CD36 Expression Contributes to Age-Induced Cardiomyopathy in Mice

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Background—Cardiac remodeling and impaired cardiac performance in the elderly significantly increase the risk of developing heart disease. Although vascular abnormalities associated with aging contribute to the age-related decline in cardiac function, myocardium-specific events may also be involved.

Methods and Results—We show that intramyocardial lipid accumulation, as well as a reduction in both fatty acid and glucose oxidation and a subsequent deterioration in cardiac ATP supply, also occurs in aged mice. Consistent with an energetically compromised heart, hearts from aged mice display depressed myocardial performance and cardiac hypertrophy. Associated with this is a dramatic increase in the fatty acid transport protein CD36 in aged hearts compared with young hearts, which suggests that CD36 is a mediator of these multiple metabolic, functional, and structural alterations in the aged heart. In accordance with this, hearts from aged CD36-deficient mice have lower levels of intramyocardial lipids, demonstrate improved mitochondria-derived ATP production, have significantly enhanced function compared with aged wild-type mice, and have a blunted hypertrophic response.

Conclusions—These findings provide evidence that CD36 mediates an age-induced cardiomyopathy in mice and suggest that inhibition of CD36 may be an approach for the treatment of the detrimental age-related effects on cardiac performance. (Circulation. 2007;116:2139-2147.)

Key Words: CD36 ■ aging ■ cardiomyopathy ■ metabolism ■ lipotoxicity

Age is a major risk factor for the development of a number of cardiovascular disorders, including left ventricular (LV) hypertrophy and heart failure. Often preceding these overt cardiovascular diseases are age-related structural and functional changes in the myocardium that result in impaired cardiac performance. As humans age, hypertension and resulting cardiac dysfunction can occur independently of atherosclerosis, although atherosclerosis and subsequent hypertension are among the major causative factors that contribute to cardiac dysfunction in the elderly. However, additional mechanisms unrelated to hypertension/increased afterload have also been proposed to contribute to contractile dysfunction in the aged heart. For example, an age-dependent decrease in myocardial fatty acid (FA) utilization has been shown to occur in humans, which suggests impaired mitochondrial FA-derived ATP production and energetically compromised hearts. In addition, accumulation of lipids within the cardiac myocyte has been observed in the aged human heart, which implicates lipotoxic cardiomyopathy as a contributor to cardiac dysfunction in the elderly. Although diminished FA oxidation (FAO) without a corresponding decrease in FA uptake can lead to lipid accumulation within the young heart, increased protein-mediated FA uptake has also been shown to contribute to lipotoxicity in the young mouse heart. As such, age-related increases in FA transport protein expression may contribute to the lipotoxicity observed in the elderly. However, whether increased expression of FA transport proteins occurs in the aged heart and whether this contributes to impaired mitochondrial FA-derived ATP production and/or promotes lipotoxicity in the aged heart have not been explored.

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In addition to the changes in myocardial FA uptake and utilization that have been proposed as potential causative components of the decline in cardiac performance observed in the elderly, other related metabolic mechanisms may also be involved. For example, mitochondrial dysfunction is observed in the skeletal muscle of aged humans, which suggests that overall mitochondrial oxidative capacity is...
decreased in the aged myocardium. As such, it is likely that glucose oxidation (GOX) is also impaired in the aged myocardium, and in association with the established decline in FAO, diminished ATP production could contribute greatly to an energetically compromised heart and a subsequent decline in myocardial function in the elderly. Although this concept has not been explored fully, it suggests that strategies designed to improve cardiac energy metabolism and subsequent ATP supply in the aged myocardium may be effective approaches that can be used to treat the age-related decline in mechanical function.

On the basis of the aforementioned rationale, we propose that increased myocardial FA uptake and decreased utilization during aging can lead to metabolic perturbations that promote a deterioration in cardiac ATP supply and subsequent contractile function in the aged myocardium. Because alterations in FA handling can be altered dramatically by increased expression of FA transport proteins in the young heart, we examined myocardial expression of the FA transport protein CD36 in the context of aging. Given that CD36 is responsible for >50% of myocardial long-chain FA uptake and subsequent oxidation, we hypothesized that altered myocardial FA uptake and utilization in the aged myocardium was due to elevated expression of CD36 with age. We further speculated that this increase in FA uptake at a time when FAO is severely depressed, as is known to occur during aging, contributes to excessive intramyocardial lipid accumulation, a further decrease in overall mitochondrial-derived ATP supply, and impaired cardiac performance. Therefore, the aim of the present study was to characterize the role of CD36 in the regulation of these cellular functions during aging and to determine whether increased expression of CD36 mediates age-induced cardiomyopathy. Furthermore, we set out to test the concepts that inhibition of CD36-mediated FA transport could offer protection from age-related cardiac lipotoxicity and that the promotion of GOX to optimize ATP production could improve mechanical function in the aged myocardium.

