Paper III

Administration of tetradecylthioacetic acid (TTA) stimulates myocardial fatty acid oxidation despite having a lipid-lowering effect.

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ABSTRACT

Myocardial fatty acid (FA) oxidation is regulated acutely by the FA supply and chronically at the transcriptional level due to FA activation of peroxisome proliferator-activated receptor-α (PPARα). In this study we have examined the cardiac response to *in vivo* administration of tetradecylthioacetic acid (TTA), a pan PPAR agonist. TTA treatment (0.5%, added to the diet, for 8 days) decreased body weight and reduced plasma concentrations of lipids (FA and triacylglycerols) and glucose. Hearts from TTA-treated mice showed an increased mRNA expression of PPARα target genes. In accordance with this observation, myocardial FA oxidation was increased 2.5-fold with a concomitant reduction in glucose oxidation. This increase in FA oxidation was abolished in PPARα-null mice. Thus, it appears that the metabolic effects of TTA on the heart must be due to a stimulatory effect on cardiac PPARα. Hearts from TTA-treated mice also showed a marked reduction in cardiac efficiency (due to a two-fold increase in unloaded myocardial oxygen consumption), and decreased recovery of ventricular contractile function following low-flow ischemia. Thus, we conclude that TTA-treatment elevated FA oxidation, in spite of a reduced plasma supply of lipids. The results obtained in this study show that increased PPARα activity overrides the impact of the plasma substrate supply on cardiac metabolism.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors regulating transcription of genes encoding proteins that affect lipid metabolism in several tissues, including the heart (21). Of the PPAR isoforms (α, β/δ, and γ), PPARα is highly expressed in the heart and is thought to play an important role in the transcriptional regulation of cardiac metabolism under both physiological and pathophysiological conditions (6; 13). Fatty acids (FA) are endogenous activators of PPARs and, accordingly, FA activation of PPARα in isolated cardiomyocytes up-regulates expression of key proteins that regulate nearly every step in the cellular FA utilization pathway, including sarcolemmal FA transport (cellular uptake), FA esterification to triacylglycerols (TG), mitochondrial transport and β-oxidation (16; 33). Genetically-engineered mouse models with altered PPARα activity have provided further insight regarding the role of these nuclear receptors. PPARα-null mice exhibited diminished rates of cardiac FA oxidation (10; 32) and reduced expression of several genes involved in FA oxidation (36), whereas cardiac-specific PPARα over-expressing (MHC-PPARα) mice showed increased FA oxidation and up-regulation of genes involved in FA uptake (14; 32) . The contrasting metabolic phenotypes of these two genetically-engineered mouse models support the notion that PPARα plays an important role in regulating cardiac energy metabolism.

Although studies employing isolated cardiomyocytes have shown that PPARα agonists lead to up-regulation of key enzymes in the FA metabolizing pathway (16; 33), experiments using *in vivo* administration of PPARα ligands are less clear; some showing up-regulation (22; 30; 37), while others report minor changes in PPARα target genes in the heart, when compared to the much more pronounced increase in the liver (4; 11; 30). The effect of *in vivo* PPARα agonist administration on cardiac substrate metabolism is not well studied. In our own laboratory, we found that *in vivo* treatment with the PPARα ligand K-111 (previously called BM 17.0744), did not alter FA oxidation in normal mice (2). In hyperlipidemic animals this treatment even reduced cardiac FA utilization (1; 2), probably a secondary response to reduced plasma FA supply to the heart.

Tetradecylthioacetic acid (TTA) is a non-β-oxidizing FA analogue. Although TTA is a pan PPAR agonist (8; 25), it regulates lipid metabolism mainly through activation of PPARα (25). TTA has been shown to increase peroxisomal proliferation and FA oxidation in the liver (8), lower the concentration of plasma lipids, improve insulin sensitivity and prevent feeding-

induced obesity (25). In this study, oral administration of TTA to mice markedly increased cardiac expression of PPARα target genes and increased FA oxidation, in spite of decreased circulating lipids. In accordance with other studies in which myocardial FA oxidation is increased, these TTA-induced metabolic changes were associated with reduced cardiac efficiency and decreased tolerance to ischemia.

