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CD36 Deficiency Rescues Lipotoxic Cardiomyopathy

John Yang, Nandakumar Sambandam, Xianlin Han, Richard W. Gross, Michael Courtois, Attila Kovacs, Maria Febbraio, Brian N. Finck and Daniel P. Kelly
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CD36 Deficiency Rescues Lipotoxic Cardiomyopathy

John Yang, Nandakumar Sambandam, Xianlin Han, Richard W. Gross, Michael Courtois, Attila Kovacs, Maria Febbraio, Brian N. Finck, Daniel P. Kelly

Abstract—Obesity-related diabetes mellitus leads to increased myocardial uptake of fatty acids (FAs), resulting in a form of cardiac dysfunction referred to as lipotoxic cardiomyopathy. We have shown previously that chronic activation of the FA-activated nuclear receptor, peroxisome proliferator-activated receptor α (PPAR α), is sufficient to drive the metabolic and functional abnormalities of the diabetic heart. Mice with cardiac-restricted overexpression of PPAR α (myosin heavy chain [MHC]-PPAR α) exhibit myocyte lipid accumulation and cardiac dysfunction. We sought to define the role of the long-chain FA transporter CD36 in the pathophysiology of lipotoxic forms of cardiomyopathy. MHC-PPAR α mice were crossed with CD36-deficient mice (MHC-PPAR α /CD36 $^{-/-}$ mice). The absence of CD36 prevented myocyte triacylglyceride accumulation and cardiac dysfunction in the MHC-PPAR α mice under basal conditions and following administration of high-fat diet. Surprisingly, the rescue of the MHC-PPAR α phenotype by CD36 deficiency was associated with increased glucose uptake and oxidation rather than changes in FA utilization. As predicted by the metabolic changes, the activation of PPAR α target genes involved in myocardial FA-oxidation pathways in the hearts of the MHC-PPAR α mice was unchanged in the CD36-deficient background. However, PPAR α -mediated suppression of genes involved in glucose uptake and oxidation was reversed in the MHC-PPAR α /CD36 $^{-/-}$ mice. We conclude that CD36 is necessary for the development of lipotoxic cardiomyopathy in MHC-PPAR α mice and that novel therapeutic strategies aimed at reducing CD36-mediated FA uptake show promise for the prevention or treatment of cardiac dysfunction related to obesity and diabetes. (*Circ Res.* 2007;100:1208-1217.)

Key Words: cardiomyopathy ■ diabetes mellitus ■ fatty acids ■ glucose ■ metabolism

We are witnessing a dramatic increase in the prevalence of obesity, which is driving a pandemic of type 2 diabetes mellitus.^{1,2} Diabetes is a lethal disease, due in large part to accelerated atherosclerosis and a unique form of myocardial dysfunction.³ Diabetic myocardial disease can occur independent of known causative factors for heart failure and confers increased susceptibility to ischemic damage.^{4–6} Indeed, the incidence of heart failure is increased in diabetic compared with nondiabetic patients following myocardial infarction.^{7–10}

Growing evidence suggests that metabolic derangements, related to the insulin resistant and diabetic state, contribute to diabetic cardiac dysfunction.¹¹ Specifically, derangements in cardiac fuel metabolism have been implicated. The normal adult heart uses both glucose and fatty acid (FA) for ATP production, switching between these energy substrate sources depending on the dietary and physiological conditions.^{12,13} The capacity of the insulin-resistant and diabetic heart to use glucose as a fuel is markedly diminished. This loss of substrate “flexibility” results in the diabetic heart relying almost exclusively on FAs as its substrate source.^{11,14} Chronically increased FA utilization is thought to contribute to

ventricular dysfunction by increasing mitochondrial oxygen consumption, generation of by reactive species attributable to increased flux through oxidative pathways, or via the toxic effects of accumulated lipid species.^{15–20} In addition, reduced capacity for glucose oxidation has also been linked to ventricular dysfunction, especially in the ischemic and postischemic heart.^{13,14,21–23}

The metabolic reprogramming of the diabetic heart is driven, in part, by gene-regulatory events. Coordinate activation of genes involved in cellular FA uptake and utilization pathways in the diabetic heart has been shown to occur via the transcription factor peroxisome proliferator-activated receptor α (PPAR α).²⁴ PPAR α is a FA-activated nuclear receptor that regulates cellular FA transport, esterification, and oxidation.²⁵ Transgenic mice with cardiac-restricted overexpression of PPAR α (myosin heavy chain [MHC]-PPAR α mice) exhibit a cardiac metabolic phenotype that is strikingly similar to that of the diabetic heart: increased FA utilization and decreased uptake and oxidation of glucose.²⁴ MHC-PPAR α mice also exhibit features of lipotoxic cardiomyopathy, including ventricular hypertrophy and dysfunction associated with myocardial lipid accumulation.²⁴ This

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From the Center for Cardiovascular Research (J.Y., N.S., R.W.G., M.C., A.K., D.P.K.) and Departments of Medicine (J.Y., N.S., X.H., R.W.G., M.C., A.K., B.N.F., D.P.K.), Molecular Biology & Pharmacology (R.W.G., D.P.K.), and Pediatrics (D.P.K.), Washington University School of Medicine, St Louis, Mo; and Department of Cell Biology (M.F.), Lerner Research Institute, Cleveland Clinic, Ohio.

Correspondence to Daniel P. Kelly, MD, Center for Cardiovascular Research, Washington University School of Medicine, 660 S Euclid Ave, Campus Box 8086, St Louis, MO 63110. E-mail dkelly@im.wustl.edu

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phenotype resembles the cardiomyopathy associated with obesity and diabetes. Several animal models of obesity and diabetes, such as the Zucker diabetic fatty (ZDF) rat, also exhibit cardiac myocyte lipid accumulation.²⁶ Cardiomyopathy in MHC-PPAR α mice is exacerbated by a high fat (HF) diet enriched in long-chain FA (LCFA) or acute insulin deficiency, states that result in increased delivery of nonesterified FA to the heart.²⁷

We sought to define the role of cellular LCFA import in the cardiomyopathic phenotype of MHC-PPAR α mice. To this end, MHC-PPAR α mice were crossed with mice deficient for CD36, a membrane-associated transporter that has been shown to facilitate uptake of LCFA in cardiac myocytes and other cell types.^{28,29} We found that CD36 deficiency completely rescues the lipotoxic cardiomyopathy of MHC-PPAR α mice. Surprisingly, however, the improvement of the lipotoxic cardiomyopathy correlated with a marked increase in myocardial glucose uptake and oxidation rather than significant changes in FA utilization.

Materials and Methods

Animal Experiments and Generation

A high-expressing MHC-PPAR α transgenic mouse line (404-3)²⁴ in C57BL/6 X CBA/J was back-crossed with C57BL/6 wild-type (Wt) mice from The Jackson Laboratory (Bar Harbor, Me) for 6 generations, resulting in >95% C57BL/6 purity. MHC-PPAR α mice were then crossed twice into the CD36-null (CD36^{-/-})²⁸ background, resulting in MHC-PPAR α /CD36^{-/-} offspring.

