Fatty Acid Translocase/CD36 Deficiency Does Not Energetically or Functionally Compromise Hearts Before or After Ischemia

Michael Kuang, BSc; Maria Febbraio, PhD; Cory Wagg; Gary D. Lopaschuk, PhD; Jason R.B. Dyck, PhD

**Background**—Evidence from humans suggests that fatty acid translocase (FAT)/CD36 deficiency can lead to functionally and/or energetically compromised hearts, but the data are equivocal, and the subject remains controversial. In this report we assessed the contribution of FAT/CD36 to overall fatty acid oxidation rates in the intact heart and determined the effect of FAT/CD36 on energy metabolism during reperfusion of ischemic hearts.

**Methods and Results**—Isolated working hearts from wild-type and FAT/CD36-knockout (KO) mice were perfused with Krebs-Henseleit solution containing 0.4 or 1.2 mmol/L [U-3H]palmitate, 5 mmol/L [U-14C]glucose, 2.5 mmol/L calcium, and 100 μU/mL insulin at a preload pressure of 11.5 mm Hg and afterload pressure of 50 mm Hg. Hearts were aerobically perfused for 30 minutes or aerobically perfused for 30 minutes, followed by 18 minutes of global no-flow ischemia and 40 minutes of aerobic reperfusion. Rates of fatty acid oxidation in FAT/CD36-KO hearts were significantly lower than in wild-type hearts at both concentrations of palmitate (0.4 or 1.2 mmol/L). In addition, hearts from FAT/CD36-KO mice displayed a compensatory increase in glucose oxidation rates. On aerobic reperfusion after ischemia, cardiac work of FAT/CD36-KO hearts recovered to the same extent as wild-type hearts.

**Conclusions**—FAT/CD36-deficient hearts are not energetically or functionally compromised and are not more sensitive to ischemic injury because glucose oxidation can compensate for the loss of fatty acid–derived ATP. (Circulation. 2004; 109:1550-1557.)

**Key Words:** metabolism ■ fatty acids ■ ischemia

In adult cardiac myocytes, the oxidation of fatty acids provides the majority of energy needed to support contractile function. Although the heart has the ability to store fatty acids, it has a limited capacity to maintain its high metabolic demand on endogenous sources alone and relies on continuous uptake from blood.1 Despite the obvious importance of fatty acid uptake to the cardiac myocyte, controversy still exists with regard to the exact process by which fatty acids enter into cells. The 2 main mechanisms proposed for transport of fatty acids into cells are (1) passive diffusion and (2) protein-mediated transport.2,3 Accumulating evidence supports a role for fatty acid translocase (FAT; also known as CD364) in protein-mediated fatty acid uptake in cardiac myocytes.

FAT/CD36 is an 88-kDa ditopic glycosylated protein that belongs to the class B family of scavenger receptors. This family also includes scavenger receptor class B type I (SR-BI), the receptor for selective cholesteryl ester uptake, and lysosomal integral membrane protein II (LIMP-II) (see Febbraio et al5 for review). Studies utilizing a nonmetabolizable fatty acid analogue, BMIPP, suggest that FAT/CD36 accounts for 50% to 80% of the total fatty acid uptake by the heart.6 Polymorphisms in human FAT/CD36 occur in Asian and African populations at a relatively high frequency, and there have been reports that these deficient individuals have reduced cardiac fatty acid uptake7–14 and cardiac abnormalities. However, considerable controversy exists with regard to the potential involvement of FAT/CD36 deficiency in the development of hypertrophic cardiomyopathy,7,15–19 and it has not been directly proven whether patients with FAT/CD36 deficiency have reduced cardiac fatty acid oxidation rates or energetically compromised hearts. To better understand the effects of FAT/CD36 deficiency, a mouse model of FAT/CD36 deficiency has been developed.20 A recent report by Irie et al21 suggested that hearts from FAT/CD36-null mice were energetically deficient. However, in that study, involvement of FAT/CD36 in regulation of fatty acid oxidation rates was determined with the
use of isolated cardiac myocytes and thus may be skewed by the fact that isolated cardiac myocytes do not perform significant amounts of contractile work and therefore have very low rates of fatty acid oxidation. It has not yet been determined whether FAT/CD36-mediated fatty acid uptake can regulate fatty acid oxidation rates in the intact working heart. In addition, the ability of carbohydrate oxidation to compensate for changes in fatty acid oxidation, such that the energy status of the heart can be determined, also needs to be assessed to fully understand the impact of FAT/CD36 deficiency.

