Overexpression of pyruvate dehydrogenase kinase 4 in heart perturbs metabolism and exacerates calcineurin-induced cardiomyopathy

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Zhao G, Jeoung NH, Burgess SC, Rosaaen-Stowe KA, Inagaki T, Latif S, Shelton JM, Anally J, Bassel-Duby R, Harris RA, Richardson JA, Kliewer SA. Overexpression of pyruvate dehydrogenase kinase 4 selectively in the heart. From PDK4 transgenic mice have a marked decrease in glucose oxidation and a corresponding increase in fatty acid catabolism. Although no overt cardiomyopathy was observed in the PDK4 transgenic mice, introduction of the PDK4 transgene into mice expressing a constitutively active form of the phosphatase calcineurin, which causes cardiac hypertrophy, caused cardiomyocyte fibrosis and a striking increase in mortality. These results demonstrate that cardiac-specific overexpression of PDK4 is sufficient to cause a loss of metabolic flexibility that exacerbates cardiomyopathy caused by the calcineurin stress-activated pathway.

fatty acid; hypertrophy; transgenic mice

There is a growing awareness that systemic perturbations in energy metabolism such as those that occur in diabetes mellitus and other forms of metabolic disease contribute to cardiovascular disease (9). The heart is a metabolic omnivore capable of switching between fatty acids and carbohydrates to match energy demands with dietary and physiological conditions (30, 31). Under conditions of pressure overload and cardiac hypertrophy, increased carbohydrate oxidation is part of the adaptive response to increased workload (21). However, in diabetes the metabolic flexibility of the heart is diminished, and it becomes more reliant on fatty acids for energy (10, 21, 29). This may contribute to functional derangements by adjusting its relative metabolism of carbohydrates and fatty acids. Loss of this metabolic flexibility such as occurs in diabetes mellitus is associated with cardiovascular disease and heart failure. To study the long-term consequences of impaired metabolic flexibility, we have generated mice that overexpress pyruvate dehydrogenase kinase (PDK)4 selectively in the heart. Hearts from PDK4 transgenic mice have a marked decrease in glucose oxidation and a corresponding increase in fatty acid catabolism. Although no overt cardiomyopathy was observed in the PDK4 transgenic mice, introduction of the PDK4 transgene into mice expressing a constitutively active form of the phosphatase calcineurin, which causes cardiac hypertrophy, caused cardiomyocyte fibrosis and a striking increase in mortality. These results demonstrate that cardiac-specific overexpression of PDK4 is sufficient to cause a loss of metabolic flexibility that exacerbates cardiomyopathy caused by the calcineurin stress-activated pathway.

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MATERIALS AND METHODS

Transgenic mice. The α-MHC-PDK4 transgene consists of a 5.5-kb segment of the α-MHC gene promoter, a 1.3-kb mouse PDK4 cDNA, and the 0.6-kb human growth hormone (hGH) gene polyadenylation signal. Two independent lines were established. Mice were genotyped by PCR on DNA samples extracted from mouse tails. PDK4/CnA double transgenic mice were generated by breeding α-MHC-PDK4 mice with α-MHC-CnA mice (25). All animal experiments were approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

RNA preparation and real-time quantitative PCR analysis. Mice were killed and the hearts were dissected into left and right ventricles. Samples were flash-frozen in liquid nitrogen and stored at −80°C until RNA preparation. Total RNA was extracted with Qiagen fibrous RNA mimikits. All real-time quantitative PCR primers were designed with Primer Express Software (Applied Biosystems, Foster City, CA) based on GenBank sequence data. Primer sequences are available on request. For reverse transcriptase reactions, 2 μg of total RNA was reverse transcribed into cDNA with Superscript II RNA polymerase. Each real-time quantitative PCR reaction contained 25 ng of cDNA, each primer at 150 nM, and 5 μl of SYBR Green PCR Master Mix (Applied Biosystems) in a total volume of 10 μl. Each sample was performed in triplicate on an Applied Biosystems Prism 7900HT Sequence Detection System, and the relative mRNA levels were calculated by the comparative threshold cycle method with cyclophilin as an internal control.

