In Vivo Metabolic Phenotyping of Myocardial Substrate Metabolism in Rodents

Differential Efficacy of Metformin and Rosiglitazone Monotherapy

Kooresh I. Shoghi, PhD; Brian N. Finck, PhD; Kenneth B. Schechtman, PhD; Terry Sharp; Pilar Herrero, MS; Robert J. Gropler, MD; Michael J. Welch, PhD

Background—Cardiovascular disease is the leading cause of death among diabetic patients, with alteration in myocardial substrate metabolism being a likely contributor. We aimed to assess noninvasively the efficacy of metformin and rosiglitazone monotherapy in normalizing myocardial substrate metabolism in an animal model of type 2 diabetes mellitus.

Methods and Results—The study used 18 male ZDF rats (fa/fa) with 6 rats in each group: an untreated group; a group treated with metformin (16.6 mg/kg/d), and a group treated with rosiglitazone (4 mg/kg). Each rat was scanned at age 14 weeks (baseline) and subsequently at 19 weeks with small-animal positron emission tomography to estimate myocardial glucose utilization (MGU) and myocardial utilization (MFAU), oxidation (MFAO), and esterification (MFAE). Treatment lasted for 5 weeks after baseline imaging. At week 19, rats were euthanized and hearts were extracted for expression analysis of select genes encoding for GLUT transporters and fatty acid transport and oxidation genes. In addition, echocardiography measurements were obtained at weeks 13 and 18 to characterize cardiac function. Metformin had no significant effect on either MGU or MFAU and MFAO. In contrast, rosiglitazone tended to enhance MGU and significantly reduced MFAU and MFAO. Rosiglitazone-induced increase in glucose uptake correlated significantly with increased expression of GLUT4, whereas diminished MFAO correlated significantly with decreased expression of FATP-1 and MCAD. Finally, changes in fractional短ening as a measure of cardiac function were unchanged throughout the study.

Conclusions—Treatment with rosiglitazone enhanced glucose utilization and diminished MFAO, thus reversing the metabolic phenotype of the diabetic heart. (Circ Cardiovasc Imaging. 2009;2:373-381.)

Key Words: metabolic imaging ■ type 2 diabetes ■ gene expression ■ response to therapy

Cardiovascular disease is the leading cause of death among diabetic patients.1,2 There is increased evidence suggesting that diabetic patients have a predisposition to heart failure resulting from impairment in heart muscle contraction, particularly abnormalities in diastolic function.3 This condition, termed diabetic cardiomyopathy, is often independent of vascular abnormalities and hypertension and is evident in both type 1 and type 2 diabetic patients. Although several theories have been put forth to explain diabetic cardiomyopathy, evidence has emerged that diabetic cardiomyopathy is at least partly a consequence of severe alterations in myocardial energy metabolism.4,5 In particular, insulin resistance in type 2 diabetes mellitus shifts the balance of substrate utilization such that the diabetic heart relies almost exclusively on fatty acids for its energy needs.6 High rates of fatty acid utilization result in accumulation of myocardial lipids and lipid intermediates, leading to lipotoxicity of the heart.7,8

Antidiabetes therapies such as metformin and rosiglitazone attempt to normalize substrate availability and to restore glycemic control by enhancing insulin sensitivity in the periphery.9 Metformin, a biguanide, is the most commonly prescribed oral antidiabetic drug to treat type 2 diabetes mellitus in humans.10 It is thought to act by decreasing hepatic glucose production through activation of AMP-activated protein kinase.11 Rosiglitazone, on the other hand, targets the peroxisome proliferator-activated receptor-γ (PPAR-γ) nuclear receptor. PPAR-γ agonists have profound effects on glucose and lipid metabolism. This class of drugs has been shown to improve insulin sensitivity in various animal models.12 In humans, rosiglitazone reduces whole-body insulin resistance by its insulin-sensitizing effects on liver, skeletal muscle, and adipose tissue.13–15 Recent studies
have shown that rosiglitazone but not metformin enhances myocardial glucose uptake in diabetic patients by enhancing insulin sensitivity. Little is known, however, about the in vivo efficacy of either metformin or rosiglitazone on myocardial fatty acid utilization.

