Improved skeletal muscle fatigue resistance in experimental autoimmune myositis mice following high-intensity interval training

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Abstract

Background

Muscle weakness and decreased fatigue resistance are key manifestations of idiopathic inflammatory myopathies (IIMs). We here examined whether high-intensity interval training (HIIT) improves fatigue resistance in skeletal muscle of experimental autoimmune myositis (EAM) mice, a widely used animal model for IIM.

Methods

Female BALB/c mice were randomly assigned to control (CNT) or EAM groups (n = 28 in each group). EAM was induced by immunization with three injections of myosin emulsified in complete Freund's adjuvant. The plantar flexor (PF) muscles from mice with EAM were exposed to either an acute bout or 4 weeks of HIIT (a total of 14 sessions).

Results

The fatigue resistance of PF muscles was lower in the EAM than in the CNT group ($P < 0.05$). These changes were associated with decreased activities of citrate synthase and cytochrome c oxidase and increased expression levels of the endoplasmic reticulum stress proteins (glucose-regulated protein 78 and 94, and PKR-like ER kinase) ($P < 0.05$). HIIT restored all these alterations and increased the peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) and the mitochondrial electron transport chain complexes (I, III, and IV) in muscles from EAM mice ($P < 0.05$).

Conclusions

HIIT improves fatigue resistance in an IIM mouse model and this can be explained by restoration of mitochondria oxidative capacity via inhibition of the ER stress pathway and PGC-1α-mediated mitochondrial biogenesis.

Background

Patients with idiopathic inflammatory myopathies (IIMs), including polymyositis (PM), dermatomyositis (DM), and juvenile DM, suffer from muscle weakness and reduced fatigue resistance [1], which are associated with debility and increased mortality [2, 3]. The decreased endurance exercise capacity in patients with PM/DM is accompanied by a lower maximal oxygen uptake (VO_2 max) [2, 4]. Moreover, mitochondrial dysfunction has been observed in skeletal muscle from PM/DM patients [2, 5, 6], suggesting a low mitochondrial oxidative capacity as an important mechanism contributing to the reduced fatigue resistance in these patients.

The mechanisms that lead to impaired mitochondrial function in IIMs are not fully clarified. Although mitochondrial dysfunction has been shown to be associated with a proinflammatory microenvironment [7], there is growing evidence showing that other factors are also involved [8]. In this regard, the endoplasmic reticulum (ER) stress pathways are chronically activated in IIMs [9] and are associated with impaired mitochondrial function [10, 11]. Previous study has suggested a potential mechanistic link between sustained ER stress and mitochondrial dysfunction, meditated by reactive oxygen/nitrogen species generation due to augmented Ca^{2+} transfer through the mitochondrial-associated ER membrane (MAMs) [8, 11, 12].

Over a period of years, physical exercise was not recommended to patients with IIMs due to fear of exacerbating muscle inflammation. However, since the safety and benefits of exercise in IIM patients were first shown in 1993 [13, 14], exercise training emerged as a non-pharmacological therapy to improve muscle function and prevent disease progression [15–17]. For instance, endurance training improved VO_2 max and mitochondrial enzyme activities with reduced disease activity and down-regulation of genes related to ER stress in PM/DM patients [2, 18]. Following a resistance exercise training program, improved muscle strength and increased VO_2 max was seen in patients with PM/DM, and these improvements were accompanied by a reduction in gene expression associated with inflammation and fibrosis [19].

A growing body of evidence demonstrates that high-intensity interval training (HIIT) can serve as an effective alternate to traditional endurance training in healthy individuals and diseased populations [20]. Improvements in aerobic capacity are linked to enhanced peripheral oxygen extraction by skeletal muscle especially after a few weeks of HIIT [21, 22]. By using *in vivo* neuromuscular electrical stimulation, we recently demonstrated that the HIIT-induced increase in fatigue resistance is larger with high-intensity than with low-intensity contractions in mouse skeletal muscle, and this effect was linked to improved mitochondria content and function [23].

Peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) is regarded as an important regulator of mitochondrial biogenesis and function [24]. The AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and p38 mitogen-activated protein kinase are well known modulators of PGC-1α expression in skeletal muscle [24–27]. Previous studies have demonstrated that HIIT increases the phosphorylation levels of these signaling molecules and hence increase the expression of PGC-1α [23, 28]. Moreover, activation of AMPK has been shown to inhibit ER stress and inflammation in skeletal muscle [29].

