Cardiac-Specific Deletion of Acetyl CoA Carboxylase 2 Prevents Metabolic Remodeling During Pressure-Overload Hypertrophy

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Rationale: **Decreased fatty acid oxidation (FAO) with increased reliance on glucose are hallmarks of metabolic remodeling that occurs in pathological cardiac hypertrophy and is associated with decreased myocardial energetics and impaired cardiac function. To date, it has not been tested whether prevention of the metabolic switch that occurs during the development of cardiac hypertrophy has unequivocal benefits on cardiac function and energetics.**

Objective: **Because malonyl CoA production via acetyl CoA carboxylase 2 (ACC2) inhibits the entry of long chain fatty acids into the mitochondria, we hypothesized that mice with a cardiac-specific deletion of ACC2 ($ACC2H^{-/-}$) would maintain cardiac FAO and improve function and energetics during the development of pressure-overload hypertrophy.**

Methods and Results: **ACC2 deletion led to a significant reduction in cardiac malonyl CoA levels. In isolated perfused heart experiments, left ventricular function and oxygen consumption were similar in $ACC2H^{-/-}$ mice despite an ≈60% increase in FAO compared with controls (CON). After 8 weeks of pressure overload via transverse aortic constriction (TAC), $ACC2H^{-/-}$ mice exhibited a substrate utilization profile similar to sham animals, whereas CON-TAC hearts had decreased FAO with increased glycolysis and anaplerosis. Myocardial energetics, assessed by ^{31}P nuclear magnetic resonance spectroscopy, and cardiac function were maintained in $ACC2H^{-/-}$ after 8 weeks of TAC. Furthermore, $ACC2H^{-/-}$-TAC demonstrated an attenuation of cardiac hypertrophy with a significant reduction in fibrosis relative to CON-TAC.**

Conclusions: **These data suggest that reversion to the fetal metabolic profile in chronic pathological hypertrophy is associated with impaired myocardial function and energetics and maintenance of the inherent cardiac metabolic profile and mitochondrial oxidative capacity is a viable therapeutic strategy. (*Circ Res*. 2012; 111:728-738.)**

Key Words: cardiac metabolism ■ cardiac hypertrophy ■ cardiovascular physiology ■ cardiac contractility and energetics ■ metabolism

Fatty acid oxidation (FAO) is a major energy source for the adult mammalian heart. Decreased FAO contributes to the reappearance of the fetal metabolic pattern in hypertrophied and failing hearts that leads to increased reliance on glycolysis combined with upregulation of anaplerosis to maintain tricarboxcylic acid cycle (TCA) flux.[1–5] Although a shift toward carbohydrate metabolism slightly improves myocardial oxygen efficiency, this metabolic profile is inefficient in utilizing carbon substrates for ATP production during increased energy demand, leading to impaired myocardial energetics and depletion of contractile reserve.[6,7] Restoration of FAO with peroxisome proliferator-activated receptor-alpha (PPARα) agonists in hypertrophied and failing hearts has been unfruitful,[8,9] probably because of broad effects of these compounds on lipid uptake and metabolism. However, recent reports showed the benefit of high fat diets in certain models of heart failure.[10,11] Since increased myocardial FAO has been implicated in the development of metabolic cardio-

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myopathy in obesity and diabetes,[12,13] it remains controversial whether enhancing FAO during the development of pathological cardiac hypertrophy will prevent metabolic remodeling and preserve myocardial energetics and function.

The rate-limiting step of FAO is the import of long chain fatty acids (FA) across the mitochondrial membrane through carnitine palmitoyl transferase I (CPT1). This action is strongly inhibited by malonyl CoA, which is formed by the carboxylation of acetyl CoA via acetyl CoA carboxylase (ACC).[14] Deletion of ACC2, the primary ACC isoform in oxidative tissues, led to increases in whole-body and tissue specific FAO.[15–17] Although originally shown to improve insulin sensitivity and resist against diet-induced obesity and diabetes,[18,19] follow-up studies from 2 independent groups found that deletion of ACC2 upregulated mitochondrial FAO without altering overall energy homeostasis.[17,20] Nonetheless, these observations are consistent with past reports demonstrating that malonyl CoA produced by ACC2, is a target for modulating substrate preference of oxidative tissues.[21,22]

A prior report showed hearts of ACC2-null mice displayed increased oxidation of both glucose and FA with reduced heart size but unaltered cardiac function.[16] This strain of ACC2-null mice were leaner with altered whole body energy homeostasis.[15,19] It is unclear whether the cardiac phenotype in those studies is the result of altered systemic metabolism or due to elevated cardiac FAO. We generated a mouse model with a cardiac-specific deletion of ACC2 in order to interrogate the specific effects of reduced cardiac malonyl CoA levels on cardiac substrate metabolism in normal and hypertrophied hearts. Our results show that deletion of ACC2 results in a shift of substrate oxidation to FAO without negatively impacting cardiac function in the long term. Moreover, ACC2 deletion prevents metabolic reprogramming and sustains myocardial energetics and function in pathological cardiac hypertrophy.

Non-standard Abbreviations and Acronyms

AA	amino acids
ACC	acetyl CoA carboxylase
CON	control
FA	fatty acids
FAO	fatty acid oxidation
FS	fractional shortening
NMR	nuclear magnetic resonance
PPARα	peroxisome proliferator-activated receptor alpha
RPP	rate-pressure product
TAC	transverse aortic constriction
TCA	tricarboxylic acid cycle
TCAI	TCA cycle intermediates

Methods

An expanded Methods section is provided in the Online Data Supplement.

Animal Model

Animal studies were approved by the Harvard Medical Area Standing Committee on Animals or University of Washington Institutional Animal Care and Use Committee. ACC2 flox/flox ($ACC2^{f/f}$) mice on a C57/129 background[20] were mated with αMHC-Cre mice to yield 4 genotypes: $ACC2^{f/WT}$, $ACC2^{f/f}$, $ACC2^{f/WT\text{-}Cre+}$ ($ACC2^{-/+}$), and $ACC2^{-f/f\text{-}Cre+}$ ($ACC2H^{-/-}$). $ACC2H^{-/-}$ were mated with $ACC2^{f/f}$ to produce both study and control littermates. Mice were kept on a 12-hour light/dark cycle with water and food ad libitum.

Isolated Heart Perfusions and Nuclear Magnetic Resonance Spectroscopy

Methods for isolated Langendorff-perfused heart experiments and nuclear magnetic resonance (NMR) spectroscopy have been previously published.[23–27] Additional details are provided in the Online Supplement.

Malonyl CoA, Acylcarnitines, and Global Metabolite Profiling

Detection and quantification of malonyl CoA levels was adapted from previously described methods using LC-MS/MS.[28,29] For acylcarnitine species, frozen ventricular tissue from nonperfused hearts were powdered and homogenized in deionized water.[30] Acylcarnitines were quantified using commercially available labeled standards and normalized to protein concentration. For global metabolite profiling, ventricular tissue was extracted in 2:1 chloroform: methanol and analyzed by GC×GC-TOFMS.[31] The previously developed *F*-ratio method was used to analyze all samples at each mass channel. Detection of additional metabolic features was made via Peak Table analysis (LECO ChromaTOF software version 3.2, LECO Corp, St Joseph, MI). Peak areas of target analytes were measured by PARAFAC analysis[32] (see Online Supplement for additional details).

