PGC-1β Deficiency Accelerates the Transition to Heart Failure in Pressure Overload Hypertrophy

Christian Riehle,* Adam R. Wende,* Vlad G. Zaha, Karla Maria Pires, Benjamin Wayment, Curtis Olsen, Heiko Bugger, Jonathan Buchanan, Xiaohui Wang, Annie Bello Moreira, Torsten Doenst, Gema Medina-Gomez, Sheldon E. Litwin, Christopher J. Lelliott, Antonio Vidal-Puig, E. Dale Abel

Rationale: Pressure overload cardiac hypertrophy, a risk factor for heart failure, is associated with reduced mitochondrial fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) proteins that correlate in rodents with reduced PGC-1α expression.

Objective: To determine the role of PGC-1β in maintaining mitochondrial energy metabolism and contractile function in pressure overload hypertrophy.

Methods and Results: PGC-1β deficient (KO) mice and wildtype (WT) controls were subjected to transverse aortic constriction (TAC). Although LV function was modestly reduced in young KO hearts, there was no further decline with age so that LV function was similar between KO and WT when TAC was performed. WT-TAC mice developed relatively compensated LVH, despite reduced mitochondrial function and repression of OXPHOS and FAO genes. In nonstressed KO hearts, OXPHOS gene expression and palmitoyl-carnitine-supported mitochondrial function were reduced to the same extent as banded WT, but FAO gene expression was normal. Following TAC, KO mice progressed more rapidly to heart failure and developed more severe mitochondrial dysfunction, despite a similar overall pattern of repression of OXPHOS and FAO genes as WT-TAC. However, in relation to WT-TAC, PGC-1β deficient mice exhibited greater degrees of oxidative stress, decreased cardiac efficiency, lower rates of glucose metabolism, and repression of hexokinase II protein.

Conclusions: PGC-1β plays an important role in maintaining baseline mitochondrial function and cardiac contractile function following pressure overload hypertrophy by preserving glucose metabolism and preventing oxidative stress. (Circ Res. 2011;109:00-00.)

Key Words: mitochondria ■ cardiac hypertrophy ■ heart failure ■ gene expression

PGC-1α and PGC-1β are primarily expressed in oxidative tissues, including heart and skeletal muscle, and regulate mitochondrial biogenesis and genes encoding enzymes of mitochondrial metabolism and proteins that compose the electron transport chain. In nonstressed hearts from PGC-1α-KO mice, impaired fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) gene expression were observed, but cardiac function was preserved. Repression of PGC-1α and its transcriptional partners has been suggested as a potential mechanism responsible for the shift away from FAO toward glucose oxidation and impaired ATP production in pressure overload hypertrophy (POH) and heart failure. Mice lacking PGC-1α show accelerated cardiac dysfunction and heart failure following POH. Interestingly, loss of PGC-1α expression in nonstressed hearts reduced expression of FAO and OXPHOS genes to the same extent as aortic banding in control mice and in both instances without overt cardiac dysfunction. However, in banded PGC-1α-deficient rodents with reduced PGC-1α, impaired fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) gene expression were observed, but cardiac function was preserved.

*These authors contributed equally to this work.

© 2011 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.111.243964
hearts there was further repression of OXPHOS and FAO gene expression that coincided with the onset of heart failure. PGC-1α-deficient hearts also exhibit increased oxidative stress in response to POH, which contributes to accelerated heart failure.⁹ These observations suggest that a component of the mitochondrial dysfunction that occurs in the transition from compensated hypertrophy to heart failure might be PGC-1α independent and that a threshold expression of PGC-1α might be required to sustain mitochondrial function in compensated cardiac hypertrophy.

The role of PGC-1β and its contribution to cardiac mitochondrial and metabolic adaptations in nonstressed hearts and in response to POH is incompletely understood. We previously reported that PGC-1β deficiency reduces OXPHOS gene expression in the heart and reduced mitochondrial size.

In isolated soleus muscle, absence of PGC-1β reduced electron transport chain gene expression, decreased mitochondrial volume density, and impaired mitochondrial oxygen consumption and ATP synthesis.¹⁰ We therefore sought to determine whether PGC-1β contributed to the maintenance of mitochondrial and contractile function in response to POH induced by transverse aortic constriction (TAC). We observed that PGC-1β deficiency represses OXPHOS but not FAO gene expression in nonstressed hearts. PGC-1β-deficient hearts develop accelerated heart failure and greater mitochondrial dysfunction following POH. This is associated with increased oxidative stress and a more rapid decline in myocardial glucose utilization. A subset of OXPHOS genes was also identified whose expression levels are further repressed during POH in hearts that lack PGC-1β. Thus, PGC-1α and PGC-1β play complementary but partially nonoverlapping roles in maintaining mitochondrial function in the hypertrophied and failing heart.

Non-Standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO</td>
<td>fatty acid oxidation</td>
</tr>
<tr>
<td>GLUT1</td>
<td>glucose transporter- isoform 1</td>
</tr>
<tr>
<td>GLUT4</td>
<td>glucose transporter- isoform 4</td>
</tr>
<tr>
<td>KO</td>
<td>knockout</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricle</td>
</tr>
<tr>
<td>LVSP</td>
<td>LV systolic pressure</td>
</tr>
<tr>
<td>LVEDP</td>
<td>LV end diastolic pressure</td>
</tr>
<tr>
<td>MVO₂</td>
<td>myocardial oxygen consumption rate</td>
</tr>
<tr>
<td>OXPHOS</td>
<td>oxidative phosphorylation</td>
</tr>
<tr>
<td>PC</td>
<td>palmitoyl carnitine</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>PPAR gamma coactivator 1 alpha</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>PPAR gamma coactivator 1 beta</td>
</tr>
<tr>
<td>PPARα</td>
<td>peroxisome proliferator activated receptor alpha</td>
</tr>
<tr>
<td>POH</td>
<td>pressure overload hypertrophy</td>
</tr>
<tr>
<td>Pyr</td>
<td>pyruvate</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcriptase–polymerase chain reaction</td>
</tr>
<tr>
<td>TAC</td>
<td>transverse aortic constriction</td>
</tr>
<tr>
<td>WT</td>
<td>wildtype</td>
</tr>
</tbody>
</table>

Methods

Animals
PGC-1β⁻/⁻ germ-line KO (βKO) mice and wildtype (WT) littermate controls were generated as previously described.¹⁰ The animals were housed at 22°C with a 12-hour light, 12-hour dark cycle with free access to water and standard chow (composition of chow is in the Online Supplement, available at http://circres.ahajournals.org). Studies were performed in mice of both genders as indicated in the figure legends. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Utah.

Surgical
Transverse aortic constriction (TAC) was performed at the age of 8 to 10 weeks or at 23 to 30 weeks using methods previously described.¹¹ Animals were euthanized either 3 or 8 weeks after surgery. See Online Supplement for surgical details.

Transmural Echocardiography
Mouse echocardiography was performed as previously described.¹² Efficacy of the aortic ligation was determined by comparing flow velocity in right and left carotid arteries using pulsed-wave Doppler. Full details are provided in the Online Supplement.

Hemodynamic Studies
Heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and the maximal rate of pressure change (+dP/dt and −dP/dt) were recorded and analyzed as previously described,¹³ following insertion of a micromanometer-tipped pressure catheter (Millar Instruments, Houston, TX) that was retrogradely introduced into the left ventricle (LV) via the right carotid artery. Studies were performed in nonstressed mice before and after dobutamine¹⁰ and following 3 or 8 weeks of TAC or sham surgery. Detailed methods are summarized in the Online Supplement.

Isolated Working Hearts
Cardiac substrate metabolism, left ventricular developed pressure, cardiac output, cardiac power, oxygen consumption, and cardiac efficiency were measured in isolated working hearts obtained after 3 weeks of banding as previously described.¹⁴ Hearts were perfused with 5 mmol/L glucose and 0.4 mmol/L palmitate without insulin.

Mitochondrial Function
Mitochondrial oxygen consumption and ATP production rates were measured in ventricular muscle fibers as described before, with palmitoyl carnitine or pyruvate as substrate.¹⁵ See Online Supplement for details.

RNA Extraction and Quantitative RT-PCR
Total RNA was isolated, reverse transcribed to cDNA, which was quantified by real-time PCR as previously described and transcript levels normalized to cyclophilin A.¹⁶ Primer sequences and accession numbers are listed in Online Table I. See Online Supplement for detailed methods.

Western Blot Analysis
Immunoblotting was performed with heart homogenates as described in the Online Supplement.

Electron Microscopy
Left ventricular samples were prepared for electron microscopy as previously described.¹⁷ Mitochondrial number and volume density were assessed in a blinded fashion using point quantification counting.¹⁸ For volume density, 2 pictures per sample were analyzed using 2 grids per picture. For mitochondrial number, 3 pictures per sample were analyzed.
Measurement of ROS Levels
Reactive oxygen species levels were measured by 2′,7′-dichlorofluorescein-diacetate (DCFDA) fluorescence as described in the Online Supplement.

Histology and Stereology
Myocardial fragments were stained by hematoxylin-eosin, Masson’s trichrome, TUNEL, and DAPI stains. Stereological analysis was performed as described in the Online Supplement.

Statistical Analysis
Statistical analysis is described in the Online Supplement. Data are presented as means ± SEM. RT-PCR results are presented as fold change versus WT sham. A probability value of <0.05 was considered significantly different.

Results
Inotropic Reserves in PGC-1β−/− Hearts
LV hemodynamics was determined in PGC-1β−/− (βKO) and wildtype (WT) hearts as a function of age. As shown in Figure 1A, peak rates of ventricular contraction (+dP/dt) were significantly higher in 7-week-old WT hearts, after which an age-dependent decline was observed. By contrast, despite an initial reduction, no age-related decline in +dP/dt was observed in βKO hearts, so that by 17 and 27 weeks of age, respectively, there were no significant differences between the genotypes. Similar patterns were observed for LV developed pressure (LVDevP) and peak rates of ventricular relaxation (−dP/dt) (data not shown). To determine whether the reduction in contractility in 7-week-old βKO hearts was associated with decreased contractile reserve, hemodynamic measurements were performed following administration of a graded infusion of dobutamine. Heart rates were similar at baseline, but βKO mice developed a blunted chronotropic response (Figure 1B). By contrast, the fold change (from respective baselines) in LVDevP and −dP/dt was similar in βKO and WT hearts (Figure 1C and 1D). Because baseline values were lower in βKO than WT, absolute values remained lower in βKO throughout the dobutamine infusion.