One of the widely used animal model for IIMs is experimental autoimmune myositis (EAM) mouse [30]. EAM is induced by immunization with three injections of myosin emulsified in complete Freund's adjuvant. Muscle function is impaired at the end of the immunization period where muscular inflammation is already established [31, 32]. Intriguingly, we recently have reported that resistance training starting one day after the last immunization inhibits ER stress and restores muscle strength in mice with EAM [31]. In the present study, we tested the following hypotheses: 1) fatigue resistance is

decreased in muscle from mice with EAM due to the decreased mitochondrial oxidative capacity induced by ER stress; 2) HIIT combats these deleterious effects of EAM.

Methods

Ethical approval

All experimental procedures were approved by the Committee on Animal Experiments of Sapporo Medical University (No. 18-030). Animal care was in accordance with institutional guidelines.

Induction of experimental autoimmune myositis

Female BALB/c mice (8-week-old, n=28) and male Wistar rats (9-week-old, n=1) were supplied by Sankyo Lab Service (Sapporo, Japan). Mice were given food and water ad libitum and housed in an environmentally controlled room (24 ± 2°C) with a 12-h light-dark cycle. Health was monitored by weight and general assessment of animal activity (every other day). EAM was induced by immunizing mice with partially purified myosin, including myosin-binding protein C, as reported previously [30, 33]. Briefly, skeletal muscle (30 g) obtained from a Wistar rat was minced and washed four times in 30 mM KCl/150 mM sodium phosphate buffer (pH 7.5), 1 mM EDTA, and 1mM DTT. Myosin was extracted by incubation of muscle sample with 90 ml chilled 300 mM KCl/150 mM phosphate buffer containing 5 mM $MgCl_2$, 5 mM ATP, 1 mM DTT, and 1 mM EDTA on ice for 45 min with constant agitation. The homogenate was centrifuged for 30 min at 4°C at 2200 *g*. For myosin precipitation, the supernatant was collected, filtered, and diluted with 15 volumes of chilled ultrapurewater. The precipitate was recovered via centrifugation for 10 min at 4°C at 10,000 *g*, dissolved in 500 mM KCl, and stored at −80°C. Purified rat myosin (10 mg/ml) was emulsified with an equal amount of complete Freund's adjuvant (Difco) with 3.3 mg/ml *Mycobacterium butyricum* (Difco). BALB/c mice were each immunized intracutaneously with 50-100 μl of an emulsion into three to four locations (a total of 200 μl) on the back on days 0, 7, and 14. One hour after the first immunization, pertussis toxin (500 ng in 100 μl saline; List Biological Laboratories) was intraperitoneally injected into each animal. In the present study, all treated animals underwent successful EAM, defined by a significant increase in spleen weight. Further details can be found in the Supplementary Materials and Methods.

Experimental design

To assess the molecular and physiological adaptations induced by HIIT in skeletal muscle from EAM mice, we performed two separate experiments. The primary outcome of this study will be fatigue resistance. Secondary outcomes constitute mitochondrial enzyme activity, the amount of mitochondrial respiratory complexes and ER stress-related proteins, myosin heavy chain (MyHC) isoforms, and the phosphorylation levels of signaling proteins.

Experiment 1.

We first examined the effect of HIIT on muscle fatigability and ER/mitochondrial adaptation in EAM mice. Female BALB/c mice (n=12) were randomly assigned to CNT (n=6) and EAM (n=6) groups. Random numbers were generated using the standard = RAND() function in Microsoft Excel. In the EAM group, HIIT was performed on the left leg (referred to as the EAM + HIIT group), and the right leg served as a non-training EAM control. HIIT was started 24 hours after the last immunization and was carried out every other day for a total of 14 sessions (Figure 1A). The training order was randomized daily, with each animal trained at a different time each training day. Under isoflurane anesthesia, mice were placed supine on a platform with the foot secured to a footplate connected to a torque sensor (S-14154, Takei Scientific Instruments) at an angle of 0° dorsiflexion (i.e., 90° relative to the tibia). The plantar flexor muscles were activated by supramaximal (45 V, 0.5-ms) monophasic rectangular current pulses via a pair of surface electrodes. The stimulation scheme was designed to mimic the activation pattern during all-out cycling bouts, i.e. 0.25 s contractions produced every 0.5 s [23, 34]. Each session consisted of six sets of 60 contractions at 4-min intervals. Twenty-four hours after the last HIIT session, *in vivo* fatigue resistance of the plantar flexor muscles in each group was measured by 80 repeated 350 ms, 70 Hz tetani given at an interval of 3 s. This was done by an investigator unaware of treatment side. Twenty-four hours after the measurement of fatigue resistance (i.e., 48 hours after the last HIIT session), mice were killed by cervical dislocation under isoflurane anesthesia and the gastrocnemius (GAS) and plantaris muscles were used for skinned muscle fiber experiments and for biochemical analyses (see below).

