CD36 Protein Influences Myocardial Ca\(^{2+}\) Homeostasis and Phospholipid Metabolism

**CONDUCTION ANOMALIES IN CD36-DEFICIENT MICE DURING FASTING**

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**Background:** Myocardial function during fasting was examined in wild-type and CD36\(^{-/-}\) mice.

**Results:** CD36\(^{-/-}\) mice have abnormalities of myocardial Ca\(^{2+}\), phospholipid composition, and cAMP levels and manifest electrical anomalies during fasting.

**Conclusion:** CD36 influences myocardial adaptation by impacting Ca\(^{2+}\) dynamics and phospholipid metabolism.

**Significance:** Inter-dependence of lipid metabolism and Ca\(^{2+}\) homeostasis can contribute to cardiac dysfunction during metabolic stress.

Sarcolemmal CD36 facilitates myocardial fatty acid (FA) uptake, which is markedly reduced in CD36-deficient rodents and humans. CD36 also mediates signal transduction events involving a number of cellular pathways. In tissue cells and macrophages, CD36 signaling was recently shown to regulate store-responsive Ca\(^{2+}\) flux and activation of Ca\(^{2+}\)-dependent phospholipases A\(_2\) that cycle polyunsaturated FA into phospholipids. It is unknown whether CD36 deficiency influences myocardial Ca\(^{2+}\) handling and phospholipid metabolism, which could compromise the heart, typically during stresses. Myocardial function was examined in fed or fasted (18–22 h) CD36\(^{-/-}\) and WT mice. Echocardiography and telemetry identified conduction anomalies that were associated with the incidence of sudden death in fasted CD36\(^{-/-}\) mice. No anomalies or death occurred in WT mice during fasting. Optical imaging of perfused hearts from fasted CD36\(^{-/-}\) mice documented prolongation of Ca\(^{2+}\) transients. Consistent with this, knockdown of CD36 in cardiomyocytes delayed clearance of cytosolic Ca\(^{2+}\). Hearts of CD36\(^{-/-}\) mice (fed or fasted) had 3-fold higher SERCA2a and 40% lower phospholamban levels. Phospholamban phosphorylation by protein kinase A (PKA) was enhanced after fasting reflecting increased PKA activity and cAMP levels in CD36\(^{-/-}\) hearts. Abnormal Ca\(^{2+}\) homeostasis in the CD36\(^{-/-}\) myocardium associated with increased lysophospholipid content and a higher proportion of 22:6 FA in phospholipids suggests altered phospholipase A\(_2\) activity and changes in membrane dynamics. The data support the role of CD36 in coordinating Ca\(^{2+}\) homeostasis and lipid metabolism and the importance of this role during myocardial adaptation to fasting. Potential relevance of the findings to CD36-deficient humans would need to be determined.

The membrane fatty acid (FA)\(^{2}\) translocase (FAT/CD36) is abundant in the heart (1) and plays an important role in facilitating myocardial uptake of circulating unesterified FA in mice (2–4) as well as humans (5, 6). The importance of CD36 in lipid metabolism and the associated cardiovascular risk are supported by recent genetic studies. Haplotypes that tag common variations in the CD36 gene associate with serum FA and with increased risk of diabetes-linked cardiovascular disease (7). Association with left ventricular mass (8), a predictor of cardiovascular events (9), has also been reported. Common single nucleotide polymorphisms in the CD36 gene influence blood lipoprotein levels (10) and risk of the metabolic syndrome (11–13). The metabolic syndrome, a cluster of factors that include dyslipidemia, increases risk of heart disease and death from a heart attack (14).

Studies of the role of CD36 in myocardial physiology and pathophysiology have focused on its potential role in myocardial “lipotoxicity.” CD36\(^{-/-}\) mice are protected from myocardial dysfunction consequent to excessive FA supply or impaired mitochondrial capacity, suggesting that targeting CD36 may be beneficial under these conditions (15–19). Conversely, CD36 deficiency eliminates the fuel flexibility that is characteristic of the healthy myocardium (20), and whether this impacts myo-

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\† This article contains supplemental Tables 1 and 2.

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Cardiac capability to adapt to certain stresses remains unknown. The heart of the CD36-deficient rodent (21) and human (22) manifests, together with the markedly impaired FA extraction, a compensatory increase in glucose uptake. This metabolic shift is similar to the one observed in the failing human heart. Indeed, down-regulation of CD36 is observed to occur in heart failure in proportion to disease severity and the increase in left ventricular mass (23). CD36 level and FA oxidation also positively correlate with ejection fraction in the infarcted rat heart (24). Whether causative effects underlie these associations remains unclear.

