HIGHLIGHTED TOPIC | Regulation of Protein Metabolism in Exercise and Recovery

Endurance exercise is protective for mice with mitochondrial myopathy

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Endurance exercise is protective for mice with mitochondrial myopathy. J Appl Physiol 106: 1712–1719, 2009. First published March 12, 2009; doi:10.1152/japplphysiol.91571.2008.—Defects in the mitochondrial ATP-generating system are one of the most commonly inherited neurological disorders, but they remain without treatment. We have recently shown that modulation of the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) level in skeletal muscle of a mitochondrial myopathy mouse model offers a therapeutic approach. Here we analyzed if endurance exercise, which is known to be associated with an increased PGC-1α level in muscle, offers the same beneficial effect. We subjected male and female mice that develop a severe mitochondrial myopathy due to a cytochrome-c oxidase deficiency at 3 mo of age to endurance exercise training and monitored phenotypical and metabolic changes. Sedentary myopathy and wild-type mice were used as controls. Exercise increased PGC-1α in muscle, resulting in increased mitochondrial biogenesis, and successfully stimulated residual respiratory capacity in muscle tissue. As a consequence, ATP levels were increased in exercised mice compared with sedentary myopathy animals, which resulted in a delayed onset of the myopathy and a prolonged lifespan of the exercised mice. As an added benefit, exercise induced mitochondrial enzymes. The overall protective effect of endurance exercise delayed the onset of the mitochondrial myopathy and increased life expectancy in the mouse model. Thus stimulating residual oxidative phosphorylation function in the affected muscle by inducing mitochondrial biogenesis through endurance exercise might offer a valuable therapeutic intervention for mitochondrial myopathy patients.

restricted ATP provision due to defective mitochondrial function is a common source for neuromuscular disorders. With an incidence of 1:5,000, these disorders are one of the most commonly inherited neurodegenerative diseases and predominantly affect high-energy-demand tissue like heart, skeletal muscle, liver, and brain (19). There are presently no effective treatments for these mitochondrial diseases (10). Endurance exercise has been shown to provide an improvement in symptoms and quality of life in patients with mitochondrial myopathies caused by mitochondrial DNA (mtDNA) mutations (12, 21–23). While these studies included significant numbers of patients with the same genetically defined defect, differences in genetic background and age in the studied individuals might affect the outcome of the study. To separate these factors from the effect of the endurance exercise, we performed a study on aerobic exercise training in a genetically homogeneous and age-matched mouse model of a mitochondrial myopathy caused by a cytochrome-c oxidase (COX) deficiency. In our mouse model, the COX10 gene, which encodes for an essential COX assembly factor (4), is ablated in skeletal muscle using the Cre-LoxP system. In this conditional knockout mouse, Cre recombination occurs over time in the muscle nuclei, resulting in a progressive and segmental COX deficiency and a feature commonly observed in human mitochondrial myopathies associated with mtDNA mutations. The mouse model develops a severe myopathy characterized by muscle weakness, increased number of falls on a treadmill at 2.5–3 mo, and premature death at 3–6 mo of age with a more severe phenotype observed in the female COX10 knockout (KO) mice (6). We took advantage of this model to assess if endurance exercise offers a potential therapeutic approach to the presently incurable mitochondrial myopathies.

Endurance exercise enhances oxidative phosphorylation (OXPHOS) function by increased mitochondrial biogenesis. This process is mediated by the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), the master regulator of mitochondrial biogenesis (14). PGC-1α is upregulated as a response to exercise to regulate metabolic adaptations (15, 17). We have shown recently that increased expression of PGC-1α in skeletal muscle of COX10 KO animals stimulates residual OXPHOS function in the affected muscle, resulting in delayed onset of the mitochondrial myopathy and increased life expectancy (25). Importantly, we found that increasing PGC-1α level by external stimuli shortly before the myopathy is sufficient to achieve a therapeutic effect (25). We now asked whether endurance exercise and the associated increase in PGC-1α level offer a similar protective effect in the mitochondrial myopathy mouse model.