Given the insulin-sensitizing properties of rosiglitazone, we hypothesized that improved insulin-stimulated glucose utilization would result in reduced myocardial fatty acid utilization in the diabetic heart. To that end, we performed multiparameter small-animal positron emission tomography (PET) imaging to assess myocardial glucose and fatty acid utilization in the diabetic heart of ZDF rats longitudinally after treatment with metformin and rosiglitazone. Additionally, we monitored changes in cardiac function by echocardiographic measurements. Finally, to validate PET findings and to provide a mechanistic insight, we performed expression analysis of genes encoding proteins involved in glucose and fatty utilization.

Methods
All chemicals, unless otherwise stated, were purchased from Aldrich Chemical Co Inc. Radioactive samples were counted on a Beckman 8000 γ-counter. Small-animal PET was performed on either the microPET Focus-120™ or Focus-220™ (Siemens Inc).

Synthesis of Radiopharmaceuticals
FDG is produced routinely in our laboratory with a commercially available module (CTI Molecular Imaging). [11C]Palmitate was synthesized according to published methods. [11C]Acetate is produced routinely in our laboratory with a commercially available acetate module (CTI).

Animal Preparation
Animal were prepared for small-animal PET imaging as described previously. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University’s Animal Studies Committee.

Echocardiographic Measurements
Noninvasive ultrasound examination of the heart was performed using a Vevo770 Ultrasound System (VisualSonics Inc) at ages 13 and 18 weeks, as described previously. Measures of left ventricular structure were used for partial volume corrections performed in conjunction with kinetic modeling (see below).

Small-Animal PET Imaging Protocol
Five seconds after a bolus injection of the radiopharmaceutical via tail vein, dynamic PET acquisition was started. Each rat was imaged at 2 time points: once at the age of 14 weeks and again at the age of 19 weeks. The imaging protocol consisted of a 60-minute dynamic acquisition PET with 18F-FDG (0.5 to 0.8 mCi) to characterize glucose utilization as described earlier and a 20-minute dynamic PET acquisition with [11C]acetate (0.6 to 0.8 mCi) to quantify myocardial blood flow (MBF) and myocardial oxygen consumption (MVO2), followed by a 20-minute acquisition with [11C]palmitate (0.6 to 0.8 mCi) to quantify myocardial fatty acid metabolism. During each imaging session, 5 to 6 whole-blood arterial samples were collected from the femoral artery to measure whole blood glucose (5 μL), free fatty acid (FFA; 20 μL), and insulin (5 μL) levels as well as to correct for the presence of 11C-metabolites, as described below and in detail by Sharp et al. Finally, heart rates were recorded at baseline and throughout the study. In total, the imaging protocol lasted 3 to 5 hours. Dynamic images were reconstructed using filtered back projection with a 2.5 zoom on the heart and 40 frames per imaging session.

Substrate Analysis
All substrate measurements were performed using commercially available, well-documented methods that have been validated in small animals. Substrate and insulin values reported within correspond to values obtained at baseline, just before PET imaging.

Metabolite Measurements
During [11C]acetate and [11C]palmitate imaging session, arterial whole blood samples were taken at 1, 2, 5, 10, 15, and 20 minutes after administration of radiopharmaceutical to correct for the contribution of CO2 to total blood radioactivity counts (11CO2, %).

Data Analysis
Extraction of Input Function
The input function was reconstructed by applying the hybrid-image and blood-sampling algorithm using whole-blood samples used for [11C]acetate metabolite correction.

Partial Volume Correction
Partial-volume corrections were derived as described previously by constructing a digital phantom of the heart for each study, based on echocardiographic measurements.

Estimation of MBF
MBF rates were characterized by fitting early [11C]acetate kinetics (typically up to 1 minute after injection) to the flow model, as described in an earlier work.

Estimation of MVO2
MVO2 was estimated by fitting a monoeponential (kmono) to the clearance phase of [11C]acetate kinetics, which has been shown to correlate to MVO2 by the relation MVO2=(kmono−0.018901)(0.0008992).

Estimation of Glucose Utilization
Glucose uptake rate and utilization were characterized by performing Patlak graphical analysis on anterolateral myocardial FDG volume of interest time activity curve (TAC). After some time, t>τ* (typically, last 30 minutes of image acquisition), a linear regression model (of order 1), was optimized against the normalized plasma TAC versus myocardial tissue volume of interest. The slope of the linear regression line provides the myocardial glucose uptake rate, MGU_{upr} of FDG. Myocardial glucose utilization (MGU) is calculated by MGU=LC^∗MGU_{upr}^∗[GLU]_p, where [GLU]_p denotes the peripheral concentration of glucose with a lumped constant (LC) LC=1.