Statistical Analysis

All data are presented as mean±SEM. The Student *t* test was used for 2 group comparisons. One-way ANOVA was performed for multiple-group comparisons at single time point. Two-way ANOVA was used for multiple group comparisons with 2 factors (ie, substrate utilization). ANOVA with repeated measures was used for multiple group comparisons over multiple time points. Bonferroni post hoc analysis was used for all ANOVAs. Analyses were performed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). All results were tested at the $P<0.05$ level of significance.

Results

Animal Model

$ACC2^{f/f}$ mice were generated as previously described,[20] and cardiac-specific deletion of ACC2 ($ACC2H^{-/-}$) was achieved by cross-breeding $ACC2^{f/f}$ with αMHC-Cre mice. No differences were found in serum levels of glucose, insulin, free FA, and triglycerides across all genotypes (Online Table I). Furthermore, $ACC2H^{-/-}$ had no significant differences in heart weight (HW) or HW relative to body weight (BW) or tibial length (TL) (Online Table II), suggesting a possible systemic and/or strain effect in the previously observed smaller heart size in ACC2-null mice.[16,20] Western blotting confirmed the cardiac-specific deletion of ACC2 (Figure 1A and 1B). Since physical characteristics and cardiac protein levels of ACC2 in both $ACC2^{f/WT}$ and $ACC2^{f/f}$ were similar to wild-type C57BL6 mice, $ACC2^{f/f}$ mice were used as controls (CON). Because the ACC1 isoform is present in the heart at low levels (≈10%) and is difficult to detect via Western blotting,[33] we measured changes in gene expression to confirm a negligible effect of ACC2 deletion on ACC1 (Figure 1C). Deletion of ACC2 resulted in a significant decrease (≈50%) in cardiac malonyl CoA levels (Figure 1D).

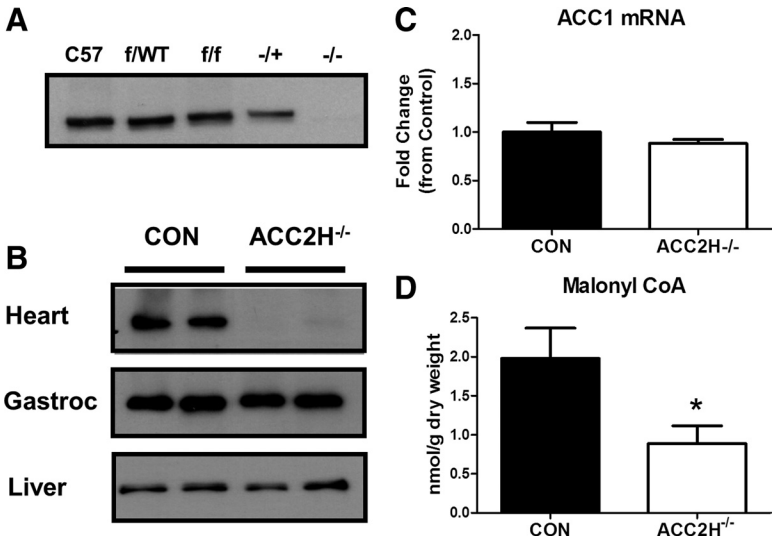

Figure 1. ACC2 deletion is cardiac-specific and leads to decreased malonyl CoA levels. A, Western blotting of ACC2 in heart in heterozygous and homozygous ACC2 floxed mice that are Cre^{-} (f/WT, f/f) or $Cre^{+}(-/+, -/-)$. C57BL6/J (C57) included as control. **B**, Western blotting analysis of heart, gastrocnemius, and liver tissue in $ACC2^{f/f}$ (CON) and $ACC2H^{-/-}$. **C**, ACC1 mRNA levels. Values expressed as fold change over CON, n=3 each group. **D**, Cardiac malonyl CoA levels assessed by LCMS. *$P<0.05$, n=5 each group.

Deletion of ACC2 Increases Cardiac Fatty Acid Oxidation Without Overloading Mitochondria

In isolated Langendorff-perfused heart experiments, $ACC2H^{-/-}$ demonstrated an ≈60% increase in FAO as compared with CON at 8 to 10 weeks of age ($P<0.05$; Figure 2A) that was concomitant with a reciprocal reduction in the contribution of glucose oxidation ($P<0.05$; Figure 2A). Despite enhanced FAO, cardiac triacylglycerol and glycogen levels remained unchanged in $ACC2H^{-/-}$ (Figure 2B and 2C). A recent report suggested that increased FAO is associated with