Increased Left Ventricular Dysfunction After TAC in PGC-1β−/− Hearts
TAC was performed in mice at 2 ages (8 to 10 weeks of age and 23 to 30 weeks of age). The first cohort was studied after 8 weeks of TAC (age 16 to 18 weeks) and the second cohort after 3 weeks of TAC (age 26 to 33 weeks of age). At these ages, there were no differences in in vivo hemodynamics between KO and WT sham-operated mice (Figure 1A).
sham-operated hearts, heart weights of βKO mice were similar to those of WT mice and exhibited no histological abnormalities. After 8 weeks of TAC, WT and βKO hearts developed comparable degrees of hypertrophy (Figure 2A through 2C and Online Table II), and similar increases in mean cardiomyocyte cross-sectional surface area (Figure 2D through 2F). However, βKO hearts developed increased fibrosis, independent of any increase in TUNEL-positive nuclei (Figure 2E and 2G through 2I). We also analyzed left ventricular function at 3 and 8 weeks following TAC. Three weeks following TAC, WT and βKO hearts exhibited modest degrees of left ventricular (LV) dysfunction as evidenced by LV dilation. However, these changes were not statistically different between banded WT and banded βKO (Figure 3B). Moreover, fractional shortening was not different from that in sham-operated animals (Figure 3B and Online Table III). LV function progressively declined as evidenced by decreased ejection fraction (EF) and fractional shortening (FS) in βKO and WT mice between 3 and 8 weeks post-TAC (Figure 3B and 3C). Thus after 8 weeks of TAC, the degree of reduction of EF and FS was greater in PGC-1β KO hearts, which developed overt heart failure as evidenced by decreased left ventricle systolic pressure (LVSP), impaired peak rates of ventricular contraction, and relaxation (±dp/dt) and increased left ventricular cavity dimensions (Figure 3A, 3C, and 3D).

Mitochondrial Dysfunction Following TAC in WT and PGC-1β KO Hearts

Mitochondrial function was determined after 8 weeks of TAC (Figure 4A and 4B). With pyruvate as a substrate, maximal ADP-stimulated mitochondrial oxygen consumption (VADP) and ATP synthesis rates were unchanged in sham-operated βKO and were decreased equivalently following TAC in both WT and βKO mice. Palmitoyl-carnitine (PC)–supported VADP and ATP synthesis were decreased in sham-operated βKO mice, and TAC reduced PC-supported VADP and ATP synthesis in WT hearts to levels that were similar to sham βKO. TAC modestly reduced VADP in βKO further so that in relation to WT-TAC, VADP was significantly reduced. ATP synthesis rates followed a similar pattern, but the decline in ATP synthesis in βKO after TAC was more pronounced, being lowest in βKO-TAC mice in relation to all other groups (Figure 4B). Mitochondrial dysfunction was not associated with any noticeable change in mitochondrial ultrastructure, number, or volume density (Online Figure I). To determine whether the decline in mitochondrial function induced by TAC was associated with independent evidence of energetic stress, we determined AMPK phosphorylation, which was significantly increased in the banded βKO after 8 weeks of TAC, but not in banded WT (Figure 4C).
Oxidative Stress Is Increased in Banded PGC-1\(\beta^{\alpha-}\) Hearts

To gain additional insight into the mechanisms that increased heart failure progression in banded \(\beta^{\alpha-}\) KO mice, we evaluated the potential contribution of oxidative stress. Total levels of tissue ROS were increased in banded \(\beta^{\alpha-}\) hearts 3 and 8 weeks after TAC, respectively (Figure 5A and 5B). Pressure overload was associated with repression of MnSOD protein after 3 weeks of banding in \(\beta^{\alpha-}\) KO but not in banded WT hearts. After 8 weeks of banding, MnSOD was equivalently repressed in banded mice of both genotypes (Figure 5C). Thus loss of antioxidant capacity occurs at an earlier stage after pressure overload in \(\beta^{\alpha-}\) KO mice. Uncoupling proteins, such as UCP3, mediate reduction of membrane potential under conditions of increased ROS.\(^1\) UCP3 protein levels were increased in sham \(\beta^{\alpha-}\) KO mouse hearts in relation to sham WT. Banding equivalently repressed UCP3 in both genotypes at 3 and 8 weeks following TAC, which paralleled changes in UCP3 mRNA (Figure 7 and Online Figure II).

Substrate Metabolism and Cardiac Efficiency in PGC-1\(\beta^{\alpha-}\) Hearts Following TAC

Substrate metabolism was determined in isolated working hearts, 3 weeks after sham or banding surgery. In relation to WT shams, TAC increased glucose oxidation and glycolysis rates in WT hearts. Rates of glucose oxidation and glycolysis were also increased in nonstressed \(\beta^{\alpha-}\) hearts. However, in response to banding there was a significant reduction both in glucose oxidation and glycolysis to levels that were lower than WT-TAC. Palmitate oxidation rates were not altered by banding in WT hearts. By contrast, FAO was increased in \(\beta^{\alpha-}\) KO hearts and tended to fall with banding (\(P<0.08\)), but did not fall below WT levels (Figure 6). Left ventricular developed pressure, cardiac output, cardiac power, oxygen consumption, and cardiac efficiency were also determined in isolated working hearts. In sham-operated hearts, there were no differences in cardiac function, but myocardial oxygen consumption rates (MVO\(_2\)) were increased in \(\beta^{\alpha-}\) hearts, resulting in decreased cardiac efficiency. LV-developed pressure declined with banding in both genotypes but to a greater extent in \(\beta^{\alpha-}\). Cardiac output and cardiac power declined equivalently in banded mice of both genotypes. MVO\(_2\) remained persistently increased in banded \(\beta^{\alpha-}\) hearts leading to a greater reduction in cardiac efficiency in banded \(\beta^{\alpha-}\) in relation to banded WT. Thus, PGC-1\(\beta\) deficiency leads to diminished glucose utilization and decreased cardiac efficiency following TAC.
Changes in Expression of Metabolic Regulators Following Banding in PGC-1β−/− Hearts

Targeted gene expression analysis was performed to evaluate the impact of TAC versus loss of PGC-1β on mitochondrial and metabolic genes (Figure 7 and Online Figure II). In WT mice, TAC leads to reduced expression of PGC-1α, OXPHOS, and FAO genes as early as 3 weeks after banding and reduced expression of PGC-1β by 8 weeks. Similar reductions of PGC-1α and PGC-1β mRNA expression, and their downstream targets, involved in OXPHOS and FAO was also observed in rat hearts following 2 weeks of TAC (Online Tables IV and V), indicating a conserved transcriptional response of mitochondrial regulatory genes to POH in 2 rodent species. After 3 weeks of TAC, expression levels of PGC-1β were increased in WT, and expression of PPARα and PGC-1α were equivalently reduced by 30% and 60%.

Figure 4. Mitochondrial function of male and female WT and KO hearts (age 16 to 18 weeks) following 8 weeks of TAC or sham surgery (4 to 7 hearts per group). Mitochondrial respiration, ATP synthesis rates, and ATP/O ratios were measured in saponin-permeabilized cardiac fibers. Pyruvate–malate (A) or palmitoyl–carnitine–malate (B) were used as substrates. C, Western blot analysis and densitometric ratios of AMPK phosphorylation 8 weeks post surgery (n=5 to 6). Data are presented as fold change versus WT sham (=1.0). *P<0.05 versus sham same genotype. †P<0.05 versus WT same treatment. ‡P<0.05 versus KO sham (Wilcoxon test).

Figure 5. Reactive oxygen species levels were measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) fluorescence in whole-tissue extract 3 (A) and 8 (B) weeks after sham or TAC surgery, n=4 to 7. C and D, Representative Western blots showing protein levels of MnSOD and UCP3 3 and 8 weeks after surgery and densitometric analysis normalized to α-tubulin (n=5 to 11). Data are presented as fold change versus WT sham. *P<0.05 versus sham same genotype. †P<0.05 versus WT same treatment. ‡P<0.05 versus same genotype 3 weeks postsurgery. TAC 3 weeks and TAC 8 weeks were 8 and 13 weeks of age, respectively, at the time of sacrifice.
respective, in \( \beta \)KO and WT, OXPHOS gene expression was globally reduced in sham-operated \( \beta \)KO, which mirrored the pattern observed in WT-TAC, suggesting redundant roles for PGC-1\( \alpha \) and PGC-1\( \beta \) in the regulation of these genes. For the majority of the OXPHOS genes examined, there was no further reduction in expression levels following TAC in \( \beta \)KO mice despite additional reductions in PGC-1\( \alpha \) expression. By contrast, 3 OXPHOS genes (\( ndf ua 9 \), \( cox 4 i \), and \( cox 5b \)) were additionally repressed in \( \beta \)KO after TAC, suggesting synergistic interactions of PGC-1\( \alpha \) and PGC-1\( \beta \) in the regulation of these genes. KO of PGC-1\( \beta \) had no impact on FAO gene expression in nonstressed hearts. In contrast, FAO gene expression was reduced equivalently in WT and \( \beta \)KO hearts after 3 weeks of TAC. Thus, impaired FAO gene expression following TAC is most likely the consequence of reduced levels of PGC-1\( \alpha \) and PPAR\( \alpha \). Similar patterns of gene expression were noted following 8 weeks of TAC, but in addition, reduced expression of PGC-1\( \beta \) occurred in WT-TAC. In nonstressed \( \beta \)KO hearts, expression levels of most glucose regulatory genes were unchanged, with the exception of the alpha subunit of PDH (PDHA1), which was reduced in sham \( \beta \)KO hearts. With the exception of HIF-1\( \alpha \) mRNA, which was induced in banding in WT and \( \beta \)KO, most genes involved in glucose metabolism were equivalently repressed by banding in WT and \( \beta \)KO. GLUT1 exhibited a biphasic response, initially being repressed by banding at 3 weeks, but induced by 8 weeks in both WT and \( \beta \)KO. We also examined the protein levels of GLUT4 and hexokinase II, and assessed the phosphorylation state of PDH. Banding repressed GLUT4 content in WT and \( \beta \)KO hearts (Figure 7B). The phosphorylation of the PDH-E1\( \alpha \) subunit was unchanged in all groups, with the exception of banded \( \beta \)KO hearts at 3 and 8 weeks, respectively, where the phosphorylation of this subunit was reduced (Figure 7C and Online Figure II). Hexokinase II (HKII) protein was induced in WT hearts after 8 weeks of banding and was increased in sham \( \beta \)KO hearts in the 8-week cohort. However, after 8 weeks there was a significant reduction in HKII protein in \( \beta \)KO following TAC (Figure 7D).

**Discussion**

The present study identified important roles for PGC-1\( \beta \) in the maintenance of cardiac OXPHOS gene expression and mitochondrial function in nonstressed hearts and cardiac function following pressure overload hypertrophy (POH). PGC-1\( \beta \) plays an important role in maintaining cardiac function in compensated POH, as evidenced by the increased susceptibility of PGC-1\( \beta \)-deficient hearts to heart failure. The mechanisms by which PGC-1\( \beta \) contributes to the maintenance of cardiac function in response to pressure overload are complex and cannot be completely accounted for by changes in the expression of nuclear genes that regulate mitochondrial OXPHOS, FAO, or glucose metabolism, given the similarity in the transcriptional responses of banded WT and \( \beta \)KO mice. Moreover, we identify some circumstances in which changes in gene expression do not necessarily correlate with changes at the protein levels as exemplified by hexokinase II in banded PGC-1\( \beta \) KO hearts. Rather, a number of defects became apparent with banding in PGC-1\( \beta \)-deficient hearts that include increased oxidative stress that may be precipitated in part by an early loss of antioxidant defenses, a precipitous reduction in glucose utilization, and reduced cardiac efficiency that precede the subsequent decline in cardiac function. By the time that heart failure ensues, additional defects were noted in \( \beta \) KO hearts such as decreased palmitoyl carnitine ATP generation, increased AMPK phosphorylation, and decreased HKII content. It is, however, challenging to discern whether these additional molecular defects are a cause or a consequence of the more severe heart failure in \( \beta \) KO hearts.