Experiment 2.

To investigate cellular signaling that underlies the HIIT-induced physiological adaptations, female BALB/c mice (n=16) were randomly divided into the same groups as in *Experiment 1* (n=8 in each group). Immediately after one HIIT session, mice were killed by rapid cervical dislocation under isoflurane anesthesia and muscles were subsequently isolated. The phosphorylation levels of AMPKα Thr172, CaMKII Thr286, ACC Ser79, and p38 MAPK The180/Tyr182 were investigated in GAS muscles from each animal.

Myosin heavy chain isoforms separation

Aliquots of GAS muscle extracts containing 5 μg protein were used for myosin heavy chain (MyHC) electrophoresis as previously described [35]. Using a 6.8% polyacrylamide slab gel, electrophoresis was run at 4ºC for 24 h at 160 V and stained with Coomassie brilliant blue. Images of gels were densitometrically evaluated with ImageJ.

Measurement of Ca^{2+}-activated force in skinned muscle fibers

Chemically skinned muscle fibers were prepared and Ca^{2+}-activated force was measured as described previously [36]. The gastrocnemius (GAS) muscle was pinned out at resting length under paraffin oil kept at 4°C. Single muscle fibers were dissected under a stereomicroscope. Four to six skinned fibers were obtained from one whole muscle. A segment of the skinned fiber was connected to a force transducer (Muscle tester, World Precision Instruments) and then incubated with a *N*-2-hydroxyethylpiperazine-*N*-2-

ethanesulfonic acid (HEPES) buffered solution (see below) containing 1% (vol/vol) Triton X-100 for 10 min in order to remove membranous structures. Fiber length was adjusted to optimal length (2.5 μm) by laser diffraction as described previously [37] and the contractile properties were measured at room temperature (24°C).

All solutions were prepared as described in detail elsewhere [38]. They contained (in mM) 36 Na^+, 126 K^+, 90 HEPES, 8 ATP and 10 creatine phosphate, and had a pH of 7.09–7.11 and a free Mg^{2+} concentration set at 1.0 mM. The maximum Ca^{2+} solution contained 49.5 mM Ca-EGTA and 0.5 mM free EGTA, whereas the relaxation solution contained 50 mM free EGTA. Various pCa (-log free Ca^{2+} concentration) solutions (pCa 6.4, 6.2, 6.0, 5.8, 5.6, 5.4, and 4.7) were prepared by mixing the maximum Ca^{2+} solution and the relaxation solution in appropriate proportions [39]. The contractile apparatus was directly activated by exposing the skinned fiber to the various pCa solutions and force was measured. Isometric force produced at each pCa was expressed as a percentage of the corresponding maximum force and analysed by fitting a Hill curve using SigmaPlot 13.0 software to establish the pCa_{50} (pCa at half-maximum force). The cross-sectional area of fibers was calculated from measurements of their diameters. The maximum Ca^{2+}-activated force per cross-sectional area (F_{max}) is expressed as mN/mm^2.

Mitochondrial enzyme activity

The maximal activities of citrate synthase (CS) and cytochrome c oxidase (COX) were determined in whole muscle homogenates. In brief, whole plantaris muscles were homogenized in ice-cold 100 mM potassium phosphate buffer (100 μl/mg wet wt) and maximal CS and COX activities were measured spectrophotometrically as described previously [40, 41].

