Effects of Concurrent Training on Oxidative Capacity in Rat Gastrocnemius Muscle

REGULA FURRER[1], ARNOLD DE HAAN[1,3], NATHALIE BRAVENBOER[2], DORIEN KOS[1], PAUL LIPS[4], and RICHARD T. JASPERS[1]

[1]Faculty of Human Movement Sciences, MOVE Research Institute Amsterdam, VU University Amsterdam, THE NETHERLANDS, [2]Department of Clinical Chemistry, MOVE Research Institute Amsterdam, VU University Medical Center Amsterdam, THE NETHERLANDS, [3]Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, Manchester, UNITED KINGDOM; and [4]Department of Endocrinology, MOVE Research Institute Amsterdam, VU University Medical Center Amsterdam, THE NETHERLANDS

ABSTRACT

FURRER, R., A. DE HAAN, N. BRAVENBOER, D. KOS, P. LIPS, and R. T. JASPERS. Effects of Concurrent Training on Oxidative Capacity in Rat Gastrocnemius Muscle. *Med. Sci. Sports Exerc.*, Vol. 45, No. 9, pp. 1674–1683, 2013. **Purpose**: Training for improvement of oxidative capacity of muscle fibers may be attenuated when concurrently training for peak power. However, because of fiber type-specific recruitment, such attenuation may only account for high-oxidative muscle fibers. Here, we investigate the effects of concurrent training on oxidative capacity (as measured by succinate dehydrogenase (SDH) activity) by using task-specific recruitment of the high- and low-oxidative compartment of rat medial gastrocnemius muscle (GM). **Methods**: Forty rats were subjected to 6 wk of peak power training (PT, $n = 10$), endurance training (ET, $n = 10$), concurrent peak power and endurance training (PET, $n = 10$), or no training (control, $n = 10$). SDH activity, mRNA expression of SDH, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), receptor-interacting protein 140, and BCL2/adenovirus E1B 19 kDa-interacting protein 3 as well as PGC-1α protein levels were analyzed in the low- and high-oxidative region of the GM. **Results**: In the low-oxidative compartment, PT and PET induced a 30% decrease in SDH activity of Type IIB fibers compared with controls and ET ($P < 0.001$) without changes in mRNA or protein levels. In the high-oxidative compartment, after ET, SDH mRNA levels were 42% higher and RIP140 mRNA levels 33% lower compared with controls, which did not result in changes in SDH activity. **Conclusion**: These results indicate that in compartmentalized rat GM, peak power on top of endurance training attenuated transcription of mRNA for mitochondrial proteins in high-oxidative muscle fibers. In low-oxidative Type IIB fibers, peak power training substantially decreased SDH activity, which was not related to lower SDH mRNA levels. It is concluded that PT and PET enhanced mitochondrial degradation in the low-oxidative compartment of rat GM. **Key Words**: STRENGTH TRAINING, RESISTANCE EXERCISE, ENDURANCE EXERCISE, FIBER TYPE, SDH ACTIVITY, MITOCHONDRIAL BIOSYNTHESIS

Skeletal muscle has the ability to undergo large adaptations in muscle size or oxidative capacity, which is evident from the difference in muscle phenotype of power and endurance athletes. However, many sports athletes require both high peak power and endurance and, hence, concurrent training for peak power and endurance is indicated. Comparison of size and oxidative capacity of different types of muscle fibers over a wide range of species has shown that muscle fiber size and the average mitochondrial enzyme activity per unit of volume are inversely related (28). This inverse relationship suggests that limitation of oxygen diffusion within a muscle fiber causes a size constraint and that an increase in mitochondrial density and muscle fiber hypertrophy are mutually exclusive (30).

This relationship implies that performing peak power and endurance training concurrently (further called concurrent training) may result in both an attenuated increase in maximal force and oxidative capacity. Indeed, in healthy young men and women, concurrent training attenuates the increase in maximal muscle force compared with strength training only (9,12,15). Such interaction effects are conceivable from recent insights that signaling pathways for protein synthesis and mitochondrial biosynthesis are distinct and interfere with one another (1,2,5,30). However, when humans were subjected to concurrent training, the increase in whole-body oxidative capacity (measured as $\dot{V}O_{2max}$) does not seem limited compared with endurance training only (9,12,15,22, 23,25). Recently, additional resistance exercise on top of endurance exercise even enhanced the transcription of several genes involved in mitochondrial biosynthesis (i.e., peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), PGC-1-related coactivator and pyruvate dehydrogenase kinase 4) compared with endurance exercise only (31). However,

this was only shown after a single bout of exercise, and it is unknown whether in the long term, this results in an increased oxidative capacity of muscle fibers. These concurrent training effects are not in line with predictions according to the inverse relationship between fiber size and oxidative capacity (30). This discrepancy raises the question whether the reported increase in $\dot{V}O_{2max}$ after concurrent training (9,12,15,22,23,25) is explained by an increase in the oxidative capacity of muscle fibers although maintaining their size or whether other mechanisms are involved.

A possible explanation for how concurrent peak power and endurance training may increase muscle oxidative capacity without whole muscle atrophy is by using training-specific recruitment of different muscle fibers. Generally, during low-intensity endurance exercise, high-oxidative muscle fibers (Types I and IIA) are mainly recruited, whereas during peak power training, larger low-oxidative fibers (Types IIX and IIB, the latter only in rodents) are mostly responsible for the generated power (7). Human studies are usually performed on whole muscle function or homogenized protein extracts and, as such, may not take into account muscle fiber type-specific effects of training. As basal expression levels of oxidative genes differ between high- and low-oxidative muscles (17,26,30), effects of concurrent training for peak power and endurance may apply to different subsets of muscle fibers. How concurrent training affects the oxidative capacity of different muscle fiber types within one muscle is not well known. Therefore, the overall aim of this study was to investigate the effects of concurrent training on muscle fiber oxidative capacity using task-specific recruitment of high- and low-oxidative muscle fibers within one muscle.

