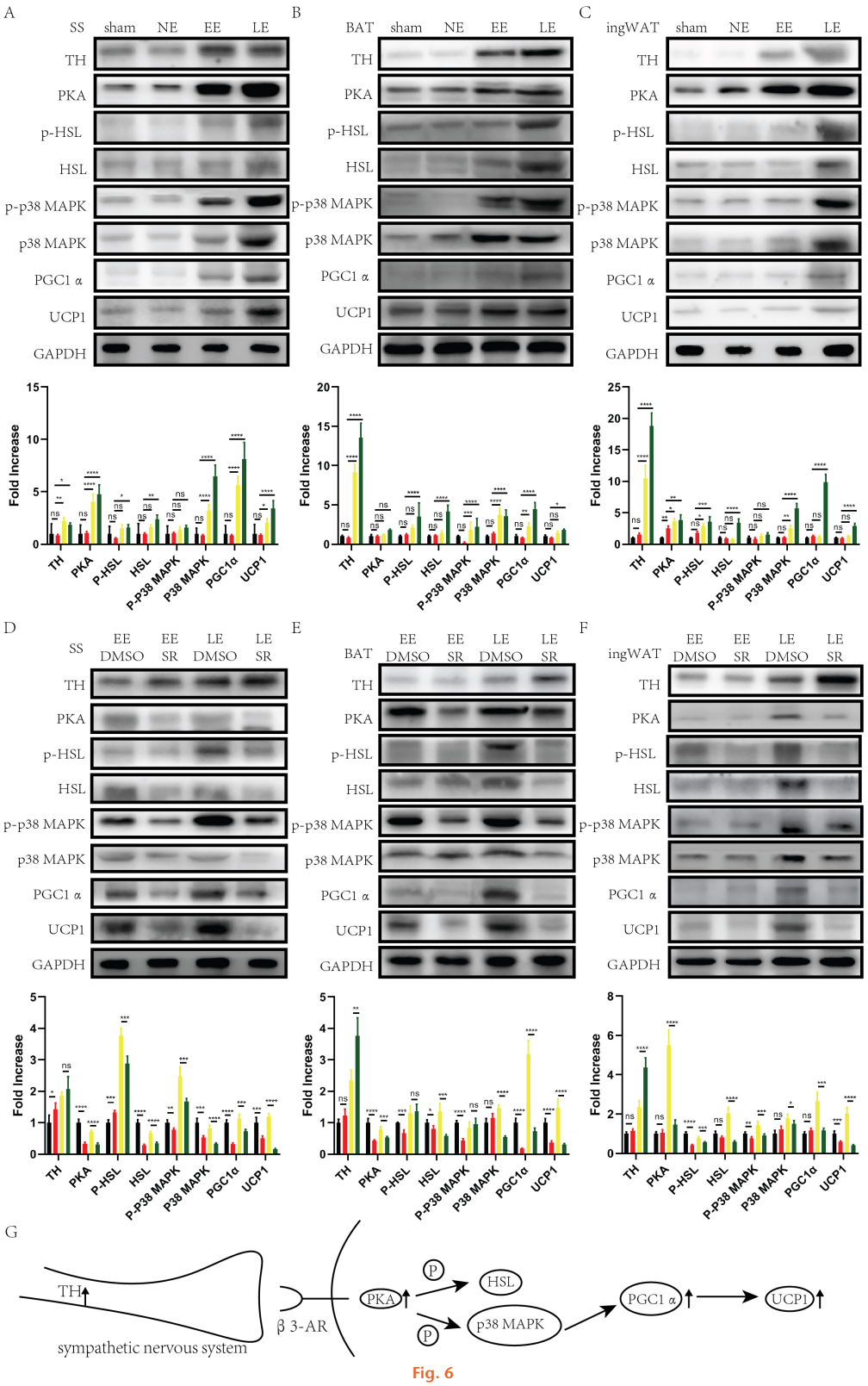

Fig. 6

Western blot of critical proteins in β3 adrenegic receptor (AR) thermogenesis pathway (n = 5 per group). We compared proteins from the sham, no exercise, early exercise, and late exercise groups in a) supraspinatus (SS) muscle, b) brown/beige-like adipose tissue (BAT), and c) inguinal subcutaneous white fat (ingWAT). We compared proteins from the early exercise (EE) dimethyl sulfoxide (DMSO), EE SR, late exercise (LE) DMSO, and LE SR groups in d) SS,e) BAT, and f) ingWAT. g) The signal pathway. For tyrosine hydroxylase (TH), PKA, HSL, P38 MAPK, PGC1α, and UCP1, fold change was calculated as target protein divided by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). For P-HSL and P-P38 MAPK, fold change was calculated as phosphorylated target protein divided by total target protein. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

