Chronic activation of PPARα is detrimental to cardiac recovery after ischemia

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FAO has been proposed to impair functional recovery of the heart during reperfusion after ischemia (6, 18). Pharmacological interventions that inhibit FAO and stimulate glucose oxidation have been shown to improve cardiac functional recovery during reperfusion (19, 42).

Fatty acid metabolism in the heart is regulated by substrate availability and allosteric modulation of enzyme activity at the level of transcription of enzymes involved in cellular uptake and oxidation of fatty acids. AMP-activated protein kinase (AMPK) is one enzyme that controls this process, secondary to phosphorylating and inhibiting acetyl-CoA carboxylase (ACC) (21). ACC produces malonyl CoA, which is a potent inhibitor of mitochondrial fatty acid uptake. After ischemia, FAO rates are high, in part because of AMPK activation, resulting in an increase in ACC phosphorylation and a decrease in malonyl CoA control of FAO (11, 21). Another important site of FAO control is peroxisome proliferator-activated receptor (PPAR)α. PPARα, a member of the nuclear receptor superfamily, is activated by lipid ligands including long-chain fatty acids.

Recently we demonstrated (9) that mice lacking PPARα (PPARα−/− mice) have profoundly diminished cardiac FAO and increased glucose utilization. The expression of PPARα and its target genes is increased in the diabetic heart with an accompanying increase in FAO (14). Further, cardiac-specific overexpression of PPARα in transgenic mice (MHC-PPARα mice) exhibits a cardiac phenotype characterized by a marked increase in fatty acid uptake and oxidation rates with a concomitant decrease in glucose uptake and oxidation rates, a profile similar to that of the diabetic heart (12, 14, 17).

Despite this link between PPARα and FAO, the role of cardiac PPARα pathway in response to ischemia-reperfusion injury is still unclear. We hypothesize that PPARα-driven increases in FAO rates, as occurs in the diabetic heart, lead to enhanced susceptibility to ischemic injury. To test this hypothesis, MHC-PPARα and PPARα−/− mice were subjected to ischemia-reperfusion in isolated working heart perfusions.

METHODS

All studies were approved by the Health Sciences Animal Policy and Welfare Committee at the University of Alberta and the Animal Studies Committee of Washington University School of Medicine. Transgenic MHC-PPARα mice (C57BL/6 × CBA/J F1) were produced with FLAG epitope-tagged PPARα cDNA with cardiac α-my-
osin heavy chain (MHC) promoter as described previously (13). Of the three different lines described previously, we used a low-expressing MHC-PPARα line (line 404-4) because this level of overexpression exhibited a metabolic phenotype without any differences in cardiac function (14). Production of PPARα-null (PPARα−/−) and wild-type (WT) control (PPARα+/+) mice of the SV129 strain was described previously (23, 24).

**Isolated Mouse Working Heart Perfusion**

Isolated mouse working heart perfusions were based on a previously described procedure (7, 9). Adult male mice (11–12 wk old with 25–30 g body wt) fed ad libitum were heparinized (100 U ip) 10 min before anesthesia. Animals were then deeply anesthetized with pentobarbital sodium (5–10 mg ip), and hearts were excised and placed in an ice-cold Krebs-Henseleit bicarbonate (KHB) solution (in mM: 118 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 2.5 CaCl2, and 5.0 glucose, pH 7.4). Hearts were cannulated first via the aorta with a 15-gauge plastic cannula and perfused retrogradely by the Langendorff method with KHB solution (gassed with 95% O2-5% CO2). During this time, the left atrium was cannulated through a pulmonary opening with a 16-gauge steel cannula. Once the cannulation for working heart mode was complete, the Langendorff line was closed and the left atrial and aortic lines were opened and perfused with KHB solution containing 5.0 mM glucose, 1.2 mM palmitate bound to 3% fatty acid-free BSA and 600 pM insulin with a preload pressure of 11.5 mmHg and an afterload pressure of 50 mmHg.

