Cardiac-Specific Induction of the Transcriptional Coactivator Peroxisome Proliferator-Activated Receptor γ Coactivator-1α Promotes Mitochondrial Biogenesis and Reversible Cardiomyopathy in a Developmental Stage-Dependent Manner

Laurie K. Russell, Carolyn M. Mansfield, John J. Lehman, Attila Kovacs, Michael Courtois, Jeffrey E. Saffitz, Denis M. Medeiros, Maria L. Valencik, John A. McDonald, Daniel P. Kelly

Abstract—Recent evidence has identified the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) as a regulator of cardiac energy metabolism and mitochondrial biogenesis. We describe the development of a transgenic system that permits inducible, cardiac-specific overexpression of PGC-1α. Expression of the PGC-1α transgene in this system (tet-on PGC-1α) is cardiac-specific in the presence of doxycycline (dox) and is not leaky in the absence of dox. Overexpression of PGC-1α in tet-on PGC-1α mice during the neonatal stages leads to a dramatic increase in cardiac mitochondrial number and size coincident with upregulation of gene markers associated with mitochondrial biogenesis. In contrast, overexpression of PGC-1α in the hearts of adult mice leads to a modest increase in mitochondrial number, derangements of mitochondrial ultrastructure, and development of cardiomyopathy. The cardiomyopathy in adult tet-on PGC-1α mice is characterized by an increase in ventricular mass and chamber dilatation. Surprisingly, removal of dox and cessation of PGC-1α overexpression in adult mice results in complete reversal of cardiac dysfunction within 4 weeks. These results indicate that PGC-1α drives mitochondrial biogenesis in a developmental stage-dependent manner permissive during the neonatal period. This unique murine model should prove useful for the study of the molecular regulatory programs governing mitochondrial biogenesis and characterization of the relationship between mitochondrial dysfunction and cardiomyopathy and as a general model of inducible, reversible cardiomyopathy. (Circ Res. 2004;94:525-533.)

Key Words: mitochondria ■ metabolism ■ transgenic mice ■ cardiomyopathy ■ transcription factors

The heart has an extraordinarily high capacity for mitochondrial ATP production to meet the rigorous and dynamic energy demands of the postnatal environment. The importance of the mitochondrion for cardiac function is underscored by the development of cardiomyopathy in inherited and acquired forms of mitochondrial dysfunction in humans.1–3 Because mitochondria contain proteins encoded by both nuclear and mitochondrial genomes, mitochondrial biogenesis requires the coordinate regulation of these 2 genomes. The transcriptional regulatory network controlling the expression of nuclear and mitochondrial genes includes nuclear respiratory factor (NRF)-1 and NRF-2 and mitochondrial transcription factor A (mtTFA). The regulatory pathways upstream of these factors are a focus of intense investigation.4–7 Recently, the peroxisome proliferator-activated receptor (PPAR) γ coactivator-1α (PGC-1α) has been identified as an inducible upstream regulator of mitochondrial number and function.8–10 PGC-1α is a transcriptional coactivator that lacks DNA-binding activity but interacts with and coactivates numerous transcription factors, including nuclear receptors such as PPARγ and PPARα, estrogen receptor α, thyroid hormone, retinoid receptors, and hepatocyte nuclear factor-4α.8,11–16 PGC-1α also coactivates nonnuclear receptor transcription factors, such as NRF-1, to activate the expression of known target genes, including mtTFA, a factor that is critical for the initiation of mitochondrial DNA replication.8,17 Some of these interactions are ligand-dependent, whereas others are ligand-independent; furthermore, cell signaling events can influence the activity of PGC-1α through the actions of kinases.18–23 Therefore, PGC-1α likely

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mediates the physiological control of mitochondrial capacity through NRF-1 and its downstream targets.