The report by Irie et al also suggested that hearts from FAT/CD36-null mice were less tolerant to ischemia, primarily as a result of deficiency in fatty acid oxidation and decreased ATP supply. However, we have previously shown that excessively high fatty acid oxidation rates contribute to ischemic injury by inhibiting glucose oxidation. As a result, it is possible that depressed fatty acid oxidation rates, as seen in FAT/CD36-KO mice, may actually protect the heart from ischemic damage. Therefore, the question remains as to whether FAT/CD36 deficiency leads to energetically deficient hearts and/or whether an alteration in fatty acid oxidation, due to FAT/CD36 deficiency, is detrimental or beneficial to the ischemic heart.

Using isolated working hearts from wild-type and FAT/CD36-null mice, we directly measured fatty acid oxidation rates in hearts perfused with both low and high levels of fatty acids. We also investigated the relationship between fatty acid oxidation and glucose oxidation rates in FAT/CD36-deficient hearts and determined whether FAT/CD36 deficiency was beneficial or detrimental to the ischemic heart.

**Methods**

**Mice**
CD36-KO mice and a littermate control line were created as previously described. Mice were backcrossed ×6 to C57Bl/6. Mice were maintained in a fully accredited facility on a 12-hour dark/light schedule with ad libitum access to food and water. All experiments were performed with the approval of the institutional animal care and use committee.

**Heart Perusions**
Hearts from wild-type and FAT/CD36-KO male mice, aged 10 to 12 weeks, were perfused in the working mode as described previously. Briefly, mice were anesthetized with 12 mg of pentobarbital sodium.

**Measurement of Cardiac Function**
Heart rate and pressure measurements were recorded with a pressure transducer in the aortic outflow line (Harvard Apparatus). Data were collected with an MP100 system from AcqKnowledge (BIOPAC Systems, Inc). Cardiac output and aortic flows were obtained by measuring the flow into the left atrium and from the afterload line with Transonic flow probes. Cardiac work was calculated as the product of peak systolic pressure and cardiac output. Coronary flow was calculated from the difference of the cardiac output and aortic flows. Frozen hearts were powdered, and approximately 20 mg (wet weight) was dried at 60°C overnight to remove all water (dry weight). The ratio of this sample (dry/wet weight) was used to calculate the total dry mass of the heart.

**Glucose Oxidation Rates**
Glucose oxidation rates were determined by measuring 14CO2 released from the metabolism of [U-14C]glucose, as described. Briefly, released 14CO2 was trapped with the use of 1 mol/L hyamine hydroxide and collected by continuously bubbling outflow gases from the perfusion apparatus through 15 mL of hyamine hydroxide. A 300-μL sample of hyamine hydroxide was taken every 10 minutes. The 14CO2 trapped in the perfusion buffer was released by the addition of 9N H2SO4 to 1 mL of perfusion buffer in sealed test tubes. The flasks were shaken overnight, and the released 14CO2 was trapped by the center well containing 300 μL of hyamine hydroxide.

**Fatty Acid Oxidation Rates in Hearts Perfused**
Fatty acid oxidation rates were determined from the release of 3H2O, derived from the metabolism of [9,10-3H]palmitate, as described. Briefly, H2O was separated from 9,10-[3H]palmitate by mixing 0.5 mL of the perfusion buffer samples with 1.88 mL of a 1:2 vol/vol ratio of chloroform and methanol. Next 0.25 mL of chloroform was added, followed by the addition of 0.625 mL of 1.1 mol/L KC1, dissolved in 0.9 mol/L HCl. Samples were allowed to separate into polar and nonpolar phases, and the polar phase was removed. The polar phase was mixed with 1 mL chloroform, 1 mL methanol, and 0.9 mL of KC1/HCl mixture. Again, the polar and nonpolar phases were allowed to form, and a 0.5-mL aliquot of the polar phase was removed and counted for 3H.

