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

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Running Head - Cardiac effects of PPARγ agonism in ZDF rats

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ABSTRACT

**Background:** It is suggested that insulin resistance and metabolic maladaptation of the heart are causes of contractile dysfunction. We tested the hypothesis whether systemic PPARγ activation, by changing the metabolic profile in a model of insulin resistance and type 2 diabetes (the ZDF rat) *in vivo*, improves contractile function of the heart *in vitro*. **Methods and Results:** Male Zucker diabetic fatty (ZDF) and Zucker Lean (ZL) rats, at 53-56 days of age, were treated with either GI262570 (a non-TZD PPARγ agonist; A) or vehicle (V) for one week. Agonist treatment resulted in correction of hyperglycemia and dyslipidemia, as well as in reduced hyperinsulinemia. The accumulation of triacylglycerols in the myocardium, characteristic of the ZDF rat, disappeared with treatment. Cardiac power and rates of glucose oxidation in the isolated working heart were significantly reduced in ZDF-V rats, but both parameters increased to non-diabetic levels with agonist treatment. In ZDF-V hearts, transcript levels of PPARα-regulated genes and of MHCβ were upregulated, while GLUT-4 was downregulated compared to ZL. Agonist treatment of ZDF rats reduced PPARα-regulated genes, and increased transcripts of GLUT-4 and GLUT-1. **Conclusion:** By changing the metabolic profile, reducing myocardial lipid accumulation and promoting the downregulation of PPARα-regulated genes, PPARγ activation leads to an increased capacity of the myocardium to oxidize glucose and to a tighter coupling of oxidative metabolism and contraction in the setting of insulin resistance and type-2 diabetes.

**Keywords:** diabetes mellitus, obesity, insulin, myocardial contraction, metabolism
INTRODUCTION

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 independent from 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 dysfunction in diabetes(13, 20). Changes in gene expression, energy substrate metabolism and in ultrastructure occur early in the course of the disease(45). While the exact mechanism for the pathogenesis of diabetic cardiomyopathy is not understood, some of the first changes appear to be at the level of myocardial energy substrate metabolism(4, 36).

Diabetes is as much a disease of dysregulated fatty acid metabolism as it is of dysregulated glucose metabolism(31). High rates of fatty acid uptake in the diabetic myocardium results in the accumulation of myocardial lipid and lipid intermediates, mitochondrial/peroxisomal generation of reactive oxygen species and excessive oxygen consumption(18, 50, 52). These findings contrast to the metabolic characteristics of the normal heart.

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 of multiple substrates simultaneously) may be lost in insulin resistance and diabetes, producing a state of metabolic inflexibility. We have proposed that the loss of metabolic flexibility is a feature of hypertrophy and heart failure(43). 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 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.

**MATERIALS AND METHODS**

**Animals and Experimental Model**

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, age 60-63 days of age for heart perfusions) and age-matched male Zucker Lean (ZL, +/?) litter mates 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-h light/12-h 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 week by oral gavage with either vehicle (V, polyethylene glycol) or with GI262570 at 8mg/kg, a non-TZD PPARγ agonist, dissolved in polyethylene glycol (A). GI262570 was supplied by GlaxoSmithKline, Research Triangle Park, NC(24).

**Working Heart Preparation and Perfusion Protocol**

The working heart preparation has been described earlier(44). Briefly, rats were anesthetized with sodium pentobarbital (5 mg/100 g body weight IP). Hearts were initially perfused in the Langendorff mode with Krebs-Henseleit buffer containing 5mM D-glucose. After the heart was switched to the working mode, the subsequent perfusion protocol consisted of two consecutive perfusion periods of 20 min each (see Fig. 1). The Krebs-Henseleit buffer contained the following additions. For the first 20- minute period, the perfusion buffer consisted of KH with 5 mM glucose (plus 20 μCi/L [U-14C]-glucose). The buffer in the second 20-minute period consisted of both 5 mM glucose (plus 20 μCi/L [U-14C]-glucose) and 0.4 mM sodium oleate (plus 30 μCi/L [9, 10,-3H]-oleate) bound to 1% BSA (Cohn fraction V, fatty acid free; Serologicals; Norcross, GA). Pre- and afterload were 15cm and 100cm of H2O, respectively. Throughout the experiment insulin (40 μU/mL) was present and the buffer was equilibrated with 95% O2 and 5% CO2. Aortic and coronary flows 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, 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 (isofluorane and 100% O2) at the start of dosing (53-56 days of age) and post dosing (60-63 days of age). Variables measured were plasma glucose, lactate, hematocrit, HbA1c, serum insulin, cholesterol, triglycerides, glycerol and non-esterified fatty acids.