Estimation of Fatty Acid Utilization
The kinetics of [11C]palmitate is characterized by the compartmental model depicted in Figure 1, 18-20. The model includes 4 compartments characterizing the uptake, β-oxidation with a rate constant k_{5} (min^{-1}), and esterification with a rate constant k_{6} (min^{-1}) of palmitate in tissue. Kinetic estimates k_{1}-k_{6} were determined by optimizing 0 to 15 minutes of PET data against myocardial tissue TAC. Myocardial fatty acid oxidation (MFAO, mmol/g/min) is defined as MFAO=k_{5}C_{2}^{(ss)}, whereas myocardial fatty acid esterification (MFAE, mmol/g/min) is defined as MFAE=k_{2}C_{2}^{(ss)}, where C_{2}^{(ss)} denotes the steady-state
concentration in the interstitial and cytosolic compartment (C2) with baseline concentration of FFA ([FFA]0) in plasma substituted for the baseline concentration of [11C]palmitate. Total myocardial fatty acid utilization (MFAU) is given by MFAU = MFAO + MFAUpR defined by MFAUpR = MFAU/M[FFA], respectively.

RNA Isolation and Real-Time Reverse-Transcription Polymerase Chain Reaction

Hearts were frozen at −80°C until RNA was isolated for gene expression analysis. Total RNA was isolated from the heart by using RNAzol B (Tel-test) according to the manufacturer’s instructions to quantify gene expression of genes encoding for GLUT1, GLUT4, medium-chain acyl-CoA dehydrogenase (MCAD), mitochondrial carnitine palmitoyltransferase-I (mCPT-I), CD36, and fatty acid transport protein (FATP-I). RNA concentration and purity were determined by spectrophotometric absorbency at two dilutions. First-strand cDNA was generated by reverse transcription using 500 ng total RNA and the Applied Biosystems reverse transcription kit. RNA Isolation and Real-Time Reverse-Transcription Polymerase Chain Reaction

![Figure 1. Compartmental model depicting the kinetics of [11C]palmitate in tissue.](image)

**Table 1. Descriptive Data, Hemodynamics, and Plasma Substrate Levels of Untreated and Treated ZDF (fa/fa) Rats**

<table>
<thead>
<tr>
<th></th>
<th>W14</th>
<th>Δ</th>
<th>W14</th>
<th>Δ</th>
<th>W14</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight, g</strong></td>
<td>316.35±17.66</td>
<td>27.73±11.63*</td>
<td>330.32±50.35</td>
<td>32.08±46.53</td>
<td>328.17±17.56</td>
<td>184.50±32.14††</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td>207.17±13.29</td>
<td>−0.75±4.99</td>
<td>216.67±26.34</td>
<td>−22.50±17.80</td>
<td>223.33±34.21</td>
<td>−1.33±13.25</td>
</tr>
<tr>
<td><strong>HbA1C, %</strong></td>
<td>7.72±0.66</td>
<td>−0.33±0.45</td>
<td>6.98±1.36</td>
<td>−1.52±0.55†</td>
<td>7.67±0.50</td>
<td>−3.27±0.39‡‡</td>
</tr>
<tr>
<td><strong>Insulin, mU/mL</strong></td>
<td>26.24±6.97</td>
<td>−8.17±12.58</td>
<td>28.24±15.01</td>
<td>6.31±32.37</td>
<td>42.18±22.13</td>
<td>−14.05±32.92</td>
</tr>
<tr>
<td><strong>Glucose, mmol</strong></td>
<td>18.28±4.97</td>
<td>4.30±4.85</td>
<td>22.94±3.38</td>
<td>−1.52±5.33</td>
<td>16.91±5.23</td>
<td>4.89±6.69</td>
</tr>
<tr>
<td><strong>FFA, mmol</strong></td>
<td>1.73±0.68</td>
<td>0.19±1.32</td>
<td>2.95±1.12</td>
<td>0.26±1.24</td>
<td>2.91±1.24</td>
<td>−0.88±1.19</td>
</tr>
<tr>
<td><strong>MBF, mL/g/min</strong></td>
<td>5.30±0.92</td>
<td>0.12±1.37</td>
<td>5.12±1.66</td>
<td>0.33±2.84</td>
<td>5.42±2.60</td>
<td>−0.45±2.51</td>
</tr>
<tr>
<td><strong>MVO₂, mmol/g/min</strong></td>
<td>33.89±6.12</td>
<td>1.46±11.03</td>
<td>30.60±6.14</td>
<td>3.44±8.25</td>
<td>34.31±3.75</td>
<td>8.24±12.15</td>
</tr>
</tbody>
</table>