In nonstressed hearts, no differences in heart weights were observed between WT and \( \beta \)KO mice, and expression of...
natriuretic peptide precursor types A and B were unchanged. At the ages at which the banded hearts were analyzed, there were no differences in contractile function between sham-operated βKO and WT, which is consistent with previous reports.10,21 Interestingly, however, in young βKO mice a reduction in basal contractility was observed, suggesting that deficiency of PGC-1β led to a constitutive defect in contractile function that did not progress with age. Importantly, inotropic reserve in response to dobutamine was relatively preserved in young βKO mouse hearts, which supports observations by Lai et al, who reported that LV contractile function following exercise was not impaired in βKO mice of similar age.21 We confirmed a defect in chronotropic reserve in young βKO mice that we previously observed in an older cohort of mice.10 However, there were no differences in heart rate following banding in β KO mice. Taken together, these data suggest that PGC-1β plays an important role in preserving LV function in response to pressure overload. However, we cannot completely rule out the possibility that this constitutive defect could contribute in part to the more rapid decline in cardiac function when pressure overload was imposed. Cardiac phenotypes have been described for 2 independently generated lines of PGC-1β KO mice. One model showed normal contractile function under basal conditions4,22 and the other exhibited age-dependent contractile dysfunction.23 Deletion of PGC-1α, results in impaired expression of both FAO and OXPHOS genes in nonstressed hearts.4 In contrast, we observed that whereas

![Figure 7. A, Gene expression 8 weeks after surgery (n=8, same mice as Figure 2). B–D, Western blot analysis and densitometric ratios of GLUT4 protein expression, PDH-E1α phosphorylation, and hexokinase II protein expression in WT and KO hearts 8 weeks postsurgery (n=5 to 6, same mice as Figure 5). *P<0.05 versus sham same genotype. †P<0.05 versus WT same treatment. Gene names are shown in Online Table III.](http://circres.ahajournals.org/doi/abs/10.1161/CIRCRESAHA.111.241493)
PGC-1β plays an important role in the regulation of OXPHOS gene expression, it does not appear to be necessary for regulating FAO genes, suggesting divergent roles for PGC-1 isoforms in regulating FAO gene expression. Similar changes in mitochondrial gene expression were noted in the hearts of 2 other independently generated PGC-1β deficient mouse models.\textsuperscript{21-24} Importantly, repression of OXPHOS and FAO gene expression in these models is not sufficient to consistently impair cardiac function in the nonstressed heart.

Deficiency of PGC-1α in the heart reduces maximal palmitoyl−carnitine (PC)−supported \( V_{\text{ADP}} \) respiration and increases pyruvate-supported \( V_{\text{ADP}} \) respiration.\textsuperscript{4} In the present study, we observed impaired PC-supported \( V_{\text{ADP}} \) respiration and ATP synthesis in nonstressed hearts lacking PGC-1β. But in contrast to PGC-1α-KO (α KO) hearts, KO of PGC-1β had no effect on pyruvate-supported mitochondrial function. The basis for the differences in pyruvate-supported respiration in α versus β KO mice is not fully understood. We speculate that because PGC-1α deficiency significantly represses OXPHOS and FAO gene expression, there is a greater reliance of α KO mitochondria on glucose carbons, which drives adaptive responses that increase pyruvate utilization. In this regard, we observed a reduction in expression levels of the PDHα1 subunit in sham β KO hearts, which could also limit the induction of pyruvate utilization. In contrast, there was no repression of FAO genes in β KO hearts, nor was there any defect in FA utilization in isolated working hearts. PC-supported respiration and ATP synthesis rates were reduced in β KO mitochondria. However, the reductions were not as striking as we previously reported in α KO mitochondria in which PC-supported ATP generation was reduced by 65%,\textsuperscript{4} which contrasts with the 30% reduction in β KO mitochondrial ATP synthesis observed in the present study. These findings are consistent with the hypothesis that the greater decline in mitochondrial function with FA substrates in α KO mice reflects the combined effects of reduced OXPHOS and FAO capacity. Electron microscopy revealed normal mitochondrial volume density and number for nonstressed PGC-1β deficient hearts, which is consistent with previous reports that examined younger PGC-1β KO hearts.\textsuperscript{21} It is important to note, though, that mitochondrial volume density declines with age in PGC-1β KO hearts.\textsuperscript{10} Thus analyses of nonstressed βKO hearts reveal divergent effects for PGC-1α versus PGC-1β on the expression of mitochondrial metabolic genes and mitochondrial function. However, both of these models suggest that mitochondrial dysfunction that develops on the basis of loss of either PGC-1α or PGC-1β is not sufficient to impair cardiac function throughout life in the absence of a superimposed stress. They also support the existence of overlapping or redundant roles for both of these transcriptional regulators in the regulation of nuclear-encoded genes that regulate mitochondrial metabolism and suggest that loss of PGC-1α may compensate for the loss of PGC-1β in maintaining contractile function under nonstressed conditions, and vice versa. Support for redundant roles for PGC-1α and PGC-1β in maintaining basal mitochondrial function in the heart was also provided by analysis of mice that lack both PGC-1α and PGC-1β, which die of heart failure shortly after birth with bradycardia, small hearts, and reduced cardiac output.\textsuperscript{21}

We examined the impact of PGC-1β deletion on pressure-overload cardiac hypertrophy (POH). PGC-1β KO mice progressed more rapidly to heart failure than did banded WT mice, and this was clearly associated with evidence of energetic stress, namely increased phosphorylation of AMPK. An earlier study demonstrated that mice lacking PGC-1α developed heart failure 8 weeks following TAC. This was associated with additional repression of FAO and OXPHOS genes in PGC-1α-KO mice in response to TAC, versus WT-TAC or sham-operated PGC-1α-KO mice.\textsuperscript{8} It is important to note that the additional repression of OXPHOS and FAO gene expression in banded PGC-1α-KO mice occurred despite normal expression of PGC-1β. These data suggest that PGC-independent mechanisms must exist that account for reduced expression of mitochondrial target genes in pressure-overload hypertrophy. They also suggest that there is a critical threshold of mitochondrial activity that is required to cope with increased workload, which cannot be substituted for or sustained by PGC-1β. The transition to heart failure was not associated with striking changes in mitochondrial morphology in β KO hearts. Although unexpected, it is probable that ultrastructural studies were performed at a time point shortly after the transition to heart failure and that a longer period of observation might be needed before changes in mitochondrial morphology become apparent.

It is also likely that energetic limitations might not be the only mechanism that contributed to heart failure in PGC-1α and PGC-1β KO hearts following TAC. A recent study in banded PGC-1α-KO mice suggested that increased oxidative stress partially drove the LV dysfunction that was observed.\textsuperscript{9} In the present study we also obtained evidence of significant oxidative stress that developed in PGC-1β KO hearts and was evident as early as 3 weeks post-TAC, prior to any differences in LV function. In this regard, it is interesting to note that protein levels of MnSOD were significantly repressed as early as 3 weeks in banded PGC-1β KO hearts but not in banded WT hearts in which repression of MnSOD was only evident after 8 weeks. It is also noteworthy that preserved MnSOD content, 3 weeks post-TAC, correlated with increased PGC-1β expression, despite reduced PGC-1α expression in WT hearts, whereas at 8 weeks, the expression of PGC-1α and β were both reduced in banded WT hearts. Taken together, these data indicate that PGC-1β plays an important role in supporting antioxidant mechanisms that may limit oxidative stress in the compensated state of pressure-overload cardiac hypertrophy and represents another function that overlaps with that of PGC-1α.

Interesting changes were observed in the expression levels and content of uncoupling proteins, which could also contribute to the cardiac responses observed. UCP3 protein and mRNA were increased in sham β KO hearts, and were repressed by TAC in KO and WT hearts. UCP2 mRNA also exhibited a similar pattern. Uncoupling proteins have been proposed to play a role in reducing mitochondrial membrane potential under conditions in which ROS is increased.\textsuperscript{20} Thus the increase in UCP2 and UCP3 in the PGC-1β-deficient...
hearts could reflect an adaptation to low levels of oxidative stress that might also be present in nonstressed β KO hearts. The increase in UCPs could also contribute to the increase in MVO₂ and reduced cardiac efficiency that characterized these hearts. ROS are potent activators of uncoupling proteins; thus the persistently elevated MVO₂ in banded β KO hearts could represent ROS-mediated activation of UCPs in the face of progressive oxidative stress, despite falling levels of UCPs following TAC. Isolated working hearts revealed significant differences in substrate utilization between PGC-1β-deficient hearts and WT controls. As reported by others, compensated LVH in wildtype hearts was associated with increased glucose utilization. Unexpectedly, glucose and FA utilization was increased in nonstressed β KO hearts. The molecular mechanisms for these adaptations are not immediately apparent, and will require additional studies in the future. Importantly, these adaptations were not sustained in β KO hearts following TAC. The global reduction in glucose utilization is not accounted for by differences in GLUT4 protein or by increased phosphorylation of PDH. There is the suggestion that hexokinase II (HKII) levels might fall more rapidly in banded β KO hearts and could contribute to decreased glucose utilization, although it is likely that additional mechanisms are also involved. An association between HKII and VDAC in mitochondria plays an important role in cardioprotection. We did not measure mitochondrial localization of HKII in response to banding in β KO hearts. If the decline in HKII in whole-heart homogenates parallels a decline in mitochondrial HKII, this could potentially contribute to exacerbated mitochondrial dysfunction.

Our results support a model that PGC-1α and PGC-1β play partially overlapping but somewhat distinct roles in maintaining cardiac mitochondrial energetics in the unstressed heart with both pathways regulating the expression of OXPHOS genes, whereas PGC-1α predominantly regulates FAO capacity. Both PGC-1 isoforms likely contribute to the maintenance of cardiac function in the context of pressure overload. Although it is clear that PGC-1α expression is repressed much earlier in the course of POH than is PGC-1β, it seems that the heart can at least in the short term tolerate a 50% reduction of PGC-1α and complete absence of PGC-1β, with relative preservation of LV function as was observed at 3 weeks. However, changes in patterns of substrate utilization and evidence of oxidative stress were already present at this stage, implying that maladaptation as a result of reduced expression of these coactivators likely contributed to the subsequent development of heart failure. The time course for the development of overt heart failure in PGC-1α and PGC-1β KO hearts after TAC are remarkably similar, implying that there is reserve mitochondrial capacity that is capable of maintaining myocardial energetics and contractile function in the face of reduced expression or activity of these transcriptional coactivators. Moreover, our study underscores the potential role of additional mechanisms such as oxidative stress that is exacerbated by deficiency of these transcriptional coactivators that likely contributes to the accelerated transition to decompensated heart failure.