Immunoblotting

Immunoblots were performed as previously described [42] using: anti-PGC-1α (ab54481, Abcam), anti-total OXPHOS rodent WB antibody cocktail (ab110413, Abcam), anti-glucose-regulated protein (Grp) 78 (ADI-SPA-826, Enzo Life Sciences), anti-Grp94 (ADI-SPA-851, Enzo Life Sciences), anti-inositol-requiring transmembrane kinase/endoribonuclease 1α (IRE1α) (#3294, Cell signaling), anti-PKR-like endoplasmic reticulum kinase (PERK) (#5683, Cell signaling), anti-phospho-AMPKα Thr172 (#2531, Cell signaling), anti-AMPKα (#2532, Cell signaling), anti-phospho-CaMKII Thr286 (#12716, Cell signaling), anti-CaMKII (611292, BD Biosciences, San Jose, CA), anti-phospho-ACC Ser79 (#3661, Cell signaling), anti-ACC (#3662, Cell signaling), anti-phospho-p38 MAPK (#4511, Cell signaling), anti-p38 MAPK (#9212, Cell signaling).

Muscle pieces were homogenized in ice-cold homogenizing buffer (40 μl/mg wet wt) consisting of (mM): Tris maleate, 10; NaF, 35; $NaVO_4$, 1; 1% Triton X 100 (vol/vol), and 1 tablet of protease inhibitor cocktail (Roche) per 50 ml. The protein content was determined using the Bradford assay [43]. Aliquots of the whole muscle homogenates (20 μg) were diluted with Laemmli buffer (mM): Urea, 4000, Tris/HCl, 250; SDS, 3.5; 20% glycerol (vol/vol); 0.0005% bromophenol blue (wt/vol). Proteins were applied to a 4–15%

Criterion Stain Free gel (BioRad). Gels were imaged (BioRad Stain Free imager) and then proteins were transferred onto polyvinylidine fluoride membranes and were blocked in 3% (wt/vol) non-fat milk, Tris-buffered saline containing 0.05% (vol/vol) Tween 20, followed by incubation with primary antibody overnight at 4°C. Membranes were then washed and incubated for 1 h at room temperature with secondary antibody (1:5000, donkey-anti-rabbit or donkey-anti-mouse, BioRad). Images of membrane were collected following exposure to chemiluminescence substrate (Millipore) using a charge-coupled device camera attached to ChemiDOC MP (BioRad), and Image Lab Software (BioRad) was used for detection as well as densitometry. The levels of protein expression were normalized to the total proteins from stain-free image.

Statistics

Data are presented as mean ± SEM. Data normality was examined with the Shapiro-Wilk test. Power calculations were performed to estimate an adequate sample size in order to detect a meaningful difference. In *Experiment 1*, for normally distributed data (the distribution of the MyHC isoforms, CS activity, COX activity, the expression levels of PGC-1α, NDUFB8, SDHB, UQCRC, MTC01, ATP5, Grp78, Grp94, IRE1α, and PERK, F_{max}, pCa_{50}), one-way ANOVA was used to determine the mean differences among the three groups (CNT, EAM, and EAM+IT group). Fatigue resistance (group x repetitions) and specific force-pCa relationship (group x pCa) were assessed by two-way repeated measures ANOVA. In *Experiment 2*, for normally distributed data (the phosphorylation levels of AMPK, CaMKII, and p38MAPK), one-way ANOVA was used to determine the mean differences between the groups. When these ANOVA tests showed significance, Bonferroni or Tukey *post hoc* test was performed. If data exhibited a non-normal distribution (the phosphorylation levels of ACC), a Kruskal-Wallis one-way ANOVA was used on ranks. A P value less than 0.05 was regarded as statistically significant. Statistical testing was performed with SigmaPlot (version 13, Systat Software, Inc).

Results

HIIT improves fatigue resistance in skeletal muscle from EAM mice

In experiment 1, there was no difference in body weight between CNT (n = 6) and mice with EAM (n= 6) (mean ± SEM 21.4 ± 0.4 g versus 19.1 ± 0.7 g; $P > 0.05$). In contrast, the spleen weight was 2.6-fold higher in the EAM than in the CNT group (mean ± SEM 289 ± 20 mg versus 110 ± 5 mg, $P < 0.05$). The GAS muscle weight was 25% lower in the EAM than in the CNT group (mean ± SEM 70.9 ± 2.2 mg versus 94.2 ± 2.6 mg, $P < 0.05$) and this was not ameliorated by HIIT (mean ± SEM 75.9 ± 3.5 mg, $P > 0.05$).

