

Cardiac Insulin-Resistance and Decreased Mitochondrial Energy Production Precede the Development of Systolic Heart Failure After Pressure-Overload Hypertrophy

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Background—Cardiac hypertrophy is accompanied by significant alterations in energy metabolism. Whether these changes in energy metabolism precede and contribute to the development of heart failure in the hypertrophied heart is not clear.

Methods and Results—Mice were subjected to cardiac hypertrophy secondary to pressure-overload as a result of an abdominal aortic constriction (AAC). The rates of energy substrate metabolism were assessed in isolated working hearts obtained 1, 2, and 3 weeks after AAC. Mice subjected to AAC demonstrated a progressive development of cardiac hypertrophy. In vivo assessment of cardiac function (via echocardiography) demonstrated diastolic dysfunction by 2 weeks (20% increase in E/E'), and systolic dysfunction by 3 weeks (16% decrease in % ejection fraction). Marked cardiac insulin-resistance by 2 weeks post-AAC was evidenced by a significant decrease in insulin-stimulated rates of glycolysis and glucose oxidation, and plasma membrane translocation of glucose transporter 4. Overall ATP production rates were decreased at 2 and 3 weeks post-AAC (by 37% and 47%, respectively) because of a reduction in mitochondrial oxidation of glucose, lactate, and fatty acids that was not accompanied by an increase in myocardial glycolysis rates. Reduced mitochondrial complex V activity was evident at 3 weeks post-AAC, concomitant with a reduction in the ratio of phosphocreatine to ATP.

Conclusions—The development of cardiac insulin-resistance and decreased mitochondrial oxidative metabolism are early metabolic changes in the development of cardiac hypertrophy, which create an energy deficit that may contribute to the progression from hypertrophy to heart failure. (*Circ Heart Fail.* 2013;6:1039-1048.)

Key Words: abdominal aortic constriction ■ cardiac insulin-resistance ■ cardiomegaly ■ electron transport chain ■ energy metabolism ■ myocardial lipid accumulation

Sustained cardiac remodeling in the form of hypertrophy is a powerful predictor of heart failure (HF). Alterations in cardiac energy substrate metabolism are also recognized as important hallmarks of the remodeling process.¹ A decline in fatty acid use, attributable to a reduction in the expression of genes involved in fatty acid metabolism, has been proposed to represent a key event in the development of HF.^{2,3} The decrease in fatty acid oxidation is accompanied by a reversion to the fetal metabolic profile, and is characterized by an increased reliance on glycolysis for ATP generation.^{1,4} A reduction in fatty acid oxidation without a concomitant increase in carbohydrate (glucose or lactate) oxidation to support ATP production can lead to an energy deficit in the failing heart. Such an energy deficit has been suggested to correlate with overall disease severity.⁴

Clinical Perspective on p 1048

The high energy demands of the heart are primarily met by the mitochondrial oxidation of fatty acids and carbohydrates

(glucose and lactate).¹ The amount of ATP produced depends on overall mitochondrial oxidative capacity, oxygen supply to the myocardium, and the supply of substrates for oxidative metabolism.¹ In hypertrophy and HF, a decrease in high-energy phosphates in the heart has been observed.^{5,6} However, it is not clear whether this is attributable entirely to a decrease in mitochondrial oxidative capacity, a switch in energy substrate preference, or a less efficient use of energy. The question also arises as to whether these metabolic changes are a consequence of HF, per se, or whether they are an early event that may contribute to the development and progression of HF.

Glucose use in the heart is highly dependent on insulin, and any decrease in responsiveness of the heart to insulin can create a state of cardiac insulin-resistance.⁷ Insulin facilitates glucose entry by inducing the translocation of glucose transporter 4 (GLUT4) from intracellular storage vesicles to the sarcolemmal membrane.⁸ Decreasing GLUT4 availability exacerbates

Received August 17, 2012; accepted July 9, 2013.

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The online-only Data Supplement is available at <http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.112.000228/-/DC1>.

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DOI: 10.1161/CIRCHEARTFAILURE.112.000228

cardiac insulin-resistance, and cardiac insulin-resistance attributable to GLUT4 deficiency is associated with the development of HF.⁹ As a result, myocardial insulin-resistance is an established risk factor for the development of cardiovascular diseases.^{1,10} Chronic hyperinsulinemia associated with whole-body insulin-resistance can stimulate angiotensin II–induced pathological hypertrophy.¹¹ In addition, insulin resistance is often observed in patients with hypertension-induced hypertrophy.¹² Because the development of cardiac insulin-resistance can potentially contribute to the development of HF, it is important to understand whether cardiac insulin-resistance occurs early during the development of HF.

The aims of this study were to characterize the profile of energy substrate metabolism in the presence and absence of insulin in the normal and hypertrophied heart. Our hypothesis is that altered energy substrate metabolism is an early event that occurs with the development of cardiac hypertrophy induced by pressure-overload attributable to an abdominal aortic constriction (AAC) in mice. These early changes will decrease myocardial energetics that contributes to the development of hypertrophy-induced contractile dysfunction. Thus, optimizing energy metabolism may be an approach to slowing HF progression.

Methods

Animals

All mice received care and treatment according to the Canadian Council on Animal Care, and all protocols on mice were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee.

Experimental Protocol

Male C57BL/6 mice (n=66; 8 weeks old) were randomly assigned to undergo either a sham surgical procedure (n=25), or an AAC (n=41). Echocardiography was performed at 1, 2, or 3 weeks postsurgery. After echocardiography, glucose tolerance tests were performed at 2 or 3 weeks post-AAC in mice from the respective groups (n=10 for the sham group and for the AAC group). Subsequently, hearts were isolated and perfused as working preparations as described below.

AAC Procedure

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

Echocardiography and Tissue Doppler Imaging

Mice were anesthetized with 0.75% isoflurane for the duration of the transthoracic echocardiographic procedure, which was performed using a Visualsonic Vevo 700 instrument as previously described.¹³

Isolated Working Heart Perfusions

Hearts were aerobically perfused with Krebs-Henseleit solution containing 1 mmol/L lactate, 5 mmol/L glucose, 0.8 mmol/L palmitate prebound to 3% bovine serum albumin, and 2.5 mmol/L free Ca²⁺, in the absence or presence of 100 μU/mL insulin for measurement of cardiac function and energy metabolism as previously described.¹⁴

Measurement of High-Energy Phosphates

Neutralized perchloric acid extracts from frozen ventricular tissue (n=5/group) were used for measurements by high-energy phosphates using high performance of liquid chromatography (Waters).¹⁵ Values of ATP and phosphocreatine (PCr) obtained from high performance

liquid chromatography analysis were used to calculate the PCr/ATP ratio. Values of PCr and cytosolic creatine (Cr) obtained from high performance of liquid chromatography analysis were used for calculating cytosolic ADP and AMP. To calculate the cytosolic contents of ADP and AMP, the equilibrium reactions PCr+ADP+H⁺↔ATP+Cr with a Keq=1.66×10⁹, whereas 2ADP↔ATP+AMP with a Keq=1.05, were used to calculate ADP and AMP, respectively, as described previously.¹⁶

Glucose Tolerance Test

At 2 and 3 weeks postsurgery, mice from the sham (n=10) and AAC (n=10) groups were fasted for 16 hours. After obtaining a fasting blood sample, glucose (2 g/kg body weight) was administered orally via gastric lavage. Blood glucose was measured with Accu-Chek Advantage (Roche) at 15-minute intervals.