Consistent with its emerging role as a central regulator of energy metabolism, PGC-1α is abundantly expressed in mitochondrial-rich tissues such as heart, skeletal muscle, and brown adipose tissue.8 Its expression is inducible in these tissues in response to physiological stimuli. In brown adipose tissue and skeletal muscle, PGC-1α mRNA expression is induced in response to cold exposure, likely through β-adrenergic stimulation. Upregulation of PGC-1α in brown adipose tissue induces expression of uncoupling protein-1 and increases uncoupled mitochondrial respiration, consistent with a role for PGC-1α in adaptive thermogenesis.8,9 In the liver, PGC-1α is induced during fasting and regulates gluconeogenesis through coactivation of the glucocorticoid receptor, hepatocyte nuclear factor-4α, and FOXO1.16,17,24 In heart and skeletal muscle, physiological stimuli, including fasting and exercise, lead to an increase in PGC-1α gene expression10,25; conversely, pressure overload decreases PGC-1α expression.26 Recent studies by our group and others have implicated PGC-1α in the activation of the mitochondrial biogenesis program in both heart and skeletal muscle.9,10,27 Forced expression of PGC-1α in a skeletal muscle–derived cell line leads to mitochondrial biogenesis and an increase in uncoupled respiration.9 In contrast, in vitro overexpression of PGC-1α in neonatal cardiac myocytes induces mitochondrial proliferation with a concomitant increase in coupled respiration,10 suggesting that there are tissue-specific differences in the gene regulatory pathways activated by PGC-1α in heart and skeletal muscle. In vivo studies in transgenic mice have confirmed the importance of PGC-1α in mitochondrial biogenesis in skeletal muscle and heart. In skeletal muscle, PGC-1α overexpression induces mitochondrial biogenesis and drives muscle fibers toward a more oxidative phenotype, thus regulating fiber-type determination.27 We have shown that constitutive overexpression of PGC-1α in the heart in transgenic mice via the α-myosin heavy chain promoter (MHC-rtTA) results in uncontrolled mitochondrial proliferation in cardiac myocytes.10 However, because of the development of severe dilated cardiomyopathy, it is not possible to study the molecular pathways driving mitochondrial biogenesis in this transgenic system.

To circumvent the problems associated with constitutive overexpression of PGC-1α in heart, we have developed a double transgenic mouse model for inducible, cardiotype-specific overexpression of PGC-1α. Using this inducible system (tet-on PGC-1α), we show that overexpression of PGC-1α in the hearts of neonatal mice results in mitochondrial proliferation. In long-term studies in adult mice, overexpression of PGC-1α drives the development of cardiomyopathy associated with derangements of mitochondrial ultrastructure. Remarkably, the cardiomyopathy in these animals is fully reversible, at least in the short term. The conditional expression system we report here will be a useful tool to study the regulatory programs governing mitochondrial biogenesis and as a mouse model for inducible yet reversible cardiomyopathy.

Materials and Methods

Transgenic Mice

Transgenic mice expressing a codon-optimized reverse tetracycline transactivator from the α-myosin heavy chain promoter (MHC-rtTA) were previously described.28 To create the PGC-1α transgene, a PGC-1α cDNA with a C-terminal myc-his tag was inserted into the pTRE2 vector (Clontech). This construct was linearized with Xho I and injected into fertilized mouse eggs to generate the tetracycline response element (TRE)-PGC-1α lines. Mice carrying the transgene were identified by a transgene-specific PCR assay. These 2 strains, both in the FVB/N genetic background, were crossed to generate double transgenic mice (Tet-on PGC-1α). To activate the TRE-PGC-1α transgene, mice were administered 2 g/L doxycycline (dox, Sigma) supplemented with 5% (wt/vol) sucrose in their drinking water. Dox was kept in dark bottles and was changed 3 times a week. Dox inductions were continued for 5 days to 7 weeks as described in Results. Mice were euthanized by carbon dioxide inhalation, and the hearts were harvested for subsequent analysis. All animal experiments and euthanasia protocols were conducted in accordance with the National Institutes of Health guidelines for human treatment of laboratory animals and were reviewed and approved by the Animal Care Committee of Washington University.

mRNA and Protein Analysis

Total RNA was isolated by the Rnazol method (Bio101) as described.10,11 For Western blots, nuclear-enriched protein extracts were isolated from the heart.29 Proteins were separated by electrophoresis, transferred onto Protean II membranes (Schleicher & Schuell), and immunoblotted with a rabbit anti-PGC-1α antibody.10 Detection was by enhanced chemiluminescence (Amersham).