Typical torque traces during a HIIT session are shown in Figure 1B. Note that the torque was decreased much faster in the later sets than in the early sets. Figure 2A-C show representative torque records during *in vivo* fatiguing stimulations of plantar flexor muscles from CNT and EAM mice with or without HIIT. EAM muscles were less fatigue resistance than control muscles (Figure 2D, $P < 0.05$). Importantly, HIIT

significantly improved fatigue resistance in EAM muscles ($P < 0.05$). The differences in fatigue resistance were not due to any changes in muscle fiber type composition (Figure 2E and F).

Figure 3A shows the typical traces of Ca^{2+}-activated force in skinned fibers from GAS muscles in each group. The fiber diameter was smaller in the EAM (37.9 ± 0.7 μm [n = 31 fibers], $P < 0.05$) and the EAM + HIIT (34.9 ± 1.0 μm [n = 31 fibers], $P < 0.05$) groups than in the CNT group (43.6 ± 1.0 μm [n = 28 fibers]). Ca^{2+}-activated specific force production was lower in skinned fibers from EAM muscles compared to those from control muscles (Figure 3B, $P < 0.05$). Notably, this was restored by HIIT to the control level ($P < 0.05$). The F_{max} was 21% lower in EAM muscle fibers than in CNT muscle fibers (278 ± 11 mN/mm^2 versus 349 ± 11 mN/mm^2, $P < 0.05$), which was recovered by HIIT (353 ± 13 mN/mm^2, $P < 0.05$) (Figure 3C). The Ca^{2+} sensitivity (pCa_{50}) was similar in the three groups (Figure 3D, $P > 0.05$).

HIIT increases mitochondrial respiratory complexes in skeletal muscle from EAM mice

Compared to the CNT group, CS and COX activities were lower in the EAM group (Figure 4A and B, $P < 0.05$). Notably, these EAM-induced deleterious alterations were restored by HIIT ($P < 0.05$). Moreover, HIIT markedly increased the protein expression of PGC-1α and mitochondrial respiratory complexes I, III, and IV in the EAM group (Figure 4C-F, $P < 0.05$).

HIIT alleviates ER stress in skeletal muscle from EAM mice

Previous studies suggest that the ER stress pathways are chronically activated and may play an aetiological role in IIM [9]. Accordingly, GAS muscles from EAM mice showed significantly increased expression of the unfolded protein response proteins Grp78, Grp94, and PERK, but not IRE-1α (Figure 5A-H, $P < 0.05$). Notably, HIIT attenuated the increased expressions of these ER stress-related proteins.

The phosphorylation levels of signaling proteins are increased after a single bout of HIIT

In experiment 2, the body weight was slightly higher in mice with EAM (n= 8) than in mice with CNT (n = 8) (mean ± SEM 19.4 ± 0.3 g versus 18.0 ± 0.3 g; $P < 0.05$). The spleen weight was 5-fold higher in the EAM than in the CNT group (mean ± SEM 432 ± 11 mg versus 87 ± 4 mg, $P < 0.05$). The phosphorylation levels of AMPK Thr172, ACC Ser79, and p38 MAPK Thr180/Tyr182 did not differ between the CNT and the EAM group, while the phosphorylation levels of these molecules were increased immediately after one HIIT session compared to the CNT group (Figure 6A, B, D, E, F, $P < 0.05$). On the other hand, CaMKII Thr286 phosphorylation was higher in the EAM group than in the CNT group (Figure 6A and C, $P < 0.05$), which was not affected by an HIIT session.

Discussion

In accordance with our hypothesis, we show a reduced fatigue resistance during *in vivo* fatiguing stimulation in skeletal muscle from EAM mice, an animal model for acute IIMs [30]. The decreased fatigue resistance was accompanied by increased expression of ER stress-related proteins and reduced

activities of mitochondrial oxidative enzymes. Importantly, these deleterious events were restored by HIIT starting 24 hours after the last immunization where muscle function is impaired.