RESEARCH DESIGN AND METHODS

Animals. Twelve weeks old male and female Balb/c ABomTac mice were purchased from M & B Taconic (Ry, Denmark). In addition, male SVEV129 wild-type (WT) and PPARα-null mice were received as a gift from Dr. Frank J. Gonzalez (National Cancer Institute, Bethesda, MD). The latter groups were bred and housed in the animal facilities at the University of Oxford, UK. All mice were housed in rooms maintained at 21 °C and 55% humidity with a 12-h light/dark cycle with free access to food and water and treated according to the guidelines on accommodation and care of animals formulated by the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes. The experimental groups received food mixed with 0.5% (w/w) TTA for a period of 8 days. Age-matched untreated mice served as controls.

Plasma parameters. Plasma glucose, non-esterified FA and TG were determined in blood samples taken from the chest cavity of the animals (non-fasted) at the day of sacrifice, using commercial kits from Boehringer Mannheim (Mannheim, Germany), Wako Chemicals (Neuss, Germany), and ABX Diagnostics (Montpellier, France), respectively.

Real-time quantitative RT-PCR. Fresh samples from unperfused liver and heart were immersed in RNAlater (Qiagen, Hilden, Germany) and stored at 4 °C until RNA extraction. Total RNA was extracted according to the RNeasy Fibrous Tissue Protocol kit (Qiagen Nordic-Norway). RNA concentration was measured spectrophotometrically (NanoDrop, Witec, Switzerland), and stored at -80 °C before use. cDNAs were obtained from 1 μg total RNA according to iScript cDNA Synthesis Kit (BioRad, Sundbyberg, Sweden). Real-time PCR (qPCR) was performed in an ABI PRISM 7900 HT Fast real-time thermal cycler using a 1:4 dilution of the cDNA and the TaqMan Fast Universal PCR master mix (Applied Biosystems, Foster City, CA). The primer/probe sequences (Eurogentec, Seraing, Belgium, or Roche Universal ProbeLibrary, Roche Diagnostics GmbH, Mannheim, Germany) for the genes studied are given in the appendixPrimers and TaqMan probes (2 μl of cDNA) were

used in a 20 µl final volume. A negative control without cDNA template was included in every assay. The PCR efficiency for all genes was determined by performing a dilution series of a pool of all samples. Housekeeping genes were selected on the basis of the average expression stability values determined with geNorm Normalisation kit (35) out of a selected pool of 5-6 candidate genes. The expression of target gene mRNAs was normalised to the geometric mean of the three best housekeeping genes. For quantification of cardiac mRNA content, we used the geometric mean of *cyclo* (cyclophilin), *gapdh* (glyceraldehyd-3-phosphate dehydrogenase) and *hprt* (hypoxanthine-guanine phosphoribosyl transferase); the mean of *cyclo*, *gapdh* and *sdha* (succinate dehydrogenase complex subunit A) was used for quantifying hepatic mRNA content.

Western blot analysis. Levels of cardiac UCP3 protein were measured in 30 µg of whole heart protein extracts resolved on 10% SDS-PAGE and transferred to a nitrocellulose membrane. UCP3 was detected by an indirect method, using a primary antibody (rabbit anti-hUCP3, AB3046 from Chemicon intl, Temecula, CA, USA) and a secondary horseradish peroxidase-linked antibody (anti-rabbit IgG, AB7074 from Cell Signalling Technology, Danvers, MA, USA). The blots were developed using CPS-1 Chemoluminescent Peroxidase Substrate (Sigma-Aldrich CO, St Louis, MO, USA) and visualized on a KODAK Image Station 1000 (Eastman Kodak Company, Scientific Imaging Systems, Rochester, NY, USA.

Heart perfusion. Mice were given an intraperitoneal injection of heparin (100 U) and anesthetized with sodium pentobarbital (10 mg, i.p.). The heart was quickly excised and the aorta cannulated for perfusion in working mode; the left atrium was connected to a preload reservoir (12.5 mm Hg), while the left ventricle was ejecting against an afterload column with a height corresponding to a pressure of 55 mmHg. A modified KHB buffer supplemented with 0.7 mM palmitate (bound to 3% BSA) and 5 mM glucose was used as perfusion medium.

