The role of PGC-1α on mitochondrial function and apoptotic susceptibility in muscle

Peter J. Adhiketty,2 Giulia Ugoccioli,1 Lotte Leick,4 Juan Hidalgo,5 Henrickette Pilegaard,4 and David A. Hood1,2,3

1School of Kinesiology and Health Science, 2Department of Biology, and 3The Muscle Health Research Centre, York University, Toronto, Ontario, Canada; 4Copenhagen Muscle Research Centre and Centre of Inflammation and Metabolism, Department of Biology, University of Copenhagen, Denmark, and 5Institute of Neurosciences and Department of Cellular Biology, Physiology and Immunology, Autonomous University of Barcelona, Barcelona, Spain

Submitted 12 February 2009; accepted in final form 5 May 2009

Adhiketty PJ, Ugoccioli G, Leick L, Hidalgo J, Pilegaard H, Hood DA. The role of PGC-1α on mitochondrial function and apoptotic susceptibility in muscle. Am J Physiol Cell Physiol 297: C217–C225, 2009. First published May 13, 2009; doi:10.1152/ajpcell.00070.2009.—Mitochondria are critical for cellular bioenergetics, and they mediate apoptosis within cells. We used whole body peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) knockout (KO) mice to investigate its role on organelle function, apoptotic signaling, and cytochrome-c oxidase activity, an indicator of mitochondrial content, in muscle and other tissues (brain, liver, and pancreas). Lack of PGC-1α reduced mitochondrial content in all muscles (17–44% \( P < 0.05 \)) but had no effect in brain, liver, and pancreas. However, the tissue expression of proteins involved in mitochondrial DNA maintenance [transcription factor A (Tfam)], import (Tim23), and remodeling [mitofusin 2 (Mfn2) and dynamin-related protein 1 (Drp1)] did not parallel the decrease in mitochondrial content in PGC-1α KO animals. These proteins remained unchanged or were upregulated (\( P < 0.05 \)) in the highly oxidative heart, indicating a change in mitochondrial composition. A change in muscle organelle composition was also evident from the alterations in subsarcolemmal and intermyofibrillar mitochondrial respiration, which was impaired in the absence of PGC-1α. However, endurance-trained KO animals did not exhibit reduced mitochondrial respiration. Mitochondrial reactive oxygen species (ROS) production was not affected by the lack of PGC-1α, but subsarcolemmal mitochondria from PGC-1α KO animals released a greater amount of cytochrome c than in WT animals following exogenous ROS treatment. Our results indicate that the lack of PGC-1α results in 1) a muscle type-specific suppression of mitochondrial content that depends on basal oxidative capacity, 2) an alteration in mitochondrial composition, 3) impaired mitochondrial respiratory function that can be improved by training, and 4) a greater basal protein release from subsarcolemmal mitochondria, indicating an enhanced mitochondrial apoptotic susceptibility.

endurance training; exercise; mitochondrial biogenesis; reactive oxygen species

The importance of PGC-1α in muscle mitochondrial biogenesis has been clearly demonstrated by overexpressing PGC-1α in C2C12 muscle cells, and with muscle-specific PGC-1α overexpression in transgenic animals. These in vivo and in vitro studies showed that enhanced PGC-1α levels significantly induced nuclear- (cytochrome c, COXIV) and mitochondrial (COXII) gene expression, elevated mitochondrial DNA (mtDNA) copy number, and increased mitochondrial volume (39). To complement overexpression studies, muscle and heart tissue from PGC-1α knockout (KO) animals exhibit marked deficiencies in mitochondrial gene expression and reductions in overall muscle function (15, 21).

While many studies have convincingly shown the potent mitochondrial biogenesis-inducing effect of PGC-1α overexpression, few studies have investigated the implications of reduced PGC-1α expression on mitochondrial maintenance in muscle, and on varying nonmuscle tissues. Thus, one purpose of the present study was to investigate the tissue-specific role of PGC-1α ablation in organelle biogenesis using PGC-1α KO mice. Given the role of PGC-1α in mitochondrial homeostasis, we hypothesized that tissues with the highest metabolic demand would be the most affected by the absence of PGC-1α.