Equipment used for clinical chemistries and hormone assays were: Glucose and lactate – YSI model 2700 Analyzer; glycated hemoglobin and calculated HbA1c – Column Mate® I Analyzer; clinical chemistries – Olympus AU640 automated analyzer; insulin – by electrochemiluminescence (Origen analyzer IGEN, Inc., 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 non-conserved 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’-TGCAATCGCCATAGGATA-3’, (Reverse primer): 5’-CCAGGAGCGGGAAGAGATT-TA-3’, (Probe): 5’-FAM-AAGCAATCGCCATAGGATA-3’, for Peroxisome proliferator gamma co-activator-1-alpha (PGC-1α), (Forward primer): 5’GCCATTGTAAGACGGTTTCA-TAMRA-3’, (Reverse primer): 5’-GGGACGTCTTTTGTC-3’, (Probe): 5’-FAM-TGGAGCAATCGCCATAGGATA-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/number of β-actin molecules.

Histology.

In a further group of ZL and ZDF rats (n=4/group, ~60-63 days of age), heart tissue was removed, 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 (40x magnification) and Trichrome (10x magnification) stained sections were taken on a Zeiss Axiophot using a Leaf Microlumina digital camera. A total of 20 images of Trichrome stained sections were analyzed with Image Pro Plus software using color-cubed based selection criteria to ensure only Trichrome-stained regions were counted. The area of Trichrome staining from all fields per photomicrograph was averaged with background subtracted (non-tissue regions).

Semiquantification of lipid deposition within the cardiomyocyte was performed by oil red O staining. Oil red O staining was performed on heart sections using standard procedures(50). Photomicrographs of oil red O stained sections were taken at 20x 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 (non-tissue regions).
**Statistical Analysis**

The time course data for the isolated working heart perfusion studies are presented as means ± SE. Statistical analysis on this data was performed in SAS v9.0 (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 the mean ± SE. Statistically significant differences between groups were calculated by the 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 lean controls (*P*<0.05). During the experimental protocol, ZDF-V rats had gained 9.5g of total body weight per day. When animals were treated with the agonist, ZDF-A rats had gained an average of 16g per day (*P*<0.05).

The levels of serum insulin, plasma glucose, HbA\textsubscript{1C}, serum triglycerides, non-esterified fatty acid and glycerol in the vehicle-treated ZDF rats (ZDF-V) were all significantly higher (*P*<0.05) than those in the ZL rats. Plasma parameters for vehicle-treated ZL and ZDF groups did not significantly differ from untreated age-matched animals (data not shown). In the ZDF-A group, the levels of glucose and HbA\textsubscript{1C}, glycerol and non-esterified 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. While PPAR\textsubscript{γ} 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
HDL-C levels were ~1.5-fold higher than in ZL rats. In the ZDF-A rats, these levels were
further increased to ~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
the ZL-V 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 (~10%, p=0.08) heart weights and heart
weight: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 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 non-diabetic littermates (compare ZDF-A with ZL-V in Fig 3A). There
was a trend (P = NS) for an increased oxygen consumption with agonist treatment in both lean
and obese rats, both in the presence of 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 agonist treated (ZL-A) compared to the ZDF vehicle-
treated (ZDF-V) (Fig 3C). There were no differences between ZL-V and ZL-A. When oleate was added, rates of glucose oxidation decreased 3 fold (P < 0.0001) in hearts of ZDF-V rats (Fig. 3C). Agonist treatment increased rates of glucose oxidation to levels similar to non-diabetic littermates (compare ZDF-A with ZL-V). Rates of oleate oxidation were not significantly different between ZDF-V versus ZL-V (Fig. 3D). However, rates of oleate oxidation were decreased significantly (P = 0.0172) in both agonist-treated groups (Fig. 3D). Cardiac power may be decreased in ZDF-V because of decreased glucose oxidation rates (compare Fig. 3A and 3C). However, we were not able to determine whether the decrease in cardiac power was due to a decrease in glucose oxidation or vice versa.

