Agonist-Induced Hypertrophy and Diastolic Dysfunction Are Associated With Selective Reduction in Glucose Oxidation
A Metabolic Contribution to Heart Failure With Normal Ejection Fraction

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Background—Activation of the renin-angiotensin and sympathetic nervous systems may alter the cardiac energy substrate preference, thereby contributing to the progression of heart failure with normal ejection fraction. We assessed the qualitative and quantitative effects of angiotensin II (Ang II) and the α-adrenergic agonist, phenylephrine (PE), on cardiac energy metabolism in experimental models of hypertrophy and diastolic dysfunction and the role of the Ang II type 1 receptor.

Methods and Results—Ang II (1.5 mg·kg^{-1}·day^{-1}) or PE (40 mg·kg^{-1}·day^{-1}) was administered to 9-week-old male C57/BL6 wild-type mice for 14 days via implanted microosmotic pumps. Echocardiography showed concentric hypertrophy and diastolic dysfunction, with preserved systolic function in Ang II- and PE-treated mice. Ang II induced marked reduction in cardiac glucose oxidation and lactate oxidation, with no change in glycolysis and fatty acid β-oxidation. Tricarboxylic acid acetyl coenzyme A production and ATP production were reduced in response to Ang II. Cardiac pyruvate dehydrogenase kinase 4 expression was upregulated by Ang II and PE, resulting in a reduction in the pyruvate dehydrogenase activity, the rate-limiting step for carbohydrate oxidation. Pyruvate dehydrogenase kinase 4 upregulation correlated with the activation of the cyclin/cyclin-dependent kinase-retinoblastoma protein-E2F pathway in response to Ang II. Ang II type 1 receptor blockade normalized the activation of the cyclin/cyclin-dependent kinase-retinoblastoma protein-E2F pathway and prevented the reduction in glucose oxidation but increased fatty acid oxidation.

Conclusions—Ang II- and PE-induced hypertrophy and diastolic dysfunction is associated with reduced glucose oxidation because of the cyclin/cyclin-dependent kinase-retinoblastoma protein-E2F–induced upregulation of pyruvate dehydrogenase kinase 4, and targeting these pathways may provide novel therapy for heart failure with normal ejection fraction. (***Circ Heart Fail***. 2012;5:493-503.)

Key Words: angiotensin II ■ cardiac metabolism ■ diastolic dysfunction ■ heart failure ■ phenylephrine
■ pyruvate dehydrogense 4

The renin-angiotensin system is known to play a key role in cardiac hypertrophy and heart failure (HF), and the renin-angiotensin system blockade is effective in animal models and patients with cardiac hypertrophy and HF.[1,2] Angiotensin II (Ang II), a major bioactive effector of the renin-angiotensin system, is aberrantly activated in heart disease and plays a key role in pathological remodeling and HF.[3,4] Activation of the sympathetic nervous system and stimulation of α-adrenergic receptors are also key mediators of pathological remodeling and HF progression.[5] Pathological hypertrophy with diastolic dysfunction is the hallmark feature of HF with normal ejection fraction, a condition with adverse outcomes for which there is no approved therapies.[6–9]

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HF induces perturbation in cardiac energy metabolism,[10,11] with mitochondrial oxidation (fatty acid oxidation and glucose oxidation) eventually becoming decreased in advanced HF.[12] Ang II is involved in the regulation of cardiac metabolism. Ang II (and its precursor angiotensinogen) has been shown to decrease fatty acid oxidation rates and reduce the expression of peroxisome proliferator-activated receptor (PPAR) isoforms.[13,14] It also induces membrane translocation of glucose transporter 4 and increases glucose use, accompanied by downregulation of AMP-activated protein kinase.[15] Glucose oxidation rates are controlled by the pyruvate dehydrogenase (PDH) complex, with PDH kinase 4

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(PDK4)–mediated phosphorylation and inhibition of the PDH complex being a central control mechanism. Given the key and fundamental role of ATP in myocardial relaxation,[16,17] metabolic perturbations may play a key role in Ang II-induced hypertrophy and diastolic dysfunction.

In the present study, we assessed Ang II- and phenylephrine (PE)-induced alterations in cardiac metabolism in models of pathological hypertrophy and diastolic dysfunction and show that Ang II and PE decrease glucose oxidation in association with an upregulation of PDK4. Furthermore, we demonstrate that the cyclin/cyclin-dependent kinase (CDK)-retinoblastoma protein (pRB)-E2F pathway plays a critical role in Ang II-induced alterations in cardiac metabolism. The Ang II type 1 receptor (AT1R) blocker, irbesartan, prevented the metabolic perturbations mediated by Ang II. These findings suggest that Ang II induces the reduction in carbohydrate oxidation in cardiac energy metabolism via the AT1R, and the cyclin/CDK-pRB-E2F pathway plays a critical role in Ang II-induced alterations in cardiac metabolism.

Methods

Detailed methodology is described in the online-only Data Supplement Expanded Methods section.

Experimental Animals and Protocol

Microosmotic pumps (model 1002; Alzet Corp, Palo Alto, CA) were implanted subcutaneously at the dorsum of the neck to infuse a pressor dose of Ang II (1.5 mg·kg^{-1}·day^{-1}) or PE (40 mg·kg^{-1}·d^{-1}) for 14 days in 9-week-old male C57/BL6 wild-type mice, as previously described.[18] A subgroup of Ang II-infused mice was also simultaneously treated with irbesartan, an AT1R blocker (50 mg·kg^{-1}·day^{-1}), or vehicle. The investigation conforms to the Care and Use of Laboratory Animals published by the US National Institutes of Health (Publication 85-23, revised 1996) and to the guidelines of the Canadian Council on Animal Care.