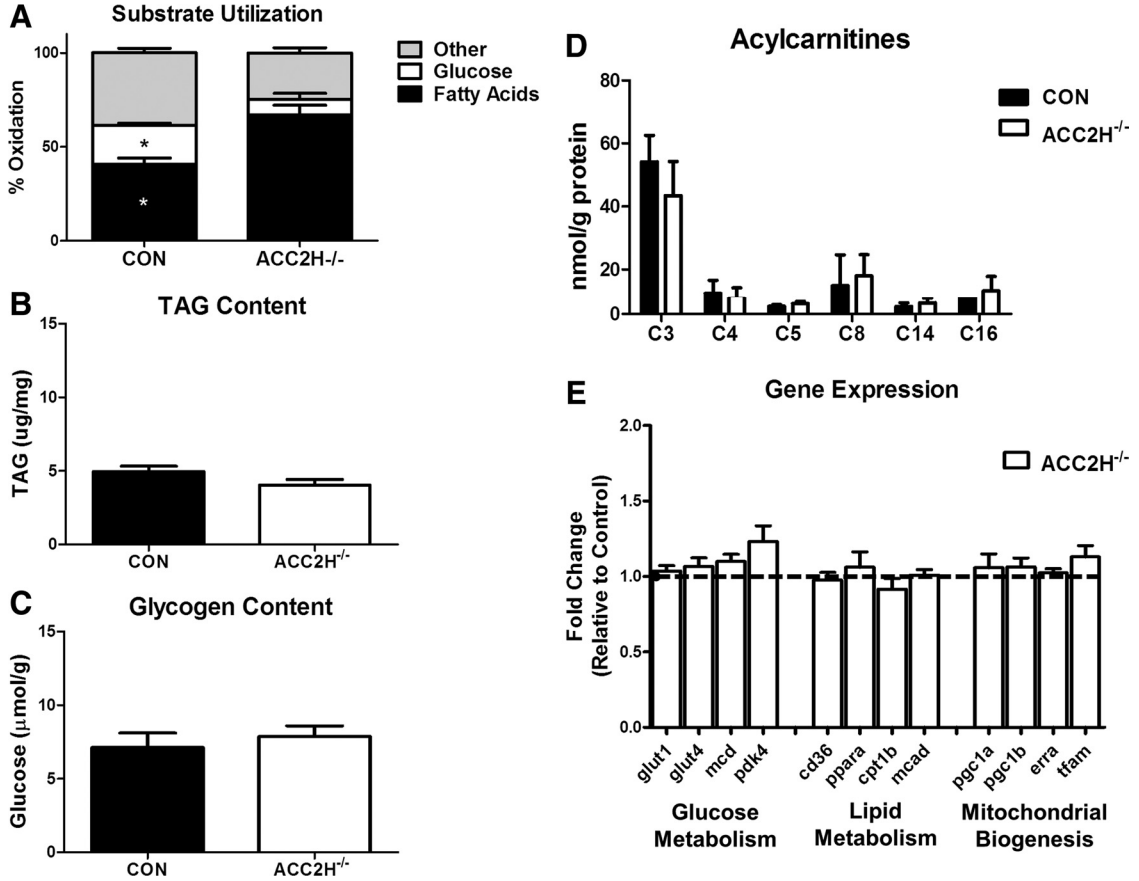

Figure 2. Effects of ACC2 deletion on cardiac metabolism. A, Relative contribution of fatty acids, glucose, and other substrates (lactate, endogenous) to the TCA cycle in hearts perfused with ^{13}C-labeled substrates, n=4 to 5 each group. **B**, Cardiac triacylglycerol (TAG) content normalized to tissue weight, n=5 to 7 each group. **C**, Glycogen content normalized to tissue weight, n=8 to 9 each group. **D**, Fatty acylcarnitine species from heart extracts of 2-month-old mice assessed by LCMS, n=5 each group. **E**, Expression of genes involved in glucose metabolism, lipid metabolism, and mitochondrial biogenesis reported as fold change over CON, n=3 to 5 each group. *$P<0.05$ versus $ACC2H^{-/-}$.

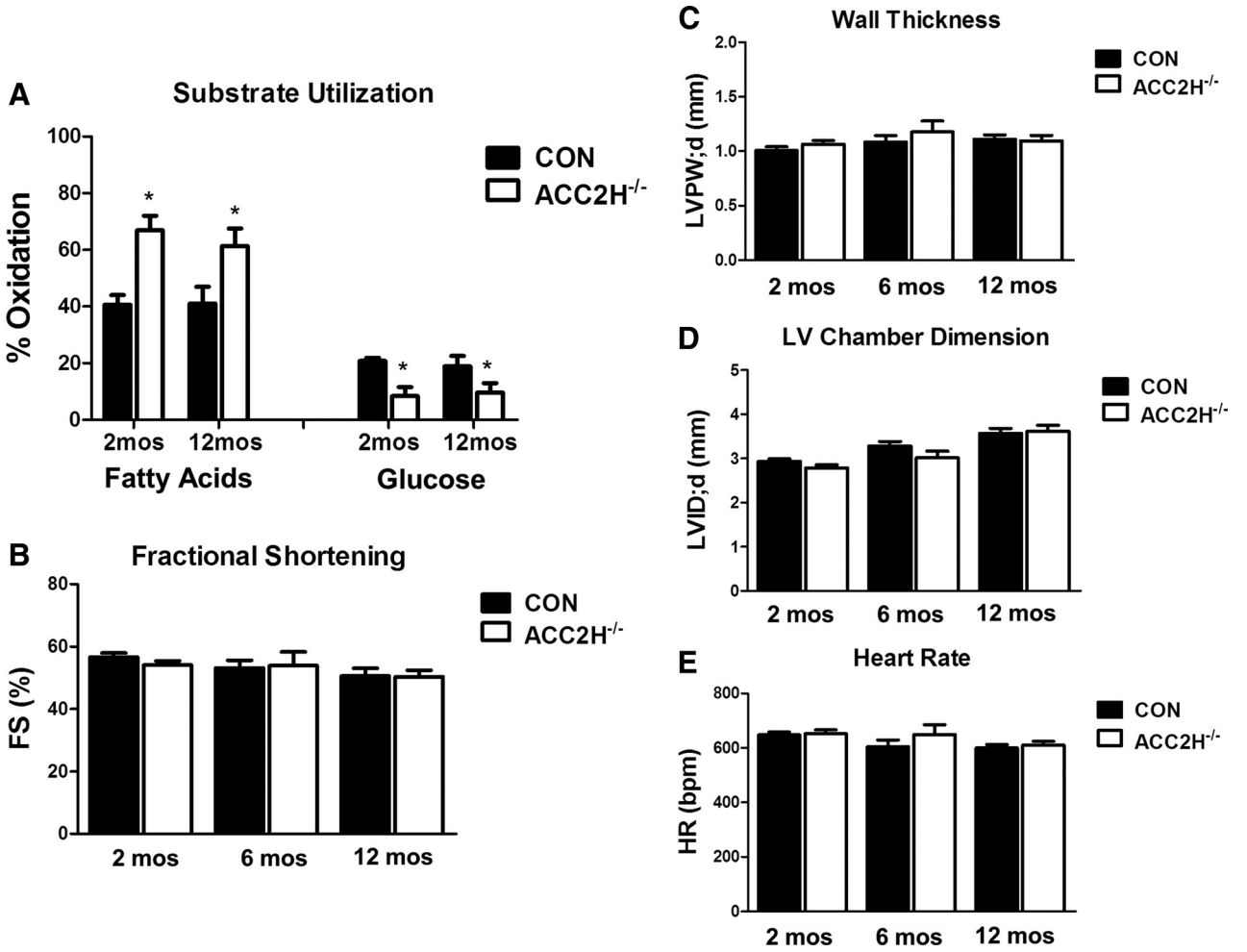

Figure 3. Cardiac fatty acid metabolism is maintained with normal function and morphology at 12 months of age. A, Relative contribution of glucose and fatty acids to TCA cycle in hearts perfused with ^{13}C-labeled substrates. *$P<0.05$ versus CON, n=4 to 5. **B**, **C**, **D**, and **E**, Fractional shortening; left ventricular posterior wall thickness in diastole (LVPW;d); left ventricular internal diameter in diastole (LVID;d); and heart rate assessed by echocardiography in CON and ACC2H$^{-/-}$ at 2 months (n=28 to 33 each group), 6 months (n=6 to 7 each group), and 12 months (n=8 to 9 each group) of age.

mitochondrial overload of acylcarnitines and decreased TCA cycle intermediates that contribute to the development of insulin resistance in skeletal muscle of mice fed a high-fat diet.[30] Thus, we assessed acylcarnitine species in nonperfused heart extracts from CON and ACC2H$^{-/-}$. No significant differences were noted in the concentrations of short-chain (C3, C4, C5), medium-chain (C8), or long-chain (C14, C16) acylcarnitine species (Figure 2D). In addition, expression of genes involved in glucose metabolism, FA metabolism, and mitochondrial biogenesis were not significantly different in ACC2H$^{-/-}$ as compared with CON (Figure 2E). Overall, these results suggest that cardiac-specific deletion of ACC2 increases cardiac FAO without overwhelming changes of substrate metabolism.