*Significantly higher than week 14.
†Treatment response significantly higher than no treatment.
‡P<0.05 was considered significant.

Real-time reverse transcription–polymerase chain reaction was performed using the ABI PRISM 7500 Fast sequence detection system and Taqman Fast Universal master mix (Applied Biosystems). Arbitrary units of target gene mRNA were corrected to 36B4 RNA content to control for loading.

**Statistical Analysis**

**Efficacy of Treatment**

Treatment effects of metformin and rosiglitazone on descriptive data, echocardiographic measurements, and PET outcome measures were determined by calculating change from baseline (W14) to follow-up (W19) and using 1-way ANOVA for each measure with 3 groups (untreated, metformin-treated, rosiglitazone-treated). Statistical contrasts were subsequently used to perform all pairwise comparisons. Differences in gene expression were evaluated by a 1-way ANOVA on follow-up measures. Unless otherwise stated, all comparisons are reported as differences in outcome measures from baseline (ie, W19 to W14) between groups, that is, untreated, metformin-treated, and rosiglitazone-treated. A value of P<0.05 was considered statistically significant. Statistical calculations were performed using SAS. Data are denoted as mean±SD.

**Correlation Between Echocardiographic Measurements**

The Pearson cross-correlation between echocardiographic measurements was assessed in the ZDF+metformin and ZDF+rosiglitazone group as well as for the combined dataset.

**Correlation Between PET Measures and Gene Expression**

PET measures at W19 and corresponding gene expression data were grouped into 3 groups. In the first and second groups, untreated data were combined separately with metformin and rosiglitazone treatment data to form 2 groups, that is, ZDF/ZDF + metformin and ZDF/ZDF + rosiglitazone, primarily to enhance the dynamic range of the data. The third group combined all data at W19, that is, ZDF/ZDF + metformin/ZDF + rosiglitazone. The correlation between PET measures and gene expression data were subsequently evaluated by the Pearson correlation, r. A value of P<0.05 was considered significant. Correlations were performed with the statistical package SPSS (SPSS Inc).

**Results**

**Descriptive, Hemodynamic, and Blood Substrate Levels**

Untreated ZDF rats are characterized diabetic at W14, as indicated by elevated levels of glycosylated hemoglobin (HbA1C). Treatment with either metformin or rosiglitazone...
significantly lowered HBA1C levels by approximately 18% (P=0.0005) and 39% (P<0.0001) relative to untreated rats. Moreover, treatment with rosiglitazone lowered HbA1C significantly more than metformin (P<0.0001). Despite the reduction in HbA1C after treatment, we did not observe a concomitant reduction in circulating glucose levels after treatment (P>0.05). Rosiglitazone-treated ZDF rats gained significantly more weight than untreated ZDF rats (P<0.0001) and metformin-treated rats (P<0.0001). There were no statistical differences in heart rate, insulin, FFA, MBF, and MVO$_2$ measures between age-matched ZDF rats and after treatment (Table 1).

**Echocardiographic Measurements**

Left ventricular mass (LVM) measures increased significantly (P<0.05) in both the metformin and rosiglitazone groups relative to untreated rats. Additionally, rosiglitazone significantly enhanced diastolic measures of LVID (LVIDd) in comparison to untreated and metformin-treated ZDF rats (P=0.0127 and P=0.025, respectively), although LVIDd correlated significantly with LVM (P<0.0001). In general, we did not observe a significant correlation between LVM and weight of ZDF rats, except in the ZDF+rosiglitazone group, in which the correlation was attributed to clustering of data by weight and LVM (data not shown). When LVM was normalized by weight (LVMI), changes in LVMI were insignificant after rosiglitazone treatment. On average, changes LVMI after metformin treatment were significantly higher than rosiglitazone treatment (P=0.007) and no treatment (P=0.042), albeit the latter is marginally significant. Finally, percent fractional shortening (FS), a measure of systolic function, was not altered significantly throughout the time-course of the study (Table 2).

