PGC-1 Coactivators in Cardiac Development and Disease

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Abstract: The beating heart requires a constant flux of ATP to maintain contractile function, and there is increasing evidence that energetic defects contribute to the development of heart failure. The last 10 years have seen a resurgent interest in cardiac intermediary metabolism and a dramatic increase in our understanding of transcriptional networks that regulate cardiac energetics. The PPAR-γ coactivator (PGC)-1 family of proteins plays a central role in these pathways. The mechanisms by which PGC-1 proteins regulate transcriptional networks and are regulated by physiological cues, as well as the roles they play in cardiac development and disease, are reviewed here. (Circ Res. 2010;107:825-838.)

Key Words: PGC-1 ■ metabolism ■ heart failure ■ mitochondria

The heart consumes tremendous amounts of energy. ATP consumption, per weight of tissue, is the highest in the body. Energy reserves in the heart are relatively limited, and a heart starved of its fuel and oxygen supply can only beat 20 to 40 times (a few seconds) before succumbing to energy deficiency. Despite this narrow window, the healthy heart will contract billions of times in the average human life. The dynamic range of cardiac activity is large, both acutely (exercise) and chronically (development, especially postnatal). Bioenergetic programs in the heart must therefore be tightly regulated.

ATP is the currency of energy in the cell. Oxidative consumption of fuels in mitochondria is by far the most efficient means of generating ATP, yielding >30 ATP per molecule of glucose, compared to a net 2 ATP via anaerobic glycolysis and lactate production. It is not surprising then that the heart is highly aerobic and sustains >95% of its ATP output via oxidative breakdown of fuels. Oxidative phosphorylation of ATP occurs strictly in mitochondria, and the heart therefore maintains a high mitochondrial content. The energetic requirements of the heart increase dramatically at birth, and mitochondrial density accordingly increases sharply during the perinatal period.1–3 Mitochondrial mass makes up fully one-third of the adult heart.

The PPAR-γ coactivator (PGC)-1 transcriptional coactivators have recently emerged as powerful regulators of mitochondrial biology in the heart, by broadly regulating gene expression from both nuclear and mitochondrial genomes. The expression of PGC-1α is repressed in numerous models of heart failure, and this has been implicated as an important contributor to the maladaptive energetic profile of failing hearts. This review focuses on the PGC-1s and their role in cardiac biology.

PGC-1 Coactivators

Coactivators are proteins that bind to nuclear receptors or other transcription factors and increase their ability to stimulate transcriptional activity. Most transcription factors likely require coactivators. A subset of coactivators are highly regulated and transduce extra- and intracellular cues to
changes in gene expression. PGC-1α is the best-studied example of such a regulated coactivator.

PGC-1α was originally identified in a 2-hybrid screen, using a cDNA library from brown fat cells, looking for brown fat–specific interactors of the nuclear receptor PPARγ (the target of the antidiabetic agents thiazolidinediones).4 Ectopic expression of PGC-1α in white fat cells conferred on them some of the properties of brown fat, including induction of uncoupling protein (UCP)1, a mitochondrial uncoupler and dissipator of heat. Since then, it has become clear that PGC-1α can coactivate a large repertoire of transcription factors, including most members of the nuclear receptor family.5,6 On binding to transcription factors, PGC-1α acts directly with the core transcriptional Trap/Mediator complex,7 as well as with chromatin-modifying enzymes such as the p300 and SRC-1 histone acetyl transferases,8 and with specific factors like MEF2,24,25 calcineurin signaling,11,26,27 metabolic sensors like the AMP-activated protein kinase (AMPK),28 nitric oxide,29–31 p53, calcium/calmodulin-dependent protein kinase,27,32 and autoregulatory positive feedback by PGC-1α itself.37 PGC-1α expression is thus affected by numerous signals, integrating important metabolic and neurohormonal states (Figure 2).

PGC-1α is also significantly regulated posttranslationally. PGC-1α protein has a relatively short half-life (20 minutes) and is ubiquitylated and degraded by the proteasome.33–35 p38 mitogen-activated protein kinase phosphorylates PGC-1α on 3 conserved sites and inhibits its degradation.33 AMPK also directly phosphorylates PGC-1α protein, stimulating its transactivation activity.28 Conversely, phosphorylation by the Akt kinase inhibits PGC-1α activity.36 In addition, 13 conserved arginines in PGC-1α are sites of inhibitory acetylation by the GCN5 acetyl transferase (and likely other acetyl transferases as well). Deacetylation and reactivation of PGC-1α is mediated by the longevity-associated, NAD-dependent Sirt1 type III histone deacetylase.37–39 PGC-1α is also methylated40 and can be modulated by various protein–protein interactions, including with p160myb, bcl3, prox1, and lipidin.41–44 PGC-1α protein therefore directly senses key metabolic states, including ADP/ATP levels (AMPK), redox state (Sirt1), and stress pathways (p38). Most of the studies demonstrating these
biochemical modifications were not performed in cardiac cells, but it is likely that the same modifications also occur in cardiomyocytes. Posttranslational modifications of PGC-1β and PRC have been much less extensively studied.45 The PGC-1 proteins, and PGC-1α in particular, are thus sensitive to a vast range of signals and are well-suited to transmit extracellular and physiological cues to the regulation of broad genetic programs. A variety of metabolic programs in different tissues are regulated by PGC-1 coactivators, including responses to fasting and high-fat feeding in liver,14,15,46 exercise-induced changes in skeletal muscle,21,22,47,48 oxidative insults in the brain,49 and the thermogenic response to cold in brown fat.4 This review focuses on the PGC-1s in the heart, where PGC-1α and -β play critical roles in maintaining energy balance.