Western blot analysis. To prepare total heart protein, frozen hearts were thawed on ice and homogenized in 500 μl of muscle lysis buffer [mM: 140 NaCl, 10 Tris•HCl (pH 8.1), 1 CaCl2, and 1 MgCl2 with 10% glycerol, 1% NP-40, and miniComplete proteinase inhibitor cocktail (Roche)] with a Polytron homogenizer. The homogenate was incubated on ice for 30 min with agitation and then spun down at 10,000 rpm for 10 min at 4°C. For mitochondrial protein, mice were killed by cervical dislocation and heart samples were removed quickly at 4°C and then homogenized using a polytron homogenizer. After weighing, hearts were incubated in room temperature Krebs-Henseleit solution (mM: 118 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, 11 glucose) as a relaxant for 30 min and then transferred to Carson’s buffer formalin solution for overnight fixation at 4°C. Heart specimens were paraffin processed, and the resulting sections were stained with hematoxylin and eosin or Masson’s trichrome. For quantification of fractional shortening was calculated from the M-mode images as the left ventricular end-systolic dimension (LVESD) divided by the LVEDD. Fractional shortening was calculated from the M-mode images as the left ventricular end-diastolic dimension (LVEDD) minus the left ventricular end-systolic dimension (LVEDSD) divided by the LVEDD.

Echocardiography. Noninvasive echocardiograms were obtained on unsedated mice. Transthoracic echocardiographic examination was performed by using a General Electric Vivid7 Pro machine equipped with a 12-MHz transducer. Motion mode (M mode) and two-dimensional echo images were obtained in the parasternal short-axis view. Fractional shortening was calculated from the M-mode images as the left ventricular end-diastolic dimension (LVEDD) minus the left ventricular end-diastolic dimension (LVEDSD) divided by the LVEDD.

Histology and morphometric analysis. Mice were euthanized via pentobarbital overdose. Hearts were quickly removed and rinsed in PBS buffer lacking calcium and magnesium. After weighing, hearts were incubated in room temperature Krebs-Henseleit solution (mM: 118 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, 11 glucose) as a relaxant for 30 min and then transferred to Carson’s buffered formalin solution for overnight fixation at 4°C. Heart specimens were paraffin processed, and the resulting sections were stained with hematoxylin and eosin or Masson’s trichrome. For quantification of dehydration, H2O added to 100 ml, pH 9.0) and 70 μl of β-NADH solution (30 mg/ml in H2O). After addition of 20 μl of lactate dehydrogenase solution (10 mg/ml in 10 mM Tris-Cl-0.1 M EDTA buffer, pH 7.5), absorbance at 340 nm (A340) was measured immediately and again at 10 min. Pyruvate concentrations were calculated from the change in A340 and a standard curve derived from reactions performed with serial dilutions of L-(-)-lactate (Sigma).

Pyruvate and lactate measurements. Mice were killed under pentobarbital sodium anesthesia, and hearts were removed and immediately freeze-clamped in liquid nitrogen. To extract pyruvate and lactate, frozen heart tissue was pulverized in liquid nitrogen and the powdered tissue was mixed with ice-cold 3 M perchloric acid. The tissue-perchloric acid mixture was incubated with agitation for 10 min at 4°C and then homogenized using a polytron homogenizer. After centrifugation at 5,000 g for 10 min at 4°C, the supernatant was collected and adjusted to pH 6 with 1 M NaHCO3. Pyruvate and lactate concentrations were measured by modification of previously described procedures (3, 13). For the pyruvate assay, 50 μl of perchloric acid extract was mixed with 1.4 ml of β-NADH solution (0.13 mM in 100 mM sodium phosphate buffer, pH 7.5). After addition of 50 μl of lactate dehydrogenase solution (10 mg/ml in 10 mM Tris-Cl-0.1 M EDTA buffer, pH 7.5), absorbance at 340 nm (A340) was measured immediately and again at 10 min. Pyruvate concentrations were calculated from the change in A340 and a standard curve derived from reactions performed with serial dilutions of sodium pyruvate (Sigma). For the lactate assay, 90 μl of perchloric acid extract was mixed with 820 μl of hydrazine-glycine buffer (3.8 g glycine, 8.3 ml hydrazine hydrate, H2O added to 100 ml, pH 9.0) and 70 μl of β-NADH solution (30 mg/ml in H2O). After addition of 20 μl of lactate dehydrogenase solution (10 mg/ml in 10 mM Tris-Cl-0.1 M EDTA buffer, pH 7.5), A340 was measured immediately and again at 1 h. Lactate concentrations were calculated from the change in A340 and a standard curve derived from reactions performed with serial dilutions of L-(-)-lactate (Sigma).