The perfusion protocol included a 30-min aerobic perfusion of spontaneously beating hearts followed by an 18-min global ischemia and then a 40-min reperfusion with oxygenated buffer solution. The spontaneous recovery of heart function was monitored. To determine palmitate oxidation, glucose oxidation, and glycolytic rates, trace amounts of [9,10-3H]palmitate (0.1 μCi/ml) and [U-14C]glucose (0.1 μCi/ml) or [5-3H]glucose (0.1 μCi/ml) were used, respectively. For Western blot analysis for phospho-AMPK (p-AMPK), phospho-ACC (p-ACC), AMPK, and ACC expression, the following antibodies were used: anti-p-AMPK (Thr172) and AMPK (Cell Signaling Technology, Beverly, MA); p-ACC (Ser79) and ACC (Upstate Technology, Lake Placid, NY); and goat anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

**Biochemical Measurements**

Detection and quantification of nucleotides were performed by extracting them from powdered tissue into 6% perchloric acid and measuring with a modified high-pressure liquid chromatography procedure, as described previously (30). Triglycerides were extracted from heart tissue according to the method of Folch et al. (15) and measured by an enzymatic method with the Wako L-Type TG H kit (Wako Pure Chemical Industries, Richmond, VA) as detailed previously (4, 9).

For Western blot analysis for phospho-AMPK (p-AMPK), phospho-ACC (p-ACC), AMPK, and ACC expression, the following antibodies were used: anti-p-AMPKs (Thr172) and AMPK (Cell Signaling Technology, Beverly, MA); p-ACC (Ser79) and ACC (Upstate Technology, Lake Placid, NY); and goat anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Western blots were performed according to standard methods, using antibody dilutions as described previously (9). Results are expressed as a percentage of the optical density of a standard sample loaded on all membranes.

**Statistical Analysis**

Values are expressed as means ± SE. A Student’s t-test or one-way ANOVA on ranks was performed to determine the statistical difference between groups. In some cases repeated-measures ANOVA was performed. P < 0.05 was considered significantly different.

**RESULTS**

**Myocardial Substrate Utilization During Pre- and Postischemic Aerobic Perfusions in MHC-PPARα Mice**

**Glucose oxidation and glycolysis.** Figure 1, A and B, shows the steady-state glucose oxidation and glycolytic rates from hearts isolated from MHC-PPARα and nontransgenic (NTG) control mice. The substrate utilization rates were linear throughout the aerobic and reperfusion perfusion periods (data not shown). Both glycolysis (Fig. 1B) and glucose oxidation (Fig. 1A) rates were markedly lower compared with NTG hearts during preischemic aerobic perfusion. During reperfusion, although glucose oxidation and glycolytic rates recovered back to the preischemic values in both groups, the rates remained lower in MHC-PPARα mouse hearts.
Palmitate oxidation. Steady-state palmitate oxidation rates are shown in Fig. 1C. Consistent with our previous findings (9), palmitate oxidation rates during the preischemic period were significantly greater in MHC-PPARα mouse hearts compared with NTG controls. During reperfusion after the global ischemia, palmitate oxidation recovered back to significantly higher rates compared with the NTG littermate group.

