Metabolic Gene Remodeling and Mitochondrial Dysfunction in Failing Right Ventricular Hypertrophy Secondary to Pulmonary Arterial Hypertension

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Background—Right ventricular (RV) dysfunction (RVD) is the most frequent cause of death in patients with pulmonary arterial hypertension. Although abnormal energy substrate use has been implicated in the development of chronic left heart failure, data describing such metabolic remodeling in RVD remain incomplete. Thus, we sought to characterize metabolic gene expression changes and mitochondrial dysfunction in functional and dysfunctional RV hypertrophy.

Methods and Results—Two different rat models of RV hypertrophy were studied. The model of RVD (SU5416/hypoxia) exhibited a significantly decreased gene expression of peroxisome proliferator-activated receptor-γ coactivator-1α, peroxisome proliferator-activated receptor-α and estrogen-related receptor-α. The expression of multiple peroxisome proliferator-activated receptor-γ coactivator-1α target genes required for fatty acid oxidation was similarly decreased. Decreased peroxisome proliferator-activated receptor-γ coactivator-1α expression was also associated with a net loss of mitochondrial protein and oxidative capacity. Reduced mitochondrial number was associated with a downregulation of transcription factor A, mitochondrial, and other genes required for mitochondrial biogenesis. Electron microscopy demonstrated that, in RVD tissue, mitochondria had abnormal shape and size. Lastly, respirometric analysis demonstrated that mitochondria isolated from RVD tissue had a significantly reduced ADP-stimulated (state 3) rate for complex I. Conversely, functional RV hypertrophy in the pulmonary artery banding model showed normal expression of peroxisome proliferator-activated receptor-γ coactivator-1α, whereas the expression of fatty acid oxidation genes was either preserved or unregulated. Moreover, pulmonary artery banding-RV tissue exhibited preserved transcription factor A mitochondrial expression and mitochondrial respiration despite elevated RV pressure-overload.

Conclusions—Right ventricular dysfunction, but not functional RV hypertrophy in rats, demonstrates a gene expression profile compatible with a multilevel impairment of fatty acid metabolism and significant mitochondrial dysfunction, partially independent of chronic pressure-overload. **(*Circ Heart Fail*. 2013;6:136-144.)**

Key Words: fatty acids ■ metabolism ■ mitochondria ■ pressure ■ pulmonary heart disease

Pulmonary arterial hypertension (PAH) is a severe and often rapidly progressive group of diseases that are characterized by a chronically and frequently progressive increase in the right ventricular (RV) afterload.[1] Increased RV afterload is partially compensated by RV hypertrophy but eventually leads to RV dysfunction (RVD), RV failure, and untimely death, regardless of medical treatment.[1] Given the prognostic importance of RVD in PAH, the cellular and molecular mechanisms underlying RVD need to be investigated, because they can be potentially reversible. Human and experimental chronic left heart dysfunction is characterized by decreased oxidative metabolism,[2] abnormal mitochondrial respiration,[3] and impaired mitochondrial biogenesis.[4] These changes have in part been explained by a deregulated expression of critical transcription factors, such as the peroxisome proliferator-activated receptor (PPAR)-α,[5] the estrogen-related receptor (ERR)-α,[6] and the master regulator of oxidative metabolism, the PPAR-γ coactivator-1α (PGC-1α).[7]

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Akin to left heart failure, it has been postulated that RVD is characterized by abnormal energy metabolism.[8,9] Studies in animal models have demonstrated that RVD exhibits an increased expression of glycolysis-related genes[10] and increased enzymatic glycolysis rate.[11] However, it is largely unknown to what extent this switch in cardiac bioenergetics (also known as metabolic remodeling)[12] involves changes in fatty acid oxidation (FAO), whether metabolic remodeling is an adaptive response to chronic pressure overload, or to what extent mitochondrial structure and function are also compromised in RVD. Thus, we sought to do the following: (1) characterize the metabolic gene expression profile associated with RVD; (2) determine whether pressure-overload is sufficient to explain metabolic gene remodeling; and (3) assess the structure and function of mitochondria in the dysfunctional right ventricle.

Here, we report that RVD, as assessed in rat and human RV tissue samples, is characterized by a downregulation of PGC-1α expression and decreased expression of several PGC-1α target genes encoding key enzymes that regulate FAO, as well as other genes involved in mitochondrial biogenesis. In addition, we present evidence for impaired mitochondrial structure and function (respiration). Some of these findings have been reported previously in abstract form.[13,14]

Methods

Animal Models

SU5416/Hypoxia Model (SuHx)

An animal model of severe angioproliferative PAH and RVD was generated in male Sprague-Dawley rats (body weight, 200 g; age, 6 weeks) with a 20-mg/kg, 1-time subcutaneous injection of a vascular endothelial growth receptor blocker (SU5416), followed by 4 weeks of 10% hypoxia, as described previously[15,16] and in the online-only Data Supplement. On return to normoxia, RV function was evaluated by transthoracic echocardiogram. The tricuspid annular plane systolic excursion was the reference parameter for RV function. For hemodynamic measurements, median sternotomy was performed, and RV systolic pressure was measured with a 4.5-mm Millar conductance catheter inserted at the RV outflow tract. As described previously, the RV in the SuHx rat model responds to pulmonary hypertension with a robust degree of hypertrophy, followed by dysfunction and failure.[17] The RV in this rat model is characterized by fibrosis, capillary rarefaction, and cardiomyocyte apoptosis,[15] which are associated with decreased cardiac output, markedly dilated RV, and decreased exercise capacity.[18] As described previously, the SuHx RVD model reproduces some features of human RVD, such as paradoxical septal movement and RV dilatation (online-only Data Supplement Movie I).

Pulmonary Artery Banding

Surgical ligation of the pulmonary artery was achieved through a left thoracotomy in male Sprague-Dawley rats weighing 180 to 200 g, with a silk suture tied around an 18-gauge needle alongside the pulmonary artery, as described previously[15] (also in online-only Data Supplement). The pulmonary artery banding (PAB) rats were killed to collect organs 6 weeks after the surgery to allow for significant hypertrophy, as reported by Bogaard et al.[15,19] As a model of strictly mechanical RV pressure overload, the PAB rat model demonstrates preserved RV function despite generating significantly high RV afterload and hypertrophy[15,19] (Figure S1F through S1H, available in the online-only Data Supplement). Thus, the PAB model was used as a model of nondysfunctional RV hypertrophy and was studied as above. The Table presents the echocardiographic and hemodynamic data illustrating the degree of pulmonary hypertension and RV function in all of the conditions studied.