In addition to its function in FA uptake, CD36 also transduces intracellular signals that impact a number of pathways and that are triggered by FA, thrombospondin 1, oxidized lipids, pathogen-derived molecules, and other ligands (25–27). Of particular interest to the myocardium is the emerging evidence connecting CD36 signaling to the regulation of cellular Ca\textsuperscript{2+} dynamics and to phospholipid metabolism. In CHO cells expressing CD36 and in macrophages, CD36-dependent signaling was required for operation of membrane Ca\textsuperscript{2+} channels responsive to depletion of endoplasmic reticulum (ER) Ca\textsuperscript{2+}. The store-operated Ca\textsuperscript{2+} flux resulted in activation of phospholipases \(A_2\) that mobilize polyunsaturated FA from membrane phospholipids to generate eicosanoids (28). The phospholipases \(A_2\) contribute to the maintenance of membrane structure and function and to the generation of bioactive lipids such as lysophospholipid and eicosanoids with pleiotropic effects on cellular functions and overall metabolism (29–31). CD36-dependent Ca\textsuperscript{2+} signaling is involved in fat perception in mouse taste bud cells (26) and in uptake of oxidized low density lipoproteins in macrophages (32). Consistent with CD36 influence on Ca\textsuperscript{2+} signaling, its membrane recruitment responds to factors that release stored Ca\textsuperscript{2+} such as thapsigargin (28) or caffeine (33, 34). CD36-mediated Ca\textsuperscript{2+} signaling in the heart and its relevance to myocardial metabolism and physiology are currently unknown. Alterations in cardiomyocyte Ca\textsuperscript{2+} flux and plasma membrane (sarcolemma) composition could compromise heart contractile function typically during cellular stresses (35, 36). We determined how CD36 deletion impacts the functional adaptation of the myocardium to fasting and whether it is associated with disturbances in the metabolism of Ca\textsuperscript{2+} and/or phospholipid. Our findings document the robust effects of CD36 deficiency on Ca\textsuperscript{2+} handling proteins in the myocardium and the susceptibility of CD36\textsuperscript{−/−} mice during fasting to conduction anomalies that include prolongation of Ca\textsuperscript{2+} transients and that increase the incidence of sudden death.

**EXPERIMENTAL PROCEDURES**

**Mice and Isolated Working Heart Preparation**—C57BL/6 WT and CD36\textsuperscript{−/−} male mice (37) were fed ad libitum or switched to cages with aspen bedding for fasting. Mice from the two genotypes were age-matched and used when 5–7 months old. Mouse working heart perfusions were performed (Mouse Cardiovascular Phenotyping Core) at 5 and 35 mm Hg. Glucose and palmitate oxidation rates were determined using [9,10\textsuperscript{3}H]palmitate and [U\textsuperscript{14}C]glucose (16). All protocols were approved by the Animal Studies Committee of Washington University.

**Surface Electrocardiographic (ECG) Recordings**—Echocardiograms were performed on resting, conscious mice using a 15 MHz transducer (Sonos 550 System, Agilent) (38). Surface ECG recordings were on anesthetized (avertin, 0.25 mg/g, intraperitoneally) mice. Needle electrodes were inserted through the skin following a three-lead scheme (left foreleg, right foreleg, and left rear leg), and ECG signals were amplified with a four-channel amplifier (1700, A-M Systems). Data were collected using a Digidata 1200 analog/digital converter with Axoscope 8 software (Axon Instrument) and analyzed using Clampfit (version 8.0, Molecular Devices) (39, 40).

**Ambulatory Electrocardiography**—Radiofrequency transmitters (PhysioTel TA10EA-F20 or TA10ETA-F20, Data Sciences International, Transoma Medical) were implanted in the posterior shoulder pockets of mice under intraperitoneal ketamine (87 mg/kg)/xylazine (13 mg/kg) anesthesia. Telemetry was performed 7 days later on unanesthetized, unrestrained mice placed on separate receivers (RPC-1, Data Sciences International). Single lead ECG signals were recorded (1 kHz) using Dataquest A.R.T. Gold 2.0 Acquisition Software (Transoma) for 180 s every hour for 6 h (base line) and for 120 s every 15 min during fasting (22 h) and re-feeding (5 h) intervals. Traces were manually and computer analyzed to document arrhythmias.