Assay of Triacylglycerol Content

Hearts perfused with [9,10-³H]-palmitate (n=5/group) were subjected to lipid extraction as previously described.⁷ Based on the specific activity of [9,10-³H]-palmitate in the perfusate, the amount of palmitate incorporated into the myocardial triacylglycerol (TAG) pool was calculated.

Assay of Myocardial Glycogen Content

Ventricular tissue from hearts perfused with [5-³H]-glucose (n=5/group) was subjected to glycogen extraction as previously described.¹⁷ Based on the specific activity of [³H]-glucose in the perfusate, the rate of glucose incorporation into the myocardial glycogen pool during the course of the 60-minute perfusion was calculated as μmol/g dry wt/h.

Activity of Hexokinase, Citrate Synthase, β-Hydroxyacyl Coenzyme A Dehydrogenase, Pyruvate Dehydrogenase, Mitochondrial Electron Transport Chain Complexes, and Superoxide Dismutase

All activities (n=5/group) were determined based on continuous kinetic changes of absorbance during 2 to 5 minutes, as described previously.^{13,18–20} Determination of pyruvate dehydrogenase activity was performed as described previously.²¹

Western Blotting

Immunoblotting was performed (n=5/group) by subjecting equal amounts of protein to SDS-PAGE as described previously.⁷ Bound antibody was visualized by incubation with enhanced chemiluminescent substrate, while Image J was used for densitometric analysis.

Statistical Analysis

Data are presented as the mean±SEM. Differences between 2 groups (sham and AAC) were evaluated using an unpaired, 2-tailed Student *t* test. Differences were considered significant when *P*<0.05.

Results

Cardiac Hypertrophy Occurs Concomitantly With the Development of Diastolic Dysfunction

To characterize the induction of cardiac hypertrophy in mice subjected to our AAC protocol, left ventricular (LV) mass was assessed by echocardiography at 1, 2, and 3 weeks post-AAC. In comparison with sham, increased LV mass was not seen at 1 week post-AAC, but was observed at 2 weeks and further progressed at 3 weeks post-AAC (Figure 1A). Echocardiographic findings were confirmed by direct measurements of heart weight-to-body weight ratios at the time of sacrifice

(Figure 1B). LV wall thickness, as reflected by the posterior wall dimension in diastole (Figure 1C), and the interventricular septal dimension (Figure 1D) were gradually increased in AAC hearts. Cardiac function was evaluated by combining mitral inflow and tissue Doppler technologies. Ventricular filling velocity was unchanged in all hearts, expressed as the ratio of mitral inflow E and A wave (E/A; data not shown). However, myocardial motion, as indicated by E/E' (Figure 1E) and E'/A' (Figure 1F) ratios, was significantly impaired 2 weeks post-AAC, indicative of diastolic dysfunction. In contrast, a decrease in percent ejection fraction (Figure 1G) and fractional shortening (Figure 1H) was only evident in mice 3 weeks post-AAC, demonstrating a time-dependent progression from diastolic dysfunction to systolic dysfunction. Cardiac function was also assessed in isolated working hearts. Rates of cardiac output, aortic outflow, and contractile performance (estimated as the product of heart rate and peak systolic pressure) were used as the indicators of the contractile state of the heart. A decrease in cardiac output, aortic outflow (Figure 2A), and the product of heart rate and peak systolic pressure (Figure 2B) at both 2 and 3 weeks post-AAC were observed, indicative of contractile abnormalities relative to sham. In addition, a mild decrease in cardiac power was observed 1 week post-AAC (Figure 2C), and progressively decreased further by 2 and 3 weeks post-AAC relative to sham.

Mitochondrial Oxidative Metabolism Decreases While Cardiac Insulin-Resistance Occurs During the Early Development of Diastolic HF

To investigate what alterations in cardiac energy substrate metabolism occur during the development of cardiac hypertrophy and progression to early HF, isolated working heart perfusions were performed 1, 2, and 3 weeks post-AAC. No differences in cardiac energy metabolism were observed 1 week post-AAC (data not shown). At 2 weeks post-AAC, basal rates of glycolysis (Figure 3A), glucose oxidation (Figure 3B), lactate oxidation (Figure 3C), and fatty acid oxidation (Figure 3D) were not significantly different from the sham hearts. In the presence of insulin, a robust increase in glycolysis and glucose oxidation was observed in hearts from the sham animals (Figure 3A–3B). However, this insulin response was blunted in hearts 2 weeks post-AAC, demonstrating the presence of cardiac insulin-resistance. As expected, insulin decreased fatty acid oxidation in hearts, but no significant difference was seen between sham and 2-week post-AAC hearts. By 3 weeks post-AAC, glycolysis was decreased both in the presence and absence of insulin (Figure 3E). The mitochondrial oxidation of glucose (Figure 3F), lactate (Figure 3G), and palmitate (Figure 3H) was also decreased, particularly in the presence of insulin.