Histology and Electron Microscopy

Hearts were fixed in formalin, embedded in paraffin, and sectioned for histological staining. Tissue sections were stained with Masson’s trichrome. For electron microscopy analysis, hearts were fixed with 2% glutaraldehyde and 1% paraformaldehyde, postfixed in 1% osmium tetroxide, embedded in plastic resin, and sectioned for electron microscopy.

Morphometric Analysis

Cardiac mitochondrial and myofibrillar volume densities were determined from electron micrographs as described previously.10 For each animal and magnification (×4000 to ×10 000), 3 different fields were quantified in a blind fashion. Data were expressed as mean volume density of mitochondria or myofibrils (μm²/μm³) in each field. Differences were determined with a Student’s two-tailed t test at an α of 0.05.

Echocardiography

Serial transthoracic echocardiography was performed as described previously.29 Tissue Doppler imaging (TDI) was used to characterize LV diastolic function. Transmitral flow velocity was obtained from a modified apical 4-chamber view with the pulse-wave Doppler sample volume positioned at the ventricular side of the mitral valve leaflets. Mitral annulus velocity measurement was performed from the same 4-chamber view by placing the TDI sample volume on the lateral portion of the mitral annulus. Digitally acquired echocardiographic images were analyzed offline by two observers blinded to the genotype of the animals.

Statistical Analysis

Unless noted otherwise, data were analyzed using ANOVA followed by multiple comparison testing to define significant differences. An expanded Materials and Methods section can be found in the online data supplement, available at http://circres.ahajournals.org.
RNA blot analysis confirms that expression of the transgene is confirmed by immunoblotting (representative result, right). C, Genotypes are denoted at the bottom. Protein expression was his tag was inserted into pTRE2 under control of a minimal CMV rtTA.28 Double transgenic mice and control (wild-type, TRE-optimized reverse tetracycline transactivator driven by MHC-mated with transgenic mice overexpressing a codon-optimized reverse tetracycline transactivator driven by MHC-mated with transgenic mice. Transgenic founders were identified and with inducible expression of the PGC-1α. The TRE-PGC-1α construct was then used to generate transgenic lines (tet-on PGC-1α) were crossed with mice carrying a C-terminal myc-his tag was inserted into pTRE2 vector downstream of the tetracycline response element to generate TRE-PGC-1α. PGC-1α-MHC promoter (MHC-rtTA)29 to generate PGC-1α-myct-his transgene in heart of 4 independent lines (left). The RNA was isolated from cardiac ventricle of mice after 2 weeks of dox administration (Figure 2A). Indeed, the marked increase in cellular volume occupied by mitochondria in these cells seemed to displace the myofibrils and the nucleus. Higher magnification demonstrated that in addition to proliferation, the mitochondria were dramatically larger in the neonatal tet-on PGC-1α hearts compared with the control hearts (Figure 2A). Although many mitochondria appeared swollen, the cristae generally seemed to be intact. Morphometric analysis of the electron micrographs (Figure 2B) confirmed a marked increase in mitochondrial volume density (0.68±0.06 versus 0.32±0.04 μm³/μm³, P<0.001) and a reduction in myofibrillar density (0.20±0.01 versus 0.37±0.02 μm³/μm³, P<0.001) in the hearts of neonatal tet-on PGC-1α mice compared with controls. When normalized to the volume density of myofibrils, there was a 3.5-fold increase in mitochondria (P<0.001). Caspase staining revealed rare apoptotic cells, suggesting that little cell death occurs in these hearts (data not shown).