Fig. 1. Generation and characterization of pyruvate dehydrogenase kinase (PDK4) transgenic mice. A: schematic representation of the α-MHC-PDK4 transgene construct, which includes the α-myosin heavy chain (α-MHC) promoter linked to the mouse (m) PDK4 coding region and the human growth hormone (hGH) intron and polyA tail. B: PDK4 mRNA levels were measured in left ventricle (LV) and right ventricle (RV) of samples pooled from wild type (WT) and PDK4 transgenic (Tg) mice (n = 5/group). C: PDK4 protein levels were measured by Western blot analysis using PDK4 antiserum and either mitochondrial (Mito) or total protein prepared from pooled heart samples of wild-type and PDK4 transgenic mice (n = 4/group).
of fibrosis, TIFF images encompassing the right and left ventricles and septum from a minimum of three mice/group were captured at ×2.5 magnification with an Optronics Microfire digital charge-coupled device camera (Optronics, Goleta, CA) with Picture Frame 2.0 imaging software. These images were separated into RGB channels, and fibrotic area and total area were quantified with NIH Image J software. For quantification of centrilobular congestion and necrosis, liver sections from a minimum of three mice/group and three sections/mouse were stained with hematoxylin and eosin, and images were captured and quantified with Image J software as described above.

Substrate utilization assay. Isolated Langendorff mouse heart perfusions (1) were carried out on fed 10 wk-old wild-type and PDK4 transgenic mice. Hearts were perfused retrogradely with Krebs-Henseleit buffer containing 23.7 μU/L insulin and a mixture of U-13C-labeled free fatty acids (FFA; 0.38 mM algal mix containing 11.6% palmitoleic, 39.1% palmitic, 14.6% linoleic, and 34.8% oleic acids bound to 2% albumin) and [1,6-13C2]glucose (8.2 mM). Non-recirculating buffer was oxygenated with a thin-film oxygenator with a 95:5 mixture of O2 and CO2 and pumped into a water-jacketed, recirculating buffer was oxygenated with a thin-film oxygenator withNous substrates (Endo). *

chain fatty acids (LCFA), and unlabeled endoge-

nous substrates (e.g., triglycerides) (24, 38).

PDH and PDK activity measurements. PDH activity was assayed by coupling the generation of acetyl-CoA with the acetylation of 4-aminoazobenzene-4-sulfonic acid (2) with recombinant arylamine N-acetyltransferase as described in detail recently (16). Briefly, hearts were removed from the mice within 10 s, freeze-clamped at the temperature of liquid nitrogen, and frozen until being used for assays. Frozen hearts were pulverized and extracted with a buffer containing Triton X-100 and protease inhibitors. The supernatant obtained by centrifugation was assayed immediately for PDHa activity with a SpectraMax190 microplate reader (Molecular Devices, Sunnyvale, CA); PDHt activity was measured after complete dephosphorylation of the complex with recombinant pyruvate dehydrogenase phosphatase 1 (16). PDHα designates the activity of PDH before activation by its phosphatase, i.e., the actual activity of the complex as it existed in the tissue before extraction and PDH the activity of the complex after complete activation by dephosphorylation with its phosphatase, i.e., the maximum activity of the complex. PDK activity was measured by determining the rate of PDH inhibition by ATP as previously described (16). Since inactivation of PDH by phosphorylation is a pseudo-first-order reaction (19), rates of kinase activity are expressed as apparent first-order rate constants (min⁻¹) calculated from least-squares linear regression analysis of Inactivation by ATP) against time of incubation (18).