MATERIALS AND METHODS

Animal husbandry. The mice (COX10 floxed had a mixed 129/SvJ and C57BL6 background; the Mlc-Cre had a C57BL6 background) were kept in a condition of 12:12-h light-dark cycle at room temperature. They were allowed a regular diet (Rodent Chow 5010, Harlan). The experiments described were approved by the University of Miami Miller School of Medicine Institutional Animal Care and Use Committee.

Treadmill experiment. Twenty female and male COX10 KO mice were exercised on a treadmill (Columbus Instruments, Columbus, OH) set at 9 m/min for 2 × 10 min intervals, with a 10-min break between the two intervals, 5 days/wk. Training started at 6 wk of age and continued until the myopathy affected adherence to the regimen. At this point, the speed was reduced to 7 m/min for the affected animals. Food and water were available before and after exercise and during the resting period. The control group included 20 sedentary COX10 KO and 20 wild-type mice of each sex. Subgroups (n = 3) were killed and analyzed at 3, 6, and 10 mo of age as described (6, 25). These time points were chosen because they correspond, in the
untrained animals, to early myopathy (3 mo) and severe myopathy (6 mo) (6). An additional time point (10 mo) was included for the exercised mice.

Isolation of mitochondria and measurement of respiratory chain complex activity. Mitochondrial preparations were obtained as described (4) and stored at −80°C until needed. Muscle homogenates were prepared by homogenizing a snap-frozen muscle piece (~50 mg) in 500 μl 10 mM HEPES, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 250 mM sucrose, and used immediately. Enzyme activities were determined spectrophotometrically (DU-640 spectrophotometer, Beckman Instruments, Fullerton, CA) as described (6). Protein concentrations were estimated by the method of Bradford using BSA as a standard.

Histochecmy. Muscle tissue was frozen in isopentane-cooled nitrogen. Cross sections (8 μm) were stained for COX, succinate dehydrogenase (SDH), and combined activities (20).

Western blot. Western blot analysis was performed as described previously (6). Antibodies against different subunits of the oxidative phosphorylation complexes (ND3, SDH, COX1, and ATP9) and VDAC were obtained from Molecular Probes, and an antibody against tubulin was obtained from Chemicon International (Temecula, CA).

Real-time quantitative PCR. Total RNA was extracted from snap-frozen muscle by TRIZOL (Life Technologies). cDNA was synthesized using the SuperScript First Strand Kit (Invitrogen). Quantitative real-time PCR reactions were performed on the cDNAs in the presence of fluorescent dye (SYBR Green, QIAGEN) in a 7300 Real Time PCR Sequence Detection system (Applied Biosystems). All results are expressed as means ± SE. The results were normalized for comparison by measuring β-actin mRNA levels in each sample. The primer sequences are described in supplementary materials available with the online version of this article.

gPCR to quantify mitochondrial DNA. DNA was isolated from tissues by chloroform-phenol extraction and incubation with RNase A before use. mtDNA was probed with primers inside ND1, and nuclear DNA was probed with primer inside β-actin. Quantitative real-time PCR reactions were performed on the cDNAs in the presence of fluorescent dye (SYBR Green, QIAGEN). All results are expressed as means ± SE.

ATP determination. Animals were euthanized and muscle tissue extracted and immediately frozen in liquid nitrogen. ATP was extracted from tissues using perchloric acid as described previously (24). ATP concentrations were determined using the luciferase-based “En-liten ATP Assay System” from Promega, and values were normalized to milligram of tissue.

Detection of oxidized proteins. Detection and quantification of carbonylated proteins was carried out using the Oxyblot Protein Carbonylation Kit (Chemicon International) according to the manufacturer recommendations.

In-gel superoxide dismutase activity. To quantify superoxide dismutase (SOD) activity in muscle mitochondria, we used an in-gel assay as described previously (10). To quantify the SOD activity staining gel, the images were digitized, and densitometric analyses were performed.

Catalase activity assay. The Amplex Red Catalase Assay Kit (Molecular Probes) was used to measure catalase activity in muscle mitochondria.

Data analysis. Data obtained are represented as mean (+ SD) from 3 mice per group, and statistical significance was determined using unpaired Student’s t-test. The P value of <0.05 was considered significant.