Previous work has established a relationship between PGC-1α and NRF-1.9 NRF-1 regulates the transcription of numerous nuclear-encoded mitochondrial genes, including many of the respiratory complex proteins.6,7 Another pivotal NRF-1 target is mtTFA, which directs both replication and transcription of the mitochondrial genome. Both NRF-1 and mtTFA are known to be important for mitochondrial biogenesis. Recent evidence supports a role for PGC-1α in the upstream regulation of NRF-1.5 PGC-1α activates NRF-1 gene expression and can also directly coactivate the NRF-1 protein (Figure 3A). Northern blot analysis demonstrated that NRF-1 and mtTFA were both upregulated in response to induction of PGC-1α (Figure 3B). These changes are consistent with induction of the mitochondrial biogenesis program. Interestingly, a reduction in β-actin mRNA expression accompanied these changes, suggesting that the expression of at least some genes encoding structural proteins is downregulated in response to PGC-1α overexpression. Surprisingly, the expression of several known PGC-1α target genes involved in mitochondrial fatty acid oxidation, including medium-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase I, was not increased in tet-on PGC-1α mice. This latter result could be attributable to the known high-level activity of other transcription factors during the postnatal
period, such as PPARα. Forced expression of PGC-1α also led to a diminution in endogenous PGC-1α expression, suggesting an autoregulatory effect.

**Overexpression of PGC-1α in the Adult Mouse Heart Results in Cardiomyopathy and Mitochondrial Ultrastructural Derangements**

Double transgenic adult mice (8 weeks old) from lines A5 and A9 were administered dox for up to 7 weeks. In line A5, overexpression of PGC-1α resulted in 50% mortality over this time period (Figure 4A). No mortality was observed in line A9 during the same time course. To additionally characterize the phenotype in the adult tet-on PGC-1α mice, cardiac structure and function were assessed after 2 or 4 weeks of dox administration. The dox-treated adult tet-on PGC-1α mice developed cardiac hypertrophy and biventricular dilatation (Figure 4B). Echocardiographic analysis of
both transgenic lines showed increased end-diastolic and end-systolic LV diameters and a mild increase in LV wall thickness 2 weeks after induction of PGC-1α overexpression (Table and Figure 4C). In addition, relative wall thickness, as determined by echocardiography, was unchanged in the adult tet-on PGC-1α mice, consistent with eccentric hypertrophy. The mean LV fractional shortening for lines A5 and A9 fell from 64% to ~42% after 4 weeks of dox administration (Figure 4C). As shown in the Table, echocardiographic parameters were not significantly different between the 2 transgenic lines. Thus, the cardiomyopathy in these animals is characterized by biventricular dilatation, eccentric hypertrophy, and reduced systolic ventricular function.

Analysis of gene expression markers after 4 weeks of dox administration revealed changes consistent with heart failure (Figure 4D). Specifically, levels of mRNA encoding both atrial natriuretic factor and brain natriuretic peptide were increased in the ventricles of mice overexpressing PGC-1α. Skeletal α-actin gene expression was also increased, but to a lesser extent, and SERCA2a gene expression was only slightly decreased in the cardiomyopathic hearts. However, there was no significant change in the expression of several known PGC-1α target genes known to be downregulated in the failing heart, such as medium-chain acyl-CoA dehydro-

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**Echocardiographic Analysis of tet-on PGC-1α Mice**

<table>
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<th>Control (10)</th>
<th>Line A5 (7)</th>
<th>Line A9 (7)</th>
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<td>LVDDd, mm</td>
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<td>FS, %</td>
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</table>

Results of echocardiographic studies performed on tet-on PGC-1α and control (αMHC-rtTA) mice administered dox in their drinking water for 4 weeks. Values represent mean±SEM. Data were analyzed by ANOVA followed by the Tukey test. HR indicates heart rate; LVPWd, left ventricle (LV) posterior wall thickness, diastole; IVSd, interventricular septum thickness, diastole; LVDDd, LV internal dimension, diastole; LVPWs, LV posterior wall, systole; IVSs, interventricular septum thickness, systole; LVDDs, LV internal dimension, systole; LVM, LV mass; RWT, relative wall thickness [(LVVPd+IVSd)/LVDDd]; and FS, fractional shortening [100*(LVDDd−LVDDs)/LVDDd]. *P<0.05 compared with controls.
genase and carnitine palmitoyltransferase I (data not shown).