Heart rates (HR) were obtained using the Data Sciences International software; PR intervals were defined from beginning of the P wave to the beginning of the QRS complex; QT intervals were defined from the beginning of the QRS to the end of the T wave, and QTc was calculated as QT/(RR/100)\textsuperscript{1/2}.

**Optical Mapping**—Following an 18-h fast, optical mapping of transmembrane potential (\(V_m\)) and calcium transients (CaT) of isolated hearts was done as described previously (41). Briefly, isolated hearts (\(n=10\) per genotype) were perfused retrogradely with Tyrode solution and stained with voltage-sensitive dye RH-237 (5 \(\mu\)L in 1 mg/mL DMSO; Invitrogen) for recording optical action potentials while immobilized with blebbistatin (10–20 \(\mu M\); Tocris Bioscience, Ellisville, MO) to reduce motion artifacts. Dual imaging (\(V_m\) and CaT) was performed on hearts (\(n=5\)) of both genotypes by additional staining with the Ca\textsuperscript{2+} indicator Rhod-2AM (Invitrogen). The following pacing protocols were used: 1) S1-S2 from the right atrium; 2) S1-S1 from the right atrium; and 3) S1-S1 from the ventricles. An S1-S1 protocol consists of continuous pacing at a constant time interval (S1) between stimuli. An S1-S2 protocol includes an additional premature stimulus and a time interval of S2. Intervals were decreased until the pacing stimulus failed to capture the tissue. Optical mapping utilized MiCAM Ultima-L CMOS cameras (SciMedia, Costa Mesa, CA), with high spatial (100 \(\times\) 100 pixels, 110 \(\pm\) 20 \(\mu\)m per pixel) and temporal (1,000–2,000 frames/s) resolution.

Data were analyzed using custom MATLAB software (41), filtered using a \(3 \times 3\) pixel spatial filter and a 0–100 Hz finite impulse response filter, and normalized. Activation times were defined as the maximum first derivative of the fluorescent signal upstroke for both \(V_m\) and CaT. Action potential duration at 80% repolarization (APD80) and CaT duration at 80% relaxation (CaD80) were calculated.

**Cell Culture and Transfection**—HL-1 cardiomyocytes, a kind gift from Dr. Claycomb (Louisiana State University Health Sci-
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FIGURE 1. Cardiac hypertrophy and impaired metabolic response of working CD36⁻/⁻ hearts to a hemodynamic challenge. A, body weight of WT and CD36⁻/⁻ mice. B, wet heart weight. C, dry biventricular weight (n = 32). D, mRNA for atrial natriuretic factor (ANF) determined by quantitative real time PCR (n = 5). E, fractional shortening evaluated by transthoracic ECG in conscious fed or fasted mice (n = 8–9). F, working heart preparation showing oxidation of glucose and palmitate in WT and CD36⁻/⁻ hearts at low (5 mm Hg) and high (35 mm Hg) workloads (LW and HW, n = 5). Data are means ± S.E. *, p < 0.05.

Intracellular Ca²⁺ Imaging—Confluent HL-1 cultures with well formed gap junctions were used for the studies. Intracellular calcium measurements were conducted as described previously (28). Briefly, cells on 35-mm glass-bottom dishes were split onto 35-mm glass-bottom dishes and were left to settle for 2 h before using the PepMute™ Plus siRNA Transfection Reagent (SignaGen). The siRNAs against mouse CD36 (Invitrogen) were 5'-AAACCCAGATGACGTGGCAAA-3' (CD36-1) and 5'-AACGACATGATTAGCAGCACA-CACCTGTCTC-3' (CD36-2). Transfection of siRNA (20 nM final) was performed together with siRNA against GFP (28) as a negative control. Transfection was repeated after 24 h, and assays were performed 24 h later. No effect of transfection was observed (GFP transfected and untransfected cells).

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... was conducted for nonbiased evaluation of lipidomic data. All data are presented as means ± S.E. Differences were considered significant at \( p < 0.05 \).

