Cardioprotective effect of the PPAR ligand tetracosylthioacetic acid in type 2 diabetic mice

Ahmed M. Khalid,1*, Anne Dragøy Hafstad,1*, Terje S. Larsen,1 David L. Severson,3 Neoma Boardman,1 Martin Hagve,1 Rolf K. Berge,2 and Ellen Aasum1

1Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø; 2The Lipid Research Group, Institute of Medicine, University of Bergen, Norway; and 3Department of Physiology and Pharmacology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

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Tetradecylthioacetic acid (TTA) is a novel peroxisome proliferator-activated receptor (PPAR) ligand for hyperlipidemic and insulin-sensitizing effects in obese models. TTA has recently been shown to attenuate dyslipidemia in patients with type 2 diabetes, corroborating the potential for TTA in antidiabetic therapy. In a recent study on normal mice, we showed that TTA increased myocardial fatty acid (FA) oxidation, which was associated with decreased cardiac efficiency and impaired postschismic functional recovery. The aim of the present study was, therefore, to elucidate the effects of TTA treatment (0.5%, 8 days) on cardiac metabolism and function in a hyperlipidemic type 2 diabetic model. We found that TTA treatment increased myocardial FA oxidation, not only in nondiabetic (db/+), but also in diabetic (db/db) mice, despite a clear lipid-lowering effect. Although TTA had deleterious effects in hearts from nondiabetic mice (increased efficiency and impaired mitochondrial respiratory capacity), these effects were not observed in db/db hearts. TTA improved ischemic tolerance, an effect that is most likely related to the antioxidant property of TTA. The present study strongly advocates the need for investigation of the cardiac effects of PPAR ligands used in antidiabetic/hypolipidemic therapy, because of their pleiotropic properties.

HEART DISEASE IS THE LEADING cause of death in diabetic patients. These patients have higher incidence of acute myocardial infarction (AMI), as well as higher mortality following AMI (17, 36). In addition, asymptomatic diabetic patients are predisposed to heart failure, suggesting a specific cardiomyopathy (13). Although the etiology of diabetic cardiomyopathy is not clear, there is strong evidence for a causal link to altered myocardial metabolism (5, 12). The db/db mouse is a model of type 2 diabetes with cardiomyopathic features, notably reduced mechanical function, and reduced functional recovery following ischemia-reperfusion (3, 16, 19). In addition, measurements of myocardial substrate utilization show a higher reliance on fatty acid (FA) oxidation for energy production (3), accompanied by reduced cardiac efficiency (8, 22). A causal link between altered metabolism, reduced efficiency, and tolerance to ischemia in diabetes is supported by the fact that improving myocardial substrate utilization (inhibition of FA oxidation and stimulation of glucose oxidation), both acutely and following lipid-lowering treatment, can improve cardiac efficiency and postschismic functional recovery in obese/type 2 diabetic models (4, 19, 23).

Tetradecylthioacetic acid (TTA) is a non-β-oxidizable FA analog that increases peroxisomal proliferation and FA oxidation in the liver via peroxisome proliferator-activated receptor (PPAR) activation (7). Accordingly, TTA treatment decreases plasma lipids (7), prevents feeding-induced obesity, and improves insulin sensitivity in rodent models of hyperlipidemia (26). In a recent open-labeled 4 wk clinical study by Løvås et al., TTA attenuated dyslipidemia in type 2 diabetic patients (25), corroborating the potential for TTA in antidiabetic therapy (31).

In a recent study from our group, it was found that TTA treatment of normal BalbC mice increased myocardial FA oxidation, decreased cardiac efficiency, and reduced the myocardial tolerance to ischemia-reperfusion (18). The metabolic effect of TTA is in contrast with previous studies showing that lipid-lowering in hyperlipidemic models in response to in vivo treatment with PPAR agonists is associated with decreased myocardial FA oxidation (2, 4, 23). Hence, further information about the cardiac effects of TTA treatment in type 2 diabetes is needed.

MATERIAL AND METHODS

Animals. C57BL/6J-knockout mice (db/db) and their nondiabetic heterozygote littermates (db/+) (12–15 wk old) were purchased from Harland (Bicester, England). All mice were housed in a room maintained at 23°C and 55% humidity with a 12-h:12-h light/dark cycle. The mice were given ad libitum access to food and water. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and was approved by the Norwegian National Animal Research Committee. Tetradecylthioacetic acid (TTA) was given as a 0.5% (wt/wt) dietary supplement for 8 days (18).