One-month-old mice were fed either a HF diet composed of 43% of calories from fat (TD 01381, Harlan Teklad, Madison, Wis) containing triacylglyceride (TAG) composed of LCFA (16:0 and 18:1) or the standard mouse chow (Rodent Chow 5053, Purina Mills Inc) for 4 weeks.

All animal experiments were conducted in strict accordance with NIH guidelines for humane treatment of animals and were reviewed by the Animal Studies Committee of Washington University School of Medicine.

Histologic Analyses

Immediately after harvest, a midventricular slice of myocardium was snap-frozen in a cryomold containing OCT for sectioning. To detect neutral lipid, frozen sections were stained with oil red O and counterstained with hematoxylin. Sectioning and staining was performed by the Morphology Core of the Digestive Diseases Research Core Center at the Washington University School of Medicine.

Analyses of Myocardial TAG

The quantitative analysis of myocardial TAG species by electrospray ionization mass spectrometry (ESI/MS) has been described.^{30,31}

Echocardiographic Studies

Transthoracic M-mode and 2D echocardiographs were performed on conscious mice by using the Acuson Sequoia 256 Echocardiography System (Acuson, Mountain View, Calif), as described.³² Methods for measurements and chamber size using M-mode have been described.³²

Northern and Western Blot Analyses

Total RNA was isolated by the RNazol B method (Tel-Test, Friendswood Tex). Northern blot analyses were performed as described³³ using radiolabeled cDNA probes. The cDNA for FA transport protein 1 (FATP1) was kindly provided by Jean Schaffer (Washington University School of Medicine, St Louis, Mo). Quantitation was performed on a Storm phosphorimager (GE Healthcare, Piscataway, NJ) by scanning the band of interest and normalizing it

to the level of 36B4. Western blot analyses were performed with cardiac whole-cell extracts as previously described³⁴ by using antibodies against β -actin (Sigma, St Louis, Mo) and FATP1 (Santa Cruz Biotechnology, Santa Cruz, Calif).

Quantitative Real-Time RT-PCR Analyses

First-strand cDNA was generated by reverse transcription using total RNA from cardiac ventricles. Real-time RT-PCR was performed using TaqMan and SYBR Green reagents (Applied Biosystems, Foster City, Calif) on a 7500 Fast-Real Time PCR System (Applied Biosystems). The following primer and FAM/TAMRA probe sets were used: brain-type natriuretic peptide (forward, gctgcttggggcacaagatag; reverse, gcagccaggaggtcttctca; probe, cagtgcgttacagcccaaacga); skeletal α -actin (forward, tatgtgctatccagcggcgtg; reverse, cccagaatccaacacgatgc); sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase 2a (SERCA2a) (forward, ggagatgcacctggaagat; reverse, ccacacagccgacgaaa; probe, tcatcaaatagagaccaactgact); uncoupling protein 3 (UCP3) (forward, tgctgagatggtgacctaga; reverse, ccaagcgagagacaaagtga; probe, aagtgtcagtaaacaggtgagactccagc); UCP2 (forward, tcatcaagatactctctgaaagc; reverse, tgacggtggtcagaagc; probe, tgacagacgacctccctgacct); muscle-type carnitine palmitoyltransferase 1 (forward, tctaggaatgccgttac; reverse, gagcacatgggaccatac; probe, tcaagccggtcatggcactgg); acyl-CoA oxidase (forward, ggatggtatgctcggagaaca; reverse, agtctggatcgtcagaatcaag; probe, tctcgattctcgacggcggc); GLUT4 (forward, atcatccggaacctggagg; reverse, gtca-gacacatagcccagc; probe, ctgcccgaagagtctaaagcggc); pyruvate dehydrogenase kinase 4 (PDK4) (forward, ccgctgcatgaagca; reverse, gcagaaaagcaaaggacgtt; probe, tgctggacttggttcagaaaaatg); PPAR α (forward, actacggagttcagcatgtg; reverse, ttgctgacaccagctcagc; probe, aggctgaagggtctcttctggcg).

Serum Chemistry

Serum TAG, free FAs, and total cholesterol levels were determined using colorimetric assays by the Core Laboratory of the Clinical Nutrition Research Unit Core Center at Washington University School of Medicine.

Mouse-Isolated Working Heart Preparation

Mouse working heart perfusions were performed as previously described.³⁵ Working hearts were perfused with Krebs-Henseleit solution containing 5 mmol/L glucose, 10 μ U/mL insulin, and 1.2 mmol/L palmitate. Myocardial FA and glucose oxidation rates were determined by quantitative collection of ³H₂O or ¹⁴C₂ produced by the hearts perfused with buffer containing [9,10-³H]palmitate or [U-¹⁴C]glucose.³⁵

Statistical Analyses

Statistical comparisons were made using one-way and 2-way ANOVA with subsequent post hoc Tukey's pair-wise analyses. All data are presented as means \pm SEM, with statistically significant differences as probability value of <0.05.

Results

Generation of CD36-Deficient MHC-PPAR α Mice

MHC-PPAR α mice (high-expressing line)²⁴ were back-crossed into a pure C57BL/6 background for 6 generations, yielding Wt and MHC-PPAR α mice that were >95% pure C57BL/6. Next, the MHC-PPAR α mice were crossed with CD36^{-/-} (C57BL/6) mice to generate 4 experimental groups that were strain matched: Wt; CD36^{-/-}; MHC-PPAR α ; and MHC-PPAR α mice in a CD36-deficient background (MHC-PPAR α /CD36^{-/-}). Appropriate and similar levels of transgene expression²⁴ and the absence of CD36 in the heart were confirmed in each genotype on the gene expression at RNA (Figure I in the online data supplement, available at <http://circres.ahajournals.org>) and protein levels (data not shown).