**RESULTS**

**CD36 Deletion Compromises Myocardial Function in Overnight Fasted Mice**—We had previously shown that CD36\(^{-/-}\) hearts do not transition to more FA use during fasting (20). This reflected the fact that CD36 facilitates myocardial uptake of FA from the two major sources available during fasting, the nonesterified FA pool and FA released by lipoprotein lipase from hydrolysis of very low density lipoprotein (VLDL) triglycerides (51). We determined whether CD36 deletion impacts contractility of the myocardium and its functional response to fasting. In the basal fed state, the CD36\(^{-/-}\) mice weigh 15% less than wild-type controls (Fig. 1A) and exhibit mild cardiac hypertrophy (Fig. 1, B and C) with increased myocardial Anf mRNA (Fig. 1D), a marker of ventricular hemodynamic compromise (52). Echocardiography of conscious fed mice showed reduced fractional shortening (measure of systolic ventricular function) in CD36\(^{-/-}\) hearts (Fig. 1, A). At a basal workload (5 mm Hg), CD36\(^{-/-}\) hearts utilized significantly less palmitate and more glucose than WT hearts. Increasing workload to 35 mm Hg induced a 2-fold increase in palmitate oxidation in WT but not in CD36\(^{-/-}\) hearts (Fig. 1F). Thus CD36\(^{-/-}\) hearts have impaired metabolic transition in response to hemodynamic stress consistent with their lack of fuel flexibility.

Surface ECG of fed, anesthetized WT and CD36\(^{-/-}\) mice were similar (data not shown). However, marked electrical anomalies were observed in CD36\(^{-/-}\), but not in WT, mice after fasting (18–22 h) (Fig. 2, A and B). These included altered QRS amplitudes (Fig. 2A), characteristic of abnormal Ca\(^{2+}\)-induced Ca\(^{2+}\) release (53), high degree atrioventricular (AV) block, and premature ventricular complexes (PVCs) (Fig. 2B). Overall, severe AV block and/or PVCs were observed in 7 of 15 CD36\(^{-/-}\) mice, 2 of which died during recordings. Fasting significantly decreased HR (Fig. 2C) in both WT and CD36\(^{-/-}\) mice, although the effect was less pronounced in the latter group. QT intervals (measured from the start of the Q wave to the end of T wave) corrected for HR (QTc) were increased with fasting in both WT and CD36\(^{-/-}\) mice (Fig. 2D). In contrast, PR intervals (measured from the start of the P wave to the start of the QRS wave) (Fig. 2E) were increased only in fasted CD36\(^{-/-}\) mice, consistent with the increased incidence of AV block in these animals.

**Time Course for Conduction Anomalies during Fasting and Their Rapid Reversal by Feeding**—To determine that conduction abnormalities in CD36\(^{-/-}\) mice were not induced by the anesthetic and to monitor their temporal progression, electrical changes were examined in conscious animals during a feeding-fasting-refeeding protocol using implanted transmitters (Fig. 3). The changes in QTc, HR, and PR are shown in Fig. 3A. The QTc interval gradually increased during fasting in CD36\(^{-/-}\) mice reaching significantly higher values than in the WT group at the 9th hour. Heart rate markedly decreased in all mice consistent with the decrease in sympathetic tone and increase in parasympathetic tone that are associated with fasting (54). PR intervals were significantly prolonged in CD36\(^{-/-}\) mice within 9–10 h of fasting, peaking at 20 h. Re-feeding rapidly (within 2 h) reversed the electrical changes. Conduction defects (RR variability) (as illustrated in Fig. 3, B and C), long sinus pauses, followed by ventricular escape beats (Fig. 3D), premature ven-
tricular contractions, and 2nd degree AV block (Fig. 3E), were observed only in CD36−/− hearts. One cause of AV dysfunction in small rodents during nutrient shortage is hypothermia (55), but this was ruled out. Body temperature, similar for fed WT and CD36−/− mice, dropped after an overnight fast by 3.5 and 2.6 °C, respectively (data not shown), so hypothermia did not contribute to the increased incidence of AV block and electrical instability in CD36−/− mice.