**Calculation of Tricarboxylic Acid Cycle Activity**
Contribution of both glucose and palmitate oxidation to the tricarboxylic acid (TCA) cycle was calculated as the product of the respective rates of oxidation and the amount of acetyl-CoA derived from glucose and palmitate, respectively. A value of 2 acetyl-CoA per molecule of glucose oxidized and 8 acetyl-CoA per molecule of palmitate oxidized was used.

**Measurement of ATP Levels**
Frozen mouse heart tissue (20 mg) was homogenized in a 6% perchloric acid/0.5 mmol/L EGTA solution. The homogenate was incubated on ice for 10 minutes and centrifuged at 10 000g for 2 minutes. The supernatant was collected, and ATP concentrations were determined by HPLC as described.

**Statistical Analysis**
Data are expressed as mean±SE. Comparisons between wild-type and FAT/CD36-deficient hearts were performed with the unpaired Student 2-tailed t test. Differences were judged to be significant at P<0.05.

**Results**
Fatty Acid Oxidation Rates in Hearts Perfused Aerobically With 0.4 mmol/L Palmitate
To examine whether FAT/CD36-mediated fatty acid uptake is rate limiting for fatty acid oxidation, the rates of palmitate oxidation were determined from the release of 3H2O, derived from the metabolism of [9,10-3H]palmitate, as described. Briefly, H2O was separated from 9,10-[3H]palmitate by mixing 0.5 mL of the perfusion buffer samples with 1.88 mL of a 1:2 vol/vol ratio of chloroform and methanol. Next 0.25 mL of chloroform was added, followed by the addition of 0.625 mL of 1.1 mol/L KC1, dissolved in 0.9 mol/L HCl. Samples were allowed to separate into polar and nonpolar phases, and the polar phase was removed. The polar phase was mixed with 1 mL chloroform, 1 mL methanol, and 0.9 mL of KC1/HCl mixture. Again, the polar and nonpolar phases were allowed to form, and a 0.5-mL aliquot of the polar phase was removed and counted for 3H.
oxidation in the intact working heart were measured. In the presence of low levels of fatty acids (0.4 mmol/L palmitate), rates of fatty acid oxidation in wild-type hearts paralleled rates seen in other studies. Palmitate oxidation rates in FAT/CD36-KO hearts were significantly lower than those in wild-type hearts (Figure 1), supporting a role for FAT/CD36 as a fatty acid transporter in cardiac myocytes. Importantly, this did not result in compromised cardiac function (Table 1). Heart rate, coronary flow, cardiac work, and peak systolic pressure measurements were not significantly different between wild-type and FAT/CD36-KO hearts, and these values compared well with those published for wild-type hearts in previous studies.

**Fatty Acid Oxidation and Glucose Oxidation Rates in Hearts Perfused Aerobically With 1.2 mmol/L Palmitate**

To determine whether FAT/CD36-deficient hearts were energetically and/or functionally compromised during conditions in which the heart relies heavily on fatty acid oxidation for ATP, wild-type and FAT/CD36-KO hearts were perfused with a high concentration of palmitate (1.2 mmol/L). Under these conditions, rates of palmitate oxidation in wild-type hearts were 6-fold higher than rates measured in the presence of 0.4 mmol/L palmitate. Interestingly, fatty acid oxidation rates in FAT/CD36-KO hearts perfused with 1.2 mmol/L palmitate were also elevated approximately 5-fold but remained significantly lower than those in the wild-type hearts (Figure 2A). Because palmitate oxidation was significantly decreased in FAT/CD36-KO hearts, we determined whether an increase in glucose oxidation was compensating for the loss of palmitate-derived ATP. We found that the reduction in the rate of fatty acid oxidation was accompanied by a 3-fold increase in the rate of glucose oxidation in FAT/CD36-KO hearts (Figure 2B). In wild-type hearts perfused with 1.2 mmol/L palmitate, the majority of TCA cycle acetyl-CoA originated from palmitate (80%), and the remainder originated from glucose (20%) (Figure 2C). In contrast, FAT/CD36-KO hearts derived 62% of the total acetyl-CoA from glucose and only 38% from palmitate (Figure 2C). Despite this dramatic switch in energy substrate utilization, the total amount of TCA cycle acetyl-CoA derived from both palmitate and glucose oxidation was similar in both wild-type and FAT/CD36-KO hearts (Figure 2C), demonstrating that FAT/CD36-deficient hearts were not energetically compromised. In addition, as was the case when the hearts were perfused with 0.4 mmol/L palmitate, there were either no changes or no negative effects of FAT/CD36 deficiency on heart rate, peak systolic pressure, coronary flow, cardiac output, or cardiac work in hearts perfused with 1.2 mmol/L palmitate (Table 2), demonstrating that FAT/CD36-deficient hearts were also not functionally compromised.

