Exercise Stimulates Pgc-1α Transcription in Skeletal Muscle through Activation of the p38 MAPK Pathway*

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Peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) promotes mitochondrial biogenesis and slow fiber formation in skeletal muscle. We hypothesized that activation of the p38 mitogen-activated protein kinase (MAPK) pathway in response to increased muscle activity stimulated Pgc-1α gene transcription as part of the mechanisms for skeletal muscle adaptation. Here we report that a single bout of voluntary running induced a transient increase of Pgc-1α mRNA expression in mouse plantaris muscle, concurrent with an activation of the p38 MAPK pathway. Activation of the p38 MAPK pathway in cultured C2C12 myocytes stimulated Pgc-1α promoter activity, which could be blocked by the specific inhibitors of p38, SB203580 and SB202190, or a dominant negative p38. Furthermore, the p38-mediated increase in Pgc-1α promoter activity was enhanced by increased expression of the downstream transcription factor ATF2 and completely blocked by ATF2ΔN, a dominant negative ATF2. Skeletal muscle-specific expression of a constitutively active activator of p38, MKK6E, in transgenic mice resulted in enhanced Pgc-1α and cytochrome oxidase IV protein expression in fast-twitch skeletal muscles. These findings suggest that contractile activity-induced activation of the p38 MAPK pathway promotes Pgc-1α gene expression and skeletal muscle adaptation.

Adult skeletal muscle is remarkably plastic (1, 2). Increased contractile activity, such as endurance exercise, elicits multiple signals to activate a large set of genes, leading to phenotypic changes in skeletal muscle, including Iib-to-Iia fiber type switching, enhanced mitochondrial biogenesis, and angiogenesis, to match physiologic capability to functional demand. To date, the signaling pathways that link the neuromuscular activity to the gene regulatory machinery are not fully understood.

PGC-1α, a transcriptional co-activator cloned from a differentiated brown fat cell line (3), was recently identified as an important regulator of adaptive thermogenesis, glucose metabolism, mitochondrial biogenesis, and muscle fiber type specialization (4). Several lines of evidence are consistent with the notion that PGC-1α functions in promoting oxidative capacity in skeletal muscle. Firstly, overexpression of PGC-1α in cultured myoblasts increases mitochondrial biogenesis and oxidative respiration (5), and muscle-specific overexpression of PGC-1α in transgenic mice results in enhanced mitochondrial biogenesis and more slow-twitch (type I) fiber formation (6). Secondly, endurance exercise induces PGC-1α mRNA and protein expression in rats and humans (7–9). Thirdly, more slow-twitch fiber formation evoked by genetic or pharmacologic means is associated with enhanced expression of PGC-1α mRNA and protein in skeletal muscle (10, 11). Finally, PGC-1α mRNA and protein are highly expressed in slow-twitch, oxidative (type I), and fast-twitch oxidative fibers (type IIa) compared with the fast glycolytic fibers (6, 11, 12). It is currently unknown whether exercise-induced PGC-1α expression in skeletal muscle is essential for enhanced mitochondrial biogenesis and/or Iib-to-Iia fiber type switching, the latter being different from the reported function of PGC-1α in transgenic mice in promoting more type I fiber formation (6).

Exercise-induced expression of PGC-1α in skeletal muscle is, at least in part, because of increased transcription (9). Human, mouse, and rat PGC-1α promoters share an overall 80–90% identity, suggesting conserved regulation of the PGC-1α gene. The functional roles of several sequence elements within the PGC-1α promoter have been tested in cultured myocytes (13, 14) and in mature skeletal muscle in vivo (15). PGC-1α itself regulates the promoter activity in a positive autoregulation loop, through myocyte enhancer factor 2 (MEF2) at a binding site (−1539) (14), possibly through its interaction with MEF2 (16). A dominant negative form of the cAMP-response element-binding protein (CREB) or mutation of the CRE site (−222) reduces the activation of PGC-1α promoter by an activated Ca²⁺/calmodulin-dependent protein kinase (CaMK) (14) (Fig. 7). We have recently confirmed the importance of the MEF2 and CRE binding sites for contractile activity-induced activation of the Pgc-1α promoter in living mice (15). These findings reveal the importance of regulatory factors that bind the CRE and MEF2 consensus sites in Pgc-1α gene regulation.

The p38 MAPK pathway in skeletal muscle has been a focus of recent research. In particular, p38 MAPK activity is necessary for myogenic cell differentiation (17) and plays an important role in glucose metabolism and energy expenditure (18, 20). MAPK, mitogen-activated protein kinase; MCK, muscle creatine kinase; MHC, myosin heavy chain; BT, reverse transcription; CMV, cytomegalovirus; COXIV, cytochrome oxidase IV; M KK, mitogen-activated protein kinase kinase.