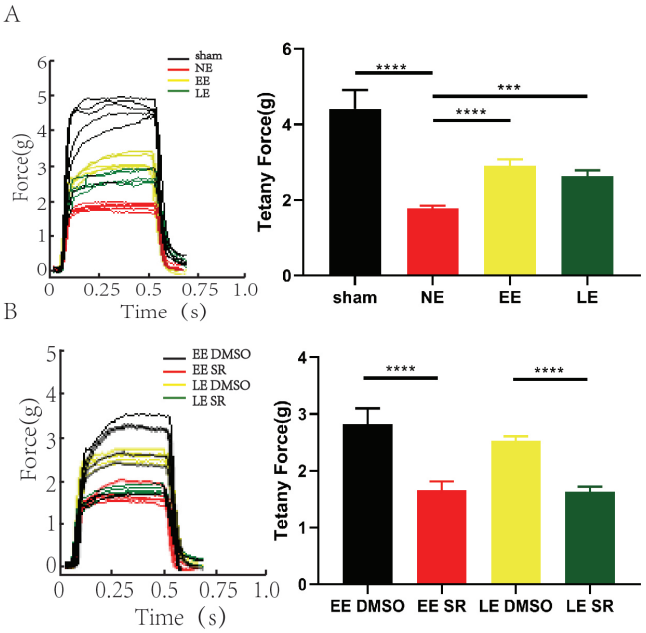

Fig. 7

Contractile test of supraspinatus muscle (n = 5 per group). a) High-intensity interval training improved tetany force in the early exercise (EE) and late exercise (LE) groups. b) SR59230A inhibited the improvement of tetany force. ***p < 0.001; ****p < 0.0001. DMSO, dimethyl sulfoxide; NE, no exercise.

stimulation in humans.[42,43] Many studies have demonstrated the benefits of HIIT in metabolic diseases. It helps to reduce adipose aggregation and local inflammation in humans.[41,44] Resistance training – another kind of high-intensity training – benefits aged skeletal muscle in mice. It increases satellite cells and type IIa muscle fibres in the hindlimb skeletal muscles of middle-aged (approx. 48 weeks old) mice.[45] Previous studies showed complex crosstalk between muscle and adipose tissue.[37,46] Irisin, Metrnl, and β-aminoisobutyric acid released by myocytes induce white fat browning. White fat secretes adiponectin and leptin: adiponectin has antioxidative, anti-inflammatory, and promyogenic effects on skeletal muscles, while leptin appears to promote glucose usage in skeletal muscle. Brown/beige-like adipose tissue secretes interferon regulatory factor 4 (IRF4) and fibroblast growth factor 21 (FGF21). IRF4 contributes to exercise capacity and mitochondrial function in muscle; FGF21 mediates the formation of cristae in mitochondria in skeletal muscle and brown/beige-like adipose tissue. Exercise increases irisin, Metrnl, β-aminoisobutyric acid, and adiponectin in plasma.