The rat medial gastrocnemius muscle (GM), which is a major calf muscle, is a compartmentalized muscle and composed of a high- and low-oxidative compartment, which are recruited according to specific tasks (6,7). During low-intensity activities, the high-oxidative compartment is active, whereas only during high-intensity activities, the low-oxidative compartment consisting of Types IIX and IIB fibers is recruited (7). Besides its important function in plantarflexion and the easily distinguishable compartments, such task-specific recruitment in rat GM allows investigation of fiber type-specific effects of concurrent training within one muscle. Here, we investigated 1) how in compartmentalized GM basal levels of succinate dehydrogenase (SDH) activity are related to key regulators of mitochondrial biosynthesis and 2) how, by using task-specific recruitment of the high- and low-oxidative compartment, concurrent training changes the oxidative capacity and the key regulatory factors. Quantification of SDH activity, using calibrated histochemistry, was used as a measure for muscle fiber oxidative capacity (3,8,29). This SDH activity measure has been shown to be proportional to $\dot{V}O_{2max}$ of isolated muscle fibers under hyperoxic conditions (8,29). For the second aim of this study, we hypothesized that in the low-oxidative compartment of GM, concurrent training and peak power training induce similar changes in SDH activity, and in the high-oxidative compartment of GM, concurrent peak power and endurance training attenuates the increase in SDH activity compared with endurance training only.

MATERIALS AND METHODS

Animal care and experimental design. The experiment was approved by the Animal Experiments Committee of the VU University Amsterdam, and animals were kept according to the guidelines of animal care. The study was conducted with 40 female Wistar rats at the age of 10 wk with food and water provided *ad libitum*. The rats were subjected to either peak power training (PT, n = 10), endurance training (ET, n = 10), concurrent peak power and endurance training (PET, n = 10), or no training (control, n = 10). To train the rats during their active period of the day, the 12-h light/12-h dark cycle was reversed. During a 3-wk acclimatization period, rats of the training groups were familiarized with running on a motor-driven treadmill. After this, the 6-wk training period started, during which the rats of the PT and ET group performed five training sessions per week (one session per day) and the PET group performed 10 training sessions per week (two sessions per day). For studying effects on bone mineralization (not reported here) 9 and 2 d before sacrificing, a very small dose of tetracycline (25 mg·kg^{-1}) was injected intraperitoneally. To standardize for the gene expression and exclude acute training effects, the last training was performed approximately 22 h before sacrificing (peak power training for the PT group and endurance training for the ET and PET group).

Training protocols and maximal running performance tests. *Peak power training* consisted of 10 sprints of 15 s at maximal attainable velocity with 3 min of rest between the sprints and was performed on a treadmill with progressively increasing slope up to 40%. Once in gallop, resembling explosive jumps of the hind limbs, the speed of the treadmill was increased up to a velocity at which the rat could just keep up. Jump-like exercises, consisting of short and powerful contractions at a high velocity, were shown to increase maximal leg extensor muscle force in humans (21). For inclusion in this study, during at least four training sessions per week, maximal velocities had to be achieved. The endurance training consisted of treadmill running in which the duration was increased progressively up to 45 min after 6 wk, and the slope and velocity were increased progressively up to 10% and 26 m·min^{-1} (trotting), respectively. The PET group performed both training sessions on the same day with 8-h rest in between. Rats unable to sustain the training duration at the given inclination and speed during three or more training sessions were excluded from this study.

Maximal running performance was defined as either maximal running velocity performed at a 40% inclination for PT and PET or maximal running duration at 26 m·min^{-1} and 10% inclination performed for ET and PET (with a

maximum of 1 h). Because treadmill running required practice, the control group did not perform the maximal running velocity or duration tests to prevent measuring only learning effects. For the same reason, the first maximal running performance test was performed after 2 wk of training.

Histochemistry. After cutting cross-sections (10 μm thick) from the middle of the GM, containing muscle fibers from both the high- and low-oxidative compartments, they were mounted on Vectabond (Vector Laboratories, Burlingame, CA)-coated slides. Sections to be stained for SDH were air-dried for 15 min and immediately assayed (see succeeding part of this article). The other sections were stored in −80°C for further analysis.

For SDH staining, the sections were first incubated for 15 min at 37°C in 37 mM sodium phosphate buffer (pH 7.6), 74 mM sodium succinate, and 0.4 mM tetranitroblue tetrazolium and then 3 s in 0.01 M HCl to stop the reaction (29). To determine the different fiber types (Types I, IIA, IIX, and IIB) in both compartments, sections were air-dried and stained for myofibrillar adenosine triphosphatase (ATPase) using acid (pH 4.625 for 10 min at room temperature) or alkaline (pH 10.4 for 20 min at 37°C) preincubation (4,10,18). In both high- and low-oxidative compartments, muscle fibers (a minimum of 250 fibers) were classified by eye into the four types according to their staining characteristics for myofibrillar ATPase. Fiber type distribution was determined on the basis of these counts.