Compared with sham, tricarboxylic acid cycle (TCA) activity was decreased by 30% 2 weeks post-AAC (Figure 4A),

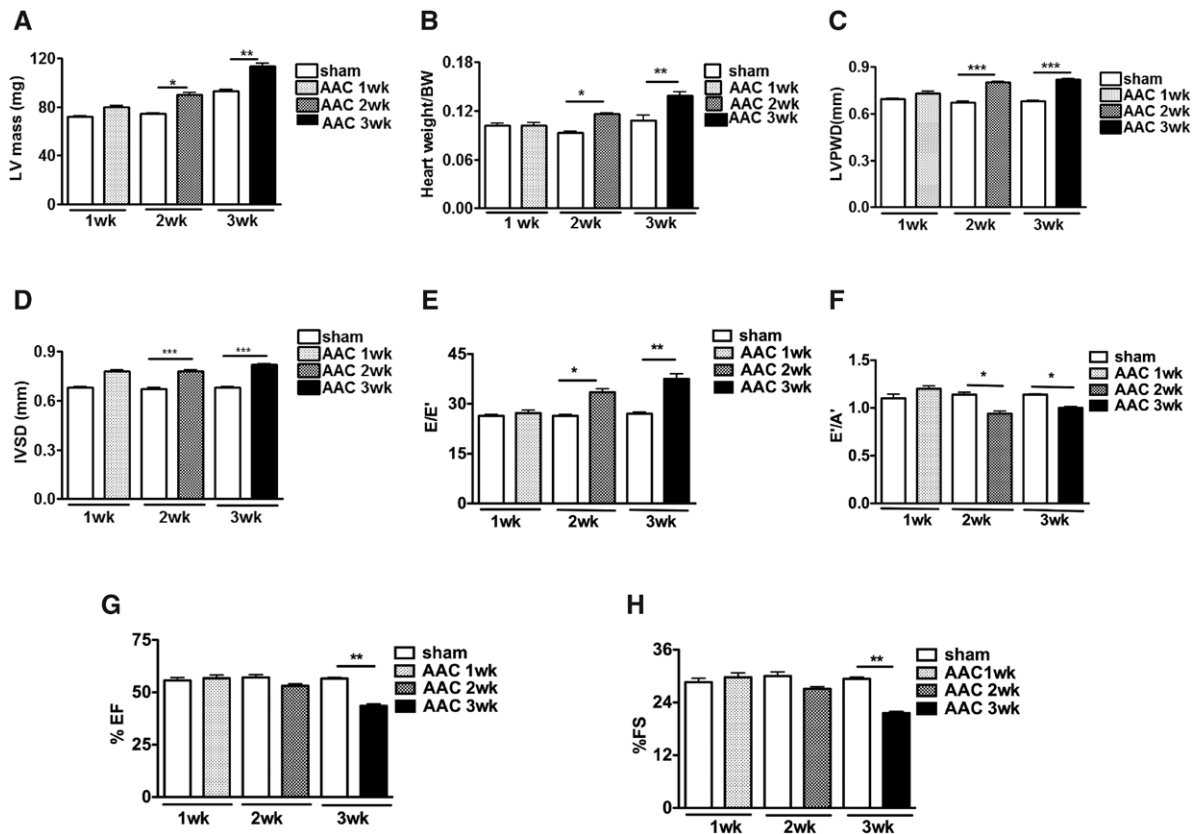


Figure 1. Characterization of cardiac hypertrophy in mice subject to 1, 2, and 3 weeks after abdominal aortic constriction (AAC). **A**, Left ventricle (LV) mass assessed by echocardiography. **B**, Ratio of dry weight of LV mass to body weight (BW) for mice. **C**, LV end-diastolic posterior wall dimension (LVPWD). **D**, Interventricular septal dimension (IVSD). **E**, Ratio of early transmitral flow velocity to early diastolic mitral valve annulus velocity (E/E'). **F**, Ratio of early and late ventricular filling velocity measured by (E'/A') tissue Doppler imaging. **G**, Ejection fraction (%EF). **H**, Fractional shortening (%FS). Values represent mean±SEM (n=10/group). **P*<0.05, ***P*<0.01, ****P*<0.001, significantly different from sham group.

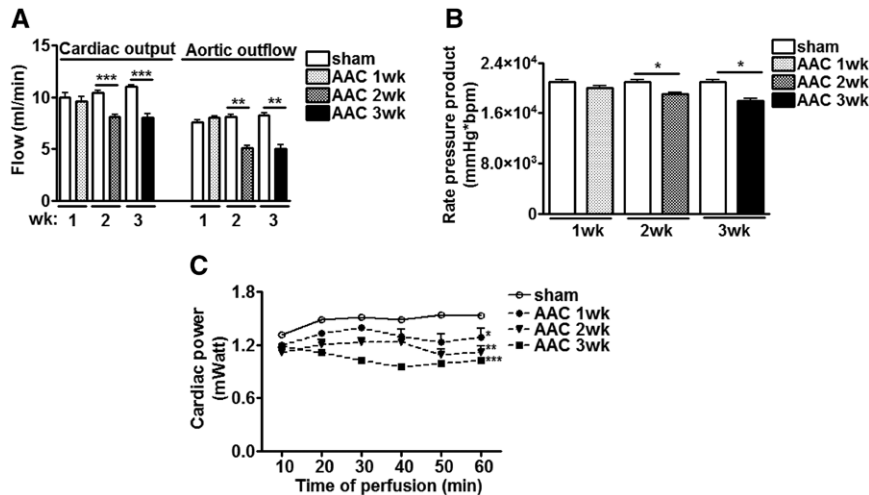


Figure 2. Cardiac contractile performance assessed by aerobic perfusion in mice subject to 1, 2, and 3 weeks of abdominal aortic constriction (AAC). **A**, Rates of cardiac output and aortic outflow. **B**, Rate pressure product. **C**, Cardiac power measured in isolated working hearts. Values represent mean±SEM (n=10/group). **P*<0.05, ***P*<0.01, ****P*<0.001, significantly different from sham group.

and decreased further by 3 weeks post-AAC (Figure 4B). This decrease in mitochondrial TCA cycle activity was not compensated by an increase in glycolysis, resulting in significant decreases in ATP production by 2 weeks (Figure 4C) and 3 weeks post-AAC (Figure 4D). The contribution of the various pathways to overall ATP production did not vary significantly either 2 weeks or 3 weeks post-AAC relative to sham, although the presence of insulin did increase the contribution of glucose oxidation to ATP production (Figure 4E and 4F). Of interest, glycolysis was a minor contributor to overall ATP production in AAC hearts and did not increase as the severity of hypertrophy increased.