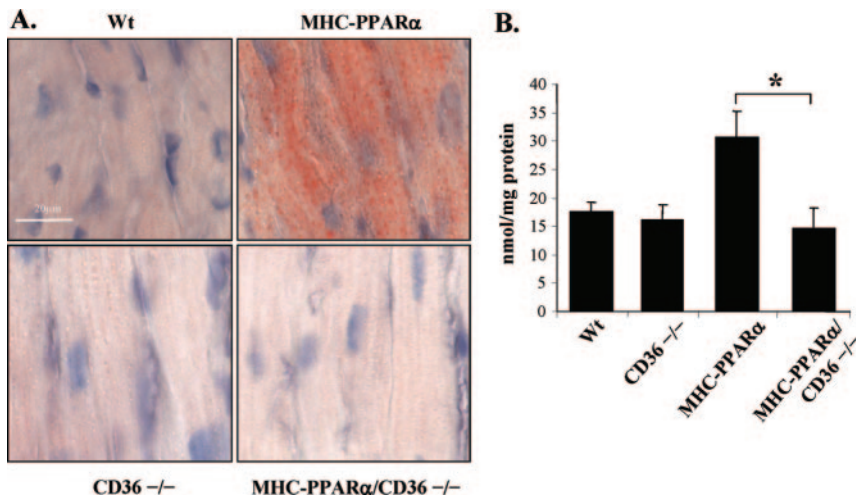


Figure 1. Reversal of myocardial lipid accumulation in MHC-PPAR α /CD36 $^{-/-}$ mice on HF diet. **A**, Representative photomicrograph depicting oil red O-stained ventricular tissue samples prepared from Wt, CD36 $^{-/-}$, MHC-PPAR α , and MHC-PPAR α /CD36 $^{-/-}$ male mice following 4 weeks of HF diet. Red droplets indicate neutral lipid staining (Wt, n=5; CD36 $^{-/-}$, n=5; MHC-PPAR α , n=3; MHC-PPAR α /CD36 $^{-/-}$, n=5). **B**, Mean (\pm SEM) myocardial TAG levels determined by ESI/MS for each genotype after four weeks of HF diet (Wt, n=3; CD36 $^{-/-}$, n=4; MHC-PPAR α , n=5; MHC-PPAR α /CD36 $^{-/-}$, n=3). * P <0.05.

Animals of each genotype group were born in the expected Mendelian ratios, and there were no significant differences in blood pressure, heart rate, or body weight between the genotypes (data not shown).

CD36 Deficiency Prevents Myocardial Lipid Accumulation in MHC-PPAR α Mice

To determine the role of the LCFA transporter CD36 in the myocardial lipid accumulation that occurs in MHC-PPAR α mice, the effects of a 4-week course of HF diet were analyzed. As expected, oil red O staining demonstrated a large accumulation of neutral lipid in the MHC-PPAR α mice hearts compared with Wt controls (Figure 1A). In stark contrast, neutral lipid staining of the hearts of both CD36 $^{-/-}$ and MHC-PPAR α /CD36 $^{-/-}$ mice was not different than that of the Wt mice (Figure 1A). Serum-free FA and TAG levels were not significantly different among the 4 genotypes, but serum cholesterol levels were slightly but significantly higher in the two CD36-deficient groups (Table 1).

Myocardial TAG content was characterized and quantified by ESI/MS in the 4 experimental groups. These data were consistent with the oil red O-staining patterns demonstrating significantly higher TAG levels in the MHC-PPAR α hearts compared with that of MHC-PPAR α /CD36 $^{-/-}$ (Figure 1B). ESI/MS profiling of myocardial TAG species revealed that CD36 deficiency reversed the increased levels of esterified palmitic, stearic, oleic, and linoleic acids in the hearts of MHC-PPAR α mice hearts (Figure 2A and 2B). These lipid

species are enriched in the HF chow and their plasma levels are known to be highly influenced by diet.³⁶ In contrast, there were no differences in the levels of other lipid species such as myristic, palmitoleic, and arachidonic acids, which are present in low levels in the HF chow (data not shown). These results, which are consistent with the oil red O-staining data, demonstrate that CD36 is necessary for the diet-induced TAG accumulation in MHC-PPAR hearts.

In addition to CD36, 2 other proteins, FATP1 and fatty acyl-CoA synthetase 1 (ACS1), have been shown to serve as LCFA transporters in the heart.³⁷ Previously, we found that the expression of the FATP1 gene is higher in MHC-PPAR α mice compared with Wt controls.²⁴ Cardiac expression of the FATP1 gene remained modestly elevated in the MHC-PPAR α /CD36 $^{-/-}$ mice (Figure 3). ACS levels were not different among the genotypes (data not shown). Thus, the absence of CD36 in cardiac myocytes can prevent LCFA accumulation even in the context of increased FATP1 expression.

CD36 Deficiency Rescues Cardiac Dysfunction but not Cardiac Hypertrophy in MHC-PPAR α Mice

Diabetic cardiomyopathy is characterized by ventricular hypertrophy, a phenotype that is also observed in the MHC-PPAR α mice (Figure 4A).³⁸ Interestingly, the biventricular to body weight ratio of 2-month old male CD36 $^{-/-}$ mice was mildly but significantly increased compared with that of the Wt mice on standard or HF chow for four weeks. The biventricular to body weight of the MHC-PPAR α /CD36 $^{-/-}$ mice was not significantly different than that of the MHC-PPAR α mice (Figure 4A). Consistent with the observed hypertrophic response, the expression of hypertrophic growth marker genes encoding brain-type natriuretic peptide and skeletal α -actin was increased in the MHC-PPAR α , CD36 $^{-/-}$, and MHC-PPAR α /CD36 $^{-/-}$ mice compared with Wt controls (Figure 4B).

Echocardiography was performed on 2-month-old male Wt, MHC-PPAR α , CD36 $^{-/-}$, and MHC-PPAR α /CD36 $^{-/-}$ mice receiving standard or HF chow to assess the impact of the CD36-deficient state on cardiac function. As expected, MHC-PPAR α mice exhibited left ventricular (LV) systolic

TABLE 1. Serum Lipid Levels

Mouse	Free FAs (mg/dL)	Triacylglycerol (mg/dL)	Total Cholesterol (mg/dL)
Wt	0.68 \pm 0.06	87 \pm 6.5	87 \pm 3.2
CD36 $^{-/-}$	0.62 \pm 0.05	103 \pm 9.4	105 \pm 4.4*
MHC-PPAR α	0.54 \pm 0.05	84 \pm 8.1	81 \pm 3.5
MHC-PPAR α /CD36 $^{-/-}$	0.73 \pm 0.08	105 \pm 12	108 \pm 6.7*

Age-matched Wt, CD36 $^{-/-}$, MHC-PPAR α , and MHC-PPAR α /CD36 $^{-/-}$ mice of both sexes were fasted for 3 hours, and blood was taken from the inferior vena cava for analysis. Values are mean \pm SEM. * P <0.05 vs Wt (Wt, n=12; CD36 $^{-/-}$, n=8; MHC-PPAR α , n=10; MHC-PPAR α /CD36 $^{-/-}$, n=9).