Plasma parameters. Plasma glucose, free fatty acids (FA), and triacylglycerols (TG) were determined in blood samples taken from the cavity of the animals at the day of euthanization, using commercial kits from Boehringer Mannheim (Mannheim, Germany), Wako Chemicals (Neuss, Germany), and ABX Diagnostics (Montpellier, France), respectively.

Cardiac metabolism. The hearts were perfused in working mode using a modified Krebs-Henseleit bicarbonate buffer supplemented with 0.4 mM palmitate bound to 3% BSA (with endogenous FA concentration of 0.3 mM, resulting in a final FA concentration of 0.7 mM) and 5 mM glucose. All hearts were allowed to beat spontaneously in the presence of low FA concentration (0.04 mM), which allows a view in both oxidation and the effects of FA oxidation on cardiac function and efficiency.
Glucose and palmitate oxidation were calculated by simultaneously measuring $^4$CO$_2$ and H$_2$O released by the oxidation of [U-$^{14}$C]-glucose and [9,10-$^3$H]-palmitate, respectively (3).

Postischemic functional recovery. Hearts were perfused in working mode, and intraventricular pressure was obtained by inserting a 2 F micromanometer-tipped catheter (Millar, SPR 407) into the left ventricle via the atrial cannula (1.3 mm inner diameter). After 30 min preischemic perfusion, hearts were subjected to 40 min low-flow ischemia (3.1 ml·g dry wt$^{-1}$·min$^{-1}$), followed by 5 min reperfusion in Langendorff mode and 30 min in working mode. Hearts that did not produce pressure exceeding that of the afterload column were perfused in an assisted mode (19). Postischemic recovery of ventricular function was measured after 35 min reperfusion relative to baseline (preischemic) values.

Cardiac efficiency and ventricular function. Cardiac efficiency was determined in a separate series of perfused hearts, by assessment of the relationship between cardiac work [pressure-volume area (PVA)] and myocardial oxygen consumption (MV$\dot{O}_2$) (19, 21). PVA was measured by means of a micromanometer-conductance catheter (1.4 F; Millar Instruments, Houston, TX), which was inserted through the apex into the left ventricle, and oxygen partial pressure was recorded by fiber-optic oxygen probes (FOXY-AL300; Ocean Optics, Duiven, Netherlands), placed before the left atrial cannula and in the pulmonary trunk. Electrodes were connected to the right atrium for electrical pacing, and hearts were exposed to different workloads by changing preload and afterload. Steady-state values of PVA and MV$\dot{O}_2$ were calculated at each workload, and regression analysis of the PVA: MV$\dot{O}_2$ relationship allowed determination of unloaded MV$\dot{O}_2$ (y-intercept) and contractile efficiency (inverse slope). MV$\dot{O}_2$ was also measured in potassium-arrested hearts, which were perfused in an unloaded retrograde mode to determine the oxygen cost of basal metabolism (MV$\dot{O}_{2\text{basal}}$), as described previously (8).

Transcriptional changes. Fresh samples from unperfused hearts were immersed in RNAlater (Qiagen, Hilden, Germany), and total RNA was extracted according to the RNeasy Fibrous Tissue Protocol kit (Qiagen Nordic, Norway). Real-time PCR (quantitative PCR) was performed in an ABI PRISM 7900 HT Fast real-time thermal cycler (18). Primers/probe sequences for housekeeping genes, transcription regulators, and PPAR-$\gamma$ target genes are given in Hafstad et al. (18). Primer sequences for antioxidant enzymes were as follows: catalase, forward: 5'-CAT-GCTTCCAAGCAAATCTCC-3' and reverse: 5'-TCTGGTCTGTCCTCAGATGT-3'; superoxide dismutase, Sod1 forward: 5'-CACCATTFTTTGCACCACCT-G3' and reverse: 5'-TTAACCTTCTCAGACCCAGATCT-3'; glutathione peroxidase, Gspx: forward: 5'-GGCTTCTTCTTCTCAACCT-G3' and reverse: 5'-CCC-ACC-TGG-TACG-TAT-CT-3'; and for the FA transporters, CD36 antigen (also referred to as FA translocase) Cd36: forward: 5'-ATGGCTGTGATCGAAGACT-G3' and reverse: 5'-GCCTTCCAAGAACTCCTCCT-3'; FA transport protein, Fatp: forward: 5'-TCCCCCTCAGAATGTCCTC-3'; and plasma membrane FA binding protein, Fabp: forward: 5'-ACCTGGAAGCTGAGAGCAG-3' and reverse: 5'-TGATGTTAGTAGGTGTCAT-3'. Housekeeping genes were selected on the basis of the average expression stability values, determined with geNorm Normalization kit (38) out of a selected pool of candidate genes.