For each fiber type, SDH activity was determined by measuring the absorbance values at 660 nm (A$_{660}$) using the 20× objective of a DMRB microscope (Leica, Wetzlar, Germany). Calibrated images were recorded with a Sony XC-77CE camera (Towada, Japan) connected to an LG-3 frame grabber (Scion, Frederick, MD) in an Apple Power Macintosh computer. Recorded images were analyzed with ImageJ 1.44p (National Institutes of Health, Bethesda, MD). SDH activity was expressed as A$_{660}$ per micrometer section thickness and seconds of incubation (A$_{660}$ $\mu m^{-1} \cdot s^{-1}$). Morphometry was calibrated using a slide micrometer and the set scale option in ImageJ, taking the pixel-to-aspect ratio into account.

For each rat, SDH activity and fiber cross-sectional area (FCSA) per fiber type per compartment were determined by averaging the absorbance and FCSA of 20 fibers. Average SDH activity for the high- and low-oxidative compartments was determined by taking into account the fiber type distribution as well as the FCSA and SDH activity per fiber type.

Quantitative polymerase chain reaction. RNA was extracted from the proximal (high oxidative) and distal (low oxidative) ends of GM. RiboPure kit (Applied Biosystems, Foster City, CA) was used to isolate RNA from the muscle tissue according to the manufacturer's protocol. RNA concentration and purity (260/280-nm mean ratio, 2.03; range, 1.79–2.16) were measured using spectroscopy (NanoDrop Technologies, Wilmington, DE). Using the high-capacity RNA-to-cDNA kit (Applied Biosystems) containing random primers in a 20-μL total reaction volume, 500 ng of total

RNA per muscle compartment was reverse transcribed. Tubes were heated at 25°C for 5 min, followed by 42°C for 20 min. Finally, to stop the reaction, the tubes were heated at 85°C for 5 min and stored at −80°C until used in the quantitative polymerase chain reaction (PCR) (method described previously) (27). Expression levels of mRNA were assessed for SDH, PGC-1α, receptor-interacting protein 140 (RIP140), and BCL2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) (see Table 1 for primer sequences). Melting curve analysis showed specific amplification, and amplification efficiencies of the primers used in this study ranged from 91.3% to 102%. Expression levels were expressed relative to 18S using the $2^{-\Delta Ct}$ method. 18S was analyzed in triplicate and the other samples in duplicate.

Protein isolation and Western blotting. For protein extraction of the high- and low-oxidative compartments of GM, 50 sections (20 μm thick) were cut from the proximal and distal ends, respectively. The muscle tissue from both regions was homogenized in ice-cold radioimmunoprecipitation assay buffer (Sigma-Aldrich, St. Louis, MO) containing protease and phosphatase inhibitor cocktail tablets (Roche, Mannheim, Germany). After centrifuging for 10 min at 12,000 rpm at 4°C, the supernatant was stored at −80°C. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL). After denaturing the samples in SDS-PAGE sample buffer for 5 min at 90°C, 5 μg of protein was subjected to SDS-PAGE and transferred to a nitrocellulose membrane (GE Healthcare, Little Chalfont, UK). After this, the membrane was blocked with 5% ECL Advance Blocking Agent (Amersham, GE Healthcare) in tris-hydroxymethyl aminomethane with 0.01% Tween 20, incubated overnight at 4°C with primary antibody against PGC-1α corresponding to amino acid 777–797 (1:1000; Calbiochem, Darmstadt, Germany) and actin (1:1000, Cell Signaling Technology), and followed by a specific horseradish peroxidase-conjugated polyclonal goat antirabbit secondary antibody (1:2000, DakoCytomation). Enhanced chemiluminescence kit (ECL Advance, Amersham, GE Healthcare) was used to detect the antibody. PGC-1α data were normalized to actin.

Statistical analysis. SPSS version 18.0 (SPSS Inc., Chicago, IL) was used for data analyses. Differences between groups in maximal running velocity or duration were tested using a mixed design two-way repeated-measures ANOVA (time point of measurement × group). One-way ANOVA with Bonferroni *post hoc* correction was performed

TABLE 1. Sequences of the specific primers used in the quantitative PCR analyses.

Target mRNA	PCR Primer Sequence 5′→ 3′ Forward	Reverse
18S rRNA	CGAACGTCTGCCCTATCAACTT	ACCCGTGGTCACCATGGTA
SDH	CAGAGAAGGGATCTGTGGCT	TGTTGCCTCCGTTGATGTTC
PGC-1α	ATGAGAAGCGGGAGTCTGAA	GCGCTCTTCAATTGCTTTCT
RIP140	TCAGGGCGAGACAGACGATACT	CTTCTGCTCTTCGCCAAACGC
BNIP3	GTCACTTCCCAGGCCTGTCGC	TACCCAGGAGCCCTGCAGGTTCT

PCR, polymerase chain reaction; SDH, succinate dehydrogenase; PGC-1, peroxisome proliferator-activated receptor- coactivator 1; RIP140, receptor-interacting protein 140; BNIP3, BCL2/adenovirus E1B 19 kDa-interacting protein 3.

to test for differences in SDH activity and gene expression between the four groups. Paired samples t-tests were performed to test for differences in the high- and low-oxidative compartment in the control situation. When data were not normally distributed (mRNA expression of SDH, PGC-1α, RIP140, and BNIP3 in the low-oxidative compartment), Wilcoxon signed-rank tests were used to test for differences between the high- and low-oxidative compartment of controls and Kruskal–Wallis test was performed to test for differences between the four groups. When a significant main effect was observed, Mann–Whitney U-test was performed to test for differences between groups. The P value was adjusted using the Bonferroni correction method. The results are presented in mean ± SE. Significance was reported as $P < 0.05$.