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1 The abbreviations used are: PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1α; Pgc-1αL, Pgc-1α-luciferase; MEF2, myocyte enhancer factor 2; CRE, cAMP-response element; CREB, CRE-binding protein; CaMK, Ca²⁺/calmodulin-dependent protein kinase;
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19. It has been proposed that the p38 MAPK pathway plays a functional role in skeletal muscle adaptation to changing patterns of contractile work (20–24). Based on several previous findings, we propose that the functional role of the p38 MAPK pathway in skeletal muscle adaptation is mediated through regulation of PGC-1α expression and activity. For example, p38 MAPK can directly stimulate upstream transcription factors of the Pgc-1α gene, such as ATF2 and MEF2 (14, 25, 26). p38 MAPK can also de-repress PGC-1α activity by inhibiting the repressor p160 Myb-binding protein (27) and promote PGC-1α function (18, 28). However, there has been no direct evidence that p38 activity stimulates Pgc-1α gene transcription in skeletal muscle, and it is not known whether activation of this pathway is sufficient to induce, and necessary for, skeletal muscle adaptation.

In this study, we subjected mice to voluntary running, a physiological model of exercise that mimics many aspects of endurance exercise-induced adaptation, such as fiber type switching, mitochondrial biogenesis, and angiogenesis (12, 29). We demonstrated that voluntary running activates the p38 MAPK pathway and stimulates Pgc-1α mRNA expression. A link between an activated p38 MAPK pathway and the expression of PGC-1α was described previously (12). Antibodies used for immunoblot analysis were PGC-1α (Purina Chow) and IIb as described previously (12). MAPK can directly stimulate upstream transcription factors of the Pgc-1α gene, such as ATF2 and MEF2 (14, 25, 26). p38 MAPK can also de-repress PGC-1α activity by inhibiting the repressor p160 Myb-binding protein (27) and promote PGC-1α function (18, 28). However, there has been no direct evidence that p38 activity stimulates Pgc-1α gene transcription in skeletal muscle, and it is not known whether activation of this pathway is sufficient to induce, and necessary for, skeletal muscle adaptation.

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pathways that are potentially involved in skeletal muscle adaptation, we performed Western immunoblot analysis using phospho-specific antibodies against signaling molecules in the MAPK pathways. We observed significant increases in phosphorylation of MAP kinase kinase 3 (MKK3), MKK6, p38, and the p38 MAPK pathway is sufficient to stimulate pathways that are potentially involved in skeletal muscle adaptation. To determine whether activation of the p38 MAPK pathway is sufficient to stimulate Pgc-1α transcription, C2C12 cells were transfected with a Pgc-1α-luciferase reporter gene together with constitutively active p38 activating kinase MKK3E or MKK6E (31, 32, 34), with or without p38α, p38β, p38γ, or p38δ, the different isoforms of p38. Overexpression of MKK3E or wild-type p38 isoforms stimulated Pgc-1α promoter activity significantly (Fig. 5A). When MKK3E was co-transfected with wild-type p38 isoforms, Pgc-1α promoter activity was further enhanced. The stimulatory effect of MKK3E was completely blocked by co-transfection with non-phosphorylatable p38δAF (Fig. 5A) and by the specific inhibitors of p38 MAPK, SB203580 and SB202190 (Fig. 5B).

To further examine the stimulatory effects of constitutively active MKKs in differentiated myotubes, we transfected C2C12 myoblasts with MKK6E under the control of the MCK promoter and induced myogenic differentiation by switching from serum-rich growth medium to low serum differentiation medium. We chose MKK6E over MKK3E in this experiment because of preliminary findings that MKK6E is more potent than MKK3E in stimulating Pgc-1α promoter in myotubes (not shown). FLAG-tagged MKK6E (under the control of MCK promoter) could only be detected in differentiated myotubes, whereas FLAG-tagged MKK6E (under the control of the con-
DISCUSSION

Fiber type composition and mitochondrial content of skeletal muscles were determining factors for the efficiency of locomotion and metabolism. Physical inactivity, endemic in the Western lifestyle, contributes to a decrease in the percentage of oxidative fibers and correlates with epidemic emergence of chronic disorders, such as coronary heart disease, obesity, and type 2 diabetes (43). Exercise-induced adaptation in skeletal muscle is an effective means of curbing the increasing health care costs caused by this metabolic syndrome. Understanding the signaling and molecular mechanisms of skeletal muscle adaptation will not only promote a correct and efficient use of exercise as a therapeutic measure but also may facilitate the discovery of new drug targets.