Lipid content and lipid peroxidation in the heart. Lipids were extracted from samples of ventricular tissue from unperfused hearts by the method of Folch, dried, and thereafter dissolved in a test-butil and Triton X-100/methyl alcohol mixture. The TG concentration of the extracts was measured with a Triglyceride 25 kit from ABX Diagnostics (Montpellier, France). Malondialdehyde (MDA) and 4-hydroxy-2-enal (4-HNE) content, indicators of lipid peroxidation, were measured in ventricular tissue using a TBARS assay kit (Cayman Chemical) and a 4-HNE ELISA kit (Cell Biolabs), respectively.

Western blot analysis and protein activity measurements. SDS-PAGE, 15% polyacrylamide gels were loaded with 10 mg of protein, and the proteins were electrophoretically transferred to nitrocellulose membranes. Membranes were probed overnight with anti-MnSOD (BD Transduction Laboratories) at 4°C and subsequently for 1 h with horseradish peroxidase conjugated anti-mouse IgG (GE Healthcare). Immunopositive bands were developed with Immobilon chemoluminescent reagent (Millipore) and detected with a Kodak Image Station 1000 (Kodak, Rochester, NY). Ponceau S staining (Sigma, St. Louis, MO) confirmed equal loading. Total superoxide dismutase activity was measured using a Superoxide Dismutase Assay Kit (Cayman Chemical).

Mitochondrial respiration and citrate synthase activity. Mitochondrial respiration was measured in saponin-permeabilized cardiac fibers by high-resolution respirometry, using a two-chambered oxygen (Oxygraph-2k; Oroboros Instruments GmbH, Innsbruck, Austria). Hearts were excited and rinsed in ice-cold relaxing solution (BIOPS; Oroboros, Innsbruck, Austria). A piece of myocardial tissue (~15 mg) was cut out from the endocardial free wall of the left ventricle with fine scissors and transferred to a Petri dish containing ice-cold BIOPS. Individual fiber bundles were separated with two pairs of sharp forceps, and pieces of bundles weighing 2–4 mg were permeabilized in BIOPS containing 50 μg/ml saponin (30 min at 4°C), washed three times for 10 min in respiration medium (MiRO5; Oroboros Innsbruck, Austria), and blotted on filter paper. Individual bundles were weighed and transferred into each chamber containing air-saturated respiration medium at 37°C, so that all measurements were made in duplicate. Respiration was assayed following addition of two different substrate mixtures: malate (2 mM) and pyruvate (10 mM) or malate (2 mM) and palmitoyl-carnitine (25 μM). Respiration was measured after addition of 2.5 mM ADP ($V_{\text{ADP}}$) and after addition of 1 μg/ml F$_0$V$_{\text{ADP}}$ ATPase inhibitor oligomycin ($V_{\text{Oligo}}$). O$_2$ flux was calculated from the negative time derivative of the oxygen concentration signal, using DatLab 4 software from Oroboros Instruments (15).

A sample of ventricular tissue from each heart (7–10 mg) was homogenized in phosphate buffer, and the activity of citrate synthase (CS), a mitochondrial enzyme, was measured spectrophotometrically according to the method of Srere (34).

Statistical analysis. Data are expressed as means ± SE. Differences were determined by a two-way ANOVA followed by Holm-Sidak’s test or Fisher method. Student’s $t$-test was used when appropriate. The overall significance level was 0.05.

RESULTS

Biometric data. Diabetic db/db mice exhibited the typical characteristics of a severe diabetic phenotype, including marked obesity, hyperglycemia, and dyslipidemia (Table 1). Eight days treatment with TTA markedly reduced body weight.