**Optical Mapping Identifies Abnormal AV-node Conduction and Prolonged Ca\(^{2+}\) Transients in Hearts from Fasted CD36−/− Mice**—To explore the electrical mechanisms underlying the conduction anomalies in hearts from fasted CD36−/− mice, simultaneous optical mapping of transmembrane potential (\(V_{m}\)) and calcium transients (CaT) was performed concurrently. An S1-S2 pacing protocol (see “Experimental Procedures” for detail on pacing protocols) from the right atrium determined the effective refractory period (ERP) of the AV-node. Significantly longer AV-node ERP was measured in CD36−/− as compared with WT hearts (96 ± 7 ms versus 60 ± 10 ms; \(p = 0.02\)) indicative of abnormal AV-node conduction (Fig. 4A). Representative S2 cycle lengths (Fig. 4, B and C) show AV-node transmission to the ventricles (left) and AV-node filtering of signals, preventing ventricular capture (right). S1-S1 pacing on the ventricles determined the functional refractory period (FRP) of the tissue. CD36−/− hearts had significantly longer ventricular FRP (Fig. 4D) as compared with WT hearts (89 ± 11 ms versus 57 ± 6 ms; \(p = 0.01\)) indicative of slow signal propagation. Representative \(V_{m}\) values and CaT signals at pacing cycle lengths that induce electrical and Ca\(^{2+}\) alternans (Fig. 4, E and F) show the shortest S1 cycle length before loss of 1 to 1 capture.

To further characterize ventricular tissue, the durations of action potentials (APD80) and Ca\(^{2+}\) transients (CaD80) (at time from maximum first derivative of signal upstroke to 80% return to base line) (Fig. 5A) were calculated at several S1-S1 pacing cycle lengths (160, 140, 120, and 100 ms). Fig. 5B shows representative APD80 and CaD80 maps of the entire epicardial surface. White dotted boxes in Fig. 5B indicate regions where APD80 and CaD80 were evaluated, and the measurements are shown in Fig. 5C. CD36−/− hearts had significantly increased APD80 at each cycle length as compared with WT hearts (78.5 ± 3.1, 71.8 ± 3.8, 66.1 ± 3.7, 57.8 ± 3.4 versus 66.5 ± 2.6, 59.5 ± 2.3, 55.6 ± 1.7, 48.6 ± 1.1 ms). Ca\(^{2+}\) transient duration (CaD80) was also significantly increased compared with WT (except at pacing cycle length of 100 ms) (82.9 ± 2.5, 78.9 ± 2.8, 73.1 ± 1.3, 65.5 ± 1.2 versus 71.6 ± 1.2, 66.4 ± 1.6, 62.6 ± 2.2, 60.1 ± 2.2). Taken together, the optimal imaging data identified abnormal conduction in CD36−/− hearts and showed that prolongation of Ca\(^{2+}\) transients underlies the longer action potentials measured in these hearts.

**CD36 Knockdown Impairs SR Ca\(^{2+}\) Uptake in Cardiomyocytes—Prolongation of Ca\(^{2+}\) transients in CD36−/− hearts suggested a defect in removal of cytosolic Ca\(^{2+}\). To determine whether CD36 down-regulation influences cytosolic Ca\(^{2+}\) clearance in cardiomyocytes, we used HL-1 cells (AT-1 mouse atrial tumor lineage) that form clusters that contract spontaneously and synchronously (42). HL-1 cardiomyocytes express CD36 (data not shown) so its knockdown (70%) was obtained (Fig. 6A) using two siRNAs. Control or CD36 knockdown HL-1 cells were loaded with Fluo4-AM and treated with caffeine to release SR Ca\(^{2+}\). As shown in Fig. 6, B and C, SR Ca\(^{2+}\) release was similar in control versus CD36 knockdown cells (CD36-1); however, decay (time to remove 50%) of cytosolic Ca\(^{2+}\) was significantly longer in the latter group. This suggested that CD36 depletion reduces SR Ca\(^{2+}\) reuptake delaying Ca\(^{2+}\) clearance. This would diminish ability of cells to regulate and synchronize beating frequency (35, 36).

**CD36 Deficiency Markedly Alters Levels of Myocardial Proteins Involved in SR Ca\(^{2+}\) Handling—Cytosolic Ca\(^{2+}\) reuptake by the SR in preparation for the next excitation is accomplished by the ATPase (SERCA) enzyme, which is tightly regulated via interaction with its inhibitor PLN (35). Phosphorylation of PLN by protein kinase A (PKA) reverses the inhibition and increases SERCA activity and the rate of SR Ca\(^{2+}\) uptake (56). Expression levels of these key Ca\(^{2+}\)-handling proteins were examined in hearts from CD36−/− and WT mice. Fig. 7, A and B, shows that
Microarrays of WT and CD36−/− hearts showed altered gene expression of a number of Ca2+ regulatory proteins in addition to Serca2 and Pln. These included genes involved in calcium transport (Atp2a1, Atp2a3, and Atp2b1), regulators of calcium transport (Sln and Calr), regulators of calcium binding (S100a9, S100a8, S199a16, and Smoc2), and phosphatase regulatory proteins (Ppp1r3g, Ppp1r16a, Ppp1r15a, and Pak1) (Table 1). Together, the protein and gene expression data indicated dysregulation of signaling pathways controlling Ca2+ handling in the CD36−/− myocardium.