Although mitochondria primarily regulate cellular metabolism, they are also key participants in programmed cellular death (apoptosis) since they 1) contain several proapoptotic proteins (i.e., cytochrome c) that, on release, can lead to cell death; and 2) produce reactive oxygen species (ROS) that can initiate apoptotic signaling (1, 27). Upon sufficient apoptotic stimuli, proapoptotic factors are released through a specialized channel termed the mitochondrial permeability transition pore (mtPTP), the opening of which is regulated by Bcl-2 family member proteins (1, 11, 32, 38).

Our previous data indicate that PGC-1α expression is significantly reduced following chronic muscle inactivity that coincides with not only a reduction in overall mitochondrial content and function, but also with enhanced mitochondrial apoptotic susceptibility in muscle (3). Other studies have also correlated reduced PGC-1α expression levels with decreased mitochondrial function and increased vulnerability to apoptosis (13, 26, 30). Furthermore, reports have indicated that ROS-detoxifying enzymes are dependent on levels of PGC-1α (17, 34, 35, 37). Thus, given that reduced PGC-1α expression is associated with apoptosis and ROS dysregulation, the second purpose of this study was to investigate whether the absence of PGC-1α alters mitochondrially mediated apoptotic signaling events/factors in various tissues, particularly in subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondrial subfrac-
tions of skeletal muscle. We hypothesized that apoptotic susceptibility and proapoptotic signaling markers would be enhanced in tissues from PGC-1α KO animals and that the magnitude of these changes correlate with the oxidative capacity of the tissue.

METHODS

Mice. The generation and characterization of the PGC-1α KO black C57 mice have been described in detail previously (24). PGC-1α KO and wild-type (WT) mice were produced by crossing heterozygote mice. Tail pieces were cut and DNA was extracted using the phenol-chloroform-isooamyl alcohol method. Genotype was determined by the presence of either a KO- or WT-specific DNA fragment using traditional PCR analyses. The survival of PGC-1α KO animals was approximately half of the expected Mendelian ratio. Mice were housed in a 12:12-h light-to-dark cycle, fed normal rodent chow (Altromin no. 1324, Chr. Pedersen, Ringsted, Denmark), and were killed at 4–5 mo of age. Experiments were approved by the Danish Animal Experimental Inspectorate and complied with the European convention for the protection of vertebrate animals used for experiments and other scientific purposes (Council of Europe, no. 123, Strasbourg, France, 1985).

Endurance training protocol. A subgroup of WT and PGC-1α KO mice (n = 8/group) were treadmill trained as described previously by Leick et al. (20). Briefly, mice were endurance trained over a 5-wk period consisting of 5 × 60-min sessions per week at 14 m/min on a 10% incline (Exer 4 treadmill, Columbus Instruments, Columbus, OH). The training group of WT and KO mice were also given free access to running wheels (Minimitter Activitycage), and distances run between genotypes were monitored (Sigma Sport, Neustadt, Germany). Mice were killed ~36 h after the last bout of exercise, and mitochondria were isolated from mixed muscle from both WT and KO animals.