Human samples were obtained from patients diagnosed with PAH who underwent cardiac transplantation. Oxygen consumption by intact mitochondria was measured with Clark-type oxygen electrodes. Immunohistochemistry and gene and protein expression studies were performed with standard procedures, as outlined in the online-only Data Supplement Methods section.

Statistical Analysis

Differences between groups were assessed with 1-way or 2-way ANOVA or Kruskal–Wallis tests. Bonferroni and Dunn post hoc tests were used to assess significant differences between groups. A P value <0.05 was accepted as significant. Correlation analysis was done with a Spearman test. Results are reported as mean±SEM or fold-change mean±SEM unless specified otherwise. Four to 6 rats were used per group, unless otherwise specified. Statistical analysis was done with PASW V.18 (IBM, Armonk, NY) and GraphPad Prism (La Jolla, CA). For detailed information regarding echocardiography, hemodynamic measurements, gene expression, protein expression, human samples, mitochondrial isolation, measurement of mitochondrial respiration, chromatography electrospray ionization tandem mass spectrometry and immunohistochemistry, see the online-only Data Supplement.

Results

RVD Is Characterized by a Load-Independent Downregulation of PGC-1α, PPAR-α, and ERR-α

We have reported previously that dysfunctional RVs from SuHx-rats differentially express multiple gene signaling pathways when compared with nondysfunctional hypertrophied RVs.[20] Importantly, we reported that RVD is associated with gene expression changes that suggest an abnormal metabolism, with a particularly strong signal relating to the PPAR signaling pathways. Therefore, as a first step, we measured the expression of PGC-1α, a direct coactivator and master regulator of the

Table. Rat Characteristics and Echocardiographic and Hemodynamic Measurements in Control, SuHx, and PAB Animals

Variable	BW, g	RV, μg	RV/LV+S, %	RVW/BW, %	RVSP, mm Hg	TAPSE, mm	HR, bpm	Lung Vascular Remodeling
Controls	340±08	204±23	25±01	0.6±0.03	24±3.12	3.46±0.12	315±25	No
SuHx	296±19	543±63†	65±02†	1.83±0.10*	92±2.5†	1.60±0.16*	246±28	Yes
PAB	395±6.5‡	500±52†	56±03†	1.26±0.05	76±2.5†	3.25±0.27‡	257±23‡	No

Four to six rats were included per group. BW indicates body weight; HR, heart rate at the time of catheterization; LV, left ventricular weight; PAB, pulmonary artery banded; RV, right ventricular weight; RVSP, right ventricular systolic pressure; S, interventricular septum weight; SuHx, SU5416/hypoxia; and TAPSE, tricuspid annular plannar systolic excursion measured by echocardiogram. Data are shown as mean±SEM.

*P<0.05 vs controls.

†P<0.0001 vs controls.

‡P<0.05 vs SuHx.

§P<0.05 vs PAB.

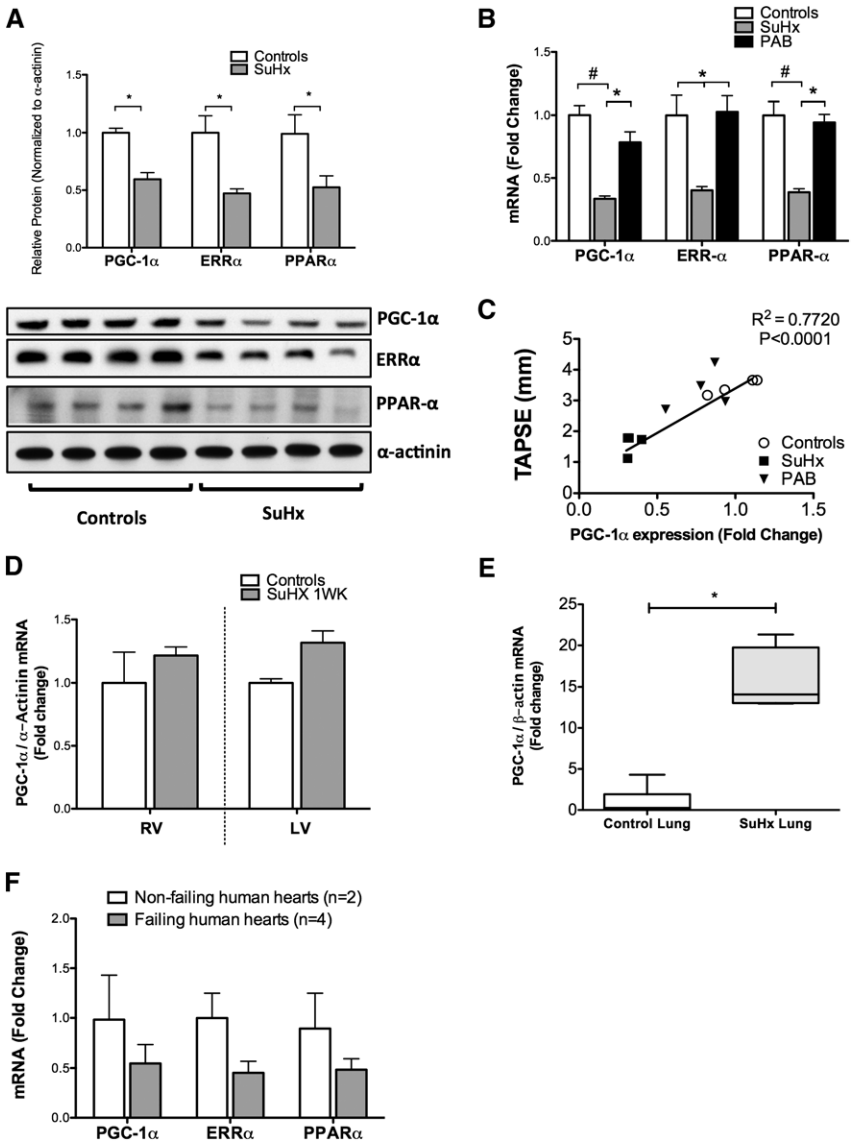

Figure 1. **A**, Western blots on SU5416/hypoxia (SuHx) right ventricular (RV) whole tissue lysates show a downregulation of peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α), estrogen-related receptor (ERR)-α, and PPAR-α on the protein level. **B**, Quantitative reverse transcription-polymerase chain reaction mRNA expression analysis from right ventricles of control, SuHx, and pulmonary artery banding (PAB) animals. Compared with controls and PAB, SuHx-RV tissue exhibits decreased PGC-1α transcript levels along with a decreased expression of the nuclear receptors ERR-α and PPAR-α. **C**, PGC-1α correlation with right ventricular function (tricuspid annular plannar systolic excursion [TAPSE]). **D**, Quantitative polymerase chain reaction analysis shows no change in PGC-1α expression in RV and left ventricle (LV) of SuHx rats at 1 week after SuHx had been initiated. **E**, mRNA expression of PGC-1α in SuHx lung tissue. **F**, PGC-1α, ERR-α, and PPAR-α mRNA transcript levels in human RV samples. Data are shown in fold changes±SEM over controls; ***P*<0.001; #*P*<0.0001.

