PGC-1α plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle

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Endurance exercise stimulates peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) expression in skeletal muscle, and forced expression of PGC-1α changes muscle metabolism and exercise capacity in mice. However, it is unclear if PGC-1α is indispensable for endurance exercise-induced metabolic and contractile adaptations in skeletal muscle. In this study, we showed that endurance exercise-induced expression of mitochondrial enzymes (cytochrome oxidase IV and cytochrome c) and increases of platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31)-positive endothelial cells in skeletal muscle, but not Iib-to-IIa fiber-type transformation, were significantly attenuated in muscle-specific Pgc-1α knockout mice. Interestingly, voluntary running effectively restored the compromised mitochondrial integrity and superoxide dismutase 2 (SOD2) protein expression in skeletal muscle in Pgc-1α knockout mice. Thus, PGC-1α plays a functional role in endurance exercise-induced mitochondrial biogenesis and angiogenesis, but not Iib-to-IIa fiber-type transformation in mouse skeletal muscle, and the improvement of mitochondrial morphology and antioxidant defense in response to endurance exercise may occur independently of PGC-1α function. We conclude that PGC-1α is required for complete skeletal muscle adaptations induced by endurance exercise in mice.

The understanding of the signaling and molecular mechanisms for endurance exercise-induced skeletal muscle adaptation has been significantly improved in the past decades. Many signal transduction pathways and gene transcriptional machineries have been implicated in skeletal muscle remodeling, including calcineurin (14, 40, 41), Ca2+/calmodulin-dependent protein kinases (CaMK) (50, 54), p38 mitogen-activated protein kinase (MAPK) (3, 49), AMP-activated protein kinase (AMPK) (17, 39, 48, 54), protein kinase D (PKD) (2, 29), myocyte enhancer factor-2 (MEF-2) (35, 51), peroxisome proliferator-activated receptorγ coactivator-1α (PGC-1α) (23, 33, 44), histone deacetylases (HDAC) (35, 44), and peroxisome proliferator-activated receptor δ (PPARδ) (39, 46). These findings clearly indicate the complexity and the importance of orchestrated signaling cascades and gene regulatory events in skeletal muscle adaptation and laid a solid foundation for a complete elucidation of the regulatory network underlying skeletal muscle plasticity in the future.

Up to date, numerous studies have suggested a pivotal function of PGC-1α in endurance exercise-induced skeletal muscle adaptation. First, both endurance exercise and motor nerve stimulation that mimic the neuromuscular activities in endurance exercise promote Pgc-1α mRNA and protein expression in skeletal muscle (3, 5, 9, 21, 42) as well as PGC-1α activity through posttranslational modification (13). Functionally, forced expression of PGC-1α promotes glycolytic-to-oxidative fiber-type transformation and mitochondrial biogenesis in skeletal muscle (33) with enhanced endurance exercise capacity (12). Consistently, both global and muscle-specific targeted disruptions of the Pgc-1α gene led to a reduced oxidative phenotype in skeletal muscle (23, 32, 34). Muscle-specific deletion of the Pgc-1α gene also resulted in enhanced proinflammatory cytokine expression (24), increased susceptibility to injury (23), and impaired angiogenesis in response to hypoxic stimuli (7). These findings prompted us to employ genetic approach to ascertain the functional role of PGC-1α in endurance exercise-induced skeletal muscle adaptation in a skeletal muscle-specific loss-of-function manner in mice. To this end, we generated skeletal muscle-specific Pgc-1α knockout mice, subjected them and their wild-type littermates to long-term voluntary running, and examined the phenotypic changes in skeletal muscles.

METHODS

Animals. Mice were housed in temperature-controlled (21°C) quarters with a 12:12-h light-dark cycle with free access to water and normal chow (Purina Chow). Muscle-specific Pgc-1α knockout mice (MKO) were generated by crossing breeding myogenin-Cre mice (generous gift from Dr. Eric Olson) with loxP flanked-Pgc-1α mice (generous gift from Dr. Bruce Spiegelman). Wild-type littermates (WT) were used as control. All animal protocols were approved by the Duke University Institutional Animal Care and Use Committee.