**PGC-1 Transcriptional Networks in the Heart**

Overexpression of PGC-1α in cardiac cells induces hundreds of genes, encoding for key enzymes in all major metabolic programs needed for high-efficiency ATP production (Figure 3). More than 70% of the subunits of the 4 respiratory chain and the ATPase complexes are induced by PGC-1α, as are all 8 enzymes of the Krebs cycle. All key enzymes in fatty acid metabolism are increased by PGC-1α, as are all 8 enzymes of the Krebs cycle and many of the other enzymes required for high-efficiency ATP production. The PGC-1α protein is therefore critical for maintaining energy balance in the heart.

**Figure 2. Transcriptional networks and regulation of PGC-1α.** Multiple stimuli activate PGC-1α, leading to the coactivation of key transcription factors involved in fatty acid oxidation and import, angiogenesis, electron transport chain assembly, membrane biogenesis, and mitochondrial DNA replication and transcription.

**Figure 3. Substrate utilization in the heart and metabolic enzymes induced by PGC-1α.** Neonatal rat ventricular myocytes (NRVM) were infected with adenovirus expressing PGC-1α vs GFP control, and 48 hours later, gene expression was measured by quantitative PCR. Metabolic pathways are noted in green. Induced genes are noted in red boxes. ATPase indicates acetyl-CoA acetyltransferase; ACO, aconitase; ALDA, aldolase A; ANT, adenine nucleotide translocator; BDH, β-hydroxybutyrate dehydrogenase; CD36, fatty acid translocase; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; CS, citrate synthase; CT, carnitine translocase; DGAT, diacylglycerol O-acyltransferase; ENO, enolase; FABP, fatty acid binding protein; FATP, fatty acid transport protein; FH, fumarase; GAPD, glyceraldehyde-3-phosphate dehydrogenase; GLUT4 indicates glucose transporter 4; FATP, fatty acid transport protein; FATP, fatty acid transport protein; FH, fumarase; GAPD, glyceraldehyde-3-phosphate dehydrogenase; GLUT4 indicates glucose transporter 4; GPI, glucosephosphate isomerase; GYS, glycogen synthase; HK, hexokinase; I, complex I; IDH, isocitrate dehydrogenase; II, complex II; III, complex III; IV, complex IV; LCFA, long-chain fatty acid; LDH, lactate dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; MCFA, medium-chain fatty acid; MCT, monocarboxylic acid transporter; MDH, malate dehydrogenase; OGDH, α-ketoglutarate dehydrogenase; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; PGAM, phosphoglycerate mutase; SCAD, short-chain acyl-CoA dehydrogenase; SDH, succinate dehydrogenase; SUCL, succinate-CoA-ligase; TPI, triosephosphate isomerase; V, complex V; VLCAD, very long-chain acyl-CoA dehydrogenase.
β-oxidation are induced, including fatty acid transport proteins and enzymes responsible for turnover of the intracellular triglyceride pool. Enzymes that mediate metabolism of lactate and ketone bodies are also induced. Induced proteins are located both in the mitochondria and in the cytosol. Overexpression of PGC-1α in cardiomyocytes in cell culture and in vivo markedly increases oxygen consumption capacity and fatty acid oxidation. Glucose oxidation is repressed, likely secondary to both product inhibition by fatty acids and ketones on the PDH (pyruvate dehydrogenase complex), as well as induction of pyruvate dehydrogenase kinase (PDK)4, a potent inhibitor of PDH activity. PGC-1α thus coordinates a complete program that allows cardiac cells to markedly increase fuel consumption and output of ATP. PGC-1α carries out this remarkable transcriptional induction by coactivating key families of transcription factors, each of which predominantly regulates specific subsets of genes involved in cardiac metabolism, as discussed below (Figure 2 and Table).

**Mitochondrial Biogenesis**

Constitutive overexpression of PGC-1α in the heart of intact mice, under control of the cardiac-specific α-myosin heavy chain (α-MHC) promoter, leads to profound mitochondrial biogenesis, to the point of significant replacement of myofibrillar apparatus with mitochondrial matrix. This was the first unequivocal demonstration that PGC-1α can activate cardiac mitochondrial biogenesis in vivo. Heart failure developed early in these animals, perhaps as a consequence of myofibrillar displacement. Tetracycline-inducible PGC-1α overexpression, again under control of the α-MHC promoter, was subsequently generated to test the role of PGC-1α specifically in adult cardiac muscle. Induction of PGC-1α shortly after birth recapitulated the findings with constitutive expression: profound mitochondrial proliferation and ensuing heart failure. Remarkably, these dramatic changes were completely reversed by subsequent repression of the PGC-1α transgene. Hence, PGC-1α, in this context, was needed for both induction and maintenance of increased mitochondrial mass.

Interestingly, induction of PGC-1α in adult mouse hearts did not activate mitochondrial proliferation, indicating that the perinatal period is uniquely permissive for PGC-1α-induced mitochondrial proliferation. Why this is remains unclear. Despite the absence of increasing mitochondrial mass, cardiac dysfunction still occurred in the adult mice, revealing the existence of other cardiotoxic consequences of supraphysiologic doses of PGC-1α. On the other hand, mild cardiac overexpression of PGC-1α, seen in transgenic mice that express PGC-1α under control of the MCK (muscle creatine kinase) promoter (Arany, unpublished results, 2010), or in BAC PGC-1α transgenic mice, do not develop heart failure, indicating that tempered PGC-1α overexpression in the heart is not toxic. It will be of interest to determine whether this mild overexpression of PGC-1α in the heart can be beneficial under conditions of cardiac stress.