PPAR family of transcription factors. PGC-1α regulates oxidative metabolism and many aspects of mitochondrial biology.[21]

Western blots of protein samples obtained from SuHx RV tissues showed a significantly decreased amount of PGC-1α protein (Figure 1A). Quantitative polymerase chain reaction analysis revealed a significant downregulation of PGC-1α mRNA levels (Figure 1B), indicating that the change in PGC-1α expression also occurred on the transcription level. Decreased PGC-1α gene expression was accompanied by a decreased expression of the ERR-α and PPAR-α genes (Figure 1B). To evaluate whether pure mechanical RV pressure-overload was sufficient to downregulate PGC-1α expression, we measured PGC-1α transcript levels in PAB-RV tissues and found that the expression of PGC-1α, ERR-α, and PPAR-α was not significantly decreased in the nonfailing hypertrophied RVs of PAB rats, despite the high RV pressure and RV hypertrophy (Figure 1B). Figures 1C and Figure S2A and S2B (available in the online-only Data Supplement) illustrate that PGC-1α (R^2=0.72; p=0.001), PPAR-α (R^2=0.72, P=0.001), and ERR-α (R^2=0.76; P=0.002) transcript levels strongly correlated with the tricuspid annular plane systolic excursion, a heart rate–independent variable of RV function.

We have described previously that SU5416 treatment alone does not induce RVD and has a limited impact on gene expression.[20] However, because decreased expression of PGC-1α could be a direct effect of the combination of SU5416 and hypoxia rather than a consequence of RVD, we measured the expression of PGC-1α 1 week after the SuHx protocol had been initiated. At 1 week no RVD is present and, indeed, as shown in Figure 1D, PGC-1α expression is not decreased in the right ventricle or in the left ventricle. In addition, we measured the expression of PGC-1α in rat lung tissues after 4 weeks of SuHx, a time point where plexiform-like lesions have already formed, and we found a significant increase in PGC-1α expression (Figure 1E). To further evaluate a potential toxic effect of SU5416 in the setting of RV pressure overload, we examined the RV gene expression of PAB animals exposed to SU5416 and found no change in the PGC-1α, ERR-α, or PPAR-α mRNA transcript levels (Figure S2C). Next, we measured the expression of PGC-1α in LV tissue

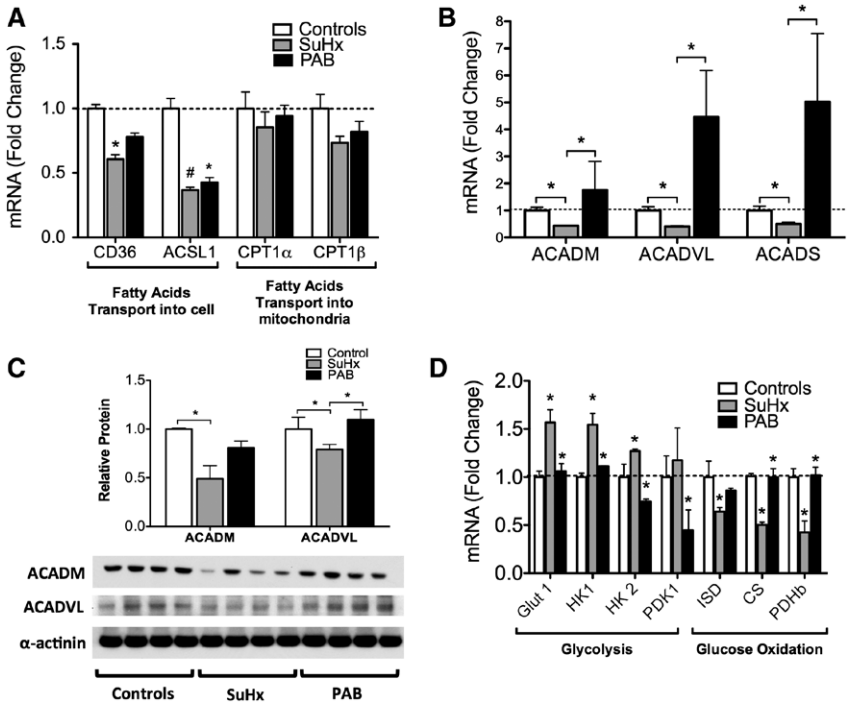

Figure 2. **A**, mRNA levels of genes encoding key rate-limiting enzymes involved in fatty acid transport into the cell (*CD36*) and transport into the mitochondria (*ACSL1*, *CPT1*α, *CPT1*β, and *CPT2*) were decreased. **B**, Genes encoding a family of acyl-coenzyme A (CoA) dehydrogenases specific for fatty acid β-oxidation (*ACADS*, *ACADM*, and *ACADVL*) also were downregulated in dysfunctional SU5416/hypoxia (SuHx) right ventricles but significantly increased in pulmonary artery banding (PAB) right ventricular (RV) tissue. **C**, Western blotting on cytosolic protein extracts obtained from RV tissue lysate shows decreased protein expression of ACADM and ACADVL in SuHx-RV tissue. **D**, Genes encoding key rate-limiting enzymes for aerobic glucose oxidation (Kreb cycle) were downregulated, whereas genes encoding enzymes necessary for glycolysis were upregulated. Data are shown in fold changes±SEM over controls; **P*<0.01; #*P*<0.0001.

obtained from animals killed 4 weeks after initiation of the SuHx protocol. Although somewhat decreased, the SuHx-LV tissue did not show a significant change in PGC-1α expression (Figure S2D). In the aggregate, the data indicate that the decreased expression of PGC-1α is unlikely attributed to a toxic effect of SU5416.