**Effects of Ischemia on Wild-Type and FAT/CD36-Deficient Hearts**

To determine how altered energy substrate preference affects functional recovery from ischemia, wild-type and FAT/CD36-KO hearts were subjected to 30 minutes of aerobic perfusion and 18 minutes of global no-flow ischemia, followed by 40 minutes of aerobic reperfusion. During the preischemic aerobic period, FAT/CD36-deficient hearts exhibited significantly higher levels of cardiac work than wild-type hearts (Figure 3A). Although preischemic work in the FAT/CD36-KO hearts was significantly elevated compared with that in wild-type hearts, both the wild-type and FAT/CD36-KO hearts exhibited similar levels of cardiac work after ischemia (Figure 3A, Table 3). When expressed as percent recovery of cardiac work, the recoveries of FAT/CD36-KO and wild-type hearts were not significantly different (Figure 3B).

**Fatty Acid Oxidation and Glucose Oxidation Rates in Hearts Perfused Aerobically With 1.2 mmol/L Palmitate After Ischemia**

A recent study suggested that FAT/CD36-KO hearts recovered poorly after ischemia as a result of decreased fatty acid metabolism. To determine whether this was the case, we...
directly examined energy metabolism during reperfusion in wild-type and FAT/CD36-deficient hearts. On aerobic reperfusion after ischemia, fatty acid oxidation rates in FAT/CD36-KO hearts were significantly lower (37%) than those in wild-type hearts (Figure 4A). However, similar to the preischemic period, the decrease in fatty acid oxidation rates in FAT/CD36-deficient hearts was compensated by a 2-fold increase in the rate of glucose oxidation (Figure 4B). During reperfusion, wild-type hearts derived 66% of TCA cycle acetyl-CoA from palmitate. In contrast, FAT/CD36-KO hearts derived only half as much TCA cycle acetyl-CoA from palmitate (36%). There was a concomitant increase in TCA cycle acetyl-CoA from glucose in hearts from FAT/CD36-KOs: 34% versus 64% of TCA cycle acetyl-CoA from glucose in wild-type and FAT/CD36-KO hearts, respectively. Despite the continued depressed rates of fatty acid oxidation in FAT/CD36-KO hearts during reperfusion, the total amount of acetyl-CoA derived from palmitate and glucose oxidation was similar in both wild-type and FAT/CD36-KO hearts (Figure 4C). Furthermore, ATP levels measured in hearts at the end of reperfusion were similar in both groups (10.9 ± 1.2 versus 13.8 ± 1.2 nmol/g dry wt in wild-type and FAT/CD36-KO hearts, respectively), confirming that FAT/CD36-KO hearts were not energetically deficient during reperfusion after ischemia.

**Discussion**

FAT/CD36 has been proposed to be a major regulator of fatty acid uptake by hearts. Evidence in support of this includes studies using the nonmetabolizable fatty acid analogue BMIPP in FAT/CD36-deficient humans and KO mice. These studies showed a decrease in fatty acid transport in heart, but the importance of this in the intact functioning heart remains unknown. In the present study, we examined whether a decrease in FAT/CD36-mediated fatty acid uptake resulted in depressed rates of fatty acid oxidation in ex vivo working mouse hearts. Our data show that FAT/CD36-mediated fatty acid uptake accounts for 40% to 60% of fatty acid oxidation in the heart under conditions of low and high palmitate.