Genotyping. Mouse DNA was isolated with a phenol-chloroform-based DNA extraction protocol and used in PCR with primers for the Cre allele (5'-AGGTTTCTTACATGGA-3' and 5'-TGGACGGTTTAGTACC-3') and the loxP-flanked Pgc-1α allele (5'-TCCAGTTAGCAGAGATTATGC-3' and 5'-TGTCGTGTTTGCAACCTGCG-3'). The PCR reaction for the Cre allele was 4 min of initial denaturation at 94°C, 30 cycles of denaturation (94°C for 30 s), annealing (56°C for 30 s) and extension (72°C for 45 s), and a final 7 min of extension at 72°C. The PCR for the loxP-flanked Pgc-1α allele was 4 min of initial denaturation at 94°C, 40 cycles of denaturation (94°C for 30 s), annealing (58°C for 30 s), and extension (72°C for 45 s)

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Universal master mix (4304437, Applied Biosystems), were purified by cyclic reactions of 95°C for 15 s and 60°C for 1 min. The reagents initial hold temperature of 50°C for 2 min, 95°C for 10 min, followed by 20 cycles with all the muscle samples harvested within 1 min.

Real-time PCR. Total RNA was extracted from skeletal muscles using Trizol (Invitrogen) and reverse-transcribed into cDNA as described previously (53). Real-time PCR was performed on an ABI Prism 7000 sequence detection system (Applied Biosystems) with an initial hold temperature of 50°C for 2 min, 95°C for 10 min, followed by 20 cycles with the runnig wheels locked. Running activities in the exercise group were recorded and quantified using a desktop PC computer and Dataquest ART Gold Acquisition Software.

RESULTS

Pgc-1α MKO mice have normal IIb-to-IIa fiber-type transformation in response to voluntary running. To determine if PGC-1α is essential for endurance exercise-induced skeletal muscle adaptation, we obtained MKO and WT mice by cross-breeding (Fig. 1A). The efficacy of the gene deletion was established by PCR amplification from genomic DNA from WT and MKO mice according to the method described previously (47). The results are presented as means ± SE. Comparisons among different treatments were analyzed for statistical significance using two-way ANOVA followed by the Student-Newman-Keuls test with P < 0.05 as statistically significant.

Determination of capillary density. Similar procedures were followed as described previously (47) with the exceptions that different primary and secondary antibodies were used: rat anti-CD31 (MCA1364; Serotec, Raleigh, NC) followed by rhodamine red-X-conjugated goat anti-rat IgG (The Jackson Laboratory). Total number of capillaries and the surface area of the entire cross section for each muscle were measured with Scion Image software (Scion, Frederick, MD) and presented as capillary density (capillaries/m²).

Transmission electron microscopy. Transmission electron microscopy analysis was performed at Duke Electron Microscopy Service for plantaris muscle longitudinal sections from sedentary and exercised WT and MKO mice according to the method described previously (36). Defective mitochondria out of total mitochondria were counted in ≈14 randomly acquired TEM images for each group of mice.

Fig. 1. Voluntary exercise training in muscle-specific peroxisome proliferator-activated receptor γ coactivator-1α gene (Pgc-1α) knockout (MKO) and wild-type (WT) mice. A: gel images of PCR products amplified from genomic DNA from WT (Pgc-1α +/+; Myog-Cre +/+) or Pgc-1α fl/fl; Myog-Cre Cre+/-) and MKO (Pgc-1α fl/fl; Myog-Cre Cre+) mice. B: total RNA from plantaris muscles of WT and MKO mice were assayed for mRNA expression of Pgc-1α, Pgc-1β, estrogen-related receptor α (Esrα), NADH dehydrogenase (ubiquinone) Fe-S protein 1 (Ndufs1), and vascular endothelial growth factor (Vegf) by real-time PCR. 18S ribosomal RNA was used to normalize the expression (n = 6). *P < 0.05, **P < 0.01.