Theoretically, the fatigue resistance of muscle fiber depends on the fiber type, which is defined by the MyHC isoform. However, the impaired endurance performance was not accompanied by an alteration in MyHC isoforms in skeletal muscle from EAM mice. In line with this, untreated newly diagnosed patients with PM/DM had a similar fiber type composition to healthy individuals, although patients with chronic PM/DM display fewer slow-twitch type I fibers [44]. A fiber type transition can therefore be excluded as a factor causing reduced fatigue resistance at disease onset of IIMs. On the other hand, previous studies suggest a low mitochondrial respiratory capacity as an important mechanism contributing to the impaired endurance performance in patients with IIMs [2, 5, 6]. In agreement, our data show that the reduced fatigue resistance is accompanied by decreased activities of CS and COX in skeletal muscle from mice with EAM.

The mechanisms underlying the impaired mitochondrial function in IIMs remain uncertain, although non-immune-mediated pathways are thought to be involved. Indeed, despite recommended treatment with conventional immunosuppressive agents, few IIM patients regain full muscle endurance performance [2]. In this regards, there is growing evidence to suggest that ER stress pathways are chronically activated in IIMs [9] and are linked to the mitochondrial dysfunction [10, 45]. Recently, Thoma et al. [11] have shown that the ER stress inducer, tunicamycin, promotes mitochondrial dysfunction in a human skeletal muscle cell line. Moreover, it has been demonstrated that PERK, a key ER stress sensor of the unfolded protein response, resides in MAMs and plays a critical role in mitochondrial dysfunction [12]. Accordingly, our findings of the decreased activities of mitochondrial oxidative enzymes in combination with the increased ER stress proteins Grp78, Grp94, and PERK suggested that sustained ER stress underlies mitochondrial dysfunction in skeletal muscle from EAM mice.

The improvement of fatigue resistance by HIIT in EAM can be explained by increased muscle aerobic capacity as judged from upregulation of mitochondrial respiratory complexes (I, III, and IV) and increased activities of CS and COX. The increased CS activity and fatigue resistance have also been reported in patients with PM/DM who performed a 12 weeks of endurance training [2, 18]. Notably, one HIIT session in skeletal muscle from EAM mice was followed by phosphorylation of AMPK, ACC, and p38 MAPK, which was associated with increased PGC-1α protein expression after 4 weeks of HIIT. These data indicate that the PGC-1α-dependent augmentation of mitochondrial oxidative capacity can be effectively induced by exercise training even under inflammatory conditions such as IIMs.

In addition to the mitochondrial biogenesis, HIIT may improve muscle aerobic capacity by ameliorating mitochondrial dysfunction due to ER stress in EAM mice. Indeed, our data show that HIIT inhibited the increased amount of ER stress proteins Grp78, Grp94, and PERK in skeletal muscle from EAM mice. This is in line with a previous study from our lab where EAM-induced upregulation of ER stress proteins, including Grp78 and Grp94, was attenuated by 4 weeks of high-intensity eccentric contraction training in EAM mice [31]. Taken together, our findings promote exercise as an important non-pharmacological

approach for relieving ER stress and improving mitochondrial function. Although the mechanisms underlying this beneficial effect of exercise remains unresolved, previous studies suggest that AMPK functions as a suppressor of ER stress [29, 46].

Conclusion

We here show reduced fatigue resistance in skeletal muscle from a mouse model of acute IIM. This functional defect was due to decreased mitochondria oxidative capacity, which was at least in part caused by activation of ER stress-dependent pathway. HIIT-mimicking electrical stimulation reversed these alterations and markedly improved fatigue resistance without any signs of deleterious effects in skeletal muscle. Thus, our findings highlight the clinical importance of HIIT as a safe and effective way to treat the increased muscle fatiguability in patients with IIMs.

Declarations

Disclosures

The authors declare no conflict of interest.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

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Author contributions

TY contributed to the conception and design of the work. All authors contributed to the acquisition, analysis, and interpretation of the data along with drafting and revising the work. All author(s) read and approved the final manuscript.

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Figures

Figure 1

Schematic overview of experimental design. (**A**) In experiment (*Exp*) *1*, the fatigue resistance and intracellular events were evaluated under control conditions (CNT) and after induction of experimental autoimmune myosistis (EAM) with and without subsequent high-intensity interval training (HIIT). HIIT was initiated the day after the last immunization to induce EAM and was performed with electrical stimulation of the left leg (EAM + HIIT) every other day for a total of 14 sessions. The right leg served as a non-training EAM control. In *Exp 2*, cellular signaling that underlies the HIIT-induced physiological adaptations was investigated after an acute single bout of HIIT. (**B**) Typical torque traces of a HIIT session.