Table 1. Effect of TTA on body, heart, and liver weights and on plasma concentrations of glucose, FA, and TG in diabetic (db/db) and nondiabetic (db+/+) mice

<table>
<thead>
<tr>
<th></th>
<th>db/db</th>
<th>TTA</th>
<th>db+/+</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>28.0 ± 0.5</td>
<td>25.1 ± 0.4*</td>
<td>44.0 ± 0.9†</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>27.7 ± 0.4</td>
<td>26.9 ± 0.6</td>
<td>24.5 ± 0.4†</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>2.2 ± 0.1†</td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>15.6 ± 0.8</td>
<td>12.9 ± 0.6*</td>
<td>52.9 ± 2.7†</td>
</tr>
<tr>
<td>Plasma TG, mM</td>
<td>0.74 ± 0.05</td>
<td>0.49 ± 0.03*</td>
<td>0.96 ± 0.08†</td>
</tr>
<tr>
<td>Plasma FA, mM</td>
<td>0.55 ± 0.04</td>
<td>0.41 ± 0.08*</td>
<td>0.88 ± 0.08†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 21–25 mice in each group. TTA, tetradecylthioacetic acid; TG, triacylglycerol; FA, fatty acid. *$P < 0.05$ vs. untreated within the same group; †$P < 0.05$ vs. untreated db+/+.
in db/+ mice, but not in db/db mice. TTA significantly reduced plasma concentrations of FA, TG, and glucose in both db/+ and db/db mice. The hypolipidemic effect of TTA is partly due to hepatic PPAR-α activation; in accordance with this we found a marked increase in hepatic Pdk4 expression in both db/db and db/+ mice (3.9 ± 0.8 and 10.5 ± 2.4-fold, respectively). There were no changes in liver or heart weight following TTA treatment.

Myocardial substrate utilization and expression of metabolic genes. Hearts from untreated db/db mice exhibited reduced rates of glucose oxidation and increased rates of FA oxidation compared with db/+ hearts (Fig. 1A). The increased FA oxidation was associated with a significant upregulation of the mRNA content (untreated db/db vs. control db/+ ) of several PPAR-α target genes: muscle carnitine palmitoyl transferase (Mcpt1), PDH-kinase4 (Pdk4), uncoupling protein 3 (Ucp3), mitochondrial and cytosolic thioredoxin (Cd36), and FA binding protein (Fabp) (Table 2). Diabetic hearts also showed significant upregulation of PPAR-γ coactivator-α (Pgc1-α) and Ppar-δ, whereas there was a small but significant downregulation of Ppar-α (Table 2).

TTA treatment resulted in a 1.5 and 1.1 μmol·min⁻¹·g dry wt⁻¹ increase in myocardial FA oxidation in db/+ and db/db hearts, respectively, and a concomitant decrease in glucose oxidation (Fig. 1A). In hearts from db/+ mice, the TTA-induced increase in FA oxidation was accompanied by an increased PPAR-α target gene expression (Mcpt1, Pdk4, Ucp3, Cd36, and Ppar-α; Table 2). In db/db hearts, TTA produced less marked changes in PPAR-α target genes; increased, decreased, and unchanged gene levels were observed (Table 2).

In accordance with previous findings in Balbc mice (unpublished), TTA treatment reduced the myocardial expression of Pgc1-α, Ppar-α, and Ppar-δ in both db/+ and db/db hearts (Table 2), which may suggest that persistent PPAR-α activation will downregulate receptor and coactivator expression through negative feedback.

<table>
<thead>
<tr>
<th>Target</th>
<th>+TTA</th>
<th>-TTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mcpt1</td>
<td>1.00±0.07</td>
<td>1.85±0.13*</td>
</tr>
<tr>
<td>Pdk4</td>
<td>1.00±0.14</td>
<td>2.63±0.51*</td>
</tr>
<tr>
<td>Cd36</td>
<td>1.00±0.07</td>
<td>0.93±0.05</td>
</tr>
<tr>
<td>Fabp</td>
<td>1.00±0.09</td>
<td>0.69±0.08*</td>
</tr>
<tr>
<td>Ucp3</td>
<td>1.00±0.09</td>
<td>1.30±0.06*</td>
</tr>
<tr>
<td>Mte1</td>
<td>1.00±0.09</td>
<td>1.34±0.13*</td>
</tr>
<tr>
<td>Mcpt1</td>
<td>1.00±0.07</td>
<td>13.38±1.06*</td>
</tr>
<tr>
<td>Fatp</td>
<td>1.00±0.05</td>
<td>4.25±0.29*</td>
</tr>
<tr>
<td>Ucp3</td>
<td>1.00±0.05</td>
<td>0.77±0.06*</td>
</tr>
<tr>
<td>Ppar-α</td>
<td>1.00±0.06</td>
<td>0.47±0.05*</td>
</tr>
<tr>
<td>Ppar-δ</td>
<td>1.00±0.08</td>
<td>0.90±0.17*</td>
</tr>
<tr>
<td>Pgc1-α</td>
<td>1.00±0.05</td>
<td>0.47±0.06*</td>
</tr>
</tbody>
</table>