Transcriptional regulation of mitochondrial biogenesis and nuclear genes encoding respiratory chain subunits has been extensively studied and reviewed elsewhere. The nuclear respiratory factor (NRF) and estrogen-related receptor (ERR) families of transcriptional factors are key players in the induction of these genes, and PGC-1α modulates mitochondrial biogenesis by directly coactivating these transcription factors. Binding sites for the NRF-1 monomer and NRF-2 heterotrimer (also known as GABP [GA-binding protein]) are found in the promoters of most respiratory chain genes. The effect of overexpressing either NRF-1 or NRF-2 in cardiac tissue has not been evaluated to date, but targeted overexpression of NRF-1 in skeletal muscle did increase genes of oxidative phosphorylation (OXPHOS). PGC-1α physically interacts with both NRF-1 and -2 and stimulates their activity on mitochondrial genes.

The ERRs are orphan nuclear receptors, named for their sequence similarity to the estrogen receptor (although they are not thought to bind to estrogen). Structural studies suggest that the mechanism of transcriptional activation by ERRs may differ from that of other nuclear receptors, and that ERRs may have no natural ligands. All 3 ERRs (α, β, and γ) are highly expressed in oxidative tissues, such as heart, muscle, and kidney. Most of the genes induced by PGC-1α also contain ERR-binding sites. ERRα has been studied most extensively. Overexpression of ERRα in rat neonatal cardiomyocytes results in strong induction of genes involved in glucose utilization (eg, PDK4, HK2, GLUT4), fatty acid oxidation

<table>
<thead>
<tr>
<th>Transcription Factor or Coactivator</th>
<th>Gain-of-Function Cardiac Phenotype</th>
<th>Loss-of-Function Cardiac Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>↑FAO, lipotoxicity, HF-induced CM, ↓tolerance to I/R</td>
<td>Mild. Resistance to I/R, ↓work capacity</td>
<td>83–87, 192</td>
</tr>
<tr>
<td>PPARβ/δ</td>
<td>↑Glucose utilization, resistance to I/R, ↑glycogen content</td>
<td>↓FAO, lipotoxic CM, early lethality</td>
<td>81, 89, 90</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Lipotoxic CM</td>
<td>Cardiac hypertrophy</td>
<td>91, 92</td>
</tr>
<tr>
<td>ERRα</td>
<td>ND</td>
<td>↓Respiratory efficiency, ↓PCr, TAC-induced CM</td>
<td>154</td>
</tr>
<tr>
<td>ERRγ</td>
<td>ND</td>
<td>Perinatal lethality</td>
<td>68, 69</td>
</tr>
<tr>
<td>TFAM</td>
<td>Protects from MI-induced heart failure</td>
<td>Spontaneous CM</td>
<td>77–79</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>↑Mitochondrial proliferation, reversible CM</td>
<td>Energy deficiency (↓ATP, ↓PCr), TAC-induced CM, ↓work capacity</td>
<td>16, 49, 52, 153, 157</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>ND</td>
<td>Mild energy deficiency; perinatal lethality and mitochondrial dysfunction when combined with PGC-1α KO</td>
<td>163–165</td>
</tr>
</tbody>
</table>

CM indicates cardiomyopathy; FAO, fatty acid oxidation; HF, heart failure; I/R, ischemia/reperfusion; MI, myocardial infarction; ND, not determined; PCr, phosphocreatine; TAC, transverse aortic constriction.
(MCAD, CD36), and the OXPHOS program (ATP5b, CYCS).63 PGC-1α binds to and coactivates PPARα in cardiomyocytes, as well as other cells.59,64,65 The mRNA expression of ERRα is also induced by PGC-1α, and ERRα also induces PPARα (see below),63 underscoring the multiple positive-feedback loops that drive metabolic transcription pathways in the heart. Germline disruption of ERRα results in significant declines in the expression of genes involved in OXPHOS and fatty acid and glucose metabolism.

In contrast to ERRα, less is known of the role of the other ERRs in cardiac tissue. Genome-wide Chip-chip studies provided evidence that ERRγ and ERRα share many targets in cardiac tissue,66 and targeted overexpression of ERRγ in skeletal muscle induces genes of fatty acid oxidation and OXPHOS (although interestingly without leading to frank increases in mitochondrial density).67 Germline disruption of ERRγ resulted in mice that die postnatally, which was attributed in part to a cardiac defect.58,68 Thus, ERRγ may have some redundancy with ERRα in vivo but also plays a separate and vital role for survival. Germline disruption of ERRβ is embryonic lethal,69 resulting from placental defects. The contribution of ERRβ to cardiac physiology is thus currently unknown. To date no gain-of-function mouse models have been developed to address the effects of overexpressing ERRs within cardiac tissue.