D: representative running activity recording. Six continuous voluntary running activity recordings are shown for WT and MKO mice during the 4 wk of voluntary running. Each of the clusters of running activities (spikes) that correspond to a duration of half-day represents the nocturnal running activity at night. D: daily voluntary running distance in WT and MKO mice during the 4 wk of voluntary running (P = 0.99). Values are means ± SE (n = 7). E: heart weight (normalized by body weight) in sedentary (Sed) and 4-wk endurance exercise-trained (Ex) WT and MKO mice (n = 6–7). *P < 0.05.
confirmed by real-time PCR analysis of total RNA from plantaris muscles, showing that MKO mice only had trace amount of Pgc-1α mRNA expression (Fig. 1B). Similar to the findings from previous studies (23, 24, 32), MKO mice showed a trend of decreased expression of Esrra, Ndufs1, and Vegf mRNA with no appreciable changes in Pgc-1β mRNA in skeletal muscle (Fig. 1B). When we subjected MKO and WT mice (male, 8 wk of age) to 4 wk of voluntary running, the daily running distance for each group increased gradually, reaching a steady-state level after 2 wk (Fig. 1C). No significant differences in running distance were observed between MKO and WT mice (Fig. 1D). Both MKO and WT mice had moderate but significant increases in the heart weight (normalized by body weight) following endurance exercise training (Fig. 1E), indicating similar degrees of endurance exercise-induced cardiac hypertrophy. Neither the body weight ([24.8 ± 0.5, 23.7 ± 0.9, 24.6 ± 1.4, and 23.2 ± 0.4 g for the WT sedentary (WT-Sed), WT endurance exercise-trained (WT-Ex), MKO-Sed, and MKO-Ex groups, respectively, P > 0.05]) nor plantaris muscle weight ([0.62 ± 0.01, 0.59 ± 0.02, 0.59 ± 0.02, and 0.61 ± 0.01 mg/g body wt for WT-Sed, WT-Ex, MKO-Sed and MKO-Ex groups, respectively, P > 0.05]) showed any significant difference among different groups.

Since plantaris muscles are actively recruited during voluntary running (51), and fiber-type analysis could be done precisely for the cross section in the middle portion of the muscle, we performed immunofluorescence-based fiber-type analysis in plantaris muscles following 4 wk of voluntary running as described (4). Our results showed that endurance exercise induced a significant increase in the percentage of type Ila myofibers in WT mice with a concurrent decrease in the percentage of type Iib fibers (Fig. 2A and Table 1). The percentage of type I fiber is extremely low in plantaris muscles, and endurance exercise training with the duration had no effect on the percentage of type I fibers in either genotypes. The increases in the percentage of type Ila myofibers in plantaris muscles were similar between MKO and WT mice, indicating