RESULTS

Metabolic consequences of PDK4 overexpression in heart. To investigate the effects of chronic PDK4 overexpression in the heart, a cDNA encoding full-length mouse PDK4 was inserted downstream of the α-MHC gene promoter (Fig. 1A) and two independent α-MHC-PDK4 transgenic mouse lines

![Fig. 2. Altered substrate utilization in PDK4 transgenic mice. A: PDH activity was measured in heart extracts prepared from either wild-type or PDK4 transgenic mice (n = 8/group). *P < 0.01. B: active and total pyruvate dehydrogenase levels (PDHα and PDHt, respectively) were measured in heart extracts prepared from either wild-type or PDK4 transgenic mice (n = 8/group) and are shown on left. Percent PDH activity for hearts of wild-type and PDK4 transgenic mice is shown on right. *P < 0.05 compared with equivalent wild-type group. C: 13C-NMR spectra (150 MHz) of the C4 of glutamate from wild-type (n = 8/group) and PDK4 transgenic (n = 4/group) heart extracts. The quartet due to C4 coupling with both C3 and C5 (Q) and the doublet due to C4-C5 coupling (D45) report relative oxidation of free fatty acids (FFA), while the doublet due to C4-C3 coupling (D34) reports relative glucose oxidation. D: relative oxidation rates of [1,6-13C2]glucose (glucose), [U-13C]long-chain fatty acids (LCFA), and unlabeled endogenous substrates (Endo). *P < 0.01.](http://ajpheart.physiology.org/ by 10.220.33.3 on June 29, 2017)
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**PDK4 OVEREXPRESSION EXACERBATES CARDIOMYOPATHY**

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were generated. Both PDK4 transgenic strains had comparable increases in PDK4 mRNA and protein concentrations (Fig. 1, B and C, and data not shown). Analysis of the intracellular localization of PDK4 showed that it was correctly targeted to the mitochondria (Fig. 1 C).

PDK activity was increased approximately sixfold in hearts of PDK4 transgenic mice, and there was a corresponding decrease in PDH activity (Fig. 2, A and B). To determine the effect of PDK4 overexpression on substrate utilization, hearts were isolated and perfused with [1,6-13C2]glucose and [U-13C]long-chain fatty acid. As predicted, NMR isotope analysis of heart extracts from PDK4 transgenic mice showed a significant decrease in glucose oxidation and a corresponding increase in long-chain fatty acid oxidation (Fig. 2, C and D). Oxygen consumption trended lower in hearts from PDK4 transgenic mice compared with control mice (16/11006 vs. 20/11006 g dry wt/11002), but did not reach significance. These data demonstrate that overexpression of PDK4 in heart is sufficient to alter substrate utilization.

**PDK4 overexpression exacerbates calcineurin-induced cardiomyopathy.** Despite their altered metabolism, hearts from 8-wk-old PDK4 transgenic mice showed no overt abnormalities or hypertrophy (Fig. 3) or changes in left ventricular function as measured by echocardiography (Table 1). Metabolic alterations are among a number of stress-induced events that contribute collectively to cardiovascular disease. To examine whether metabolic changes in PDK4 transgenic mice exacerbate preexisting cardiomyopathy caused by other stress-related pathways, PDK4 transgenic mice were crossed with transgenic mice expressing a constitutively active form of the calcineurin catalytic subunit (CnA), also under the control of the α-MHC promoter (25). Calcineurin is a calcium-dependent serine/threonine phosphatase that regulates cardiac growth during development and disease (32). The α-MHC-CnA model