PGC-1α also indirectly modulates the mitochondrial genome, in addition to its direct regulation of nuclear genes. Although >98% of the >1500 genes that encode for mitochondrial proteins are encoded by the nucleus, mitochondria do contain their own genome, encoding for 22 tRNAs, 2 rRNAs, and 13 proteins that contribute to complexes I and III to V of the electron transport chain (but not II). Mitochondrial biogenesis thus requires replication of mitochondrial DNA and the coordinated transcription of the mitochondrial genome. Crosstalk between the nuclear and mitochondrial genomes is carried out, at least in part, via the nuclear-encoded proteins TFAM (transcription factor A, mitochondrial), TFB1M, and TFB2M.71,72 The genes for all 3 of these proteins are induced by PGC-1α, via the induction and coactivation of NRF-1 and NRF-2.73,74 TFAM, a nuclear-encoded high-mobility group transcription factor, is responsible for both the replication and transcription of mitochondrial DNA.75,76 Targeted disruption of TFAM specifically within cardiac tissue resulted in a significant decrease in electron transport capacity, spontaneous cardiomyopathy, and heart failure.77,78 Conversely, increasing the expression of TFAM within cardiac tissue offered protection from heart failure induced by myocardial infarction.79 These observations underscore the importance of maintaining mitochondrial integrity and copy number as a protection against heart disease.

**Fatty Acid Import and Utilization**

Mitochondrial function also requires various ancillary, non-mitochondrial programs, such as the transport and initial breakdown of nutrients. Normal adult cardiac muscle relies primarily on fatty acids as an energy source. Interestingly, most fatty acids are shuttled through the intracellular triglyceride pool before being transported to mitochondria for oxidation.50 Fatty acid import, shuttling, and oxidation must thus be coordinated, under both basal and stimulated conditions. Overexpression of PGC-1α in cardiomyocytes in cell culture and in vivo markedly increases fatty acid oxidation.16 The PPAR family of transcription factors plays a prominent role in this process. There are 3 isoforms of PPARs (α, β, δ, and γ), all of which form heterodimers with retinoic acid–activated receptors (RXRs) to bind DNA. Fatty acids and their catabolic byproducts act as low/medium-affinity ligands for PPARs, thus likely transmitting information about the intracellular lipid milieu to gene regulation. Natural, high-affinity, physiological ligands for the PPARs have yet to be identified and might not exist. On the other hand, high-affinity pharmacological agents that can modulate the activity of the PPARs exist, including fibrates for PPARα and thiazolidinediones for PPARγ.80

PPARα has been studied more extensively in cardiac tissue than the other PPARs. PPARα expression is high in tissues that consume fatty acids (heart, brown adipose tissue, kidney, liver). PGC-1α binds to, and coactivates PPARα in cardiac cells, thereby inducing numerous genes critical for fatty acid handling, including CD36 (import into cell), CPT1b (import into mitochondria), PDK4 (reciprocal inhibition of pyruvate entry into mitochondria), and MCAD (rate-limiting step in medium chain fatty acid β oxidation).81,82 Hearts of PPARα-null mice exhibit a decrease in fatty acid oxidation and concomitant increase in glucose oxidation, coincident with decreased expression of fatty acid oxidation genes.83,84 The animals showed improved tolerance to ischemia/reperfusion injury ex vivo, likely, in part, because of decreased fatty acid oxidation/import.85 Conversely, overexpression of PPARα within cardiac tissue, using the MHC promoter, resulted in induced fatty acid oxidation and repressed glycolysis.86,87 High expression of PPARα in the heart led to spontaneous left ventricular dysfunction and lipotoxicity,86 suggesting that increases in fatty oxidation were insufficient to match the increase in fatty acid uptake. Consistent with this notion, feeding a chow enriched in long-chain fatty acids to lower-expressing transgenic animals also precipitated cardiac dysfunction.86,87 Crossing PPARα overexpressing mice with mice lacking cardiac CD36 or lipoprotein lipase, both of which are critical for import of fatty acids into cardiomyocytes, rescued the cardiac toxicity of PPARα overexpression.82,88 Together, these data support the notion that PPARα overexpression leads to intracellular lipid accumulation that is toxic to cardiac function.

PPARβ/δ has also been relatively well studied in the heart. Overexpression of PPARβ/δ in neonatal cardiomyocytes, or transgenic overexpression in the heart, induced genes involved in fatty acid oxidation, and this was further enhanced with the addition of PPARβ/δ ligands.51,89 In contrast to PPARα overexpressors, however, the MHC-PPARβ/δ hearts exhibited increased glucose utilization, did not develop cardiomyopathy even on high fat diet, and were resistant to myocardial infarct. The difference between these 2 transgenics may reflect the lack of induction of fatty acid import genes (eg, FATP, CD36, ACS, FAS) in the PPARβ/δ overexpressors,84 thus averting toxic accumulation of intracellular lipids. Conversely, cardiac-specific disruption of PPARβ/δ led to a decrease in genes involved in fatty acid oxidation90 but also...
increased cardiac lipid accumulation and lipotoxic cardiomyopathy. Again, this contrast with the PPARα knockout (KO) mice is probably the result of unchanged fatty acid import in the PPARβ/δ mice, in combination with decreased fatty acid oxidation.

The third PPAR, PPARγ, is expressed at substantially lower levels in the heart than in other tissues like fat and liver, and its role in the heart remains somewhat controversial. Clinical modulation of PPARγ activity with thiazolidinediones can reduce fatty acid oxidation in the heart, but this likely occurs in large part indirectly by sequestering fatty acids in fat depots and reducing the exposure of the heart to circulating fatty acid levels. Nevertheless, cardiomyocyte-specific deletion of PPARγ does result in cardiac hypertrophy, indicating an important role for PPARγ in these cells. Overexpression of PPARγ under the control of the αMHC promoter resulted in increased lipid uptake and oxidation in the heart, increased expression of glucose metabolism genes, and systolic dysfunction. PGC-1α is a potent activator of PPARγ activity in brown fat biology, and this is likely true in cardiomyocytes as well.