**Methods**

**Mice**

CD36 knockout (KO) and wild-type (WT) male mice of either 10 to 12 or 52 to 54 weeks of age derived from littermates that were bred inhouse and were 6X backcrossed to a C57Bl/6 background strain were used in the present study. Mice were maintained on a standard chow diet (MCT Oil Rodent Diet 10%; Harlan Teklad, Madison, Wis) and were housed in a normal light/dark cycle relatively undisturbed for either 10 to 12 or 52 to 54 weeks. All experiments involving mice were performed with the approval of The University of Alberta Animal Policy and Welfare Committee.

**In Vivo Assessment of Cardiac Function and Blood Pressure**

Transthoracic echocardiography was performed on mildly anesthetized mice as described previously with a Vevo 770 high-resolution imaging system equipped with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Canada). M-mode images were obtained for measurements of LV wall thickness (LVWT), LV end-diastolic diameter (LVEDD), and LV end-systolic diameter (LVESD). LV fractional shortening (FS), a measure of systolic function, was calculated with the following equation: 

\[
\text{FS} \% = \left( \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \right) \times 100.
\]

Isovolumic relaxation time and deceleration time of the early (E) filling wave were determined with pulsed-wave Doppler. Isovolumic relaxation time was calculated as the time from closure of the aortic valve to initiation of the E wave, in milliseconds. The deceleration time of the E wave was determined by measuring the time (in milliseconds) taken for the downslope of the peak of the E wave to reach the baseline. Noninvasive blood pressure measurements were made with the CODA 2 tail-cuff system (Kent Scientific Corp, Torrington, Conn).

**Graded Exercise Test**

Graded exercise tests were performed on a calibrated, motor-driven treadmill with incremental increases in treadmill belt speeds until the mouse exhibited signs of exhaustion. Time to exhaustion was defined as the mouse spending >50% of the time or >15 consecutive seconds on the shock grid.

**Heart Perfusions**

Hearts from WT and CD36 KO male mice were perfused in the working mode as described previously with 5 mmol/L [U-14C] glucose, 1.2 mmol/L [9,10-3H] palmitate prebound to 3% FA free bovine serum albumin, and 50 μU/mL insulin. Hearts were perfused aerobically for 30 minutes at a constant preload pressure of 11.5 mm Hg and an afterload set at 50 mm Hg (normal workload). Hearts were subjected to an increase in workload by increasing the afterload to 80 mm Hg and adding isoproterenol (300 nmol/L) to the perfusate for an additional 30 minutes (high workload). A similar protocol was also used for the aged mice except for the high-workload condition, which involved the afterload being increased to 70 mm Hg. GOX and palmitate oxidation rates, along with acetyl coenzyme A (CoA) production, were measured and calculated as described previously. At the end of the experiment, hearts were frozen in liquid nitrogen and stored at -80°C.

**Myocardial Triacylglycerol and Long-Chain Acyl CoA Measurements**

Intramyocardial lipids were extracted from 5 mg of frozen unperfused heart tissue and dried under N2 atmosphere at 60°C as described previously. The dried lipids were redissolved in 50 μL of 3:2 tert-butyl alcohol:Triton X-100/methyl alcohol (1:1, vol/vol) mixture, and cardiac triacylglycerol content was measured with the Wako L-Type TG-H kit (Wako Diagnostics, Osaka, Japan). Identification and quantification of the major long-chain acyl CoA molecular species were performed by high-performance liquid chromatography, as we have described previously. The sum of the major peaks identified by high-performance liquid chromatography (C16:0, C18:0, C18:1, and C18:2) was referred to as total long-chain fatty acyl CoAs.