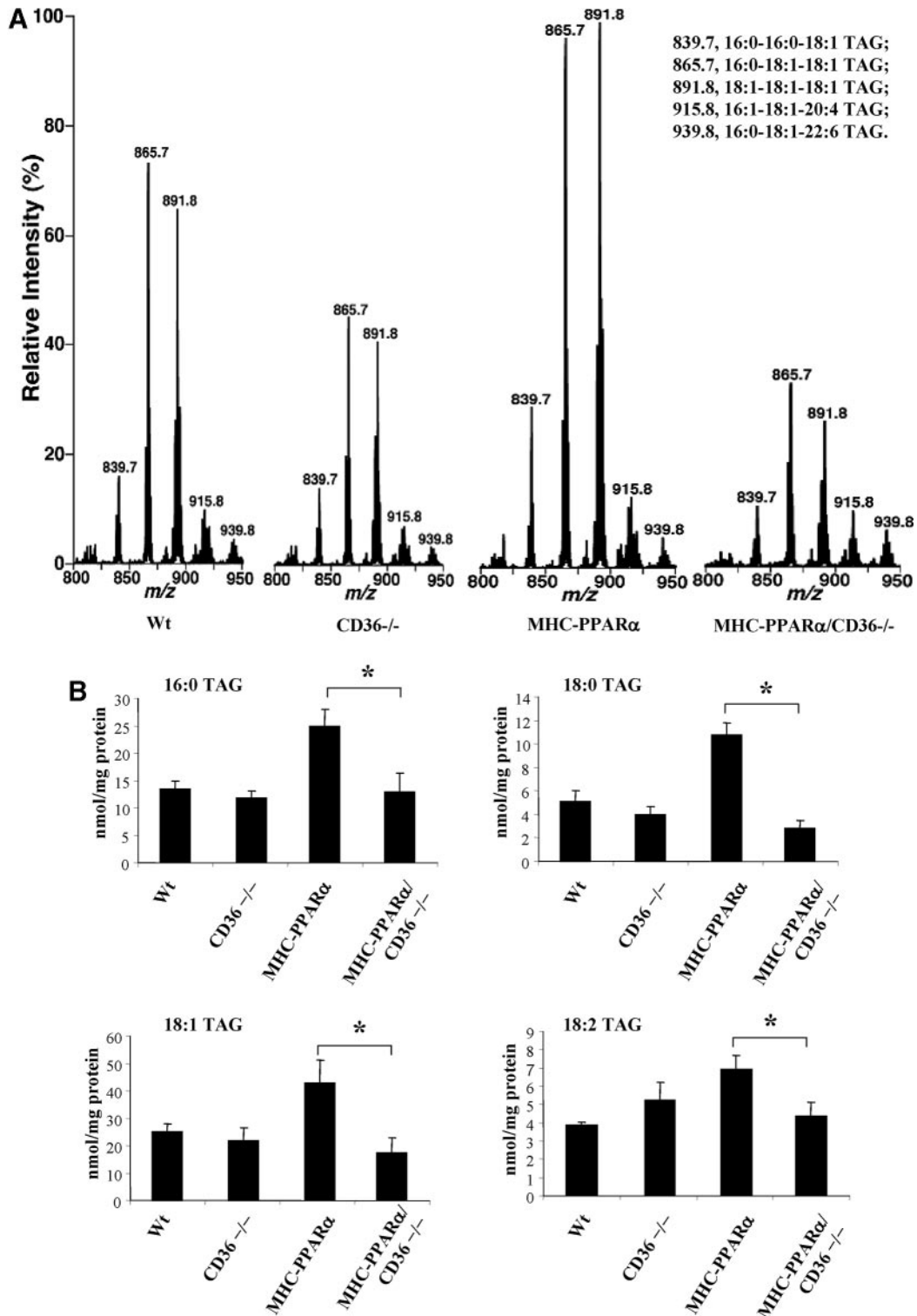


Figure 2. Decreased levels of myocardial LCFA species in MHC-PPAR α /CD36^{-/-} mice on HF diet. A, Representative neutral loss mass spectra based on ESI/MS analyses of oleic acid-containing TAG molecular species in lipid extracts prepared from the mouse lines indicated. Each spectrum is displayed normalized to the corresponding internal standard. The most abundant TAG molecular species as identified by “shotgun” lipidomics using multidimensional mass spectrometric analyses^{30,32} are indicated. B, Bars represent mean(\pm SEM) (Wt, n=3; CD36^{-/-}, n=4; MHC-PPAR α , n=5; MHC-PPAR α /CD36^{-/-}, n=3) levels of TAG-associated FAs with chain length of 16:0, 18:0, 18:1, and 18:2 in the hearts of male mice of each genotype after 4 weeks of HF diet, as determined by ESI/MS. **P*<0.05.

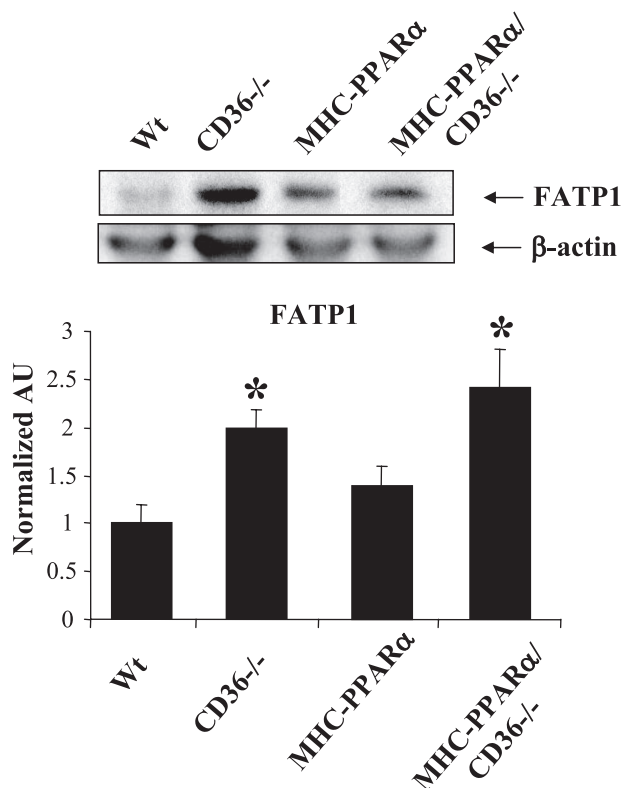


Figure 3. Increased expression of FATP1 in CD36-deficient myocardium. The bars represent mean (\pm SEM) mRNA levels of FATP1 from male mice quantified by phosphorimager analysis of Northern blot studies ($n=6$ per group). Values shown are corrected to 36B4 signal intensity and normalized (1.0) to values of Wt mice. * $P<0.05$ vs Wt mice. AU, arbitrary units. Inset at top shows a representative autoradiograph of protein vs analyses for FATP1. β -Actin signal is shown as the loading control.

dysfunction (reduced LV fractional shortening and increased chamber size), which was worsened following administration of HF diet (Figure 5A and 5B). In striking contrast, LV function of the MHC-PPAR α /CD36^{-/-} mice was not different from that of the Wt control mice on standard or HF chow (Figure 5A and 5B; Table 2). Consistent with this finding, the expression of the gene encoding SERCA2a, which is known to be reduced in the failing heart, was downregulated in the MHC-PPAR α hearts, but not in the MHC-PPAR α /CD36^{-/-} (Figure 4B). Taken together with the results of the hypertrophic growth data, these results indicate that the ventricular dysfunction but not the hypertrophic growth phenotype in MHC-PPAR α mice requires CD36.