Increased FAO by ACC2H Deletion Is Sustained in Normal Aging With No Alteration of Cardiac Function

Because the 60% increase of FAO in ACC2H$^{-/-}$ renders a substrate oxidation profile similar to a diabetic heart,[12] we determined whether chronic elevation of mitochondrial FAO led to detrimental consequences during normal aging. First, we verified that 12-month-old ACC2H$^{-/-}$ mice maintained elevated FAO and had a similar substrate utilization profile as 2 months of age (Figure 3A). Next we performed serial echocardiography to evaluate cardiac function and morphology in ACC2H$^{-/-}$ and CON up to 12 months of age. Fractional shortening and heart rate were similar in CON and ACC2H$^{-/-}$ at 2, 6, and 12 months of age (Figure 3B and 3E). Likewise, increased FAO via ACC2 deletion did not affect gross cardiac morphology, as both wall thickness and internal chamber dimensions were comparable in CON and ACC2H$^{-/-}$ up to 12 months of age (Figure 3C and 3D). Thus, chronic elevation of cardiac FAO is not harmful to overall cardiac performance.

Myocardial Energetics and Performance Are Improved in ACC2H$^{-/-}$ Hearts During High Work Load Challenge

To determine efficiency of ACC2H$^{-/-}$ hearts, we simultaneously measured myocardial oxygen consumption (MVO_2) and contractile function in isolated Langendorff-perfused

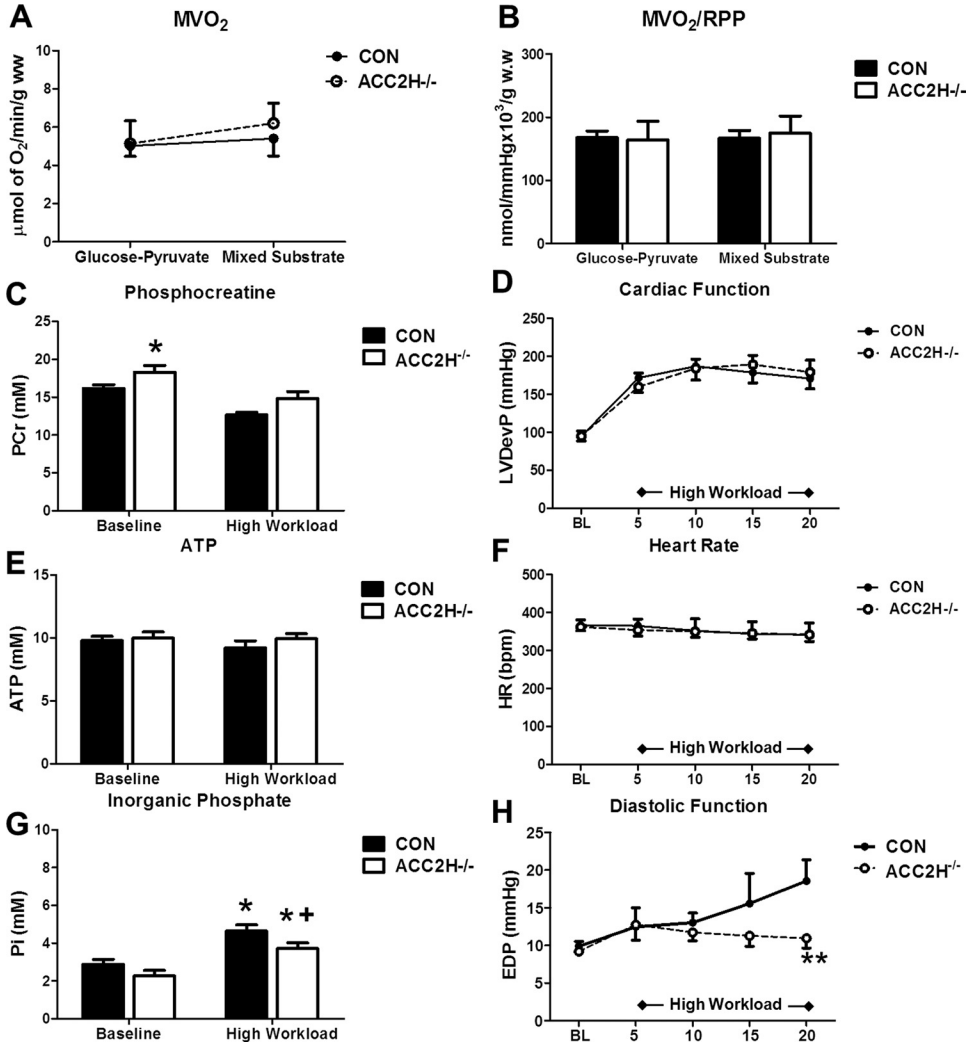

Figure 4. Myocardial energetics and cardiac function of ACC2H$^{-/-}$. A, Myocardial oxygen consumption assessed in isolated perfused hearts. Hearts were perfused with a buffer containing glucose and pyruvate followed by a buffer containing fatty acids, glucose, lactate, and insulin (mixed substrate). Serial measures were made in each heart, n=3 each group. **B**, Oxygen efficiency for contractile function, estimated as amount of oxygen used per unit of contractile performance (MVO_2/RPP), during perfusion with glucose and pyruvate or mixed substrate buffer. Serial measures were made in each heart, n=3 each group. **C**, **E**, and **G**, Phosphocreatine (PCr), ATP, and inorganic phosphate (Pi) by ^{31}P NMR spectroscopy in isolated perfused hearts during baseline and high work load conditions. *P<0.05 versus respective baseline, +P<0.05 versus control at high work load, n=6 to 10 each group. **D, F,** and **H,** LV developed pressure (LVDevP), heart rate (HR), and end-diastolic pressure (EDP) response of isolated perfused hearts subjected to a high work load challenge. **P<0.05 versus CON, n=6 each group.

hearts (Figure 4A and 4B). MVO_2 and contractile performance were similar in CON and ACC2H$^{-/-}$ when hearts were perfused with a buffer consisting of glucose and pyruvate as the substrates (Figure 4A). When the perfusate was switched to a buffer consisting of glucose, FA, and lactate, both MVO_2 and contractile function, assessed by rate-pressure product (RPP), increased slightly in ACC2H$^{-/-}$, although this was not statistically significant. The oxygen efficiency, estimated by MVO_2/RPP, was not different between the groups (Figure 4B).