Because nuclear receptors other than PGC-1α have been implicated in phenotypic metabolic changes that occur during normal cardiac development, we decided to evaluate the expression of the ERR-γ and of the transcription factors COUP-TF1 and SP1.[22,23] Quantitative polymerase chain reaction analysis showed that the expression of these transcription factors was not affected in dysfunctional RV tissues (Figure S2E). Lastly, to examine whether the changed expression of the metabolic gene pattern was also observed in human myocardium, we examined the gene expression of PGC-1α, PPAR-α, and ERR-α in RV tissue samples from patients with PAH. Indeed, we found a comparable expression pattern in the human RV tissue as we had observed in the SuHx RV rat tissue (Figure 1F).

Dysfunctional RV Hypertrophy Is Characterized by Decreased Expression of Genes Involved in Fatty Acid and Glucose Oxidation

Figure 2A illustrates that multiple PGC-1α/PPAR-α/ERR-α target genes encoding critically important proteins, required for fatty acid transport into the cardiomyocytes and into the mitochondria, were downregulated in the SuHx dysfunctional right ventricles. In addition, the expression of genes encoding proteins required for β-oxidation (acyl-coenzyme A [CoA] dehydrogenase, C-4 to C-12 straight chain [ACADM], acyl-CoA dehydrogenase, very long chain [ACADVL], and acyl-CoA dehydrogenase, C-4 to C-8(6) short chain [ACADS]) was decreased (Figure 2B). Western blot analysis confirmed the decreased expression of ACADM and ACADVL in RVD (Figure 2C). Supporting the observation of normal expression of PGC-1α/PPAR-α/ERR-α, nonfailing hypertrophied (PAB) right ventricles demonstrated a normal expression of ACADM and a significantly increased expression of ACADS and ACADVL. These acyl-CoA dehydrogenases are required to metabolize medium, short, and long fatty acids, respectively.

In accordance with our previous reports,[20] dysfunctional RV hypertrophy in the SuHx rats was characterized by increased expression of GLUT1 and hexokinase 1; these 2 genes play an important role in glycolysis (Figure 2D). In contrast, we found a downregulation of genes that encode enzymes involved in aerobic glucose catabolism, such as the genes encoding the Krebs cycle enzymes citrate synthase and isocitrate dehydrogenase. Furthermore, the β subunit of pyruvate dehydrogenase, an important link between glycolysis and glucose oxidation, showed a 50% decreased mRNA expression, whereas the gene expression of pyruvate dehydrogenase kinase, an enzyme controlling pyruvate dehydrogenase activity, was upregulated. Conversely, nonfailing hypertrophied PAB RVs exhibited a normal expression of glycolysis-related genes, a significantly lower expression of pyruvate dehydrogenase kinase, and normal expression of isocitrate dehydrogenase and citrate synthase (Figure 2D).

RVD Is Characterized by Abnormal Mitochondrial Ultrastructure, Impaired Mitochondrial Respiration, and Abnormal Mitochondrial Biogenesis

PGC-1α regulates mitochondrial biogenesis along with oxidative metabolism.[21] Therefore, we sought to explore abnormalities in mitochondrial biology. PGC-1α exerts pleiotropic effects by direct coactivation of an array of nuclear and nonnuclear receptors used in the control of cellular metabolism.[21] Among them, transcription factor A, mitochondrial (TFAM),

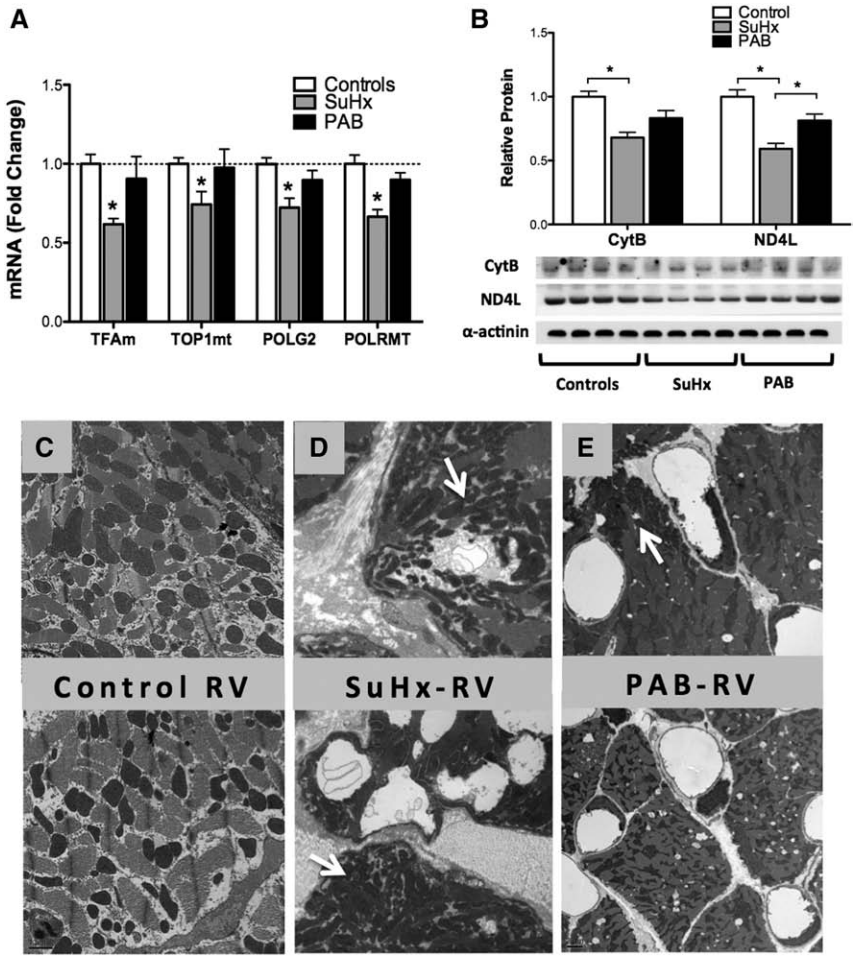

Figure 3. **A**, mRNA transcript levels of genes required for mitochondrial biogenesis and mitochondrial DNA (mtDNA) replication and transcription. **B**, Western blotting of right ventricular (RV) tissue demonstrates a significantly decreased protein expression of mtDNA-encoded proteins in SU5416/hypoxia (SuHx)-RV dysfunction. **C** and **D**, Electron microscopy demonstrates abnormal ultrastructure of mitochondrial in RV dysfunction. Data are shown in fold changes±SEM compared with controls; **P*<0.01; #*P*<0.0001.

