Activation of PPARγ enhances myocardial glucose oxidation and improves contractile function in isolated working hearts of ZDF rats

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The normal heart readily adapts to changes in the environment by switching from one substrate to another. Additionally, it has been shown that the heart is a metabolic omnivore and functions best when oxidizing multiple substrates simultaneously. The ability of the myocardium to switch from fatty acids to carbohydrate and back to fatty acids (or to use multiple substrates simultaneously) may be lost in insulin resistance and diabetes, producing a state of metabolic inflexibility. We (43) have proposed that the loss of metabolic flexibility is a feature of hypertrophy and heart failure. We now propose that prolonged exposure to the abnormal metabolic milieu of increased fatty acids in the plasma leads to a similar loss of metabolic flexibility in the heart. Loss of metabolic flexibility probably begins as an adaptive process that occurs before the onset of contractile dysfunction (14). In later stages, loss of metabolic flexibility contributes to cardiac dysfunction.

High-affinity thiazolidinedione (TZD) ligands for peroxisome proliferator-activated receptor-γ (PPARγ) have potent insulin-sensitizing properties in the setting of type 2 diabetes by increasing liver, skeletal muscle, and adipose sensitivity to insulin. Although little is known about the consequences of PPARγ activation in the heart, we speculated that metabolic flexibility might be restored. This issue becomes important, because it has recently been proposed that insulin resistance and metabolic maladaptation of the heart are associated with pacing-induced left ventricular failure in dogs (34).

We examined the effects of a novel PPARγ ligand (24) (non-TZD) on cardiac substrate metabolism and function in a rat model of type 2 diabetes. We tested the hypothesis that, in a rat model of insulin resistance and type 2 diabetes, PPARγ activation improves contractile function by increasing the capacity of the isolated heart to oxidize glucose.

DIABETES MELLITUS ADVERSELY AFFECTS the cardiovascular system both at the level of the vasculature and at the level of the myocardium (45). Diabetes is considered an independent risk factor for heart failure (27), because abnormal ventricular function occurs in diabetic patients independently of clinically overt vascular disease (17, 32). Alterations in protein synthesis, calcium handling, and contractile proteins have all been implicated as key contributors to the development of cardiac dys-
MATERIALS AND METHODS

All protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas-Houston. Male Zucker Diabetic Fatty rats (ZDF/Drt-fa, 60–63 days of age for heart perfusions) and age-matched male Zucker lean (ZL, +/+) littermates were obtained from Genetic Models (Indianapolis, IN). Animals were kept in the Animal Care Center at the University of Texas-Houston Medical School under controlled conditions (23 ± 1°C; 12:12-h light-dark cycle) and received Formula Diet 5008 (PMI Feeds, Richmond, IN) and water ad libitum. Rats were acclimated before treatment initiation. All animals were dosed twice daily for 1 wk by oral gavage with either vehicle (V, polyethylene glycol) or GI-262570, a non-TZD PPARγ agonist, at 8 mg/kg dissolved in polyethylene glycol (A). GI-262570 was supplied by GlaxoSmithKline, Research Triangle Park, NC (24).

Working heart preparation and perfusion protocol. The working heart preparation was described earlier (44). Briefly, rats were anesthetized with pentobarbital sodium (5 mg/100 g body wt ip). Hearts were initially perfused in the Langendorf mode with Krebs-Henseleit (K-H) buffer containing 5 mM glucose. After the heart was switched to the working mode, the subsequent perfusion protocol consisted of two consecutive perfusion periods of 20 min each (Fig. 1). The perfusion buffer contained the following additions. For the first 20-min period, the perfusion buffer consisted of K-H with 5 mM glucose (plus 20 μM [U-14C]glucose). The buffer in the second 20-min period consisted of both 5 mM glucose (plus 20 μM [U-14C]glucose) and 0.4 mM sodium oleate (plus 30 μM [9,10-H]oleate) bound to 1% BSA (Cohn fraction V, fatty acid free; Serologicals, Norcross, GA). Pre- and afterload were 15 and 100 cmH2O, respectively. Throughout the experiment, insulin (40 μU/ml) was present, and the buffer was equilibrated with 95% O2-5% CO2. Aortic and coronary flow rates were measured every 5 min, and the cardiac output was calculated as the sum of both values. At the end of the protocol, hearts were freeze-clamped and stored in liquid nitrogen. Dry weights, cardiac power, and rates of oxygen consumption, glucose oxidation, and oleate oxidation were determined as previously described (22).