CD36 Deficiency Rescues the Myocardial Glucose Metabolic Derangements in MHC-PPAR α Mice

MHC-PPAR α mice exhibit myocardial fuel utilization abnormalities that are similar to the diabetic heart: increased FA oxidation and reduced glucose utilization. To assess the metabolic correlates of the functional rescue conferred by CD36 deficiency, myocardial substrate oxidation rates were determined in the working mode in hearts isolated from 2-month-old mice of all 4 genotypes. Surprisingly, despite functional rescue, fatty acid oxidation rates were not significantly reduced in the MHC-PPAR α /CD36^{-/-} hearts com-

pared with MHC-PPAR α hearts (Figure 6A). In contrast, the CD36-deficient background reversed the reduction in glucose oxidation rates in MHC-PPAR α hearts. Specifically, mean glucose oxidation rates in the MHC-PPAR α /CD36^{-/-} hearts were markedly increased (approximately 5-fold) to levels of the CD36^{-/-} hearts, both being greater than the Wt hearts (Figure 6A).

The expression of genes involved in myocardial fuel utilization parallel the metabolic derangements in MHC-PPAR α and diabetic mice; PPAR α target genes involved in FA oxidation are activated, whereas myocyte glucose uptake and oxidation programs are downregulated.^{24,39,40} Therefore, we examined the expression of a subset of genes known to be involved in myocardial fuel metabolism in the MHC-PPAR α /CD36^{-/-} mice. Consistent with the results of the metabolic flux studies, the expression of PPAR α target genes involved in mitochondrial (muscle-type carnitine palmitoyl-transferase 1) and peroxisomal (acyl-CoA oxidase) fat oxidation pathways remained increased in both MHC-PPAR α and MHC-PPAR α /CD36^{-/-} hearts compared with Wt and CD36^{-/-} lines (Figure 6B). Expression of the mitochondrial UCP2 and UCP3, known PPAR α target genes, were also activated to a similar extent in the hearts of MHC-PPAR α and MHC-PPAR α /CD36^{-/-} mice (Figure 6B). In contrast, the expression of the glucose transporter GLUT4 and the negative regulator of glucose oxidation PDK4 was normalized in the MHC-PPAR α /CD36^{-/-} hearts (Figure 6C). Taken together, these results indicate that CD36 deficiency results in a reversal of the gene regulatory and metabolic derangements in glucose utilization but not FA oxidation in the CD36-deficient background. These findings also suggest that only a subset of the gene targets downstream of PPAR α are deactivated in the context of CD36 deficiency.

Discussion

The PPAR α gene regulatory pathway has been identified as a driver of the metabolic derangements contributing to insulin-resistant and insulin-deficient forms of diabetic cardiomyopathy. MHC-PPAR α mice exhibit metabolic and functional signatures of the diabetic heart, including increased myocardial uptake and oxidation of FAs, myocyte TAG accumulation, reduced glucose utilization, and cardiomyopathy. This study was performed to determine whether a genetic manipulation aimed at reducing myocardial LCFA import would affect the lipotoxic cardiomyopathic phenotype of MHC-PPAR α mice. The results were striking; the absence of CD36 completely rescued the myocardial neutral lipid imbalance and cardiac dysfunction of MHC-PPAR α mice.

One of the signatures of lipotoxic forms of cardiomyopathy is myocyte lipid accumulation. CD36 deficiency prevented myocardial TAG accumulation in MHC-PPAR α mice, even in the context of HF diet. This result was somewhat surprising given the existence of other cardiac FA transporters including FATP1 and ACS1. Our findings provide additional evidence for the importance of CD36 as a bona fide cardiac FA transporter in the heart. However, these results do not exclude the potential importance of other cardiac FA transporters. Previous studies have shown that transgenic overexpression of either ACS1 and/or FATP1 leads to increased cardiac