**Table 1. Echocardiogram data**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR, beats/min</th>
<th>ESD, cm</th>
<th>EDD, cm</th>
<th>FS, %</th>
</tr>
</thead>
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<tr>
<td>WT</td>
<td>2</td>
<td>720 ± 0a</td>
<td>0.056 ± 0.004a</td>
<td>0.219 ± 0.041a</td>
<td>73.7 ± 3.5a</td>
</tr>
<tr>
<td>PDK4</td>
<td>3</td>
<td>740 ± 20a</td>
<td>0.064 ± 0.015a</td>
<td>0.257 ± 0.014a</td>
<td>75.2 ± 5.3a</td>
</tr>
<tr>
<td>CnA</td>
<td>4</td>
<td>630 ± 30a</td>
<td>0.241 ± 0.033b</td>
<td>0.372 ± 0.034a</td>
<td>35.3 ± 3.0b</td>
</tr>
<tr>
<td>PDK4/CnA</td>
<td>4</td>
<td>525 ± 45b</td>
<td>0.234 ± 0.031b</td>
<td>0.351 ± 0.034bc</td>
<td>33.7 ± 5.1b</td>
</tr>
</tbody>
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Data are means ± SE for n 8-wk-old mice. HR, heart rate; ESD, end-systolic diameter; EDD, end-diastolic diameter; FS, fractional shortening; WT, wild type; PDK, pyruvate dehydrogenase kinase; CnA, constitutively active form of calcineurin. Values labeled with different superscripts in a column are significantly different (P < 0.05).
was chosen because it is a noninvasive and robust model of cardiac hypertrophy with a well-defined pathological progression (25, 27). Thus it avoids some of the variability associated with surgical models of cardiac pressure overload. Mice bearing both the CnA and PDK4 transgenes did not have increased cardiac hypertrophy compared with the single CnA transgenic animals (Fig. 3A). However, PDK4/CnA double transgenic mice did have pronounced multifocal cardiomyocyte necrosis, fibrosis, and mineralization in all chambers of the heart (Fig. 3, B–D). The fibrosis was much more prevalent in the double transgenic mice than in the CnA transgenic mice (Fig. 3D). Similar results were obtained with PDK4/CnA double transgenic mice generated with two independently derived PDK4 transgenic lines (data not shown).

Decreased PDH activity is predicted to result in an increase in pyruvate concentrations. Consistent with this hypothesis, pyruvate concentrations were significantly increased in hearts from PDK4 transgenic mice but not CnA transgenic animals (Fig. 4). These levels were further increased in PDK4/CnA double transgenic mice (Fig. 4). Lactate concentrations showed a similar trend (Fig. 4). These changes demonstrate that there are strong metabolic perturbations in the hearts of PDK4/CnA double transgenic mice.

The expression of a panel of genes involved in metabolism and cardiac remodeling was analyzed in the hearts of wild-type, PDK4 transgenic, CnA transgenic, and PDK4/CnA double transgenic mice. In PDK4 transgenic mice, the only gene other than PDK4 that changed significantly was H9252/MHC (Fig. 5). While these data are consistent with the lack of any evidence of cardiomyopathy in the PDK4 transgenic mice, they do not reflect the changes that occur in metabolic flux in these animals. Thus they highlight the limitations of using gene expression data to assess metabolic status. In contrast to what was seen in PDK4 transgenic mice, expression of most of the genes Fig. 5. Gene expression in left ventricles from wild-type and transgenic mice. mRNA levels of the indicated genes were measured with total RNA prepared from wild-type and PDK4, CnA, and PDK4/CnA transgenic mice (n = 5/group). ANF, natriuretic peptide precursor type A; BNP, natriuretic peptide precursor B; ACTA1, skeletal muscle actin-α1; PPAR-α, peroxisome proliferator-activated receptor-α; SERCA2a, sarcoplasmic reticulum Ca²⁺-transport ATPase isoform 2a; PLB, phospholamban; GLUT, glucose transporter; CPT1b, carnitine palmitoyltransferase 1b; MCD, malonyl-CoA decarboxylase; UCP, uncoupling protein. For PDK4, inset shows the difference in PDK4 mRNA levels between wild-type and CnA transgenic mice.
was altered in CnA transgenic mice (Fig. 5). The changes included increased mRNA levels of natriuretic peptide precursor type A (ANF), natriuretic peptide precursor B (BNP), β-MHC, and skeletal muscle actin-α1 (ACTA1) and decreased mRNA levels of PDK2, PDK4, α-MHC, PPAR-α, sarco(endo)plasmic reticulum Ca\(^{2+}\)-transport ATPase isoform 2a (SERCA2a), phospholamban (PLB), glucose transporter-4 (GLUT4), carnitine palmitoyltransferase 1b (CPT1b), malonyl-CoA decarboxylase (MCD), and uncoupling proteins (UCP)2 and UCP3. The changes in mRNA levels of ANF, β-MHC, PPAR-α, GLUT4, and CPT1b in hearts from CnA transgenic mice are similar to those previously reported (4). A similar pattern of altered gene expression also occurs in pressure overload-induced cardiac hypertrophy (21, 30).