Activation of AMP-activated protein kinase was observed in hearts at both 2 and 3 weeks post-AAC (Figure 5A). However, the overall energy-compromised state relative to sham was only observed at 3 weeks post-AAC, as reflected by an increase in the ratio of AMP/ATP (Figure 5B), ADP/ATP (Figure 5C), and a decrease in PCr/ATP (Figure 5D). The presence of cardiac insulin-resistance in AAC hearts led us to determine whether it was accompanied by a systemic insulin-resistance. Of interest, no difference in glucose tolerance was observed at 2 (Figure 5E) and 3 weeks post-AAC (Figure 5F) compared with the sham mice, suggesting that impaired insulin action in the hypertrophied hearts occurs before the

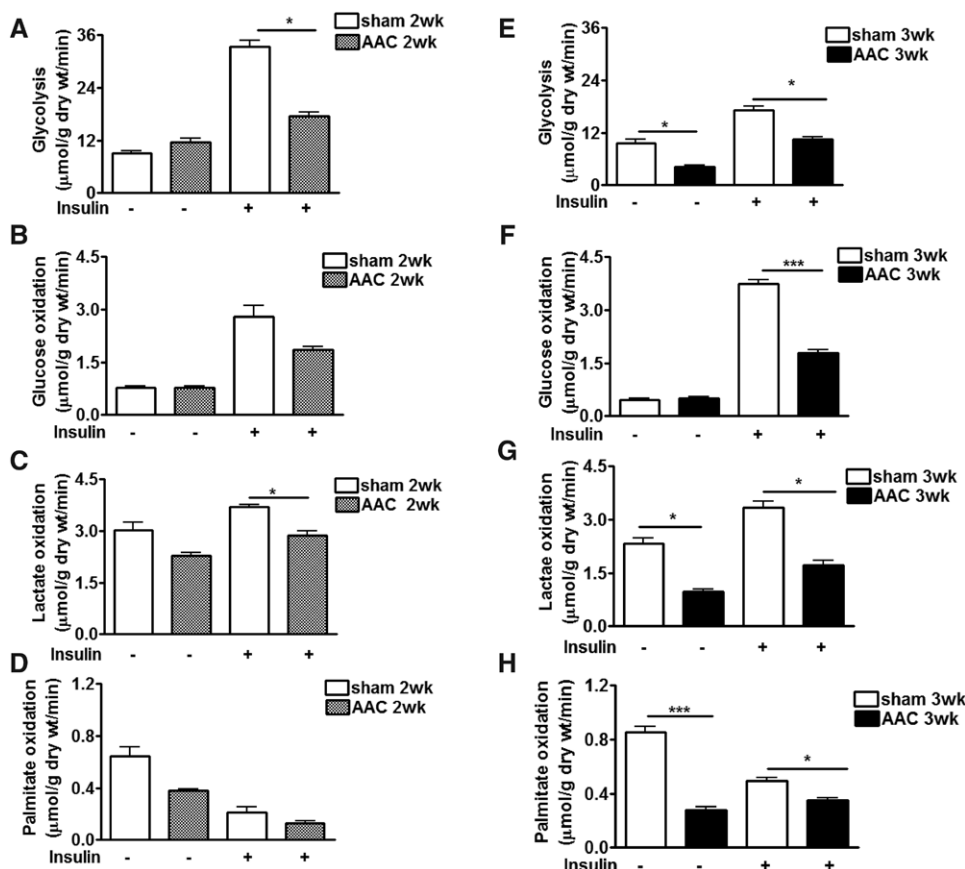


Figure 3. Cardiac energy metabolism in mice subjected to 2 and 3 weeks of abdominal aortic constriction (AAC). **A** and **E**, Rates of glycolysis in the absence (-) or presence (+) of 100 μU/mL insulin. **B** and **F**, Rates of glucose oxidation in the absence (-) or presence (+) of 100 μU/mL insulin. **C** and **G**, Rates of lactate oxidation in the absence (-) or presence (+) of 100 μU/mL insulin. **D** and **H**, Rates of palmitate oxidation in the absence (-) or presence (+) of 100 μU/mL insulin. Values represent mean±SEM (n=5). **P*<0.05, ****P*<0.001, significantly different from the corresponding sham group.

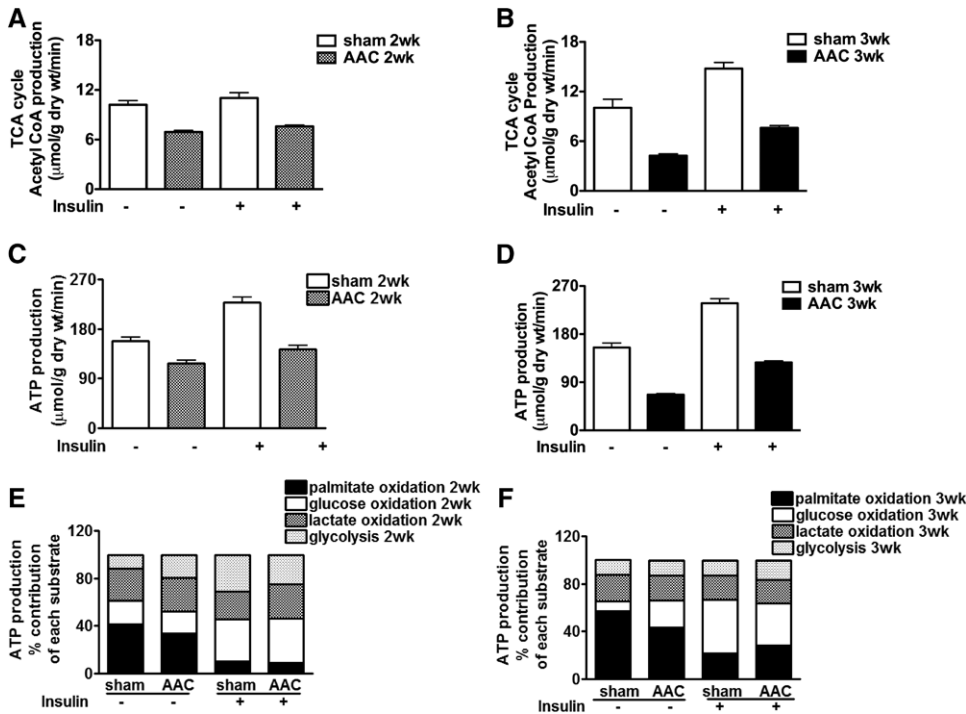


Figure 4. Cardiac tricarboxylic acid cycle (TCA) activity and ATP production rates in mice subject to 2 and 3 weeks of abdominal aortic constriction (AAC). Hearts were obtained from sham and AAC mice 2 weeks (A, C, and E) or 3 weeks (B, D, and F) post-AAC. A and B, TCA cycle acetyl coenzyme A (CoA) production rates in the absence (-) or presence (+) of 100 μ U/mL insulin. C and D, ATP production rates in the absence (-) or presence (+) of 100 μ U/mL insulin. E and F, Percentage of contribution of individual carbon substrates to ATP production in the absence (-) or presence (+) of 100 μ U/mL insulin. Values represent mean \pm SEM (n=5). * P <0.05, ** P <0.01, significantly different from the corresponding sham group.

development of systemic insulin-resistance. Coronary flow in the presence of insulin was unaffected at 2 and 3 weeks post-AAC (Figure 5G) relative to sham, indicating that impaired insulin action on energy metabolism was not accompanied by changes in insulin-mediated vascular effects on coronary flow post-AAC, and was independent of alterations in substrate (ie, glucose) delivery to the myocardium.