Body weight, blood chemistries, and harvesting of the heart. In a parallel set of fed animals, body weight and blood samples were obtained under inhalant anesthesia (isoflurane and 100% O2) at the start of dosing (53–56 days of age) and after dosing (60–63 days of age). Variables measured were plasma glucose, lactate, hematocrit, Hb A1c, serum insulin, cholesterol, triglycerides, glycerol, and nonesterified fatty acids. Equipment used for clinical chemistries and hormone assays were as follows: glucose and lactate, YSI Analyzer; clinical chemistries, Olympus AU640 automated analyzer; and insulin, by electrochemiluminescence (Origen analyzer; IGEN, Austin, Texas). Hearts from this set of rats were isolated and freeze-clamped in liquid nitrogen for subsequent RNA extraction.

RNA extraction and quantitative reverse transcription-polymerase chain reaction. RNA extraction and quantitative reverse transcription-polymerase chain reaction of samples were performed as previously described (9). Specific quantitative assays were designed from nonconserved sequences of the genes (allowing for isoform specificity), spanning sites where two exons join (splice sites), when such sites are known (preventing recognition of the assay to any potential contaminating genomic DNA). The primer and probe sequences were as follows. For acyl-CoA oxidase (ACO): forward primer 5′-TGCA-CAATCGCCATAGGATA-3′, reverse primer 5′-CAGAGCGGGGAAGAGTITTA-3′, probe 5′-FAM-AAGCAAAGCGACAGAA-CCACAGATT-TAMRA-3′; for PPARγ coactivator-1α (PGC-1α): forward primer 5′-GCCATTGTAAAGACCGAATTC-3′, reverse primer 5′-GGGACGTCTTTGTGGCTTTT-3′, probe 5′-FAM-TG- GACCAATAAGCGAAGAGATTTGTA-TAMRA-3′. The nucleotide sequences for the other primers and probes have been previously published (35, 50, 51). The level of transcripts for the constitutive housekeeping gene β-actin was used for data normalization. The expression of β-actin mRNA was not significantly different between the treatment groups (data not shown). Polymerase chain reaction data are reported as the number of transcripts per number of β-actin molecules.

Histology. In an additional group of ZL and ZDF rats (n = 4/group, ~60–63 days of age), heart tissue was removed and weighed, and portions were either frozen for Oil Red O staining or formalin fixed for Trichrome and hematoxylin and eosin (H&E) staining. Tissues were embedded in paraffin, sectioned at 1 μm thickness, and stained using H&E. Trichrome was used to visualize collagen deposits (fibrosis). Photomicrographs of H&E (∼40 magnification) and Trichrome (∼10 magnification)-stained sections were taken on a Zeiss Axioshot using a Leaf Microlumina digital camera. A total of 20 images of Trichrome-stained sections were analyzed with Image Pro Plus software using color cube-based selection criteria to ensure that only Trichrome-stained regions were counted. The area of Trichrome staining from all fields per photomicrograph was averaged with background subtracted (nontissue regions).

Semiquantification of lipid deposition within the cardiomyocyte was performed by Oil Red O staining. Oil Red O staining was performed on heart sections by use of standard procedures (50). Photomicrographs of Oil Red O-stained sections were taken at ∼20 magnification. A total of 5 images were analyzed with Image Pro Plus software using color cube-based selection criteria to ensure that only Oil Red O-stained droplets were counted and area of staining of all droplets from five fields per photomicrograph were averaged with background subtraction (nontissue regions).

Statistical analysis. The time course data for the isolated working heart perfusion studies were presented as means ± SE. Statistical analysis on these data was performed in SAS v. 9.0 (SAS Institute, Cary, NC) using PROC MIXED for repeated measures using the Huynh-Feldt covariance structure. Blood chemistry, histology, and mRNA transcript data are presented as means ± SE. Statistically significant differences between groups were calculated by Student’s t-test. A value of P < 0.05 was considered significant.

RESULTS

Body weight and blood chemistries. Table 1 shows body weights and blood chemistries of the four groups. At 60–63 days of age, body weights were significantly greater (1.4-fold) for ZDF-V rats than for the ZL controls (P < 0.05). During the experimental protocol, ZDF-V rats had gained 9.5 g of total body weight per day. When animals were treated with the
agonist, ZDF-A rats had gained an average of 16 g per day ($P < 0.05$).