**Immunoblot Analysis**

Equal amounts of protein from homogenized unperfused heart tissue were subjected to 10% SDS-PAGE, transferred to nitrocellulose, and immunoblotted with affinity-purified rabbit polyclonal antibody to CD36 and goat polyclonal anti-actin (I-19; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) and eventually visualized with the Amersham Pharmacia enhanced chemiluminescence Western blotting detection system (GE Healthcare UK Ltd, Buckinghamshire, UK). The anti-CD36 antibody was generated in-house and characterized as in a previous report and was used at a dilution of 1:500, whereas the anti-actin antibody was used at a dilution of 1:1000.

**Statistical Analysis**

Data are expressed as mean±SEM. A Mann–Whitney U test was performed for Figure 1. To test for the effects of age, genotype, and their interaction on the functional and metabolic parameters measured...
sured in Tables 1 and 2 and in Figures 2 and 3, a 2-factor ANOVA was used in combination with the nonparametric Mann–Whitney U test. To account for multiple testing with the Mann–Whitney U test, a Bonferroni-corrected probability value of 0.025 (0.05/2) was used. Difference scores between the same hearts assessed at normal and high workload were also calculated, and the Mann–Whitney U test was performed to compare the differences in scores between genotypes. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Age-Related Increases in Myocardial CD36 Protein Expression

To examine whether increased expression of CD36 was different between hearts from young (10 to 12 weeks of age) and aged (52 to 54 weeks of age) WT mice, heart homogenates from both age groups were subjected to CD36 immunoblot analysis. A significant 4.5-fold increase in CD36 protein expression was observed in aged WT mice compared with young WT mice (Figure 1A). Elevated levels of CD36 protein were associated with a 2- and 3-fold increase in triacylglycerol and long-chain fatty acyl CoA levels in aged WT mouse hearts compared with young WT mouse hearts (Figure 1B and 1C, respectively). This increase in long-chain fatty acyl CoA levels in the aged myocardium is particularly important given that it is these types of lipotoxic intermediates that have been shown to be detrimental to the cardiomyocyte and associated with myocardial contractile dysfunction.6,7 These data are consistent with our hypothesis that increased myocardial CD36-mediated FA uptake promotes lipotoxicity in aged hearts.

CD36 Mediates the Loss of Cardiac Performance in Aged Mice In Vivo

To assess the importance of CD36 in regulating cardiac function in vivo, young and aged WT and CD36 KO mice were evaluated with typical clinical diagnostic procedures. Using a graded treadmill test that simulates the commonly used submaximal stress test used in the clinic, we determined that low-stress exercise performance in fed mice was not different between the young WT and CD36 KO mice (Figure 2A). Although there was the expected decrease in exercise performance in aged WT mice compared with young WT mice, exercise performance in aged CD36 KO mice was

Table 1. Functional Parameters of Ex Vivo Perfused Working Hearts From Young WT and CD36 KO Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Young WT</th>
<th>Young CD36 KO</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal Workload</td>
<td>High Workload</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>340±13</td>
<td>523±27*</td>
</tr>
<tr>
<td>Peak systolic pressure, mm Hg</td>
<td>75.5±1.4</td>
<td>101±1.7*</td>
</tr>
<tr>
<td>Developed pressure, mm Hg</td>
<td>23.3±1.0</td>
<td>25.2±2.4</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>11.0±0.8</td>
<td>7.3±0.2</td>
</tr>
<tr>
<td>Aortic flow, mL/min</td>
<td>7.8±0.7</td>
<td>2.3±0.8*</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 4 to 8 WT and CD36 KO young (10- to 12-week-old) mice perfused ex vivo at normal and high workloads. Values shown represent averages for the 0-to-30–minute (normal workload) and 35-to-65–minute (high workload) perfusion period.

*P<0.025 for high vs normal workload values between mice of the same genotype.

Figure 1. Immunoblot analysis of CD36 expression in young and aged WT mouse hearts along with myocardial triacylglycerol and long-chain acyl CoA levels. A, Immunoblot analysis was performed on extracts from young (n=4) and aged (n=6) WT mouse hearts with anti-CD36 and anti-actin antibodies. Levels of CD36 expression were quantified by densitometry and normalized against actin as a control for protein loading. B and C, Levels of triacylglycerol (TG) and long-chain acyl CoAs (LCACoAs) in young (n=6) and aged (n=3) WT heart tissue were determined. Values are mean±SEM. *P<0.025, young vs aged WT mice.
significantly higher than in aged WT mice and was virtually identical to that of young WT mice (Figure 2A). Even though we used this low-stress exercise performance end point as an indicator of cardiac performance akin to the clinical exercise stress test, we recognize that altered systemic metabolism may influence our results. Because of this, we used transthoracic M-mode and pulsed-wave Doppler echocardiography to directly assess cardiac function in vivo.