**TABLE 2. Cardiac Function in FAT/CD36-KO Mouse Hearts Aerobically Perfused With High Levels of Fatty Acids**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type (n=13)</th>
<th>FAT/CD36 KO (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats · min⁻¹</td>
<td>246±10</td>
<td>275±7*</td>
</tr>
<tr>
<td>Peak systolic pressure, mm Hg</td>
<td>69±1</td>
<td>68±1</td>
</tr>
<tr>
<td>Coronary flow, mL · min⁻¹</td>
<td>2.9±0.3</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td>Cardiac output, mL · min⁻¹</td>
<td>8.1±0.3</td>
<td>10±0.4†</td>
</tr>
<tr>
<td>Cardiac work, mL · mm Hg · min⁻¹ · 10⁻²</td>
<td>5.5±0.1</td>
<td>6.8±0.2‡</td>
</tr>
</tbody>
</table>

Functional parameters were measured in isolated working hearts from FAT/CD36-KO and wild-type mice perfused with 5.5 mmol/L glucose, 1.2 mmol/L palmitate, 3% bovine serum albumin, and 100 μU/mL insulin, at a 11.5-mm Hg preload and 50-mm Hg afterload.

*Significantly different from wild-type value (P<0.05).
†Significantly different from wild-type value (P<0.005).
‡Significantly different from wild-type value (P<0.01).

**Figure 2.** Palmitate oxidation (A), glucose oxidation (B), and TCA acetyl-CoA production (C) in FAT/CD36-KO and wild-type mouse hearts perfused with 1.2 mmol/L palmitate. Values represent mean±SE of 5 KO and 6 wild-type mouse hearts. Rates of palmitate oxidation and of 8 KO and 7 wild-type mouse hearts for glucose oxidation. Thirteen KO and wild-type mouse hearts were used to calculate acetyl-CoA production from palmitate oxidation and glucose oxidation. We calculated TCA cycle activity from the rates of palmitate and glucose oxidation from A and B, using 8 mol of acetyl-CoA for every 1 mol of palmitate oxidized and 2 mol of acetyl-CoA for every 1 mol of glucose oxidized. *Significantly different from wild-type mouse hearts (P<0.01).
The compensatory increase in glucose oxidation rates, which depressed fatty acid oxidation rates, is most likely due to a reason(s) for this increased function in FAT/CD36-KO hearts compared with the wild-type hearts (Table 2). Although the CD36-KO hearts exhibited increased levels of cardiac work (Table 1). However, at 1.2 mmol/L palmitate, FAT/CD36-deficient hearts, supported by our data, is the potential mechanism for the improved cardiac function of FAT/CD36-KO hearts compared with wild-type hearts. One possible reason for this is that the inability of FAT/CD36-KO hearts to oxidize fatty acids resulted in an energy-deficient state, while the observed increase in glucose oxidation rates, which maintained adequate TCA cycle ATP supply in the FAT/CD36-deficient hearts. Although we did not measure ATP levels in hearts before ischemia, total acetyl-CoA production was not different in the wild-type and FAT/CD36-deficient hearts, suggesting that ATP production in the 2 sets of hearts is similar before ischemia.

In stark contrast to the study of Irie et al, which showed general poor function of FAT/CD36-KO hearts, we found cardiac function to be significantly elevated in the FAT/CD36-KO hearts compared with wild-type hearts. One potential mechanism for the improved cardiac function of FAT/CD36-deficient hearts, supported by our data, is the observed increase in glucose oxidation rates, which would result in the production of more acetyl-CoA per molecule of oxygen and subsequent increase in cardiac efficiency. In the present study, hearts were perfused with insulin. However, insulin did not appear to be present in the perfusate in the present study, hearts were perfused with insulin. However, the concentration of insulin used in our study was supraphysiological, it is possible that this high concentration of insulin may have beneficial effects on function by itself. The presence of insulin in the perfusate may have increased cardiac glucose utilization, which could play a role in the improved performance observed in the FAT/CD36-KO hearts in the present study.

Our data suggest that FAT/CD36-deficient hearts, regardless of fatty acid concentration, are not energetically or functionally compromised during basal conditions that do not stress the heart. Because it has been reported that FAT/CD36 deficiency is detrimental during ischemia, we also subjected hearts to 18 minutes of global no-flow ischemia followed by 40 minutes of aerobic reperfusion. Again, in contrast to the study by Irie et al, we found that reperfused ischemic FAT/CD36-deficient and wild-type hearts recovered to a similar degree (Figure 3). In the present study, we confirmed that FAT/CD36-deficient hearts had significantly lower palmitate oxidation rates under these conditions, but this did not affect functional recovery. In the study of Irie et al, the authors speculated that the inability of FAT/CD36-KO hearts to oxidize fatty acids resulted in an energy-deficient state.