Immunoblotting. Whole muscle protein extracts were separated by 10–15% SDS-PAGE and were subsequently transferred to nitrocellulose membranes using a semi-dry and/or wet electrophoresis apparatus. Nitrocellulose membranes were blocked (1 h) with 5% skim milk in 1× TBST solution [Tris-buffered saline with Tween 20: 25 mM Tris–HCl (pH 7.5), 1 mM NaCl, and 0.1% Tween 20]. Membranes were then incubated with primary antibodies directed against apoptosis-inducing factor (AIF; 1:1,000), cytochrome c (1:750), Tim23 (1:500), mitofusin 2 (Mfn2; 1:1,000), dynamin-related protein (Drp1; 1:500), mitochondrial transcription factor A (Tfam; 1:500), Bax (1:500), and Bel-2 (1:500) overnight at 4°C. Membranes were subsequently washed (3 × 5 min) using TBST to remove excess primary antibody and incubated with the corresponding secondary antibody at room temperature (45 min). Antibodies were obtained from Santa Cruz (Mfn2, sc-59268; Bax, sc-7475; Bel-2, sc-246; IgG, sc-2751; Drp1, sc-97032), Cell Applications (Sema3F, CA1790), Abcam (Bax, ab-22671; Bel-2, ab-29757; Bcl-2, ab-19185), BD Bioscience (TREK, 619279), and Cell Applications (Mfn2, CA1790). The cytochrome c antibody was produced in our laboratory, while the Tfam antibody was a generous donation of Dr. H. Inagaki (Nagoya, Japan; 12). Membranes were washed (3 × 5 min) in TBST to eliminate unbound secondary antibody, and detection was revealed using the enhanced chemiluminescence method. Films were scanned and analyzed using SigmaScan Pro software (version 5, Jandel Scientific, San Rafael, CA).

Mitochondrial isolation. Mixed muscle excised from the lower and upper limbs (representing approximately a 50:50 distribution of white and red fiber distribution) of WT or PGC-1α null mice were homogenized and subjected to differential centrifugation to isolate IMF and SS mitochondrial subfractions as previously described in detail (10, 25, 36). The mitochondria were resuspended in buffer 1 (100 mM KCl, 10 mM MOPS, and 0.2% BSA–fatty acid free; pH 7.4) for mitochondrial respiration, ROS production, and the mitochondrial release assay, or in buffer 2 (215 mM mannitol, 71 mM sucrose, 3 mM HEPES, and 5 mM succinate) for the mtPTP assay.

Mitochondrial respiration. Isolated SS and IMF mitochondrial respiration rates were measured in VO2 buffer (250 mM sucrose, 50 mM KCl, 25 mM Tris–HCl, and 10 mM K2HPO4), with continuous stirring at 30°C. Oxygen consumption (n atoms O2 min−1 mg−1) was assessed in the presence of 11 mM glutamate (state 4 respiration), or glutamate plus 0.4 mM ADP (state 3 respiration) using a Clark oxygen electrode (MT200A, Strathtekelvin Instruments, North Lanarkshire, Scotland), as done previously (2, 10, 25).

Cytochrome c oxidase enzyme activity. Whole muscle powdered tissues and/or isolated mitochondrial subfractions were diluted in a buffer (0.1 M KH2PO4 and 2 mM EDTA, pH 7.2) and sonicated (3 × 5 s) on ice as previously described in detail (10). Enzyme activity was determined by the maximal oxidation rate of completely reduced cytochrome c, evaluated as a change in absorbance at 550 nm using a multi-detection microplate reader (Synergy HT, Biotek Instruments, Winooski, VT).

Proapoptotic mitochondrial protein release assay. Isolated SS and IMF mitochondrial fractions (150 μg) from control and stimulated muscle were incubated with 250 μM H2O2 and 50 mM FeSO4 for 60 min at 30°C as described previously (2, 3). Reaction mixtures were subsequently centrifuged at 14,000 × g (4°C) to pellet mitochondria, and the supernatant was analyzed for AIF and cytochrome c released from mitochondria with immunoblotting.

Mitochondrial permeability transition pore assessment. mtPTP opening was measured by assessing the reduction of light scattering associated with mitochondrial swelling at 540 nm as described previously (2, 3). SS and IMF mitochondria from control and stimulated muscle were treated with 400 μM CaCl2 and 75 μM tert-butylymperoxide, and the decrease in absorbance was measured spectrophotometrically for 15 min at 30°C (Biochrom UltraSpec 2100pro).