regulates mitochondrial DNA replication and maintenance and is required for cellular and mitochondrial viability.[24,25] Associated with the decreased expression of PGC-1α, SuHx-RVD tissue had a decreased expression of TFAM (Figure 3A). Because reduced TFAM mRNA levels are associated with alterations in mitochondrial biogenesis, we measured the expression of *Top1mt*, *POGL2*, and *POLRMT*, a set of genes that encode enzymes required for the replication of mtDNA and mitochondrial biogenesis.[4,25] All 3 of the genes were downregulated in dysfunctional (SuHx) RV hypertrophy but not in PAB-induced RV hypertrophy, as illustrated in Figure 3A. To test for decreased mtDNA transcription, we measured the expression of 2 mtDNA-encoded proteins, NADH-ubiquinone oxidoreductase subunit 4L and cytochrome B. These 2 proteins are subunits of the mitochondrial electron-transport chain complexes I and III, respectively. As illustrated in Figure 3B, the relative protein expression of NADH-ubiquinone oxidoreductase subunit 4L and cytochrome B was significantly decreased in SuHx-RVs.

High-power magnification electron microscopy demonstrated that the mitochondrial ultrastructure in RVD tissue was highly abnormal. In comparison to controls (Figure 3C), mitochondria in SuHx RVs were consistently abnormal in shape and size and clumped together in clusters (Figure 3D). Although clustering of mitochondria was also present in the PAB RVs (Figure 3E, arrow), the overall distribution of mitochondria was similar to that of control RVs. On isolation, RVD tissues exhibited a significantly decreased amount of mitochondria, as evidenced by mitochondrial yield and by citrate synthase activity (Figure 4A and 4B). Isolated mitochondria were studied by respirometry to evaluate the efficiency of oxidative phosphorylation. RVF mitochondria demonstrated a significantly decreased ADP-stimulated (state 3) respiration rate when using glutamate (Figure 4C) but not when using succinate as electron donors to complexes I and II, respectively (Table S2). The complete respirometry results are depicted in Table S2.

Because mitochondrial dysfunction could contribute to the generation of reactive oxygen species (ROS), we measured the levels of 8-isoprostane (8-*iso*Prostaglandin $F_{2\alpha}$) in RVD tissues. 8-Isoprostane has been proposed as a marker of antioxidant deficiency and enhanced oxidative stress.[26] Analysis from liquid chromatography-tandem mass spectrometry of SuHx RV tissue demonstrated no change in the amount of 8-isoprostane in comparison with controls (Figure S3A) but increased levels in the lungs (Figure S3B). However, although the amount of ROS generated might have not been sufficient to cause significant lipid peroxidation in whole RV tissues, ROS could still induce damage. mtDNA is particularly susceptible to ROS-induced damage,[27] and a common marker of mtDNA damage is the formation of 7,8-dihydro-8-oxoguanine, a mutagenic base byproduct that

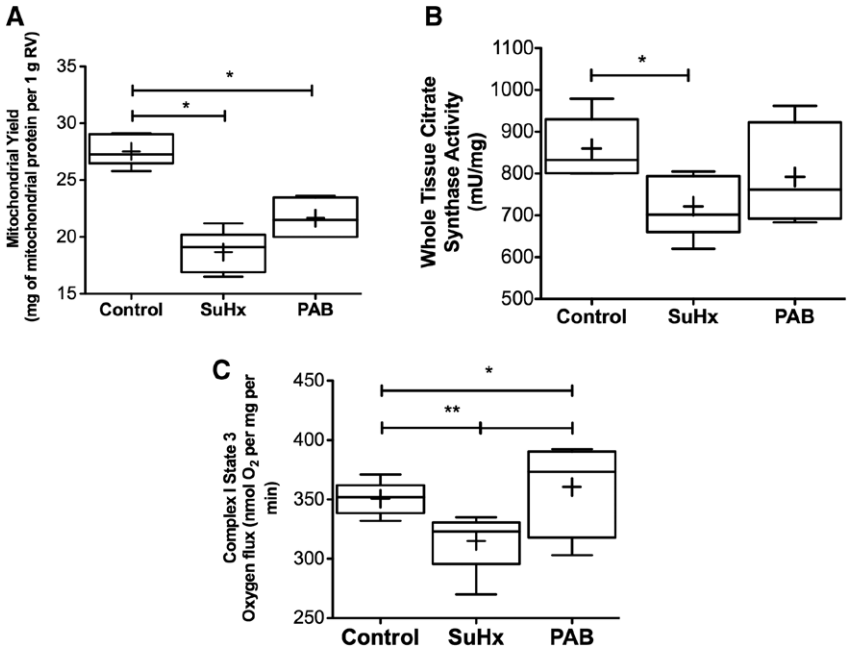

Figure 4. **A**, Amount of mitochondrial protein per 100 mg of right ventricular (RV) tissue. SU5416/hypoxia (SuHx) and pulmonary artery banding (PAB) RV tissues demonstrate significantly decreased mitochondrial yield when compared with control RV tissue. **B**, Whole tissue citrate synthase activity assay demonstrates that SuHx has a reduced oxidative capacity when compared with control RV. **C**, State 3 respiration with complex I substrate (glutamate) is significantly decreased in SuHx RV dysfunction when compared with control or PAB RV tissues. The lines in the box-and-whiskers plots illustrate the median, whereas the + sign illustrates the mean. **P*<0.01; #*P*<0.0001.

results from direct exposure of DNA to ROS.[28] Figure 5 shows 8-oxo-G–positive staining in dysfunctional RV tissues, particularly in the endomyocardial area.

Discussion

RV failure is a common consequence of severe chronic pulmonary hypertension and the most frequent cause of death in patients with PAH.[1] Although RVD plays an important prognostic role in patients with PAH,[29,30] there are relatively few experimental data shedding light on the mechanisms of chronic RVD and failure.[31,32] Although it has been proposed that RVD is associated with metabolic gene remodeling,[8] a comprehensive metabolic gene profile of the failing right ventricle is still lacking.