CD36 Deficiency Alters Levels of cAMP and PKA Activity—To gain insight into the mechanism underlying the enhanced PLN phosphorylation at the PKA site, we determined levels of cAMP and downstream PKA activity in WT and CD36−/− hearts (Fig. 7, C and D). In fed mice, levels of intracellular cAMP were significantly increased (40%) in the CD36−/− as compared with the WT myocardium (Fig. 7C). Fasting decreased myocardial cAMP (60%) in WT but not in CD36−/− mice where myocardial cAMP levels were about 5-fold higher than those measured in WT tissue (Fig. 7C). Consistent with the higher cAMP levels, PKA activity, evaluated from phosphorylation of PKA substrates (RRXS/T), was increased in the CD36−/− myocardium, and the increase was further exaggerated by fasting (Fig. 7D). These data indicated that the CD36−/− myocardium has abnormal up-regulation of the cAMP/PKA pathway, which plays an important role in modulating Ca2+ handling (57).

Increase in Lysophospholipids and Altered Polyunsaturated FA Composition of Phospholipids in the CD36−/− Myocardium—Expression of CD36 in CHO cells documented its involvement in Ca2+-dependent activation of phospholipases A2, responsible for FA cycling into phospholipids and resulting in production of bioactive eicosanoids (28). The effect of CD36 deletion on phospholipid hydrolysis and acyl composition was examined in hearts from fed or fasted WT and CD36−/− mice using shotgun lipidomics mass spectrometry (50). Under normal fed conditions, CD36−/− hearts when compared with WT hearts had similar levels of the major phospholipids phosphatidylcholine and phosphatidylethanolamine (PC and PE) (data not shown). However, lyso-PC and lyso-PE levels (Fig. 8, C and D) were increased by 15% (p = 0.02) in hearts from fed CD36−/− mice. During fasting, total PC did not change, although total PE decreased similarly (20%) in both groups (data not shown). Lyso-PE content slightly decreased in WT hearts, although it increased in CD36−/− hearts up to levels that were 80% higher than those of WT hearts (p = 0.006) (Fig. 8B). FA composition of phospholipids was significantly altered by CD36 deletion. The amount of DHA (22:6) increased resulting in significantly higher ratios of DHA/AA (Fig. 8C). PCA (58), an unbiased data processing method that evaluates the contributions of individual species to profiles, separation was used to identify the changes that define the phospholipid profile of the CD36−/− myocardium in the fed and fasted states. The first two PCA components separated mice into four distinct groups corresponding to genotype (x axis) and dietary state (y axis) (Fig. 8D). Loading bi-plot (Fig. 8E) displays association of increased lysophospholipid species in the CD36−/− genotype in both fed and fasted states. Similarly, Fig. 8F shows an increase of phospholipid species containing DHA as being strongly characteristic
of the CD36−/− myocardium. Overall, these data identified changes in Ca2+-dependent phospholipid metabolism in the CD36−/− myocardium involving FA cycling and acyl composition.

**DISCUSSION**

This study documented that sarcolemmal CD36, which facilitates FA uptake by the heart, is involved in regulating myocardial Ca2+ handling and increased heart lysophospholipid content and the proportion of 22:6 in phospholipids. These alterations were present in the myocardium of fed mice but did not appear deleterious under such conditions. However, during fasting, abnormally prolonged Ca2+ transients and action potentials were observed in CD36−/− hearts resulting in electrical anomalies and the incidence of sudden death. The evidence generated using telemetry, optical imaging, and CD36 knockdown in HL-1 cardiomyocytes supports the interpretation that CD36 down-regulation slows removal of cytosolic Ca2+, which would delay initiation of the next action potential and reduce cellular ability to regulate excitation-contraction. During telemetry, we showed in vivo a prolongation of QTc, which suggests prolongation of ventricular action potentials. This was directly confirmed by optical mapping of perfused hearts, where the prolonged action potentials were documented to reflect prolongation of Ca2+ transients. Consistent with these findings, knockdown of CD36 in HL-1 cardiomyocytes resulted in delayed removal of cytosolic Ca2+ following its release from the SR.