In conclusion, the present study describes the contribution of PGC-1β to mitochondrial function and gene expression in nonstressed hearts and identifies its contribution to maintaining contractile function under pathological increased workload. Thus, modulation of PGC-1 activity may represent a promising target for limiting the transition from pressure-overload cardiac hypertrophy to heart failure.

**Acknowledgments**

We thank Deborah Jones, Chase Andrizzi, Alfred P McQueen, Heather A. Theobald, Jamie Soto, Joseph Tuinei, and Ping Hu for important technical help during the course of these studies. PGC1β KO mice were generated and provided by the AstraZeneca Transgenics & Comparative Genomics Department, Mölndal, Sweden.

**Sources of Funding**

These studies were supported by National Institute of Health (NIH) grant RO1HL73167 to E.D.A.—who is an established investigator of the American Heart Association, the British Heart Foundation, MRC Programme Grant, and FP7-European Commission (MITIN, HEALTH-F4-2008 to 223450)—and to A.V.-P., and a VA Merit award to S.E.L. T.D. was a Heisenberg Professor of the German Research Foundation (DFG) and was supported by grants from the DFG (Do602/4-1, 6-1, and 8-1). C.R. was supported by a student fellowship from the German Academic Exchange Service (DAAD). V.Z. was supported by a postdoctoral fellowship from the American Heart Association (AHA) Western Affiliates. A.R.W. was supported by NIH Grant ST32 HL007576 and a postdoctoral fellowship from the AHA Western Affiliates. K.P. and A.B.M. were supported by scholarships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). H.B. was supported by a postdoctoral fellowship grant from the German Research Foundation (DFG).

**Disclosures**

C. Lelliott is employed by Astra Zeneca. The other authors have no conflicts of interest to disclose.

**References**


What is New Information Does This Article Contribute?

- PGC-1alpha and PGC-1beta are transcriptional coactivators that regulate mitochondrial biogenesis and oxidative capacity.
- Pressure overload cardiac hypertrophy and heart failure are associated with impaired mitochondrial function that correlates with the repression of PGC-1alpha expression.
- PGC-1alpha-deficient hearts have reduced oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) capacity and develop heart failure more rapidly in response to pressure overload.
- PGC-1beta regulates OXPHOS capacity but not the FAO capacity of cardiac mitochondria.
- PGC-1beta deficient hearts rapidly decompensate following pressure overload.
- PGC-1beta deficiency promotes oxidative stress and reduces glucose metabolism in response to pressure-overload hypertrophy.

What New Information Does This Article Contribute?

- PGC-1alpha and beta are critical mediators of mitochondrial biogenesis and oxidative capacity in the heart. Although repression of PGC-1alpha may contribute to mitochondrial dysfunction in pressure-overload hypertrophy (POH) and heart failure, the role of PGC-1beta was unknown. Using PGC-1beta deficient mice, we show that PGC-1beta regulates mitochondrial OXPHOS but not their FAO capacity, without affecting LV function. In contrast to PGC-1alpha deficiency, there is no compensatory increase in mitochondrial metabolism of glucose-derived carbon. After transverse aortic constriction (TAC), PGC-1beta KO hearts decouple more rapidly to heart failure as a result of an increase in oxidative stress, a decrease in cardiac efficiency, and a failure to upregulate glycolysis and glucose oxidation, despite only modest additional reductions in mitochondrial function. In control animals, TAC is initially associated with induction of PGC-1beta that correlates with increased glucose utilization, despite mitochondrial dysfunction. These studies show for the first time that PGC-1beta plays a critical role in maintaining antioxidant mechanisms and in activating glucose utilization in POH, both of which are believed to be important adaptations that prevent the transition from compensated POH to heart failure. Thus, modulation of PGC-1beta may represent a novel approach for retarding the transition from compensated POH to heart failure.
PGC-1β Deficiency Accelerates the Transition to Heart Failure in Pressure Overload Hypertrophy


Circ Res. published online July 28, 2011;

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/early/2011/07/28/CIRCRESAHA.111.243964

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2011/07/28/CIRCRESAHA.111.243964.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
Supplement Materials

Supplement Materials for:

**PGC-1β Deficiency Accelerates the Transition to Heart Failure in Pressure Overload Hypertrophy**

Christian Riehle, MD*, Adam R Wende, PhD*, Vlad G Zaha, MD, PhD‡, Karla Maria Pires, MS¹, Benjamin Wayment, MPH², Curtis Olsen, BS³, Heiko Bugger, MD, PhD†, Jonathan Buchanan, MD¹, Xiaohui Wang, PhD², Annie Bello Moreira, PhD¹, Torsten Doenst, MD, PhD₅, Gema Medina-Gomez, PhD⁴, Sheldon E Litwin, MD⁵, Christopher J Lelliott, PhD³, Antonio Vidal-Puig, MD, PhD⁴, E. Dale Abel, MBBS, DPhil³

**Detailed Methods**

*Mice and genotyping*

All mouse experiments were approved by the Institutional Animal Care and Use Committee of the University of Utah. PGC-1β⁻/⁻ germ-line KO mice (βKO) and wildtype littermate controls (WT) were generated and provided by the AstraZeneca Transgenics & Comparative Genomics Department, Mölndal, Sweden. βKO mice were generated using a triple LoxP targeting vector. Murine PGC-1β is alternatively spliced at the 5' end and at exon 4. Exons 4 and 5 were deleted, which encode for two of the nuclear hormone receptor interacting motifs (LXXLL). Ablation of exons 4 and 5 also introduced a premature stop codon. Following primers were used for genotyping: forward primer 1: 5’-GCACACCCGTGAATACATGTA -3’, reverse primer 1: 5’- CAAGGAGCAGGAACTGGGATT -3’, and reverse primer 2: 5’- CCTTGGGCTCCATCTCTGTGTT -3’.

*Composition of mouse chow*

Mice were fed standard chow Harland Teklad Diet 8656 (3.8 Kcal/g of gross energy) that contained 65% carbohydrate (corn and soybean meal), 24.5 % protein (Soy based), 4.4 % fat (Soybean oil), 3.4 % fiber and supplemented with vitamins and minerals.

*Surgical procedures and hemodynamic measurements (mouse experiments)*

**Aortic banding:** Mice were anesthetized (single intraperitoneal injection of 400 mg chloral hydrate / kg body weight) and placed in the supine position on a heating pad (37 °C). A topical depilatory agent was applied to the chest, and the area was cleaned. Following horizontal skin incision ~ 0.5–1 cm in length at the level of the suprasternal notch, a 2- to 3-mm longitudinal cut was made in the proximal portion of the sternum. In our early studies a 6-0 silk suture was tightened around a blunt 27-G needle, which was placed between the innominate artery and the left common carotid artery. After ligation, the needle was quickly removed, and the chest and overlying skin were closed. We subsequently modified our protocol
to use a 27-G clip, as described in the Methods. The sham procedure was identical except that the aortic arch was not ligated.²

Cardiac Catheterization: Cardiac catheterizations with dobutamine infusion surgeries were performed as previously described with the following modifications. Mice at 7-wk of age were anesthetized (single intraperitoneal injection of 400 mg chloral hydrate / kg body weight) and placed in the supine position on a heating pad (37 °C). The left jugular vein was identified and accessed by cut down method using a 25 G needle connected to a 1 mL syringe with 80 ng/µL dobutamine hydrochloride that was mounted on a standard infuse/withdraw Harvard 33 twin syringe pump (Harvard Apparatus). A Millar Mikro-Tip catheter (1.0F; Millar Instruments, Houston TX) was then inserted into the left ventricle via the right carotid artery, and hemodynamic measurements were obtained using LabChart7 Pro software (ADInstruments, Colorado Springs, CO). After obtaining baseline pressure and heart rate recordings the dobutamine infusion was commenced with readings at 4, 8, 16, 32 and 64 ng/ g BW/ min infusion rates. Each dose was maintained for 1 min and the last 10 seconds of each dose were reported. Total volume infused was monitored to not exceed 75 µL per mouse over the entire procedure. In studies that were performed in sham and TAC mice at ages of 16-18 weeks (8-week banded cohort) or at 26-33 weeks (3-week banded cohort), the right carotid artery of anesthetized mice (chloral hydrate 400mg / kg body weight i.p.) was cannulated and a 1.4 F Millar Mikro-Tip catheter was used to obtain hemodynamic measurements.

Transthoracic echocardiography

Mice were anesthetized with isoflurane, placed supine and temperature was maintained with a heating pad (37 °C). Next, the chest hair was removed with a topical depilatory agent. Two-dimensional guided M-mode images were taken in short and long axis projections using a 13 MHz linear probe (Vivid FiVe, GE Medical Systems, Milwaukee, WI). Left ventricular dimensions and wall thickness were measured in at least three beats from each projection and averaged. Fractional shortening [%] was calculated as [(LVDd - LVDs) / LVDd] * 100 and ejection fraction [%] was calculated as [(LVDd³ - LVDs³) / LVDd³] * 100; where LVDd = left ventricular diastolic dimension and LVDs = left ventricular systolic dimension. In an apical long-axis view, pulsed wave Doppler recordings were made with the sample volume placed in the left ventricular outflow tract (LVOT). Stroke volume [µl] was calculated as π * (LVOT diameter/2)² * LVOT VTI; where VTI = the velocity time integral [cm]. Cardiac output was calculated as SV * HR; where SV = stroke volume [µl] and HR = heart rate [beats/min].

Measurement of carotid velocity gradients
It is difficult to noninvasively measure the gradient across a band placed on the transverse aortic arch in mice. Therefore, we measured flow velocity in the right and left carotid arteries of sham and banded mice using pulsed wave Doppler. A 13 MHz Doppler probe was used to measure flow velocity both in the right carotid artery proximal to the aortic constriction and in the left carotid artery distal to the aortic constriction immediately after surgery. The peak flow velocity difference of the right / left carotid artery was used to determine the transaortic gradient. \(^4\) The peak flow velocities in the right and left carotid arteries in sham-operated animals were (mean±SEM) 0.46±0.021 and 0.435±0.021 m/s, respectively, \(p>0.35, n=11\). In contrast, following application of a 27 gauge clip to the aortic arch between the right innominate artery and the left carotid artery, the flow velocities in the right and left carotid arteries were 0.63±0.038 and 0.359±0.038 m/s, \(p<0.0001, n=8\). Increased flow in the vessel proximal to the clip and decreased flow distal is consistent with significant narrowing of the aorta.

**Mitochondrial Function Measurements – Overview**

Left ventricular muscle fibers were dissected from freshly excised hearts and permeabilized with saponin. Respiration and ATP synthesis were measured using palmitoyl-carnitine (20\(\mu\)M, PC), pyruvate (10mM, Pyr) as substrates, each combined with malate (2mM). Oxygen consumption was determined under three different conditions: in the presence of substrate alone \((V_0)\), following ADP-stimulation \((1\text{mM}; V_{ADP})\) and after addition of the ATP synthase inhibitor oligomycin \((1\mu\text{g/ml}; V_{Oligo})\).