Echocardiography and Hemodynamic Assessment

Transthoracic echocardiography was performed and analyzed in a blinded manner, with a Vevo 770 high resolution imaging system equipped with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Ontario, Canada), as described previously.[18] Invasive pressure measurements were made using the Millar system (MPVS-400; Millar Instruments, Houston, TX), and tail-cuff systolic blood pressure was measured using the IITC Blood Pressure Monitoring System (IITC Life Science Blood Pressure System; IITC Life Science Inc, Woodland Hills, CA).[18]

Working Heart Model, Metabolic Measurements, and PDH Activity

Isolated working hearts were perfused with modified Krebs-Henseleit buffer containing 5 mmol/L glucose, 1.2 mmol/L palmitate, 1 mmol/L lactate, 3% albumin, and 100 µU/mL insulin. Hearts were subjected to 40 minutes of perfusion with perfusate containing [U-^{14}C]glucose and [9,10-^{3}H]palmitate or [5-^{3}H]glucose and [U-^{14}C]lactate. The rates of glucose oxidation and palmitate oxidation were measured by quantitative collection of $^{14}CO_2$ and $^{3}H_2O$ from [U-^{14}C]glucose and [9,10-^{3}H]palmitate, respectively, and in a parallel series of heart, the rates of glycolysis and lactate oxidation were measured by quantitative collection of $^{3}H_2O$ and $^{14}CO_2$ from [5-^{3}H]glucose and [U-^{14}C]lactate, respectively, as described previously.[19,20] PDH activity was measured by a radiometric assay, as described previously.[21]

TaqMan Real-Time Polymerase Chain Reaction and Western Blotting Analyses

TaqMan real-time polymerase chain reaction was used to quantify mRNA expression levels, using a ABI 7900 sequence detection system, as described previously.[18,22] We extracted nuclear and cytoplasmic proteins, and Western blotting analyses were performed, as described previously.[18,23]

Histology

Picro-Sirius Red staining was performed, as described previously[18] and imaged and analyzed using fluorescence microscopy (Olympus IX81 and MetaMorph software, Center Valley, PA).

Statistical Analysis

All data are presented as mean±SEM. Statistical analysis of the data was performed with the use of either a 2-tailed Student *t* test or a 1-way ANOVA, followed by multiple comparison testing using Student Neuman-Keuls testing and, when appropriate, using the SPSS Statistics 19 software (IBM, Armonk, NY). *P*<0.05 was considered significant.

Results

Ang II Induces Pathological Hypertrophy and Diastolic Dysfunction via the AT1R

Two-dimensional/M-mode echocardiography and Doppler imaging were used to assess in vivo heart function. M-mode imaging and 2-dimensional short-axis midventricular views revealed increased wall thickness of the left ventricle (LV) induced by Ang II, but normal systolic function with no statistically significant difference in fractional shortening and ejection fraction (Figure 1A–1D; online-only Data Supplement Table I). The functional abnormality in response to Ang II was characterized by diastolic dysfunction, leading to increased A-wave amplitude accompanied by the reduction of the E/A ratio, as well as tissue Doppler imaging showing reduced E'-wave amplitude, resulting in the elevation of E/E' ratio and a reduction of the E'/A' ratio (Figure 1E–1H; online-only Data Supplement Table I). Diastolic dysfunction was confirmed by invasive hemodynamic assessment, which showed elevated LV end-diastolic pressure and reduced –dP/dt$_{max}$ in response to Ang II (online-only Data Supplement Table II). Morphometric assessment and expression analysis of pathological hypertrophy markers showed increased cardiac hypertrophy in response to Ang II (online-only Data Supplement Figure I). LV dry weight and percent LV dry weight/body weights were significantly increased in Ang II-treated mice, with markedly increased expression of atrial natriuretic factor, brain natriuretic peptide, β-myosin heavy chain, α-skeletal actin, and cardiomyocyte cross-sectional area (online-only Data Supplement Figure IA–IH). These results indicate that Ang II induces a hypertrophied heart with diastolic dysfunction and preserved systolic function. The pathological effects of Ang II are mediated predominantly by the AT1R.[4,24] We administered irbesartan to Ang II-treated mice to assess the effects of AT1R blockade on Ang II-induced heart function and morphometric changes. Irbesartan prevented the Ang II-induced concentric hypertrophy and diastolic dysfunction (Figure 1A–1H; online-only Data Supplement Tables I and II), with reversal of the morphometric and molecular correlates of pathological hypertrophy (online-only Data Supplement Figure II). These
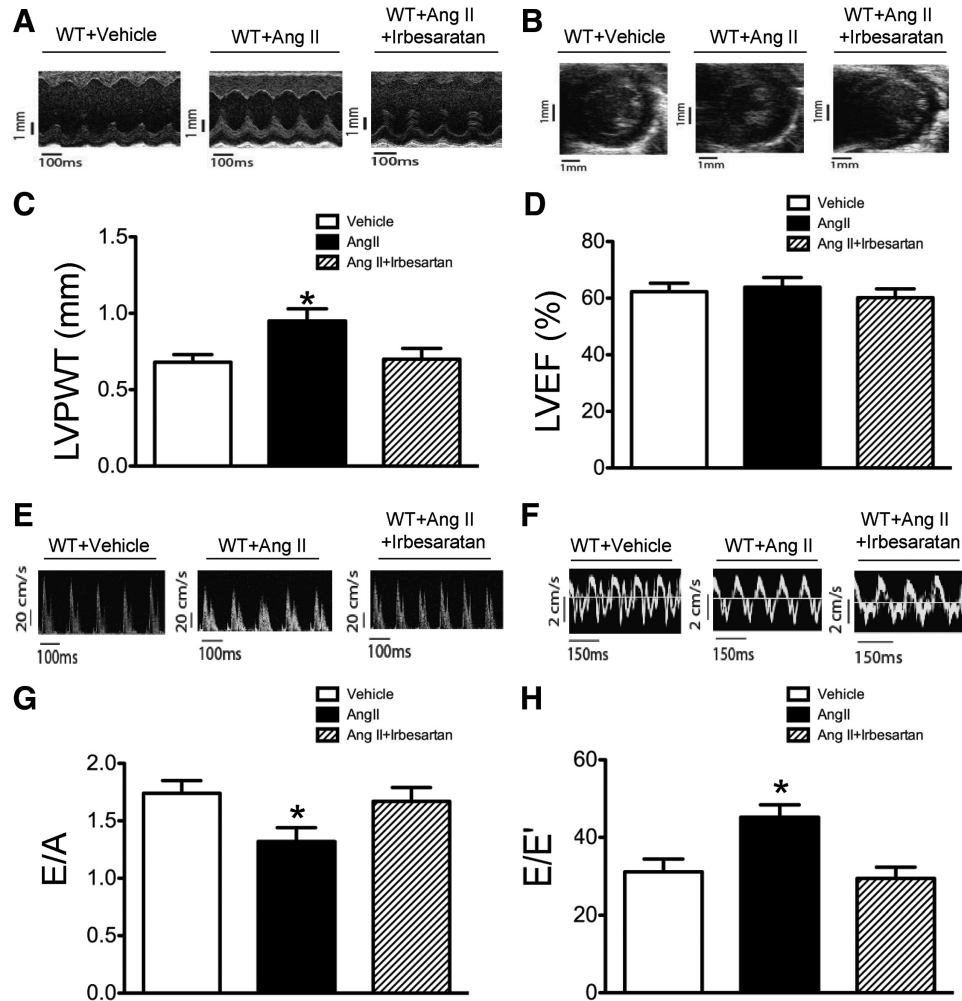
Figure 1. Angiotensin II (Ang II) induces concentric hypertrophy and diastolic dysfunction. Echocardiographic assessment showing M-mode (**A**) and short-axis view (**B**), illustrating concentric hypertrophy with normal systolic function as quantified by increased left ventricular (LV) posterior wall thickness (LVPWT) (**C**) and normal LV ejection fraction (LVEF) (**D**). Transmitral mitral Doppler flow (**E**) and tissue Doppler imaging (**F**) showing diastolic dysfunction as illustrated by decreased E/A ratio (**G**) and increased E/E' ratio (**H**) in Ang II-treated mice. Ang II type 1 receptor blockade with irbesartan prevents Ang II-induced concentric hypertrophy and diastolic dysfunction (**A–H**). Values are mean±SEM of n=9 to 12 in each group; *P<0.05 compared with all groups. E-wave indicates peak early transmitral inflow mitral E velocity; A-wave, transmitral inflow velocity because of atrial contraction; E', early diastolic tissue Doppler velocity; WT, wild-type.