To determine whether altered substrate utilization observed in ACC2H$^{-/-}$ hearts affected ATP production and myocardial energetics, we measured dynamic changes of high-energy phosphate content in isolated hearts during a high work load challenge with ^{31}P NMR spectroscopy. Under normal work load conditions, the concentration of the energy reserve compound, phosphocreatine ([PCr]), was slightly higher in ACC2H$^{-/-}$ (P<0.05), with insignificant differences of inorganic phosphate ([Pi]) or ATP concentrations between the 2 genotypes (Figure 4C, 4E, and 4G). At the end of the high work load challenge, [PCr] tended to be higher in ACC2H$^{-/-}$ (P=0.08; Figure 4C). While [ATP] was maintained in ACC2H$^{-/-}$, this measure decreased ≈10% in CON (P=0.10; Figure 4E). Although [Pi] was significantly increased in both groups at the end of the high work load challenge, it was lower in ACC2H$^{-/-}$ (P<0.05; Figure 4G). Assessment of cardiac function showed that left ventricular developed pressure (LVDevP) and heart rate (HR) were similar in ACC2H$^{-/-}$ and CON under normal work load (Figure 4D, 4F). Both ACC2H$^{-/-}$ and CON had equivalent increases in LVDevP at similar HR in response to a high work load challenge (Figure 4D and 4F). However, EDP increased significantly in CON suggesting an impairment of relaxation during the high work load challenge, which was not observed in ACC2H$^{-/-}$ (Figure 4H). These observations suggest that elevated FAO provides equivalent if not slightly advantageous energetic support to cardiac function in ACC2H$^{-/-}$ mice.

ACC2H$^{-/-}$ Hearts Are Resistant to Metabolic Remodeling During Pressure-Overload–Induced Hypertrophy

To test the metabolic and functional response of cardiac-specific ACC2 deletion during the development of pressure-overload hypertrophy, CON and ACC2H$^{-/-}$ mice underwent sham or transverse aortic constriction (TAC) surgery. Acute mortality, defined as any death within 48 hours after surgery,

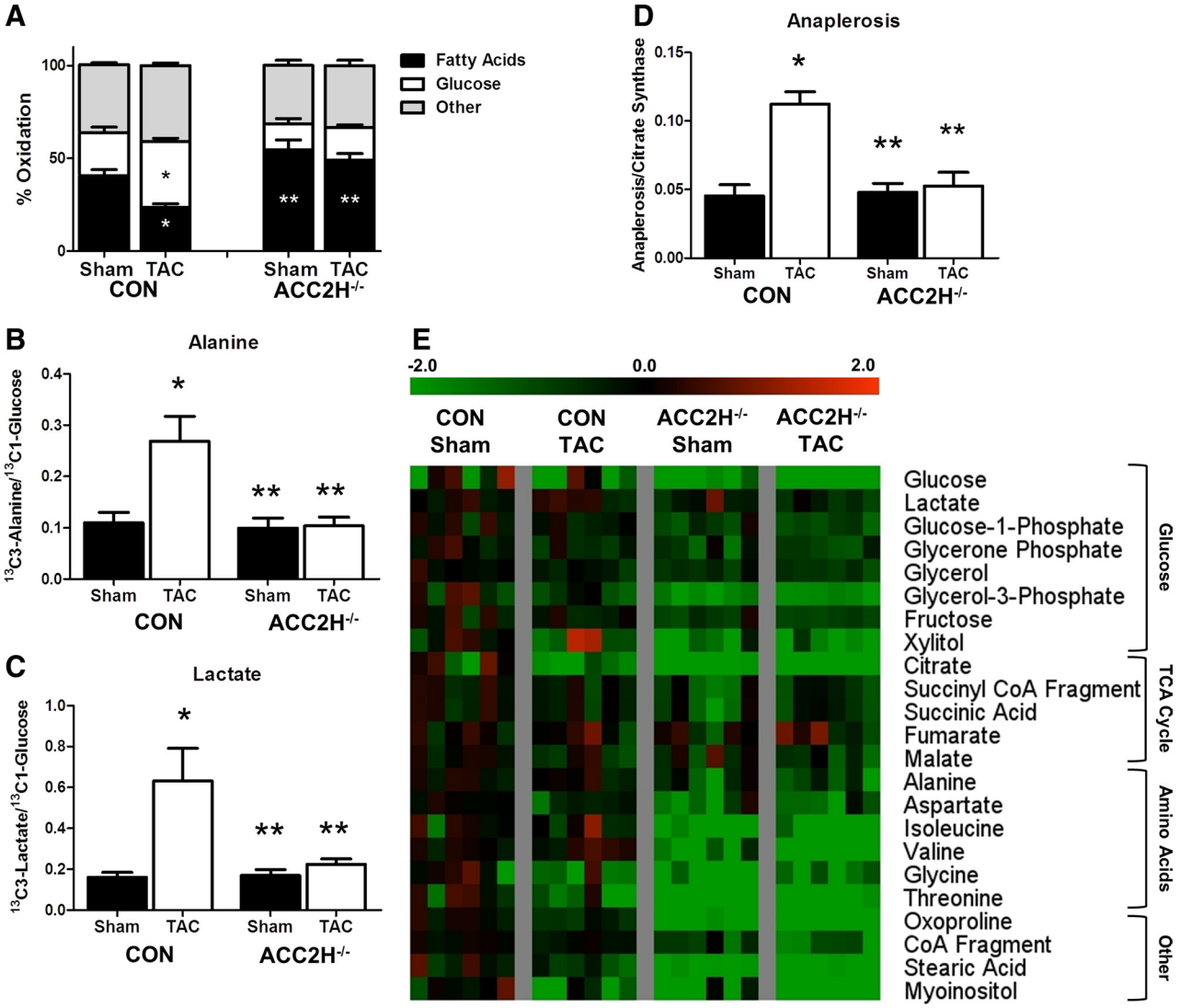

Figure 5. Metabolic remodeling in pressure-overload hypertrophy. A, Relative contribution of glucose, fatty acids, and other substrates (lactate, endogenous) to TCA cycle in TAC hearts perfused with ^{13}C-labeled substrates (n=8 to 10). **B**, Enrichment of ^{13}C3-alanine of hearts perfused with ^{13}C labeled glucose. Data presented as ratio of ^{13}C3-alanine peak area to ^{13}C1-glucose peak area (n=8 to 10). **C**, Enrichment of ^{13}C3-lactate of hearts perfused with ^{13}C labeled glucose. Data presented as ratio of ^{13}C3-lactate peak area to ^{13}C1-glucose peak area (n=8 to 10). **D**, Total anaplerosis determined from isotopomer analysis of C4 and C3 glutamate peaks in hearts perfused with ^{13}C labeled substrates. Data presented as percent anaplerosis divided by TCA cycle flux (n=8 to 10). **E**, Heat map represents metabolites from glucose metabolism, TCA cycle intermediates, amino acids, and other significantly different metabolites in CON and ACC2H$^{-/-}$ hearts 4 weeks after TAC or sham surgery. Color coding for each metabolite was assigned using a log2 fold change versus the mean value of CON (n=6 each group). *P<0.05 versus CON-sham; **P<0.05 versus CON-TAC.

was not significantly different in CON-TAC or ACC2H$^{-/-}$-TAC (26.3% versus 26.2%). No deaths occurred as a result of sham surgery. To confirm that the pressure overload achieved during surgery was comparable in both groups, pressure gradient measures were obtained using pulsed wave (PW) Doppler 24 hours after surgery in a cohort of hearts. No significant differences were noted between CON-TAC and ACC2H$^{-/-}$-TAC (Online Figure I).