**Myocardial Substrate Metabolism**

Neither untreated nor metformin-treated ZDF rats exhibited a significant change in MGU and uptake rate (MGU$_{UpR}$), although there is a trend for an increase in uptake and utilization with age (Figure 2). The rosiglitazone-induced increase in MGU$_{UpR}$ was only marginally insignificant compared with untreated ZDF rats (P=0.06) (Figure 2), with a net relative increase in MGU$_{UpR}$ of 142% compared with untreated rats. Similar to the pattern of glucose metabolism, neither untreated nor metformin-treated ZDF rats displayed a significant change in MFAU$_{UpR}$ (Figure 3A). Rosiglitazone-treated rats, however, exhibited a significant reduction in MFAU$_{UpR}$ compared with untreated ZDF rats (P=0.016) and metformin-treated rats (P=0.024). The relative difference in reduction of MFAU$_{UpR}$ compared with untreated and metformin-treated rats was 76% and 89%, respectively. We did not observe a significant change in MFAE between groups (Figure 3B) suggesting that the fraction of fatty acids that are stored is unchanged. However, MFAO$_{UpR}$ was

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**Table 2. Echocardiographic Measurements for Untreated and Treated ZDF (fa/fa) Rats**

<table>
<thead>
<tr>
<th></th>
<th>ZDF</th>
<th>ZDF + Metformin</th>
<th>ZDF + Rosiglitazone</th>
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<tbody>
<tr>
<td></td>
<td>W13 (n=6)</td>
<td>W13 (n=6)</td>
<td>W13 (n=6)</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>9.16±0.32</td>
<td>9.13±0.16</td>
<td>8.98±0.13</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>5.53±0.29</td>
<td>5.78±0.39</td>
<td>5.25±0.48</td>
</tr>
<tr>
<td>LVM, g</td>
<td>1.00±0.07</td>
<td>1.17±0.10</td>
<td>1.05±0.07</td>
</tr>
<tr>
<td>LVMI</td>
<td>3.15±0.17</td>
<td>3.61±0.57</td>
<td>3.21±0.28</td>
</tr>
<tr>
<td>FS, %</td>
<td>39.72±1.72</td>
<td>36.70±4.41</td>
<td>41.60±5.44</td>
</tr>
</tbody>
</table>

*Values are represented as mean±SD. W14 indicates average baseline; Δ, change from baseline.

†Treatment response significantly higher than no treatment.

‡Treatment response significantly higher than metformin treatment.

*Treatment effect is marginally insignificant compared with untreated ZDF rats (P=0.06).

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**Figure 2.** MGU$_{UpR}$ (A) and MGU (B) in untreated (ZDF), metformin-treated (ZDF + MET), and rosiglitazone-treated (ZDF + ROSI) rats at week 14 (W14) and week 19 (W19). MGU$_{UpR}$ represents the intrinsic capacity of the heart to uptake glucose independent of the plasma concentration of glucose ([Glu]$_p$), whereas MGU is derived by the relation $MGU = MGU_{UpR} * [Glu]_p$. All results are presented as mean±SD. *Treatment effect is marginally insignificant compared with untreated rats (P=0.06).
significantly lower in ZDF rats treated with rosiglitazone compared with untreated ZDF rats ($P=0.0189$) and metformin-treated rats ($P=0.01$). The combined effect of enhanced glucose uptake rate and reduction in myocardial fatty utilization rate resulted in a significant ($P<0.001$) net gain in fractional glucose utilization as depicted in Figure 4.