results suggest that Ang II induces pathological hypertrophy and diastolic dysfunction via the AT1R.

Ang II-Induced Alterations in Cardiac Energy Metabolism and Efficiency: Effects of AT1R Blockade

Metabolic perturbations may represent a key driver of diastolic abnormalities in the setting of pathological hypertrophy.[16,17,25] We, therefore, subjected hearts to ex vivo aerobic perfusions to measure the cardiac function and rates of cardiac energy metabolism (fatty acid oxidation, glucose oxidation, lactate oxidation, and glycolysis). The overall mechanical function between vehicle- and Ang II-treated hearts during ex vivo aerobic perfusions was similar (online-only Data Supplement Table III), which is consistent with the echocardiographic and invasive hemodynamic assessment, and collectively confirms that Ang II-treated hearts maintained normal systolic function. Ang II resulted in reduced cardiac work

(Figure 2A), in association with a 45% decrease in glucose oxidation (vehicle, 946±75 nmol·g dry wt^{-1}·min^{-1}; Ang II, 518±36 nmol·g dry wt^{-1}·min^{-1}; $P<0.05$; Figure 2B) and a 31% decrease in lactate oxidation (Figure 2C). In contrast, no statistically significant change in either palmitate oxidation (Figure 2D) or glycolysis (vehicle, 14.96±2 versus Ang II, 10.81±2.1 μmol·g dry wt^{-1}·min^{-1}; $P=0.32$) occurred in response to Ang II. The resultant decrement in myocardial glucose and lactate oxidation lowered total acetyl coenzyme A (CoA) production (Figure 2E) and ATP production (online-only Data Supplement Table IV) in response to Ang II. These data indicated that Ang II selectively reduces carbohydrate oxidation and its contribution to tricarboxylic acid cycle activity, resulting in impaired ATP production. We next assessed the effects of AT1R blockade on these cardiac energy perturbations. Ang II-mediated reduction in glucose oxidation and lactate oxidation was prevented by treatment with irbesartan (Figure 2B and 2C). In contrast, the rate of
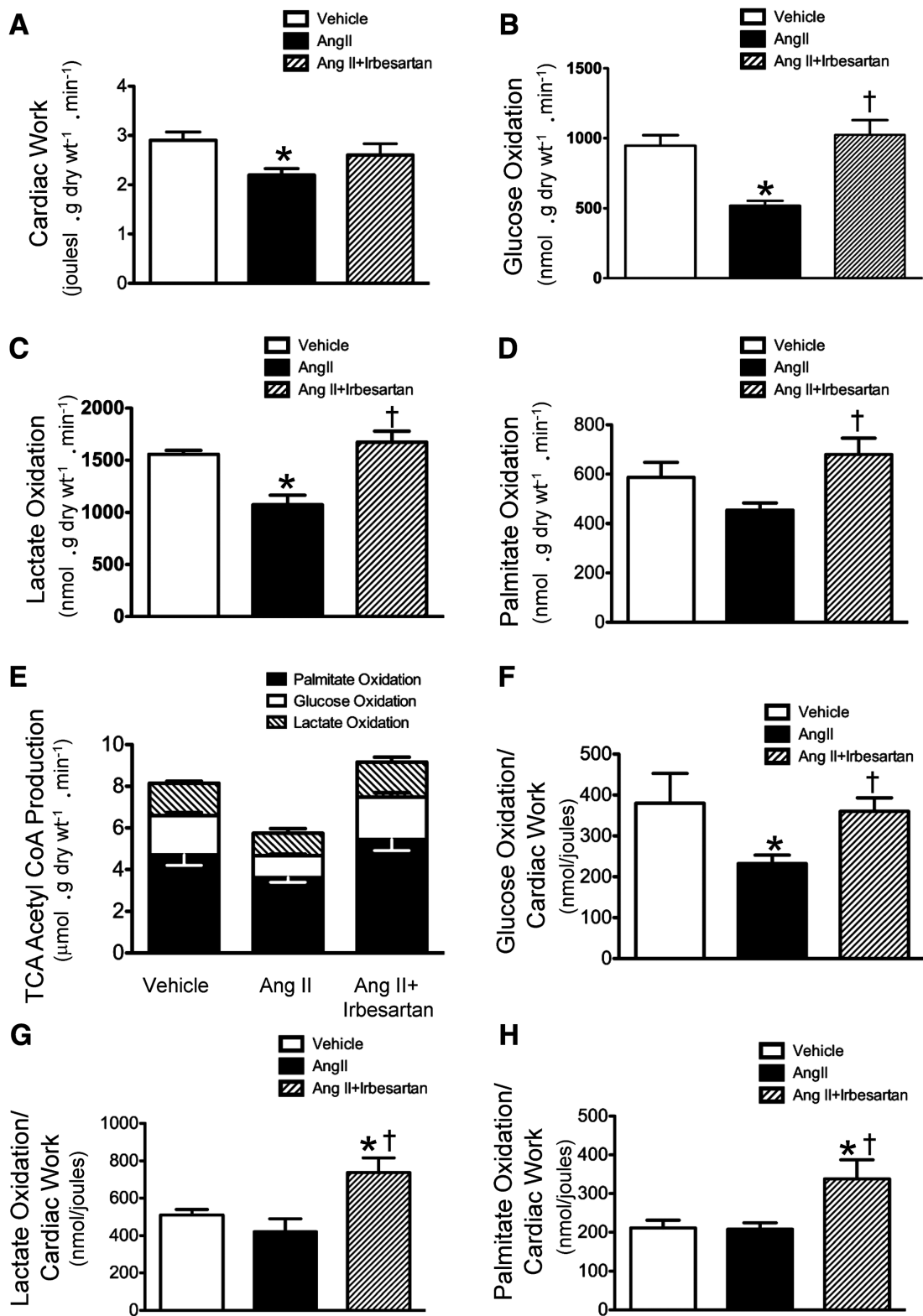
Figure 2. Angiotensin II (Ang II) mediates metabolic perturbations in the heart characterized by reduced carbohydrate oxidation. Ang II reduced cardiac work (**A**), with reduction in glucose oxidation (**B**) and lactate oxidation (**C**), whereas palmitate oxidation (**D**) was not significantly decreased. The contributions of acetyl coenzyme A (CoA) production from glucose oxidation, lactate oxidation, and palmitate oxidation are shown for all groups (**E**). Ang II significantly reduced glucose normalized by cardiac work (**F**), but neither palmitate oxidation normalized by cardiac work (**G**) nor lactate oxidation normalized by cardiac work (**H**). Ang II type 1 receptor (AT1R) blockade with irbesartan prevents Ang II-induced metabolic perturbations in the heart. Glucose oxidation (**B**) and lactate oxidation (**C**) are significantly increased in response to irbesartan. Irbesartan treatment also significantly increased palmitate oxidation (**D**), while restoring acetyl CoA production (**E**). Although AT1R normalized glucose oxidation per cardiac work (**F**), both palmitate and lactate oxidation per cardiac work were significantly increased above baseline values (**G** and **H**). Values are mean±SEM of n=10 to 13 in each group; *P<0.05 compared with vehicle-treated group; †P<0.05 compared with Ang II-treated group. TCA indicates tricarboxylic acid.