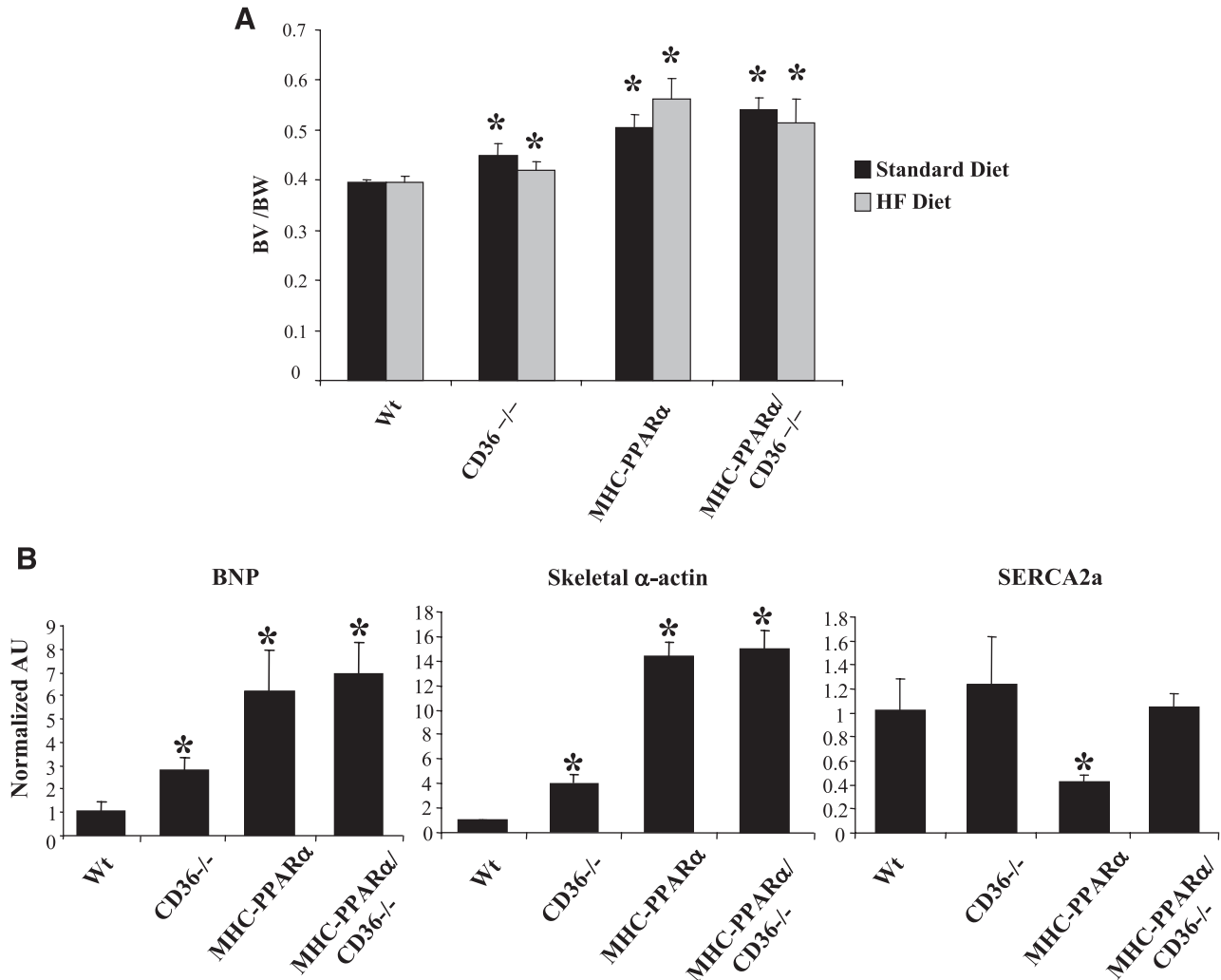


Figure 4. CD36 deficiency does not rescue the cardiac hypertrophic phenotype of MHC-PPAR α mice. **A**, Bars represent mean(\pm SEM) biventricular to body weight (BV/BW) ratio for 2-month-old male Wt, CD36^{-/-}, MHC-PPAR α , and MHC-PPAR α /CD36^{-/-} mice fed standard or HF diet for 4 weeks (Wt on standard diet, n=18; CD36^{-/-} on standard diet, n=15; MHC-PPAR α on standard diet, n=10; MHC-PPAR α /CD36^{-/-} on standard diet, n=10; Wt on HF diet, n=15; CD36^{-/-} on HF diet, n=15; MHC-PPAR α on HF diet, n=13; MHC-PPAR α /CD36^{-/-} on HF diet, n=10). **P*<0.05 vs Wt on matched corresponding diet. **B**, Hypertrophy gene markers are activated in the hearts of the CD36^{-/-}, MHC-PPAR α , and MHC-PPAR α /CD36^{-/-} mice. Graphs represent the mean(\pm SEM) (Wt, n=4; CD36^{-/-}, n=4; MHC-PPAR α , n=8; MHC-PPAR α /CD36^{-/-}, n=7) mRNA levels of brain-type natriuretic peptide (BNP), skeletal α -actin, and SERCA2a from the hearts of each genotype, as determined by real-time RT-PCR. Values shown are corrected to 36B4 and normalized (1.0) to Wt values. **P*<0.05 vs Wt. AU, arbitrary units.

myocyte lipid import.^{16,41} It is possible that each transporter mediates FA import in response to a specific physiologic or nutritional milieu. Alternatively, CD36 could function in series rather than in parallel with the other proteins implicated in the transport process including FATP1 and ACS1. In this latter model, CD36 would serve as an indispensable component of the FA cellular transport system. Future loss-of-function studies aimed at each of the other cellular transport proteins alone and in combination with CD36 deficiency will be necessary to further define the physiological role and relevant interactions among the FA transporters.

Lipotoxic cardiomyopathy has been described in animal models of obesity and diabetes.^{15,16} In addition, humans with inborn errors in mitochondrial FA oxidation also develop cardiomyopathy associated with myocyte lipid accumulation.⁴² Proposed mechanisms for the development of cardiac

dysfunction secondary to myocyte lipid overload include: (1) lipopoptosis via ceramide-dependent pathways, (2) generation of reactive oxygen species via increased flux through mitochondrial and peroxisomal pathways, and (3) increased rates of oxygen consumption related to excessive mitochondrial FA oxidation. The results shown here provide additional evidence for a tight association between excessive lipid uptake and cardiac dysfunction. The rescue of the cardiomyopathy phenotype was associated with reversal of myocyte TAG accumulation. Surprisingly, however, the functional rescue did not significantly change FA oxidation rates; rather, it correlated with increased rates of glucose oxidation. This latter finding was also reflected at the gene expression level. Previous studies have demonstrated a link between reduced cardiac glucose oxidation and diabetic cardiac dysfunction, an association that is particularly relevant following ischemic

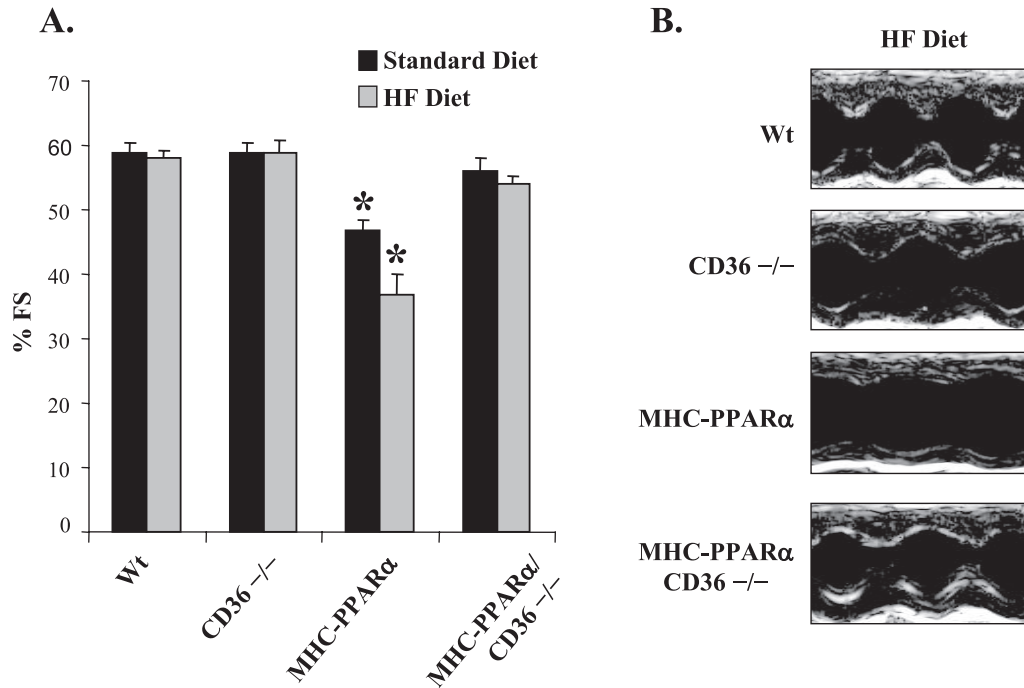


Figure 5. CD36 deficiency rescues the ventricular dysfunction of MHC-PPAR α mice. A, Bars represent mean percent LV fractional shortening (FS), as determined by echocardiographic analyses for 4 male and 4 female mice 2 months of age on either standard or HF diet for 4 weeks. * $P < 0.05$ vs Wt on matched diet ($n = 8$ per group). B, Representative M-mode echocardiographic images of the LV performed on each genotype after 4 weeks of HF diet.