Angiogenesis

The vasculature transports oxygen and nutrients and thus plays a critical role in mitochondrial metabolism (Figure 4). The heart is highly vascular, consistent with its high oxidative and metabolic needs. Recent findings in skeletal muscle have implicated PGC-1α in the regulation of blood vessel formation. PGC-1α expression is induced by ischemia-like conditions in cell culture, and in turn PGC-1α activates a broad program of angiogenic factors, including vascular endothelial growth factor (VEGF). The induction of VEGF occurs independently of the canonical hypoxia-inducible factor (HIF) pathway and instead is mediated by coactivation of ERRα on a novel enhancer in the first intron of the VEGF gene. Transgenic overexpression of PGC-1α in skeletal muscle induces robust angiogenesis and accelerates recovery of blood flow after surgically induced hindlimb ischemia, demonstrating the functional capacity of newly formed vessels. Endurance exercise induces both mitochondrial proliferation and new blood vessel formation in skeletal muscle, and is one of the few examples of physiological angiogenesis in adult tissues. PGC-1α is strongly induced in skeletal muscle by exercise in rodents and humans, likely, in part, by adrenergic activation and induction of the alternative promoter of PGC-1α discussed above. Mice lacking PGC-1α specifically in skeletal muscle fail to expand their vascular network in response to exercise, demonstrating that PGC-1α mediates exercise-induced angiogenesis. PGC-1α thus coordinates the delivery of oxygen and nutrients to myocytes (angiogenesis) with their transport across the cell and their consumption in mitochondria. It will now be of great interest to determine whether PGC-1α plays a similar role in the heart. Microvascular rarefaction has recently been implicated in the development of heart failure. Decreased activity of PGC-1α may thus be contributing to this decrease in vessel density and subsequent heart failure.

NRFs, ERRs, and PPARs thus form together a coordinated transcriptional network orchestrated by PGC-1α to regulate energy homeostasis within cardiac tissue. Each transcription factor controls a subset of genes, encompassing specific metabolic pathways. PGC-1α orchestrates these various metabolic pathways by directly regulating each of the transcription factors. A large number of other transcription factors can also be bound by PGC-1 coactivators, including MEF2 and FOXO1 family members, and most nuclear receptors. These factors have mostly not been studied in the context of cardiac metabolism. As outlined in the previous section, PGC-1α is also exquisitely sensitive to various physiological cues, both at the level of mRNA expression and posttranslational modification. PGC-1α thus likely acts as an integrator of metabolic and neurohormonal information and uses that information to appropriately modulate metabolic pathways in cardiomyocytes (Figure 2). It will be of great interest to investigate whether, and how, upstream signals can confer specificity on PGC-1α and differently influence its pleiotropic downstream pathways.

PGC-1β has been investigated less extensively but likely shares many of the properties of PGC-1α. NRFs, PPARs, and ERRs can all be targeted by PGC-1β. Certain posttranslational modifications seen on PGC-1α, including acetylation and deacetylation, are also present on PGC-1β. Pathways downstream of PGC-1β appear to be quite similar to those of PGC-1α, although important subtleties can be discerned. For example, overexpression of PGC-1β in skeletal myotubes leads to increased respiration, as does PGC-1α, but less proton leak is seen than with PGC-1α. How this occurs remains unclear. As noted above, PGC-1β gene expression is generally less easily modulated than that of PGC-1α. PRC has been much less investigated (in fact not at all in cardiomyo-
cytes), but PRC can coactivate NRFs,10 and knockdown of PRC by small interfering RNA in human osteosarcoma cells significantly inhibits mitochondrial genes and activity,105 suggesting that the same might be true in cardiac cells or tissue.

PGC-1s and Cardiac Energetic Failure
Human genetic diseases poignantly demonstrate the impact of energetic deficiencies on cardiac function. More than 50% of disease-causing mutations in mitochondrial DNA lead to cardiomyopathy in humans,68,106–114 many in the context of complex syndromes like Kearns–Sayre and Leigh syndromes. Mutations in nuclear-encoded genes of the respiratory chain also lead to cardiac dysfunction.115–119 In addition, genetic defects in mitochondrial fuel handling, most notably in fatty acid transport and β-oxidation, can also lead to profound cardiomyopathy.116,120–124 The use of genetic manipulations in mice has extensively supported these clinical observations. Targeted mutations affecting processes including fatty acid transport and oxidation, high-energy phosphate transport and shuttling, protection from mitochondrial reactive oxygen species (ROS), and mitochondrial DNA proofreading activity, all lead to often profound cardiac dysfunction.76,78,123,125–130

These observations leave little doubt, therefore, that defects in mitochondrial function can lead to heart disease. Most cases of heart failure, however, are not caused by rare genetic mutations. Mounting evidence points to the "generically" failing heart, of diverse etiologies, as also energy-starved.109–111,131 ATP concentrations are decreased by up to 25% in failing hearts.110,132,133 Normally, cardiomyocytes tightly maintain their ATP concentration, and a 25% decrease in ATP marks advanced disease, much like elevated glucose levels do in diabetes. This “snapshot” value also likely belies much more profound decreases in fluxes of ATP generation, as well as increases in ADP (and thus loss of the phosphorylation potential, the driving force for ATPases). Phosphocreatine/ATP ratios, a measure of high-energy phosphate-buffering capacity and an indirect measure of ADP, is decreased as much as 60% in heart failure.110 and ATP flux through the creatine kinase reaction has also been shown to be reduced.134 In fact, the phosphocreatine/ATP ratio is a better predictor of cardiovascular mortality than is ejection fraction.135 The efficiency of ATP production, as defined by ATP flux/oxygen consumption, also declines in heart failure.136