**TABLE 3. Cardiac Function in FAT/CD36-KO Mouse Hearts Aerobically Perfused With High Levels of Fatty Acids and Reperfused After Ischemia**

<table>
<thead>
<tr>
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<th>Wild Type (n=13)</th>
<th>FAT/CD36 KO (n=13)</th>
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<td>245±10*</td>
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<td>63±2</td>
<td>59±2</td>
</tr>
<tr>
<td>Coronary flow, mL·min⁻¹</td>
<td>2.9±0.4</td>
<td>4.2±0.9</td>
</tr>
<tr>
<td>Cardiac output, mL·min⁻¹</td>
<td>5.6±0.5</td>
<td>6.4±1</td>
</tr>
</tbody>
</table>

Functional parameters were measured in isolated working hearts from FAT/CD36-KO and wild-type mice perfused with 5.5 mmol/L glucose, 1.2 mmol/L palmitate, 3% bovine serum albumin, and 100 μU/mL insulin, at a 11.5-mm Hg preload and 50-mm Hg afterload. Hearts were perfused for a 30-minute aerobic period, followed by 18 minutes of global no-flow ischemia and 40 minutes of aerobic reperfusion. Cardiac function was measured at the end of the 40 minutes.

*Significantly different from wild-type value (P<0.05).
thereby compromising functional recovery. However, energy metabolism or energy status of the hearts during reperfusion was not measured in that study. Our data show that the loss of energy from decreased rates of palmitate oxidation was completely compensated by increased rates of glucose oxidation in FAT/CD36-deficient hearts. After ischemia, there is an overall decrease in oxidative metabolism for both wild-type and FAT/CD36-KO hearts. However, in the FAT/CD36-KO hearts, the relative contribution to overall acetyl-CoA production by glucose and fatty acids is not different between preischemic and postischemic hearts. Furthermore, ATP levels at the end of reperfusion in FAT/CD36-KO and wild-type hearts were similar (10.9±1.2 versus 13.8±1.2 nmol/g dry wt, respectively). Therefore, our results clearly show that FAT/CD36-deficient hearts are not energetically compromised during reperfusion and that the hypothesis of Irie et al. 21 is not supported by measurements of energy metabolism and status.

The discrepancy between our data and those of Irie et al. 21 may be explained by differences in the overall health of the hearts before experimentation. We noted, for example, the low end-diastolic pressures in the perfused hearts used in the study of Irie et al., 21 which suggests that the hearts used in their study exhibited poor function before ischemia. This is consistent with the fact that their hearts were not able to tolerate >6 minutes of ischemia and that all hearts (wild type and FAT/CD36 KO) failed to function after 12 minutes of ischemia. In our study, hearts were able to recover to approximately 50% of their preischemic values after 18 minutes of ischemia, whereas 12 minutes of ischemia resulted in 100% recovery of all hearts (data not shown). Another explanation for the differences in our data may be the apparent absence of albumin in the fatty acid–free perfusate used in the study of Irie et al. 21 Hearts perfused without fatty acids and albumin were compared with hearts perfused with fatty acids and albumin. It has previously been shown that albumin protects the heart against ischemic injury and that hearts perfused without albumin exhibit increased tissue edema. 22 Therefore, the absence of albumin in hearts perfused without fatty acids in the study of Irie et al. 21 could provide an alternative explanation for decreased function in the glucose-perfused FAT/CD36-KO hearts reperfused after ischemia. It is not unreasonable to predict that the FAT/CD36-KO hearts perfused in the absence of fatty acids and albumin, as in the study of Irie et al., 21 would be energetically deficient, but the effect cannot necessarily be related to absence of FAT/CD36 due to the artificial conditions used (ie, the total lack of fatty acids as a major source of myocardial energy). Although FAT/CD36 is responsible for 40% to 60% of cardiac fatty acid uptake, other mechanisms provide the heart with the remainder, and this could be an important nutritional resource that provides protection. The total lack of a major source of myocardial energy supply in the study of Irie et al. 21 makes conclusions with regard to FAT/CD36 function impossible. It is important to note that in the clinical situation of ischemia and reperfusion (ie, after myocardial infarction or after surgery), the heart is normally exposed to high levels of fatty acid. 29–31