The levels of serum insulin, plasma glucose, Hb A1c, serum triglycerides, nonesterified fatty acids, and glycerol in the ZDF-V rats were all significantly higher ($P < 0.05$) than those in the ZL rats. Plasma parameters for ZL-V and ZDF-V groups did not significantly differ from untreated age-matched animals (data not shown). In the ZDF-A group, the levels of glucose and Hb A1c, glycerol, and nonesterified fatty acids were all significantly lower than those of the ZDF-V rats (see Table 1 for significances) and were not significantly different from those of the ZL rats. Although PPARγ agonist treatment for 7 days significantly reduced serum insulin and triglyceride levels in the ZDF rats (by 31 and 64%, respectively), the levels were still higher than in ZL rats. In addition, serum cholesterol and high-density lipoprotein fraction C (HDL-C) levels were $\sim$1.5-fold higher than in ZL rats. In the ZDF-A rats, these levels were further increased to $\sim$1.6-fold relative to the ZL rats.

Intramyocardial lipid accumulation. The ZDF-V rats exhibited severe intramyocardial lipid accumulation (Fig. 2). Treatment of the ZDF rats with the PPARγ agonist markedly lowered the lipid content to levels similar to those in the ZL rats. There was no fibrosis in any of the groups, as assessed by Trichrome staining (data not shown).

The ZDF-V rats tended to have higher ($\sim$10%, $P = 0.08$) heart weights and heart weight-to-tibia length ratios compared with the ZL-V rats (Table 2). Treatment of ZDF rats with agonist increased both heart weight and heart weight/tibia length ratio compared with vehicle-treated rats (Table 2).

Cardiac function and metabolism in vitro. Cardiac power (CP) was significantly reduced ($P = 0.0277$) in ZDF-V hearts when glucose and oleate were provided as substrates (Fig. 3A). Agonist treatment improved cardiac function to the level of nondiabetic littermates (compare ZDF-A with ZL-V in Fig. 3A). There was a trend ($P = 0.02$) toward an increased oxygen consumption with agonist treatment in both lean and obese rats in the presence of both glucose alone or glucose plus oleate as substrates (Fig. 3B), suggesting tight coupling of glucose oxidation and oxygen consumption.

When glucose alone was provided as the substrate for oxidation, glucose oxidation rates were significantly higher ($P < 0.003$) in the ZL-A compared with the ZDF-V rats (Fig. 3D). CP may be decreased in ZDF-V because of decreased glucose oxidation rates (Fig. 3, compare A with C). However, we were not able to determine whether the decrease in CP was due to a decrease in glucose oxidation or vice versa.

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Table 1. Body weight and blood chemistries of fed ZL and ZDF rats before and after 7 days of treatment with either vehicle or the agonist GI-262570