**Transcripts of proteins regulating fatty acid metabolism.**

Increased free fatty acid levels in diabetes results in the activation of PPARα 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 palmitoyl transferase (mCPT1).

There were no significant differences in the expression of PPARα (Fig. 4A) or PGC-1α (Fig. 4B) or mCPT1 (Fig. 4C) from hearts of either ZL or ZDF rats. In contrast, the expression of MCD (Fig. 4D), MCAD (Fig. 4E), LCAD (Fig. 4F) and ACO (Fig. 4G) were all significantly increased (1.3-1.5-fold (P < 0.05)) in hearts from ZDF-V rats compared to the ZL-V group. Administration of the agonist in ZDF rats significantly reduced the expression of these genes to levels of expression similar to lean rats.
**Transcripts of proteins that regulate glucose metabolism**

GLUT-4 is inducible and is the main glucose transporter isoform found in the normal adult heart, whereas the predominant isoform in fetal hearts is GLUT-1, which is constitutively expressed(37). Although there were no significant differences in GLUT-1 expression (Fig. 5A) in hearts from ZDF-V rats compared to leans, GLUT-4 expression (Fig. 5B) was significantly reduced (~30% (P < 0.05)) in ZDF-V. Agonist treatment of ZDF rats significantly increased the expression of both GLUT-1 and GLUT-4 to levels similar to or greater than those of the ZL-V hearts. In hearts of ZL-V rats, pyruvate dehydrogenase kinase-4 (PDK4), 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 PDK4 in ZL-A vs. ZL-V rats, it did so in the ZDF-A vs. the ZDF-V rats. The expression of PDK4 in ZDF-A rat hearts was significantly lower (P < 0.05) than in the ZL-V rats. Although we did not interrogate 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. While 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. the 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 were still significantly higher (P < 0.01) than in hearts of ZL-V rats.

The sarcoplasmic/endoplasmic 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 to the observation that SERCA2a activity is downregulated in type 1 diabetes(20).

**DISCUSSION**

The main findings of our study are: 1) In ZDF rats, *in vitro* cardiac function is impaired. While 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) PPAR\(\gamma\) 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 PPAR\(\gamma\) agonist reverses myocardial lipid accumulation, improves contractile function, increases glucose oxidation and restores the myocardial transcriptional profile similar to that of non-diabetic animals.

**Choice of Model**

Type 2 diabetes is a chronic and progressive disease of insulin resistance accompanied by pancreatic \(\beta\)-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 beta cell (46), this rat model manifests a relatively rapid and predictable progression from pre-diabetic (severe
hyperinsulinemia, impaired glucose tolerance and dyslipidemia) to the diabetic state (relative hypoinsulinemia, insulin resistance, with progressively worsening of the dyslipidemia and hyperglycemia). This progression is evidenced in the ZDF vehicle-treated group by worsening glucose, HbA1C, insulin and triglyceride levels. Untreated animals show the same progression of the disease (data not presented). By greater than 9 weeks, male rats become more severely hypertriglyceridermic, 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 beta 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 weeks(8).