Here we demonstrate that dysfunctional RV hypertrophy, in rats and patients with PAH, exhibits a significant reduction in the expression of PGC-1α and its corresponding nuclear receptors (PPAR-α and ERR-α). Interestingly, the change in PGC-1α expression seems to be largely independent of the RV pressure-overload and hypertrophy. Moreover, multiple PGC-1α target genes encoding proteins required for fatty acid metabolism were significantly decreased in expression in RVD tissues. Particularly, the expression of genes encoding the acyl-CoA dehydrogenases, which are specific for fatty acid β-oxidation, was significantly decreased in dysfunctional SuHx-RV hypertrophy but not in adaptive PAB-RV hypertrophy. Conversely, functional PAB-RV hypertrophy was associated with a high expression of ACADS and ACADVL, the latter being the most important heart acyl-CoA dehydrogenase for FAO. Altogether, the gene and protein expression data suggest that, in RVD, FAO is impaired on multiple levels. Along with the metabolic gene remodeling, we show evidence for an abnormal mitochondrial ultrastructure and decreased mitochondrial respiration at the level of complex I of the electron transport chain. Moreover, RVD is characterized by decreased expression of genes encoding proteins required for mitochondrial biogenesis, such as TFAM, *Top1mt*, *POGL2*, and *POLRMT*. RVD also demonstrated a significantly low mitochondrial yield in comparison with control RVs. Finally

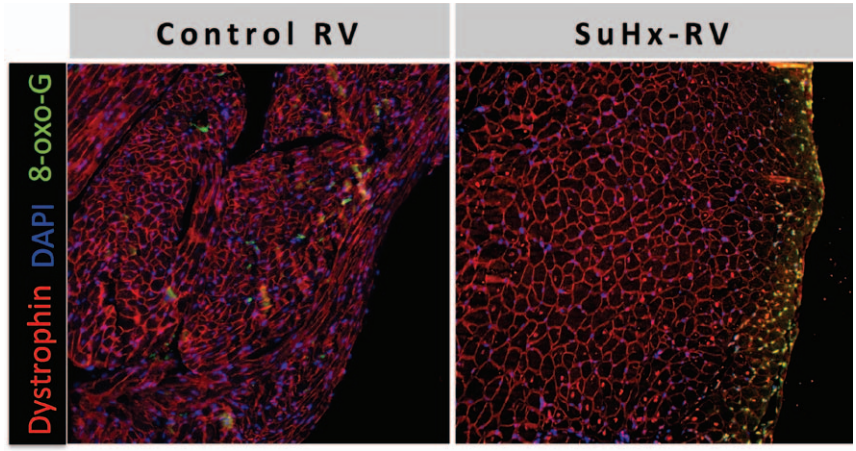

Figure 5. Immunofluorescence shows increased 7,8-dihydro-8-oxoguanine (green) in right ventricular dysfunction (RVD) tissue vs SU5416/hypoxia (SuHx)-RVD.

we demonstrate that RVD exhibits high levels of 7,8-dihydro-8-oxoguanine, consistent with ROS-induced DNA damage.

We decided to focus on central transcriptional regulators, such as PGC-1α and its corresponding nuclear receptors ERR-α and PPAR-α, because multiple gene knockout studies have illustrated that these proteins play an important role during the functional bioenergetic adaptation of the heart-to-pressure overload.[21,29,30] PGC-1α is preferentially expressed in tissues with high oxidative capacity and coordinates several biological processes of mammalian energy metabolism by activating genes involved in cellular uptake and mitochondrial oxidation of fatty acids.[33] Heart tissue obtained from PGC-1α knockout mice displays a reduced palmitoyl-L-carnitine state 3 respiration, suggesting reduced FAO, and a reduction in the amount of ATP generated per oxygen consumed.[34] Of equal importance, in the absence of PGC-1α, the expression of mitochondrial genes in the heart is suppressed, the activities of mitochondrial enzymes are altered, and ATP production is reduced.[35] As has been shown in models of left heart failure,[7,35,36] the SuHx model of severe PAH and RVD is characterized by reduced PGC-1α expression (Figure 1A-B). We consider this reduced expression as a central component of RV metabolic remodeling. Although downregulation of PGC-1α is a feature of dysfunctional hypertrophy, it remains unclear what drives the downregulation of PGC-1α expression during RVD. Because the SuHx model is characterized by capillary rarefaction,[15] ischemia and hypoxia could potentially drive the metabolic remodeling. However, PGC-1α expression is not decreased until RVD occurs. Because PGC-1α expression is a hypoxia-inducible factor–independent hypoxia-inducible gene,[37] it is unlikely that the downregulated expression of PGC-1α and the associated metabolic remodeling profile would be entirely explained by hypoxia or by hypoxia-inducible factor activation. Although decreased PGC-1α mRNA expression has been reported in human left heart failure,[38] recent studies using samples of left ventricles obtained from patients with heart failure have demonstrated a relatively normal expression of PGC-1α.[4] Perhaps these discrepant results may be explained by different drug treatments of the patients with LV failure.

Although impaired glucose oxidation has been well characterized in the monocrotaline-injury model of RVD, changes in fatty acid metabolism are less clear.[39] In our study, the downregulation of PGC-1α, ERR-α, and PPAR-α expression was coupled to a decreased expression of genes encoding fatty acid transport proteins and FAO, which suggests to us that fatty acid catabolism in the failing RVs is likely compromised on the levels of regulation, transport, and catabolism. Others have reported that changes in FAO occur in the monocrotaline-injury model of PH, mainly in carnitine palmitoyltransferase-1β expression,[40] and few case reports have shown reduced uptake of radiolabeled fatty acid analogues in the RV of patients with PAH.[41] However, it remains unclear whether the changes in fatty acid metabolism are beneficial or detrimental in the overall function of the right ventricle. In the left ventricle, multiple studies have shown that the rate FAO is preserved or increased in physiological/adaptive hypertrophy and that FAO decreases during the progression of heart failure.[42] Similarly, we demonstrate a normal/increased expression of ACADM, ACADS, and ACADVL in adaptive PAB-RV hypertrophy. These results are supported by the data of Fang et al,[43] who demonstrated that rats with PAB-RV hypertrophy exhibit higher rates of FAO. We postulate that, along with capillary rarefaction, fibrosis, and ROS-induced damage, mitochondrial metabolic remodeling in RVD is pathological. It has been reported that FAO inhibition may have a therapeutic potential[43]; however, it will remain to be tested whether further inhibition of FAO is beneficial in the model of SuHx RVD.