<table>
<thead>
<tr>
<th></th>
<th>ZL-V Before</th>
<th>ZL-V After</th>
<th>ZL-A Before</th>
<th>ZL-A After</th>
<th>ZDF-V Before</th>
<th>ZDF-V After</th>
<th>ZDF-A Before</th>
<th>ZDF-A After</th>
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<tr>
<td>Body weight, g</td>
<td>178±3</td>
<td>224±3</td>
<td>166±14</td>
<td>219±19</td>
<td>241±4</td>
<td>308±5</td>
<td>218±19</td>
<td>331±6*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>147±4</td>
<td>153±5</td>
<td>153±7</td>
<td>136±2.8</td>
<td>181±9</td>
<td>229±15</td>
<td>185±9</td>
<td>146±2†</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.19±0.04</td>
<td>5.15±0.04</td>
<td>5.21±0.03</td>
<td>5.18±0.07</td>
<td>5.47±0.05</td>
<td>6.43±0.08</td>
<td>5.82±0.16</td>
<td>5.52±0.05†</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>0.21±0.01</td>
<td>BQL</td>
<td>0.23±0.05</td>
<td>BQL</td>
<td>2.30±0.27</td>
<td>3.84±0.56</td>
<td>2.89±0.35</td>
<td>2.00±0.44*</td>
</tr>
<tr>
<td>NEFA, mg/dl</td>
<td>0.39±0.06</td>
<td>0.30±0.02</td>
<td>0.36±0.03</td>
<td>0.09±0.01</td>
<td>0.52±0.03</td>
<td>0.60±0.06</td>
<td>0.53±0.03</td>
<td>0.16±0.02†</td>
</tr>
<tr>
<td>Glycerol, mg/dl</td>
<td>84±1</td>
<td>78±1</td>
<td>86±1</td>
<td>86±2</td>
<td>112±2</td>
<td>117±2</td>
<td>113±3</td>
<td>133±2†</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>50±1</td>
<td>48±0</td>
<td>50±1</td>
<td>56±1</td>
<td>65±1</td>
<td>62±1</td>
<td>66±2</td>
<td>96±2†</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>38±5</td>
<td>36±5</td>
<td>37±5</td>
<td>35±5</td>
<td>40±5</td>
<td>39±5</td>
<td>41±5</td>
<td>39±5</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 12 animals for each group. All groups are described in METHODS, Animals and experimental model. ZL, Zucker lean; ZDF, Zucker diabetic fatty; V, vehicle treated; A, agonist treated; Hb A1C, calculated %hemoglobin A1C fraction; NEFA, nonesterified fatty acids; HDL-C, high-density lipoprotein fraction C; BQL, below quantification level. *P < 0.05, †P < 0.01 vs. ZDF-V.
Transcripts of proteins regulating fatty acid metabolism. Increased free fatty acid levels in diabetes results in the activation of PPARγ and its coactivator PGC-1α (15). Both induce the expression of the genes of fatty acid metabolism, including medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCAD), malonyl-CoA dehydrogenase (MCD), acyl-CoA oxidase (ACO), and muscle carnitine palmitoyltransferase (mCPT I).

There were no significant differences in the expression of PPARγ (Fig. 4A) or PGC-1α (Fig. 4B) or mCPT I (Fig. 4C) from hearts of either ZL or ZDF rats. In contrast, the expressions of MCD (Fig. 4D), MCAD (Fig. 4E), LCAD (Fig. 4F), and ACO (Fig. 4G) were all significantly increased (1.3- to 1.5-fold, \( P < 0.05 \)) in hearts from ZDF-V rats compared with the ZL-V group. Administration of the agonist in ZDF rats significantly reduced the expression of these genes to levels of expression similar to ZL-V and ZL-A rats.

Transcripts of proteins that regulate glucose metabolism. GLUT4 is inducible and is the main glucose transporter isoform found in the normal adult heart, whereas the predominant isoform in fetal hearts is GLUT1, which is constitutively expressed (37). Although there were no significant differences in GLUT1 expression (Fig. 5A) in hearts from ZDF-V rats compared with ZL-V rats, GLUT4 expression (Fig. 5B) was significantly reduced (\( \sim 30\% \), \( P < 0.05 \)) in ZDF-V. Agonist treatment of ZDF rats significantly increased the expression of both GLUT1 and GLUT4 to levels similar to or greater than those of the ZL-A hearts. In hearts of ZL-V rats, pyruvate dehydrogenase kinase-4 (PDK-4), a key regulator of glucose and lactate oxidation through inhibitory phosphorylation of the pyruvate dehydrogenase complex (25), was significantly higher than in the ZDF rats (Fig. 5C). Although agonist treatment did not significantly lower the expression of PDK-4 in ZL-A vs. ZL-V rats, it did so in the ZDF-A vs. the ZDF-V rats. The expression of PDK-4 in ZDF-A rat hearts was

Table 2. Heart weights, body weights, and heart weight:tibia length ratios of ZL and ZDF rats at 60–63 days of age

<table>
<thead>
<tr>
<th></th>
<th>ZL-V</th>
<th>ZDF-V</th>
<th>ZDF-A</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>256±13</td>
<td>326±11‡</td>
<td>368±4§</td>
</tr>
<tr>
<td>Heart wet weight, mg</td>
<td>990±27</td>
<td>1,075±31*</td>
<td>1,270±13§</td>
</tr>
<tr>
<td>HW/TL, mg/cm</td>
<td>214±6</td>
<td>243±5†</td>
<td>285±1§</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 independent observations. HW/TL, heart weight-to-tibia length ratio. *\( P < 0.08 \), †\( P < 0.01 \), ‡\( P < 0.001 \) vs. ZL-V; §\( P < 0.001 \) vs. ZDF-V.