Mitochondrial ROS production. SS and IMF mitochondria (50 μg) from WT and KO muscle were incubated with 50 μM dichlorodihydrofluorescein diacetate (H2DCFDA) and VO2 buffer at 37°C for 80 min in a 96-well plate. The fluorescence emission between 480 and 520 nm measured with a fluorescence microplate reader (Fluoroscan Ascent Them Labsystems) is directly related to ROS production. Data were acquired and interpreted using KC4 (version 3.0) software. ROS production was assessed during state 4 and state 3 respiration by adding 11 mM glutamate, or glutamate plus 0.4 mM ADP, respectively, immediately before the addition of H2DCFDA.

Statistical analyses. Data are expressed as means ± SE. Student’s t-tests were used for comparison of data between WT and KO tissues. Two-way analyses of variance (ANOVA) were performed when WT and KO conditions were being compared between SS and IMF mitochondria, followed by Bonferroni post test when appropriate. Statistical differences were considered significant if P < 0.05.

RESULTS

Cytochrome c oxidase activity and mitochondrial yield. COX activity was significantly decreased by 17% and 30% in red quadriceps (RQ) and white quadriceps (WQ) muscle, respectively, from KO compared with WT animals (Fig. 1A). In PGC-1α KO mice, COX activity was reduced to a greater extent in the more oxidative tissues of diaphragm (33%) and heart (44%) muscle than in less oxidative tissues (P < 0.05; Fig. 1A). Within striated muscle, the suppression of COX activity due to the lack of PGC-1α was correlated (r2 = 0.89) to the basal WT COX activity of the particular tissue (Fig. 1A, inset). However, COX activity in the metabolically active nonmuscle tissues such as brain, liver, and pancreas was unaltered by the absence of PGC-1α (Fig. 1B). As anticipated on the basis of the suppressed COX activity of striated muscle, SS and IMF mitochondrial yield was 36% and 27% lower, respectively, in mixed muscle from KO mice than in WT
counterparts (Table 1). COX enzyme activity of isolated SS and IMF mitochondria from mixed muscle was not different between KO and WT animals (Fig. 1C). Since mitochondrial content differences between KO and WT animals only existed in WQ, RQ, and heart muscle between KO and WT animals, we focused our subsequent experiments on those particular tissues.

**Mitochondrial protein expression.** Expression of the mitochondrial proteins AIF, cytochrome c, and Tim23 was assessed in heart, WQ, and RQ whole tissue extracts from WT and PGC-1α KO animals (Fig. 2). No effect of genotype was evident in AIF protein content in any of these tissues (Fig. 2A). Cytochrome c, a mitochondrial marker, was unchanged in heart but significantly reduced by 36% and 47% in RQ and WQ muscles, respectively, in KO, compared with WT animals ($P < 0.05$; Fig. 2B). Expression of the mitochondrial import protein Tim23 was not different in heart, but it tended to be reduced in

<table>
<thead>
<tr>
<th>Table 1. Mitochondrial yield of SS and IMF subfractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SS</td>
</tr>
<tr>
<td>IMF</td>
</tr>
</tbody>
</table>

Values are means ± SE. SS, subsarcolemmal; IMF, intermyofibrillar; WT, wild type; KO, knockout. *$P < 0.05$ ($n = 7$/group) versus WT.
RQ and was significantly lower by 32% in WQ muscle of KO mice (P < 0.05; Fig. 2C).

Expression of proteins involved in mitochondrial regulation and restructuring. To examine whether mtDNA gene regulation might be affected by the absence of PGC-1α, we assessed the tissue levels of the important mitochondrial DNA transcription factor Tfam. Despite differences in mitochondrial content exhibited in striated muscle, Tfam expression was similar in heart (Fig. 3) and skeletal muscle (data not shown) from KO and WT animals. To assess whether PGC-1α might be involved in gross mitochondrial remodeling/restructuring, we measured two key proteins involved in fusion and fission, mitofusin 2 (Mfn2) and dynamin-related protein (Drp1), respectively. Expression of both Mfn2 and Drp1 was significantly increased by 110% and 83%, respectively, in heart of KO animals (Fig. 3), while no differences existed in WQ and RQ (data not shown).