Collectively, these results indicate that although the hypertrophic gene regulatory program is activated in adult tet-on PGC-1α hearts, the expression of certain metabolic genes remains normal, possibly through the influence of chronically activated PGC-1α.

Mitochondrial Ultrastructural Abnormalities in Adult tet-on PGC-1α Hearts

The ultrastructural appearance of the tet-on PGC-1α adult hearts after 2 or 4 weeks of induction was strikingly different from that of the induced neonates. The dramatic expansion of mitochondria that occurred in neonates after 2 weeks of PGC-1α overexpression was not evident in the adult hearts at the same time point, although mitochondrial number was modestly increased in a patchy distribution (Figure 5A). Furthermore, the myofibrillar structure was less disorganized compared with the tet-on PGC-1α neonatal hearts. After 4 weeks of PGC-1α overexpression in the adult heart, mitochondrial number was increased additionally, although still to a lesser extent than in the neonatal hearts, and some myofibrillar degeneration was observed. Mitochondrial proliferation was most apparent in the intermyofibrillar areas, where large aggregates of mitochondria were observed. Adult hearts at both time points exhibited various mitochondrial ultrastructural abnormalities, including vacuoles that seemed to be replacing mitochondria and intramitochondrial alterations ranging from the appearance of small electron-dense particles to numerous large granular inclusions (Figure 5A). Trichrome staining revealed myocyte hypertrophy without significant fibrosis in the hearts from the adult tet-on PGC-1α mice, suggesting that cell death with fibrotic replacement was not the mechanism of disease in these animals (Figure 5B). Caspase staining showed very few apoptotic nuclei, indicating that cell death is not the primary cause of the cardiac phenotype (data not shown).

Cardiomyopathy in Induced Adult tet-on PGC-1α Mice Is Reversible

Double transgenic mice from line A9 were administered dox for 4 weeks and then returned to drinking water for 4 weeks, with echocardiography performed at both time points. As expected, M-mode echocardiograms revealed mild LV dilation and depressed ventricular function 4 weeks after induction (Figures 6A and 6C; online Table 1, available in the online data supplement at http://circres.ahajournals.org). In addition, Doppler recordings of transmitral blood flow velocities and tissue Doppler imaging of mitral annular velocities demonstrated characteristic patterns of diastolic dysfunction with elevated LV filling pressure, as evidenced by increased
function, also resolved. Remarkably, electron microscopy confirmed that after 4 weeks of recovery, most of the abnormalities of mitochondrial and myofibrillar structure returned to normal (Figure 6D).

Discussion

In this report, we describe a transgenic model for inducible, cardiac-specific overexpression of the transcriptional coactivator PGC-1α. Overexpression of PGC-1α promotes cardiac mitochondrial biogenesis in a manner that is markedly influenced by developmental stage; we observed robust mitochondrial proliferation during the neonatal period but only a modest increase in cellular mitochondrial density in adult stages. Moreover, chronic induction of PGC-1α expression in adult mice results in the development of a reversible cardiomyopathy.