**Saponin-permeabilized cardiac fibers**

Mitochondrial function was measured in saponin-permeabilized cardiac muscle fibers. \(^7\) Small pieces (2 to 5 mg) of left ventricular cardiac muscle were dissected from freshly excised hearts and permeabilized for 30 minutes at 4 °C in buffer A (50 \(\mu\)g/mL saponin, 7.23 mmol/L \(K_2\)EGTA, 2.77 mmol/L \(K_2\)CaEGTA, 6.56 mmol/L MgCl\(_2\), 20 mmol/L imidazole, 0.5 mmol/L dithiothreitol, 53.3 mmol/L K-methanS, 20 mmol/L taurine, 5.3 mmol/L \(Na_2\)ATP, 15 mmol/L PCr, and 3 mmol/L KH\(_2\)PO\(_4\), pH 7.1 adjusted at 25 °C). Next, fibers were washed twice for 10 minutes at 4 °C in buffer B (7.23 mmol/L \(K_2\)EGTA, 2.77 mmol/L \(K_2\)CaEGTA, 1.38 mmol/L MgCl\(_2\), 20 mmol/L imidazole, 0.5 mmol/L dithiothreitol, 100 mmol/L K-methanS, 20 mmol/L taurine, 3 mmol/L KH\(_2\)PO\(_4\), 2 mg/ml BSA, 2 mmol/L malate, and 20 \(\mu\)mol/L Palmitoyl-carnitine or 10 mmol/L pyruvate as substrate, pH 7.1 adjusted at 25 °C).

**Mitochondrial oxygen consumption**

The respiratory rates of cardiac fibers were measured using a oxygen sensor probe (Ocean Optics, Dunedin, FL) in 2 ml of KCl buffer at 25 °C (125 mmol/L KCl, 20 mmol/L HEPES, 3 mmol/L Mg-Acetate, 0.4 mmol/L EGTA, 2 mg/ml BSA, 5 mmol/L KH\(_2\)PO\(_4\) and 0.3 mmol/L Dithiothreitol, 2 mmol/L
Supplement Materials

malate, and 20 µmol/L Palmitoyl-carnitine or 10 mmol/L pyruvate as substrate, pH 7.1 adjusted at 25 °C). Oxygen consumption was determined under three different conditions: in the presence of substrate alone (V₀), following ADP-stimulation (1mM; V_{ADP}) and after addition of the ATP synthase inhibitor oligomycin (1µg/ml; V_{Oligo}). The solubility of oxygen in KCl buffer was 246.87 nmol of O₂/mL. Oxygen consumption rates were expressed as nmol of O₂ * min⁻¹ * mg dry fiber weight⁻¹.

Mitochondrial ATP production

For measurement of ATP production, ADP was added to 2 ml of buffer B to a final concentration of 1 mmol/L. Next, 10 µl Buffer B from the respiration chamber were added to 190 µl DMSO every 10 seconds for a 1-min time period. ATP production was determined by a bioluminescence assay based on the luciferin/luciferase reaction with the ATP assay kit (Promega Corporation, Madison, WI).

Isolated working mouse hearts

Hearts were perfused in the isolated working heart mode with Krebs Henseleit Buffer (118.5 mmol/L NaCl, 25 mmol/L NaHCO₃, 4.7 mmol/L KCl, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 0.5 mmol/L EDTA, and 5 mmol/L glucose, gassed with 95% O₂ and 5% CO₂ and supplemented with 0.4 mmol/L palmitate bound to 3% BSA) using a perfusion apparatus totally sealed to prevent loss of CO₂. Throughout the 60-min perfusion, measurements of flow and pressure (Millar pressure catheter, Millar Instruments, Houston, TX) were obtained every 20-min. Using a fiber-optic oxygen sensor (Ocean Optics, Orlando, FL), the oxygen content of freshly oxygenated buffer (arterial partial pressure of oxygen [PaO₂]) and oxygen concentration in pulmonary artery effluent, collected using a capillary tube (venous partial pressure of oxygen [PvO₂]) was measured.

Palmitate oxidation was measured in one set of hearts and glycolytic flux and glucose oxidation rates were measured simultaneously in a second set of hearts. Glucose oxidation was assessed by measuring ¹⁴CO₂ released by the metabolism of [U-¹⁴C] glucose (specific activity = 296 Mbq/mol). The amount of ³H₂O released from the metabolism of exogenous [5-³H] glucose (specific activity = 177 Mbq/mol) was used to determine glycolytic flux. Palmitate oxidation rates were measured by determining the amount of ³H₂O released from [9,10-³H] palmitate (specific activity = 42 Gbq/mol).⁸

The following formulas were used to determine myocardial oxygen consumption, cardiac hydraulic work and cardiac efficiency:

\[ \text{MVO}_2 [\text{ml} \times \text{min}^{-1} \times \text{g}^{-1} \text{WHW}] = \left(\frac{\text{PaO}_2 \times \text{PvO}_2}{100}\right) \times \left(\frac{\text{Coronary flow}}{\text{WHW}}\right) \times \left(\frac{725}{760}\right) \times \left(1000 \times \text{C}\right); \]

where PaO₂ = arterial partial pressure of oxygen [mmHg], PvO₂ = venous partial pressure of oxygen [mmHg], WHW = wet heart weight [g], 725 and 760 are atmospheric pressures at the University of Utah and at sea level respectively [mmHg], and C = Bunsen Coefficient for plasma i.e. 0.0212.
Cardiac hydraulic work \[ J \cdot \text{min}^{-1} \cdot \text{g}^{-1} \cdot \text{WHW} \] = \( CO \cdot \text{DevP} \cdot 1.33 \cdot 10^{-4} / \text{g WHW} \); where \( CO \) = Cardiac output [ml/min], and \( \text{DevP} \) = Developed pressure [mm Hg].

Cardiac efficiency [%] = Hydraulic work / \( \text{MVO}_2 \cdot 100 \). \( \text{MVO}_2 \) [ml/min] was converted to \( \mu \text{mol/min} \) by multiplying by the conversion factor 0.0393, and then to Joules [J/min] using the conversion of 1\( \mu \text{mol} \text{O}_2 \) = 0.4478 J as previously described.\(^9\)

**Measurement ROS levels**

A fluorometric assay based on the conversion of non-fluorescent 2', 7'-dichlorofluorescein-diace-tate (DCFDA) to the highly fluorescent DCF in the presence of ROS was used to measure ROS levels in heart homogenates. Following homogenization in homogenization buffer (1 mM EDTA, 50 mM phosphate buffer, 1 tablet Phosphatase Inhibitor Cocktail 1 and 2 (Sigma-Aldrich, St. Louis, MO) 1 tablet Complete Mini Protease Inhibitor Cocktail (Roche, Nutley, NJ), pH 7.4) samples were centrifuged at 900 g for 15 min. Next, the supernatant was centrifuged at 12,000 g for 15 min. The oxidation-sensitive carboxy-H\(_2\)DCFDA (C400, Molecular Probes, Carlsbad, CA) or the oxidation-insensitive carboxy-DCFDA (C369, Molecular Probes) were added to 100 \( \mu \text{g} \) of supernatant protein with 25 \( \mu \text{M} \) final concentration at 37 °C. The change in fluorescence intensity was measured at 0 and 30 min using a fluorescence plate reader (485 nm excitation/530 nm emission). Data were expressed as C400 / C369 ratios and results were compared to sham operated wildtype mice.\(^10\)

**Immunoblotting analysis**

For immunoblotting analysis, \( \sim 50 \text{ mg} \) of frozen tissue was homogenized in 100 \( \mu \text{l} \) Lysis buffer (50 mmol/L Hepes, 150 mmol/L NaCl, 10% Glycerol, 1% Triton X-100, 1.5 mmol/L MgCl\(_2\), 1 mmol/L EGTA, 10 mmol/L Sodium Pyrophosphate, 100 mmol/L Sodium Fluoride and 100 \( \mu \text{mol/L} \) Sodium Vanadate, 1 mmol/L PMSF, 10 \( \mu \text{g/mL} \) Aprotinin, and 10 \( \mu \text{g/mL} \) Leupeptin) using a motor-driven tissue homogenizer. Tissue lysates were resolved on SDS-PAGE and transferred to PVDF membranes (Millipore Corp., Billerica, MA). Primary and secondary antibodies used are summarized in the table below.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Company</th>
<th>Catalog #</th>
<th>~Size (kDa)</th>
<th>2°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospho-AMPKa (Thr172)</td>
<td>Cell Signaling, Danvers, MA</td>
<td>CS-2531</td>
<td>62</td>
<td>Rabbit</td>
</tr>
<tr>
<td>AMPKa</td>
<td>Cell Signaling</td>
<td>CS-2793</td>
<td>62</td>
<td>Mouse</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Cell Signaling</td>
<td>CS-2118</td>
<td>37</td>
<td>Rabbit</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Millipore, Billerica MA</td>
<td>MP 04-1404</td>
<td>50</td>
<td>Mouse</td>
</tr>
</tbody>
</table>
Supplement Materials

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Supplier, Location</th>
<th>Catalog No.</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKII (hexokinase)</td>
<td>Chemicon, Temecula CA</td>
<td>AB3279</td>
<td>Rabbit</td>
</tr>
<tr>
<td>MnSOD</td>
<td>BD Biosciences, San Jose CA</td>
<td>BD-611580</td>
<td>Mouse</td>
</tr>
<tr>
<td>Phospho-PDH (Ser293)</td>
<td>Calbiochem, San Diego CA</td>
<td>AP1062</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase E1-a</td>
<td>Abcam, Cambridge MA</td>
<td>ab67592</td>
<td>Mouse</td>
</tr>
<tr>
<td>a-Tubulin</td>
<td>Sigma Aldrich, St. Louis MO</td>
<td>S-T5168</td>
<td>Mouse</td>
</tr>
<tr>
<td>UCP3</td>
<td>ABR Affinity BioReagents, Golden CO</td>
<td>ABR PA1-055</td>
<td>Rabbit</td>
</tr>
<tr>
<td>VDAC</td>
<td>ABR Affinity BioReagents</td>
<td>ABR PA1-954</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Anti-Rabbit-AlexaFluor 680</td>
<td>Invitrogen, Carlsbad CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Mouse IRDYE800</td>
<td>Li-COR, Lincoln NE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IRDye 800CW anti-Mouse (LICOR, Lincoln, NE) and Alexa fluor anti-Rabbit 680 (Invitrogen, Carlsbad, CA) were used as secondary antibodies and fluorescence was quantified using the LICOR Odyssey imager.

**RNA extraction and quantitative RT-PCR (mouse experiments)**

Total RNA was extracted from hearts with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA) and purified with the RNAeasy kit (Qiagen Inc., Valencia, CA). RNA concentration was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (NanoDrop 1000, NanoDrop products, Wilmington, DE). Total RNA (~ 3 µg) was reverse transcribed (SuperScript™ III Reverse Transcriptase Kit, Invitrogen, Carlsbad, CA). The resulting cDNA, Platinum Taq DNA polymerase (Invitrogen), primers, and SYBR-green (Invitrogen) fluorescent dye were transferred to a 384-well plate in triplicate and real-time polymerase chain reaction was performed with an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA). The following cycle profile was used: 1 cycle at 95 °C for 10 min, 40 cycles of 95 °C for 15 sec, 59 °C for 15 sec, 72 °C for 30 sec, and 78 °C for 10 sec, 1 cycle of 95 °C for 15 sec, 1 cycle of 60 °C for 15 sec, and 1 cycle of 95 °C for 15 sec. Data were normalized to Cyclophilin and results were compared to sham operated wildtype mice. See online-only Data Supplement Table S3 for primer sequences.