M-mode echocardiographic assessment did not detect significant differences in percent fractional shortening between young WT and young CD36 KO mice (Figure 2B), which indicates that LV function and cardiac output are similar between both groups of mice. Furthermore, M-mode echocardiography did not detect any differences in LVWT between young WT and CD36 KO mice (Figure 2C), which was confirmed by an examination of gross morphology after the animals were euthanized (not shown). However, consistent with the reduced performance on the exercise stress test, M-mode echocardiographic analysis in aged WT mice revealed that percent fractional shortening was significantly lower in aged WT mice than in aged CD36 KO mice (Figure 2B). In addition, noninvasive indices of diastolic dysfunction, such as isovolumic relaxation time and deceleration time of the E wave, were increased in aged WT mice but were normal in aged CD36 KO mice (WT versus CD36 KO mice: isovolumic relaxation time 26.8±2.0 ms versus 18.1±0.3 ms, P<0.05; deceleration time 25.6±2.9 ms versus 18.3±3.7 ms, P=NS). These changes were not due to increased

Table 2. Functional Parameters of Ex Vivo Perfused Working Hearts From Aged WT and CD36 KO Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aged WT Normal Workload</th>
<th>Aged WT High Workload</th>
<th>Aged CD36 KO Normal Workload</th>
<th>Aged CD36 KO High Workload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>295±15.4</td>
<td>422±50.4</td>
<td>280±20.0</td>
<td>471±35.1*</td>
</tr>
<tr>
<td>Peak systolic pressure, mm Hg</td>
<td>56.6±1.2</td>
<td>41.7±7.1</td>
<td>63.6±1.4</td>
<td>64.0±10.0</td>
</tr>
<tr>
<td>Developed pressure, mm Hg</td>
<td>16.9±1.3</td>
<td>8.9±1.7*</td>
<td>25.1±1.6</td>
<td>22.3±3.9</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>7.9±1.4</td>
<td>1.9±0.6*</td>
<td>13.1±1.0</td>
<td>8.2±1.4</td>
</tr>
<tr>
<td>Aortic flow, mL/min</td>
<td>5.1±0.9</td>
<td>0</td>
<td>8.8±1.3</td>
<td>3.5±1.2</td>
</tr>
</tbody>
</table>

Data are mean±SEM of 5 WT and 5 CD36 KO hearts from aged (52- to 54-week-old) mice perfused ex vivo at normal and high workloads. Values shown represent averages for the 0- to 30-minute (normal workload) and 35- to 65-minute (high workload) perfusion period.

*P<0.025 for high vs normal workload values between mice of the same genotype.

Figure 2. Effects of CD36 ablation on myocardial performance and structure in young and aged WT and CD36 KO mice. A, Time to exhaustion in young and aged WT and CD36 KO mice with a rodent treadmill. Echocardiographic assessment of percent fractional shortening (%FS; B) and LVWT (C) from young and aged WT and CD36 KO mice at rest. D, Heart weight/tibia length ratio (HW/TL) in young and aged WT and CD36 KO mice. E, Representative M-mode echocardiographic recordings of LV performance in aged WT and CD36 KO mice at rest. F, Representative cross section of aged WT and aged CD36 KO hearts confirming changes in LV wall thickness. Values are mean±SEM of n=3 to 8 hearts in each group. *P<0.025, WT vs CD36 KO mice.
because aged WT mice were hypertensive and actually had slightly lower systolic and diastolic systemic blood pressures than aged CD36 KO mice (systolic blood pressure: WT 105 ± 3.0 versus CD36 KO 132 ± 2.4 mm Hg, P < 0.01; diastolic blood pressure: WT 84 ± 4.3 versus CD36 KO 113 ± 3.2 mm Hg, P < 0.01).