<table>
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<tr>
<th>Fiber-type composition</th>
<th>WT (n = 6)</th>
<th>Ex (n = 5)</th>
<th>MKO (n = 6)</th>
<th>Ex (n = 7)</th>
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<tr>
<td>MHC I</td>
<td></td>
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<td>%Total fibers</td>
<td>1.42 ± 0.49</td>
<td>1.49 ± 0.59</td>
<td>3.32 ± 0.73*</td>
<td>3.51 ± 0.65‡</td>
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<tr>
<td>%Area</td>
<td>0.46 ± 0.17</td>
<td>0.92 ± 0.54</td>
<td>1.50 ± 0.35‡</td>
<td>1.39 ± 0.27</td>
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<td>MHC IIa</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>%Total fibers</td>
<td>21.1 ± 1.1</td>
<td>29.5 ± 2.5†</td>
<td>22.5 ± 1.1</td>
<td>29.6 ± 1.3†</td>
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<tr>
<td>%Area</td>
<td>15.1 ± 1.6</td>
<td>26.3 ± 5.0</td>
<td>20.1 ± 1.0</td>
<td>32.4 ± 2.5†</td>
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<td>MHC IId/x</td>
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<tr>
<td>%Total fibers</td>
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<td>30.0 ± 2.6</td>
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<td>%Area</td>
<td>20.7 ± 1.2</td>
<td>26.8 ± 5.2*</td>
<td>24.9 ± 1.3</td>
<td>28.6 ± 2.2</td>
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<tr>
<td>%Total fibers</td>
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<td>%Area</td>
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<td>46.0 ± 9.6*</td>
<td>53.5 ± 2.0</td>
<td>37.6 ± 3.9*</td>
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Values are means ± SE. MKO, muscle-specific Pgc-1α gene knockout mouse; WT, wild-type littermates; Sed, sedentary; Ex, endurance exercise trained; MHC, myosin heavy chain; %Total fibers, fiber type as percentage of total fibers counted; %Area, fiber type area as percentage of total area. *P < 0.05, †P < 0.01 vs. Sed group of the same genotype. ‡P < 0.05 vs. WT group of the same treatment.
important question, we performed immunoblot analysis to quantify the expression of different isoforms of MHC proteins. As shown in Fig. 2, B and C, voluntary running induced significant increases in MHC IIa protein expression in plantaris muscles with a trend of reduction of MHC IIb protein; these changes were not affected by muscle-specific disruption of the Pgc-1α gene. Consistent with the immunofluorescence-based fiber type analysis, we could only detect trace amount of MHC I protein expression, and it was not affected by voluntary running. We observed the same MHC IIa protein induction by immunoblot analysis in two other predominantly glycolytic muscles, gastrocnemius (GA) and extensor digitorum longus (EDL), independent of the genotype (see Supplementary Figure S1, available with the online version of this article).

Muscle-specific deletion of the Pgc-1α gene attenuates endurance exercise-induced mitochondrial biogenesis. PGC-1α is a pivotal regulator of mitochondrial biogenesis by interacting with and stimulating the expression of transcription factors that function in control of nuclear-mitochondrial interactions, such as nuclear respiratory factor 1 (NRF1) and NRF2 (52). Despite the fact that overexpression of PGC-1α in cultured muscle cells and adult skeletal muscle induces mitochondrial biogenesis (33, 52), it was not known whether PGC-1α required for endurance exercise-induced mitochondrial biogenesis, particularly in light of the recent finding that some exercise-responsive genes, including genes that function in mitochondrial oxidative phosphorylation, were not affected in mice with global knockout of the Pgc-1α gene (31). To address this important question, we performed immunoblot analysis for COX IV and Cyt c in skeletal muscles from MKO and WT mice following 4 wk of endurance exercise. These two proteins are critical components of the mitochondrial electron transport chain, and their expression levels are indicative of mitochondrial biogenesis (15, 26, 45).

Following 4 wk of voluntary running, WT mice showed a 1.8-fold increase in COX IV expression (P < 0.001) while MKO mice showed a significantly less increase (1.3-fold, P < 0.05) (Fig. 3, A and B). The postraining level of COX IV protein in MKO was significantly lower than that in WT mice (P < 0.001). Similar results were obtained for Cyt c protein; endurance exercise training induced a significant (1.6-fold) increase of Cyt c protein in WT mice (P < 0.001), but not in MKO mice (1.3-fold; P = 0.10) (Fig. 3, A and B). Interestingly, endurance exercise-induced expression of myoglobin, which is known to be upregulated in cultured muscle cells and in skeletal muscle in transgenic mice where the Pgc-1α gene is overexpressed (25, 33), was not affected by muscle-specific deletion of the Pgc-1α gene (P < 0.05 and P < 0.01 for WT and MKO mice, respectively). Similar observations for these proteins were made in three other glycolytic muscles, GA, EDL, and white vastus lateralis (WV) muscles (see Supplementary Figure S1, available with the online version of this article).