In neonatal mice, overexpression of PGC-1α results in a marked increase in cardiac myocyte mitochondrial number and activation of known markers of the biogenic response, including the PGC-1α target NRF-1 and its downstream target mtTFA. Electron microscopic studies of cardiac myocytes of tet-on PGC-1α mice demonstrated a dramatic increase in the number and cellular volume density of mitochondria within 2 weeks of dox administration. The mitochondrial proliferation was associated with a marked widening of the space between myofibrils, consistent with an expansion of the intermyofibrillar mitochondrial fraction. Mitochondrial size was also increased in the neonatal tet-on PGC-1α mice. These results are similar to the original findings by our group and others demonstrating mitochondrial proliferation in the hearts and skeletal muscle of transgenic mice with chronic overexpression of PGC-1α. However, transgenic mice with forced expression of PGC-1α in heart (MHC-PGC-1α mice) die because of the development of cardiomyopathy. Accordingly, in contrast to MHC-PGC-1α mice, the tet-on PGC-1α mice should prove to be a valuable system to investigate the early events in postnatal cardiac mitochondrial biogenesis. Moreover, this system will allow the study of mitochondrial functional capacity at precise temporal points in the mitochondrial biogenic process. Depending on the results of future mitochondrial function studies, this experimental system may prove useful for the evaluation of rescue strategies of energy deprivation states, including ischemic or hypoxic insults.

Surprisingly, the mitochondrial biogenic response induced by PGC-1α is dependent on developmental stage. Although overexpression of PGC-1α in the neonatal heart promotes a robust mitochondrial biogenic response, in adult mice, forced expression of PGC-1α triggers a modest degree of mitochondrial proliferation accompanied by mitochondrial ultrastructural abnormalities and the development of cardiomyopathy. These results suggest that certain components of the mitochondrial biogenesis program may be inactive in the adult cardiac myocyte. In contrast, a recent report demonstrated that chronic overexpression of PGC-1α in skeletal muscle led to an adaptive mitochondrial biogenic response in adult mice. Specifically, overexpression of PGC-1α in skeletal muscle increased oxidative capacity and the fatigue index of muscle. The difference in the PGC-1α response in these two

Figure 6. Cardiomyopathy and myocardial ultrastructural changes in the tet-on PGC-1α mice are reversible. Echocardiography was performed at baseline, after 4 weeks of dox administration, and 4 weeks after dox removal (recovery). A, M-mode images of LV function. B, Doppler measurements of transmitral flow and mitral annular TDI. C, Graphic representation of fractional shortening corresponding to preinduction (Baseline) and 4 weeks of dox (Induced) followed by 4 weeks of dox withdrawal (Recovered). D, Control; □, tet-on PGC-1α; *P<0.05 compared with control at the same time point (ANOVA). D, Electron photomicrographs of cardiac ventricle from control (Baseline), tet-on PGC-1α after 4 weeks of PGC-1α overexpression (4 Week Induction), and tet-on PGC-1α after 4 weeks of dox administration followed by 4 weeks of dox withdrawal (4 Week Recovery). M indicates region of mitochondrial proliferation.
tissues could be attributable to tissue-specific differences in the availability of transcription factor partners for PGC-1α, the state of cellular differentiation, or cell signaling.

The adult tet-on PGC-1α mice developed a cardiomyopathy as early as 2 weeks after initiation of dox. Electron microscopic evaluation of the adult tet-on PGC-1α mice revealed patchy aggregates of mitochondria in the intermyofibrillar spaces. In addition, various mitochondrial ultrastructural abnormalities were observed in the adult mice, including intramitochondrial granules and mitochondrial vacuolar degeneration. Some myofibrillar degeneration was also noted. The mitochondrial ultrastructural abnormalities of the adult tet-on PGC-1α mice were associated with ventricular dilatation and hypertrophy. Surprisingly, the cardiomyopathy of the tet-on mice was reversible, at least during a 4-week time frame; parameters of ventricular systolic and diastolic dysfunction returned to normal after withdrawal of dox. Moreover, mitochondrial ultrastructural abnormalities, including mitochondrial proliferation and myofibrillar degeneration, improved almost to preinduction levels after the 4-week recovery period. Although it was not possible to track the fate of individual mitochondria during the recovery phase, it was clear that most of the mitochondrial ultrastructural abnormalities resolved.