The 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 on 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 non-esterified fatty acids as quickly as 6 hours post-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 beta 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 accordance 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 in our study suggest decreased insulin signaling in the myocardium. Although not a direct measure of substrate oxidation, the use of tracers (glucose analog, $^{18}$F-FDG, and a fatty acid analog, $^{125}$I-BMIPP) can provide indirect evidence of relative preferences for glucose and fatty acid oxidation in vivo. Pharmacologic studies in db/db mice (compared to lean control mice) treated with vehicle or a PPAR$\gamma$ agonist for 12 days showed that uptake of $^{18}$F-FDG was severely depressed in the vehicle treated diabetic mice and restored to levels equivalent to lean control littermates when treated with a PPAR$\gamma$ agonist for 12 days. BMIPP uptake in lean controls and vehicle treated db mice were equivalent but decreased by treatment, as was incorporation of BMIPP into intracellular lipid triacylglycerol pools(KK 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 have proposed that metabolic inflexibility also occurs in heart muscle(43). 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 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 which favors enhanced mitochondrial free fatty acid uptake and oxidation which is demonstrated by the profound reduction in carbohydrate oxidation when supplied as the sole metabolic substrate. The ability to oxidize glucose was further decreased when oleate was added to the media. 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 GLUT-4 transcript levels. In addition to perturbed fatty acid metabolism, decreased glucose uptake is thought to be a result of decreased GLUT-4 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 GLUT-4 transcript levels are consistent with that observed in type 1 diabetes (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 GLUT-4 protein was associated with a decrease in insulin-stimulated glucose uptake
with decreased insulin receptor phosphorylation and blunted PI3K kinase activity after insulin
stimulation. We have shown earlier in patients with heart failure and diabetes that MEF2C
protein, MEF2C-regulated transcripts and GLUT-4 are decreased, while GLUT1 (a non-MEF2C-
regulated gene) remains unaltered(35).

In our study, surprisingly, cardiac PDK4 mRNA transcript levels were not increased in
the hearts of untreated ZDF rats compared to leans. Our results are, however, consistent with
findings in skeletal muscle where high insulin levels alone are sufficient to suppress PDK4
expression(29). In contrast with our present findings, are reduced PDK4 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 directly
measure 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 IDDM, MHCα expression is decreased early after diabetes induction and was associated with a rapid and concomitant up-regulation in MHCβ expression(10, 13, 20). We have also recently shown that heart failure in humans in the presence of obesity or diabetes leads to an increase in MHCβ expression(38). In our study, we show that MHCβ expression is significantly higher (~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 sarcoplasmic reticular Ca\(^{2+}\)-pump(20) which 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 the occurrence of remodeling of both sarcoplasmic reticulum and also sarcolemmal 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 down regulation 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\(^+\)-K\(^+\)-ATPase, sarcolemmal calcium pump, effectors (e.g. phospholamban), or post-transcriptional 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⁺ channels (K_{ATP}) 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 versus 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 K_{ATP} channels requires further investigation.

CONCLUSIONS

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 matched with 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 one another, our study underscores the importance of early diagnosis and treatment of insulin resistance for the heart.

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REFERENCES


**LEGEND FOR TABLES**

**Table 1.** Body weight and blood chemistries of fed Zucker Lean and Zucker Diabetic Fatty rats before and after 7 days of treatment with either vehicle (V) or the agonist, GI262570 (A).

Values are means ± SE, 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; HbA1C, calculated (%) hemoglobin A1C fraction; NEFA, non-esterified fatty acids; HDL-C, high-density lipoprotein-fraction C; BQL below quantification level. * P < 0.05, ** P < 0.001 versus ZDF-V.

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

Values are mean ± SE for 4 independent observations. ZL – Zucker lean; ZDF – Zucker Diabetic Fatty; V – Vehicle; A – Agonist; HW/TL – Heart Weight Tibia Length ratio. * P = 0.08, ** P < 0.01, *** P < 0.001 vs. ZL-V; † P < 0.001 vs. ZDF-V.

**LEGENDS FOR FIGURES**

**Figure 1.** Perfusion Protocols.

Hearts from all groups of fed rats (60-63 d old) were perfused in the working mode at physiological workload (100cm H2O) and preload (15cm H2O) with Krebs-Henseleit buffer (K-H) for two consecutive aerobic perfusion periods of 20 minutes each. The K-H buffer in the first 20-minute period contained glucose (5 mM) and in the second 20-minute period, the buffer contained both glucose (5 mM) and sodium oleate (0.4 mM bound to 1% BSA). Insulin (40 µU/ml) was present throughout the experiment.
**Figure 2.** Myocardial Lipid Deposition.

Representative photomicrographs (A) showing oil red O staining in sections isolated from hearts of an untreated Zucker Lean rat (ZL-V), an untreated Zucker Diabetic Fatty rat (ZDF-V), and an agonist-treated Zucker Diabetic Fatty rat (ZDF-A) (60-63 d old). Semiquantification of oil red O staining (B). Values are means ± SE of four independent observations in each group.