In this study, we showed that HIIT significantly improved muscle quality. Supraspinatus muscle in the early exercise and late exercise groups had less fatty infiltration, fibrosis, and wider myofibres, and had better contractile force. The results from the early exercise group indicate that HIIT helps to prevent fatty infiltration, and the results from the late exercise group indicate that HIIT helps to improve muscle quality, since statistically significant fatty infiltration, atrophy, and fibrosis occur in supraspinatus muscle at six weeks after tendon tear surgery.[38] We attribute these effects to activated UCP1 expression. Previous studies have confirmed that UCP1 plays a critical role in decreasing fatty infiltration.[30,47] In our study, HIIT significantly increased the expression of TH and UCP1. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of norepinephrine, a sympathetic neurotransmitter.[48] Increased expression of tyrosine hydroxylase indicates activation of local sympathetic nerve fibres.[40,48] Increased expression of UCP1 indicates white fat browning or activation of brown/beige-like adipose tissue.[17] We also detected tyrosine hydroxylase and UCP1 expression in other beige/brown fat depots by immunofluorescence to confirm this HIIT effect. The results were similar to those in the supraspinatus. Similar results between fat depots and fatty infiltration in muscle suggest a similar mechanism. This finding indicates that the effect of HIIT on fatty infiltration is a localized reflection of the systemic effect rather than a result of shoulder exercise. Since β3AR plays a critical role in brown/beige-like adipose tissue activation, we injected SR59230A, a selective β3AR antagonist, into mice before HIIT and examined the phenotypes of supraspinatus atrophy and fatty infiltration. The SR59230A groups showed worse muscle quality and function, similar to those who did not exercise. However, local tyrosine hydroxylase expression was as high as that in the DMSO groups, so HIIT activated local sympathetic nervous fibres when mice underwent SR59230A injection. The muscle and other beige/brown fat depots showed similar expression tendencies for tyrosine hydroxylase and UCP1 between groups. Therefore, the effect of HIIT on muscle quality and fat depots depended on β3AR. Similarly, other factors can also affect fat depots by activating the sympathetic nervous system. For example, calorie restriction affects sympathetic outflow in different fat depots of mice.[49] Leptin acts in the brain and promotes systemic lipolysis dependent on sympathetic nerve fibres innervated in adipose depots.[50] Besides brown/beige-like adipose tissue and white fat depots,[51,52] previous studies also reported sympathetic nerve fibres innervating the supraspinatus.[53] These studies support a systemic sympathetic effect on adipocytes in different tissues. We further examined related proteins in the β3AR thermogenesis pathway by western blotting. The expression was as expected: HIIT activated the pathway, while SR59230A inhibited HIIT. Adipose tissue in the supraspinatus and other fat depots reacted similarly to HIIT, all showing higher sympathetic nervous activity than the no exercise groups. Inhibition of β3AR increased supraspinatus fatty infiltration, decreased UCP1 expression, and downregulated the β3AR thermogenesis pathway. These results indicate that the effect of HIIT on fatty infiltration resulted from systemic activation of sympathetic nerve fibres near adipocytes.

Despite the promising results that we observed, some limitations exist in this study. First, we should consider the difference between species. Many previous studies have

studied β3AR in adipose tissue in mice. However, some studies found low expression of β3AR in human adipose tissue, and relatively high β2AR expression instead.[54] Nonetheless, a similar outcome might be expected as long as the local sympathetic nerve fibres are activated. Whether HIIT and its mechanism also works in humans requires further research. Second, SR59230A is an antagonist of β3AR with a relatively small effect on β1AR and β2AR. If it works on β1AR and β2AR, it might improve the potential for translation from mice to humans as described in the first limitation. HIIT plays a role through β-adrenoceptors in this research. We will use some β-adrenoceptor knockout mice in our future study. Third, we used acute tendon transaction in this rotator cuff tear model, but chronic rotator cuff tears are also present in a large portion of clinical cases.[55] Despite the difference between our model and the clinical environment, the muscle degeneration seen is similar, so the effect of HIIT is meaningful. Fourth, the mice were forced to perform HIIT – such forced interventions may affect the nervous system. However, in a clinical environment, patients under HIIT also need supervision. This supervision may have similar effects on the nervous system.

In conclusion, we discovered that HIIT improved fatty infiltration and muscle quality after rotator cuff tears. This finding is likely due to white fat browning in muscle, by stimulating the β3AR-dependent pathway. HIIT may be an effective prevention method of fatty infiltration for patients with a mild rotator cuff injury and preoperative preparation for patients with severe fatty infiltration.

Supplementary material

Two-way analysis of variance of Figure 4f and quantitative real-time polymerase chain reaction results for supraspinatus muscle.

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