The relative contributions of glucose oxidation, glycolysis, and palmitate oxidation to the total production of ATP are shown in Fig. 1D. ATP production was calculated based on the stoichiometry that each mole of glucose produces 30 moles of ATP during complete oxidation and 2 moles of ATP during glycolysis, and each mole of palmitate produces 105 moles of ATP during complete oxidation. Although the theoretical level of ATP does not account for any uncoupled oxidation that might have occurred, it gives a good perspective on the relative contribution of different substrates toward total ATP pools in the myocardium. In NTG control mouse hearts, during preischemic aerobic perfusions palmitate oxidation contributed 55% of total ATP (90.2 ± 15.0 μmol·min⁻¹·g dry wt⁻¹), with the rest of the ATP originating from glucose oxidation and glycolysis (74.4 ± 6.0 μmol·min⁻¹·g dry wt⁻¹). In MHC-PPARα hearts, >90% of ATP (237.7 ± 33.5 μmol·min⁻¹·g dry wt⁻¹) was derived from palmitate and <10% from glucose metabolism (15.2 ± 2 μmol·min⁻¹·g dry wt⁻¹). During reperfusion after global ischemia, the relative contributions of glucose and palmitate to total ATP production did not change in either NTG control or MHC-PPARα hearts compared with their preischemic levels (NTG: ATP from glucose 86.3 ± 6, from palmitate 77.5 ± 9.0; MHC-PPARα: ATP from glucose metabolism 19.8 ± 2, from palmitate oxidation 148.8 ± 60 μmol·min⁻¹·g dry wt⁻¹). When the cardiac metabolic efficiency was calculated as a ratio of total ATP produced from FAO and glucose oxidation over cardiac power, MHC-PPARα hearts exhibited a poor efficiency compared with that of NTG littermates during aerobic (MHC-PPARα 2.4 ± 0.5, NTG 1.4 ± 0.2 μmol·g dry wt⁻¹·J⁻¹) and postischemic (MHC-PPARα 6.0 ± 1.7, NTG 2.1 ± 0.3 μmol·g dry wt⁻¹·J⁻¹) periods.

Myocardial Substrate Utilization During Pre- and Postischemic Aerobic Perfusions in PPARα⁻/⁻ Mice

As expected, the steady-state glucose oxidation rates were significantly higher in PPARα⁻/⁻ hearts compared with those of WT controls. This increase in glucose oxidation was observed during both the pre- and postischemic aerobic perfusions (Fig. 2A). On the other hand, palmitate oxidation was significantly lower in PPARα⁻/⁻ mice during pre- and postischemic perfusions compared with WT littermates (Fig. 2B). Calculated ATP production from glucose and palmitate oxidation indicates that the PPARα⁻/⁻ mouse heart derives its energy mainly from glucose (Fig. 2C). The contribution of palmitate to total ATP during reperfusion remained low in PPARα⁻/⁻ hearts (ATP from glucose 96.8 ± 20.2, from palmitate 26.4 ± 3.2 μmol·min⁻¹·g dry wt⁻¹). WT control hearts derived their energy equally from glucose and palmitate metabolism both during the pre- and postischemic perfusions, similar to the NTG control hearts in the MHC-PPARα study (during preischemia ATP from glucose 32.4 ± 4.3 and from...
palmitate $57.0 \pm 9.7$ and during postischemia ATP from glucose $47.3 \pm 6.2$ and from palmitate $44.6 \pm 4.9$ $\mu$mol·min$^{-1}·g$ dry wt$^{-1}$). Metabolic efficiency in PPARα$^{-/-}$ mice was comparable to that in WT littermates both during preischemic (PPARα$^{-/-}$ 1.0 $\pm$ 0.3 and WT 1.1 $\pm$ 0.2 $\mu$mol·g dry wt$^{-1}·J^{-1}$) and postischemic (PPARα$^{-/-}$ 1.3 $\pm$ 0.2 and WT 1.7 $\pm$ 0.4 $\mu$mol·g dry wt$^{-1}·J^{-1}$) perfusions.