Effect of PGC-1α on SS and IMF mitochondrial respiration and ROS production. To evaluate mitochondrial function, oxygen consumption was measured in SS and IMF mitochondria from muscles of WT and KO animals. In SS mitochondria, the absence of PGC-1α caused a significant reduction in state 4 (basal) and state 3 (active) respiration of 48% and 45%, respectively, compared with WT animals (P < 0.05; Fig. 4A). Basal and active IMF respiration rates were 12% and 30% lower, respectively, in PGC-1α KO compared with WT animals (P < 0.05; Fig. 4B). To assess whether impaired oxygen consumption affected free radical production, we measured mitochondrial ROS production. Despite reduced oxygen consumption, ROS production was unaltered in both SS and IMF mitochondria from KO animals (Fig. 4C). However, in agreement with our previous results, ROS per natom of oxygen consumed in IMF was significantly lower than that of the SS subtraction (P < 0.05; Fig. 4C; 1).

Expression of pro- and antiapoptotic proteins regulating mtPTP opening. To determine whether lack of PGC-1α imposed a greater susceptibility to mtPTP opening, we assessed the levels of proapoptotic Bax and antiapoptotic Bcl-2. Bax was not significantly elevated in WQ, RQ, or heart of KO animals (Fig. 5A). However, Bcl-2 was increased by 65–75% (P < 0.05) in RQ and heart of KO mice compared with WT controls, while no significant difference was observed in WQ muscle (Fig. 5B).

SS and IMF expression of cytochrome c and H2O2-induced cytochrome c release. To further characterize mitochondrial apoptotic susceptibility, isolated SS and IMF subfractions from WT and PGC-1α KO muscle were treated with exogenous hydrogen peroxide (H2O2) to determine the extent of proapoptotic cytochrome c release. Endogenous levels of mitochondrial cytochrome c were not statistically different in SS and IMF subfractions from KO mice compared with WT controls (Fig. 6A). In accordance with our previous results, IMF mitochondria displayed significantly elevated levels of endogenous cytochrome c relative to the SS subtraction, and H2O2 trig-

---

**Fig. 3.** Expression of mitochondrial proteins involved in regulating mitochondrial remodeling [mitofusin 2 (Mfn2) and dynamin-related protein (Drp1)] and gene expression [mitochondrial transcription factor A (Tfam)] from heart muscle of PGC-1α KO mice compared with WT animals. Representative Western blots are shown with graphical quantification expressed as percentage of WT (n = 7–9; *P < 0.05 vs. WT).

**Fig. 4.** SS and IMF mitochondrial respiration rates and reactive oxygen species (ROS) production from mixed muscle comparing PGC-1α KO and WT animals. A: SS state 4 and state 3 respiration rates in KO compared with WT animals (n = 12–13; *P < 0.05 vs. WT). B: IMF state 4 and state 3 respiration rates in KO compared with WT animals (n = 13–15; *P < 0.05). C: SS and IMF mitochondrial ROS production expressed per natom of oxygen consumed in IMF was not different in PGC-1α KO compared with WT animals (n = 11–14; *P < 0.05 vs. SS; AFU, arbitrary fluorescence units).
gered an increased release of cytochrome c relative to non-treated mitochondria (2). The absence of PGC-1α significantly increased the basal and H2O2-induced release of cytochrome c from SS mitochondria by 52% and 56%, respectively. No effect was observed in the IMF mitochondrial subfraction.

Mitochondrial transition pore kinetics. The mitochondrial release of cytochrome c is regulated by opening of the mtPTP. Therefore, SS and IMF mtPTP kinetics were also measured to determine whether functional differences between WT and PGC-1α KO muscle existed in the maximal rate of pore opening ($V_{\text{max}}$) and time to reach $V_{\text{max}}$ of both mitochondrial subfractions. In agreement with previous work, the $V_{\text{max}}$ was significantly greater in IMF, relative to SS mitochondria in both genotypes (2). In SS mitochondria, the lack of PGC-1α led to a significant reduction in $V_{\text{max}}$ compared with WT controls. No difference in $V_{\text{max}}$ was observed in the IMF mitochondria. In addition, there was no difference in the time to $V_{\text{max}}$ in either the SS or the IMF mitochondria between the WT and KO animals (Table 2).