Strong up-regulation of the SERCA2a protein and down-regulation of PLN mRNA were observed in CD36−/− hearts and may represent compensatory mechanisms aiming to stimulate removal of cytosolic Ca2+. Similarly, the enhanced PKA-induced phosphorylation of PLN during fasting, which would abolish PLN’s inhibitory activity against SERCA, would further facilitate SR Ca2+ reuptake. The chronic up-regulation of SERCA observed in the myocardium of fed CD36−/− mice would be predicted to shorten the Ca2+ transient and enhance its amplitude, effects that improve contractility (59). Possibly SERCA2 up-regulation may have contributed to the protective effect of CD36 deletion against aging-associated depression of myocardial performance when measured under basal conditions (15). However, SERCA operation, which consumes an important fraction of cellular ATP (60), may contribute to compromising the CD36−/− myocardium during fasting. Normally, fasting increases sarcolemmal...
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CD36 content, which enhances FA extraction by the WT heart (20), but the CD36\(^{-/-}\) heart cannot extract FA during the fasted state because FA uptake from both VLDL and the nonesterified FA pool is CD36-dependent (51). This would explain why fasting increases the susceptibility of the CD36\(^{-/-}\) heart to conduction anomalies and failure. Fasting prolongs Ca\(^{2+}\) transients and APD (61), and these electrical changes are exaggerated in the CD36\(^{-/-}\) heart resulting in conduction anomalies such as severe atrioventricular block and bradycardia.

**TABLE 1**

Changes in expression of Ca\(^{2+}\) homeostasis genes

mRNAs of genes related to SR calcium homeostasis in the myocardium of fed versus 18-h fasted WT and CD36\(^{-/-}\) mice (\(n = 4\) per genotype per condition) were determined by microarray analysis. Complete microarray data for most increased and decreased genes with CD36 deficiency or fasting are presented in supplemental Tables 1 and 2.

| WT fast/fed | KO fast/fed | KO/WT fed | KO/WT fast | Gene | Gene description |
|-------------|-------------|-----------|------------|------|------------------|
| 0.069       | 0.719       | 0.615     | 5.821      | Atp2a1 | SERCA1-ATPase, Ca\(^{2+}\) transport, cardiac muscle |
| 0.511       | 0.922       | 1.088     | 2.047      | Atp2a3 | ATPase, Ca\(^{2+}\) transport, ubiquitous, heart, brain |
| 0.083       | 0.384       | 0.497     | 2.949      | Atp2b1 | PMCA1, plasma membrane ATPase 1, Ca\(^{2+}\) extrusion |
| 3.614       | 4.529       | 2.767     | 2.47       | Ppp1r3g | Protein phosphatase 1 regulatory (inhibits) subunit 3G, important for basal cardiac function |
| 0.088       | 0.523       | 1.036     | 4.061      | Ppp1r16a | Protein phosphatase 1, regulatory sub-16A, inhibits activity towards phosphorylase, myosin lc, myosin substrates |
| 0.292       | 2.13        | 0.749     | 7.92       | Ppp1r15a | Regulates pp1, also called myeloid differentiation primary response |
| 0.09        | 0.246       | 0.845     | 2.812      | Calr   | Calreticulin, endoplasmic reticulum, regulates calcium and L-type Ca\(^{2+}\) channel activity. Overexpression causes AV block |
| 1.743       | 1.913       | 0.356     | 0.158      | Slc17a4 | Sarcolipin, small proteolipid that inhibits SERCA2 |
| 0.359       | 1.532       | 1.248     | 2.277      | Pak1   | Regulates phosphatase 2A p21 (CDKN1A)-activated kinase |
| 16.748      | 5.328       | 0.644     | 0.229      | S100a9 | S100 calcium-binding protein A9 (calgranulin B) |
| 25.766      | 7.272       | 0.763     | 0.233      | S100a8a | S100 calcium-binding protein A8 (calgranulin A) |
| 0.995       | 0.349       | 1.022     | 6.021      | S100a16 | S100 calcium-binding protein A16 |
| 0.041       | 0.25        | 0.866     | 4.126      | Smoc2   | SPARC-related modular calcium binding 2 |