Biochemical Characteristics of MHC-PPARα and PPARα$^{-/-}$ Hearts After Ischemia-Reperfusion

The levels of triglycerides (TG) were not statistically different between the hearts of NTG and MHC-PPARα mice (36.8 $\pm$ 4.9 vs. 33.4 $\pm$ 5.2 $\mu$mol/g dry wt). However, the cardiac TG content was significantly decreased in PPARα$^{-/-}$ mice compared with WT (12.66 $\pm$ 0.73 vs. 27.8 $\pm$ 2.4 $\mu$mol/g dry wt; $P < 0.05$). AMPK is induced in response to metabolic stress. Previous studies have shown that AMPK could be a key player in the metabolic alterations during ischemia-reperfusion (22, 32, 36). Therefore, we measured phosphorylation of AMPK in the heart homogenates frozen at the end of reperfusion. Figure 3A shows that the ratio of p-AMPK to total AMPK (tot-AMPK) was greater, although not statistically significant, in cardiac tissues of MHC-PPARα compared with NTG (798 $\pm$ 63 vs. 701 $\pm$ 38) littermates. Ser79 phosphorylation of ACC, the downstream target of AMPK, was increased by 2.7-fold in MHC-PPARα hearts. The ratio of p-ACC to total ACC (tot-ACC) was 169.7 $\pm$ 29.5 in MHC-PPARα compared with 63.9 $\pm$ 10.9 in NTG (Fig. 3B, left; $P < 0.05$) hearts. In PPARα$^{-/-}$ hearts, p-AMPK/tot-AMPK was decreased by 57% compared with WT hearts (293 $\pm$ 32 vs. 513 $\pm$ 67; $P < 0.05$; Fig. 3A, right) without a significant change in percentage ratio of p-ACC to tot-ACC compared with that of WT littermates (51 $\pm$ 12 vs. 155 $\pm$ 56; $P > 0.05$; Fig. 3B).

Cardiac Function in MHC-PPARα and in PPARα$^{-/-}$ Mice

Table 1 lists various parameters of cardiac function in MHC-PPARα and NTG control mouse hearts perfused at 50-mmHg afterload and 11.5-mmHg preload. The values are the averages of 10-, 20-, and 30-min measurements of aerobic perfusion. Under the aerobic perfusion conditions, there were no significant differences in heart rate, peak systolic pressure, developed pressure, coronary flow, and rate-pressure product (heart rate $\times$ peak systolic pressure) between MHC-PPARα and NTG littermates (Table 1). As summarized in Table 2, except for cardiac output and cardiac power, other hemodynamic parameters like peak systolic pressure, developed pressure, coronary flow, and rate-pressure product were not different in PPARα$^{-/-}$ mice compared with WT littermates during aerobic perfusion.

Recovery of Cardiac Function During Postischemic Perfusion in MHC-PPARα Mice and PPARα$^{-/-}$ Mice

During 18 min of no-flow ischemia, the heart ceases to function. When reperfusion begins after ischemia, heart function starts to recover spontaneously from zero function. Except for coronary flow and developed pressure, recovery of heart rate, peak systolic pressure, cardiac output, and rate-pressure product was significantly lower in the MHC-PPARα group compared with NTG control hearts (Table 1). Recovery of cardiac power was markedly reduced compared with NTG controls during reperfusion after global ischemia (Fig. 4A). Because changes in heart rate could have contributed to the decrement in cardiac work, we calculated stroke work ($g \times m$),

Fig. 2. Steady-state glucose oxidation (A) and palmitate oxidation (B) rates and contribution of glucose oxidation and palmitate oxidation to the production of total ATP pool (C) in isolated working hearts from PPARα null (PPARα$^{-/-}$) and wild-type (WT) littermate control mice. Values are means $\pm$ SE of 5 PPARα$^{-/-}$ and 10 WT control hearts for glucose oxidation and palmitate oxidation rates. *Significantly different from WT control hearts ($P < 0.05$, 1-way ANOVA). Moles of ATP were calculated as in Fig. 1.
which is the product of stroke volume and peak systolic pressure. Table 1 shows that the mean stroke work for 40 min of postischemic reperfusion was significantly lower in MHC-PPAR\textsuperscript{α} hearts. Although mean cardiac power recovered back to 71 ± 6% and stroke work to 75 ± 7% of preischemic performance in NTG controls, MHC-PPAR\textsuperscript{α} hearts recovered to 45 ± 9% and 52 ± 10% of cardiac power and stroke work, respectively, during reperfusion.