RESULTS

Endurance exercise delays the onset of the a mitochondrial myopathy. We submitted mice with a conditional muscle deletion of a gene essential for COX assembly, COX10, to daily endurance training on a treadmill (9 m/min, 2 × 10 min with a 10-min break). Due to the observed sex difference in sedentary COX10 KO mice (6), we studied the effect of endurance training in both female and male animals.

Endurance exercise increased the lifespan of COX10 KO mice: all of the male and female exercised COX10 KOs were alive up to 5 and 4 mo, respectively, while the first death for the sedentary myopathy mice was observed ~6 wk before (Fig. IA). In addition to a delay in death, the survival curve in the untrained COX10 KO mice for both sexes was steeper. While 50% of the sedentary male COX10 KO mice where dead at 4.5 mo, 50% of the exercised myopathy mice reached 8 mo of age, representing a doubling in lifespan. An even more beneficial effect was seen in exercised female COX10 KO mice: compared with the sedentary myopathy mouse, 50% of the untrained COX10 KO mice died before reaching 3.5 mo of age, while 50% of the exercised COX10 KO mice where still alive at 9 mo of age, which equals about a threefold increase in life expectancy.

Onset of the disease was monitored by performance on a submaximal, short treadmill test (7 m/m, 5 min). Here we also saw a improvement in performance for the exercised vs. sedentary COX10 KO mice at 3 mo of age, only 6 wk after starting the endurance exercise, both male and female exercise COX10 KO behaved comparably to the wild-type control animals, whereas the sedentary COX10 KO showed increased number of falls for both sexes, indicating onset of the myopathy (Fig. IB). At 6 mo of age, falls for the sedentary COX10 KO increased even further, indicating late stages of the myopathy. In contrast, the exercised COX10 KO showed only a small increase in number of falls, indicating a prolonged period without a marked worsening of the myopathy symptoms. A marked increase in falls could only be observed at 10 mo for the sedentary COX10 KO.

Exercise mice have increased OXPHOS capacity per muscle volume. To evaluate the effect of the endurance exercise on the underlying COX deficiency in the COX10 KO animals, we analyzed COX activity in muscle homogenates and muscle mitochondria. We observed an increase of COX activity in muscle homogenates of the exercised COX10 KO mice compered with the sedentary myopathy mice at 3 and 6 mo of age. At 3 mo of age, sedentary COX10 KOs have ~40% of the wild-type COX activity in muscle homogenates. Age-matched exercised COX10 KO showed a marked improvement: after only 6 wk of training, the COX activity in muscle homogenates increased to ~60% of the wild-type control (Fig. 2A). A more remarkable improvement was observed at the 6-mo time point. Here, sedentary COX10 KO mice showed only ~10–15% of the control COX activity, representing a drop to nearly one-fourth of the 3-mo value. In contrast, exercised COX10 KO mice maintained ~50% of the control COX activity in muscle homogenates, which represents only a minor decrease of the value obtained at 3 mo (60% of control). The endurance exercise did not affect the COX defect at the mitochondrial levels as indicated by equally decreased COX activity in mitochondria in exercised and sedentary COX10 KO mice (Fig. 2B). The partially rescued and maintained COX activity in the exercised COX10 KO correlates with the less severe course of the myopathy as indicated by treadmill performance and increased survival compared with the faster onset and more severe course of the disease in the sedentary COX10 KO. Unchanged COX10 deletion in muscle showed that the molec-
ular changes induced by the endurance exercise did not interfere with the COX10 ablation (data not shown).

The partial rescue of the COX defect in muscle homogenates of exercised COX10 KO mice was also evident in Western blots. While COX subunit I (COXI) was below detection limits for 6-mo-old sedentary COX10 KO mice, the signal for COXI in the exercised mice reached ~30–40% of the wild-type level, whereas COXI of the untrained COX10 KOs remained below detection limit (Fig. 2C). In Western blots of muscle mitochondria of both exercised and sedentary COX10 KO, COXI was below the detection limit for both groups (Fig. 2C), and only faint bands were visible after overexposure (data not shown). This finding confirmed the COXI deficiency at the mitochondrial level was not altered by the endurance exercise, in agreement with the COX activity measurements (Fig. 2B).