Table 2. Effect of TTA on mRNA expression of PPAR-α targets and transcription regulators in hearts from diabetic (db/db) and nondiabetic (db+/+) mice.

Values are means ± SE of 6–8 hearts in each group. mRNA levels were expressed relative to the geometric mean of the housekeeping genes Hmbs, Gapdh, and Hprt and is presented as fold expression as compared with untreated db+/+, PPAR, peroxisome proliferator-activated receptor. *P < 0.05 vs. untreated within the same group; †P < 0.05 vs. untreated db/db.

CS activity and mitochondrial respiration. Diabetes has been associated with increased cardiac mitochondrial biogenesis, which is supported in the present study by a small increase in CS activity (145 ± 4 vs. 123 ± 3 U/mg wwt, in db/db and db/+, respectively; P < 0.002) and in pgc1-α expression (Table 2) in db/db hearts. Although the significance of these changes is unclear, CS activity was used for normalization of mitochondrial respiration. Maximum ADP-stimulated respiration rates (V_{ADP}) were measured in saponin-permeabilized cardiac fibers from unperfused hearts, using pyruvate/malate or palmitoyl-carnitine/malate as substrates (Fig. 1B). Cardiac fibers from db/db hearts showed significantly decreased V_{ADP} when measured with pyruvate, but not with palmitoyl-carnitine. The latter observation is in accordance with Boudina et al., who showed unchanged V_{ADP}in fibers isolated from palmitate and glucose-perfused db/db hearts (10). In contrast with their study, however, we did not observe any difference in the respiratory state between db/+ and db/db fibers in the presence of oligomycin (V_{oligom}) (data not shown).

TTA treatment did not alter CS activity in db/+ (127 ± 7 U/mg wwt) and db/db (139 ± 6 U/mg wwt) hearts. In myocardial fibers from TTA-treated db/+ mice, we found reduced contractile efficiency (dimensionless), and r² is the square of the regression coefficient. *P < 0.05 vs. untreated within the same group; †P < 0.05 vs. untreated db/+.

Table 3. Regression analysis of the MVO₂-pressure-volume area relationships in hearts from untreated and TTA-treated db/+ and db/db mice.

<table>
<thead>
<tr>
<th>db/+</th>
<th>db/db</th>
</tr>
</thead>
<tbody>
<tr>
<td>+TTA</td>
<td>-TTA</td>
</tr>
<tr>
<td>y-intercept</td>
<td>4.80±0.46</td>
</tr>
<tr>
<td>Slope</td>
<td>2.34±0.18</td>
</tr>
<tr>
<td>r²</td>
<td>0.96±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. The y-intercept represents unloaded MVO₂ (expressed as Joule heat -1·g wet wt⁻¹·10⁻⁷), the inverse of the slope represents contractile efficiency (dimensionless), and r² is the square of the regression coefficient. *P < 0.05 vs. untreated within the same group; †P < 0.05 vs. untreated db/+.

Fig. 1. A: myocardial fatty acid and glucose oxidation in hearts of db/+ (white bars) and db/db (gray bars) mice. Results are means of 8 to 9 hearts in each group. B: mitochondrial respiratory maximal capacity measured in skinned cardiac fibers from db/+ and db/db mice. Results are obtained from 8–12 hearts in each group. Hatching indicates tetradecylthioacetic acid (TTA) treatment. CS, citrate synthase. *P < 0.05 vs. untreated within the same group; †P < 0.05 vs. untreated db/+.
V_{ADP} both when using pyruvate and palmitoyl-carnitine as substrates (Fig. 1B). In cardiac fibers from TTA-treated db/db mice, however, there were no changes in V_{ADP}. TTA treatment did not change V_{oligo} in db/+ or db/db fibers (data not shown).