Fig. 3. Cardiac function and rates of substrate oxidation. A: cardiac power in the presence of 5 mM glucose and 5 mM glucose + 0.4 mM olate (shaded area) as substrates. B: myocardial oxygen consumption (MV˙O2) with 5 mM glucose and 5 mM glucose + 0.4 mM olate present as substrates. C: glucose oxidation (Ox) rates in the in the presence of 5 mM glucose and 5 mM glucose + 0.4 mM olate as substrates. D: olate oxidation rate. Functions were assessed in isolated perfused working hearts from fed ZL-V (◦), ZL-A (●), ZDF-V (○), and ZDF-A (■) rats (60–63 days old) during 40 min of aerobic perfusion. Values are means ± SE for 10–13 independent observations in each treatment group.

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significantly lower ($P < 0.05$) than in the ZL-V rats. Although we did not investigate the other isoforms of PDK, the results suggest that there is impaired glucose oxidation in diabetes and restoration of glucose oxidation with improved insulin sensitization.

Transcripts of sarcomeric and calcium cycling proteins. Myosin heavy chains (MHC), the main component of myosin, exist in two distinct isoforms (1). In adult rat heart the predominant isoform is MHCα; in fetal rat heart the predominant isoform is MHCβ. Although the expression of MHCα did not differ between ZL and ZDF rats (Fig. 5D), the expression of MHCβ (Fig. 5E) was significantly higher ($P < 0.001$) in hearts from ZDF-V rats vs. ZL-V rats. Although MHCβ in ZDF-A rat hearts tended to be lower than in hearts of ZDF-V rats, its expression in hearts of ZDF-A rats was still significantly higher ($P < 0.01$) than in hearts of ZL-V rats.

The sarco(endo)plasmic reticulum ATPase 2a protein (SERCA2a) maintains and regulates the Ca$^{2+}$ content of the sarcoplasmic reticulum (16). Surprisingly, SERCA2a expression did not differ among the ZL or ZDF rats (Fig. 5F). This finding contrasts with the observation that SERCA2a activity is downregulated in type 1 diabetes (20).

**DISCUSSION**

The main findings of our study are as follows. 1) In ZDF rats, in vitro cardiac function is impaired. Although reversible contractile dysfunction of the heart has been demonstrated before in vivo (52), the present results in vitro provide new evidence for the reversible metabolic dysregulation of the heart. 2) PPARγ activation restores the deranged metabolic profile in ZDF rats at a time when the diabetic phenotype of the ZDF rat is still reversible. 3) Treatment with PPARγ agonist reverses myocardial lipid accumulation, improves contractile function, increases glucose oxidation, and restores the myocardial transcriptional profile similar to that of nondiabetic animals.

**Choice of model.** Type 2 diabetes is a chronic and progressive disease of insulin resistance accompanied by pancreatic β-cell decompensation and failure. The male ZDF (ZDF-Drt/fa) rat is obese, dyslipidemic, and insulin resistant at an early age and has been used extensively as a model of type 2 diabetes. Because of the genetic defects in the pancreatic β-cell (46), this rat model manifests a relatively rapid and predictable progression from prediabetic (severe hyperinsulinemia, impaired glucose tolerance, and dyslipidemia) to the diabetic state (relative hypoinsulinemia, insulin resistance, with progressive worsening of the dyslipidemia and hyperglycemia). This progression is evidenced in the ZDF vehicle-treated group by worsening glucose, Hb A1c, insulin, and triglyceride levels. Untreated animals show the same progression of the disease (data not presented). By greater than 9 wk, male rats become more severely hypertriglyceridemic and hyperglycemic, and serum insulin levels begin to decline precipitously due to pancreatic failure and insufficiency (5). We chose to focus our studies on a stage in their disease at which sufficient pancreatic β-cell function remains (60–63 days of age), because beyond this age insulin resistance is complicated by severe hyperglycemia and insulin deficiency (48, 52). Other investigators found changes in cardiac substrate metabolism preceding changes in contractile function by 7 and 11 wk (8).