FIGURE 7. Changes in SERCA2a, phospholamban, cyclic AMP levels, and PKA activity in fed and fasted WT and CD36\(^{-/-}\) hearts. A, levels of SERCA2a, SERCA2, phospho(Ser-16) and total PLN proteins from hearts of WT and CD36\(^{-/-}\) fed and fasted mice. B, immunoblot densitometric analysis with samples normalized either to the control protein Ran (Serca2a, total phospholamban) or to total phospholamban (Ser-16(P)-phospholamban). AU, arbitrary units. C, cytosolic cyclic AMP levels from WT and CD36\(^{-/-}\) fed and fasted hearts determined by ELISA. Data are expressed relative to WT fed. D, Western blotting documenting increased PKA activity (PKA-specific phosphorylated substrates RR\(X(S/T^*)\)) in CD36\(^{-/-}\) hearts. Data are means ± S.E. *, \(p < 0.05\), \(n = 3\), representative of three experiments.
The altered remodeling of myocardial phospholipid in CD36−/− mice may have contributed to the conduction anomalies. Myocardial phospholipases regulate FA cycling into phospholipids and membrane composition facilitating adaptive responses to nutritional stresses. Previously, calcium-independent phospholipase A2 overexpression in the heart resulted in fasting-induced hemodynamic dysfunction (62). Changes in FA composition of phospholipids modulate functioning of membrane proteins (63), intracellular signal transduction, and gene transcription (64, 65). There is evidence to support the importance of shifts toward lower n3/n6 FA content in phospholipids during myocardial adaptation to energy shortage such as with hibernation (66), which may serve to boost SR Ca2+ uptake and Ca2+ transients (67, 68). In this context, the increase in n3/n6 FA ratio observed in heart phospholipids from CD36−/− mice could be maladaptive during fasting. The mechanisms underlying the altered DHA to AA ratios in PC from the CD36−/− myocardium are likely to be complex. The change in DHA/AA content was not reflected in similar changes in the unesterified FA pool (data not shown) and thus was not a direct consequence of the defect in FA uptake. We suggest it reflects at least in part the altered activities of phospholipases with different substrate preferences based on the observed increase in lysophospholipid content in the CD36−/− myocardium and the previously documented role of CD36 signaling in regulating phospholipase localization and activity (28). However, the contribution of changes in activities and localization of acyltransferases and transacylases with selective substrate specificities cannot be ruled out and will need to be explored in future studies.

The influence of CD36 on SR Ca2+ handling involves at least in part the modulation of the cAMP/PKA signaling pathway, an important regulator of myocardial function under stress (57). We measured fasting-induced increases in cAMP levels, PKA activity, and PKA-induced PLN phosphorylation in the CD36−/− myocardium indicating abnormal regulation of this pathway. Emerging evidence links CD36 to cAMP/PKA signaling with divergent effects reported in different cells. In platelets, TSP-1-induced activation involves CD36-mediated inhibition of the cAMP/PKA pathway (69). In enteroendocrine cells, long chain fatty acids induce robust CD36-dependent increases in cAMP levels and PKA activity. In 3T3-L1 adipocytes, knockdown of CD36 reduces hormone-sensitive lipase phosphorylation and triglyceride lipolysis in part via altering cellular cAMP (70). cAMP levels and signaling are regulated by phosphodiesterases and PKA-anchoring proteins with tissue-specific expression that provides compartmentalization of signals mediating the effects of various regulators (71). Further work is needed to explore how CD36 influences the cAMP/PKA pathway that may vary with cell type and interacting ligand.

The relevance of our findings in mice to humans with CD36 deficiency or with common polymorphisms in the gene that influence CD36 expression level (10) is currently unknown.

3 S. Sundaresan and N. A. Abumrad, unpublished data.
Extrapolating the data obtained in mice would suggest that the CD36-deficient heart as a result of its compromised FA metabolism and Ca\(^{2+}\) handling might be vulnerable to dysfunction in response to particular stresses. This would be supported with the findings of genetic studies. For example, in families selected via a hypertensive proband, CD36 single nucleotide polymorphisms are associated with left ventricular mass (8), a predictor of cardiovascular events (9). Single nucleotide polymorphism association with heart failure was reported in the Cardiovascular Health Genome-wide Association Study. Additional support may be deduced from the clinical findings that reduced \textit{in vivo} myocardial uptake of the palmitate analog \(\beta\)-methylidoo-phenylpentadecanoic acid, which strongly correlates with CD36 levels (5, 72), identifies subjects with a high risk of cardiovascular events among those with coronary artery disease (73). Coronary artery disease morbidity is also higher in CD36-deficient subjects (74). These observations suggest that CD36 deficiency could be deleterious for compromised hearts during stresses.

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