Figure 2

HIIT improves fatigue resistance in skeletal muscle from EAM mice. (**A-C**) Representative torque records during the *in vivo* fatigue protocol (70 Hz, 350 ms tetani every 3 s) of the plantar flexor muscles from control (CNT) and EAM mice with or without high-intensity interval training (HIIT). (**D**) Mean (±SEM) relative tetanic torque during fatiguing stimulation. Torque in the first tetanus was set to 100% in each muscle. Two-way repeated measures ANOVA with Bonferroni *post hoc* test was performed. $*P < 0.05$ CNT vs EAM, $^{\#}P < 0.05$ EAM vs EAM + HIIT. (**E**) Blots showing electrophoretically separated myosin heavy chain (MyHC) isoforms in the gastrocnemius muscles in each group. (**F**) Distribution of MyHC isoforms. Data show mean and SEM results from 6 muscles per group. One-way ANOVA was performed.

Figure 3

HIIT restores the decrease in myofibrillar force production in skeletal muscle from EAM mice. (**A**) Representative original records of *in vitro* Ca^{2+}-activated force in chemically skinned fibers of gastrocnemius muscles from control (CNT) and EAM mice with or without high-intensity interval training (HIIT). Fibers were exposed to solutions with progressively higher free Ca^{2+} concentration: pCa 6.4, 6.2, 6.0, 5.8, 5.6, 5.4, and 4.7. (**B**) Specific force-pCa relationships. Two-way repeated measures ANOVA with Tukey *post hoc* test was performed. (**C**) Maximum Ca^{2+}-activated force per cross-sectional area (F_{max}). (**D**) pCa50 (pCa at half-maximum force). Data presented as mean and SEM from 28-31 fibers per group. One-way ANOVA with Bonferroni *post hoc* test was performed. *P < 0.05 CNT vs EAM, #P < 0.05 EAM vs EAM + HIIT.