Table 1. Cardiac functions of NTG and MHC-PPAR\textsuperscript{α} mice

<table>
<thead>
<tr>
<th></th>
<th>NTG (n = 19)</th>
<th>MHC-PPAR\textsuperscript{α} (n = 15)</th>
<th>NTG (n = 19)</th>
<th>MHC-PPAR\textsuperscript{α} (n = 15)</th>
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<tr>
<td>HR, bpm</td>
<td>305±7</td>
<td>268±9</td>
<td>270±8</td>
<td>185±17*\±</td>
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<td>PSP, mmHg</td>
<td>71±1</td>
<td>64±2</td>
<td>67±1</td>
<td>56±2*\±</td>
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<td>Developed pressure, mmHg</td>
<td>32±1</td>
<td>36±4</td>
<td>26±2*\±</td>
<td>24±5*\±</td>
</tr>
<tr>
<td>HR × PSP, bpm/mmHg × 10\textsuperscript{3}</td>
<td>22±1</td>
<td>18±1</td>
<td>18±1</td>
<td>12±1*\±</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
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<td>4±0.5</td>
<td>3.6±0.4</td>
<td>2.9±0.7</td>
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<tr>
<td>Cardiac output, ml/min</td>
<td>12.3±0.6</td>
<td>10.3±0.6</td>
<td>8.6±0.8</td>
<td>5.0±1.0*\±</td>
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<tr>
<td>Cardiac power, J/min</td>
<td>97±5</td>
<td>84±6</td>
<td>71±6</td>
<td>37±8*\±</td>
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<tr>
<td>Stroke work, g·m × 10\textsuperscript{-2}</td>
<td>3.9±0.2</td>
<td>3.6±0.3</td>
<td>3.0±0.3</td>
<td>2.1±0.4*\±</td>
</tr>
</tbody>
</table>

Values are means ± SE for n hearts for 30-min aerobic period and 40-min reperfusion period. Functional parameters were measured throughout the perfusion period with a preload of 11.5 mmHg and an afterload of 50 mmHg. NTG, nontransgenic; MHC-PPAR\textsuperscript{α}, transgenic with peroxisome proliferator-activated receptor \(\alpha\) and myosin heavy chain promoter; HR, heart rate; PSP, peak systolic pressure; bpm, beats per minute. *Significantly different from NTG littermate; \(\dagger\)different from own preischemic values. \(P < 0.05\) is considered significant.
Postischemic recovery of cardiac power during reperfusion was significantly higher in PPARα−/− mice compared with WT controls (Table 2 and Fig. 4B; P < 0.05). The mean rate of recovery of stroke work during 40 min of reperfusion was not significantly different between the two groups (Table 2). Recovery of heart rate was 75 ± 9% of preischemic levels in WT controls, whereas recovery was 80 ± 8% of preischemic levels in PPARα−/− hearts (Table 2). Although not statistically significant, PPARα−/− mice exhibited a better recovery of other parameters like peak systolic pressure and coronary flow compared with age-matched WT controls. It is interesting to note that mean cardiac power over the 40-min reperfusion period was significantly greater in PPARα−/− hearts compared with MHC-PPARα hearts (95 ± 8 vs. 37 ± 8 J/min; P < 0.05, one-way ANOVA followed by Fisher’s least significant difference multiple comparison). There is no significant difference between respective control groups (NTG littermates 71 ± 6 vs. WT littermates 63 ± 6 J/min).