Translocation of GLUT4, Glycogen Metabolism, and TAG Metabolism After AAC

To understand the mechanism underlying the impaired myocardial insulin action, we investigated membrane translocation of myocardial GLUT4. Although the total expression of GLUT4 at 2 weeks post-AAC was not affected relative to sham (Figure 6A), sarcolemmal GLUT4 content was decreased, whereas cytosolic GLUT4 content was increased in hypertrophied hearts. This impaired GLUT4 translocation in the hypertrophied hearts is indicative of potential decreases in glucose uptake, which is reflected by decreased glycogen content (Figure 6B), and decreased glucose incorporation into the myocardial glycogen pool (Figure 6C). Combined with the decrease in glycolysis (Figure 3A and 3E), these data are consistent with the development of cardiac insulin-resistance. In addition, hexokinase activity and pyruvate dehydrogenase (the rate-limiting enzyme for carbohydrate oxidation) activity remained normal in the hypertrophied hearts (Table in the online-only Data Supplement).

Relative to sham, TAG levels were significantly increased in hearts 2 and 3 weeks post-AAC (Figure 6D), as was fatty acid incorporation into TAG during the perfusion (Figure 6E). This suggests that sarcolemmal fatty acid uptake was not impaired in the hypertrophied hearts, and that the observed decrease in fatty acid oxidation in the hypertrophied hearts was accompanied by preferential partitioning of fatty acids

into the endogenous TAG pool, as opposed to mitochondrial oxidative metabolism. The content of malonyl coenzyme A (CoA), an endogenous inhibitor of mitochondrial long chain fatty acid uptake, was unchanged 3 weeks post-AAC (Table in the online-only Data Supplement), suggesting that the decrease in fatty acid oxidation rates in early HF are not attributable to alterations in malonyl CoA control of mitochondrial fatty acid uptake. Rather, a decrease in the fatty acid oxidative enzyme, β -hydroxyacyl CoA dehydrogenase, may have contributed to decreased fatty acid oxidation in hearts 3 weeks post-AAC (Figure 6F).

Mitochondrial Electron Transport Chain Changes During the Development of Cardiac Hypertrophy

Apart from the reduced influx of energy substrates for mitochondrial oxidation, decreased ATP production in AAC hearts could also be a result of defects in the TCA cycle, mitochondrial biogenesis, or the electron transport chain (ETC). However, neither citrate synthase activity (Figure 7A) nor the expression of mitochondrial biosynthesis markers, such as nuclear peroxisome proliferator-activated receptor- γ coactivator (PGC-1 α) (Figure 7B) and nuclear estrogen-related receptor alpha (ERR α) (a downstream target of PGC-1 α ; Figure 7C), were altered in mice 2 weeks post-AAC compared with sham. In contrast, the activities of mitochondrial complex I (Figure 7D) and complex II (Figure 7E) were significantly decreased 2 weeks post-AAC, whereas the activities of complex V (Figure 7F) were unchanged. Thus, alterations in mitochondrial ETC may, in part, have contributed to the decrease in mitochondrial ATP production observed in the presence of cardiac hypertrophy. Decreased nuclear content of both PGC-1 α (Figure 7B) and ERR α (Figure 7C) were evident 3 weeks post-AAC relative to sham. However, an increase in activities of complex I (Figure 7D) and complex II (Figure 7E), but a

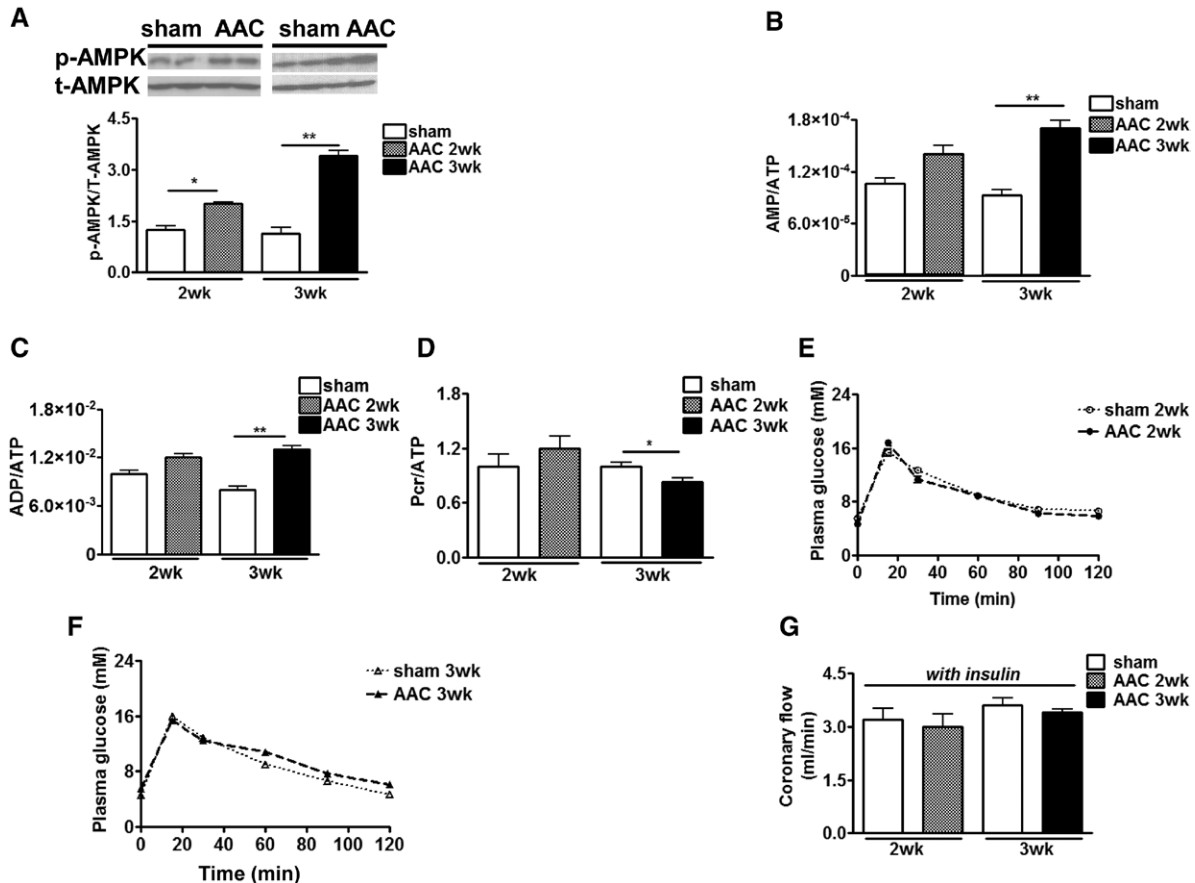


Figure 5. Phosphorylation of AMP-activated protein kinase (AMPK), high-energy phosphates and glucose tolerance test (GTT) at 2 and 3 weeks after abdominal aortic constriction (AAC). **A**, Representative blots of p-AMPK normalized to total AMPK at 2 and 3 weeks post-AAC. **B**, Ratio of AMP/ATP. **C**, Ratio of ADP/ATP. **D**, Ratio of phosphocreatine (PCr)/ATP. **E**, GTT at 2 weeks post-AAC. **F**, GTT at 3 weeks post-AAC. **G**, Rates of coronary flow measured in the presence of insulin at 2 and 3 weeks post-AAC. Values represent mean \pm SEM (n=5–10), * P <0.05, ** P <0.01, significantly different from the corresponding sham group.