Histological analyses of skeletal muscle of exercised and sedentary COX10 KO confirmed the beneficial effect of endurance exercise. The biceps femoris of 3- and 6-mo-old exercised COX10 KO mice was also evident in Western blots. While COX subunit I (COXI) was below detection limits for 6-mo-old sedentary COX10 KO mice, the signal for COXI in the exercised mice reached ~30–40% of the wild-type level, whereas COXI of the untrained COX10 KOs remained below detection limit (Fig. 2C). In Western blots of muscle mitochondria of both exercised and sedentary COX10 KO, COXI was below the detection limit for both groups (Fig. 2C), and only faint bands were visible after overexposure (data not shown). This finding confirmed the COXI deficiency at the mitochondrial level was not altered by the endurance exercise, in agreement with the COX activity measurements (Fig. 2B).

Histological analyses of skeletal muscle of exercised and sedentary COX10 KO confirmed the beneficial effect of endurance exercise. The biceps femoris of 3- and 6-mo-old exercised COX10 KO showed less COX-deficient fibers than age-matched sedentary COX10 KO mice (Fig. 3), whereas the muscle from 3-mo-old untrained COX10 KO showed ~60–70% COX-negative fibers. The muscle from age-matched exercised COX10 KO animals did not only show less COX-negative fibers (~20–30%), but the COX-positive fibers had a more intense staining than the COX-positive fibers in the untrained mice. The difference was even more evident in the 6-mo-old animals, in agreement with the treadmill performance and COX activity. In the sedentary COX10 KO mice, less than 10% of the fibers were COX positive, and most of those had a weak COX staining. In contrast, the muscle from 6-mo-old exercised COX10 KO mice showed ~30–40% COX-negative fibers with the COX-positive fibers more intensely stained than in the sedentary sample. At 10 mo of age, the muscle from the exercised COX10 KO seemed not to be as affected by the COX deficiency as the 3-mo-old untrained COX10 KO. They also had ~60–70% COX-negative fibers as judged from succinate dehydrogenase (SDH)/COX staining, with the COX staining of the remaining fibers being more intense than in the 3-mo-old sedentary COX10 KO. Interestingly, we observed very intense staining in single fibers that were surrounded by COX-negative fibers.

Endurance exercise induces mitochondrial proliferation and a shift to oxidative muscle fiber type. The partially rescued COX activity in muscle homogenates along with the persistent COX defect at the mitochondrial level in exercised COX10 KO animals indicated increased mitochondrial biogenesis. In histological analyses of the biceps femoris, we saw a very intense SDH activity stain for the exercised COX10 KO animals compared with the sedentary COX10 KO and wild-type ani-
mals, suggesting increased mitochondrial mass (Fig. 3). Also comparing steady-state levels of OXPHOS proteins in muscle homogenates from exercised and sedentary COX10 KO showed an upregulation of other OXPHOS complexes as judged from increased signal intensity for the subunits ND39, SDH, and ATPβ (Fig. 2C).

To analyze the degree of mitochondrial proliferation, we examined the activity of citrate synthase (CS), a marker for mitochondrial proliferation. (Fig. 3). Exercised COX10 KO mice have less COX-deficient fibers than age-matched sedentary COX10 KO mice. Histology of the biceps femoris muscle from mice at different ages (20× magnification) showing the development of the COX deficiency of sedentary and exercised COX10 KO mice. Shown are SDH, COX, and combined COX/SDH staining. COX and COX/SDH staining highlight the degree of COX deficiency. Scale bar, 100 μm.
mitochondrial mass. The activity of CS was increased ~2-fold in muscle homogenates of exercised COX10 KO mice at 3, 6, and 10 mo compared with wild-type animals, indicating increased mitochondrial proliferation (Fig. 4A). Consistent with the increased CS activity in muscle homogenates, we saw a ~2-fold increased expression of CS as revealed by RT-PCR (Fig. 4C). The magnitude of the increase is similar to the ones previously reported for exercised rodents (2, 8, 13).