palmitate oxidation was not significantly decreased in Ang II-treated hearts and was normalized by irbesartan (Figure 2D). The reduction in total acetyl CoA production was also normalized (Figure 2E). Because cardiac work is an important determinant of oxidative metabolism, we determined whether Ang II alterations in cardiac energy metabolism are because of decreases in cardiac work by normalizing cardiac energy metabolism for differences in cardiac work (Figure 2F–2H). The significant reduction in glucose oxidation in response to Ang II treatment remained when corrected for differences in cardiac work (Figure 2F). On the contrary, Ang II did not reduce either lactate oxidation or palmitate oxidation normalized for cardiac work (Figure 2G and 2H). Interestingly, although irbesartan normalized glucose oxidation per cardiac work (Figure 2F) in the Ang II-treated hearts, both lactate and palmitate oxidation normalized for cardiac work were significantly increased above baseline values (Figure 2G and 2H). These results show that Ang II induces selective reduction in glucose oxidation, regardless of decreases in cardiac work. Furthermore, to assess cardiac energy efficiency, we normalized cardiac work for tricarboxylic acid acetyl CoA production, which confirmed no statistically significant difference between vehicle-treated and Ang II-treated groups (online-only Data Supplement Table III). Interestingly, AT1R blockade significantly decreased cardiac work per tricarboxylic acid acetyl CoA production. These results show that AT1R blockade induces cardiac energy inefficiency.

Ang II-Induced Upregulation of PDK4

We hypothesized that treatment with Ang II activates PDK4, which is the predominant PDK isoform responsible for phosphorylating and inhibiting the PDH complex in the heart, the rate-limiting enzyme for mitochondrial carbohydrate oxidation.[26,27] Western blotting analysis showed that cardiac expression of PDK4 protein was significantly elevated in response to Ang II (Figure 3A). In contrast, expression of PDK2 was unaltered between vehicle- and Ang II-treated hearts (online-only Data Supplement Figure IIIA). Active PDH activity in Ang II-treated hearts was significantly decreased (Figure 3B) but the total PDH activity remained unaltered (Figure 3C), which is likely driven by increased phosphorylation of PDH (Figure 3D) in response to the upregulation of PDK4. Consistent with a lack of change in palmitate oxidation rates, the activity of β-hydroxylacyl CoA, a key enzyme of fatty acid β-oxidation, did not show significant change in response to Ang II (online-only Data Supplement Figure IIIB). These results provide a mechanism for the reduction of glucose (and lactate) oxidation by Ang II in so far as Ang II-induced upregulation of PDK4 phosphorylates and inactivates PDH, leading to a reduction in carbohydrate oxidation. Consistent with the restoration of glucose oxidation by AT1R blockade, PDK4 expression was decreased, resulting in upregulation of active PDH activity (Figure 3E and 3F), leaving total PDH activity unchanged with decreased phospho-PDH level in response to AT1R blockade (Figure 3G and 3H). These results show a critical role of AT1R in mediating the Ang II-induced metabolic perturbations.

Mechanism of Ang II-Induced Upregulation of PDK4

Next, we explored the mechanism of Ang II-induced upregulation of PDK4 by examining the expression of transcription factors known to modulate PDK4.[28–30] The transcription factors estrogen-related receptor-α (ERRα) and PPARα positively regulate PDK4 expression. However, nuclear levels of PPARα (online-only Data Supplement Figure IIIC) and ERRα (online-only Data Supplement Figure IIID) were reduced in response to Ang II. The cyclin/CDK-pRB-E2F pathway is implicated in the metabolic adaptive response triggered by G-protein–coupled receptor agonist and growth factors.[29] The nuclear protein level of nuclear E2F1 (Figure 4A) and the expression of pRB (Figure 4B), an indicator of nuclear translocation of E2F1, were increased in Ang II-treated hearts. The nuclear expression of CDK4 and the cytosolic expression of phospho-cyclin D1 were enhanced in Ang II-treated hearts (Figure 4C and 4D), providing further mechanistic insight into the upregulation of PDK4. Ang II also reduced complex I levels without affecting mitochondrial density as assessed by citrate synthase activity (online-only Data Supplement Figure IIIE and IIIF). Consistent with a key role of the cyclin/CDK-pRB-E2F pathway, increased protein expression of E2F1, phospho-Rb, CDK4, and phospho-cyclin D1 in response to Ang II was reduced by treatment with irbesartan (Figure 4E–4H). Irbesartan also reversed the protein expression of complex I, PPARα, and ERRα (online-only Data Supplement Figure IVA–IVC), which is likely responsible for the normalization of total acetyl CoA production (Figure 2E). These results show that the cyclin/CDK-pRB-E2F pathway is one of the possible candidates responsible for the Ang II-induced upregulation of PDK4.