In addition to cardiac dysfunction in the aged WT mice, M-mode echocardiographic analysis revealed cardiac remodeling with increased LVWT (Figure 2C) and dilatation and poor function of the LV in aged WT mice but not in aged CD36 KO mice (Figure 2E). Moreover, there was a significant effect of age on both percent fractional shortening and LVWT, with evidence of an interaction with genotype (Data Supplement Table I). Furthermore, the ability of CD36 ablation to significantly reduce increased LVWT in the aged mouse was confirmed subsequently by measurements of heart weight after euthanasia. Because aged WT mice had significantly increased body weight compared with aged CD36 KO mice (WT 43.2 ± 1.6 versus CD36 KO 35.0 ± 0.7 g, P < 0.01), heart weight was expressed as a ratio to tibia length (Figure 2D). Two-factor ANOVA indicated that both age and genotype significantly affected heart weight/tibia length ratio, with evidence of an interaction with genotype (Data Supplement Table I). Finally, an examination of gross cardiac morphology also indicated increased LVWT (Figure 2F). Interestingly, cardiac histology from both aged WT and aged CD36 KO mice looked similar, with no evidence of interstitial fibrosis in either group (not shown), and the amount of poly(ADP-ribose) polymerase (PARP) or caspase 3 cleavage was not different in aged WT and aged CD36 KO hearts (not shown), which suggests no signs of increased apoptosis in the aged WT heart. Taken together, the noninvasive in vivo data provide direct evidence that the aged CD36 KO mice were protected from age-dependent diastolic and systolic dysfunction at rest. In addition, aged CD36 KO hearts were protected from increases in LVWT associated with pathological hypertrophy and remodeling.

CD36 Mediates the Loss of Cardiac Performance and the Age-Related Deterioration in Cardiac ATP Supply in Aged Mice

Germline deletion of CD36 causes significant alterations in systemic metabolism in young mice,19 which were also evident in systemic levels of relevant substrates in aged mice (fasted plasma glucose: WT 6.6 ± 0.9 versus CD36 KO 4.8 ± 0.4 mmol/L, P < 0.01; fasted plasma free FA: WT 0.7 ± 0.06 versus CD36 KO 1.8 ± 0.04 mmol/L, P < 0.01). Therefore, to determine cardiac effects that resulted from germine deletion of CD36 in a model in which substrate supply, insulin levels, and both preload and afterload were consistent between WT and CD36 KO hearts, an ex vivo perfused working heart model was used. Baseline parameters of ex vivo cardiac function of WT and CD36 KO hearts from young and aged mice were measured in hearts aerobically perfused for 30 minutes at a preload of 11.5 mm Hg and 50 mm Hg of afterload (normal workload). To mimic the increase in cardiac workload induced by a graded exercise test, hearts from WT and CD36 KO mice were also subjected to increased workload via adrenergic stimulation and increased afterload (high workload). Although high workload significantly increased heart rate and peak systolic pressure in WT and CD36 KO hearts above their baseline values (Table 1), neither of the functional parameters measured were significantly different between young WT hearts and young CD36 KO hearts at normal or high workloads (Data Supplement Table II).
Supplement Table I). However, baseline functional parameters of WT hearts from aged mice were dramatically depressed compared with young WT hearts (Tables 1 and 2), and many of the indices measured, such as developed pressure, cardiac output, and aortic flow, were higher in aged CD36 KO hearts than in aged WT hearts (Table 2). Despite these dramatic changes, difference scores indicated that only developed pressure was significantly different between aged WT and CD36 KO hearts at normal and high workloads (data not shown). In addition, there was an effect of both workload and genotype on cardiac output and aortic flow in aged mice but no evidence of interaction (Data Supplement Table II).

Consistent with improved function in aged CD36 KO hearts in vivo, cardiac power was maintained in aged CD36 KO hearts compared with their young counterparts, whereas cardiac power was significantly depressed in aged WT hearts compared with young WT hearts (Figure 3A; Data Supplement Table III). When subjected to increased workload, hearts from young WT and young and aged CD36 KO mice maintained cardiac power, whereas hearts from aged WT mice almost completely failed, likely as a result of decreased stroke volume that resulted from the increased heart rate and subsequent failure to meet the increase in afterload (Figure 3E; Tables 1 and 2). Because cardiac power was maintained in aged CD36 KO hearts at high workload, this finding confirmed our in vivo finding that cardiac performance was not compromised in aged CD36 KO mice. This may have contributed to improved exercise performance in the aged CD36 KO mice compared with the aged WT mice.