RESULTS

In total, 30 rats were trained. Seven rats (one control, three of the PT group, one of the ET group, and two of the PET group) had to be excluded either because they did not successfully complete the training or because of problems with the tissue preparation. Therefore, the number of rats used for the analyses was for controls ($n = 9$), PT ($n = 7$), ET ($n = 9$), and PET ($n = 8$). Final body weight of the rats included in this study ($n = 33$) was on average 256.6 ± 2.8 g, and this was not different between the four groups.

Effect of 6-wk training on maximal running performance. Throughout the training period, maximal running velocity of PT and PET increased significantly by 13.5%, with no difference observed in the maximal running velocity between the PT and PET group (Fig. 1A). Maximal running duration of ET and PET increased significantly throughout the training period (144% from week 2 to week 4 and 32% from week 4 to week 6). The increase was similar for ET and PET (Fig. 1B).

Basel SDH activity and expression of regulatory factors in the high- and low-oxidative compartment of untrained rats. The high-oxidative compartment was composed of 23.7% ± 1.0% Type I, 22.0% ± 1.7% Type IIA, 34.8% ± 2.2% Type IIX, and 19.5% ± 1.2% Type IIB fibers, whereas the low-oxidative compartment contained only Type IIX and Type IIB fibers (17.8% ± 3.1% and 82.2% ± 3.1%, respectively). To obtain insight in the mechanisms underlying the differences in SDH activity between high- and low-oxidative regions, we assessed the average SDH activity per region as well as mRNA and protein levels of key factors involved in mitochondrial biosynthesis. Because FCSA and SDH activity differ substantially between fiber types (Fig. 2A and B), average SDH activity per compartment was calculated taking into account the fiber type distribution as well as the fiber type-related differences in FCSA and SDH activity. Basal average SDH activity in the high-oxidative compartment was 2.2-fold higher ($P < 0.001$) than that in the low-oxidative compartment (Fig. 2C). Similarly, SDH mRNA content was also 2.2-fold higher ($P = 0.008$) in the high- than that in