In this study, we subjected wild-type sedentary mice to voluntary running and performed comprehensive phenotypic analysis to determine the effects of long-term exercise on skeletal muscle. We confirmed functional, morphological, and biochemical adaptations in mouse skeletal muscle following 4 weeks of voluntary running. The running distance during the nocturnal activity increased ~2-fold following the training, indicating an increase in overall running capacity. Fiber type analysis with simultaneous staining of three MHC isoforms (I, IIA, and IIB) in plantaris muscles provided morphological evidence of a 2-fold increase in the percentage of type IIA fibers, with a concurrent decrease in type IIB fibers. Finally, quantitative analysis of proteins related to skeletal muscle contractile function and mitochondrial biogenesis showed significantly increased expression of MHC IIA and PGC-1α proteins in trained plantaris muscles. Taken together, these results provided direct and comprehensive evidence, complementary to previous reports (30, 44), that long-term voluntary running induces significant skeletal muscle adaptation in mice.

Voluntary running has been employed in rodents as a model of exercise to induce skeletal muscle adaptation (30, 45). Although the muscle activity pattern (intermittent bursts of activities) is different from human endurance exercise, such as long-distance running, the observed functional adaptations in skeletal muscle are similar between the animal models and humans in many aspects, which have been characterized in mouse skeletal muscle using various morphological and biochemical analyses (12, 29, 30). A combination of this physiological exercise model with transgenic or targeted mutation approaches provides an excellent opportunity for elucidation of molecular and signaling mechanisms of skeletal muscle adaptation that otherwise would not be feasible in other animal models or in humans.

The molecular mechanisms that regulate skeletal muscle fiber type composition and mitochondrial biogenesis in response to exercise are not completely understood. It has recently been reported that endurance exercise induces PGC-1α mRNA and protein expression (7–9), suggesting that PGC-1α protein plays a functional role in exercise-induced skeletal
Activation of the p38 MAPK pathway stimulates Pgc-1α promoter activity in cultured myocytes. A, C2C12 myoblasts were co-transfected with the Pgc-1α reporter gene and wild-type p38 isoforms or dominant negative p38αAF with or without MKK3E. Empty plasmid pCI-neo was used as control (Con). Wild-type p38 isoforms are p38α, p38β, p38γ, or p38δ under the control of the constitutively active CMV promoter. Non-phosphorylatable p38αAF is under the control of the CMV promoter. Normalized luciferase activities are presented as fold changes compared with the control without MKK3E. Empty plasmid pCI-neo was used as control (Con). Wild-type p38 isoforms are p38α, p38β, p38γ, or p38δ under the control of the constitutively active CMV promoter. Non-phosphorylatable p38αAF is under the control of the CMV promoter. Normalized luciferase activities are presented as fold changes compared with the control without MKK3E (n = 6–10; *, p < 0.05 versus −MKK3E). B, C2C12 myoblasts were co-transfected with PGC-1α and MKK3E (+MKK3E) or pCI-neo (−MKK3E) followed by incubation with 10 μM SB203580, 10 μM SB202190, or the vehicle dimethyl sulfoxide (DMSO). Normalized luciferase activities are presented as fold changes compared with cells treated with dimethyl sulfoxide (n = 6; *, p < 0.05 versus −MKK3E; +, p < 0.05 versus dimethyl sulfoxide). C, C2C12 myoblasts were co-transfected with PGC-1αL and MCK-MKK6E or pCI-neo (Con). Luciferase activity was determined in myoblasts (MB) and after induction of myogenic differentiation into myotubes (MT) for 4 days. Normalized luciferase activities are presented as fold changes compared with myoblasts transfected with pCI-neo (n = 3; *, p < 0.05 versus myoblast; +, p < 0.05 versus control). D, C2C12 myoblasts were co-transfected with PGC-1αL and MKK3E (+MKK3E) or pCI-neo (−MKK3E) together with pCI-neo control plasmid (Con), ATF2, ATF2(T69A/T71A), or ATF2ΔN under the control of the CMV promoter. Normalized luciferase activities are presented as fold changes compared with the control (n = 6–10; *, p < 0.05 versus −MKK3E).

FIG. 6. Muscle-specific expression of MKK6E enhances PGC-1α and COXIV protein expression in fast-twitch muscles. A, PCR and RT-PCR analyses in tail genomic DNA and muscle total RNA samples from three F0 transgenic mice (TG171, TG1123, and TG554) for the presence of the FLAG-tagged human MKK6E transgene (hMKK6E transgene) and mRNA (hMKK6E mRNA) and the endogenous Mkk6 mRNA (Mkk6 mRNA). B, Western blot analysis for PGC-1α and COXIV proteins in white vastus lateralis muscles of MCK-MKK6E transgenic mice (line TG1123, TG) and the wild-type (WT) littermates. Actin protein was used as a control for loading. C, quantification of PGC-1α and COXIV proteins after normalization by the abundance of actin protein. The mean values in the control mice were set as references for comparison (n = 7 and 13 for PGC-1α and COXIV, respectively; **, p < 0.01 versus WT).