How does the failing heart become energy starved? Acquired defects in mitochondrial respiration likely play a large part. Mitochondrial dysfunction is seen in human cardiomyopathy134,137–141 and in most animal models of heart failure.77,142–145 Mutations in mitochondrial DNA have been demonstrated in humans after treatment with cardiototoxic therapies like doxorubicin or nucleoside reverse transcriptase inhibitors112,146 and in rodents after myocardial infarction.142 Replication of the mitochondrial genome is blunted in human heart failure.147 In addition, declines in the expression of numerous nuclear genes encoding for mitochondrial proteins are seen in both human heart failure and in rodent models.148–151 Therefore, there appears to be a coordinated downregulation of mitochondrial pathways during heart failure. How this occurs, however, is not clear. The notion that dysregulation of PGC-1 coactivators may play a role was first suggested by the observation in rodents that the expression of PGC-1α is reduced in pressure overload–induced cardiac hypertrophy.149 Since that initial observation, repression of PGC-1α has been seen in a number of rodent models of cardiac hypertrophy or failure,152–156 suggesting that decreased PGC-1α is a common signature of acquired cardiac disease. The consequences of decreased PGC-1 activity in the heart have been investigated extensively, using genetically modified mouse models. Two groups generated PGC-1α KO mice, with slightly different results. Spiegelman and colleagues reported pronounced brain defects in PGC-1α−/− mice, with a strong sensitivity to neurotoxins, possibly attributable to decreased protection against ROS.49,157 At baseline, these animals did not exhibit an overt cardiac phenotype, and the hearts had normal mitochondrial content.158 However, gene expression analyses did reveal reactivation of “embryonic” markers, including ANP, BNP, and β-MHC, suggesting the presence of cardiac dysfunction. Nuclear magnetic resonance studies revealed pronounced decreases in ATP concentrations, suggesting significant decreases in energy reserves. Consistent with this, PGC-1α−/− hearts were unable to increase work output in response to inotropic stimulation.158 With aging, the animals spontaneously developed mild cardiac dysfunction. More strikingly, hemodynamic challenge in the form of transverse aortic banding led to pronounced cardiac failure in PGC-1α−/− mice.153 Cardiac failure only occurred after 2 months, indicating that PGC-1α is dispensable for the acute response to aortic banding, but it is critical for maintaining the subsequent compensated state. Interestingly, ERRα−/− mice revealed a very similar phenotype after transverse aortic banding: impaired energetics, including decreased levels of phosphocreatine and ATP, and the progressive development of heart failure,154 underscoring the central role that ERRα plays in PGC-1α biology.

The PGC-1α−/− mice generated by the Kelly group did not display the same extensive neurological defects described above.159 This difference may stem from the retention in these mice of alternatively splice forms of PGC-1α. For example, a short form of PGC-1α that is highly expressed in the brain was recently described.160,161 Nevertheless, cardiac inotropic and chronotropic responses to exercise were both blunted in these PGC-1α−/− mice, and cardiac hemodynamics measured in isolated working hearts demonstrated mild but significantly decreased cardiac work and output. A subsequent meticulous examination of cardiac fuel preference and ATP-producing capacity in these PGC-1α−/− hearts revealed significant defects.162 Permeabilized myocardial fibers isolated from PGC-1α−/− hearts had reduced fatty acid oxidation, decreased ATP synthesis rates, and blunted efficiency (ie, ATP produced per oxygen consumed). Isolated working hearts from PGC-1α−/− mice had decreased fatty acid oxidation and diminished cardiac power. Structural studies showed abnormal mitochondrial cristae density and cytoplasmic accumulation of neutral lipids, suggesting that the reduction in fatty acid consumption was not matched by a reduction in fatty acid import.

Both PGC-1α−/− models thus show significantly impaired bioenergetics and moderate defects in cardiac function, which are markedly worsened by hemodynamic and metabolic...
challenges. PGC-1α is thus felt to be critical for myocardial metabolic flexibility. Interpretations from both of these mouse models must be tempered by the fact that PGC-1α was absent in the entire body and that PGC-1α clearly has pleiotropic roles in numerous extracardiac tissues. Cardiac-specific deletion of PGC-1α has not yet been reported.

The cardiac phenotype of PGC-1β−/− mice appears to be significantly less pronounced, although it has been studied less extensively. Three groups have generated mice either lacking or bearing deletions in PGC-1β.163–165 Kelly and colleagues reported mild reductions in cardiac mitochondrial density in PGC-1β−/− mice, and a mild chronotropic defect in response to dobutamine stimulation in vivo. Cardiac contractility appeared normal.163 The other 2 groups reported no cardiac abnormalities,164,165 and the response of PGC-1β−/− mice to hemodynamic stresses like transverse aortic constriction have not been reported.