The cardiomyopathic phenotype observed in tet-on PGC-1α mice bears resemblance to human cardiomyopathies caused by genetically determined forms of mitochondrial dysfunction. For example, patients with cardiomyopathy attributable to mutations in mitochondrial DNA often exhibit cardiomyopathy with hypertrophic and dilated features associated with skeletal myopathy. Histological studies of skeletal muscle and heart of such patients often reveal mitochondrial proliferation and an array of mitochondrial ultrastructural abnormalities, likely a compensatory response to the cellular energy deficit caused by mitochondrial dysfunction. However, it is possible that secondary mitochondrial proliferation contributes to the pathogenesis of the cardiomyopathy caused by primary disorders of mitochondrial function. Our results with the adult tet-on PGC-1α mice suggest that chronic activation of the mitochondrial proliferative response can lead to mitochondrial abnormalities and cardiomyopathy. Interestingly, the cellular phenotype of the tet-on PGC-1α mice also resembles the changes reported in skeletal muscle in the adenine nucleotide transporter 1 knockout (Ant1−/−) mouse. In the Ant1−/− model, skeletal muscle displays histological evidence of mitochondrial proliferation similar to the ragged red fibers seen in humans with mitochondrial myopathies. Ant1−/− mice also develop cardiac hypertrophy associated with modest mitochondrial proliferation and alterations in myofibrillar structure. Recently, Exil et al demonstrated that mice lacking very long-chain acyl-CoA dehydrogenase, a mitochondrial fatty acid oxidation enzyme, develop cardiac mitochondrial proliferation in association with increased expression of PGC-1α. Collectively, these results suggest that the conditional expression system for PGC-1α described here may prove useful in the investigation of human mitochondrial diseases and that PGC-1 is a candidate regulatory factor involved in the secondary mitochondrial proliferation of mitochondrial myopathies in humans.

We suggest 3 possible mechanisms for the pathologic phenotype associated with PGC-1α overexpression. First, mitochondrial function may be impaired. Under this hypothesis, the failure to upregulate the expression of a subset of key metabolic enzymes in accord with mitochondrial number could lead to a state of energy depletion. A second possibility is that increased mitochondrial flux could result in the increased production of reactive oxygen species, triggering myocyte injury or apoptosis. The latter seems unlikely, because the changes in cardiac function are reversible. Finally, induction of a metabolic program in the heart may alter expression of structural and sarcomeric proteins. For example, it is possible that the gene regulatory programs driving mitochondrial biogenesis and sarcomeric proteins cannot be simultaneously activated. Given the reversible nature of the cardiomyopathy, we favor the first proposed mechanism, although the potential mechanisms are not mutually exclusive.

In conclusion, we have developed a transgenic model for inducible overexpression of PGC-1α in heart. This mouse model exhibits tightly regulated, nonleaky expression of PGC-1α. Given that the system is comprised of 2 independent transgenes, the TRE-PGC-1α component will allow the evaluation of conditional overexpression of PGC-1α in extracardiac tissues combined with the appropriate tissue specific transactivator system. Short-term induction of the transgene in neonatal mice will be useful for the analysis of the mitochondrial biogenesis program or for rescue of mitochondrial abnormalities. Induction in adult animals results in the development of an inducible cardiomyopathy that should provide a useful model for genetic and acquired forms of human metabolic cardiomyopathy or as a murine model for heart failure.