**Electron Microscopy**

Left ventricular samples were fixed in 2.5 % glutaraldehyde and 1 % paraformaldehyde for at least 1 day. Samples were post-fixed in 2 % osmium, stained en bloc with aqueous uranyl acetate, and dehydrated
through a graded series of ethanol washes (50% up to 100%). Next, samples were embedded in Spurr’s plastic and processed for electron microscopy. Mitochondrial morphology was assessed at 10,000 x and 40,000 x magnifications. Mitochondrial number and volume density were assessed in a blinded fashion using the point quantification counting. For volume density, 2 pictures per sample were analyzed using 2 grids per picture. For mitochondrial number, 3 pictures per sample were analyzed.

**Histology and Stereology:**

Myocardial fragments were stained by hematoxylin-eosin, Masson’s trichrome, TUNEL, and DAPI stains. Light microscopy was performed using an Olympus LX81 inverted microscope that was connected to an Olympus Microfire Digital Camera (New York, NY). In the 3 µm-thick sections, myocardium was analyzed with a 36-point test-system. From each sample, ten microscopic fields were analyzed at random by the same observer, the stage of the microscope being moved blindly. Volume density was estimated for cardiomyocytes (cmy) and interstitium (int): \( Vv[\text{structure}] = \frac{P_p[\text{structure}]}{P_T} \) (\( P_p \) is the number of points that hit the structure; \( P_T \) is the total test-points inside the frame). The mean cross-sectional area was estimated for cardiomyocytes, \( A[\text{cmy}] = \frac{Vv[\text{cmy}]}{2} \). \( N[\text{cmy}] \cdot A_T^{-1} \) (µm²; 1/mm², \( N \) is the number of profiles counted in the test-area, the frame \( A_T \), considering the forbidden line or its extensions). The absolute stereological indices of interstitial volume was estimated by the product of \( Vv[\text{int}] \) and the respective cardiac volume.

**Surgical procedures (rat experiments)**

Male Sprague-Dawley rats (380 – 430 g) were obtained from Charles River (Sulzfeld, Germany). The use of animals and the experimental protocol were approved by the German Animal Welfare Committee of Baden-Wuerttemberg, Freiburg jurisdiction. Rats were operated in an analogous manner as described for mice (anaesthesia: 50 mg ketamine / kg body weight and 10 mg xylazine / kg body weight). A 3-0 silk suture was tightened around a blunt 20-G needle, which was placed between the innominate artery and the left common carotid artery. The sham procedure was identical except that the aortic arch was not ligated. The rats were sacrificed two weeks after surgery.

**RNA extraction and quantitative RT-PCR (rat experiments)**

RNA extraction, RNA purification and determination of RNA concentration (spectrophotometer Ultrospec 2100 pro, Amersham Pharmacia Biotech) were performed as described for mouse experiments. The resulting cDNA, primers, probes, and LightCycler® 480 Probes Master Mix (Roche Applied Science, Mannheim, Germany) were transferred to a 384-well plate in triplicate and real-time polymerase chain reaction was performed with a LightCycler® 480 instrument (Roche Applied Science, Mannheim,
Germany). The following cycle profile was used: 1 cycle at 95 °C for 5 min, 45 cycles of 95 °C for 10 sec, 60 °C for 30 sec, 72 °C for 30 sec, 1 cycle at 40 °C for 5 min. Data were normalized to S29 and results were compared to sham operated rats. See online-only Data Supplement Table S6 for primer and probe sequences.

Statistical analysis
All results are expressed as means ± SEM. Unpaired Student’s t tests were used to determine p-values when comparing two groups. For multi-group comparisons, differences were analyzed by ANOVA, and significance was assessed by Fisher’s protected least significant difference test. Data that were non-normally distributed were analyzed by the non-parametric Wilcoxon Rank Sums test. Analysis of covariance (ANCOVA) was used to evaluate the effect of genotype and dobutamine infusion for cardiac catheterization studies. Statistical calculations were performed using the StatView 5.0.1 or JMP software packages (SAS Institute, Cary, NC). Differences were considered statistically significant for p-values < 0.05.
Online Figure I:
Representative longitudinal electron microscopy images of left ventricular wall eight weeks after Sham or TAC surgery (A). For mitochondrial number (B) and mitochondrial volume density (C) no significant difference was observed between the groups. Data were obtained from 4 hearts per group.
Online Figure II:

(A) Gene expression three weeks post surgery (n = 8). (B, C) Western blot analysis and densitometric ratios of PDH-E1α phosphorylation and Hexokinase II protein expression in WT and KO hearts three weeks after surgery (n = 5-6). * = p<0.05 vs. Sham same genotype, † = p<0.05 vs. WT same treatment. Gene names are shown in Online Table III.
### Supplemental Online Tables

#### Online Table I: Primer Sequences used for Quantification of mRNA and DNA levels by RT-PCR (mouse)

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Sequence of forward and reverse primers (5’→3’)</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2)</td>
<td>CGGGAATGGATCTACACTGG GGAGAGCAAAGTGAGGGATG</td>
<td>NM_008825</td>
</tr>
<tr>
<td>Actin, alpha 1, skeletal muscle (ACTA1)</td>
<td>CCTGTATGCCAACAAACGTCA CTCGTCGTACTCCTGCTTGG</td>
<td>XM_134551</td>
</tr>
<tr>
<td>Acyl-CoA thioesterase 10 (Acate3)</td>
<td>CAGAAGCCTGTTGAAGTTGG TGACTTGAAATGTCGCTGTCC</td>
<td>NM_022816.2</td>
</tr>
<tr>
<td>ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (Atp2a2)</td>
<td>TGGGGAGATATCTGCTGCTG GTTCGGGTTTGTCCAGGATG</td>
<td>NM_001110140</td>
</tr>
<tr>
<td>Calcium channel, voltage-dependent, L type, alpha 1C subunit (Cacna1c)</td>
<td>AAGAGGGAGATCCAGCCATC TGGGGAATGTGGTAGGAGA</td>
<td>NM_001159535</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase 1 beta – muscle (CPT1β)</td>
<td>TGCCCTACATCGTCTCACA AGACCCCCGATCCATCATC</td>
<td>NM_009948</td>
</tr>
<tr>
<td>Citrate synthase (CS)</td>
<td>CTCACAGTGGGGTGCTGCTG CTGGATCTCCCCCCATTTTACCC</td>
<td>NM_026444</td>
</tr>
<tr>
<td>Creatine kinase, mitochondrial 2 (CKMT2)</td>
<td>AGAACTGCGGCTCCAAAAG CACTTCCGTCCAACACTGAGG</td>
<td>NM_198415.2</td>
</tr>
<tr>
<td>Cyclophillin A (CHPN)</td>
<td>AGCAGCTGGAGAGAAAGGATTTG</td>
<td></td>
</tr>
</tbody>
</table>
Supplement Materials

TCTTCTTGCTGGTCTTGCCATT
NM_008907

Cytochrome c oxidase subunit IV isoform 1 (Cox4i1)
CGCTGAAGGAGGAAGGAGAAG
GCAGTGAAGCCAATGAAGAA
NM_009941

Cytochrome c oxidase, subunit Vb (Cox5b)
TGGAGGTGAGTCCTCATG
CTCTTGTTGCTGATGGATGG
M_009942
Electron transferring flavoprotein, dehydrogenase (ETF DH)
CCTCTGTGGCTTTGAGTGGT
TCGAAATCCATCACCTTGTT
NM_025794

Fatty acid binding protein 3 (FABP)
GACGGGAAACTCATCCTGAC
TCTCCAGAAAATCCCACCC
NM_010174.1

Hexokinase II (HK II)
CCTTGGATCTTGGAGGAC
CAAGGGAAGGAAGGATGA
NM_013820.3

Hydroxyacyl-CoA Dehydrogenase - alpha subunit (HADHα)
TCAGGAGGCTCAAAGAATA
GAAAGCCAAGCCCAAAGAC
XM_131963

Hydroxyacyl-CoA Dehydrogenase - beta subunit (HADHβ)
GCCAACAGACTGAGGAAGGA
ACACTGGCAAGGCTGATT
NM_145558

Hypoxia inducible factor 1, alpha subunit (HIF-1α)
TCACCGACAGACGAGGAAA
CTTGAAAAGGGACCGCATCA
NM_010431

Isocitrate dehydrogenase 2 (NADP+), mitochondrial (IDH2)
CCCTATTGCCAGCATTTG
TGTCAGGAAGTCTGTGGTG
NM_173011

Isocitrate dehydrogenase 3 (NAD+) alpha (IDH3α)
CCCCATCCAGTTTGTGGTC
GCATCATCAGCACTAAGCA
NM_029573
Medium chain acetyl-Coenzyme A dehydrogenase (MCAD)
ACTGACGCGGTTTCAGATT
GCTTAGTTACACGAGGGTGATG
NM_007382

Mitochondrial acyl-CoA thioesterase 1 (MTE1)
GACCTCCCCAAGAGCATAGA
TCCCTTGTAGGATGGTGTT
NM_134188

NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 9 (Ndula)
ATCCCTTACCTTTGCCACT
CCGTAGCACCTCAATGGACT
NM_025358

NADH dehydrogenase (ubiquinone) flavoprotein 1 (Ndufv1)
TGTGAGACCGTGCTAATGGA
CATCTCCCTTCACAAATCGG
NM_133666

Natriuretic peptide precursor type A (NPPA)
ATGGGCTCCTTCTCCATCA
CCTGCTTCCTCAGTCTGCTC
K02781

Natriuretic peptide precursor type B (NPPB)
GGATCCTCCTGAAGGTGCTGT
TTCTTTTGTGAGGCCTTGGT
D16497

Peroxisome proliferator activated receptor alpha (PPAR-α)
GAGAATCCACGAAGCCTACC
AATCGGACCTCTGCCTCTTT
NM_011144

Peroxisome proliferator activated receptor gamma co-activator 1 alpha (PGC-1α)
GTAAATCTGCGGGATGATGG
AGCAGGGTCAAAATCGTCTG
NM_008904

Peroxisome proliferator activated receptor gamma co-activator 1 beta (PGC-1β)
TGAGGTGTTCGGTGAGATTG
CCATAGCTCAGGTGGAAGGA
NM_133249

Pyruvate dehydrogenase E1 alpha 1 (PDHA1)
GGGACGTCTGTGAGAGAC
TGTGTCATGGTAGCGGTA
NM_008810.2
Pyruvate dehydrogenase kinase 4 (PDK4)
GCTTGCCAATTTCTCGTCTC
CTTCTCCTTCGCCAGGACTT
NM_013743

Solute carrier family 2 (facilitated glucose transporter), member 1 (GLUT1)
GTCCCTGCTGATTGCTGTG
GCCTTTGGTCTCAGGGACTT
NM_011400

Solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20 (CACL)
CCTGCCAGTGGAATGATTT
TCGGATCAGCTCTCAACA
NM_020520

Solute carrier family 27 (fatty acid transporter), member 1 (FATP1)
CCATCTTTCTGCCTTCTG
GTGTCAGGCTCCAGGTTCTC
NM_011977.3

Solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (CTR)
TGATGTGAGTGGGGTAAGG
AGGACCAGCACCATTTCATC
NM_133987

Transcription factor A, mitochondrial (TFAm)
CAAAAAGACCTCGTTCAGCA
CTTCAGCCATCTGCTCTC
NM_009360

Uncoupling protein 2 (UCP2)
TCTCCTGAAAGCCAACCTCA
CTACGTTCCAGGATCCCAAG
NM_011671.4

Uncoupling protein 3 (UCP3)
TTTGGAGCTGGCTTCTG
AAGGCCCTCTTCAGTTGCTC
NM_009464.3

Primer pairs were designed based on GenBank reference sequences. We used the WWW interface Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with default settings. To avoid non-specific amplifications, primer sequences were blasted against mouse genes. Dissociation curves were analyzed for all primer-pairs to ensure single product amplification.
### Online Table II: Weights of WT and KO hearts 8 weeks after TAC or sham surgery

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>BW [g]</th>
<th>HW [mg]</th>
<th>TL [mm]</th>
<th>HW / BW</th>
<th>HW / TL [mg/mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Sham (12)</td>
<td>28.8 ± 0.7</td>
<td>141.7 ± 2.4</td>
<td>17.20 ± 0.08</td>
<td>4.93 ± 0.10</td>
<td>8.24 ± 0.13</td>
</tr>
<tr>
<td>WT TAC (12)</td>
<td>29.0 ± 0.8</td>
<td>255.0 ± 21.9*</td>
<td>17.28 ± 0.10</td>
<td>8.91 ± 0.91*</td>
<td>14.73 ± 1.23*</td>
</tr>
<tr>
<td>KO Sham (12)</td>
<td>27.9 ± 0.7</td>
<td>142.3 ± 5.1</td>
<td>16.75 ± 0.10 †</td>
<td>5.11 ± 0.17</td>
<td>8.49 ± 0.29</td>
</tr>
<tr>
<td>KO TAC (12)</td>
<td>27.2 ± 1.0</td>
<td>270.8 ± 17.0*</td>
<td>16.75 ± 0.13 †</td>
<td>10.29 ± 0.98*</td>
<td>16.16 ± 0.98*</td>
</tr>
</tbody>
</table>

BW, Body weight; HW, Heart weight; TL, Tibia length; HW / BW, Heart weight/Body weight ratio; HW / TL, Heart weight/Tibia length ratio

* = p<0.05 vs. Sham same Genotype; † = p<0.05 vs. WT same Treatment
**Online Table III**: Cardiac Function by Echocardiography in WT and PGC-1β<sup>−/−</sup> 8 weeks following TAC or Sham Surgery

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>WT Sham (8)</td>
<td>4.05 ± 0.13</td>
<td>2.64 ± 0.16</td>
<td>0.99 ± 0.07</td>
<td>0.96 ± 0.08</td>
<td>35.4 ± 2.3</td>
<td>72.3 ± 2.8</td>
<td>28 ± 1</td>
<td>456 ± 14</td>
<td>12.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>WT TAC (13)</td>
<td>4.16 ± 0.11</td>
<td>2.66 ± 0.12</td>
<td>1.04 ± 0.04</td>
<td>1.01 ± 0.07</td>
<td>36.4 ± 1.9</td>
<td>73.3 ± 2.4</td>
<td>31 ± 1</td>
<td>458 ± 12</td>
<td>14.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>KO Sham (9)</td>
<td>4.22 ± 0.09</td>
<td>2.64 ± 0.08</td>
<td>1.04 ± 0.05</td>
<td>1.10 ± 0.04</td>
<td>37.1 ± 1.1</td>
<td>74.9 ± 1.3</td>
<td>30 ± 1</td>
<td>435 ± 14</td>
<td>12.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>KO TAC (10)</td>
<td>4.34 ± 0.16</td>
<td>2.80 ± 0.15</td>
<td>1.08 ± 0.06</td>
<td>1.01 ± 0.07</td>
<td>36.0 ± 1.7</td>
<td>72.9 ± 1.8</td>
<td>33 ± 2</td>
<td>447 ± 16</td>
<td>14.6 ± 1.1</td>
</tr>
<tr>
<td>1</td>
<td>WT Sham (8)</td>
<td>3.60 ± 0.12</td>
<td>2.35 ± 0.16</td>
<td>1.03 ± 0.06</td>
<td>1.11 ± 0.08</td>
<td>35.4 ± 2.3</td>
<td>72.6 ± 2.6</td>
<td>26 ± 2</td>
<td>497 ± 16</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>WT TAC (13)</td>
<td>4.08 ± 0.15*</td>
<td>2.72 ± 0.19</td>
<td>1.13 ± 0.05</td>
<td>1.17 ± 0.06</td>
<td>34.0 ± 2.5</td>
<td>69.8 ± 3.1</td>
<td>23 ± 2</td>
<td>520 ± 19</td>
<td>12.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>KO Sham (9)</td>
<td>4.20 ± 0.14†</td>
<td>2.70 ± 0.17</td>
<td>1.07 ± 0.07</td>
<td>1.02 ± 0.07</td>
<td>36.2 ± 2.9</td>
<td>72.8 ± 3.5</td>
<td>28 ± 1</td>
<td>460 ± 12</td>
<td>13.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>KO TAC (9)</td>
<td>3.77 ± 0.15</td>
<td>2.24 ± 0.21</td>
<td>1.27 ± 0.09*</td>
<td>1.30 ± 0.12*</td>
<td>41.5 ± 3.8</td>
<td>78.1 ± 3.5</td>
<td>23 ± 2*</td>
<td>532 ± 14*</td>
<td>12.2 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>WT Sham (8)</td>
<td>3.75 ± 0.16</td>
<td>2.20 ± 0.18</td>
<td>0.96 ± 0.05</td>
<td>0.96 ± 0.08</td>
<td>41.5 ± 3.6</td>
<td>78.3 ± 3.7</td>
<td>30 ± 1</td>
<td>479 ± 12</td>
<td>14.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>WT TAC (13)</td>
<td>4.23 ± 0.10</td>
<td>2.89 ± 0.11*</td>
<td>1.12 ± 0.04</td>
<td>1.24 ± 0.06*</td>
<td>31.4 ± 1.5*</td>
<td>67.3 ± 2.0*</td>
<td>23 ± 1*</td>
<td>494 ± 14*</td>
<td>11.4 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>KO Sham (9)</td>
<td>4.19 ± 0.14</td>
<td>2.72 ± 0.21</td>
<td>1.01 ± 0.07</td>
<td>0.94 ± 0.06</td>
<td>37.0 ± 1.7</td>
<td>75.0 ± 2.4</td>
<td>28 ± 2</td>
<td>467 ± 8</td>
<td>12.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>KO TAC (9)</td>
<td>4.57 ± 0.32</td>
<td>3.03 ± 0.33</td>
<td>1.19 ± 0.08</td>
<td>1.16 ± 0.07*</td>
<td>43.7 ± 3.0</td>
<td>70.6 ± 4.2</td>
<td>23 ± 2*</td>
<td>490 ± 22</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>WT Sham (8)</td>
<td>3.75 ± 0.17</td>
<td>2.31 ± 0.18</td>
<td>0.98 ± 0.04</td>
<td>0.93 ± 0.05</td>
<td>39.1 ± 2.5</td>
<td>76.8 ± 2.7</td>
<td>25 ± 2</td>
<td>468 ± 5</td>
<td>11.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>WT TAC (13)</td>
<td>4.37 ± 0.19*</td>
<td>2.95 ± 0.25</td>
<td>1.18 ± 0.04*</td>
<td>1.11 ± 0.05*</td>
<td>33.7 ± 3.2</td>
<td>68.5 ± 4.0</td>
<td>27 ± 2</td>
<td>484 ± 11</td>
<td>13.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>KO Sham (9)</td>
<td>4.04 ± 0.20</td>
<td>2.53 ± 0.19</td>
<td>1.06 ± 0.06</td>
<td>1.01 ± 0.06</td>
<td>37.7 ± 2.8</td>
<td>74.7 ± 2.6</td>
<td>27 ± 2</td>
<td>460 ± 13</td>
<td>12.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>KO TAC (9)</td>
<td>4.59 ± 0.24</td>
<td>3.23 ± 0.26</td>
<td>1.06 ± 0.05</td>
<td>1.13 ± 0.04</td>
<td>30.0 ± 2.2</td>
<td>65.0 ± 3.1</td>
<td>25 ± 2</td>
<td>451 ± 23</td>
<td>10.9 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>WT Sham (11)</td>
<td>4.48 ± 0.05</td>
<td>3.43 ± 0.06</td>
<td>0.62 ± 0.02</td>
<td>0.64 ± 0.02</td>
<td>24.3 ± 1.0</td>
<td>55.1 ± 1.7</td>
<td>49 ± 1</td>
<td>422 ± 23</td>
<td>20.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>WT TAC (10)</td>
<td>5.03 ± 0.19*</td>
<td>4.33 ± 0.25*</td>
<td>0.76 ± 0.06*</td>
<td>0.79 ± 0.05*</td>
<td>17.9 ± 2.5*</td>
<td>41.6 ± 4.1*</td>
<td>48 ± 5</td>
<td>470 ± 22</td>
<td>22.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>KO Sham (11)</td>
<td>4.37 ± 0.10</td>
<td>3.31 ± 0.12</td>
<td>0.69 ± 0.04</td>
<td>0.67 ± 0.03</td>
<td>24.4 ± 1.1</td>
<td>56.6 ± 1.9</td>
<td>45 ± 2</td>
<td>474 ± 24</td>
<td>21.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>KO TAC (9)</td>
<td>5.62 ± 0.19†</td>
<td>5.01 ± 0.22†</td>
<td>0.66 ± 0.02</td>
<td>0.81 ± 0.05*</td>
<td>11.1 ± 1.1†</td>
<td>29.6 ± 2.6†</td>
<td>41 ± 4</td>
<td>435 ± 18</td>
<td>18.0 ± 2.4</td>
</tr>
</tbody>
</table>

LVDd, Left ventricular cavity diameter at diastole; LVDs, Left ventricular cavity diameter at systole; IVSd, Interventricular septum diameter at diastole; LVPWd, Left ventricular posterior wall thickness at diastole; FS, Fractional shortening; EF, Ejection fraction; SV, Stroke volume; HR, heart rate; CO, Cardiac output;

* = p<0.05 vs. Sham same Genotype; † = p<0.05 vs. WT same Treatment
**Online Table IV**: Weights of rat hearts 2 weeks after TAC or sham surgery

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>BW [g]</th>
<th>HW [g]</th>
<th>HW / BW [mg/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (8)</td>
<td>437 ± 22</td>
<td>1.13 ± 0.08</td>
<td>2.59 ± 0.26</td>
</tr>
<tr>
<td>TAC (8)</td>
<td>411 ± 27</td>
<td>1.24 ± 0.10*</td>
<td>3.03 ± 0.22*</td>
</tr>
</tbody>
</table>