decrease in complex V activity (Figure 7F), was observed 3 weeks post-AAC. These results suggest that a combination of defects in mitochondrial ETC activity and mitochondrial biosynthesis exacerbate energy deficiency.

Defects in mitochondrial ETC activity have the potential to increase the levels of reactive oxygen species, and can lead to ventricular dysfunction.^{22,23} However, neither cytosolic superoxide dismutase nor mitochondrial superoxide dismutase activity (Figure 8A and 8B) and its protein expression (Figure 8C) were altered in the hypertrophied hearts.

The expression of nuclear hypoxia-inducible factor 1 α , a transcription factor important in upregulating enzymes involved in glycolysis,²⁴ was decreased (Figure 8D) at 3 weeks post-AAC compared with sham, suggesting that diastolic dysfunction is not associated with alterations in hypoxia-inducible factor 1 α , but that decreased hypoxia-inducible factor 1 α expression may be associated with the transition from diastolic to early systolic dysfunction.

Discussion

This study demonstrates several key issues. First, cardiac hypertrophy was evident 2 weeks post-AAC, and was accompanied by the development of diastolic dysfunction. Accompanying the development of diastolic dysfunction

in the hypertrophied heart was a marked reduction in insulin-stimulated glucose use, and impaired plasma membrane translocation of GLUT4. As diastolic dysfunction progressed to systolic dysfunction further decrements in glycolysis and insulin-stimulated glycolysis and glucose oxidation became evident. Second, a depression of fatty acid oxidation occurred in conjunction with a transition from diastolic dysfunction to systolic dysfunction, in which a reduction in mitochondrial complex V activity was seen, and the ratio of PCr/ATP was decreased. Taken together, our data are consistent with alterations in energy substrate metabolism leading to a depression of overall mitochondrial oxidative metabolism that contributed to the progression from cardiac hypertrophy to HF.

Of importance is the demonstration that insulin-stimulated glucose oxidation is suppressed in the hypertrophied heart with underlying diastolic dysfunction at 2 weeks post-AAC. This cardiac insulin-resistance precedes any alterations in basal energy substrate metabolism, and it is not associated with systemic glucose intolerance. Therefore, impaired myocardial insulin action is an early event in the development of cardiac hypertrophy. Impaired insulin-stimulated myocardial GLUT4 translocation at 2 weeks post-AAC suggests a potential decrease in glucose uptake, which may contribute to impaired diastolic function. This is also supported by previous

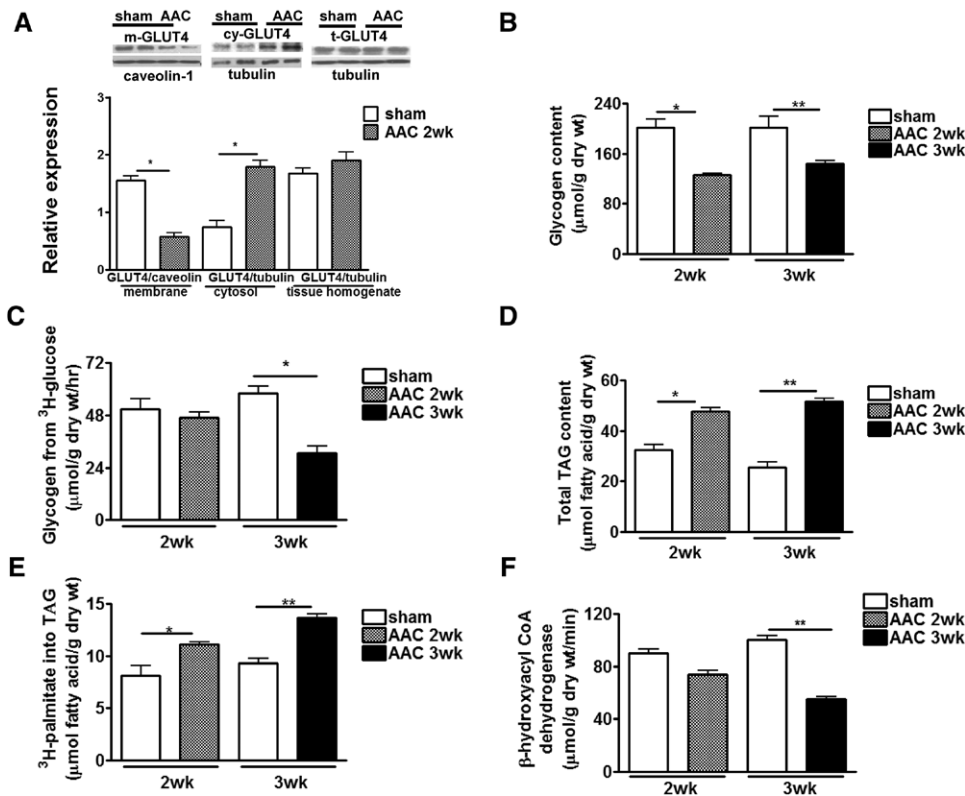


Figure 6. Alterations in cardiac GLUT4 translocation, triacylglycerol, and glycogen content in mice subject to 2 and 3 weeks after abdominal aortic constriction (post-AAC). **A**, Representative immunoblots of glucose transporter 4 (GLUT4), and densitometric analyses of membrane GLUT4 (m-GLUT4) to caveolin-1, cytosolic GLUT4 (cy-GLUT4) to tubulin, and total tissue expression of GLUT4 (t-GLUT4) to tubulin. **B**, Myocardial glycogen content. **C**, Radiolabeled glycogen content during the 1 hour perfusion. **D**, Myocardial triacylglycerol (TAG) content. **E**, Incorporation of [3 H]-palmitate into TAG. **F**, β -Hydroxyacyl coenzyme A (CoA) dehydrogenase activity. Values represent mean \pm SEM (n=5). * P <0.05, ** P <0.01, significantly different from the corresponding sham group.