For measurements of cardiac metabolism and susceptibility to ischemia-reperfusion, the hearts were allowed to beat spontaneously, and peak systolic pressure (PSP) was recorded in the aortic line with a Codman Micro Sensor. Coronary flow (CF) was measured by timed collections of the effluent dripping from the heart, while aortic flow (AF) was determined by a drop counter at the outlet of the afterload line. After 30 min perfusion baseline (pre-ischemic) functional parameters were recorded, and thereafter hearts were subjected to 40 min low-flow ischemia (1.68 µl/g dry wt/min) followed by 35 min reperfusion (5 min in Langendorff and 30 min in working mode). Post-ischemic recovery of ventricular function was calculated based on values obtained at the end of reperfusion relative to pre-ischemic baseline values. Glucose

and palmitate oxidation were determined in the pre-ischemic perfusion period by collection of $^{14}CO_2$ and $^{3}H_2O$ released by the oxidation of [U-^{14}C]-glucose and [9,10-^{3}H]-palmitate, respectively (3; 7).

In a separate series of hearts cardiac performance was measured using a 1.4 F micromanometer-conductance catheter (Millar Instruments, Houston, TX) inserted into the left ventricle through the apex. Myocardial oxygen consumption (MVO_2) (19; 20)was measured by placing fibre-optic oxygen probes (FOXY-AL300, Ocean Optics ltd., Duiven, Netherlands) in the preload line and in the pulmonary trunk to monitor the oxygen partial pressure of the buffer entering the heart ($PO_{2\ buffer}$) and the coronary effluent ($PO_{2\ effluent}$), respectively. MVO_2 was calculated by the following equation: $MVO_2 = [PO_{2\ buffer} - PO_{2\ effluent}]$ • Bunsen solubility coefficient of O_2 • coronary flow. Electrodes were connected to the right atrium for electrical pacing of the heart. In order to determine cardiac efficiency the hearts were exposed to different workloads by changing preload (from 5 to 10 mmHg) and afterload (from 35 to 50 mmHg). Steady-state values of PVA and MVO_2 were calculated at each workload. Analysis of the relationship between PVA and MVO_2 allowed determination of unloaded MVO_2 (y-intercept of the PVA-MVO_2 relationship), as well as of contractile efficiency (the inverse slope of the PVA-MVO_2 relationship).

Statistical analysis. Data are expressed as mean ± SEM; mRNA data are normalized to untreated controls. Differences between groups with respect to cardiac mRNA and protein expression, myocardial substrate oxidation and cardiac efficiency were analysed using an unpaired t-test. Differences in substrate oxidation between hearts from TTA-treated and non-treated WT and PPAR-null mice were analysed by two-way ANOVA, followed by Holm-Sidak´s test. Finally, differences in post-ischemic functional recovery were analysed using repeated measures ANOVA followed by an unpaired t-test. The overall significance level was 0.05.

RESULTS

Biometric data. Eight days treatment with TTA reduced body weight by 8%, as shown in Table 1; by comparison, untreated control mice exhibited a 4% increase in body weight. TTA-treatment also resulted in a significant reduction in plasma lipid and glucose levels. Heart weight and liver weight in TTA-treated mice were the same as in controls (Table 1).

Transcriptional changes in liver and heart and cardiac UCP3 protein expression. In agreement with previous studies (8), TTA-treatment resulted in up-regulation of PPARα target genes in the liver, including *lcpt1* (1.9±0.4 fold, p=0.013)*, pdk4* (4.2±1.2 fold, p=0.006) and *ucp2* (2.2±0.4 fold, p=0.008). Analysis of myocardial mRNA levels also showed a significant up-regulation of PPARα-target genes in hearts from TTA-treated mice, including *mcpt1, pdk4*, *ucp3, mte1, cte1*, and *mcd* (Fig. 1). Despite the fact that mRNA expression of *ucp3* was markedly increased, UCP3 protein content was unchanged (Fig. 2).