**DISCUSSION**

A strong association between elevated myocardial FAO rates and reperfusion recovery of cardiac function after ischemia has been well documented in experimental and clinical studies (2, 13, 16, 29, 37). Furthermore, partial inhibition of FAO has proven beneficial in improving cardiac function after ischemia and in chronic heart failure (2, 13, 16, 29, 33, 37, 40). PPARα controls FAO by regulating the expression of genes involved in the uptake and oxidation of fatty acids. Despite this role of PPARα in the regulation of cardiac fatty acid metabolism, the role of PPARα in ischemia-reperfusion injury is not clear. The existing literature on the cardioprotective effects of PPARα agonists in ischemia-reperfusion is inconsistent (1, 8, 34, 44). Whereas some studies have shown that PPARα agonists are cardioprotective (8, 43, 44), other studies have not demonstrated any beneficial effect during postischemic recovery (1, 34). The differences may be related to the effects of specific agonists, duration of treatment, and differences in conditions to which the heart is exposed (i.e., the presence or absence of high levels of fatty acids), duration of ischemia, and type of ischemia (global vs. local). In the present study, we have clearly demonstrated that cardiac-restricted PPARα overexpression leads to increased FAO rates during aerobic and postischemic reperfusion, coinciding with a significant decrement in functional recovery of heart function during reperfusion. This decrease in heart function after ischemia in PPARα-overexpressing hearts was due to both a decreased recovery of heart rate and a decreased recovery of stroke work. Conversely, when the hearts from the mice lacking PPARα were subjected to ischemia-reperfusion, recovery of stroke work was improved compared with control hearts subjected to similar insults. This suggests that alterations in PPARα expression in the heart can have profound effects on functional recovery of ischemic hearts. Not surprisingly, hearts from PPARα−/− mice metabolized carbohydrates more favorably than fatty acids even during reperfusion after no-flow ischemia. These obser-

### Table 2. Cardiac functions of WT and PPARα−/− mice

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 10)</th>
<th>PPARα−/− (n = 5)</th>
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<th>PPARα−/− (n = 5)</th>
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<tr>
<td>HR, bpm</td>
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<td>74±2</td>
<td>73±2</td>
<td>66±3†</td>
<td>70±2†</td>
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<td>Developed pressure, mmHg</td>
<td>34±2</td>
<td>30±2</td>
<td>25±3†</td>
<td>27±2</td>
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<td>HR × PSP, bpm × mmHg × 10⁴</td>
<td>21±1</td>
<td>22±1</td>
<td>16±2</td>
<td>20±1</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
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<td>5.3±0.7</td>
<td>4.6±0.6</td>
<td>6.3±0.8</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>10.6±0.4</td>
<td>14.7±0.9*</td>
<td>8.5±0.6</td>
<td>12.4±1.2*</td>
</tr>
<tr>
<td>Cardiac power, J/min</td>
<td>88.1±4.4</td>
<td>119.7±7.1*</td>
<td>63.4±6.5</td>
<td>95.3±8.0*</td>
</tr>
<tr>
<td>Stroke work, g·m×10⁻²</td>
<td>3.8±0.2</td>
<td>4.6±0.2</td>
<td>3.3±0.1</td>
<td>4.1±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE for n hearts. Functional parameters were measured as in Table 1. WT, wild type; PPARα−/−, PPARα null. *Significantly different from WT littermate, †different from own preischemic values. P < 0.05 is considered significant.

![Fig. 4. Recovery of cardiac power in MHC-PPARα (A) and PPARα−/− (B) isolated working mouse hearts reperfused after no-flow global ischemia. Hearts were subjected to aerobic perfusion for 30 min, ischemia for 18 min, and reperfusion for 40 min. Cardiac power was calculated as the product of developed pressure and cardiac output. A conversion factor of 1.33 × 10⁻⁴ was used to convert cardiac power values from millimeters of mercury to joules (7). Values are means ± SE of 19 hearts from NTG control, 10 hearts from MHC-PPARα, 5 hearts from PPARα−/−, and 10 hearts from WT control mice. *Significantly different from NTG control hearts (P < 0.05, 1-way repeated-measures ANOVA).](http://ajpheart.physiology.org/)

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vations corroborate the already existing large body of literature that elevated FAO rates are indeed detrimental to the heart during reperfusion and that partial inhibition of FAO could be beneficial in the recovering heart.