In pathological cardiac hypertrophy, FAO is reduced with increased reliance on carbohydrate sources for energy production. However, when ACC2H$^{-/-}$ were exposed to 8 weeks of pressure overload by TAC, glucose and FAO remained similar to sham hearts (Figure 5A). Conversely, CON hearts undergoing 8 weeks of TAC had a significant decrease in FAO as compared with CON sham animals, accompanied by an increase in glucose oxidation (Figure 5A). Glycolytic activity was assessed from the ^{13}C enrichment patterns of alanine and lactate, derived from the glycolytic end product pyruvate, in extracts from isolated hearts perfused with ^{13}C-labeled glucose. CON-TAC hearts had significant increases of both ^{13}C-alanine and ^{13}C-lactate, which was abrogated in ACC2H$^{-/-}$-TAC (Figure 5B and 5C). Furthermore, isotopomer analysis in extracts from hearts perfused with ^{13}C labeled fatty acids and glucose revealed an ≈2-fold increase in anaplerosis in CON-TAC, which was also not observed in ACC2H$^{-/-}$-TAC (Figure 5D). All together, these data indicate that preservation of FAO prevents the metabolic shift toward increased reliance on glycolysis and anaplerosis in hypertrophied hearts.

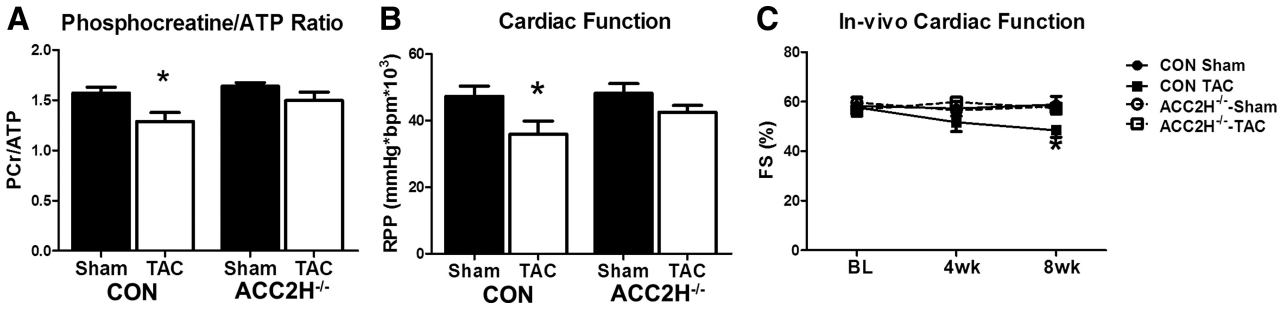

Figure 6. Myocardial energetics and cardiac function after pressure-overload hypertrophy. A, Phosphocreatine to ATP ratio (PCr/ATP) assessed by ^{31}P NMR spectroscopy in isolated perfused hearts (n=8 to 10). **B**, Rate-pressure product (RPP, the product of LVDevP and heart rate) during isolated heart perfusion experiments (n=8 to 10). **C**, Fractional shortening (FS %) assessed by echocardiography before (BL) and at 4 and 8 weeks after TAC (n=8 to 10). *$P<0.05$ versus CON-sham.

We performed global metabolite profiling in CON and ACC2H$^{-/-}$ hearts to explore potential changes in the metabolic network in response to chronic elevation of FAO under normal and pressure overload conditions. Heart extracts were analyzed by GC×GC-TOFMS and chromatographic data were processed by the Fisher ratio (*F*-ratio) algorithm.[34] Two dimensional (2D) Sum of *F*-ratio plots were produced to compare the 4 groups (Online Figure II), and more than 800 hits were sorted in descending order and plotted against a null-distribution (Online Figure III). With this approach, we observed minimal effects of TAC and moderate effects of ACC2 deletion on the global metabolite profile. Identification of the top 15 hits yielded relatively few known metabolites. PARAFAC analysis did not show significant differences in nearly half of the hits (Online Tables III–VI). These findings suggest that there was no global cardiac metabolite shift during pressure-overload hypertrophy and/or with increased FAO as a result of ACC2 deletion.

We also incorporated a targeted approach specifically examining metabolites involved in FA, glucose, and amino acid (AA) metabolism as well as TCA cycle intermediates. We found significant decreases in a number of AAs and several metabolites in glucose metabolism with minor changes in TCA cycle intermediates in ACC2H$^{-/-}$-sham hearts (Figure 5E), suggestive of decreased glucose reliance and increased AA consumption in ACC2H$^{-/-}$ hearts. These results propose that relative amounts of metabolites are maintained during the early development of pressure-overload hypertrophy despite a shift toward increased glucose utilization. Furthermore, adaptations to increased FAO in ACC2H$^{-/-}$ probably include reduced glucose uptake and utilization with increased consumption of AAs, which may contribute to the resistance to metabolic remodeling during pathological hypertrophy.

Sustained Myocardial Energetics and Function and Attenuation of Hypertrophy and Fibrosis in ACC2H$^{-/-}$ With Pressure Overload

A hallmark of pathological cardiac hypertrophy is impaired myocardial energetics as reflected by significant decreases in the [PCr] despite minimal changes in [ATP].[35,36] In ACC2H$^{-/-}$-TAC hearts, PCr/ATP ratios were maintained, whereas a significant decrease in PCr/ATP was observed in CON-TAC relative to sham controls (Figure 6A). Cardiac function, assessed by the RPP in isolated perfused hearts, or assessed in vivo via echocardiography, was sustained in ACC2H$^{-/-}$-TAC hearts, whereas CON-TAC showed a significant decline 8 weeks after surgery (Figure 6B and 6C, and Online Table VII).

To investigate the development of hypertrophy from pressure overload, hearts from CON and ACC2H$^{-/-}$ mice were harvested 4 weeks after TAC. Both CON and ACC2H$^{-/-}$ hearts had a significant increase in brain natriuretic peptide (BNP) mRNA levels compared with sham counterparts (Figure 7A). However, the relative increase of BNP in ACC2H$^{-/-}$-TAC hearts was approximately 50% less than CON-TAC. More importantly, cardiac hypertrophy, assessed by the heart weight–to–tibial length ratio (HW:TL), was ≈50% less in ACC2H$^{-/-}$-TAC compared with CON-TAC (Figure 7B). The attenuation of pathological hypertrophy was further evidenced by attenuation in myocyte cross-sectional area and decreased fibrosis in ACC2H$^{-/-}$-TAC versus CON-TAC (Figure 7C through 7F). This pattern remained after 8 weeks of pressure overload as molecular markers of hypertrophy, BNP, and atrial natriuretic peptide (ANP), as well as a physical marker of hypertrophy, HW:TL, were significantly elevated in CON-TAC hearts with attenuation in ACC2H$^{-/-}$-TAC (Online Figure IV). Consistent with our findings of decreased energetics, expression of genes associated with mitochondrial biogenesis (PGC1α) and mitochondrial ATP synthase activity (ATP5b) were significantly downregulated in CON-TAC, but not in ACC2H$^{-/-}$-TAC (Online Figure IV), which was not associated with changes in mitochondrial volume as estimated by citrate synthase activity (Online Figure IV).