Gene expression in the PDK4/CnA double transgenic mice generally followed one of two distinct patterns compared with wild-type and CnA transgenic mice. Expression of the first group of genes, including PDK2, α-MHC, SERCA2a, PLB, PPAR-α, GLUT4, CPT1b, MCD, and UCP3, was decreased in the CnA single transgenic mice and further decreased in the PDK4/CnA double transgenic animals (Fig. 5). Since several of these gene products are involved in fatty acid and carbohydrate metabolism, the changes are consistent with generalized metabolic dysfunction as previously hypothesized for CnA transgenic mice (4). A second set of genes, including BNP, β-MHC, and ACTA1, were most highly expressed in the CnA transgenic mice, with lower expression levels in PDK4/CnA transgenic animals (Fig. 5). Although no synergy between the PDK4 and CnA transgenes was seen in the gene expression studies, the cumulative effect of all these changes may contribute to the metabolic perturbations and cardiomyopathy in the double transgenic mice.

Given the changes in heart histology and metabolite profile in the PDK4/CnA transgenic mice (Figs. 3 and 4), we next investigated whether the double transgenic animals have compromised cardiac function beyond that previously reported for CnA mice (25, 27). Although PDK4/CnA double transgenic mice had a significantly reduced heart rate compared with the single transgenic mice, no additional deterioration in left ventricular function was detected by echocardiography performed on 8-wk-old double transgenic mice compared with CnA transgenic mice (Table 1). However, the CnA transgenic mice already have severe left ventricular dysfunction that may mask any further deterioration (22, 25, 27). Importantly, longevity studies showed that double transgenic mice have a marked increase in mortality compared with single transgenic animals; at 130 days of age all of the double transgenic mice had died, whereas 60% of the CnA transgenic mice were still alive (Fig. 6A). No deaths occurred in either wild-type or PDK4 transgenic mice (data not shown). Histology performed on liver sections from PDK4/CnA double transgenic mice revealed centrilobular congestion consistent with heart failure that was not seen in either the PDK4 or CnA single transgenic animals (Fig. 6, C and D). Moreover, gross histological examination of hearts from several dead PDK4/CnA double transgenic mice revealed prominent mural thrombi that were located in all cardiac chambers (Fig. 6B and data not shown). It is concluded that the metabolic alterations caused by PDK4 overexpression exacerbate cardiomyopathy and increase mortality caused by activation of the calcineurin stress-response pathway.

**DISCUSSION**

PDK4 plays a crucial role in dictating substrate utilization throughout the body (29). Its importance in whole body energy homeostasis is underscored by the recent description of global
PDK4-knockout mice, which have a range of metabolic defects during starvation including lower concentrations of blood glucose and gluconeogenic intermediates in liver and a faster rate of pyruvate oxidation in muscle (17). In this report, we demonstrate that selective overexpression of PDK4 in heart results in significant changes in energy metabolism, including increased cardiac fatty acid utilization and decreased carbohydrate consumption with a corresponding increase in pyruvate concentrations. These data demonstrate that overexpression of PDK4 is sufficient to markedly alter substrate utilization in the heart.