studies indicating that targeted myocardial-GLUT4 deficiency induces cardiac hypertrophy, and that insulin-resistant cardiomyopathy is accompanied by decreased GLUT4/GLUT1 ratios.²⁵ In addition, short-term high-fat feeding-induced insulin resistance in mice predisposes to adverse LV remodeling,²⁶ in which insulin-stimulated myocardial glucose uptake was significantly impaired.²⁶ It is unclear whether myocardial insulin-resistance is a cause or consequence of LV remodeling. Clinical evidence suggests that cardiac hypertrophy is associated with an increase in insulin resistance.²⁷ Thus, decrements in membrane GLUT4 at 2 weeks post-AAC, at least in part, may contribute to diminished rates of glucose oxidation in the hypertrophied heart with diastolic dysfunction. Our finding that glycolysis is decreased in AAC hearts seems to be discordant with previous reports that glycolysis is elevated in the hypertrophied heart.^{17,28} Comparisons between studies are difficult because of differences in experimental protocols, such as different animal models resulting in different magnitudes of pressure overload, different phenotypic factors, or varying degrees of afterload, and variation in the inclusion of insulin during perfusion.²⁹ Despite the observation that promoting glucose use in the setting of established HF does have salutary effects,³⁰ directly targeting glucose supply may not be physiologically feasible. Whether targeting cardiac insulin-resistance could attenuate the progression from diastolic dysfunction to HF remains to be assessed. Thus, focusing on restoration of fatty acid oxidation during the development of cardiac hypertrophy may be a preferred approach.

A reduction in fatty acid oxidation has been repeatedly demonstrated in models of cardiac hypertrophy, including transverse aortic constriction (TAC) in both the mouse²⁸ and rat.³¹ Time-dependent alterations in TAC-rats reveal that fatty acid

oxidation is decreased early during hypertrophy development when heart function is near-normal with preserved wall stress and E/E'.³¹ Decreased fatty acid oxidation in our AAC mice was accompanied by the development of systolic dysfunction, whereas Kolwicz et al²⁸ showed a reduced fatty acid oxidation in mice subjected to TAC that had diastolic dysfunction. It should be pointed out that they were primarily examining alterations in fatty acid oxidation in hearts with established hypertrophy, and they did not examine overall energetic status or whether cardiac insulin-resistance was occurring in TAC-mice. In contrast, we were interested in examining the energetic changes that occurred during the development of cardiac hypertrophy, as well as whether cardiac insulin-resistance occurred. The common observation between our study and the Kolwicz et al²⁸ study is that reduction of fatty acid oxidation occurred in conjunction with a decreased PCr/ATP ratio, suggesting that decreased fatty acid oxidation is associated with the energy deficit. Whether enhancing fatty acid oxidation would attenuate the development of cardiac hypertrophy and preserve cardiac function is still controversial. However, it is important to recognize the occurrence of decreased fatty acid oxidation in association with the development of cardiac hypertrophy. Improving cardiac function through restoration of fatty acid oxidation with peroxisome proliferator-activated receptor- α agonists in hypertrophied and failing hearts has not provided beneficial results³² because the broad effects of peroxisome proliferator-activated receptor- α agonists on lipid metabolism may hamper its impact on recovery of cardiac function.^{28,32} Thus, exploring the changes of myocardial TAG in the hypertrophied heart could help our understanding in designing the appropriate therapeutic strategy toward improving cardiac function. In the current study, accompanying the decrease in fatty acid β -oxidation was an

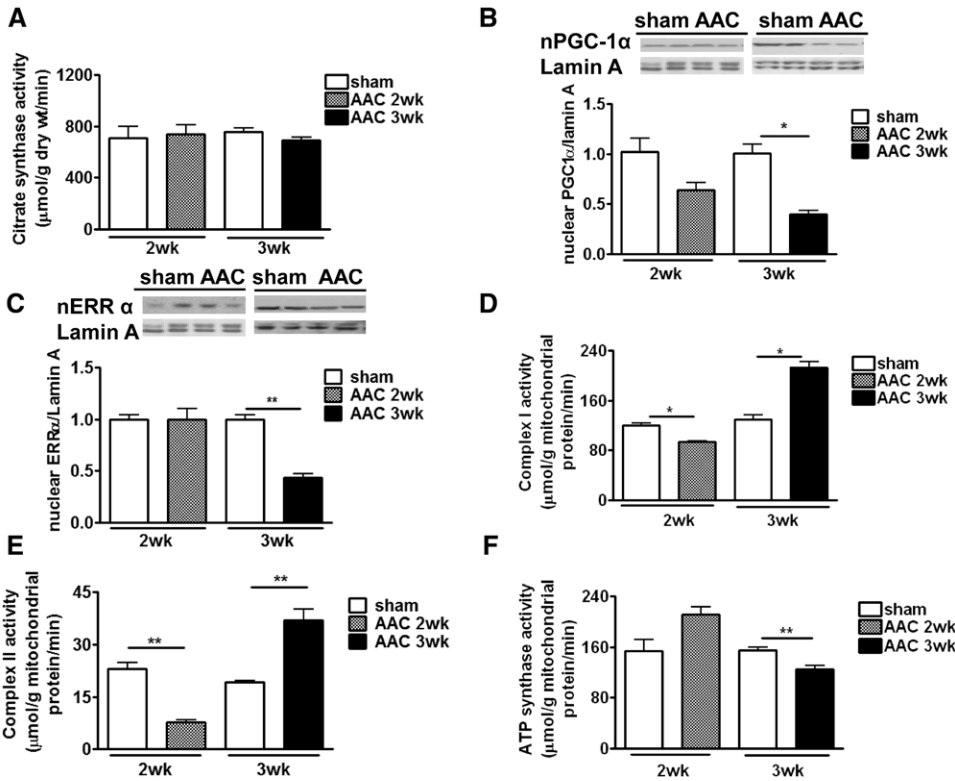


Figure 7. Alterations of cardiac mitochondrial capacity and electron transport chain activity 2 and 3 weeks after abdominal aortic constriction (post-AAC). **A**, Citrate synthase activity. **B**, Content of nuclear peroxisome proliferator-activated receptor-γ coactivator (PGC-1α; nPGC-1α). **C**, Content of nuclear estrogen-related receptor alpha (ERRα; nERRα). **D**, Mitochondrial complex I activity. **E**, Mitochondrial complex II activity. **F**, Mitochondrial complex V (ATP-synthase) activity. Values represent mean±SEM (n=5). **P*<0.05, ***P*<0.01, significantly different from the corresponding sham group.

increase in myocardial TAG content, and an increased incorporation of exogenous fatty acids into the myocardial TAG pool (effects that were evident 2 weeks post-AAC). However, the role of alterations in TAG content remains to be resolved as both increased³³ and decreased^{34,35} myocardial TAG content have both been reported in pressure overload or HF.