**Gene Expression Analysis**

ZDF rats treated with rosiglitazone exhibited a marginally significant ($P=0.04$) increase in expression of GLUT1 and a $>2$-fold increase in the expression of GLUT4 ($P=0.0028$). In contrast, metformin did not alter expression of GLUT1 and GLUT4 significantly ($P>0.05$) (Figure 4A). Treatment with metformin also did not significantly alter gene expression of fatty acid transporters CD36 and FATP1 ($P>0.05$). Treatment with rosiglitazone, however, significantly downregulated expression of FATP1 relative to untreated and metformin-treated ZDF rats ($P=0.014$ and $P=0.0375$, respectively), whereas gene expression of CD36 was unchanged. Additionally, rosiglitazone-treated rats exhibited a significant decrease in expression of MCAD relative to untreated ZDF rats ($P=0.03$) and metformin-treated ZDF rats ($P=0.0388$). We did not observe significant differences ($P>0.05$) in the expression of the gene encoding for mCPT-I. Finally, the expression of PPAR-α and its cardiac-enriched coactivator protein PGC-1α were not significantly altered ($P>0.05$) by either rosiglitazone or metformin treatment (Figure 5).

**Correlation Between PET Measures and Gene Expression**

In the combined ZDF/ZDF+metformin dataset, MGU$_{Upr}$ significantly correlated with GLUT1 ($P=0.027$), whereas MFAO$_{Upr}$ measures significantly correlated with gene expression of CD36 ($P=0.018$) and mCPT-I ($P=0.007$). In the combined ZDF/ZDF+rosiglitazone dataset, MGU$_{Upr}$ highly correlated with gene expression of GLUT4 ($\tilde{n}=0.71, P=0.007$). In addition, MFAO$_{Upr}$ significantly correlated with gene expression of FATP1 ($\tilde{n}=0.74, P=0.004$) and MCAD ($\tilde{n}=0.71, P=0.007$), with a high correlation between gene expression of FATP1 and MCAD ($\tilde{n}=0.87, P=0.001$) (not shown). Finally, when all 3 groups (ZDF/ZDF+metformin/ZDF+rosiglitazone) were combined, we observed no significant correlation between MGU$_{Upr}$ and GLUT1 gene expression ($\tilde{n}=0.40, P=0.07$). In contrast, MGU$_{Upr}$ remained significantly correlated with gene expression of GLUT4 ($\tilde{n}=0.55, P=0.017$), whereas MFAO$_{Upr}$ remained significantly correlated with FATP1 ($\tilde{n}=0.48, P=0.03$), albeit the correlation is slightly lower compared with values obtained using the ZDF/ZDF+rosiglitazone data set (Table 3).

**Discussion**

The metabolic phenotype of the diabetic heart is characterized by impairments in myocardial glucose uptake and oxidation and overreliance on fatty acids for energy production as reflected in the complex interplay in insulin resistance in both peripheral tissues and heart. The detrimental effects of this metabolic shift include an increased susceptibility to ischemia, enhanced reactive oxygen species production, and the potential for accumulation of myocardial lipids and lipid intermediates leading to lipotoxicity of the heart.7

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**Figure 4.** The fractional net gain in glucose utilization uptake rate in untreated (ZDF), metformin-treated (ZDF+MET) and rosiglitazone-treated (ZDF+ROSI) ZDF rats at week 14 (W14) and week 19 (W14). Y-axis is in logarithmic scale. All results are presented as mean±SD. $P<0.05$ was considered significant. *Treatment is significantly better than no treatment; †rosiglitazone treatment is significantly better than metformin treatment.
Therapeutic interventions are designed primarily to achieve a level of glycemic control and treatment of other comorbidities such as hyperlipidemia and hypertension. However, even though several classes of antidiabetic agents may improve glucose homeostasis and insulin sensitivity, their impact on myocardial substrate may differ. In this work, we used small-animal PET to noninvasively assess the efficacy of human dose-equivalence of metformin and rosiglitazone in reversing alterations in myocardial glucose and fatty acid utilization in the ZDF rat.

Rosiglitazone-induced myocardial glucose uptake in ZDF rat (Figure 2) is consistent with observations in diabetic patients and perfused heart studies. Because MGU_{UpR} is independent of plasma glucose levels, it provides a lumped measure for the machinery involved in glucose utilization, such as levels of GLUT. We showed that the decline in MGU_{UpR} was associated with low expression of gene encoding for GLUT4. Indeed, several studies have shown that decreased glucose uptake is linked to decreased GLUT4 protein and mRNA levels in type 1 diabetes and various models of type 2 diabetes. In agreement with PET data, gene expression analysis suggests that rosiglitazone-induced PPAR-γ activation resulted in elevated GLUT4, and to a lesser extent, GLUT1 (Figure 5A), confirming previous reports in which rosiglitazone was shown to increase myocardial gene expression of GLUT1 and GLUT4 in ZDF rats. The significant correlation between MGU_{UpR} and gene expression measures of GLUT further supports their association (Table 3).