Although many factors could account for the decreased cardiac performance in aged WT mice and maintained cardiac performance in aged CD36 KO mice, we investigated whether alterations in cardiac energy metabolism were involved. Consistent with rat models of aging in which mitochondrial oxidation of FA is decreased in the aged myocardium,12,13 FAO rates were reduced ≈2.5-fold in aged WT hearts compared with young WT hearts (Figure 3F; Data Supplement Table III). However, because hearts from CD36 KO mice have less FAO at baseline, there was no further inhibition of FAO in the aged CD36 KO hearts at either workload (Figure 3B and 3F; Data Supplement Table III). Despite this equivalent decrease in FAO, unperfused hearts from aged CD36 KO mice had reduced accumulation of long-chain fatty acyl CoA levels compared with hearts from aged WT mice (WT 16.8±0.7 versus CD36 KO 10.8±1.6 nmol/g wet weight, P<0.05), as would be predicted in hearts that lack a major FA transport protein.

Notwithstanding the profound decreases in FAO at both normal and high workloads in hearts from young CD36 KO mice (Figure 3B and 3F), these hearts are not energetically compromised even at elevated workloads because of the corresponding increase in GOX rates (Figure 3C and 3G). These increased rates of GOX in the young CD36 KO hearts can account for adequately maintained acetyl CoA−derived ATP production for proper cardiac function at both normal and high workloads (Figure 3D and 3H). Furthermore, in accordance with a reduction in overall mitochondrial function associated with aging, GOX rates were reduced ≈4-fold in the aged WT hearts compared with the young WT hearts at both workloads (Figure 3C and 3G). Interestingly, GOX rates were not different in young CD36 KO hearts compared with aged CD36 KO hearts at normal workload (Figure 3C; Data Supplement Table III) and were only slightly lower in aged CD36 KO hearts compared with young CD36 KO hearts at high workload (Figure 3G; Data Supplement Table III). This maintained GOX in the aged CD36 KO hearts indicates an improvement in acetyl CoA−derived ATP supply over aged WT hearts at both normal and high workloads (Figure 3D and 3H). This overall improvement in mitochondrial ATP supply in CD36 KO hearts compared with aged WT hearts likely contributes to the ability of CD36 KO hearts to maintain cardiac power even at elevated workloads (Figure 3A and 3E).

Discussion
Cardiac remodeling and impaired cardiac performance in the elderly significantly increase the risk of developing heart failure.20 Although altered vascular structure and function have been implicated as the major cause of the age-related decline in cardiac function,2 other unrelated processes may also be involved. Indeed, we have identified CD36 as being a major contributor to age-induced cardiomyopathy in mice. Specifically, using a mouse model of aging, we showed that CD36 expression is increased almost 5-fold in the aged WT mouse myocardium (Figure 1A). Because CD36 is a major regulator of cardiac myocyte FA uptake, we predicted that this increase would promote myocardial lipotoxicity. Indeed, we observed a significant increase in both triacylglycerol and long-chain acyl CoA levels in the aged WT heart compared with the young WT heart (Figure 1B and 1C). Because the accumulation of lipids within the cardiac myocyte from young mice has been shown to be detrimental to the heart and associated with myocardial contractile dysfunction,6,7,9 age-related increases in CD36 expression likely contribute to the lipotoxicity that has been observed in the aged human heart.4

A significant combined diastolic and systolic dysfunction was associated with elevated levels of myocardial CD36 expression in aged WT mice (Figure 2). Remarkably, however, aged CD36 KO mice were protected from age-dependent decreases in both diastolic and systolic function (Figure 2). Although early indices of cardiac dysfunction associated with the aged human heart are characterized primarily by delayed diastolic relaxation before systolic dysfunction,1 it is possible that in the present mouse model, we only observed combined diastolic and systolic dysfunction based on the rapid progression of age-related changes in mice. Nevertheless, in the majority of untreated symptomatic elderly patients, overall cardiac dysfunction is a combination of both diastolic and systolic dysfunction. Therefore, regardless of the pathogenesis of the systolic dysfunction that we observed in aged WT mice, the present findings may have considerable clinical implications given that systolic dysfunction is most clinically relevant, because it correlates with the symptoms and prognosis of patients based on a deterioration of LV ejection fraction.21 These results provide valuable
insights into the importance of CD36 in contributing to the cardiac dysfunction associated with aging.