Effect of endurance training on mitochondrial yield and function. To determine the effect of training on mitochondrial function in the absence of PGC-1α, a subgroup of both WT and PGC-1α KO mice were subjected to a 5-wk endurance training program. Animals in the training group were given free access to running wheels, and differences in voluntary running performance between WT and PGC-1α KO animals were carefully controlled for so that both genotypes had similar running volumes, as described previously (20). Endurance training increased the yield of SS mitochondria by 32% and 21% from skeletal muscle of WT and KO mice, respectively ($P < 0.05$; Fig. 7A). Basal and active respiration rates in SS mitochondria...
were 67% and 60% lower, respectively, in KO mice compared with WT controls \((P < 0.05; \text{Fig. } 7B)\), similar to the results shown in Fig. 4. In contrast to non-exercise trained KO animals, respiration rates were not depressed in endurance trained KO mice (Fig. 7B). This suggests that endurance training is able to restore mitochondrial respiratory function back to untrained, control levels in animals deficient of PGC-1α. ROS production in SS mitochondria was not different between genotypes, and there was no effect of training (Fig. 7C). Similar data were acquired from the IMF mitochondrial subfraction (data not shown).

### DISCUSSION

The importance of PGC-1α induction in mitochondrial biogenesis has been clearly established in a variety of tissues (4, 15, 16, 19, 28, 31, 39). However, the effect of decreased levels, and/or ablation of PGC-1α expression on mitochondrial content and/or function is less well documented. Previous work has indicated that targeted reduction of PGC-1α results in only subtle changes in mitochondrial morphology and volume of cardiac and skeletal muscle tissue (4). Given the profound functional and morphological alterations that occur in PGC-1α gain-of-function studies, complete loss of PGC-1α was anticipated to significantly suppress mitochondrial content and function, particularly in muscle (4, 21). However, our data indicate that this is not the case. Thus, in the present study, we wished to further elucidate the role of PGC-1α on mitochondrial regulation and function in various tissues, with particular emphasis on striated muscle.

Our previous work has shown a close parallelism between PGC-1α protein expression and the steady-state mitochondrial content in various tissues (15). On the basis of these tissue-specific differences, we hypothesized that the absence of PGC-1α would have the largest impact on tissues with the greatest inherent oxidative capacity. Contrary to our original hypothesis, however, the lack of PGC-1α and its effect on mitochondrial content was not strictly dependent on basal oxidative capacity. Instead, an important finding of the present study is that lack of PGC-1α did not alter mitochondrial content of highly metabolic tissues such as brain, liver, and pancreas, but it did significantly reduce mitochondrial content in all striated muscles examined. Indeed, mitochondrial content in PGC-1α KO animals was reduced to the greatest extent in the muscles with highest basal oxidative capacity (i.e., heart and diaphragm). The present findings are supported by our previous work showing that the mitochondrial markers, cytochrome c and cytochrome oxidase subunit I, are reduced by 20% in muscle from PGC-1α KO animals compared with WT mice (20). Furthermore, our results also corroborate a previous report showing decreased overall COX activity, citrate synthase activity, and reduced levels of ATP in the heart of PGC-1α KO animals (4).

To discriminate whether reduced whole muscle COX activity in KO muscle represented a lower overall mitochondrial number or was due to impairment in individual mitochondria, we measured COX activity of isolated skeletal muscle mitochondria. Since both whole muscle COX activity and mitochondrial yields were reduced in PGC-1α KO mice, while mitochondrial COX activity was not different between genotypes, we interpret these data to indicate that the reduced COX activity in PGC-1α KO mice is mainly a result of an overall reduction in tissue mitochondrial content.