PE-Induced Hypertrophy and Diastolic Dysfunction Are Associated With a Selective Reduction in Glucose Oxidation

The α$_1$-adrenergic agonist, PE, resulted in sustained hypertension and the development of hypertrophy (online-only Data Supplement Figure VA and VB). Echocardiography revealed increased LV wall thickness, with normal systolic function with no change in ejection fraction and velocity of circumferential shortening (Figure 5A and 5B). PE-induced hypertrophy was associated with diastolic dysfunction, characterized by a reduction in the E/A ratio, as well as tissue Doppler imaging showing reduced E'-wave amplitude, thereby decreasing the E'/A' ratio (Figure 5C–5F). Metabolic assessment revealed a 50% reduction in glucose oxidation (Figure 5G), with no statistically significant difference in palmitate oxidation (Figure 5H). Consistent with the changes seen with Ang II, treatment with PE resulted in increased PDK4 and phospho-PDH levels, leading to reduced active PDH activity (Figure 5I–5K). We conclude that α-adrenergic stimulation induces concentric hypertrophy and diastolic dysfunction, with selective impairment in glucose oxidation in response to increased PDK4 levels.

Discussion

Our study provides several important findings regarding Ang II- and PE-induced alterations in cardiac energy metabolism. We demonstrate that: (1) Ang II selectively decreases glucose
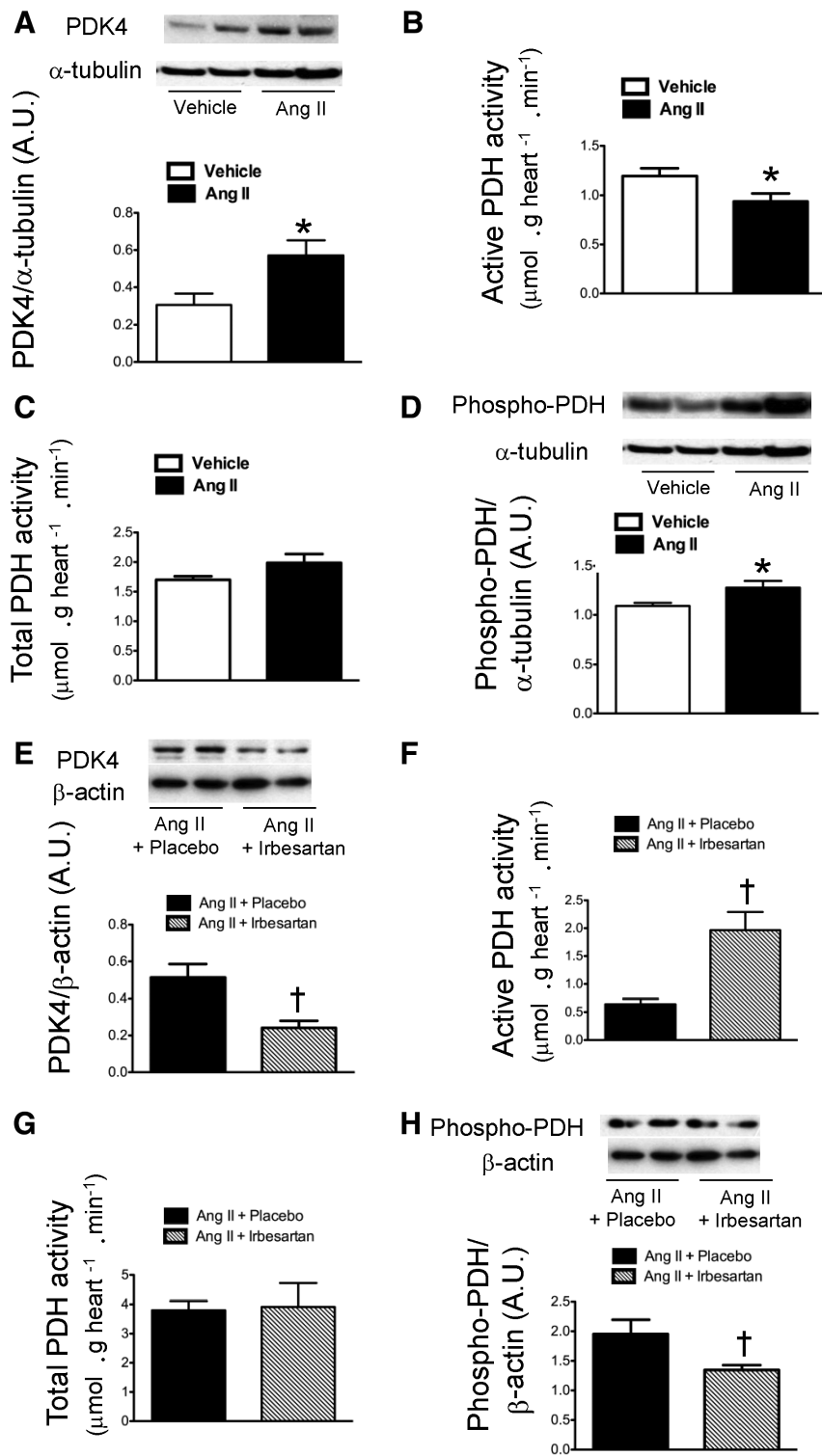
Figure 3. Mechanism of angiotensin II (Ang II)-mediated reduction in carbohydrate oxidation. Upregulation of pyruvate dehydrogenase kinase 4 (PDK4) levels (**A**), resulting in reduced active pyruvate dehydrogenase (PDH) activity (**B**) with unaltered total PDH activity (**C**) and the upregulation of phospho-PDH levels (**D**). The expression of PDK4 was reduced (**E**), resulting in elevation in active PDH activity (**F**) with no change in total PDH activity (**G**) by treatment with irbesartan. These changes correlated with reduced phospho-PDH levels in response to irbesartan (**H**). Values are the mean±SEM of n=6 to 7 in each group; *$P<0.05$ compared with vehicle-treated group; †$P<0.05$ compared with Ang II+placebo group. A.U. indicates arbitrary units.