the low-oxidative compartment (Fig. 2E). The substantially higher SDH mRNA content in the high-oxidative compartment may be an effect of higher expression levels of the regulatory coactivator PGC-1α and/or lower levels of the corepressor RIP140. Indeed, PGC-1α protein content was even 3.7-fold higher in the high- compared with that in the low-oxidative compartment (Fig. 2D, $P < 0.001$). For controls, PGC-1α mRNA content was 1.5-fold higher ($P = 0.011$) in the high- compared with the low-oxidative compartment of controls, whereas RIP140 mRNA levels were similar in the two compartments (Fig. 2E). These results suggest that basal expression levels of mitochondrial enzymes within untrained GM muscle fibers seem to be determined by PGC-1α levels rather than by RIP140.
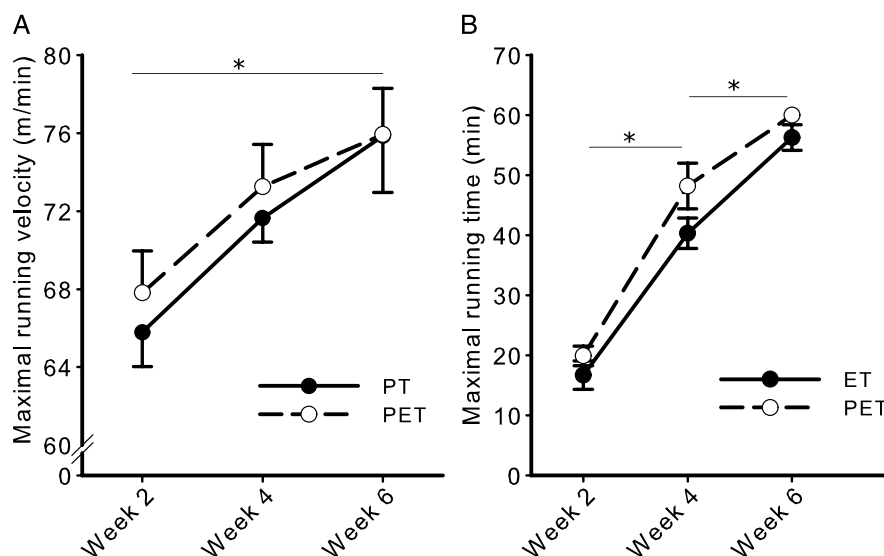
FIGURE 1—Effects of training on running performance. (A) Effects of peak power training (PT) alone and in combination with endurance training (PET) are shown on maximum running velocity. (B) Effects of PET and endurance training (ET) alone are shown on maximal running duration. Rats were trained for 6 wk, 5 d·wk^{-1}, performing PT, ET, or PET. All values are mean ± SE. *Significant difference ($P < 0.05$).
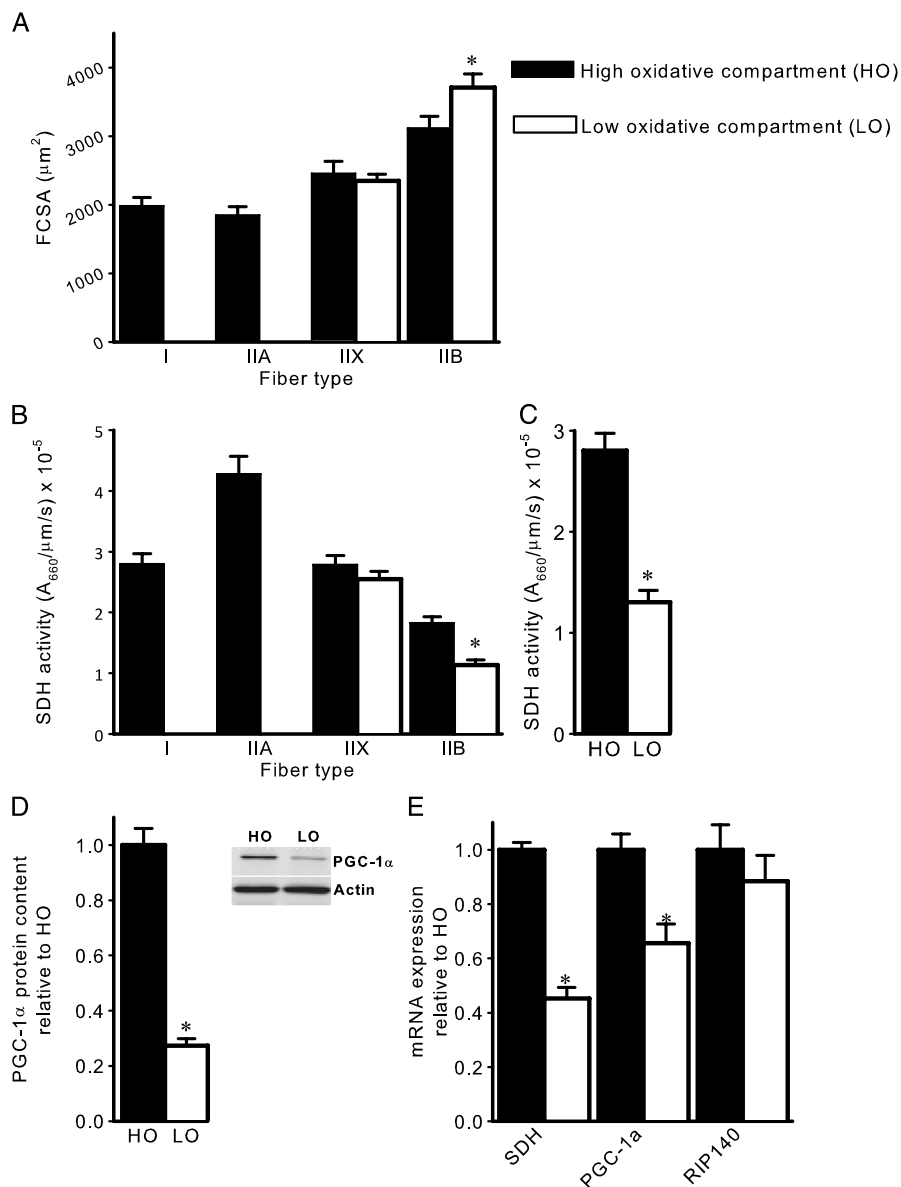
FIGURE 2—Basal SDH activity and expression levels of regulatory factors of mitochondrial biosynthesis in the high- and low-oxidative compartments of controls. Differences in FCSA, SDH activity, and basal expression levels of protein and/or mRNA within the high-oxidative (HO, *black bar*) and low-oxidative (LO, *open bar*) compartment of rat medial GM: (A) FCSA per fiber type; (B) SDH activity per fiber type; (C) average SDH activity (taking fiber type distribution and fiber type specific FCSA and SDH activity into account); (D) PGC-1α protein content; and (E) mRNA expression of SDH, PGC-1α, and RIP140. mRNA expression levels are expressed relative to 18S. Values are mean ± SE. Protein and mRNA data are expressed relative to the mean value of controls of the high-oxidative compartment. *Significant difference between the high- and low-oxidative compartment of controls ($P < 0.05$).