Myocardial metabolism. Isolated perfused hearts from TTA-treated mice showed a 2.5-fold increase in FA oxidation (Fig. 3A), with a concomitant decrease (- 47%) in glucose oxidation (Fig. 3B). In order to evaluate whether the TTA-induced changes in cardiac metabolism were reversible, we measured cardiac metabolism following addition of dichloroacetate (DCA, 1 mM) to the perfusate. DCA, which is known to activate pyruvate dehydrogenase, resulted in a 2.6-fold increase glucose oxidation (2.7±0.3 vs 1.1±0.2 μmol glucose/min/g dry wt, $p<0.05$), accompanied by a 60 % reduction in FA oxidation (1.0±0.2 vs 2.4±9.1 μmol palmitate/min/g dry wt, $p<0.05$). Finally, to find out whether the cardiometabolic effect of TTA was due to PPARα activation, FA and glucose oxidation were measured in hearts from TTA-treated WT and PPARα-null mice. In line with earlier studies (10; 32), PPARα-null mice exhibited diminished rates of cardiac FA oxidation compared with WT mice (Fig. 3C). Hearts from TTA-treated WT mice showed markedly increased values of FA oxidation combined with reduced glucose oxidation, whereas TTA had no effect on cardiac metabolism in hearts from PPARα-null mice (Fig. 3C and D).

Ventricular function and tolerance to ischemia. Assessment of mechanical function by pressure-volume analysis (Table 2) revealed that TTA-treatment did not alter cardiac function, except for an increased dP/dt_{max} (+18.7%, $p<0.02$). As increased FA oxidation has been associated with reduced myocardial tolerance to ischemia-reperfusion, hearts were exposed to 40 min low-flow ischemia followed by 35 min reperfusion. Again, we found no differences with respect to pre-ischemic cardiac function (Fig. 4). TTA-treatment, however, resulted in

reduced functional recovery, as indicated by a significant decrease in post-ischemic recovery of aortic flow, cardiac output and rate-pressure product.

Cardiac efficiency. The decreased tolerance to ischemia-reperfusion in hearts with elevated FA oxidation has been associated with and may be caused by decreased cardiac efficiency. In accordance with this we found that TTA-treatment significantly reduced cardiac efficiency (Fig. 5). This inefficiency was revealed by a 97% increase in unloaded MVO_2 (elevation of the y-intercept of the regression line of the PVA:MVO_2 relationship), while contractile efficiency (the inverse slope of the regression line) was not altered.

DISCUSSION

PPARs are ligand-activated nuclear receptors that play an important role in the transcriptional regulation of lipid utilization. In this study, we have demonstrated that *in vivo* treatment with the PPAR agonist tetradecylthioacetic acid (TTA) leads to up-regulation of PPARα target genes and increased myocardial fatty acid (FA) oxidation, despite decreased circulating levels of lipids. This study for the first time reports that administration of a synthetic PPAR ligand increases myocardial FA oxidation. The finding that TTA-treatment had no effect in PPARα KO mice, proves that TTA mediates its effect on cardiac metabolism via PPARα activation. These data support the current view of PPARα as an important regulator of cardiac metabolism as shown previously using gain-of and loss-of function approaches, i.e. MHC-PPARα mice and PPARα-null mice (10; 14; 32; 36).

TTA is a saturated FA which is modified by insertion of a sulfur atom at specific positions in the carbon backbone. The presence of the sulfur atom makes TTA-coenzyme A resistant to β-oxidation. Studies have revealed that TTA is a pan PPAR agonist, and although the degree of activation of the different PPARs depends on the cell type studied (8), its metabolic effect is most likely linked to PPARα activation. It is well documented that TTA has a hypo-lipidemic effect, which has been linked to hepatic PPARα activation resulting in decreased expression of genes involved in vascular lipid transport and increased expression of genes involved in intracellular FA metabolism (8; 9; 25; 31).