Muscle-specific deletion of the Pgc-1α gene attenuates endurance exercise-induced angiogenesis. Angiogenesis is one of the important adaptations of skeletal muscle in response to endurance exercise training, and PGC-1α has recently been shown to play an important role in hypoxia-induced VEGF expression and angiogenesis independent of hypoxia-inducible factor-1 (HIF-1) in skeletal muscles (7). Considering this and the fact that MKO mice present a trend for reduced Vegf mRNA expression (Fig. 1B), we hypothesized that PGC-1α plays an essential role in endurance exercise-induced angiogenesis and performed immunofluorescence by using specific antibodies against platelet endothelial cell adhesion molecule-1.
(PECAM-1, CD31) to evaluate capillary density in skeletal muscle following voluntary running. Consistent with our hypothesis, endurance exercise induced a \(\sim 60\%\) increase in the number of CD31-positive endothelial cells per unit cross-sectional area (capillary density) in the WT mice. This adaptation was abolished in plantaris muscles from mice with muscle-specific deletion of the \(Pgc-1\alpha\) gene \((P = 0.17)\) (Fig. 3, C and D).

Voluntary running corrects abnormal mitochondrial morphology and restores SOD2 expression in skeletal muscles in MKO mice. It has been shown previously that muscle-specific deletion of the \(Pgc-1\alpha\) gene leads to enhanced expression of IL-6 and oxidative genes and increased susceptibility to muscle injury \((24)\). We performed electron microscopy for plantaris muscle sections and found that MKO mice had significantly more vacuolated mitochondria \((18.6 \pm 3.2\%)\) in both glycolytic and oxidative myofibers than WT mice \((10.2 \pm 3.2\%)\). The vacuolated mitochondria showed reduced density of the matrix, disarray of the cristae, and significant enlargement (Fig. 4, A and B). Importantly, 4 wk of voluntary running resulted in significant reduction in the number and severity of mitochondrial vacuolation in both WT \((4.8 \pm 0.7\%, P < 0.05\) vs. WT-Sed mice) and MKO mice \((3.9 \pm 0.9\%, P < 0.01\) vs. MKO-Sed mice) (Fig. 4, A and B). Immunoblot analysis revealed that MKO mice had significantly less SOD2 protein expression in plantaris muscles compared with the wild-type littermates, which could be completely corrected by voluntary running (Fig. 4, C and D). These findings suggest that deletion of the \(Pgc-1\alpha\) gene resulted in significantly compromised mitochondrial integrity and impaired mitochondrial SOD2 expression in skeletal muscle, which could be restored by endurance exercise training in the absence of functional PGC-1\(\alpha\).

**DISCUSSION**

One of the important questions in exercise physiology is about the regulatory factor(s) that is functionally responsible for endurance exercise-induced skeletal muscle adaptation. Since the cloning of the \(Pgc-1\alpha\) gene, there have been increasing interests in its function in skeletal muscle plasticity. The most intriguing finding is the formation of significantly more oxidative myofibers when the \(Pgc-1\alpha\) gene is overexpressed in skeletal muscle in transgenic mice \((33)\). However, it was not known whether PGC-1\(\alpha\) was required for endurance exercise-induced skeletal muscle adaptation and what aspects, if not all, of muscle adaptation would be affected when the \(Pgc-1\alpha\) gene was deleted. This study confirmed the essential function of PGC-1\(\alpha\) in endurance exercise-induced adaptation based on the findings that MKO mice had significant attenuation of 1) the induction of mitochondrial electron transport protein

![Fig. 4. Transmission electron microscopy (TEM) analysis for mitochondrial morphology and immunoblot analysis for antioxidant enzyme expression in plantaris muscles of sedentary (Sed) and 4-wk endurance exercise-trained (Ex) WT and MKO mice. A: TEM images of longitudinal plantaris muscle sections of glycolytic and oxidative myofibers. Arrows show degenerative mitochondrion. To show detailed structure of some degenerative mitochondria, cropped images are shown with 2.5× magnification of the original images on one corner. Bars = 1 μm. B: quantification of abnormal mitochondria in ≥14 randomly acquired images as a percentage of total mitochondria. *\(P < 0.05\), ***\(P < 0.001\), respectively. C: Immunoblot analysis of superoxide dismutase 2 (SOD2) protein expression in plantaris muscles from sedentary (S) and endurance exercise trained mice (E) with β-actin as loading control. Each lane was loaded with 40 μg of muscle protein from whole muscle homogenates. D: Quantifications of SOD2 protein expression in plantaris muscle \((n = 5–7)\). *\(P < 0.05\).](http://ajpcell.physiology.org/fig4.jpg)
expression, and 2) new capillary formation in skeletal muscles. These two adaptations constitute the major adaptive processes that confer the oxidative metabolic phenotype leading to improved oxidative phosphorylation and microcirculation during exercise.