Despite the mild phenotype of PGC-1β−/− mice, the importance of PGC-1β in cardiac energetics was made clear by the simultaneous deletion of both PGC-1α and -β. PGC-1α and PGC-1β both powerfully induce mitochondrial biogenesis104 and regulate sets of genes that largely coincide. The coactivators thus provide extensive redundancy. To test this notion directly, Kelly and colleagues generated PGC-1α/β−/− double KO mice.103 Almost all double KO mice died in the immediate postnatal period, with evidence of significant cardiac dysfunction. Mice bearing total-body deletion of PGC-1α but only cardiac-specific deletion of PGC-1β also died perinatally with the same cardiac phenotype, elegantly demonstrating that the cause of death in these animals was cardiac dysfunction. Heart from double KO animals had markedly diminished mitochondrial mass and density.103

Interestingly, hearts from PGC-1α/β double KO animals had apparently normal mitochondrial mass until late in gestation. PGC-1α and -β are thus both largely dispensable for mitochondrial biogenesis during prenatal development. Marked proliferation of cardiac mitochondria normally occurs immediately after birth, both in rodents and larger animals, coinciding with increasing workload and a switch to fatty acid consumption at birth. At the same time, there is a marked induction of PGC-1α expression.2,16 PGC-1α and -β likely mediate this event together, because no increase in cardiac mitochondrial proliferation is seen in the double KO mice. Postnatal mitochondrial proliferation is thought to be mediated, in large part, by thyroid hormone signaling.166,167 and PGC-1α and -β can coactivate the thyroid hormone receptor,168 suggesting a likely, but untested, mechanism.

In addition to changes in mitochondrial respiratory function, cardiomyopathy and heart failure are also typically marked by important shifts in fuel use. The heart is a fuel omnivore, capable of consuming glucose, fatty acids of various lengths, lactate, pyruvate, ketones, and amino acids (Figure 3). During fetal development, when oxygen tension and fatty acid levels are lower, the heart primarily consumes glucose and lactate. Shortly after birth, coincident with sharp increases in cardiac work and with lactation, numerous genes of fatty acid transport and oxidation are induced, and cardiac substrate use largely switches to fatty acids.1,3 The adult heart generates 60% of its ATP from oxidation of fatty acids, primarily long chain. It is likely that the PGC-1α mediate, in large part, this perinatal switch in substrate use, given the strong coactivation of PPARs by PGC-1α on genes of fatty oxidation and the pronounced induction of PGC-1α expression at birth, although this remains to be formally proven. Conversely, cardiac hypertrophy and heart failure, of almost any cause, are marked by an incomplete “switch” back to glucose consumption and away from fatty acid oxidation.141,169 An increasing level of evidence points to this switch as an important adaptive response.141 Again, declines in PGC-1 activity during cardiac remodeling may contribute to this substrate switch. Interestingly, some cardiac pathologies, such as that associated with diabetes, have an early increase in fatty acid use, before the development of frank systolic dysfunction. Whether a transient increase in PGC-1 activity contributes to this observation is not known.

Taken together, these studies demonstrate the key role that PGC-1α and -β play in regulating mitochondrial mass, oxygen consumption, respiratory efficiency, and fatty acid oxidation in the normal heart. Overall, the coactivators appear dispensable for developmental processes, at least until shortly before birth; instead, they appear critical for environmental and physiological modulation of cardiac energetic balance. PGC-1α likely, for example, mediates exercise-induced mitochondrial proliferation in the heart (although this has not yet been formally tested). Conversely, a substantial body of evidence suggests that PGC-1α downregulation during cardiac failure may play an important maladaptive role.

**PGC-1α in the Vasculature**

In addition to its role in myocytes and their communication with the vasculature, PGC-1α appears to also have important functions in the vascular wall itself. Endothelial cells mediate local tissue homeostasis by regulating blood flow, coagulation, metabolic exchange between the circulation and tissue, and trafficking of inflammatory cells. Endothelium dysfunction is an early feature of chronic cardiovascular diseases170 and is usually associated with excess levels of ROS.171 Antioxidant pathways are thus critical for protection from endothelial dysfunction. A number of proteins can decrease ROS, either by limiting ROS production, such as the mitochondrial uncoupling proteins (UCP2 and -3) and the adenine nucleotide translocator (ANT), or by directly scavenging ROS, such as manganese superoxide dismutase (MnSOD), catalase, peroxiredoxin (Prx)3, Prx5, and thioredoxin 2 (Trx2). PGC-1α directly regulates this anti-ROS program, thus offsetting the increase in ROS production that would otherwise occur with mitochondrial biogenesis.49 Overexpression of PGC-1α in endothelial cells induces the expression of MnSOD, catalase, Prx3, Prx5, Trx2, and ANT,172,173 likely in part via coactivation of the O subfamily of Foxo3α (forkhead transcription factor 3a).174 Overexpression of PGC-1α in endothelial cells reduces levels of ROS and rescues ROS-mediated mitochondrial toxicity and cellular apoptosis.172,173,175 Activation of AMPK in endothelial cells also prevents oxidative cell injury through a PGC-1α-mediated mechanism.175 In intact animals, chronic angiotensin II administration induces endothelial dysfunction, as measured by reduced endothelium-dependent relaxation to...
acetycholine, and this was reversible by activation of AMPK in wild-type but not in PGC-1α−/− mice.175 The reduction of ROS by PGC-1α in endothelial cells is also associated with reduced expression of chemokines and adhesion molecules including vascular cell adhesion molecule (VCAM)-1 and monocyte chemotactrant protein-1, as well as reduced activity of the redox-sensitive transcription factor nuclear factor κB activity, suggesting a role of PGC-1α in endothelium-related inflammation.176 Finally, shear stress, a potent beneficial stimulus in endothelial cells, appears to induce PGC-1α, possibly through SIRT1 (silent mating type information regulation 2 homolog).177 Together, these data strongly suggest that PGC-1α plays an important role in endothelial redox homeostasis.