**Figure 3.** Cardiac Function and Rates of Substrate Oxidation.

Cardiac power in the presence of 5 mM glucose and 5 mM glucose and 0.4 mM oleate (shaded area) as substrates (A). Myocardial oxygen consumption with 5 mM glucose and 5 mM glucose and 0.4 mM oleate present as substrates (B). Glucose oxidation rates in the presence of 5 mM glucose and 5 mM glucose and 0.4 mM oleate as substrates (C) and oleate oxidation rate (C). Function was assessed in isolated perfused working hearts from fed ZL-V (Δ), ZL-A (▲), ZDF-V (□), and ZDF-A (■) rats (60-63 d old) during 40 min of aerobic perfusion. Values are means ± SE for 10 to 13 independent observations in each treatment group.

**Figure 4.** Gene Expression.

Transcript levels of PPARα, PGC-1α, and various PPARα-regulated genes in hearts from fed ZL-V, ZL-A, ZDF-V and in ZDF-A rats. Transcripts encoding for PPARα (A), PGC-1α (B), mCPT1 (C), MCD (D), MCAD (E), LCAD (F), and ACO (G) were measured in hearts from fed ZL-V, ZL-A, ZDF-V and in hearts of ZDF-A rats (60-63 d old). Results are normalized to the housekeeping gene β-actin. Values are means ± SE from six independent observations in each treatment group.
**Figure 5.** Gene Expression.

Transcript levels of genes encoding glucose transporters, myosin isoforms, SERCA2a, and PDK4. Transcripts encoding GLUT-1 (A), GLUT-4 (B), PDK4 (C), MHCα (D), MHCβ (E) and SERCA2a (F) were measured in hearts from fed ZL-V, ZL-A, ZDF-V and in hearts of ZDF-A rats (60-63 d old). Results are normalized to the housekeeping gene β-actin. Values are means ± SE from six independent observations in each treatment group.
Table 1.  

<table>
<thead>
<tr>
<th></th>
<th>ZL-V</th>
<th>ZL-A</th>
<th>ZDF-V</th>
<th>ZDF-A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Before</td>
<td>178 ± 3</td>
<td>224 ± 3</td>
<td>166 ± 14</td>
<td>219 ± 19</td>
</tr>
<tr>
<td>After</td>
<td>241 ± 4</td>
<td>308 ± 5</td>
<td>218 ± 19</td>
<td>331 ± 6*</td>
</tr>
<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Before</td>
<td>147 ± 4</td>
<td>153 ± 5</td>
<td>153 ± 7</td>
<td>136 ± 2.8</td>
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<tr>
<td>After</td>
<td>181 ± 9</td>
<td>229 ± 15</td>
<td>185 ± 9</td>
<td>146 ± 6**</td>
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<tr>
<td><strong>HbA1c (%)</strong></td>
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<td></td>
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<tr>
<td>Before</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>
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<tr>
<td>After</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>
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<tr>
<td><strong>Insulin (ng/mL)</strong></td>
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<tr>
<td>Before</td>
<td>0.21 ± 0.01</td>
<td>BQL</td>
<td>0.23 ± 0.05</td>
<td>BQL</td>
</tr>
<tr>
<td>After</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>
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<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before</td>
<td>69 ± 6</td>
<td>74 ± 6</td>
<td>85 ± 9</td>
<td>55 ± 2</td>
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<tr>
<td>After</td>
<td>299 ± 19</td>
<td>680 ± 55</td>
<td>348 ± 31</td>
<td>126 ± 9**</td>
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<tr>
<td><strong>NEFA (mEq/L)</strong></td>
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<tr>
<td>Before</td>
<td>0.38 ± 0.06</td>
<td>0.30 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.09 ± 0.01</td>
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<td>After</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>
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<td><strong>Glycerol (mg/dL)</strong></td>
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<tr>
<td>Before</td>
<td>10 ± 1</td>
<td>9 ± 1</td>
<td>10 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>After</td>
<td>23 ± 1</td>
<td>30 ± 2</td>
<td>23 ± 2</td>
<td>8 ± 0**</td>
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<tr>
<td><strong>Cholesterol (mg/dL)</strong></td>
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<tr>
<td>Before</td>
<td>84 ± 1</td>
<td>78 ± 1</td>
<td>86 ± 1</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>After</td>
<td>111 ± 2</td>
<td>117 ± 2</td>
<td>113 ± 3</td>
<td>133 ± 2**</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
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<td></td>
</tr>
<tr>
<td>Before</td>
<td>50 ± 1</td>
<td>48 ± 0</td>
<td>50 ± 1</td>
<td>56 ± 1</td>
</tr>
<tr>
<td>After</td>
<td>65 ± 1</td>
<td>62 ± 1</td>
<td>66 ± 2</td>
<td>96 ± 2**</td>
</tr>
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</table>