Next, we evaluated the expression of key proteins involved in mitochondrial biogenesis (cytochrome c, Tfam), mitochondrially mediated apoptosis and/or oxidative phosphorylation (AIF), mitochondrial protein import (Tim23), and mitochondrial remodeling/restructuring (Mfn2 and Drp1). We anticipated a suppressed expression of these particular proteins due to the absence of PGC-1α, and we expected that the magnitude of these alterations would be proportional to the differences
exhibited in mitochondrial content. In contradiction to our hypothesis, the changes evident in individual proteins were highly specific. For example, cytochrome c expression was similar between WT and KO in the highly oxidative tissue of the heart, but it was significantly reduced in the less oxidative muscle tissues of RQ and WQ, similar to our previous findings (19). Similar results were also observed for AIF and Tim23 expression, with a larger suppression occurring in the less oxidative tissues. In contrast, the mitochondrial fusion (Mfn2) and fission (Drp1) proteins were significantly upregulated in the heart of PGC-1α KO animals but were unchanged in less oxidative muscles. Our interpretation of these findings is that oxidative tissues, such as the heart, that normally exhibit high basal expression of PGC-1α may have a greater potential to activate compensatory responses that lead to the maintenance, or elevation, of the levels of specific mitochondrial proteins. This compensatory response may be more difficult to accomplish for multisubunit complexes such as cytochrome-c oxidase, which require coordinated changes in the expression of multiple protein subunits from both the nuclear and the mitochondrial genomes.

Elevated mitochondrial fusion proteins such as Mfn1, Mfn2, and Opa1 are typically associated with an expanding mitochondrial reticulum, while increased mitochondrial fission proteins (Drp1, Fis1) lead to fragmentation of the mitochondrial network (8, 9, 18, 29, 40). Mitochondrial homeostasis requires a tightly regulated equilibrium of fission and fusion events (8, 9, 18, 29, 40). Thus, our data suggest that elevations in both fission and fusion proteins may represent a compensatory response to increase the organelle remodeling rate in an attempt to restore the reduced mitochondrial content exhibited in the heart of PGC-1α KO mice. This result was somewhat surprising given that PGC-1α has recently been shown to regulate the expression of Mfn2 (33), and it might be anticipated that the lack of PGC-1α should reduce, rather than enhance the expression of Mfn2. However, it is likely that PGC-1α represents only one of numerous factors that can influence the expression of Mfn2 in skeletal muscle, and other regulatory factors could produce an upregulation of Mfn2 in the absence of PGC-1α. Indeed, PGC-1β has recently been shown to be important in mediating Mfn-2 expression (22). However, we (G. Ugucconi and D. Hood, unpublished observations) and others (23, 33) have failed to find an increased compensatory PGC-1β response during reductions in PGC-1α expression. Thus, further research on the nature of the relationship between PGC-1 family members and fission and fusion events is warranted. Surprisingly, the important mitochondrial DNA transcription factor, Tfam, was not different in KO compared with WT animals and did not exhibit similar compensatory responses as Mfn2 and Drp1 in KO heart tissue. Our result differs from that reported previously by Arany et al. (4), who found Tfam mRNA to be reduced by 50% in hearts of PGC-1α KO mice. This discrepancy may be due to altered posttranslational events involved in increasing the stability of the Tfam protein in KO animals. However, this remains to be established.

To assess electron transport chain function, we measured oxygen consumption from isolated skeletal muscle mitochondria. We found a significant impairment in mitochondrial respiration rates in skeletal muscle of PGC-1α KO animals. This indicates a diminished ability to produce ATP in muscle of KO animals and could contribute to the impaired muscle performance found in muscle-specific PGC-1α KO animals (7). We, and others, have previously shown that impaired mitochondrial function is associated with elevations in ROS production (3, 6) and that this could increase mitochondrial apoptotic susceptibility. Additionally, previous reports have indicated that PGC-1α is involved in regulating the expression of several important ROS-detoxifying enzymes (17, 34, 35, 37). However, despite defective oxygen consumption, mitochondrial ROS levels were unaffected in both SS and IMF subfractions of PGC-1α KO animals. This suggests that the lower oxidative capacity evident in the KO animals is matched by a reduced formation of ROS, thereby maintaining a balance between respiration and ROS production in the absence of PGC-1α.