insult.^{11,21–23,43} Metabolic modulation strategies aimed at increasing myocardial glucose oxidation have been shown to have improved functional recovery following ischemia/reperfusion.^{43–47} Thus, a possible explanation for the rescue of the LV dysfunction in the MHC-PPAR α mice is that CD36 deficiency reverses the suppression of myocardial glucose uptake and oxidation. Future studies aimed at the mechanism underlying this link are necessary to substantiate this conclusion. The observed reduction in neutral lipid stores could also be relevant. Although it would seem unlikely that the stored TAG is directly toxic to the myocyte, toxic byproducts of nonmitochondrial LCFA metabolism could be reduced by CD36 deficiency. In addition, increased import of LCFA can drive hydrogen peroxide production through the peroxisomal

pathway, resulting in oxidative stress to the cardiac myocytes.²⁷ Although the absence of CD36 rescued the cardiac dysfunction, it did not alter the cardiac hypertrophy of the MHC-PPAR α mice; in fact, CD36 deficiency itself triggers a modest hypertrophic response. Interestingly, human studies have demonstrated a link between CD36 deficiency and cardiac hypertrophy.⁴⁸ The connection between altered myocardial fuel flexibility and cardiac hypertrophic growth is well described; yet the mechanism remains elusive.

We were surprised to find that activation of PPAR α target genes involved in FA oxidation were unchanged in the MHC-PPAR α mice in a CD36-null background. Although the exact ligand for PPAR α is not known, it is likely a LCFA derivative. Accordingly, despite markedly reduced rates of

TABLE 2. Transthoracic Echocardiography Measurements

HF Diet	Wt	CD36 ^{-/-}	MHC-PPAR α	MHC-PPAR α /CD36 ^{-/-}
HR, bpm	656 \pm 13	671 \pm 19	601 \pm 14	653 \pm 17
LVPWd, mm	0.68 \pm 0.02	0.62 \pm 0.03	0.66 \pm 0.02	0.69 \pm 0.03
IVSd, mm	0.65 \pm 0.02	0.66 \pm 0.01	0.69 \pm 0.02	0.68 \pm 0.03
LVPWs, mm	1.42 \pm 0.04	1.43 \pm 0.06	1.24 \pm 0.04*	1.41 \pm 0.06
IVSs, mm	1.50 \pm 0.06	1.48 \pm 0.04	1.20 \pm 0.04*	1.60 \pm 0.03
LVIDs, mm	1.40 \pm 0.05	1.43 \pm 0.08	2.56 \pm 0.17*	1.70 \pm 0.08
LVIDd, mm	3.47 \pm 0.04	3.54 \pm 0.09	4.0 \pm 0.11*	3.60 \pm 0.09
LVM, mg	68 \pm 3.3	70 \pm 3.2	91 \pm 3.8*	81 \pm 7.0

Values represent mean \pm SEM. * $P < 0.05$ vs Wt; $n = 8$ (4 males and 4 females) per group. HR, indicates heart rate; LVPWd, left ventricular posterior wall thickness at diastole; IVSd, interventricular septal wall thickness at diastole; LVPWs, left ventricular posterior wall thickness at systole; IVSs, interventricular septal wall thickness at systole; LVIDs, left ventricular internal diameter at systole; LVIDd, left ventricular internal diameter at diastole; LVM, left ventricular mass.