Myocardial FA oxidation rate is largely determined by lipid supply to the heart. Thus, an acute increase of the plasma lipid level in intact animals (23), or the perfusate FA level in isolated heart perfusions (19), will accelerate myocardial FA oxidation (Randle cycle). It is also known that elevated plasma lipids will increase the reliance on FA as energy substrate in the heart, due to transcriptional changes of metabolic enzymes (PPARα target genes) (6; 13). Therefore, increased myocardial FA oxidation in type 2 diabetes/obesity is due to increased FA supply, as well as a FA-induced activation of cardiac PPARα. The regulatory role of PPARα in the heart is also demonstrated by increased FA oxidation in MHC-PPARα mice and decreased FA oxidation in PPARα-null mice (10; 14; 32; 36). In accordance with this, studies on isolated cardiomyocytes have shown that PPARα agonists lead to up-regulation of PPAR target genes, coincided with increased FA oxidation rates (16; 33). In contrast, studies using *in vivo* administration of PPARα ligands in normal animals are less clear; increased or unchanged expression of cardiac PPARα regulated genes has been reported (14; 22; 30; 37),

and the changes in the heart is reported to be minor when compared to the more pronounced increase in the liver (4; 11; 30). The PPAR ligand TTA, produce a large increment in cardiac expression of PPARα target genes and a 2.5-fold increase in myocardial FA oxidation. This finding is in accordance with a previous report showing increased oxidation of palmiotyl CoA in cardiac tissue from rats treated with TTA (17). The fact that treatment with TTA increased myocardial FA oxidation, despite its hypolipidemic effect, indicates that the effect on cardiac metabolism must be due to changes in gene expression rather than altered FA supply. As the TTA-induced increase in myocardial FA oxidation was abolished in hearts from PPARα-null mice, the cardiometabolic effect of TTA is due to activation of PPARα. Interestingly, hearts from TTA-treated mice showed a metabolic switch towards glucose oxidation by DCA, indicating that these hearts still exhibit metabolic flexibility.

The TTA-induced increase in myocardial FA oxidation is in fact in contrast to studies from our own laboratory; administration of the PPARα ligand K-111 (previously called BM 17.0744) did not alter FA oxidation in normal mice (2), whereas both K-111 (1) and fenofibrate (4) reduced myocardial FA oxidation in hyperlipidemic mice, an effect which was most likely linked to the lipid-lowering effect of these PPARα ligands. Our working hypothesis prior to the present study was therefore that PPARα ligands primarily affect the hearts indirectly through changes in the lipid supply, including endogenous PPAR ligands. As far as we know, this study is the first to report a synthetic PPAR ligand that following *in vivo* administration to a normal animal, promotes a switch in myocardial substrate preference towards FA. Thus, in conditions with sub-optimal energy production (34), such as hypertrophy and/or heart failure, where carbohydrates is the main energy source, TTA could potentially be used to create an optimal balance between FA and carbohydrate oxidation in the heart.

The discrepancy between the metabolic effect on the heart of TTA on the one side and K-111 and fenofibrate on the other may be related to differences in tissue uptake and/or affinity to cardiac PPARα. TTA is possibly incorporated into chylomicrons during intestinal absorption, taken up in the lymph and subsequently transported to the heart via the blood, allowing efficient uptake of the compound following lipoprotein lipase-catalysed degradation of the chylomicrons. In support of this view Asiedu *et al.* (5)reported higher concentrations of TTA in heart than in liver of TTA-treated rats.

Both *in vivo* and *ex vivo* studies have shown that increased myocardial FA oxidation and/or FA supply to the heart is associated with increased myocardial oxygen consumption (MVO_2)

and decreased cardiac efficiency (19; 23; 26), similarly to what has also been reported in diabetic hearts (20). In accordance with this, TTA-treatment reduced cardiac efficiency, due to a significant increase in unloaded MVO_2. Since increased MVO_2 may have deleterious consequences, particularly under conditions of limited oxygen supply, narrowing the window of reversible damage, increased MVO_2 is most likely the cause of the decreased post-ischemic function in hearts from TTA-treated mice.