**Role of PPARγ for the heart.** Given the low level of cardiac PPARγ expression and the absence of direct effects of PPARγ ligands on either gene expression or fatty acid oxidation in cultured myocytes (19), the actions of PPARγ agonist-induced...
changes in perfused heart metabolism are most likely indirect and related to the restoration of a normal fuel homeostasis, although direct effects of PPARγ agonists on cardiac growth cannot be excluded. PPARγ agonists have profound effects on glucose utilization, and they are also associated with improvement in lipid profiles (41). Indeed non-TZD PPARγ agonists used have been shown to decrease plasma nonesterified fatty acids as quickly as 6 h after dosing (49). It has been suggested that the improvement in insulin action in skeletal muscle is through alterations in lipid metabolism (41). However, the timing of treatment with PPARγ agonists is important. In the present study, drug dosing was initiated before the transition to severe diabetes and pancreatic-cell failure. Preliminary work established that chronic oral administration of this agonist in ZDF rats and in db/db mice is highly effective in ameliorating the diabetic phenotype as early as 4 days after the start of treatment (5).

Restoration of metabolic flexibility in the heart. Our results of depressed glucose oxidation rates in isolated working hearts of untreated ZDF rats are in accord with decreased oxidation rates reported in obese Zucker fatty rats (50), in db/db mice (4), in CIRKO mouse hearts (3), in ZDF rat hearts (8), and in insulin-resistant JCR:LA-cp rats (2). The reduced rates of glucose oxidation in the obese Zucker rats, CIRKO mice, and the animals in in our study suggest decreased insulin signaling in the myocardium. Although not a direct measure of substrate oxidation, the use of tracers [glucose analog [18F]fluorodeoxyglucose (FDG) and a fatty acid analog, 125I-BMIPP (β-methyl-p-iodophenylpentadecanoic acid)] can provide indirect evidence of relative preferences for glucose and fatty acid oxidation in vivo. Pharmacological studies in db/db mice (vs. lean control mice) treated with vehicle or a PPARγ agonist for 12 days showed that uptake of [18F]FDG was severely depressed in the vehicle-treated diabetic mice and restored to levels equivalent to those of lean control littermates when treated with a PPARγ agonist for 12 days. BMIPP uptake in lean controls and vehicle-treated db/db mice were equivalent but decreased by treatment, as was incorporation of BMIPP into intracellular lipid triacylglycerol pools (K. K. Brown, unpublished observations).

In skeletal muscle it has been demonstrated that this flexibility of substrate selection is lost with insulin resistance (28). Central to metabolic flexibility is the turnover of energy stores within the cell. A hallmark of diabetes is the accumulation of lipid stores and decreased rates of turnover. Previous studies in skeletal muscle have shown that PPARγ activation with a TZD normalized lipid and glycogen stores and increased turnover of glycogen stores by normalization of pyruvate dehydrogenase complex (PDC) activity (42). The overall result in skeletal muscle is the restoration of metabolic flexibility. We (43) have proposed that metabolic inflexibility also occurs in heart muscle. For example, insulin responsiveness is impaired in both hypertrophied and atrophied heart (14). In the present study, we provide an example of impaired metabolic flexibility in the heart, caused by an excess supply of free fatty acids, that can be reversed by PPARγ agonist treatment (Fig. 3). As demonstrated in this study, hearts of diabetic, severely insulin-resistant ZDF rats display a transcriptional shift that favors enhanced mitochondrial free fatty acid uptake, and oxidation that is demonstrated by the profound reduction in carbohydrate metabolism.
oxidation when supplied as the sole metabolic substrate. The ability to oxidize glucose was further decreased when oleate was added to the medium. Associated with impaired glucose oxidation was a decrease in contractile function. In the normal hearts as well as hearts from ZDF-A rats, the ability to oxidize glucose is maintained even when oleate is added to the perfusate, which suggests that the ability to metabolize multiple substrates simultaneously is restored in these hearts.

In diabetes and insulin-resistant states, myocardial glucose uptake and subsequent oxidation are impaired (45). The present study shows that ZDF rats exhibit significantly reduced (30%, $P < 0.05$) myocardial GLUT4 transcript levels. In addition to perturbed fatty acid metabolism, decreased glucose uptake is thought to be a result of decreased GLUT4 protein and mRNA levels (7); it has been proposed that abnormal regulation of this process has a role in the pathogenesis of cardiac dysfunction (45). Our results of decreased myocardial GLUT4 transcript levels are consistent with those observed in type 1 diabetes [streptozotocin (STZ) rat model] (6, 40). In a polygenic model of spontaneous type 2 diabetes and in obese insulin-resistant Zucker rats (11, 39, 47), decreased GLUT4 protein was associated with a decrease in insulin-stimulated glucose uptake with decreased insulin receptor phosphorylation and blunted phosphatidylinositol 3-kinase activity after insulin stimulation. We (35) showed earlier in patients with heart failure and diabetes that myocyte enhancer factor (MEF)2C protein, MEF2C-regulated transcripts, and GLUT4 are decreased whereas GLUT1 (a non-MEF2C-regulated gene) remains unaltered.