**MV\dot{O}_2 and cardiac efficiency.** Cardiac efficiency was examined by regression analysis of the PVA-MV\dot{O}_2 relationship. The major advantage of this technique is that it allows myocardial oxygen cost to be separated in two independent components: unloaded MV\dot{O}_2 (the y-intercept) and contractile efficiency (the inverse slope). In accordance with previous reports from our laboratory (19, 22), hearts from db/db mice showed impairment of both systolic and diastolic ventricular function and tolerance to ischemia-reperfusion.

The major advantage of this technique is that it allows myocardial oxygen cost to be separated in two independent components: unloaded MV\dot{O}_2 (the y-intercept) and contractile efficiency (the inverse slope). In accordance with previous reports from our laboratory (19, 22), hearts from db/db mice showed impaired function in hearts from db/db mice (Table 4), as indicated by a reduced functional recovery (Fig. 2). However, in contrast with what we have previously shown in nondiabetic mice (18), TTA treatment significantly improved postischemic functional recovery in db/db hearts (Fig. 2).

**Myocardial TG, lipid peroxidation, and antioxidant enzymes.** Lipid accumulation and increased generation of reactive oxygen species (ROS) have been suggested to result in lipotoxicity and oxidative stress in the diabetic heart (10). We therefore explored the potential of TTA to reduce lipid accumulation and oxidative stress in db/db hearts. Hearts from db/db mice showed increased TG content with markedly increased levels of MDA and 4-hydroxynonenal (4-NHE) (Fig. 4). TTA also decreased mRNA expression of catalase (Cat) and glutathione peroxidase (Gshpx) (Fig. 4). TTA treatment decreased lipid peroxidation, as indicated by a reduction of MDA content in db/db hearts, although 4-HNE did not show a similar reduction (Fig. 3). TTA has been shown to exhibit antioxidant properties (7) by scavenging superoxide anions (6, 28), and in accordance with this notion TTA treatment reduced SOD expression with a borderline reduction in SOD activity (P = 0.067; Fig. 4). TTA also decreased mRNA expression of glutathione peroxidase in db/db hearts (Fig. 4). The content of MDA or 4-HNE was not altered in db/+ hearts.

**Table 4. Ventricular function in hearts from untreated and TTA-treated db/+ and db/db mice obtained by pressure-volume analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>db/+</th>
<th>+TTA</th>
<th>db/db</th>
<th>+TTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>448 ± 11</td>
<td>442 ± 10</td>
<td>431 ± 11</td>
<td>397 ± 15</td>
</tr>
<tr>
<td>P_{es}, mmHg</td>
<td>67 ± 0.8</td>
<td>70 ± 1.5</td>
<td>63 ± 10*</td>
<td>64 ± 0.6</td>
</tr>
<tr>
<td>P_{ed}, mmHg</td>
<td>11.3 ± 0.9</td>
<td>10.3 ± 1.5</td>
<td>10.9 ± 0.8</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>56.7 ± 1.1</td>
<td>59.6 ± 1.3</td>
<td>52.5 ± 1.2*</td>
<td>56.1 ± 0.7</td>
</tr>
<tr>
<td>dP/dt_{max}, mmHg/s</td>
<td>4,752 ± 314</td>
<td>4,843 ± 253</td>
<td>3,797 ± 210*</td>
<td>4,500 ± 249</td>
</tr>
<tr>
<td>dP/dt_{min}, mmHg/s</td>
<td>−3,800 ± 290</td>
<td>−3,944 ± 195</td>
<td>−2,737 ± 159*</td>
<td>−2,915 ± 093</td>
</tr>
<tr>
<td>Tau (g), ms Weiss</td>
<td>10.4 ± 0.5</td>
<td>10.1 ± 0.6</td>
<td>12.6 ± 0.6*</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>Aortic flow, ml/min</td>
<td>12.8 ± 0.7</td>
<td>12.0 ± 0.7</td>
<td>8.9 ± 0.6*</td>
<td>8.4 ± 0.5</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>3.2 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.3 ± 0.1*</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>16.0 ± 0.8</td>
<td>14.8 ± 0.8</td>
<td>11.2 ± 0.6*</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>36.2 ± 2.1</td>
<td>33.4 ± 1.5</td>
<td>26.4 ± 1.9*</td>
<td>26.9 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Steady state parameters were obtained at pre- and afterloads of 10 and 50 mmHg, respectively. The data are obtained from 8–14 hearts in each group. P_{ed}, left ventricular end-diastolic pressure; P_{es}, left ventricular end-systolic pressure; LVDP, left ventricular developed pressure. *P < 0.05 vs. untreated db/+.