Effect of 6-wk training on the low-oxidative compartment of the GM. Within the low-oxidative compartment, SDH activity of Type IIX fibers was lower after PET compared with that in controls ($P = 0.029$), whereas SDH activity of Type IIB fibers was substantially lower after both PT and PET compared with that after ET and in the control group (Fig. 3A, $P < 0.001$). The average SDH activity of the low-oxidative compartment (taking into account fiber type distribution, FCSA, and SDH activity) after PT was 30% lower compared with that in controls (Fig. 3B, $P = 0.029$). After PET, average SDH activity was similar to that in the PT group. Because in the low-oxidative compartment, FCSAs of both Type IIX and IIB fibers were not significantly larger after peak power training compared with those of controls (data not shown), it is suggested that the reduction in SDH activity was not the result of muscle fiber hypertrophy but likely caused by a reduction in SDH activity per fiber. After training, SDH mRNA, PGC-1α mRNA, and protein and RIP140 mRNA levels were unchanged (Fig. 3C and D), suggesting that the observed decrease in SDH activity was likely not the result of a decrease in rate of SDH mRNA transcription or translation. The reduction in SDH activity seems to be due to an increase in mitochondrial degradation. Therefore, we analyzed BNIP3
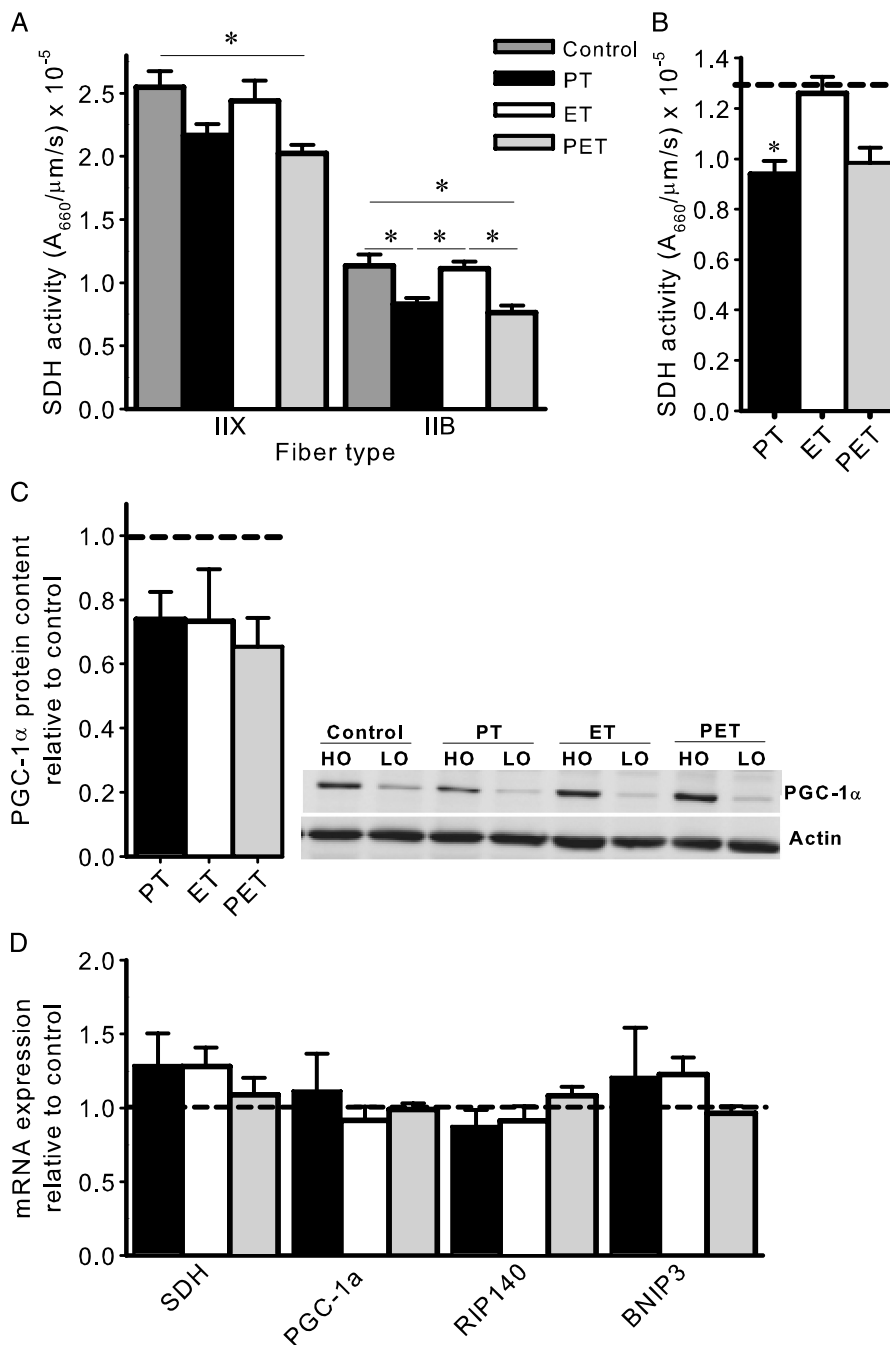
FIGURE 3—Effects of training on SDH activity and regulatory factors within the low-oxidative compartment of rat medial GM. Rats were trained for 6 wk, 5 d·wk^{-1}, performing either peak power training (PT), endurance training (ET), or concurrent peak power and endurance training (PET). Effects of PT, ET, or PET on (A) SDH activity per fiber type; (B) average SDH activity (taking into account fiber type distribution, fiber type-specific FCSA as well as SDH activity); (C) PGC-1α protein content; and (D) mRNA expression levels of SDH, PGC-1α, RIP140, and BNIP3. mRNA expression is relative to 18S. Protein and gene expression data are expressed relative to the mean value of the low-oxidative compartment of controls (*dashed line* represents the control value set at 1). Values are mean ± SE. Dashed line represents controls. HO, high-oxidative compartment; LO, low-oxidative compartment. *Significant difference between groups ($P < 0.05$). In panel B: *Significantly different from controls.