Our data indicate that in parallel to increased glucose utilization, rosiglitazone but not metformin significantly reduced MFAU (Figure 3). The observation that MFAE is unaltered after treatment suggests that the decrease is MFAU is attributed primarily to inhibition of MFAO in cardiac muscle. Interestingly, in a recent study rosiglitazone enhanced fatty oxidation in skeletal muscle, although glucose utilization in the same study was not determined. Considering the potential lipotoxic effects of accumulated fatty acids, it can be argued that stimulating fatty acid oxidation in muscle can decrease fatty acid storage and therefore increase insulin-stimulated glucose uptake. However, myocardial fatty acid and glucose utilization rates are almost always reciprocal. Randle et al originally demonstrated that high-level fatty acid utilization is associated with diminished glucose utilization in rat heart and vice versa. Thus, one interpretation of the present study is that rosiglitazone caused a metabolic shift by blunting the heightened uptake and oxidation of fatty acids, resulting in an increase in glucose utilization. However,

Table 3. Pearson Correlations Between PET Measures of MGU_{UpR}, MFAO_{UpR}, and Gene Expression at W19

<table>
<thead>
<tr>
<th></th>
<th>ZDF/ZDF + Metformin</th>
<th>ZDF/ZDF + Rosiglitazone</th>
<th>ZDF/ZDF + Metformin/ ZDF + Rosiglitazone</th>
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<tbody>
<tr>
<td></td>
<td>ρ</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>MGU_{UpR} vs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GLUT1</td>
<td>−0.66</td>
<td>0.02698*</td>
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</tr>
<tr>
<td>GLUT4</td>
<td>0.01</td>
<td>0.48850</td>
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<tr>
<td>CD36</td>
<td>0.09</td>
<td>0.01728*</td>
<td>12</td>
</tr>
<tr>
<td>FATP1</td>
<td>0.09</td>
<td>0.39339</td>
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<tr>
<td>mCPT1</td>
<td>0.68</td>
<td>0.00730*</td>
<td>12</td>
</tr>
<tr>
<td>MCAD</td>
<td>−0.05</td>
<td>0.43995</td>
<td>11</td>
</tr>
</tbody>
</table>

ρ indicates Pearson correlation; P, significance value of correlation; N, No. of data points. *Significant at P<0.05.
whether these metabolic effects are primary or secondary as the result of altered substrate flux or insulin sensitivity remains to be determined.

To provide a mechanistic insight into the observed decrease in MFAO in cardiac muscle, we characterized the expression profile of genes involved in fatty acid transport and oxidation as well as PPAR-α and PGC-1α. Both PPAR-α and PGC-1α were unaltered with treatment consistent with previous reports.31 The observation that rosiglitazone led to a downregulation of FATP-I but not FAT/CD36 (Figure 5) is somewhat surprising and may indicate a specific role for each transport system. It is possible that the different transporters direct fatty acids toward distinct intracellular pools. Recent work in transgenic mice suggested that FATP-I overexpression directed fatty acids primarily toward the oxidation pool.37 Accordingly, our data indicate that reductions in FATP-I expression correlated with diminished MFAO. On the other hand, both FAT/CD36 and MFAE are unaffected by rosiglitazone, suggesting that fatty acids entering through the FAT/CD36 transport system may be directed first toward storage pool or serve as substrates in signal transduction, as has been suggested by others.38 Thus, additional work using mouse models with specific genetic alterations in the activity of each of these pathways is required to determine the role of fatty acid transport.