In agreement with these findings, we observed an ~2-fold increase in mtDNA levels at all examined ages in the exercised COX10 KO mice (Fig. 4B). The sedentary COX10 KO animals also showed a small increase in mitochondrial mass (compared with the sedentary wild type) as indicated by small increases in both CS activity and mtDNA levels (Fig. 4, A and B). This is not surprising, as mitochondrial proliferation can occur as a response to OXPHOS defects (6, 7).

**Fig. 4.** Endurance exercise increases mitochondrial biogenesis and mediates a change to oxidative fiber types. A: citrate synthase (CS) activity as a mitochondrial marker protein in muscle homogenates from exercised, sedentary COX10 KO, and sedentary wild-type mice at different ages. *P < 0.05. B: relative quantification of mitochondrial DNA (ND1) vs. nuclear DNA (β-actin) by qPCR of DNA isolated from skeletal muscle exercised and sedentary COX10 KO at different ages (n = 3). Values are normalized to the age-matched wild-type control. *P < 0.05, **P < 0.01, ***P < 0.001. C: relative expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and -1β (PGC-1β), the 4 myosin heavy chain (MHC) isoforms (IIx, IIa, IIb, I), and the mitochondrial protein CS in skeletal muscle of 3-mo exercised and sedentary COX10 KO. Values are normalized to the wild-type control. *P < 0.05, **P < 0.01, ***P < 0.001. D: quantification of ATP in the biceps femoris muscle from 3-mo-old exercised, sedentary COX10 KO, and sedentary wild-type mice (n = 3 for each group). *P < 0.01. E: endurance exercise test. The average total distance traveled for 3-mo-old male and female exercised and sedentary COX10 KO mice compared with sedentary wild-type mice (n = 6 for each group). *P < 0.01. In A–D statistical comparisons were made between either exercised COX10 KO or sedentary COX10 KO vs. the wild type.
Increase in mitochondrial proteins is associated with increased expression of PGC-1α, which has been shown to be the master regulator of mitochondrial biogenesis (14). We analyzed changes in PGC-1α expression by RT-PCR. Exercised COX10 KO mice had an ~2.5-fold increased expression of PGC-1α in muscle, indicating that this change in PGC-1α expression mediated the mitochondrial biogenesis. No differences were observed between the sedentary COX10 KO and wild-type control. Surprisingly, we found a slight downregulation of PGC-1β in the exercised animals (Fig. 4C).

To further analyze the change induced by the endurance exercise, we examined changes in muscle fiber-type composition by analyzing expression of the different myosin heavy chain (MHC) isoforms in the biceps femoris of exercised and sedentary animals. In agreement with the observed increased oxidative capacity in the exercised mice, we observed a change to a more oxidative fiber type: MHC IIB and MHC IIX, representing glycolytic type II fibers, were significantly decreased to ~75% of the control value. Oxidative type II fibers were increased as indicated by the ~2-fold increased expression of MHC IIA. An unchanged expression of MHC I indicated that fiber type I formation remained unaffected by the endurance exercise (Fig. 4C).

**Exercised mice have increased ATP levels resulting in increased endurance.** In agreement with the increased mitochondrial mass and increased OXPHOS capacity, endurance exercise increased ATP levels in COX10 KO mice. At 6 mo of age, exercised COX10 KO mice maintained ~60% of the wild-type ATP level in muscle, whereas age-matched sedentary COX10 KO animals had only ~30% (Fig. 4D). In line with the severe course of the myopathy in the untrained mice, sedentary COX10 KO mice had only 10% of the control ATP level at 6 mo of age. In exercised COX10 KO animals of the same age as KO animals, ATP levels were 30%, reflecting the nearly unchanged OXPHOS capacity in the 3- to 6-mo time frame. At 10 mo of age, the exercised COX10 KO mice showed a marked decrease to ~30% of wild-type ATP levels in agreement with the worsened myopathy symptoms at this age.