mRNA expression, which is involved in mitophagy (elimination of mitochondria) (11). However, BNIP3 mRNA levels in the low-oxidative compartment were not altered after training.

Effect of 6-wk training on the high-oxidative compartment of the GM. In the high-oxidative compartment, for any muscle fiber type, SDH activity was not

significantly altered by training (Fig. 4A). Only Type I fibers showed a trend toward an increased SDH activity in response to ET ($P = 0.090$). Average SDH activity in the high-oxidative compartment (taking into account fiber type distribution and fiber type-specific FCSA as well as SDH activity) was unchanged after training (Fig. 4B), although ET training resulted in 42% higher ($P = 0.028$) SDH mRNA
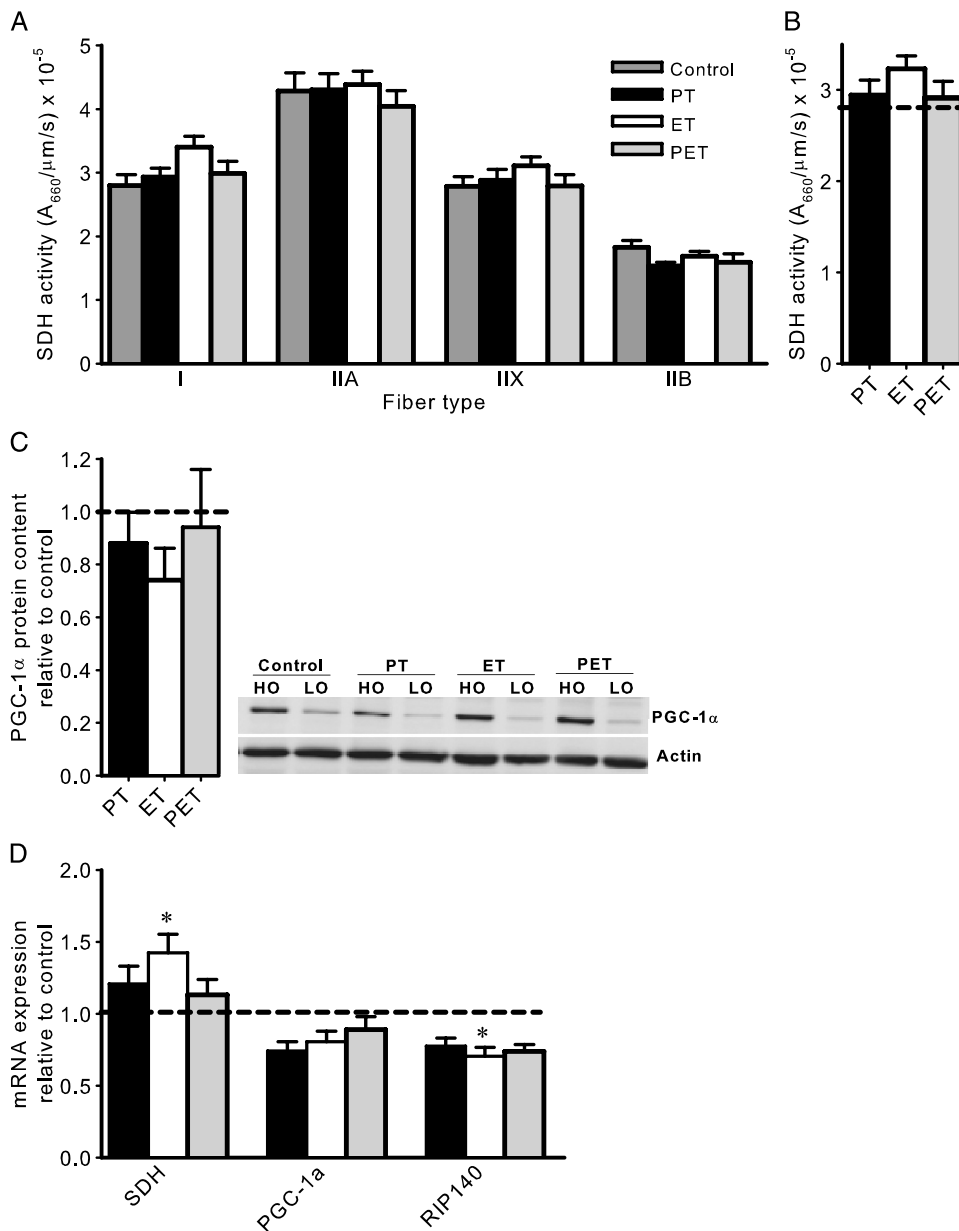
FIGURE 4—Effects of training on SDH activity and regulatory factors within high-oxidative compartment of rat medial GM. Effects of peak power training (PT), endurance training (ET), or concurrent peak power and endurance training (PET) on (A) SDH activity per fiber type; (B) average SDH activity (taking into account fiber type distribution, fiber type-specific FCSA as well as SDH activity); (C) protein content of PGC-1α; and (D) mRNA expression levels of SDH, PGC-1α, and RIP140. Rats were trained for 6 wk, 5 d·wk^{-1}, performing PT, ET, or PET. mRNA expression level is relative to 18S. Protein and gene expression data are expressed relative to the mean value of the high-oxidative compartment of controls (*dashed line* represents the control value set at 1). Values are mean ± SE. *Dashed line* represents controls. HO, high-oxidative compartment; LO, low-oxidative compartment. *Significantly different from controls ($P < 0.05$).

levels compared with those in controls (Fig. 4D). As in control muscle, basal SDH mRNA levels were largely determined by PGC-1α levels, we tested whether the increase in SDH mRNA after ET was related to an increase in this transcriptional coactivator. In response to training, PGC-1α mRNA as well as protein content remained unchanged after training (Fig. 4C and D). However, RIP140 mRNA content was 33% lower in the ET group compared with that in controls ($P = 0.009$), which was in the same order of magnitude as the changes seen in SDH mRNA (Fig. 4D).

This suggests that the endurance training-induced decrease in RIP140 mRNA may have contributed to the increase in SDH mRNA after ET.

DISCUSSION

This study showed differential effects of peak power training, endurance training, and a combination thereof on mitochondrial gene expression and enzyme activity. The

main findings were as follows: 1) peak power training caused a reduction in SDH activity in the fast fibers within the low-oxidative compartment of GM, and 2) peak power training with additional endurance training as performed by PET did not induce a different response in SDH activity within the low-oxidative compartment compared with PT alone. In the high-oxidative compartment, ET induced higher SDH mRNA and lower RIP140 mRNA expression levels, which however did not alter SDH activity. The increase in SDH mRNA could not be shown for the PET group, suggesting that additional peak power training interfered with the adaptive responses of endurance training at the transcriptional level.

Reduced SDH activity in the low-oxidative compartment after peak power training. In line with our hypothesis, in the low-oxidative compartment, peak power training caused a substantial reduction in SDH activity, and additional endurance training on top of peak power training as performed by PET did not interfere with the adaptive response of peak power training. The reduction may have been the result of different mechanisms. The training induced a decrease in rate of transcription and/or translation, an increase in mitochondrial degradation, or a combination of both. Given the observation that after peak power training, SDH mRNA levels were unaltered, and in untrained muscles, basal SDH mRNA levels were proportional to SDH activity, we conclude that the reduction in SDH activity observed after PT and PET was likely not the result of a reduction in the rate of transcription or translation. It seems that peak power training caused an increase in mitochondrial degradation.