and lactate oxidation; (2) Ang II-induced upregulation of PDK4 contributes to altered cardiac energy metabolism; (3) irbesartan prevents the Ang II-induced metabolic perturbations, confirming a key role for the AT1R but increased fatty

acid oxidation per unit of cardiac work; (4) the cyclin/CDK-pRB-E2F pathway plays a critical role in mediating the effects of Ang II on carbohydrate oxidation; and (5) PE selectively decreases glucose oxidation, with upregulation of PDK4.
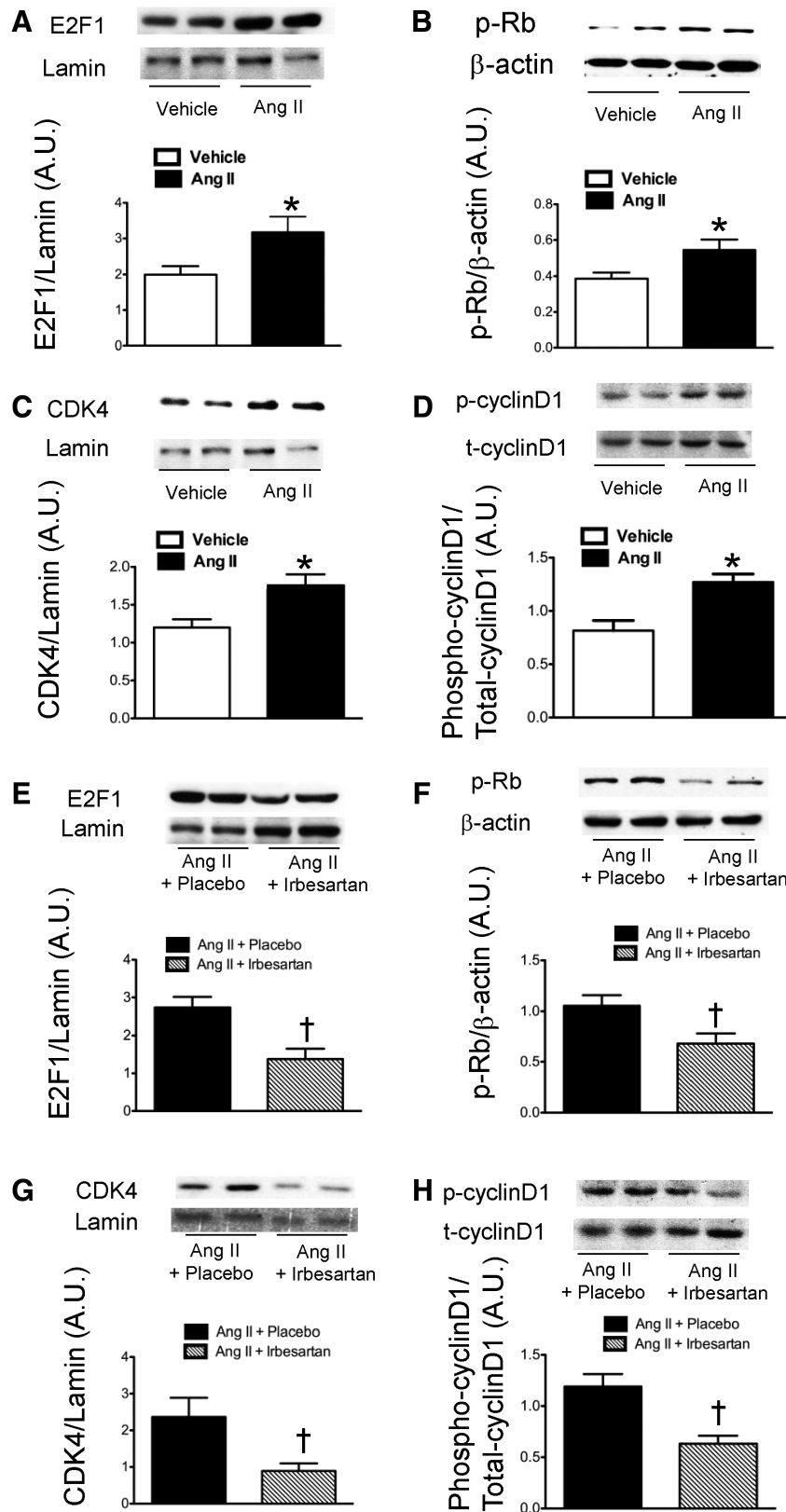
Figure 4. Cardiac expression of the cell cycle regulators, E2F1, retinoblastoma protein (p-RB), cyclin-dependent kinase 4 (CDK4), and phospho-cyclin D1 in response to angiotensin II (Ang II). Increased nuclear expression of E2F1 (**A**), cytosolic expression of pRB (**B**), nuclear expression of CDK4 in the nucleus (**C**), with increased phosphorylation of cyclin D1 in the cytosol (**D**) in response to treatment with Ang II. The expressions of E2F1 in the nucleus (**E**), pRB in the cytosol (**F**), CDK4 in the nucleus (**G**), and phospho-cyclin D1 (**H**) in the cytosol are reversed in response to treatment with irbesartan. Values are the mean±SEM of n=5 in each group; *$P<0.05$ compared with vehicle-treated group; †$P<0.05$ compared with Ang II+placebo group. A.U. indicates arbitrary units.
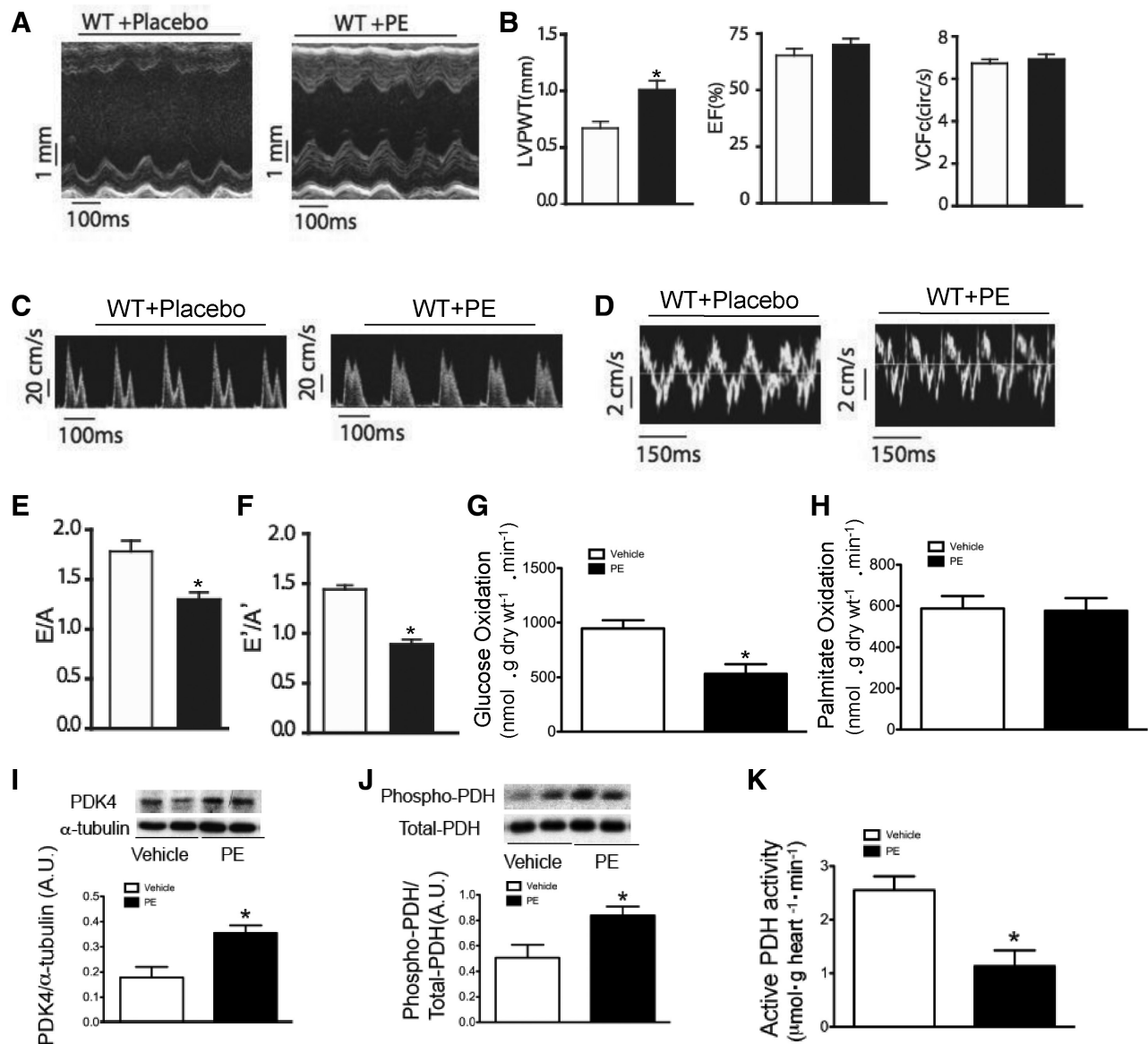
Figure 5. Phenylephrine (PE)-induced concentric hypertrophy with diastolic dysfunction is associated with selective reduction in glucose oxidation. Echocardiographic assessment showing M-mode (**A**), illustrating concentric hypertrophy with normal systolic function as quantified by increased left ventricular (LV) posterior wall thickness (LVPWT) and normal LV ejection fraction and velocity of circumferential shortening corrected for heart rate (VCFc) (**B**). Transmitral mitral Doppler flow (**C**) and tissue Doppler imaging (**D**) showing diastolic dysfunction as illustrated by reduced E/A ratio (**E**) and decreased E'/A' ratio (**F**) in response to treatment with PE. Metabolic profiling showing a selective reduction in glucose oxidation (**G**), with no change in palmitate oxidation (**H**) driven by increased pyruvate dehydrogenase kinase 4 (PDK4) levels (**I**), increased phospho-pyruvate dehydrogenase (PDH) levels (**J**), and reduced active PDH activity (**K**) in hearts from PE-treated mice. Values are mean±SEM of n=9 in each group; *$P<0.05$ compared with vehicle-treated group. E-wave indicates peak early transmitral inflow mitral E velocity; A-wave, transmitral inflow velocity due to atrial contraction; E', early diastolic tissue Doppler velocity; WT, wild-type; A.U., arbitrary units.