The therapeutic effect of endurance exercise resulting in the partial rescue of the ATP levels was also evident in an endurance test, where 3-mo-old animals were run at moderate speed until exhaustion. Sedentary COX10 KO mice showed a decreased exercise capacity and could run only ~one-fourth of the distance of the untrained wild-type control. Both male and female exercised COX10 KO showed increased endurance capacity and managed to run approximately three-fourths of the distance of the wild-type control mice (Fig. 4E).

**Endurance exercise increases antioxidant enzymes.** The increased oxygen uptake during exercise has the potential to increase oxidative stress. While acute exercise increases levels of reactive oxygen species, chronic exercise has been found to increase the cellular antioxidant defense program, resulting in a protective adaptation to prevent oxidative stress in healthy subjects (reviewed in Refs. 9, 18). To analyze if endurance exercise increases or decreases oxidative damage in the COX10 KO mouse muscle, we performed oxybloth analysis in mitochondria isolated from skeletal muscle of 3- and 6-mo-old exercised and sedentary COX10 KO mice and wild-type controls. At the 3-mo time point, there was no significant difference from the wild-type control (not shown). However, at 6 mo of age, terminal untrained COX10 KO mice showed an increase in oxidative damage to proteins as indicated by increased protein carbonylation detected by the oxybloth (Fig. 5, A and B). The age-matched trained COX10 KOs did not show such oxidative damage after 20 wk of endurance exercise (Fig. 5, A and B).

We next analyzed how this decrease in oxidative damage in the exercised mice might be mediated. Catalase activity in muscle mitochondria from exercised and sedentary COX10 KO were not significantly altered at 3 mo of age with the wild-type control. However, at 6 mo of age, the catalase activity in mitochondria from exercised COX10 KO was increased ~2-fold compared with the wild-type control, whereas the catalase activity from sedentary COX10 KO remained unchanged (Fig. 5C). A similar trend was observed for steady-state levels of mitochondrial superoxide dismutase (SOD2): while no changes for SOD steady-state levels in muscle mitochondria were observed for 3-mo-old exercised and sedentary COX10 KO animals, at 6 mo the steady-state level of SOD2 of the exercised COX10 KO animals was increased ~2.5-fold according to Western blot analysis (Fig. 5, D and E). This increase correlated with an increased in-gel SOD2 activity for the 6-mo-old trained COX10 KO, while age-matched sedentary myopathic mice showed no changes as indicated by unchanged SOD2 levels in Western blots and unchanged SOD2 activity (Fig. 5F). These findings indicate that endurance exercise upregulated SODs and catalase as part of an antioxidant response, which results in less oxidative damage in the exercised vs. sedentary animals.

**DISCUSSION**

Present therapeutic interventions for mitochondrial myopathy patients are limited and rather address symptoms and not the deficiency itself. We have shown recently that increased PGC-1α levels in skeletal muscle of a mitochondrial myopathy mouse model stimulated residual OXPHOS capacity by increasing mitochondrial mass, resulting in delayed and less severe course of the disease and increased lifespan (25). These results showed that inducing mitochondrial biogenesis might be a therapeutic approach for mitochondrial myopathies. PGC-1α is a master regulator of mitochondrial biogenesis (14) and coordinates activation of metabolic genes in human and rodent muscle in response to endurance exercise (15, 17). Hence we hypothesized that endurance exercise and its associated increase in PGC-1α level might offer a protective effect in mitochondrial myopathies by enhancing oxidative capacity and thus preventing a bioenergetic deficit that causes the progressive course of the disease and premature death.

To test this hypothesis, we subjected male and female mitochondrial myopathy mice to endurance exercise. The exercise started at 6 wk of age shortly before the initial symptoms of the myopathy start (~2.5 mo). The training regimen had a therapeutic benefit for the myopathic mice and resulted in an increased lifespan and a delayed onset of the disease in both sexes. Importantly, after only 6 wk of endurance exercise at 3 mo of age, the exercised COX10 KO mice showed no signs of the myopathy, in contrast to their age-matched sedentary COX10 KO littersmates. This beneficial effect can be attributed to an increased OXPHOS capacity per muscle volume in the exercised animals, which was shown by increased activities and steady-state levels of OXPHOS and mitochondrial enzymes in muscle homogenates. The improved OXPHOS ca-
pacity in the exercised COX10 KO animals improved ATP levels in resting skeletal muscle. The improved OXPHOS capacity in the trained animals most likely also allowed efficient ATP delivery during exercise, resulting in increased exercise performance. The stimulation of the OXPHOS activity can most likely be attributed to the increased mitochondrial biogenesis, which was probably mediated by an ~2.5-fold increase in PGC-1α expression and potential posttranslational activation as a response to the endurance exercise. Importantly, the course of the disease was less severe in the exercised animals: as judged by COX activity and ATP levels in muscle homogenates, the exercised COX10 KO maintained ~50–60% of the wild-type OXPHOS capacity between 3 and 6 mo, whereas sedentary animals showed a drop from 10% to 40%.