In parallel to diminished FATP-I gene expression, rosiglitazone significantly reduced expression of the gene encoding MCAD, a highly regulated step in mitochondrial β-oxidation of fatty acids. Accordingly, cardiac intrinsic measures of myocardial fatty acid oxidation rates, MFAO_Upr, correlated significantly with both FATP-I and MCAD, suggesting that PET measures of MFAO_Upr provide a noninvasive measure of myocardial fatty acid oxidation. Furthermore, the high correlation between expression of FATP-I and MCAD suggests that the 2 genes are coregulated after treatment with rosiglitazone. Both MCAD and FATP-I are known to be target genes that are activated by PPARs.39 The observed repression by a PPAR-γ agonist may, at first glance, seem counterintuitive. However, these results are consistent with previous work40 and suggest that rosiglitazone is eliciting its effects on cardiac substrate selection through extracardiac effects on whole animal metabolism.

Despite a significant decrease in HbA1C levels, we did not observe a concomitant decrease in peripheral glucose levels that can be attributed to the effects of the anesthetic agent on hepatic glucose production, metabolism, and disposal in the periphery.41 In addition, we did not observe reductions in plasma FFA with either treatment. Several groups observed reductions in peripheral FFA levels when prediabetic ZDF rats (≤8 weeks old) were treated with either metformin or PPAR-γ agonists (such as troglitazone and rosiglitazone)8,31,42; the abovementioned studies were designed to assess the effectiveness of the agents to delay or prevent the onset of diabetes. In contrast, we used ZDF rats at age 14 weeks, which are considered diabetic by all accounts, to assess response to treatment. Indeed, in recent investigations involving diabetic patients undergoing treatment with either metformin or rosiglitazone, the authors did not observe reductions in FFA levels, whereas improvements in glucose levels were mixed during either fasting or hyperinsulinemia states.16,17,43,44 Thus, the effectiveness of therapeutic agents in lowering peripheral substrate levels may be limited by the severity of the disease and the duration of treatment.

The echocardiographic data suggest that treatment with either metformin or rosiglitazone induced changes in LVM. However, when LVM was normalized by the weight (LVMI), changes in LVMI were insignificant after rosiglitazone treatment and only marginally significant after metformin treatment. In addition, because LVIDd correlated with LVM, changes in LVIDd can be attributed to weight gain in the rosiglitazone treatment group. FS, a measure of systolic function, did not improve with either treatment. Furthermore, in an earlier publication, we did not observe differences in FS between age-matched ZDF rats and lean littermates.21 In a recent study, however, Zhou et al8 assessed myocardial function in ZDF rats after a 13-week treatment with Troglitazone, an antidiabetic drug of the same class (thiazolidinedione) as rosiglitazone. The authors noted improvements in FS at 20 weeks compared with untreated rats. In contrast, in this work we report alterations in myocardial substrate metabolism as early as 5 weeks after treatment. Taken together, further studies are needed to characterize the time delay between metabolic alterations and cardiac function both before onset of disease and after therapy.

In summary, we used small-animal PET to assess changes in myocardial substrate metabolism after monotherapy with either metformin or rosiglitazone. Collectively, our data indicate that the PPAR-γ agonist rosiglitazone has profound effects on in vivo myocardial glucose utilization and fatty acid oxidation, which we confirmed against expression profiles of genes involved in glucose transport and fatty acid oxidation. In particular, we demonstrated noninvasively that treatment with rosiglitazone not only increased myocardial glucose utilization but reduced myocardial fatty acid oxidation, thus reversing the metabolic phenotype of the diabetic heart and resulting in significant fractional net gain in glucose utilization. Finally, our findings underscore both the translational capability and the potential use of PET in assessing the efficacy of therapies on myocardial substrate metabolism in vivo, noninvasively.

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Disclosures

None.
References


Cardiovascular disease is the leading cause of death among diabetic patients. There is increasing evidence suggesting that diabetic patients have a predisposition to heart failure resulting from impairment in heart muscle contraction, particularly abnormalities in diastolic function. One hypothesis argues that the observed abnormalities in diastolic function are a consequence of alterations in myocardial substrate metabolism such that the diabetic heart relies more on fatty acid metabolism than glucose metabolism to meet its energy needs. Increased reliance on fatty acids leads to accumulation of fatty acid intermediates as well as triglycerides resulting in lipotoxicity of the heart. In this work, we validated technology to noninvasively assess alterations in myocardial substrate metabolism, allowing observation of metabolic alterations in vivo potentially preceding development of abnormalities in diastolic function. Moreover, as treatment strategies aim to restore a level of glycemic control, the methodology allows for noninvasive monitoring of therapeutic efficacy, as we have demonstrated in this work.
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