Collectively, these metabolic changes resulted in reduced cardiac efficiency in a setting of concentric pathological hypertrophy and diastolic dysfunction, with preserved systolic function.

In response to Ang II and PE, active PDH activity is downregulated. PDH activity plays a pivotal role in the development of cardiac hypertrophy, and PDH activity is downregulated in the setting of cardiac hypertrophy.[31] We demonstrate that PDK4 levels and phosphorylation of PDH are increased in Ang II-treated hearts. PDK4 is one of the PDK isozymes, which phosphorylates PDH and reduces

the activity of PDH.[26,27,32] Consequently, Ang II-induced reduction in carbohydrate oxidation is likely because of a decreased conversion of pyruvate to acetyl CoA by reduction in PDH activity. Importantly, AT1R blockade using irbesartan prevents the Ang II-induced alterations in cardiac metabolism, while preventing pathological hypertrophy and diastolic dysfunction. Our results are consistent with findings in diabetic hearts where angiotensin-converting enzyme inhibition reduces the expression of PDK4[33] and in cardiac-specific overexpression of PDK4, which results in reduced glucose oxidation.[34]

Ang II and PE result in pathological hypertrophy, with diastolic dysfunction and preserved systolic function. Ang II-induced metabolic alterations could provide a mechanistic basis for the diastolic dysfunction. First, Ang II and PE reduce myocardial glucose oxidation, with a relative preservation of glycolysis. Although we expected the rate of glycolysis to increase, in fact the rate of glycolysis was slightly decreased (although not significant). Ang II induces insulin resistance, and many clinical studies have shown that patients with HF have insulin resistance.[35–37] As a result, it is possible that Ang II-induced insulin resistance may impair glucose uptake in the heart. Furthermore, relatively maintained fatty acid β-oxidation rates may have inhibited phosphofructokinase-1, the rate-limiting enzyme of glycolysis, thus reducing the rate of glycolysis. The resulting uncoupling between glycolysis and glucose oxidation produces protons by the hydrolysis of glycolytically derived ATP, leading to intracellular acidosis. Intracellular acidosis may lead to the accumulation of intracellular Na^+ because of the activation of the Na^+/H^+ exchanger, leading to the secondary elevation of intracellular Ca^{2+} in response to reverse mode Na^+-Ca^{2+} exchanger, which may further exacerbate the diastolic dysfunction observed in our Ang II model.[38,39] Second, ATP production is decreased in Ang II-treated hearts. Relaxation is an energy-consuming process because ATP hydrolysis is required for myosin detachment from actin, Ca^{2+} dissociation from Tn-C, and active sequestration of Ca^{2+} by the sarcoplasmic reticulum. Loss of ATP production and a rise in the ADP/ATP ratio have been linked to diastolic dysfunction in hypertrophied hearts.[16,17] Our results support a key role of Ang II-mediated diastolic dysfunction mediated by metabolic perturbations coupled with increased interstitial myocardial fibrosis.[18]