In addition to the cardiac dysfunction observed in the aged WT mouse, elevated levels of myocardial CD36 expression were also associated with increased cardiac hypertrophy (Figure 2C through 2F). Consistent with preserved cardiac function in aged CD36 KO mice, the cardiac hypertrophy that developed in aged CD36 KO mice in the present study was significantly blunted compared with that associated with aging in the aged WT mice. Interestingly, the beneficial effects of CD36 ablation on cardiac hypertrophy and function observed in the present study are not necessarily consistent with the hypertrophic cardiomyopathy that originally was proposed to be caused by the loss of CD36 in CD36-deficient patients or the hypertrophy that exists in the spontaneously hypertensive rat, which also has loss of CD36 function. However, a recent report has indicated that there is no correlation between hypertrophic cardiomyopathy and loss of CD36 in CD36-deficient patients, and thus, the original findings may have no relation to the present study. In addition, transgenic “rescue” of CD36 in the spontaneously hypertensive rat has been shown to restore certain metabolic parameters but does not rescue hypertension. As such, the spontaneously hypertensive rat likely develops hypertrophy in response to hypertension and not because of the loss of CD36. In relation to this, systemic blood pressures in the aged WT mice were at the lower end of normal, as observed in standard control mice of the same genetic background, which demonstrates that the development of cardiac hypertrophy in WT mice cannot be attributed to increased afterload, as is often observed in aged humans. Thus, it appears that this mouse model allows for examination of the direct effects of age-induced increases in myocardial CD36 expression on cardiac function in the absence of coronary artery disease that is often observed in humans.

An additional mechanism that explains why CD36 ablation offers protection from age-related cardiac dysfunction appears to be metabolic in nature. Indeed, the present data indicate that CD36 KO mice are protected from an age-related decline in cardiac function because of the prevention of an age-related deterioration in cardiac ATP supply. In support of this, ex vivo assessment of cardiac energy metabolism demonstrated that whereas aged WT and aged CD36 KO hearts had equally low levels of FAO (Figure 3B and 3F), aged CD36 KO hearts had dramatically higher rates of Gox (≈5- to 8-fold) than aged WT hearts at both normal and elevated workloads (Figure 3C and 3G). The resulting 2-fold increase in acetyl CoA–derived ATP production in aged CD36 KO hearts compared with aged WT hearts (Figure 3D and 3H) is consistent with preserved cardiac power in aged CD36 KO hearts even at elevated workloads (Figure 3A and 3E). However, ex vivo mitochondrial ATP production is calculated from acetyl CoA production, and we have not assessed in vivo ATP production directly using techniques such as phosphorus magnetic resonance spectroscopy. Because other factors such as mitochondrial uncoupling could also affect ATP production, future studies using this technique will be used to substantiate our ex vivo studies.

Nevertheless, the present ex vivo heart perfusion data indicate that there are heart-specific effects, such as improved mitochondrial ATP production from GOX, that likely contribute to maintained function in the aged CD36 KO mouse heart compared with the aged WT heart. This finding is consistent with the emerging concept in cardiovascular medicine that the optimization of cardiac energy metabolism by promotion of GOX can significantly improve mechanical function in numerous pathologies that cause cardiac dysfunction. As such, the present data suggest that drugs designed to improve cardiac energy metabolism may be a beneficial adjunctive treatment designed to improve cardiac performance in the aged patient and/or patients displaying systolic dysfunction.