Elevated unloaded MVO_2 (work independent MVO_2) reflects increased oxygen cost for non-mechanical purposes such as basal metabolism and excitation-contraction (E-C) coupling. The mechanism explaining this elevation in MVO_2 is not fully understood, but it may involve (i) increased oxygen demand related to the change in fuel consumption from glucose to FA (reduced P:O ratio) (29), (ii) induction of futile cycles, including FA-triacylglycerol cycling (28) and/or increased H^+ transport due to uncoupling of glucose oxidation from glycolysis (24), and/or (iii) mitochondrial uncoupling (27). Himms-Hagen and Harper (18) have suggested that two mitochondrial proteins, mitochondrial thioesterase I (MTE-I) and uncoupling protein 3 (UCP3) permit high rates of FA oxidation due to UCP3-mediated transport of FA anions (generated by the MTE-I reaction) from the mitochondrial matrix to the cytosol. Studies with isolated mitochondria have also indicated that UCP3 can cause an inward proton leak (12). Both processes occur at the expense of a decreased mitochondrial membrane potential and could therefore result in increased MVO_2. In support of this mechanism we observed a marked increase in the mRNA expression of MTE-1 and UCP3 following TTA-treatment. Although PPARα ligands increase the expression of these genes (22)(present study), less information is available with regard to changes in protein content and activity. Recent reports have demonstrated an association between the mRNA expression of MTE-1 and its protein and activity levels (15; 22). In accordance with King *et al*. 2007 (22), we found a PPAR-induced increase in UCP3 gene expression, but not at the protein level. Lack of functional studies makes it difficult, however, to assign a role of these proteins (UCP3 and MTE-1) in the TTA-induced increase in MVO_2. Finally, since oxygen consumption in the unloaded heart also includes oxygen cost for E-C coupling, we can not exclude the possibility that the TTA-induced increase in MVO_2 was related to altered cardiac Ca^{2+} handling. Indeed, this assumption was supported by the TTA-induced increase in dP/dt_{max}.

In conclusion, the present study has shown that *in vivo* treatment with TTA, a pan PPAR agonist, leads to a marked increase in myocardial FA oxidation and a decrease in glucose oxidation, a response which was mediated via myocardial PPARα activation. The TTA-induced increase in FA oxidation occurred in the face of decreased circulating lipid levels, demonstrating that PPARα activation can override the impact of the plasma substrate supply on cardiac metabolism. To our knowledge, this study is the first report showing that *in vivo* administration of a synthetic PPARα ligand increases myocardial FA oxidation. Thus, TTA could potentially be used to maintain an optimal balance between FA and carbohydrate oxidation in the heart, for instance in hypertrophy and/or heart failure where energy requirements are covered mainly by utilization of carbohydrates.

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TABLES AND FIGURE LEGEND

Table 1. Effect of TTA-treatment on body, heart and liver weights and plasma concentrations of glucose, fatty acids (FA) and triacylglycerol (TG).

		+ TTA
Body weight (g)	26.6±0.9	24.1±0.8 *
Body weight change (%)	4.1±0.6	-8.2±0.8 *
Heart weight (dry, mg)	128±4	132±6
Liver weight (wet, g)	1.5 ± 0.2	1.5 ± 0.2
Plasma glucose (mM)	15.3±0.7	12.6±0.6*
Plasma FA (mM)	0.72±0.07	0.41±0.04*
Plasma TG (mM)	0.92±0.07	0.65±0.07*

Body weight changes are given in % of the body weights prior to treatment. Weights are mean of 32-33 hearts in each group. Plasma analyses are mean of 26-28 in each group. *, $p<0.05$ *vs* untreated mice.

Table 2. Effect of TTA-treatment on ventricular function, evaluated by pressure-volume analysis of isolated perfused working hearts.

		+ TTA
Heart rate (beats/min)	442±29	443±29
LVEDP (mmHg)	6.5±2.2	6.2±1.2
LVSP (mmHg)	63.5±2.4	67.6±6.0
dP/dt_{max} (mmHg/sec)	4934±429	6074±694*
dP/dt_{min}(mmHg/sec)	-4579±561	-4900±287
Tau (g) msec	13.0±2.0	11.3±1.4
Cardiac output (ml/min)	13.6± 1.8	13.6±0.7
Stroke work (mmHg·µl)	1809±341	1840±193

Steady state parameters were obtained at pre- and after-loads of 10 and 50 mmHg, respectively. LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure. The relaxation constant Tau (glanz) is the regression of dP/dt versus pressure. Results are mean of 5 hearts in each group *, $p<0.05$ *vs* hearts from untreated mice.