Fig. 5. Endurance exercise protects from oxidative damage in degenerating muscle. A: blot of protein oxidation in muscle mitochondria from 6-mo-old exercised and sedentary COX10 KO mice and wild-type controls using Oxyblot. B: quantification of the blot shown in A. C indicates the nonderivated control. * P < 0.01. C: catalase activity in muscle mitochondria from 3- and 6-mo exercised, sedentary COX10 KO, and sedentary wild-type mice (n = 3 for each group). * P < 0.01. D: Western blot of superoxide dismutase (SOD2) in muscle mitochondria of 3- and 6-mo exercised, sedentary COX10 KO, and sedentary wild-type mice (n = 3 for each group). E: quantification of the ratios of SOD2/VDAC1 obtained by the blots on D. * P < 0.01. F: SOD2 activity in muscle mitochondria from 6-mo exercised, sedentary COX10 KO, and sedentary wild-type mice measured by in-gel activity assay (n = 3 for each group). * P < 0.01.
Interestingly, we observed that endurance exercise caused a significant decrease in PGC-1β expression. Decreased levels of PGC-1β after endurance exercise have been reported before (15, 16). While effects of PGC-1β are not as well characterized as those of PGC-1α, it is also implicated in OXPHOS gene expression (3). However, the difference in PGC-1α and PGC-1β gene expression in response to endurance exercise implies that regulation of PGC-1α is distinct from that of PGC-1β.

In addition to the beneficial effect of long-term endurance exercise on the OXPHOS capacity, the exercised animals also showed less oxidative stress, which in untrained animals likely results from severe muscle degeneration (11). While at 3 mo of age no significant difference in oxidative damage and antioxidant response between exercised and sedentary animals was observed, we saw a decreased protein oxidation and increased SOD and catalase activity after 20 wk of endurance exercise in the trained COX10 KO mice compared with terminal age-matched sedentary myopathy mice. It is possible that exercise-induced protection from oxidative stress added to the beneficial effect of increased OXPHOS capacity and might contribute to the positive effect of the endurance exercise. These results are in agreement with previous findings that exercise improves adaptation to the oxidative challenge accompanied with increased oxygen intake and increased mitochondrial mass upregulation of the antioxidant response and an increased rate of protein turnover to minimize oxidative damage in healthy subjects (5, 18). However, myopathy patients with mtDNA defects showed increased oxidative stress during a 14-wk course of training (1), suggesting that endurance exercise regimens, period of training, and the specificity of the defect influence the protective mechanisms exercised.

The few studies where patients with mitochondrial myopathies associated with mtDNA mutations were subjected to aerobic training showed a beneficial effect on the OXPHOS function (21–23). In these studies patients were already myopathic at the beginning of the study, which could have limited the improvement. The effect of endurance exercise has not been systematically evaluated in patients with nuclear DNA defects, but our results suggest that they may benefit as well.

In conclusion, we showed that conditioning the muscle of mitochondrial myopathy (COX deficient) mice enhances ATP production via increased mitochondrial mass, thus preventing a bioenergetic crisis. The overall protective effect of endurance exercise delayed the onset of the disease and increased life expectancy of mitochondrial myopathy mice. We believe that stimulating residual OXPHOS function in the affected muscle by inducing mitochondrial biogenesis through endurance exercise might offer a valuable therapeutic intervention for mitochondrial myopathy patients.

GRANTS

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