In summary, the present data show that CD36-mediated FA uptake and the affected cardiac energy metabolic pathways play critical roles in the pathogenesis of cardiac dysfunction associated with aging. In addition, we show that the aged CD36 KO mouse heart is protected from the accumulation of long-chain fatty acyl CoA levels, displays preserved mitochondrial function in the form of maintained glucose-derived ATP production, and improved mechanical function both in vivo and ex vivo, and has a blunted age-induced hypertrophic response. The finding that CD36 ablation can prevent accumulation of intramyocardial lipids is entirely consistent with findings by Yang et al, who recently showed that CD36 deficiency was able to prevent the development of lipotoxic cardiomyopathy in young cardiac-specific peroxisome proliferator–activated receptor–α–overexpressing transgenic mice. The present study extends that previous work to a more physiological model of myocardial lipid accumulation, namely, aging. However, like the study by Yang et al, the CD36 KO mouse has helped us address some of the issues that surround the increased expression of CD36 in the aged WT mouse heart. For example, because levels of sarcosomal membrane–associated CD36 were not examined in the aged WT hearts, it is not known whether aged WT hearts have elevated levels of FA uptake. Indeed, it is possible that lipid accumulation in the aged WT hearts may be solely due to impaired FAO. However, because hearts from CD36 KO mice have been shown to have reduced FA uptake, it is likely that FA uptake and not impaired FAO is responsible for the lipid accumulation observed in hearts from aged WT mice. Thus, the implication of CD36-mediated FA uptake and cardiac energy metabolism as contributing to cardiac dysfunction associated with aging highlights a potential new strategy to prevent or treat age-associated cardiac dysfunction. Because aging is a natural process that leads to the development of cardiac pathologies, either myocardium-specific or systemic inhibition of CD36 may be a new approach for treatment of the detrimental age-related effects on cardiac performance.

Study Limitations
Although the present study provides significant insight into the role of CD36 in age-induced cardiomyopathy, there remain some outstanding issues that need to be addressed. For instance, the degree of cardiac dysfunction of the aged WT
mice used in the present study was much more severe than that normally observed in mice of the same age and strain. Although we cannot explain the cause of this, one possibility is that the aged WT mice studied herein had a significant increase in body weight compared with other studies using the same strain of mouse. Because weight gain and increased adipose mass significantly alter circulating adipokines, which can cause cardiac insulin resistance, we propose that this may explain the more severe phenotype observed in hearts from the aged WT mice used in the present study. In relation to this, aged CD36 mice do not gain as much weight as aged WT mice, which, in addition to the direct metabolic effects in the heart that we have documented in the present study, may also contribute to the protection of aged CD36 KO mice from the severe cardiac dysfunction observed in aged WT mice. Because differences in body weights between aged WT and aged CD36 KO mice cannot be controlled for in the absence of interventions, one possible means to address this is to use a cardiomyocyte-specific CD36 KO mouse, which would presumably alleviate the complicated systemic metabolic perturbations and altered plasma energy substrate profiles of germline deletion of CD36. We are currently generating the cardiomyocyte-specific CD36 KO mouse to address these potential additional mechanisms.

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Dr Koonen is a Heart and Stroke Foundation of Canada and an Alberta Heritage Foundation for Medical Research (AHFMR) postdoctoral fellow. Dr Dyck is an AHFMR Senior Scholar and a Canada Research Chair in Molecular Biology of Heart Disease and Metabolism. Dr Michelakis is a Canada Research Chair in Pulmonary Hypertension. The authors would like to thank Dr Michael D. Schneider for his critical appraisal of the manuscript and his helpful and insightful comments and suggestions.

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Disclosures

None.

References

An age-related decline in cardiac function is a major risk factor for cardiovascular disease in the elderly. Although progressive age-related decline in cardiac function is multifactorial, derangements in myocardial energy metabolism also play important roles. In this article, we provide evidence that an age-dependent increase in myocardial expression of the fatty acid transport protein CD36 is a causative factor in the cardiomyopathy of aging. Our data indicate that increased CD36 expression in the aging mouse heart is correlated with intramyocardial lipid accumulation, impaired mitochondrial oxidative metabolism, decreased ATP production, and cardiac dysfunction, yet hearts from CD36-deficient mice are protected from these age-related effects. In addition to implying that reduction of fatty acid uptake is involved in protecting aged CD36-deficient mice from cardiac dysfunction, we show that indirect stimulation of myocardial glucose oxidation via inhibition of fatty acid oxidation also plays a role. We demonstrate that this phenotypic switch in cardiac mitochondrial substrate utilization to predominantly glucose oxidation assists in preserving mitochondrial function, thus maintaining the adequate ATP supply necessary for proper cardiac function. This finding is consistent with the concept that optimization of myocardial energy metabolism by the promotion of glucose oxidation can significantly improve contractile performance in a number of cardiac pathologies, including ischemic heart disease, ischemia-reperfusion injury, and heart failure. As such, our data support the notion that drugs that target CD36 to reduce fatty acid uptake or optimize cardiac energy metabolism may represent a beneficial adjunctive treatment that can improve cardiac performance in the aged patient.
CD36 Expression Contributes to Age-Induced Cardiomyopathy in Mice

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