Acknowledgments

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References

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**Supplementary Table 1. Echocardiography data from 4-week doxycycline induction and 4-week recovery.** Tet-on PGC-1α mice and control littermates were administered doxycycline for 4 weeks. After 4 weeks, the doxycycline was removed, and the mice were allowed to recover for an additional 4 weeks. Echocardiography data were collected at baseline, after 4 weeks of doxycycline, and 4 weeks after removal of doxycycline. Data were analyzed using two-way analysis of variance followed by the Tukey test. * indicates p<0.05 compared to control animals at baseline; † indicates p<0.05 compared to tet-on PGC-1α animals at baseline. Abbreviations: HR, heart rate; LVPWd, left ventricle (LV) posterior wall, diastole; IVSd, interventricular septum, diastole; LVIDd, LV internal dimension, diastole; LVPWs, LV posterior wall, systole; IVSs, interventricular septum, systole; LVIDs, LV internal dimension, systole; LVM, LV mass; LVMI, LV mass index; RWT, relative wall thickness ((LVPWd+IVSd)/LVIDd); FS, fractional shortening.

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<tr>
<td><strong>HR (beats per minute)</strong></td>
<td>577 ± 13</td>
<td>574 ± 4</td>
<td>636 ± 13</td>
<td>601 ± 10</td>
<td>539 ± 10†</td>
<td>670 ± 6†</td>
</tr>
<tr>
<td><strong>LVPWd (mm)</strong></td>
<td>0.82 ± 0.04</td>
<td>0.77 ± 0.01</td>
<td>0.83 ± 0.03</td>
<td>0.87 ± 0.02</td>
<td>0.87 ± 0.02</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td><strong>IVSd (mm)</strong></td>
<td>0.82 ± 0.04</td>
<td>0.82 ± 0.02</td>
<td>0.85 ± 0.00</td>
<td>0.88 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td><strong>LVIDd (mm)</strong></td>
<td>3.01 ± 0.07</td>
<td>3.15 ± 0.08</td>
<td>3.20 ± 0.10</td>
<td>3.14 ± 0.08</td>
<td>3.64 ± 0.06*†</td>
<td>3.34 ± 0.08</td>
</tr>
<tr>
<td><strong>LVPWs (mm)</strong></td>
<td>1.58 ± 0.03</td>
<td>1.57 ± 0.06</td>
<td>1.54 ± 0.02</td>
<td>1.62 ± 0.04</td>
<td>1.44 ± 0.03†</td>
<td>1.63 ± 0.04</td>
</tr>
<tr>
<td><strong>IVSs (mm)</strong></td>
<td>1.58 ± 0.02</td>
<td>1.59 ± 0.06</td>
<td>1.56 ± 0.06</td>
<td>1.65 ± 0.03</td>
<td>1.45 ± 0.03†</td>
<td>1.61 ± 0.05</td>
</tr>
<tr>
<td><strong>LVIDs (mm)</strong></td>
<td>1.12 ± 0.03</td>
<td>1.19 ± 0.04</td>
<td>1.29 ± 0.09</td>
<td>1.22 ± 0.05</td>
<td>2.30 ± 0.08*†</td>
<td>1.36 ± 0.06</td>
</tr>
<tr>
<td><strong>LVM (mg)</strong></td>
<td>75.6 ± 2.8</td>
<td>78.4 ± 2.8</td>
<td>86.6 ± 6.1</td>
<td>89.7 ± 4.5</td>
<td>116.1 ± 3.1*†</td>
<td>98.3 ± 4.7</td>
</tr>
<tr>
<td><strong>LVMI</strong></td>
<td>3.6 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>4.2 ± 0.1*†</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td><strong>RWT</strong></td>
<td>0.54 ± 0.04</td>
<td>0.50 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>0.56 ± 0.02</td>
<td>0.49 ± 0.01</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td><strong>FS (%)</strong></td>
<td>62.3 ± 0.2</td>
<td>62.3 ± 0.3</td>
<td>59.7 ± 1.6</td>
<td>61.3 ± 0.8</td>
<td>36.9 ± 1.6*†</td>
<td>59.3 ± 0.9</td>
</tr>
</tbody>
</table>
Recovery of cardiac function after discontinuation of PGC-1α overexpression. Movie loops of two-dimensional echocardiograms from a tet-on PGC-1α mouse at baseline, after 4 weeks of PGC-1α overexpression, and 4 weeks after PGC-1α overexpression was discontinued.