Muscle adaptation. Here, we report that a transient increase in Pgc-1α mRNA in skeletal muscle occurs during and following a single bout of voluntary running or motor nerve stimulation in mice. This transcriptional activation of the Pgc-1α gene may contribute directly to contractile activity-induced PGC-1α protein expression that mediates skeletal muscle adaptation.

Ca2+ has been shown to play an essential role in contractile activity-induced skeletal muscle adaptation (46). Recent studies have linked Ca2+ signaling to PGC-1α expression in both cultured myocytes (14, 47) and in skeletal muscle of intact animals (11). On one hand, Ca2+ signaling may activate PGC-1α promoter via the MEF2 site by activating the calcineurin/MEF2 signaling cascade (14). On the other hand, it may also exert a positive regulatory role on PGC-1α transcription via the CRE site by activating the CaMK pathway (14) (Fig. 7). Using optical bioluminescence imaging analysis, we have shown recently that contractile activity-induced Pgc-1α promoter activity in skeletal muscle is dependent on the MEF2 and CRE sequences for comparison (n = 7 and 13 for PGC-1α and COXIV, respectively; **, p < 0.01 versus WT).
Exercise/Neuromuscular Activity

![Figure 7: A model for the exercise-induced transcription of the Pgc-1α gene.](image)

In this study, we used phospho-specific antibodies to characterize activation of signaling molecules that are involved in skeletal muscle adaptation. The p38 MAPK pathway, including MKK3/6, p38, and transcription factor ATF2, was shown to be activated in response to running as shown by the increased phosphorylation of these signaling molecules. Activation of the p38 MAPK pathway was concurrent with a transient increase in Pgc-1α mRNA following both voluntary running and nerve stimulation, consistent with a functional role of p38 MAPK in skeletal muscle adaptation in vivo (20–23).

To determine whether activation of the p38 MAPK pathway has a role in regulating the Pgc-1α promoter activity in skeletal muscle, we employed a reporter gene assay in cultured C2C12 myoblasts by co-transfection of a muscle-specific promoter and a luciferase reporter gene. The p38 MAPK pathway can also promote Pgc-1α transcription by activating transcription factors ATF2 and MEF2 directly and inhibit p160 to de-repress Pgc-1α function, which exerts a positive autoregulatory feedback regulation of Pgc-1α transcription, possibly through interaction with and activation of MEF2.

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Finally, we tested the hypothesis that activation of the p38 MAPK pathway in adult skeletal muscle in vivo is sufficient to promote Pgc-1α expression, changes in fiber type composition, and mitochondrial biogenesis. We have obtained evidence of significantly enhanced PGC-1α and COXIV protein expression in fast-twitch white vastus lateralis muscles in the transgenic mice with skeletal muscle-specific expression of MKK6E. A similar trend of enhanced expression was observed in plantaris muscles of MKK6E transgenic mice, but it was not statistically significant. However, we did not observe significantly enhanced expression of MHC IIa in white vastus lateralis muscles in these mice. Our data suggest that transgenic activation of the p38 MAPK pathway in adult skeletal muscle results in enhanced PGC-1α expression and mitochondrial biogenesis. Together with the calcineurin and CaMK pathways, the p38 MAPK pathway represents another signaling pathway in the regulation of the Pgc-1α promoter activity through transcription factors binding to the cis-elements (Fig. 7).

Exercise exerts the most complex and powerful stimuli for functional adaptations in skeletal muscle. Multiple signaling pathways, such as the calcineurin, AMP-activated protein kinase, and protein kinase C pathways, have been reported to be activated in skeletal muscle in response to exercise (44, 50, 51). Each of these pathways has unique temporal and spatial patterns and distinct regulatory targets (6, 52). They collectively form a signaling network in the skeletal muscle that ensures the adaptability as well as the fidelity of the adaptive process, which is likely to be accomplished by coordinated interactions and cross-talks. The present study provides evidence linking activation of the p38 MAPK pathway to skeletal muscle adaptation through its regulatory control of Pgc-1α gene expression. It remains to be determined whether activation of the p38 MAPK pathway is an obligatory signaling event in skeletal muscle adaptation in response to endurance exercise.

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gene regulation in skeletal muscle