Although it has been reported that RVD is associated with mitochondrial hyperpolarization,[44] a comprehensive analysis of mitochondrial respiration in RVD is still lacking. Here we demonstrate that RVD is associated with significant mitochondrial dysfunction, reduced mitochondrial yield, and reduced overall oxidative capacity. Surprisingly, although the expression of PGC-1α and TFAM was unchanged in PAB-RV hypertrophy, mitochondrial yield and citrate synthase activity were similarly decreased in both SuHx-RVD and PAB-RV hypertrophy. Because PGC-1α regulates mitochondrial biogenesis, our results would suggest an uncoupling between PGC-1α expression and mitochondrial biogenesis in adaptive PAB-RV hypertrophy. Yet, abnormal mitochondrial biogenesis can occur in the presence of normal PGC-1 levels.[4] We speculate that preserved PGC-1α and TFAM expression could explain the better preserved mitochondrial respiration in PAB-RV hypertrophy when compared with SuHx-RVD, because both proteins play a critical role in mitochondrial function.[21,25]

Study Limitations and Future Directions

We do not know whether a decrease of PGC-1α expression is a consequence or a cause of RVF. Nonetheless, our results show that decreased PGC-1α gene expression is not explained by a toxic effect of SU5416, hypoxia, or RV pressure overload. Several mechanisms may participate in the downregulation of PGC-1α expression in RVD and the associated gene metabolic remodeling. We did not explore whether the metabolic remodeling-dependent gene and protein expression profile affects enzymatic activity.

Conclusions

Our data illustrate that RVD is associated with a complex multilevel disturbance of FAO and mitochondrial respiration that is not entirely explained by pressure-overload or hypertrophy. We propose that RV metabolic remodeling is a consequence of decreased PGC-1α expression. To what extent metabolic remodeling or mitochondrial dysfunction is of functional importance for the development of RVD remains to be investigated.

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Disclosures

None.

References

1. Sandoval J, Bauerle O, Palomar A, Gómez A, Martínez-Guerra ML, Beltrán M, Guerrero ML. Survival in primary pulmonary hypertension: validation of a prognostic equation. *Circulation*. 1994;89:1733–1744.
2. Neubauer S. The failing heart: an engine out of fuel. *N Engl J Med*. 2007;356:1140–1151.
3. Rosca MG, Vazquez EJ, Kerner J, Parland W, Chandler MP, Stanley W, Sabbah HN, Hoppel CL. Cardiac mitochondria in heart failure: decrease in respirasomes and oxidative phosphorylation. *Cardiovasc Res*. 2008;80:30–39.
4. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, del Monte F, Tian R. Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. *Circ Res*. 2010;106:1541–1548.
5. Barger PM, Brandt JM, Leone TC, Weinheimer CJ, Kelly DP. Deactivation of peroxisome proliferator-activated receptor-alpha during cardiac hypertrophic growth. *J Clin Invest*. 2000;105:1723–1730.
6. Huss JM, Imahashi K, Dufour CR, Weinheimer CJ, Courtois M, Kovacs A, Giguère V, Murphy E, Kelly DP. The nuclear receptor ERRalpha is required for the bioenergetic and functional adaptation to cardiac pressure overload. *Cell Metab*. 2007;6:25–37.
7. Arany Z, Novikov M, Chin S, Ma Y, Rosenzweig A, Spiegelman BM. Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha. *Proc Natl Acad Sci USA*. 2006;103:10086–10091.
8. Tuder RM, Davis LA, Graham BB. Targeting energetic metabolism: a new frontier in the pathogenesis and treatment of pulmonary hypertension. *Am J Respir Crit Care Med*. 2012;185:260–266.
9. Rehman J, Archer SL. A proposed mitochondrial-metabolic mechanism for initiation and maintenance of pulmonary arterial hypertension in fawn-hooded rats: the Warburg model of pulmonary arterial hypertension. *Adv Exp Med Biol*. 2010;661:171–185.
10. Drake JI, Bogaard HJ, Mizuno S, Clifton B, Xie B, Gao Y, Dumur CI, Fawcett P, Voelkel NF, Natarajan R. Molecular signature of a right heart failure program in chronic severe pulmonary hypertension. *Am J Respir Cell Mol Biol*. 2011;45:1239–1247.
11. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, Marsboom G, Zhang HJ, Haber I, Rehman J, Lopaschuk GD, Archer SL. The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: resuscitating the hibernating right ventricle. *J Mol Med*. 2010;88:47–60.
12. van Bilsen M, van Nieuwenhoven FA, van der Vusse GJ. Metabolic remodelling of the failing heart: beneficial or detrimental? *Cardiovasc Res*. 2009;81:420–428.
13. Gomez-Arroyo JG, Mizuno S, Drake JI, Hussaini Al AA, Farkas L, Farkas D, Natarajan R, Kraskauskas D, Bogaard HJ, Voelkel NF. Adrenergic receptor blocker improves metabolic remodeling in experimental right ventricular failure due to pulmonary hypertension. *Am J Respir Crit Care Med*. 2011; 183(1 meeting abstracts):A2520.
14. Gomez-Arroyo J, Szczepanek K, Syed A, Farkas L, Farkas D, Kraskauskas D, Alhussaini A, Lesnefsky EJ, Voelkel N, Bogaard HJ. Metabolic remodeling in right ventricular failure is associated with abnormal mitochondrial biogenesis. *Am J Respir Crit Care Med [Internet]*. 2012 May 14;185:A:3455. Available from: http://ajrccm.atsjournals.org/cgi/reprint/185/1_MeetingAbstracts/A3455?sid=7a5c6260-c6c7-4d03-a20a-a76749f7fe57
15. Bogaard HJ, Natarajan R, Henderson SC, Long CS, Kraskauskas D, Smithson L, Ockaili R, McCord JM, Voelkel NF. Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. *Circulation*. 2009;120:1951–1960.
16. Sakao S, Taraseviciene-Stewart L, Cool CD, Tada Y, Kasahara Y, Kurosu K, Tanabe N, Takiguchi Y, Tatsumi K, Kuriyama T, Voelkel NF. VEGF-R blockade causes endothelial cell apoptosis, expansion of surviving CD34+ precursor cells and transdifferentiation to smooth muscle-like and neuronal-like cells. *FASEB J*. 2007;21:3640–3652.
17. Oka M, Homma N, Taraseviciene-Stewart L, Morris KG, Kraskauskas D, Burns N, Voelkel NF, McMurtry IF. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats. *Circ Res*. 2007;100:923–929.
18. Bogaard HJ, Natarajan R, Mizuno S, Abbate A, Chang PJ, Chau VQ, Hoke NN, Kraskauskas D, Kasper M, Salloum FN, Voelkel NF. Adrenergic receptor blockade reverses right heart remodeling and dysfunction in pulmonary hypertensive rats. *Am J Respir Crit Care Med*. 2010;182:652–660.
19. Bogaard HJ, Mizuno S, Hussaini AA, Toldo S, Abbate A, Kraskauskas D, Kasper M, Natarajan R, Voelkel NF. Suppression of histone deacetylases worsens right ventricular dysfunction after pulmonary artery banding in rats. *Am J Respir Crit Care Med*. 2011;183:1402–1410.
20. Drake JI, Bogaard HJ, Mizuno S, Clifton B, Xie B, Gao Y, Dumur CI, Fawcett P, Voelkel NF, Natarajan R. Molecular signature of a right heart failure program in chronic severe pulmonary hypertension. *Am. J. Respir. Cell Mol. Biol*. 2011; 45:1239–1247.
21. Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest*. 2006;116:615–622.
22. Alaynick WA, Kondo RP, Xie W, He W, Dufour CR, Downes M, Jonker JW, Giles W, Naviaux RK, Giguère V, Evans RM. ERRgamma directs and maintains the transition to oxidative metabolism in the postnatal heart. *Cell Metab*. 2007;6:13–24.
23. Sack MN, Disch DL, Rockman HA, Kelly DP. A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. *Proc Natl Acad Sci USA*. 1997;94:6438–6443.
24. Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci USA*. 1994;91:1309–1313.
25. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev*. 2004;18:357–368.
26. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ 2nd. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers: smoking as a cause of oxidative damage. *N Engl J Med*. 1995;332:1198–1203.
27. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci USA*. 1994;91:10771–10778.
28. Bartz RR, Suliman HB, Fu P, Welty-Wolf K, Carraway MS, MacGarvey NC, Withers CM, Sweeney TE, Piantadosi CA. Staphylococcus aureus sepsis and mitochondrial accrual of the 8-oxoguanine DNA glycosylase DNA repair enzyme in mice. *Am J Respir Crit Care Med*. 2011;183:226–233.
29. van de Veerdonk MC, Kind T, Marcus JT, Mauritz GJ, Heymans MW, Bogaard HJ, Boonstra A, Marques KM, Westerhof N, Vonk-Noordegraaf A. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. *J. Am. Coll. Cardiol*. 2011; 58:2511–2519.
30. Voelkel NF, Gomez-Arroyo J, Abbate A, Bogaard HJ, Nicolls MR. Pathobiology of pulmonary arterial hypertension and right ventricular failure. *Eur Respir J*. 2012;40:1555–1565.
31. Voelkel NF, Quaife RA, Leinwand LA, Barst RJ, McGoon MD, Meldrum DR, Dupuis J, Long CS, Rubin LJ, Smart FW, Suzuki YJ, Gladwin M, Denholm EM, Gail DB; National Heart, Lung, and Blood Institute Working Group on Cellular and Molecular Mechanisms of Right Heart Failure. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. *Circulation*. 2006;114:1883–1891.
32. Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest*. 2009;135:794–804.
33. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev*. 2003;24:78–90.
34. Lehman JJ, Boudina S, Banke NH, Sambandam N, Han X, Young DM, Leone TC, Gross RW, Lewandowski ED, Abel ED, Kelly DP. The transcriptional coactivator PGC-1alpha is essential for maximal and efficient cardiac mitochondrial fatty acid oxidation and lipid homeostasis. *Am J Physiol Heart Circ Physiol*. 2008;295:H185–H196.
35. Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, Ahmad F, Matsui T, Chin S, Wu PH, Rybkin II, Shelton JM, Manieri M, Cinti S, Schoen FJ, Bassel-Duby R, Rosenzweig A, Ingwall JS, Spiegelman BM. Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. *Cell Metab*. 2005;1:259–271.

36. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest*. 2000;106:847–856.
37. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, Spiegelman BM. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature*. 2008;451:1008–1012.
38. Sebastiani M, Giordano C, Nediani C, Travaglini C, Borchi E, Zani M, Feccia M, Mancini M, Petrozza V, Cossarizza A, Gallo P, Taylor RW, d'Amati G. Induction of mitochondrial biogenesis is a maladaptive mechanism in mitochondrial cardiomyopathies. *J Am Coll Cardiol*. 2007;50:1362–1369.
39. Piao L, Marsboom G, Archer SL. Mitochondrial metabolic adaptation in right ventricular hypertrophy and failure. *J Mol Med*. 2010;88:1011–1020.
40. Buermans HP, Redout EM, Schiel AE, Musters RJ, Zuidwijk M, Eijk PP, van Hardeveld C, Kasanmoentalib S, Visser FC, Ylstra B, Simonides WS. Microarray analysis reveals pivotal divergent mRNA expression profiles early in the development of either compensated ventricular hypertrophy or heart failure. *Physiol Genomics*. 2005;21:314–323.
41. Kim Y, Goto H, Kobayashi K, Sawada Y, Miyake Y, Fujiwara G, Chiba H, Okada T, Nishimura T. Detection of impaired fatty acid metabolism in right ventricular hypertrophy: assessment by I-123 beta-methyl iodophenyl pentadecanoic acid (BMIPP) myocardial single-photon emission computed tomography. *Ann Nucl Med*. 1997;11:207–212.
42. Abel ED, Doenst T. Mitochondrial adaptations to physiological vs. pathological cardiac hypertrophy. *Cardiovasc Res*. 2011;90:234–242.
43. Fang YH, Piao L, Hong Z, Toth PT, Marsboom G, Bache-Wiig P, Rehman J, Archer SL. Therapeutic inhibition of fatty acid oxidation in right ventricular hypertrophy: exploiting Randle's cycle. *J Mol Med*. 2012;90:31–43.
44. Nagendran J, Gurtu V, Fu DZ, Dyck JR, Haromy A, Ross DB, Rebeyka IM, Michelakis ED. A dynamic and chamber-specific mitochondrial remodeling in right ventricular hypertrophy can be therapeutically targeted. *J Thorac Cardiovasc Surg*. 2008;136:168–78, 178.e1.

CLINICAL PERSPECTIVE

Our experiments demonstrate that in the setting of severe pulmonary arterial hypertension, load-independent alterations of cardiac energy metabolism and mitochondrial efficiency are associated with RVD. Because RV failure determines the outcome in patients with severe PAH, the cellular and molecular mechanisms that underlie RVF need to be understood, for they could be reversible. As we design RV-targeted therapies, the use of metabolic modulators should aim to restore not only substrate use but also mitochondrial function.