PPARα controls the transcription of numerous genes involved in cardiac fatty acid metabolism (uptake and utilization) (5). Cardiac-restricted overexpression of PPARα (MHC-PPARα mice) results in stimulated expression of genes involved in cardiac fatty acid uptake (fatty acid transport protein 1 and CD36), thioredoxinification (fatty acyl-CoA synthetase 1), triacylglycerol synthesis (glycerol-3-phosphate acyltransferase and diacylglycerol acyltransferase), FAO (carnitine palmitoyl transferase 1 and acyl-CoA dehydrogenases), and malonyl-CoA decarboxylase (13). Furthermore, MHC-PPARα mice hearts exhibit a profound increase in palmitate uptake and oxidation and significantly decreased glucose utilization (13), which was confirmed during preischemic aerobic perfusion in this study. During postischemic reperfusion, the oxidation rates for palmitate were significantly increased in MHC-PPARα hearts compared with NTG littermates. Glucose oxidation also recovers almost to preischemic levels in control hearts during reperfusion, which is in contrast to earlier reports on rat heart substrate utilization (18). Mouse hearts in our hands seem to be more responsive to insulin (100 μU/ml) in the perfusate than rat hearts. Thus, despite rapid recovery of FAO during reperfusion, the glucose oxidation rates also resume preischemic levels in reperfused hearts and contribute to 40–50% of total energy production in the control hearts. Nevertheless, in MHC-PPARα hearts the majority of ATP (>80%) comes from palmitate during reperfusion, overriding the favorable effects of insulin on glucose metabolism. During postischemic reperfusion, the oxidation rates for palmitate were increased in MHC-PPARα hearts compared with NTG controls. During reperfusion after ischemia, MHC-PPARα hearts also exhibited significantly higher phosphorylation of ACC at Ser79. This phosphorylation is consistent with the increase in FAO seen in these hearts. Although Ser79 of ACC is a downstream target of AMPK, phosphorylation of AMPK was not significantly altered in MHC-PPARα heart homogenates. This suggests that kinases other then AMPK (such as protein kinase A) may be stimulated in MHC-PPARα hearts. Phosphorylation of ACC at Ser79 inhibits this enzyme, leading to diminished synthesis of malonyl-CoA, a known negative modulator of FAO. Thus PPARα could also mediate allosteric control of FAO via the ACC pathway in addition to the direct role of PPARα in upregulating other fatty acid oxidizing genes.

Despite the presence of insulin in our perfusions, glucose oxidation and glycolysis rates remained low during both the pre- and postischemic perfusions in MHC-PPARα hearts. This decrease in glucose metabolism is probably due to decreased glucose uptake by MHC-PPARα hearts and fatty acid inhibition of glucose oxidation (13). Although we did not measure GLUT4 in this study, we showed previously (14) that PPARα overexpression downregulates GLUT4 mRNA and protein expressions. ATP production calculations showed that 90% of total ATP was derived from palmitate oxidation and the remaining 10% was from glucose metabolism in MHC-PPARα hearts. Recently, Hopkins et al. (17) reported a much higher contribution of glucose oxidation (20–30%) to the total ATP pool, whereas FÀO contributed the remaining 70–80% of ATP in MHC-PPARα hearts. It is important to note that the present study used a higher concentration of palmitate (1.2 mM) in the perfusion buffer, which increases FAO rates contributing to ~90% of the total ATP production. However, glucose oxidation was not severely affected in the present study because of the presence of a high concentration of insulin in the perfusate. In the control hearts, palmitate and glucose metabolism contributed equally to the total ATP production in during preischemic aerobic perfusion. Conversely, PPARα−/− mouse hearts derived a preponderance of their ATP from glucose utilization pathways. Previous and present studies demonstrated that palmitate oxidation rates in these hearts were remarkably reduced and glycolysis and glucose oxidation rates were significantly elevated (9). The above metabolic profiles in PPARα−/− mouse hearts conform to the previous findings that the various lipid metabolizing genes in these hearts were not upregulated (24). On the basis of theoretical calculation of the total number of ATP molecules derived from fatty acid and glucose oxidation over cardiac power, it is apparent that MHC-PPARα hearts exhibited the least metabolic efficiency among all the groups.