Discussion

The current study addresses several key issues in cardiac metabolism. First, it is generally noted that enhanced FAO is not well tolerated in cardiac tissues, especially in conditions with elevated lipid delivery. However, we demonstrate that the heart is very capable of sustaining chronic increases of FAO when lipid supply is maintained. Our results show that it is unlikely that FAO per se but rather, the balance of lipid supply and oxidation is important. Second, despite strong evidence suggesting metabolic derangements that occur during the development of cardiac hypertrophy contribute to the transition to failure; prevention of metabolic reprogramming

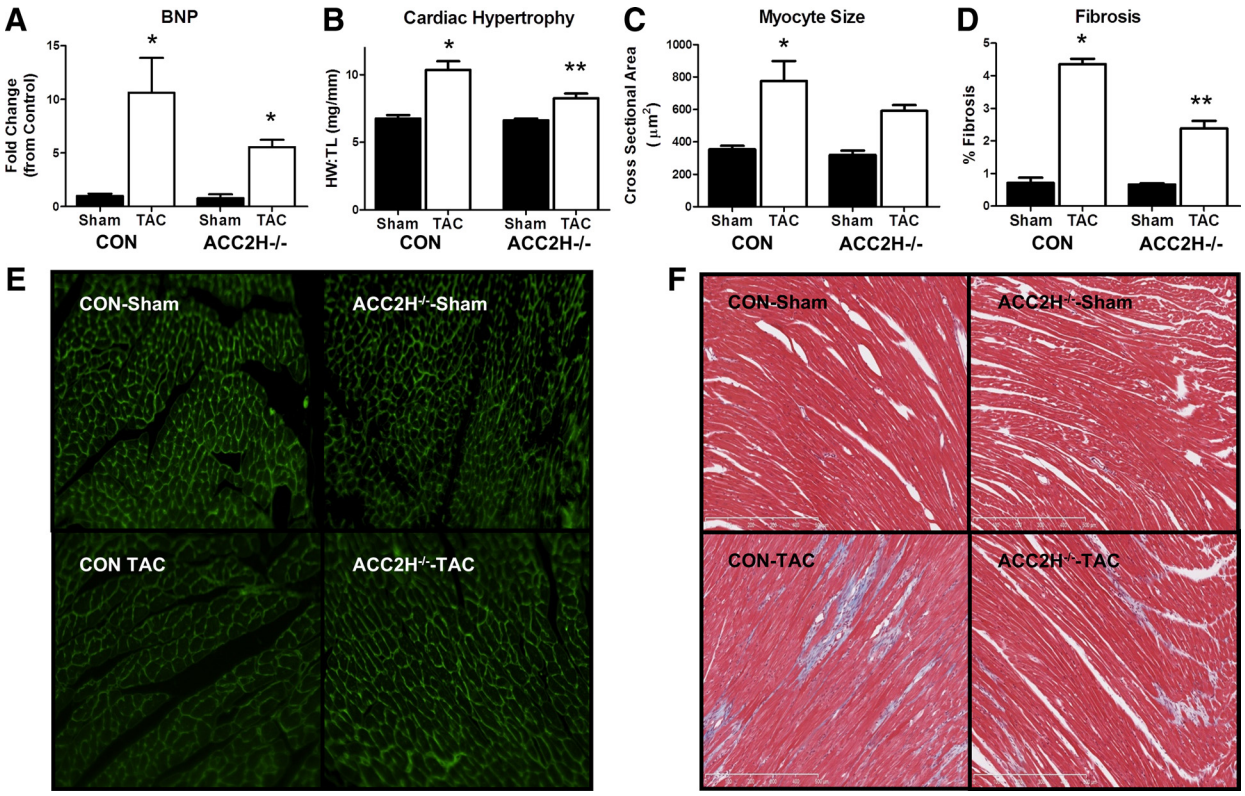

Figure 7. Reduced hypertrophy and fibrosis in ACC2H$^{-/-}$ after 4 weeks of pressure overload. A, Brain natriuretic peptide (BNP) mRNA values normalized to 18s and reported as fold change from CON-sham. *$P<0.05$ versus respective shams, n=5 to 8. **B**, Heart weight normalized to tibial length (HW:TL) of CON and ACC2H$^{-/-}$ undergoing sham or TAC surgery (n=5 to 8). **C**, Myocyte cross-sectional area in histological sections stained with wheat germ agglutinin (WGA) to assess myocyte hypertrophy (n=2 to 3). **D**, Percentage of fibrosis in histological sections stained with Masson trichrome (n=2 to 3). **E**, Representative images of WGA staining. **F**, Representative images of Masson trichrome staining. *$P<0.05$ versus CON-sham, **$P<0.05$ versus CON-TAC.

in pathological hypertrophy has not been accomplished. We show that facilitating long-chain FA entry into the mitochondria via the ACC2/malonyl CoA/CPT1 mechanism will sustain the inherent cardiac metabolic profile during the development of pathological hypertrophy. Last, the question of whether mechanical dysfunction is a cause or consequence of metabolism has been debated. Our results suggest that altered cardiac metabolism leads to impaired function and energetics and that maintenance of cardiac metabolism preserves these measures during the early development of mild pressure-overload hypertrophy.

Conditions of chronic increased lipid delivery, as in obesity and diabetes, have been linked to dysfunction in liver, skeletal muscle, and heart.[12,30,37] In the heart, increased FAO observed during reperfusion after ischemia formulated the basis that elevated FAO was the cause of poor functional recovery.[38] Studies in PPARα transgenic mice[13] or PPARα agonism in hypertrophied hearts[8,9] suggested that enhanced FAO was harmful to the myocardium. However, PPARα has widespread effects on lipid metabolism, including fatty acid uptake, which may lead to an imbalance between FA uptake and oxidation. In skeletal muscle, increasing FAO via high fat feeding was associated with reduced TCA cycle intermediates (TCAI) combined with elevations in acylcarnitine species, suggesting that a portion of FAO is incomplete and contributes to insulin resistance.[30] However, depletion of TCAI during high fat feeding was not seen in the heart,[39] suggesting that chronic elevations in cardiac FAO are not detrimental. Our present data support this as increasing cardiac FAO via reductions in malonyl CoA is well tolerated without adverse effects on cardiac function in mice up to 1 year of age. A similar finding has been reported recently as mice with overexpression of PDK4 had increased FAO with normal cardiac function.[40] Overall, these data suggest that mitochondrial capacity for FAO in heart tissue is more robust than other tissues and high FAO in the face of unchanged FA delivery is not a culprit for mechanical and mitochondrial dysfunction.