The voluntary running model has been used to induce skeletal muscle adaptation in rodents (6). While the periodic bursts of running activity are not the same as the endurance exercise in humans, the phenotypic adaptations are quite similar (4, 6, 47). The MKO mice were able to run similar distance as WT mice with a nearly twofold increase in daily running distance after 2 wk of endurance exercise. Therefore, the attenuated muscle adaptation in mitochondrial biogenesis and angiogenesis in MKO mice is clearly not due to reduced running activity in these mice. MKO had a similar degree of physiological cardiac hypertrophy (heart weight normalized by body weight) compared with WT mice, further supporting this notion. Furthermore, induced expression of other exercise responsive genes, such as myoglobin and MHC IIA, was not affected by the Pgc-1α gene deletion in skeletal muscle, providing biochemical evidence for the exercise perturbation.

The similar daily running distance between MKO and WT mice seems to be contradictory to the substantial attenuation of endurance exercise-induced COX IV and Cyt c expression as these proteins play important role in mitochondrial oxidative phosphorylation and hence endurance exercise capacity. There are at least three possible reasons for this apparently paradoxical finding. First and most likely, mitochondrial function and/or efficiency may be enhanced independent of mitochondrial biogenesis. In other words, in the absence of increased mitochondrial electron transport protein expression, mitochondrial function may still be improved through other processes, such as posttranslational modification and/or improved physical structure of the electron transport chain complexes. Our finding that MKO mice had a significant amount of abnormal mitochondria and reduced SOD2 protein expression that could be corrected by voluntary running support to a certain degree the independence of the mitochondrial remodeling process on PGC-1α function. Consistent with this notion, Adhihetty et al. (1) have recently shown that Pgc-1α gene disruption impaired mitochondrial function (state 3 and state 4 respiration) in isolated mitochondria from skeletal muscle of sedentary animals, but that endurance exercise training led to enhanced mitochondrial function regardless of the genetic background. Second, other muscle adaptive processes, such as those related to calcium handling and the contractile apparatus, may result in compensation for the Pgc-1α gene deletion-induced loss of oxidative function in skeletal muscles. Finally, although voluntary running is a great physiological exercise model to induce skeletal muscle adaptation, the daily running distance on the voluntary wheel is not an index of endurance capacity. It would be very interesting to determine if MKO mice have lower endurance capacity compared with WT mice and if endurance exercise training improves exercise capacity in these mice.

Mitochondrial biogenesis, the process by which new mitochondria are formed in the cell along with increased mitochondrial enzyme expression/activity, is not only a hallmark of skeletal muscle adaptation (20, 27, 28) but is also pivotal for the enhanced exercise capacity (10, 27). Much of the recent attentions have been paid to PGC-1α function in endurance exercise-induced mitochondrial biogenesis since activation of Pgc-1α transcription (9, 42) and activity (13) occurs upon a single bout of endurance exercise, and transgenic overexpression of the Pgc-1α gene or upstream signaling molecules in skeletal muscle resulted in enhanced mitochondrial biogenesis (3, 19, 33). While we were conducting this study in musclespecific Pgc-1α knockout mice, Leick et al. (31) employed a mouse model of global gene disruption of the Pgc-1α gene and obtained surprising results indicating that Pgc-1α is not mandatory for exercise- and training-induced adaptive gene responses.