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![Fig. 2. Postischemic functional recovery of cardiac output (CO), left ventricular developed pressure (LVDP), and cardiac power (CP) in percentage of preischemic function in isolated perfused working hearts from db/+ (white bars) and db/db (gray bars) and TTA-treated db/db (gray hatched bars) mice. Hatching indicates TTA treatment. Results are mean of 5–8 hearts in each group. *P < 0.05 vs. untreated db/db; #P < 0.05 vs. untreated db/+](http://ajpheart.physiology.org/).
DISCUSSION

In the present study, we found that treatment with the PPAR agonist TTA increased FA oxidation in hearts from both nondiabetic and type 2 diabetic mice. The TTA-induced increase in FA oxidation in nondiabetic hearts was accompanied by reduced cardiac efficiency and mitochondrial capacity. This was not the case for diabetic hearts; in contrast with the detrimental effect of TTA on ischemic tolerance in nondiabetic hearts (18), TTA improved functional recovery following ischemia-reperfusion and reduced oxidative stress in diabetic hearts.

The uptake and oxidation of FA in the heart is increased by elevated circulating FA concentration, due to Randle’s glucose-FA cycle (32), as well as transcriptional changes caused by FA-induced PPAR-α activation (14). The increased myocardial FA uptake and oxidation in type 2 diabetes/obesity has therefore been regarded as the consequence of both of these processes. This is also supported in the present study where increased FA oxidation in db/db hearts was associated with increased expression of several PPAR-α target genes including genes encoding proteins important for FA uptake and oxidation. The accompanying increase in protein-mediated FA uptake and FA oxidation has been suggested to play a central role in the development of ventricular dysfunction due to cardiac steatosis and lipotoxicity (9, 20, 41). Another hallmark of diabetic hearts (also demonstrated in the present study) is decreased cardiac efficiency (22, 27), due to increased unloaded MV\(\dot{O}_2\) (8, 22). As a consequence of increased MV\(\dot{O}_2\), diabetic hearts show increased susceptibility to ischemia-reperfusion (3, 16, 19).

TTA is a sulphur-containing FA analog, which has been regarded as a pan-PPAR ligand, with the following rank order of activation: PPAR-\(\alpha\) > PPAR-\(\delta\) >> PPAR-\(\gamma\) (24). TTA has hypolipidemic effects (7, 25, 26), which has been ascribed to PPAR-induced increase in hepatic \(\beta\)-oxidation, as well as actions on very-low-density lipoprotein and TG metabolism (7). In accordance with this, we have found TTA treatment to reduce plasma lipids both in nondiabetic (18) and diabetic mice. We have in previous studies with \(db/db\) or diet-induced obese mice shown that the PPAR-\(\alpha\) agonists fenofibrate and K-111 resulted in a reduction of the myocardial FA oxidation rate (2, 4), a finding attributed to their lipid-lowering effect. Although the lack of comparison with other PPAR ligands and the exclusive use of \(db/db\) mice in this study as a single model of obesity and type 2 diabetes must be acknowledged as a limitation, it is obvious that the effect of TTA on the heart differs from the mentioned PPAR ligands in that the lipid-lowering effect of TTA is not only due to increased uptake and oxidation in the liver, but also in the heart. Taken together, these findings suggest that TTA exerts a direct transcriptional effect on the heart, which overrides any effects related to the reduced lipid supply. Although the mechanism responsible for the increased FA oxidation rate was not investigated in the present study, the above notion is further supported by the finding that TTA-induced cardiometabolic effects are absent in PPAR-\(\alpha\) knockout mice (18). It should be noted that the increase in cardiac FA oxidation in TTA-treated \(db/db\) hearts was accompanied by no consistent change in PPAR-\(\alpha\) target genes, showing that caution must be taken when mRNA

Fig. 3. Triacylglycerols (TG), malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) content in hearts from \(db/+\) (white bars), \(db/db\) (gray bars), and TTA-treated \(db/db\) (gray hatched bars) mice. Results are mean of 5–8 hearts in each group, *\(P<0.05\) vs. untreated \(db/db\); \#\(P<0.05\) vs. untreated \(db/+\).