**Table 1.** Body weight and blood chemistries of fed Zucker Lean and Zucker Diabetic Fatty rats before and after 7 days of treatment with either vehicle (V) or the agonist, GI262570 (A).

Values are means ± SE, 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; HbA1c, calculated (%) hemoglobin A1c fraction; NEFA, non-esterified fatty acids; HDL-C, high-density lipoprotein-fraction C; BQL below quantification level. * P < 0.05, ** P < 0.001 versus ZDF-V.
Table 2. Heart weights, body weights, and heart weight:tibia length ratios of ZL and ZDF rats at 60-63 days of age.

Values are mean ± SE for 4 independent observations. ZL – Zucker lean; ZDF – Zucker Diabetic Fatty; V – Vehicle; A – Agonist; HW/TL – Heart Weight Tibia Length ratio.

* P = 0.08, ** P < 0.01, *** P < 0.001 vs. ZL-V; † P < 0.001 vs. ZDF-V.

<table>
<thead>
<tr>
<th></th>
<th>ZL-V</th>
<th>ZDF-V</th>
<th>ZDF-A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bodyweight (g)</strong></td>
<td>256 ± 13</td>
<td>326 ± 11***</td>
<td>368 ± 4†</td>
</tr>
<tr>
<td><strong>Heart Weight (wet, mg)</strong></td>
<td>990 ± 27</td>
<td>1075 ± 31*</td>
<td>1270 ± 13†</td>
</tr>
<tr>
<td><strong>HW/TL (mg/cm)</strong></td>
<td>214 ± 6</td>
<td>243 ± 5**</td>
<td>285 ± 1†</td>
</tr>
</tbody>
</table>
Figure 1. Perfusion Protocols.

Hearts from all groups of fed rats (60-63 d old) were perfused in the working mode at physiological workload (100cm H₂O) and preload (15cm H₂O) with Krebs-Henseleit buffer (K-H) for two consecutive aerobic perfusion periods of 20 minutes each. The K-H buffer in the first 20-minute period contained glucose (5 mM) and in the second 20-minute period, the buffer contained both glucose (5 mM) and sodium oleate (0.4 mM bound to 1% BSA). Insulin (40 µU/ml) was present throughout the experiment.
Figure 2. Myocardial Lipid Deposition.

Representative photomicrographs (A) showing oil red O staining in sections isolated from hearts of an untreated Zucker Lean rat (ZL-V), an untreated Zucker Diabetic Fatty rat (ZDF-V), and an agonist-treated Zucker Diabetic Fatty rat (ZDF-A) (60-63 d old). Semiquantification of oil red O staining (B). Values are means ± SE of four independent observations in each group.
Figure 3. Cardiac Function and Rates of Substrate Oxidation.

Cardiac power in the presence of 5 mM glucose and 5 mM glucose and 0.4 mM oleate (shaded area) as substrates(A). Myocardial oxygen consumption with 5 mM glucose and 5 mM glucose and 0.4 mM oleate present as substrates(B). Glucose oxidation rates in the presence of 5 mM glucose and 5 mM glucose and 0.4 mM oleate as substrates(C) and oleate oxidation rate (C). Function was assessed in isolated perfused working hearts from fed ZL-V (△), ZL-A (▲), ZDF-V (□), and ZDF-A (■) rats (60-63 d old) during 40 min of aerobic perfusion. Values are means ± SE for 10 to 13 independent observations in each treatment group.
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