Angiogenesis, the formation of new capillary from the existing vasculature, is equally important to improved functional capacity after exercise training (11, 22). It has even been postulated that angiogenesis precedes and plays a permissive role for the other adaptive processes, such as fiber-type transformation (47). The recent study by Arany et al. (7) showed that PGC-1α is necessary and sufficient for hypoxia-induced angiogenesis in skeletal muscle. This raised the possibility of a functional role of PGC-1α in endurance exercise-induced angiogenesis. In light of the findings that hypoxia-induced angiogenesis is mediated by PGC-1α independent of HIF-1, it would be interesting to determine if endurance exercise-induced angiogenesis in skeletal muscle is achieved through the same mechanism.

In addition to the phenotypic changes that confer characteristics in metabolism, endurance exercise also promotes IIb-to-IIa fiber-type transformation (16, 47). While overexpression of Pgc-1α in skeletal muscle promotes more oxidative myofiber formation (33), we showed here that voluntary exercise-induced IIb-to-IIa transformation was not affected by the dele-
tion of the Pgc-1α gene in skeletal muscle, suggesting that endurance exercise-induced fiber-type transformation in skeletal muscle could occur independent of the function of the Pgc-1α gene. These findings have several implications. First, considering the impaired angiogenesis in the muscle-specific knockout mice, one might argue that angiogenesis is not obligatory for endurance exercise-induced fiber-type switching. In other words, these findings are not consistent with the hypothesis that angiogenesis plays an instrumental role for fiber-type transformation. Second, it has previously been shown that overexpression of active upstream signaling molecules in skeletal muscle that upregulate PGC-1α expression can promote mitochondrial biogenesis without inducing shifts in fiber-type composition (3, 19). We have also obtained new evidence in skeletal muscle-specific p38 knockout mice that endurance exercise-induced mitochondrial biogenesis and angiogenesis in skeletal muscle, but not fiber-type transformation, are dependent on the upstream p38γ MAPK signaling pathway (43). The findings in this study once again in a tissue-specific loss-of-function mouse model genetically separate the endurance exercise-induced mitochondrial biogenesis and angiogenesis from the contractile adaptation, specifically IIb-to-IIa fiber-type transformation. All these findings collectively support the view that PGC-1α and its regulatory control axis play a functional role in complete skeletal muscle adaptations that lead to improved microcirculatory function and oxidative metabolism, but their function seems not absolutely required for endurance exercise-induced contractile adaptation in mice.

It should be pointed out that although we have employed skeletal muscle-specific Pgc-1α knockout mice to minimize the possibility of developmental and interorgan influences from the disruption of the gene globally, we could not rule out the possibility of other regulatory factors that function in a compensatory manner. For example, Pgc-1β has recently been shown to promote formation of type IIX fibers (8), although the decreased Pgc-1β expression in human skeletal muscle by endurance exercise training (38) appears to be contradictory to this potential function in promoting fiber-type transformation from Iib to Iia. The possibility that a redundant functional role of a different isoform of PGC-1α as a result of alternative promoter (37) in MKO mice could be ruled out as we deleted exons 3, 4, and 5 of the Pgc-1α gene and prevented the formation of this PGC-1α isoform. The fact that endurance exercise-induced mitochondrial biogenesis and angiogenesis are significantly attenuated in Pgc-1α MKO mice suggests that the essential function of PGC-1α in these fundamental adaptive processes could not be compensated adequately by redundant systems if they existed.

In summary, our studies revealed that Pgc-1α plays an important functional role for endurance exercise-induced mitochondrial biogenesis and angiogenesis but not for Iib-to-Iia fiber-type transformation in mouse skeletal muscle. Our findings provide evidence in a loss-of-function mode that some of the metabolic adaptations could be genetically separated from the contractile adaptation. The improved understanding of the molecular mechanisms by which endurance exercise training promotes skeletal muscle adaptation will be instrumental for future development of novel interventions for many chronic diseases that have been shown to be benefited from regular exercise.

DISCLOSURES
No conflicts of interest are declared by the authors.

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