Targeted metabolic profiling revealed significant differences in glucose and amino acid metabolism with maintained TCA cycle intermediates between CON and ACC2H$^{-/-}$ hearts that remained during the development of pathological hypertrophy. The overall decreased presence of metabolites related to glucose metabolism in ACC2H$^{-/-}$ hearts probably represents an overall inhibition of glucose metabolism, including glucose uptake, which results from increased FAO as proposed by the Randle or "glucose-fatty acid cyle."[41] As a result of enhanced FAO, elevations in the [acetyl-CoA]/[CoA] and [NADH]/[NAD$^+$] ratios lead to inhibition of pyruvate dehydrogenase (PDH) activity. Concurrently, accumulation of cytosolic citrate inhibits glycolysis via phosphofructokinase (PFK) in addition to glucose uptake.[42–44] One potential negative outcome of increasing FAO is the production of acetyl CoA without the additional production of TCAI

such that occurs with pyruvate.[45] In ACC2H$^{-/-}$ hearts sustained TCAI was associated with increased consumption of glucogenic amino acids which can supply TCAI. The increased amino acid catabolism becomes particularly salient in light of the demonstration that disruption of branched chain amino acids (BCAA) catabolism through loss of protein phosphatase 2Cm (PP2Cm) was associated with mitochondrial dysfunction and heart failure.[46–48] Therefore, increases in the contribution of amino acids to metabolism, especially from BCAAs, may play a significant role in cardiac metabolism during pathological stress and deserves attention in future research plans.

An unexpected observation in ACC2H$^{-/-}$ mice exposed to pressure overload was the attenuation of LV hypertrophy. Unfortunately, the exact mechanism by which increased FAO via ACC2 deletion leads to an attenuation of cardiac hypertrophy is not known at this time. Interestingly, high-fat feeding corresponded with reduced cardiac hypertrophy, although the exact mechanism was also not uncovered.[49] We speculate that the maintained metabolism and energetics in ACC2H$^{-/-}$ presents an adaptive phenotype that resists pathological stress, leading to attenuation of hypertrophic growth. However, it is also possible that increases in amino acid metabolism, including the BCAAs, could divert amino acid availability, thus, retarding the normal upregulation of protein synthesis seen in pathological hypertrophy.[50]

Decreased FAO and increased glycolysis has been repeatedly demonstrated in models of pathological cardiac hypertrophy.[1–3] Increased anaplerosis has been recently identified in hypertrophied hearts as well contributing to the metabolic phenotype.[4,5] Although glucose has the advantage of being more oxygen-efficient compared with FA, it is not as carbon-efficient, as only two-thirds of the carbon in glucose is oxidized compared with the complete oxidation of FA. In this regard, reliance on carbohydrate metabolism probably represents an energy-deficient state that predisposes the hypertrophied myocardium to contractile dysfunction.[6,7] We have previously shown that substantial increases of glucose entry via an insulin-independent mechanism was necessary to rescue impaired FAO and prevent heart failure in mice.[24] However, since achieving such increases in glucose uptake is not physiologically feasible, focusing on maintaining the inherent cardiac metabolic profile (ie, reliance on FA over glucose) during the development of cardiac hypertrophy may be a preferred approach. In the present study, since deletion of ACC2 in pathological hypertrophy maintained cardiac FAO, normalized the expected increases in glycolysis and anaplerosis and led to preserved cardiac function and energetics, metabolic therapies tailored toward this outcome may be more effective in preventing the transition to heart failure.

Previous work demonstrated partial reduction of cardiac malonyl CoA content as a result of global ACC2 deletion.[16,20] We made similar findings suggesting that total removal of cardiac malonyl CoA is not achievable by ACC2 deletion. In our model, several possibilities exist for this: (1) ACC1 is present and probably contributes to a portion of the remaining malonyl CoA pool; (2) αMHC-Cre recombinase does not result in total deletion of protein, so there is residual malonyl CoA formed from ACC2; and (3) since αMHC-Cre is myocyte-specific, malonyl CoA from nonmyocytes is still present. Nevertheless, our results clearly show that cardiac-specific deletion of ACC2 is sufficient to lower malonyl CoA levels and significantly increase mitochondrial FAO.

In summary, modulation of mitochondrial FA entry through cardiac-specific deletion of ACC2 increases FAO without adverse effects of cardiac function in mice up to 1 year of age. Furthermore, ACC2 deletion is sufficient to prevent metabolic reprogramming during pressure-overload hypertrophy with preservation of cardiac function and energetics, representing an overall state of reduced pathological stress leading to an attenuation of hypertrophic growth. These data suggest that maintenance of the inherent metabolic profile of the heart, and, as a result, maintaining mitochondrial oxidative metabolism without switching to glucose reliance is beneficial for optimal function and energetics during the development of pathological cardiac hypertrophy. Although our data clearly demonstrate the benefits of preventing metabolic remodeling in the early course of pathological hypertrophy, additional studies are needed to verify that maintaining cardiac FAO is likewise beneficial in heart failure or other cardiac pathologies.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Fatty acids are the major source of energy production for contraction in the adult heart.
- Hypertrophied and failing hearts switch their source of energy production from fatty acids to glucose, including an increase in glycolysis and anaplerosis, which is probably inefficient in sustaining cardiac contraction.
- Targeting fatty acid oxidation (FAO) with pharmacological therapies has not provided conclusive results in hypertrophy and/or heart failure models.

What New Information Does This Article Contribute?

- Deletion of acetyl CoA carboxylase 2 (ACC2) in the heart decreases malonyl CoA and increases FAO without an adverse effect on cardiac function.
- Deletion of ACC2 is sufficient to maintain FAO and prevent the metabolic remodeling that occurs during the development of pressure-overload hypertrophy.
- Targeting FAO by modulating the influx of fatty acids at the mitochondrial entry point may be a viable therapeutic strategy in the treatment of cardiac pathologies.

Metabolic remodeling in hypertrophied and failing hearts, including reduced FAO and increased reliance on glucose, has been well described in the literature. Furthermore, the shift in substrate utilization is thought to be a major contributor to the reduced myocardial energetics and impaired cardiac function. Despite this, prevention of the metabolic switch that occurs during the development of cardiac hypertrophy and/or heart failure leading to improvements of cardiac function and energetics has not been accomplished experimentally. We demonstrate that cardiac-specific deletion of ACC2 decreases malonyl CoA levels and increases FAO, which is well tolerated in mouse myocardium. Furthermore, deletion of ACC2 maintains cardiac FAO and prevents increases in glycolysis and anaplerosis (ie, prevention of metabolic remodeling) during the development of pressure-overload hypertrophy. Moreover, this metabolic phenotype is associated with preserved cardiac function and myocardial energetics with attenuated hypertrophy. These results suggest that modulation of mitochondrial substrate entry and mitochondrial oxidative capacity during the development of cardiac pathologies is a viable approach for therapeutic intervention.