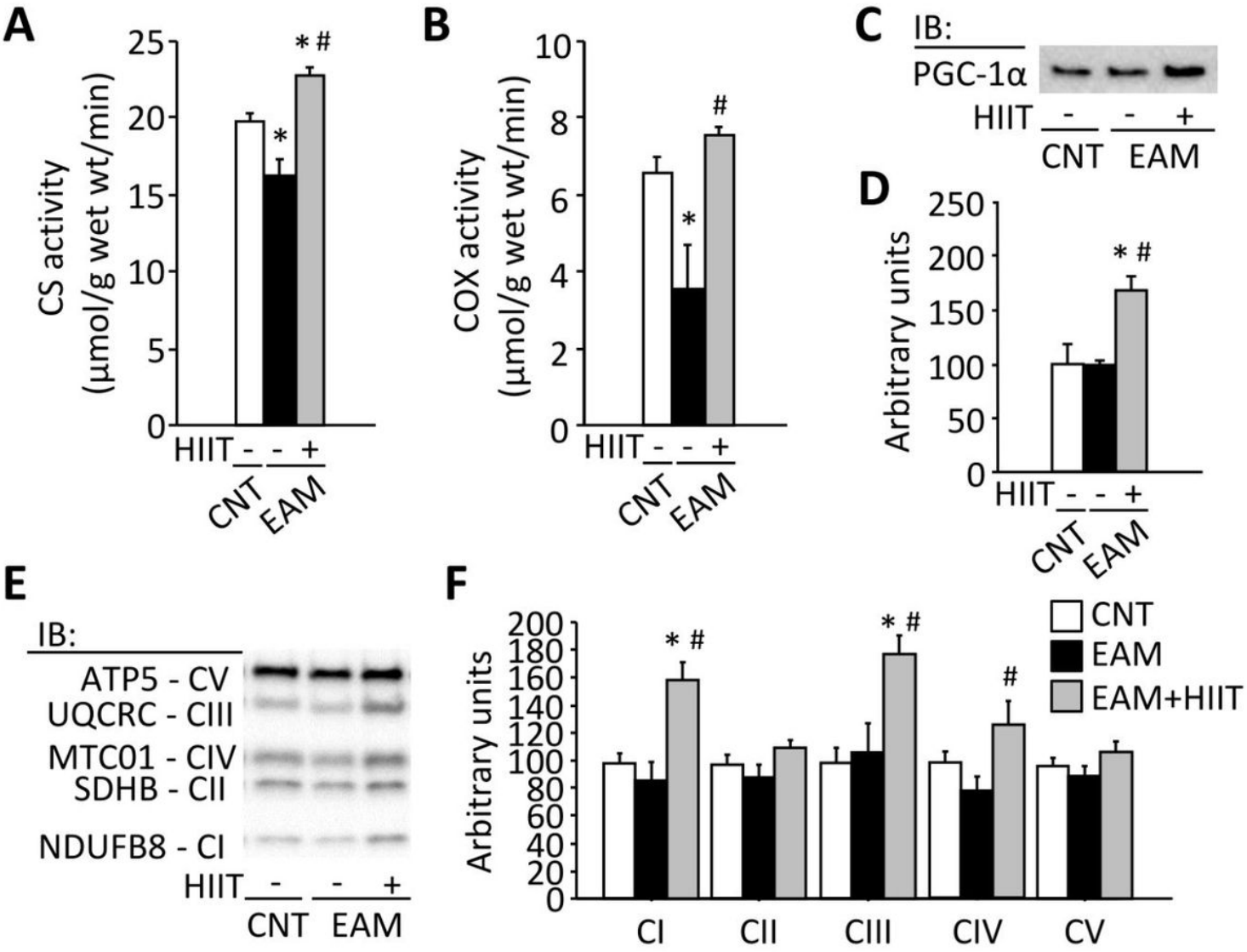

Figure 4

HIIT increases markers of mitochondrial respiration in skeletal muscle from EAM mice. Citrate synthase (CS) (**A**) and cytochrome c oxidase (COX) (**B**) activities in plantaris muscles from control (CNT) and EAM mice with or without high-intensity interval training (HIIT). Representative western blots illustrating the levels of peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α) (**C**), complex I (CI) subunit (NDUFB8), CII subunit (SDHB), CIII subunit (UQCRC), CIV subunit (MTC01), CV subunit (ATP5) (**E**) in gastrocnemius muscles. The levels of PGC-1α (**D**) and CI-V (**F**) were normalized to total protein in Stain-Free images and the mean in CNT muscles was set to 100%; n = 5-6 muscles per group. One-way ANOVA with Bonferroni *post hoc* test was performed. *P < 0.05 vs CNT, $^{\#}P$ < 0.05 vs EAM.

Figure 5

HIIT alleviates the upregulation of ER stress proteins in gastrocnemius muscle from EAM mice. Representative western blots illustrating the levels of glucose-regulated protein (Grp) 78 (**A**), Grp94 (**B**), inositol-requiring transmembrane kinase endoribonuclease-1α (IRE-1α) (**E**), and PKR-like ER kinase (PERK) (**F**) of gastrocnemius muscles in control (CNT) and EAM mice with or without high-intensity interval training (HIIT). Bars showing mean and SEM levels of these proteins (**C**, **D**, **G**, **H**). Data were normalized to the total proteins from the Stain-Free image and the mean in CNT muscles was set to 100%; n = 5-6 muscles per group. One-way ANOVA with Bonferroni *post hoc* test was performed. *P < 0.05 vs CNT, $^{\#}P$ < 0.05 vs EAM.

Figure 6

The phosphorylation levels of signaling proteins are increased after a single bout of HIIT. Representative western blots of total and phosphorylated AMPK Thr172, CaMKII Thr286 (**A**), ACC Ser79, and p38 MAPK Thr180/Tyr182 (**D**) of gastrocnemius muscles in control (CNT) and EAM mice with or without one bout of high-intensity interval training (HIIT; Exp 2). Bars showing mean and SEM of the phosphorylation levels of AMPK Thr172 (**B**), CaMKII Thr286 (**C**), ACC Ser79 (**E**), p38 MAPK Thr180/Tyr182 (**F**) relative to total protein content. Data expressed relative to the mean in CNT muscles, which was set to 100%; n = 10 muscles per group. One-way ANOVA with Tukey *post hoc* test was performed, except the phosphorylation level of ACC where a Kruskal-Wallis one-way ANOVA was used on ranks. *P < 0.05 vs CNT, $^{\#}P$ < 0.05 vs EAM.