In our present study, surprisingly, cardiac PDK-4 mRNA transcript levels were not increased in the hearts of untreated ZDF rats compared with leans. Our results are, however, consistent with findings in skeletal muscle where high insulin levels alone are sufficient to suppress PDK-4 expression (29). In contrast with our present findings are reduced PDK-4 expression and diminished glucose oxidation previously observed in hearts of Zucker fatty rats (50) and in hearts lacking the insulin receptor (CIRKO) (3). In these, as in the present study, we did not measure directly the activity of the PDC. Also, our study did not examine pyruvate dehydrogenase phosphatase activity, which has been found to be decreased in starvation and STZ-induced diabetes, which leads to PDC inactivation (26). This process will no doubt prevent the oxidation of glucose in diabetes and in insulin resistance. A limitation of our study is the fact that we did not measure pyruvate and lactate oxidation (8).

A further limitation of our work is that we did not measure directly rates of glucose uptake and glycogen turnover in the myocardium. We anticipate that the increase in glucose oxidation with agonist treatment is a marker for increased glucose uptake in the heart, but glycogen synthesis complicates the issue. We have demonstrated before an increased turnover of glycogen stores and the preferential entry of glycogen-derived glucose in the oxidative pathway in the isolated rat heart (21, 22). Further investigation is needed to understand these complexities of carbohydrate metabolism in the myocardium in the setting of insulin resistance.

Transcription of genes encoding for contractile proteins and SERCA2a. In both alloxan- and STZ-induced insulin-dependent diabetes, MHCα expression is decreased early after diabetes induction and was associated with a rapid and concomitant upregulation in MHCβ expression (10, 13, 20). We have also recently shown (38) that heart failure in humans in the presence of obesity or diabetes leads to an increase in MHCβ expression. In our present study, we show that MHCβ expression is significantly higher ($\sim 3$-fold, $P < 0.001$) in hearts from untreated ZDF rats compared with ZL-V rats. In contrast to type 1 models of diabetes, myocardial MHCα expression was not significantly different. Our results are consistent with those in obese, insulin-resistant Zucker rats (50).

In a model of alloxan-induced diabetes, a depression in the SERCA pump (20) is considered to account for diastolic dysfunction in hearts of diabetic animals. Both pressure overload-induced hypertrophy and STZ-induced diabetes are also associated with decreased expression of SERCA2a in the heart (10, 33). A large body of evidence suggests that the occurrence of remodeling of both sarcomplasmic reticulum and sarcocellular membrane occurs in the heart during the development of chronic diabetes (12). However, in the present study, and in our earlier study (50), we did not observe a downregulation of SERCA2a. In addition to SERCA2a, abnormal calcium handling may be due to altered expression of other ion channels (e.g., $Na^+/Ca^{2+}$ exchanger), $Na^+\cdot K^+\cdot$ ATPase, sarcocellular calcium pump, effectors (e.g., phospholamban), or posttranscriptional effects (e.g., fatty acyl-CoAs directly affecting the ryanodine receptor). Also, fatty acyl-CoAs have been demonstrated as modulators of potassium currents mediated by ATP-sensitive K+ (KATP) channels in the heart (30) and to a lesser extent in the pancreatic β-cell (23). The accumulation of lipids in the cytoplasm in the hearts of ZDF-V vs. ZDF-A (Fig. 2) has the potential to affect repolarization and intracellular calcium supply and handling. The role of improved fatty acid metabolism by PPARγ agonist treatment on KATP channels requires further investigation.

In conclusion, We found that PPARγ activation, by improving the metabolic profile of severely insulin-resistant ZDF rats, improves contractile function and increases the capacity of the isolated heart to oxidize glucose. Although no cause-effect relationship has been established between the metabolic profile and contractile function, these effects are associated with decreased heart triglyceride content and are matched by a transcriptional profile shift to reduced fatty acid utilization and increased glucose utilization. Because of a proposed vicious cycle, in which impaired glucose metabolism and cardiac failure worsen each other, our study underscores the importance of early diagnosis and treatment of insulin resistance for the heart.

ACKNOWLEDGMENTS

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