BW, Body weight; HW, Heart weight; HW / BW, Heart weight/Body weight ratio; * = p<0.05 vs. Sham

**Online Table V**: Gene expression in rat hearts 2 weeks following TAC

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcriptional regulators</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGC-1α</td>
<td>1.00 ± 0.09</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>1.00 ± 0.09</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>1.00 ± 0.07</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>ERRα</td>
<td>1.00 ± 0.07</td>
<td>0.71 ± 0.11</td>
</tr>
<tr>
<td>NRF1</td>
<td>1.00 ± 0.25</td>
<td>1.10 ± 0.24</td>
</tr>
<tr>
<td>NRF2α</td>
<td>1.00 ± 0.08</td>
<td>0.95 ± 0.07</td>
</tr>
<tr>
<td>TFαm</td>
<td>1.00 ± 0.12</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>Polr2a</td>
<td>1.00 ± 0.08</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td><strong>Hypertrophy markers / cardiac structure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPPA</td>
<td>1.00 ± 0.28</td>
<td>2.81 ± 0.42</td>
</tr>
<tr>
<td>αMHC</td>
<td>1.00 ± 0.09</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>βMHC</td>
<td>1.00 ± 0.12</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td><strong>OXPHOS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ndufa10</td>
<td>1.00 ± 0.07</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
<td>Sdhb</td>
<td>1.00 ± 0.08</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Uqcrce2</td>
<td>1.00 ± 0.06</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>Cox4i1</td>
<td>1.00 ± 0.07</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>Atp2a2</td>
<td>1.00 ± 0.05</td>
<td>0.81 ± 0.05</td>
</tr>
<tr>
<td><strong>FAO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCAD</td>
<td>1.00 ± 0.05</td>
<td>0.80 ± 0.06</td>
</tr>
</tbody>
</table>
### Supplement Materials

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normalized Expression</th>
<th>Change in Expression</th>
<th>p Value vs. Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAD</td>
<td>1.00 ± 0.18</td>
<td>0.57 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>CPT1β</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.09</td>
<td>p = 0.066</td>
</tr>
<tr>
<td>FABP</td>
<td>1.00 ± 0.04</td>
<td>0.74 ± 0.07</td>
<td>*</td>
</tr>
<tr>
<td>FAT</td>
<td>1.00 ± 0.06</td>
<td>0.81 ± 0.08</td>
<td>p = 0.078</td>
</tr>
</tbody>
</table>

#### Glucose metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normalized Expression</th>
<th>Change in Expression</th>
<th>p Value vs. Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDHE1α</td>
<td>1.00 ± 0.05</td>
<td>0.93 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>PDP1</td>
<td>1.00 ± 0.26</td>
<td>0.61 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>PDK4</td>
<td>1.00 ± 0.16</td>
<td>1.71 ± 0.12</td>
<td>*</td>
</tr>
<tr>
<td>GLUT1</td>
<td>1.00 ± 0.12</td>
<td>1.04 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td>1.00 ± 0.07</td>
<td>0.78 ± 0.05</td>
<td>*</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>1.00 ± 0.06</td>
<td>0.95 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>HIF-1α</td>
<td>1.00 ± 0.06</td>
<td>0.80 ± 0.08</td>
<td>p = 0.079</td>
</tr>
<tr>
<td>HK2</td>
<td>1.00 ± 0.11</td>
<td>0.93 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

n = 8, * = p<0.05 vs. Sham surgery, gene names are shown in supplementary table S6.
**Online Table VI**: Primer Sequences used for Quantification of mRNA and DNA levels by RT-PCR (rat)

**Gene Name**
**Gene Sequence of forward and reverse primers and probes (5’→3’)**
**GenBank Accession Number**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Sequence of forward and reverse primers and probes (5’→3’)</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
</table>
| ATPase, Ca++ transporting, slow twitch 2 (Atp2a2)                        | CTGGGAAGATTCTGCGAACTTCA
               |                                                                 | CCCACACAGCCAAACGAAAG     |
|                                                           | 6-FAM-CAAATATGAGACCAATCTG-MGB                                     | NM_017290                |
| Carnitine palmitoyltransferase 1 beta – muscle (CPT1β)                   | AGTGTGCCAGCCAAATTTCA
               |                                                                 | ATAGGCTTCTGTCATCCAGCA     |
|                                                           | 6-FAM-CGGTACTTGGATTCTGTGC-MGB                                     | NM_013200                |
| Cytochrome c oxidase subunit IV isoform 1 (Cox4i1)                      | GCCTTTCCAGGGATGAGAAAG
               |                                                                 | TCTCAGCGAAGCCTCCGTTAAA    |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | NM_017202                |
| Estrogen related receptor, alpha (ERRα)                                 | CCTGGTCTGTGGATGGATG     |
               |                                                                 | CACCTGTCTGGTGGCAAAG       |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | NM_001008511             |
| Fatty acid binding protein 3 (FABP)                                     | TTTGTCCGTAACCTGGAAGAG   |
               |                                                                 | CACCTGTCTGTGGCAAG         |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | AF144090                 |
| Fatty acid translocase (FAT)                                             | TTACTGGAGCCGTATG       |
               |                                                                 | CACCTGTCTGTGGCAAG         |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | AF144090                 |
| Glycogen synthase kinase 3 beta (GSK-3ß)                                | CAGCTTTTTGTAGCATGAAAGTT|
               |                                                                 | CAGAGGGTGCCACCACTGT       |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | AF144090                 |
| Hexokinase II (HK II)                                                    | CCAGCAGAACAGCCCTAGACC  |
               |                                                                 | AGATGCCCTGAATCCCTTTTG     |
|                                                           | 6-FAM-CCAAATTGTACCAGCATCC-MGB                                     | AF144090                 |
| Hypoxia inducible factor 1, alpha subunit (HIF-1α)                      | CAGAGGAAGCGAAAAATGGA    |
Supplement Materials

TTGCTGCAGTAACGTTCCAA
#18 *
NM_024359

Long chain acetyl-Coenzyme A dehydrogenase (LCAD)
GCAGTTACTTGGGAAGAGCA
GGCATGACAATATCTGAATGGA
#81 *
NM_012819

Medium chain acetyl-Coenzyme A dehydrogenase (MCAD)
GGGACTAGGGTTTAGCTTCGAG
CCGAGCAATTGTTTGAAACTC
#84 *
NM_016986

Myosin, heavy chain 6, cardiac muscle, alpha (αMHC)
CAGAAGAAACTGAAGAAAA
CCA
GCTCCGCCTCTAGCTCCT
#17 *
NM_017239

Myosin, heavy chain 7, cardiac muscle, beta (βMHC)
CAGCCTACCTCATGGGACTGA
GTGACATACTGTTGCCCATTT
6-FAM-TTGTGCCACCCTCGAGT-MGB
NM_017240

NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10 (Ndufa10)
GGATGATCGGACCTTTCACA
GACGGTCGTGTAATTCAGCA
#89 *
NM_199495

Natriuretic peptide precursor type A (NPPA)
CAACACAGATCTGATGGATT
TCA
CGCTTCATCGGTCTGCTC
#25 *
NM_012612

Nuclear respiratory factor 1 (NRF1)
GAAGATCAGCAGACAAAC
TCA
TGCCCCAGTACCAACCTG
#77 *
NM_001100708

Nuclear respiratory factor 2 alpha (NRF2a)
CTGCGGGATGATGGAGAC
GCGAAAGCGTCATCGCTGCT
6-FAM-TACGCCGACTACTATTAAG-MGB
NM_001100708

Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PGC-1α)
CTGCGGGATGATGGAGACA
GCCAAAAGCGTCACAGGTTGA
6-FAM-CTATGGTTTCATCACCTACC-MGB
NM_031347.1
Supplement Materials

Peroxisome proliferator activated receptor alpha (PPAR-α)
TGGAGTCCACGCATGTGAAG
CGCCAGCTTTA
GCCGAATAG
6-FAM-CTGCAAGGGCTTCTTTCCGCGA
NM_013196

Peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PGC-1β)
GCCCTGATGATTCCGAGTTC
TTGGTAAGCGCAGCCAAGA
6-FAM-TGACAGTGAGAATGAAGCTTA-MGB
NM_176075

Polymerase (RNA) II (DNA directed) polypeptide A (Polr2a)
CAAGTTCAACCAAGCCATT
AGAGACTGAGCAGCAAACGC
#80 *
XM_001079162

Pyruvate dehydrogenase E1α alpha subunit (PDHE1α)
TGGTCGAGGTAGGTGAGAGC
AT
TGGGGTGCACGAGAAGCT
6-FAM-CTCCACGTGCACTGG-MGB
U44125

Pyruvate Dehydrogenase Kinase 4 (PDK4)
CCAAGTTCAACCAAGCCATT
AGAGACTGAGCAGCAAACGC
#80 *
XM_001079162

Pyruvate dehydrogenase phosphatase isoenzyme 1 (PDP1)
ACAGCAGCCGGGTGCTCTACT
GAACAGTGGTAGCATGGATTTCT
6-FAM-TGCCCCGAATCC-MGB
AF062740

Ribosomal protein S29 (S29)
CAAGATGGTGTCACCAGCAG
CAGACCGTGCCGGTTAGA
#109 *
BC058150

Solute carrier family 2 (facilitated glucose transporter), member 1 (GLUT1)
CATCGCTCTTTGGATCCTTA
GAGCAGTGAGGCTTACCAAGTCT
6-FAM-AGGTGTTCCGCTTACACTCCATCATGG-MGB
BC061873

Solute carrier family 2 (facilitated glucose transporter), member 4 (GLUT4)
CATCTGGACGTTTCTCTCAT
GGCGATTTCTCCCAACATACATA
6-FAM-CGCTCTACTCAGGCTACACTCATCAGGGTTG-MGB
BC085757

Succinate dehydrogenase complex, subunit B, iron sulfur (Ip) (Sdhb)
CCAAGGGTCTGAATCCAGGA
A
TCTCCTTGTAGGTGCACCATCA
6-FAM-CAATTGCAGAAATCAA-MGB
NM_001100539

Transcription factor A, mitochondrial (TFAm)
TGATAGAATTCTGTTATGACAAATGAAATGA
AGATCACTTGGCCCAAACCTCAG
6-FAM-TCTTGGGAAGAAATCAA-MGB
BC062022

Ubiquinol cytochrome c reductase core protein 2 (Uqrc2)
AAGATCACCCGTGGGAAATGA
TTCCCTTGGTTGCAGTCACAC
#119 *
NM_001006970

* probe number, “Universal ProbeLibrary Set. Rat“ (Roche Applied Science, Mannheim, Germany)

Primers were designed using Primer Express 1.5 (Applied Biosystems, Foster City, CA) and the WWW interface www.universalprobelibrary.com with default settings. To avoid non-specific amplifications, primer sequences were blasted against rat genes. Gel analysis of the PCR products was performed for all sets of primers/probes to ensure single product amplification.
References