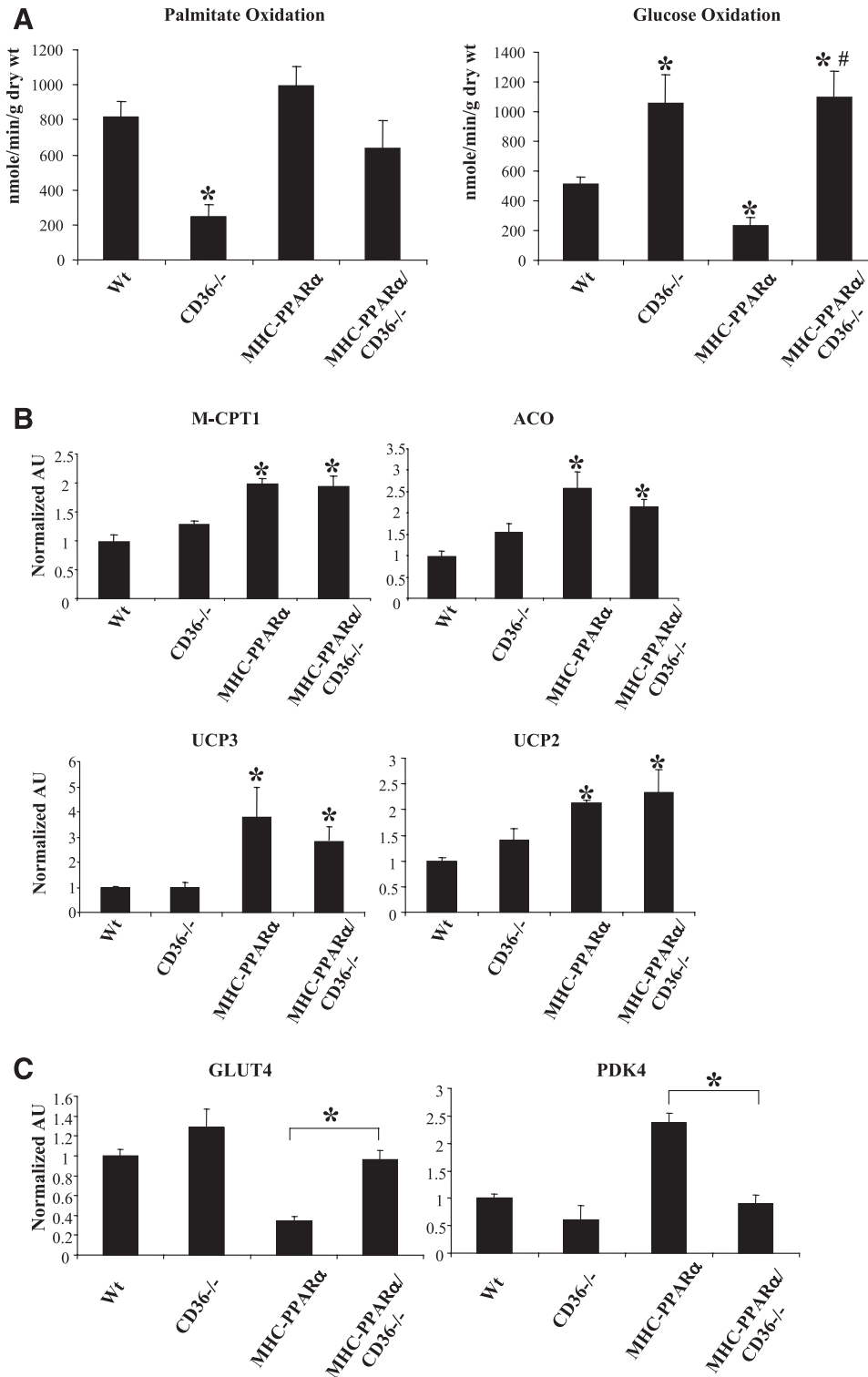


Figure 6. CD36 deficiency reverses derangements in myocardial glucose oxidation but not FA oxidation in MHC-PPAR α mice. A, The oxidation rates of [9,10-³H]palmitate and [U-¹⁴C]glucose were assessed in isolated working hearts from male and female mice (Wt, n=8; CD36^{-/-}, n=7; MHC-PPAR α , n=8; MHC-PPAR α /CD36^{-/-}, n=7). Bars represent mean(\pm SEM) oxidation rates expressed as nanomoles of substrate oxidized per minute per gram of dry weight (wt). **P*<0.05 vs Wt mice, #*P*<0.05 vs MHC-PPAR α mice. B, Cardiac FA metabolic PPAR α gene target expression remains increased in the MHC-PPAR α /CD36^{-/-} mice. Bars represent mean mRNA levels as determined by real-time RT-PCR using RNA from heart ventricles of 2-month-old Wt, CD36^{-/-}, MHC-PPAR α , and MHC-PPAR α /CD36^{-/-} male mice (Wt, n=12; CD36^{-/-}, n=15; MHC-PPAR α , n=17; MHC-PPAR α /CD36^{-/-}, n=15). Values shown are corrected by 36B4 and normalized (1.0) to values of Wt mice. **P*<0.05 vs Wt mice. AU indicates arbitrary units; Wt, wild-type. C, Bars represent mean GLUT4 and PDK4 mRNA levels as determined using RNA isolated from heart ventricles of Wt, CD36^{-/-}, MHC-PPAR α , and MHC-PPAR α /CD36^{-/-} male mice (Wt, n=8; CD36^{-/-}, n=6; MHC-PPAR α , n=7; MHC-PPAR α /CD36^{-/-}, n=8). Values shown are corrected by 36B4 and normalized (1.0) to values of Wt mice. **P*<0.05.

myocyte FA import, PPAR α ligand is still available in the CD36-deficient mice. However, the reversal of the PPAR α -mediated suppression of GLUT4 gene expression and activation of PDK4, a key negative regulator of glucose oxidation, in the MHC-PPAR α /CD36^{-/-} mice indicates that a subset of PPAR α target genes was deactivated in the absence of CD36. This interesting result suggests the existence of target gene-specific thresholds for PPAR α -mediated transcriptional control. It should be noted that the precise events involved in

PPAR α -mediated regulation of GLUT4 and PDK4 gene expression have not been delineated but the absence of known PPAR α response elements in the promoter region of either gene suggests indirect mechanisms.

The observation that palmitate oxidation rates in the hearts of MHC-PPAR α /CD36^{-/-} mice are not different than that of MHC-PPAR α mice raises the question of how FA oxidation is maintained, when presumably less LCFA is delivered in the absence of CD36. It is believed that the translocation of

LCFA across the plasma membrane is achieved by multiple mechanisms including diffusion (eg, flip-flop mechanism) and by protein-mediated transport (eg, FATP).^{49–53} Furthermore, FA metabolism influences the rate of FA uptake.^{53,54} Thus, the MHC-PPAR α /CD36^{-/-} mice could maintain high levels of FA oxidation by upregulating genes involved in the β -oxidation cycle in conjunction with CD36-independent LCFA uptake. The maintenance of palmitate oxidation in the hearts of MHC-PPAR α /CD36^{-/-} mice also raises the question of how the electron transport chain and ATP synthesis machinery copes with increased delivery of reducing equivalents. The most likely explanation is that respiratory uncoupling is increased in the hearts of the MHC-PPAR α /CD36^{-/-} mice. Consistent with this possibility, both UCP2 and UCP3 gene expression is increased in these hearts.

In conclusion, our results demonstrate that CD36 deficiency rescues a lipotoxic form of cardiomyopathy, as modeled by MHC-PPAR α mice. The functional improvement of cardiac function conferred by CD36 deficiency was mirrored by reversal of myocardial lipid accumulation and a shift to reliance on glucose as a fuel source. If our results prove relevant in humans, CD36, as a glycoprotein cell surface receptor, shows promise as a new therapeutic target for prevention and treatment of cardiac dysfunction related to metabolic diseases such as obesity and diabetes. However, given that CD36 deficiency triggered mild cardiac hypertrophy and increased serum cholesterol level, future studies aimed at understanding the mechanisms linking CD36, cardiac fuel metabolism, and cardiac hypertrophic growth are warranted.

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Disclosures

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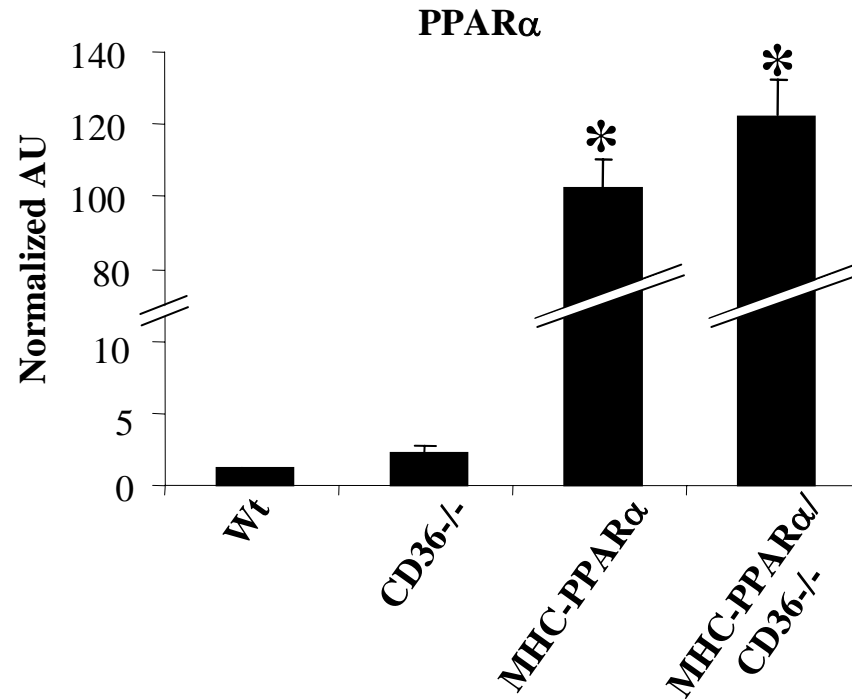


Figure S1. Characterization of PPAR α overexpression. The bars represent mean (\pm SEM) mRNA levels of PPAR α as determined by real-time RT-PCR using RNA isolated from heart ventricles of Wt, CD36^{-/-}, MHC-PPAR α , and MHC-PPAR α /CD36^{-/-} male mice (n=5 per group). Values shown are corrected by 36B4 and normalized (=1.0) to Wt mice. *, $P < 0.05$.