Our previous work has shown that decrements in PGC-1α cause not only a decrease in mitochondrial content, but are also associated with an increase in apoptotic susceptibility (3). Furthermore, PGC-1α has also been shown to be significantly downregulated in cardiomyocytes undergoing apoptosis (30). In support of a specific role for PGC-1α in apoptosis, Bianchi et al. (5) have shown that PGC-1α overexpression protects against calcium-induced apoptosis mediated by mitochondria. Given the impaired mitochondrial function and reduced mitochondrial content associated with PGC-1α ablation in skeletal muscle observed in the present study, we examined whether there was an associated increase in mitochondrially mediated apoptotic susceptibility in PGC-1α KO muscle. Thus, we treated isolated mitochondria with an exogenous ROS-stimulus (H2O2) and determined the extent of cytochrome c release. Our results indicate that both basal and H2O2-stimulated release of cytochrome c was significantly elevated in the SS mitochondrial subfraction isolated from PGC-1α KO mice. This implies that the absence of PGC-1α may increase the susceptibility of muscle to myonuclear decay, given their proximity to subsarcolemmal mitochondria. However, the increase in apoptotic susceptibility may not be severe enough to compromise muscle fiber size, at least in young animals, since fiber cross-sectional area has been reported to be similar between KO and WT mice (7). This higher cytochrome c release occurred despite evidence of a significantly lower Vmax of mtPTP opening in KO animals in the SS mitochondrial subfraction, as well as an apparent increase in the expression of Bcl-2 within fast-twitch red muscle fibers in PGC-1α KO animals. Thus it is clear that other factors, such as the mitochondrial apoptosis channel, or the expression of other channel-forming proteins, could contribute to the enhanced release of proapoptotic proteins from mitochondria in KO animals.

Chronic exercise is a well-known stimulus for muscle mitochondrial biogenesis, resulting in an enhanced capacity for ATP provision. Thus, we trained a subgroup of animals to investigate whether a program of exercise training could improve the respiratory deficit in PGC-1α KO animals. Untrained animals exhibited the typical respiratory deficit observed in the absence of PGC-1α. However, this respiratory deficiency was not observed in the trained animals, suggesting that endurance training can have beneficial influence in modifying mitochondrial respiratory function, and ATP provision, in the absence of PGC-1α. Evidently, the chronic exercise stimulus activates alternative regulatory proteins that are capable of sufficiently maintaining a normal mitochondrial respiratory chain to com-
penetrate for the lack of PGC-1α. The identity of these proteins remains to be determined.

In conclusion, our data indicate that the lack of PGC-1α results in a tissue-specific suppression of mitochondrial content, proportional to the basal oxidative capacity of the tissue in striated muscle types. However, not all mitochondrial proteins were reduced by the lack of the coactivator, suggesting the possibility of a compensatory upregulation of selective mitochondrial proteins in an attempt to preserve mitochondrial functions while organelle content is reduced. Despite this, mitochondrial function, assessed using rates of state 3 and 4 respiration, was depressed in muscle from KO animals, while cytochrome c release was elevated, indicative of increased apoptotic susceptibility in the absence of PGC-1α. Thus, we conclude that PGC-1α plays an important, but likely not exclusive, role in preserving organelle function and energy production in skeletal muscle. Endurance exercise training also appears to ameliorate the decline in mitochondrial function in the absence of PGC-1α.

**ACKNOWLEDGMENTS**

The authors thank Dr. B. M. Spiegelman (Harvard Medical School, Boston, MA) for the kind donation of heterozygous PGC-1α-knockout mice for breeding purposes. The authors are grateful to Kristian Kiliarich for assistance in training the animals. G. Ugucioni is a recipient of a Heart and Stroke MA) for the kind donation of heterozygous PGC-1α/H9251.

**REFERENCES**