The vascular wall also contains vascular smooth muscle cells (VSMCs) and pericytes, which surround endothelial cells and contribute significantly to the function of the vasculature. PGC-1α is expressed in VSMCs and can be upregulated by AMPK activators.176 Conversely, treatment of VSMCs with angiotensin II activates Akt, which phosphorylates PGC-1α on serine 570 and inhibits it,36 leading to repression of PGC-1α-dependent genes like catalase. Overexpression of PGC-1α in these cells inhibits angiotensin II–induced increases in ROS and [3H]leucine incorporation, markers of vascular hypertrophy.178 Overexpression of PGC-1α in VSMCs also reduces tumor necrosis factor–induced ROS generation, VCAM-1 and monocyte chemotactrant protein-1 level, and nuclear factor κB activity.179 Migration and proliferation of VSMCs are also key components of intimal hyperplasia following endovascular injury, such as after coronary stenting. Overexpression of PGC-1α significantly inhibits migration of VSMCs, whereas knockdown of PGC-1α enhances migration. In a carotid balloon injury rat model, PGC-1α overexpression inhibited neointimal formation, likely through upregulation of SOD2.179

There is thus a burgeoning understanding of the role of PGC-1α in endothelial and perivascular cells, with a strong indication that PGC-1α regulates redox homeostasis in these cells. No data are available on PGC-1β or PRC, and no vascular-specific mouse models of PGC-1 coactivators have yet been reported. It will be of great interest to know the effects of endothelial PGC-1 coactivators on angiogenesis and atherosclerosis, 2 critical endothelial processes of high relevance to cardiac disease. In this context, it is also important to note that PGC-1β and PPARs appear to play important roles in macrophage activation, a key step in the development of atherosclerosis. This is discussed in detail by Chawla in an accompanying contribution to this series.180

**Conclusions and Human Relevance**

It has become abundantly clear that the PGC-1 coactivators are key regulators of metabolism in the heart, both in cardiomyocytes and likely in other cardiac cell types. In the context of highly metabolically active cells like cardiomyocytes, PGC-1α coordinates broad genetic programs spanning the entire metabolism of preferred substrates: blood vessel recruitment for transport of oxygen and nutrients, transport of fatty acids into the cell and the mitochondrion, oxidation of fatty acids via β-oxidation and the TCA cycle, generation of ATP via the respiratory chain complex, transport of ATP back to the cytoplasm, and protection against ROS generated by the respiratory chain. PGC-1α appears to be more responsive to extracellular and metabolic cues than are PGC-1β and PRC, and it has been studied more extensively. Nevertheless, important redundancies exist between PGC-1α and -β. Little is known of the role PRC in the heart.

Does abnormal activity of PGC-1α and/or -β contribute to cardiac remodeling and heart failure? This was first suggested by the observation in rodents that the expression of PGC-1α and known targets of PGC-1α are reduced in a number of rodent models of cardiac dysfunction, as discussed above. The situation in humans is less clear. Some have reported decreased expression of PGC-1α in failing human hearts,January,182 whereas others have not.147,151 Interpretations of these findings are invariably difficult, because patients differ widely in treatment and other critical clinical variables. It may also be that PGC-1α expression is not altered in human heart failure, but rather that its intrinsic activity is decreased. As noted above, PGC-1α is heavily modified posttranslationally. However, evaluating this possibility has been difficult, in part, because of poor antibodies to PGC-1s and because of the short half-life of PGC-1α protein.183 What is clear is that numerous mitochondrial genes and other known targets of PGC-1 like glycolytic and fatty acid oxidation (FAO) genes are repressed in human heart failure,147,151 strongly suggesting that PGC-1α may be playing a role.

It is also possible that, in certain circumstances, PGC-1α is at first repressed but then induced in terminal heart failure as a compensatory response to accelerated energetic collapse. Indeed, the expression of PGC-1α can be induced by isch-emia and ATP wasting.28,183 Mitochondrial proliferation has been observed in certain models of cardiac dysfunction, most notably diabetic models,184–187 and in human mitochondrial myopathy.181 Mitochondrial proliferation may be mediated by PGC-1α in some cases,184 but perhaps not all.188,189

Conclusions drawn thus differ depending on the etiology of heart disease studied, and what time point in the process of cardiac adaptation and maladaptation is being investigated. In short, it remains unclear to what extent PGC-1α activity is altered in human heart failure, and, although the hypothesis is highly plausible, the jury is still out on whether alterations in PGC-1 activity play a causal role in heart failure.

Are the PGC-1 proteins possible therapeutic targets? Whether the PGC-1s contribute to heart failure or not, they represent appealing nodal points for modulation of the cardiac energy state. Transcription factors other than nuclear receptors are historically regarded as “nondruggable,” although this dogma is increasingly being contested. Small molecules that specifically modulate PGC-1α/ERRα interaction have been developed, for example.190 Pharmaceuticals that target pathways upstream of the PGC-1s may also provide interesting opportunities, without having to target PGC-1 proteins directly.191 Gene therapy may afford another approach, although targeting vectors for the heart remain a challenge. It is also important to appreciate that a blanket activation of the pleiotropic effects of PGC-1α on metabolism may not be ideal. Excessive activation of PGC-1α leads to cardiac dysfunction,52 suggesting that remaining within a
therapeutic window will be important. Alternatively, activation of specific PGC-1 pathways might avert toxic side effects. This could be done either by pharmaceutical targeting of specific PGC-1/transcription factor interactions, or by introduction of genetically modified PGC-1 molecules. Clearly, much remains to be learned. The PGC-1 coactivators are undisputedly key regulators of cardiac bioenergetic, and understanding the mechanisms by which they do so will undoubtedly continue to yield new and important insights.

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Disclosures

None.

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