Despite these selective differences in substrate use, decreased respiratory capacity in TAC-rat³¹ and reduced PCr/

ATP in TAC-mice²⁸ are also observed in AAC mice. It has been shown that the level of complex V protein is significantly correlated with PCr/ATP in the postinfarcted heart.³⁶ Hence, it is tempting to speculate that a reduction of mitochondrial complex V activity at 3 weeks post-AAC contributed to an increase in ADP/ATP, and therefore a decrease in PCr/ATP ratio. However, the present data do not prove a causal relationship between the decreased complex V activity and the

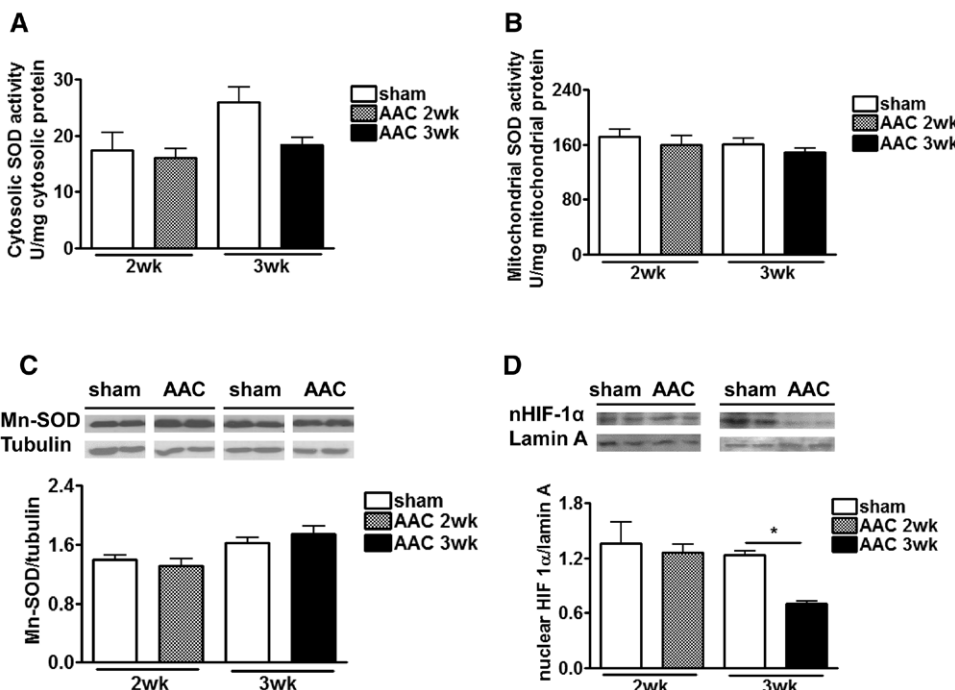


Figure 8. Alterations in cardiac antioxidant capacity and the expression of nuclear hypoxia-inducible factor 1α (nHIF-1α) 2 and 3 weeks after abdominal aortic constriction (post-AAC). **A**, Activity of cytosolic superoxide dismutase (SOD). **B**, Activity of mitochondrial SOD. **C**, Protein expression of mitochondrial SOD. **D**, Protein expression of nuclear HIF-1α. Values represent mean±SEM (n=5). **P*<0.05, significantly different from the corresponding sham group.

increased levels of ADP/ATP or decreased PCr/ATP ratios. Alternative explanations also need to be considered.

Limitation

Our study design is such that the studies cannot distinguish whether cardiac insulin-resistance is a cause or a consequence of diastolic dysfunction in hypertrophied heart. Such insights may ultimately be obtained from studies that use genetic or pharmacological manipulation of the implicated energy use pathways during the evolution of experimental pressure-overload hypertrophy. Furthermore, although we assessed diastolic function via estimates of LV filling pressure (E/E') and the ratio of early and late filling velocities, we did not assess chamber stiffness in these studies. Also, in our sham-operated controls, we observed larger-than-expected time-dependent changes in substrate use (Figure 3), and this may have affected some of our statistical comparisons. Finally, our study was performed in hypertrophied mouse hearts that transition rapidly from a compensated state to isolated diastolic dysfunction and then to mild systolic dysfunction. In humans, hypertension-induced diastolic abnormalities develop over years or decades, and are typically accompanied by chamber stiffening.³⁷ Therefore, it needs to be recognized that our model of diastolic dysfunction in the mouse may not completely recapitulate the diastolic dysfunction seen in humans.

In conclusion, cardiac insulin-resistance is an early event that accompanies the development of cardiac hypertrophy and diastolic dysfunction in hypertrophied hearts as a result of an AAC. A time-course of events provides support that the primary defect in mitochondrial energy metabolism is a specific defect in fatty acid oxidation. The metabolic perturbations elicited in the hypertrophied heart progressively worsen during the transition to systolic dysfunction and suggest that (1) limited oxidative metabolism of carbohydrates and fatty acids impairs the generation of reducing equivalents for the ETC, and that (2) alterations in the ETC may further limit oxidative-phosphorylation, and hence contribute to the well-noted energetic deficiencies observed in the failing heart.

Disclosures

This study was supported by a grant from the Heart and Stroke Foundation of Alberta (HSFA) to G.D. Lopaschuk, and a HSFA grant to M. Zaugg. G.D. Lopaschuk is an Alberta Heritage Foundation for Medical Research (AHFMR) Medical Scientist. L. Zhang is a fellow of the AHFMR.

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CLINICAL PERSPECTIVE

A significant imbalance between ATP production and use can occur in the failing heart muscle. These alterations in energy metabolism have the potential to contribute to the severity of contractile dysfunction in heart failure. Therefore, we explored the time-dependent energy metabolic changes that occur in hypertrophied hearts during the development of heart failure. Our study shows that cardiac insulin-resistance and decreased mitochondrial oxidative capacity occur coincident with the development of hypertrophy and diastolic dysfunction. Our results suggest that a dramatic impairment of insulin-stimulated glucose oxidation in combination with a decreased fatty acid oxidation causes a severe limitation of ATP production early in the development of heart failure. As a result, therapeutic strategies aimed at stimulating cardiac glucose oxidation may have potential clinical benefit in improving cardiac energy production in the failing heart, thereby improving cardiac function.