Postischemic recovery of cardiac function was very poor in MHC-PPARα hearts compared with control hearts. The poor functional recovery can be attributed to elevated levels of FAO rates during perfusions before and after ischemia. High levels of FAO have been shown to inhibit glucose oxidation at the level of pyruvate dehydrogenase (6, 25). This can lead to lactate and proton accumulation, which are detrimental to the myocardium. Myocardial damage can also occur because of generation of excessive amounts of reactive oxygen species as a result of enhanced FAO. It was shown that mitochondria from ischemic myocardium could be a source of toxic reactive oxygen species due to defective complex I activity (35). Indeed, previous studies in MHC-PPARα mice demonstrated that high fat feeding resulted in significant increases of markers of oxidative stress (e.g., hydrogen peroxide) (12). Thus poor functional recovery of MHC-PPARα mouse hearts during ischemia-reperfusion could therefore be due to a high FAO-induced oxidative stress.

The low-overexpressing MHC-PPARα (line 404-4) hearts in the present study did not exhibit hypertrophy (body wt/dry heart wt 136 ± 7 vs. 130 ± 4 for NTG littermates). Previously, Finck et al. (14) reported that low levels of PPARα overexpression caused moderate hypertrophy without alterations in cardiac function. This discrepancy between the two studies could be due to differences in age and or differences in litters that were used in the above-described studies. On the basis of our data, although we can eliminate contributions of hypertrophy to impaired functional recovery, we still must establish the causal relationship between impaired postischemic function and increased FAO. Further interventional studies are under way to examine the underlying mechanism(s).

PPARα−/− hearts performed better during both aerobic and postischemic perfusions compared with WT littermates. On the contrary, previous studies (9, 34) did not observe any alterations in basal cardiac function. This is probably due to the differences in perfusion conditions. For example, Panagia et al. (34) used 11 mM glucose without palmitate or other fatty acids whereas Campbell et al. (9) perfused the hearts with 0.4 mM palmitate and 5.0 mM glucose. In the present study, we used 1.2 mM palmitate and 5.0 mM glucose in the perfusion buffer. Postischemic functional recovery of PPARα−/− hearts was...
also greater compared with WT controls, and the percent recovery was significantly higher compared with postischemic recovery of MHC-PPARα hearts. In PPARα−/− mouse hearts, FAO rates remained low and glucose oxidation rates remained high during reperfusion after ischemia. The high rates of glucose oxidation were probably due to a compensatory mechanism in PPARα−/− hearts such that their AMP-to-ATP ratio (data not shown) and AMPK and ACC activities were not altered compared with perfusion-matched WT controls, indicating that these hearts were not energetically compromised.

In summary, cardiac-specific overexpression of PPARα favors lipid oxidation at the expense of glucose metabolism during pre- and postischemic perfusion and conversely affects functional recovery of the heart after global ischemia. Our results seem to contradict reports on the beneficial effects of PPARα agonist on ischemia-reperfusion injury to the heart (20, 44). In contrast to studies using acute systemic activation with PPARα ligands, our model is a chronic, cardiac-specific activation of PPARα that was previously characterized to exhibit a phenotype that resembles the diabetic heart (12). Therefore, although PPARα agonists by their systemic effects (both metabolic and anti-inflammatory) may improve myocardial performance, localized activation of PPARα in the heart may have deleterious effects on ischemic myocardium, especially in the presence of clinically relevant high levels of free fatty acids (3, 27). This evidence suggests that cardiac PPARα antagonism could be a potential therapeutic target to treat ischemia-reperfusion injury.

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