Figure 4. Palmitate oxidation rates (A), glucose oxidation rates (B), and TCA acetyl-CoA production (C) in FAT/CD36-KO and wild-type mouse hearts during reperfusion after ischemia. Values represent mean±SE of 5 KO and 6 wild-type hearts for palmitate oxidation and of 8 KO and 7 wild-type hearts for glucose oxidation. All hearts were used to calculate relative contributions of palmitate and glucose oxidation to acetyl-CoA production. We calculated TCA cycle activity from the rates of palmitate and glucose oxidation from A and B, using 8 mol of acetyl-CoA for every 1 mol of palmitate oxidized and 2 mol of acetyl-CoA for every 1 mol of glucose oxidized. *Significantly different from wild-type mouse hearts (P<0.01).
Irie et al. concluded that the poor functional recovery of FAT/CD36-deficient hearts on reperfusion was due to a decrease in fatty acid transport and the subsequent drop in fatty acid oxidation and energy production. They concluded that lack of palmitate was therefore detrimental to the recovery of hearts during reperfusion. In contrast to these data, previous studies have shown that decreased fatty acid oxidation during reperfusion is actually beneficial to functional recovery of the heart. Normally, acetyl-CoA derived from fatty acid oxidation stimulates pyruvate dehydrogenase kinase, inactivates the pyruvate dehydrogenase complex, and decreases the rate of glucose oxidation. Therefore, if fatty acid oxidation is decreased during reperfusion, glucose oxidation would increase, leading to improved functional recovery. Moreover, pharmacological inhibition of fatty acid oxidation (ie, inhibition of carnitine palmitoyltransferase I) has also been shown to be beneficial to the reperfused ischemic heart. Thus, our data with regard to FAT/CD36-KO hearts are more consistent with previously published data than the results of Irie et al.

One observation made in the study of Irie et al. that cannot be attributed to perfusion conditions is that nonperfused FAT/CD36-KO hearts exhibited lower levels of ATP than the wild-type hearts. This decrease in ATP may reflect lower ATP/ADP ratio under aerobic conditions or lower ATP levels produced in FAT/CD36-KO hearts may be attributed to perfusion conditions is that nonperfused hearts are more consistent with previously published data than the findings of Irie et al. that lack of palmitate is therefore detrimental to the recovery of hearts in vivo, nor do the decreases in ATP adversely affect function in wild-type hearts. This decrease in ATP may reflect lower fatty acid oxidation in FAT/CD36-KO hearts than in wild-type hearts under aerobic conditions or lower ATP levels produced in FAT/CD36-KO hearts. However, there are no reports showing that the decreases in ATP affect function in FAT/CD36-KO hearts in vivo, nor do the decreases in ATP adversely affect function in vitro if hearts are adequately perfused. It is possible that the lower ATP levels produced in FAT/CD36-KO hearts may depress cardiac function only when stressed by potentially injurious perfusions, which may rapidly deplete myocardial ATP. In this instance, hearts with a higher energy reserve (ie, wild-type hearts) would be less susceptible to ischemic injury. Indeed, our study demonstrates that, when adequately perfused, the initial decrease in total ATP does not hinder cardiac function.

Conclusion

We show that FAT/CD36-mediated fatty acid uptake is an important regulator of fatty acid oxidation in the heart. Hearts from FAT/CD36-KO mice exhibit 40% to 60% lower rates of palmitate oxidation than do wild-type hearts. However, regardless of whether perfused under aerobic conditions or during reperfusion after ischemia, FAT/CD36-deficient hearts are not functionally or energetically compromised. This is because glucose oxidation rates compensate for the depressed rates of fatty acid oxidation. Therefore, in contrast to the findings of Irie et al., the loss of FAT/CD36 is not detrimental to the heart in either the absence or presence of ischemia.

Acknowledgments

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