Despite the change in their cardiac substrate utilization profile, the PDK4 transgenic mice showed no evidence of overt cardiomyopathy. These findings highlight the remarkable metabolic flexibility of the heart. However, overexpression of PDK4 in the context of the activated calcineurin stress-response pathway resulted in profound cardiomyopathy that included fibrosis and necrosis that were not seen in the CnA single transgenic mice (Fig. 3, C and D). Moreover, the PDK4/CnA double transgenic mice showed a pronounced increase in mortality (Fig. 6A). The pathological synergy between PDK4 and CnA is reminiscent of that seen by combining banding-induced overload with either streptozotocin-induced diabetes or PPAR-α reactivation (6–8, 26, 37). Our findings are consistent with the hypothesis that disturbances in contractile function can be compensated for by changes in metabolism and vice versa, but that simultaneous disruption of both energy-producing and energy-consuming pathways causes cardiac mal-adaptation and failure (5).

While the impact of the CnA transgene on substrate utilization has not been established, cardiac hypertrophy is generally associated with an increased reliance on carbohydrate metabolism to meet energy demands (30, 31). CnA transgenic mice had decreased expression of PDK4, PDK2, and several genes involved in fatty acid oxidation including PPAR-α, CPT1b, and MCD (Fig. 5). However, CnA transgenic mice also had decreased expression of GLUT4 (Fig. 5), suggesting a more generalized metabolic dysfunction as previously reported (4). While the changes in gene expression that were observed in the PDK4/CnA double transgenic mice are generally consistent with those seen in other models of cardiac hypertrophy (21, 30), it is important to note that changes in mRNA levels may not accurately reflect changes in either the levels or activities of the proteins they encode.

Transcription of PDK4 is stimulated in heart and other tissues by the nuclear fatty acid receptor PPAR-α (15, 28, 34). Two recent studies showed that increased PPAR-α activity in heart can cause cardiomyopathy (11, 37). Finck et al. (11) showed that selective overexpression of PPAR-α in heart caused ventricular hypertrophy and systolic dysfunction. Young et al. (37) showed that PPAR-α mRNA levels decrease in the heart of mice subjected to pressure overload, suggesting that down-regulation of PPAR-α is part of the adaptive response of the hypertrophic heart. Similar effects on PPAR-α expression were observed in the CnA transgenic mice (Fig. 5). Importantly, Young et al. (37) showed that ligand-mediated reactivation of PPAR-α caused contractile dysfunction and heart failure in the pressure overload model. Among the genes induced by PPAR-α reactivation was PDK4 (37). While multiple PPAR-α target genes undoubtedly contribute to the cardiomyopathy observed in both the Finck et al. (11) and Young et al. (37) studies, our results demonstrate that overexpression of PDK4 alone is sufficient to cause metabolic inflexibility and to exacerbate preexisting cardiomyopathy caused by chronic activation of the calcineurin signaling pathway.

Limitations of study. The results presented in this article highlight the important role that PDK4 plays in determining substrate utilization in heart and, furthermore, demonstrate that PDK4 overexpression in heart increases the already elevated mortality seen in CnA single transgenic mice. Nevertheless, there are several important limitations of these studies. First, the perfusates in the heart perfusion studies did not include ketone bodies, lactate, or pyruvate. Thus the analysis of cardiac metabolism in the PDK4 transgenic mice was limited to glucose and fatty acid catabolism. Second, the only gene other than PDK4 that changed significantly in our analyses of PDK4 transgenic mice was β-MHC. While these data are consistent with the lack of any evidence of cardiomyopathy in the PDK4 transgenic mice, they do not reflect the changes that occurred in metabolic flux in these animals. Thus they highlight the inherent limitations of using mRNA measurements to assess metabolic status. A related limitation is that only PDH activity was measured in the PDK4 transgenic mice. Clearly, altering PDK4 could have secondary effects on the expression and activity of proteins other than PDH that could contribute to metabolic inflexibility and, ultimately, cardiomyopathy. Finally, we have not been able to determine the cause of the increased mortality of the PDK4/CnA double transgenic mice. PDK4/CnA double transgenic mice had prominent cardiomyocyte fibrosis and necrosis and hepatic centrilobular congestion consistent with heart failure, and postmortem cardiac examination revealed mural thrombosis. The extensive interstitial fibrosis raises the possibility of reentrant arrhythmias as a potential etiology of sudden death in these animals. However, additional studies will be required to determine the precise cause of death.

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