Although Ang II-reduced carbohydrate oxidation is expected to lead to a compensatory rise in fatty acid oxidation based on the Randle principle, palmitate oxidation was actually slightly decreased or showed no change in response to Ang II and PE, respectively. The lack of a compensatory increase in palmitate oxidation may be because of Ang II-induced impairment of mitochondria function as a result of nicotinamide adenine dinucleotide phosphate oxidase–mediated reactive oxygen species production.[18,40,41] Reductions in total acetyl CoA production and expression of complex I in response to Ang II are consistent with impaired mitochondrial function. Treatment with irbesartan significantly increased palmitate oxidation normalized for cardiac work, and AT1R blockers are known to possess AT1R-independent effects, including an increase in the expression of PPARs.[42,43] Indeed, we showed that irbesartan increased complex I, PPARα, and ERRα levels, leading to an increase in palmitate oxidation. Furthermore, under conditions where Ang II and irbesartan were coadministered, Ang II is more likely to bind and activate the Ang II type 2 receptor, which may also contribute to the observed alterations in cardiac metabolism. Metabolic modulators that aimed at shifting metabolism from fatty acid toward glucose oxidation,[11] which have recently been shown for hypertrophic cardiomyopathy treatment, may serve as a novel therapy for HF with normal ejection fraction.[44]

Although the ERRα/PPARα axis is known to upregulate PDK4 expression, consistent with previous findings, we showed Ang II-induced downregulation of PPARα and ERRα,[14] which would be expected to reduce PDK4 expression and increase glucose oxidation. In contrast, we showed that the expression of PDK4 is increased with inhibitory phosphorylation of PDH, resulting in reduced glucose and lactate oxidation rates. Importantly, we showed a critical role of the cyclin/CDK-pRB-E2F1 axis in the Ang II-induced upregulation of PDK4. E2F1 is known as a transcriptional factor and a critical regulator in the development of cardiac hypertrophy,[45,46] whereas cyclin D1 assembles with CDK4. The complex phosphorylates and inactivates Rb, which represses nuclear E2F1. In addition, the nuclear translocation of the complex of CDK4 and cyclin D1 enhances the expression of phospho-cyclin D1 in cytosol.[47,48] In the setting of cardiac hypertrophy, cyclin D1 is upregulated, thus leading to the activation of E2F1,[49,50] whereas in human HF increased expression of pRB, cyclin D1, and E2F1 is reversible in response to ventricular unloading.[51] Ang II-induced hypertrophy results in activation of E2F1 in skeletal muscle, resulting in enhancement of protein synthesis and energy production,[52] whereas E2F1 regulates the expression of PDK4 and glucose oxidation.[53] We demonstrate the upregulation of pRB, enhanced expression of cytosolic phospho-cyclin D1, and accumulation of nuclear E2F1 in response to Ang II. Human studies suggest that in HF there is a decreased fatty acid β-oxidation and increased glucose uptake in the heart.[31] However, alterations of glucose oxidation in human HF are not that well characterized, primarily because it is difficult to directly measure glucose oxidation rates in human hearts. Also, alterations in cardiac metabolism can vary depending on the severity of HF.[54] We report the specific reduction in carbohydrate oxidation in Ang II- and PE-induced preclinical models of HF with normal ejection fraction. Ang II-specific activation of PDK4 via E2F1 and AT1R blockade prevents these changes, which supports E2F1 playing a pivotal role in Ang II-induced alterations in cardiac metabolism.

In conclusion, Ang II- and PE-mediated suppression of glucose oxidation correlated with the development of pathological hypertrophy and diastolic dysfunction. Ang II induces a reduction in glucose oxidation via the cyclin/CDK-pRB-E2F axis, leading to increased PDK4 levels. Suppression of the cyclin/CDK-pRB-E2F axis and inhibition of PDK4 represent potential new therapeutic tools for treatment of HF with normal ejection fraction.

Disclosures

Dr Mori is a fellow of the Mazankowski Alberta Heart Institute. Dr Oudit is an Alberta Innovates Health Solution (AIHS) Clinician Investigator and a distinguished clinician-scientist of the Heart and Stroke Foundation of Canada (HSFC) and Canadian Institutes of Health Research. Dr Lopaschuk is an AIHS scientist, and Dr Kassiri is an AIHS scholar and HSFC New Investigator. The other authors have no conflicts to report.

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CLINICAL PERSPECTIVE

The renin-angiotensin system plays a pivotal role in cardiac hypertrophy and heart failure. This notion is supported by many studies in animals and humans, which showed that renin-angiotensin system blockade is effective in preventing cardiac hypertrophy and ameliorates heart failure. Stimulation of α-adrenergic receptors is also a key mediator of pathological remodeling and heart failure. We hypothesized that agonist-induced hypertrophy and pathological remodeling are associated with early changes in cardiac energy metabolism. Using pressor doses of angiotensin II (Ang II) and phenylephrine, we showed that Ang II and phenylephrine induced hypertrophied hearts with diastolic dysfunction and normal systolic function. Importantly, glucose oxidation was decreased by 45%, in association with an upregulation of pyruvate dehydrogenase kinase 4 in response to Ang II- and phenylephrine-induced pathological hypertrophy and diastolic dysfunction. There was minimal change in fatty acid oxidation. We demonstrated that the cyclin/cyclin-dependent kinase-retinoblastoma protein-E2F pathway plays a critical role in Ang II-induced alterations in cardiac metabolism and efficiency. The Ang II type 1 receptor blocker, irbesartan, prevented pathological hypertrophy and diastolic dysfunction. Irbesartan prevented the Ang II-mediated metabolic perturbations but increased fatty acid oxidation above baseline, which may confer a possible detrimental effect. Our results imply that suppression of the cyclin/cyclin-dependent kinase-retinoblastoma protein-E2F axis and inhibition of pyruvate dehydrogenase kinase 4 represent potential new therapeutic tools for treatment of heart failure with normal ejection fraction.