Fig. 4. Superoxide dismutase mRNA (MnSOD) and protein (MnSOD) expression, as well as activity (A), and mRNA expression of catalase (Cat) and glutathione peroxidise (Gshpx) in heart tissue from \(db/+\) (white bars), \(db/db\) (gray bars), and TTA-treated \(db/db\) (gray hatched bars) mice. mRNA levels were expressed relative to the geometric mean of the housekeeping genes Hmbs, Gapdh, and Hprt. Results are mean of 3–8 hearts in each group, *\(P<0.05\) vs. untreated \(db/db\); \#\(P<0.05\) vs. untreated \(db/+\).
expression is used as a substitute for direct measurements of metabolic rates (substrate flux). A limitation in the present study, however, is that the flux was measured ex vivo under fixed FA concentrations.

TTA treatment did not alter CS activity in hearts from nondiabetic or diabetic mice, suggesting that 8 days treatment did not induce cardiac mitochondrial proliferation. In nondiabetic db/+ mice, TTA treatment resulted in reduced maximum mitochondrial respiratory capacity, suggesting impaired mitochondrial function. In accordance with Hafstad et al. (18), TTA also decreased cardiac efficiency in nondiabetic db/+ mice as a consequence of increased unloaded MV˙O2 (18), which in turn was due to increased oxygen cost for basal metabolism. This increase in MV˙O2 can only partly be explained by the fact that FA is a more O2 consuming substrate, since the change in P-to-O ratio induced by a shift from 100% glucose to FA utilization only results in a 12% increase in MV˙O2. Thus it is clear that TTA treatment of nondiabetic hearts must induce additional oxygen consuming processes, e.g., various futile cycles (30) and/or mitochondrial uncoupling (33, 35). In contrast with its effect on normal hearts, TTA treatment did not alter mitochondrial respiratory capacity, cardiac efficiency, or MV˙O2 in hearts from db/db mice.

Previous studies have demonstrated that acute and chronic administration of PPAR agonists limit myocardial ischemia-reperfusion injury in nondiabetic (37, 40), as well as obese/type 2 diabetic, models (4, 11, 23). Because studies from our laboratory have shown that improved postsischemic functional recovery following PPAR treatment of obese/diabetic models is associated with improved myocardial substrate utilization (inhibition of FA oxidation and stimulation of glucose oxidation) (4, 23) and cardiac efficiency (23), we were surprised to find that hearts from TTA-treated db/db mice showed improved recovery of ventricular function after ischemia-reperfusion, despite elevated FA oxidation rates and unaltered cardiac efficiency. This shows that improved cardiac metabolism and efficiency are not the only predictors for increased ischemic tolerance in db/db hearts and that TTA must provide additional cardioprotective effects. TTA was found to decrease myocardial TG content, which may have provided cardioprotection since increased lipid accumulation and lipotoxicity is a more O2 consuming substrate, since the change in P-to-O ratio induced by a shift from 100% glucose to FA utilization only results in a 12% increase in MV˙O2. Thus it is clear that TTA treatment of nondiabetic hearts must induce additional oxygen consuming processes, e.g., various futile cycles (30) and/or mitochondrial uncoupling (33, 35). In contrast with its effect on normal hearts, TTA treatment did not alter mitochondrial respiratory capacity, cardiac efficiency, or MV˙O2 in hearts from db/db mice.

In conclusion, TTA treatment of nondiabetic mice has del- lierative effects on the heart in terms of reduced cardiac efficiency, impaired mitochondrial respiratory capacity, and reduced functional recovery following ischemia-reperfusion (18). These effects of TTA were blunted or absent in diabetic hearts. Of particular importance was the finding that TTA offered cardioprotection in these hearts, most likely via reduced oxidative stress. Although this cardioprotective mechanism was not fully explored, the present study strongly advocates the need for investigating the cardiac effects of PPAR ligands used in antidiabetic/hypolipidemic therapy due to their pleiotropic effects.

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DISCLOSURES

Thia Medica AS holds patents regarding effects of TTA. R. K. Berge, University of Bergen, and Haukeland University Hospital are shareholders of Thia Medica AS.

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