Similar to our observation, a decrease in SDH activity within Type IIB fibers was also reported in hypoxia-inducible factor (HIF) 1α transfected rat extensor digitorum longus muscle fibers (20). Because the low-oxidative compartment of rat GM is mainly composed of fast glycolytic fibers, the oxygen demand during peak power training may have been greater than its supply and therefore may have induced hypoxic cores within Type IIB fibers. After fast brief trains of electrical stimulation, HIF-1α protein levels were shown to be increased within rat extensor digitorum longus muscle fibers (20). Because this type of muscle activation is fairly similar to that during our peak power training, it is conceivable that the peak power training caused an elevated mitochondrial degradation in Type IIB fibers by increasing HIF-1α. A molecular mechanism by which HIF-1α could increase mitochondrial degradation is by increasing the expression of the proapoptotic factor BNIP3. BNIP3 plays an important role in the fragmentation and elimination of mitochondria (mitophagy) and was shown to be mediated by HIF-1α *in vitro* (11,33). However, because in our study, BNIP3 mRNA levels were unchanged in response to peak power training, it remains to be determined whether the reduction in SDH activity occurred because of hypoxia in Type IIB fibers during peak power training and whether hypoxia is responsible for the reduction in SDH activity. More insight into how hypoxia may have reduced the SDH activity is needed.

Recently, it has been suggested that in humans, low-volume high-intensity training is an effective training strategy to increase mitochondrial biosynthesis. By only six training sessions of 8–12 × 30 s of all-out exercise, levels of regulatory proteins involved in the mitochondrial biosynthesis were increased (19). On the basis our findings and those of the study by Lunde et al. (20), one may conclude that high-intensity training inducing hypoxia may not only increase the mitochondrial biosynthesis but also concomitantly induce mitophagy. Although this latter was primarily shown in Type IIB fibers, it is suggested that mitophagy may play a role in the adaptation of oxidative capacity of muscle fibers in response to high-intensity training and should also be taken into consideration.

Endurance training increased SDH mRNA content in the high-oxidative compartment. For the high-oxidative compartment of GM, we hypothesized that concurrent peak power and endurance training attenuates the increase in SDH activity compared with endurance training only. Although SDH activity was unchanged after training, only ET induced an increase in SDH mRNA levels, suggesting that additional peak power training attenuated the increase in SDH mRNA. The 42% higher SDH mRNA levels after ET were accompanied with a similar decrease in RIP140 mRNA levels. RIP140 has previously been shown to negatively regulate SDH activity (26). Therefore, we suggest that the training-induced reduction in RIP140 mRNA may have contributed to the enhanced transcription of SDH in high-oxidative muscle fibers of GM. Because we do not have RIP140 protein data, we cannot be conclusive about the effect of RIP140 on SDH mRNA levels in response to ET. Another key cofactor involved in the transcriptional regulation of mitochondrial proteins is PGC-1α (14,17). In untrained muscle, PGC-1α protein was abundantly expressed within the high-oxidative compartment and was mainly responsible for the difference in SDH activity between the high- and low-oxidative compartments. However, training did not change PGC-1α mRNA or its protein content. Similarly, unchanged PGC-1α protein content within the high-oxidative part of the GM was also reported after 12 wk of treadmill running in mice (16). Despite this lack of change in PGC-1α protein content, transcription of genes involved in the mitochondrial biosynthesis (i.e., nuclear respiratory factor 1) was increased and accompanied by decreased acetylation of PGC-1α by sirtuin 1 (16). Others also showed elevated mRNA expression of nuclear encoded mitochondrial enzymes before the increase in PGC-1α protein content in response to exercise (24,32), which was likely due to posttranslational modifications of PGC-1α (13). Because in untrained muscle, the difference in PGC-1α protein content between the high- and low-oxidative compartment was almost twofold higher than the difference observed in SDH mRNA between the two compartments, we suggest that the high-oxidative compartment of GM contained a redundant amount of PGC-1α protein. Therefore, it seems conceivable that PGC-1α protein abundance in high-oxidative muscle

fibers is sufficient to stimulate mitochondrial biosynthesis without an increase in its protein content. The increase in SDH mRNA observed after ET could therefore be induced by a reduction in RIP140 and/or posttranslational modifications of PGC-1α.

Although ET enhanced the transcription of SDH mRNA, indicating that the endurance training did increase the transcription of mitochondrial enzymes, the enzyme activity was not increased. This suggests that the intensity of the training may not have been high enough or the duration of the whole training period was too short to increase SDH activity. During the endurance training, the intensity as well as duration of the sessions was progressively increased. Greater increase of the inclination of the treadmill slope or running velocity during the endurance training would have induced additional recruitment of faster muscle fibers rather than increase the oxidative capacity of the ones already recruited. Maybe the duration of the training sessions was too short to increase the AMP/ATP ratio enough to activate AMP-activated protein kinase. For future studies, we recommend that the duration of the training sessions as well as total training period should be increased.

In conclusion, comparison of effects of endurance training, peak power training, and concurrent training showed that in the low-oxidative Type IIB fibers of rat GM, peak power training induced a substantial decrease in SDH activity, which was not related to a reduction in the expression of SDH mRNA. In the high-oxidative compartment of GM, only endurance training induced an increase in SDH mRNA levels, which may be the result of a reduction in RIP140 mRNA. However, this higher SDH mRNA expression did increase SDH activity. Therefore, it is suggested that in compartmentalized rat GM, peak power on top of endurance training attenuates transcription of mRNA for mitochondrial proteins in the high-oxidative muscle fibers and reduces mitochondrial activity in low-oxidative muscle fibers.

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