Figure 1. Cardiac mRNA content of mitochondrial carnitine palmitoyl transferase (*mcpt1*), pyruvate dehydrogenase (PDH) kinase 4 (*pdk4*), uncoupling protein 3 and 2 (*ucp3, ucp2*), mitochondrial and cytosolic thioesterase I (*mte1*, *cte1*), malonyl-CoA decarboxylase (*mcd*) in untreated (black bars) and TTA-treated (white bars) mice. Results are normalized to untreated controls and expressed as mean of 8 hearts in each group. The geometric mean of *cyclo*, *gapdh* and *hprt* was used as housekeeping genes. *, $p<0.05$ *vs* hearts from untreated mice.

Figure 2. UCP3 protein content in hearts from untreated (black bars) and TTA-treated (white bars) mice: representative Western blot (A) and densitometric (B) analyses. Results are mean of 7-9 hearts in each group.

Figure 3. Rates of fatty acid (palmitate) oxidation (panel A) and glucose oxidation (panel B) in isolated perfused hearts from untreated (black bars) and TTA-treated (white bars) mice. Results are mean of 7-11 hearts in each group. *, $p<0.05$ vs hearts from untreated mice. Rates of fatty acid (palmitate) oxidation (panel C) and glucose oxidation (panel D) in isolated perfused hearts from untreated (black bars) and TTA-treated (white bars) wild type (WT) and PPARα-null mice (TG). Results are mean of 6-7 hearts in each group. *, $p<0.05$ *vs* hearts from untreated mice of the same genotype. #, $p<0.05$ *vs* hearts from untreated WT mice.

Figure 4. Pre- and post-ischemic values of aortic flow, cardiac output and rate-pressure product (peak systolic pressure · heart rate) of hearts from untreated (filled circles) and TTA-treated mice (open circles). The bars to the right show percentage functional recovery after reperfusion (mean of the last three post-ischemic values relative to the mean of the last three pre-ischemic values). Results are mean of 8-9 hearts in each group. *, $p<0.05$ *vs* hearts from untreated mice.

Figure 5. Panel A; pooled scatter plot showing the linear relationship between oxygen consumption (MVO_2) and cardiac work (PVA), both expressed as J/beat/g dry wt, at increasing workloads in hearts from untreated (filled circles, dotted line) and TTA-treated (open circles, solid line) mice. Panel B; the table gives individual values and group means of the y-intercept and slope of the MVO_2-PVA relationships. The y-intercept represents unloaded MVO_2 (expressed as Joule/beat/g dry wt). The slope indicates contractile efficiency (dimensionless) and r^2 is the square of the regression coefficient. *, $p<0.05$ *vs* hearts from untreated mice.

FIG 1

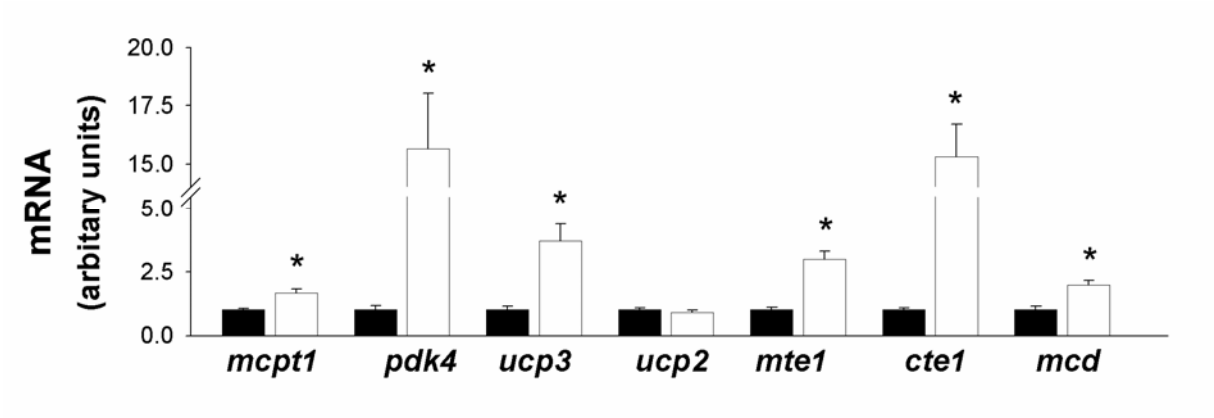

mRNA
(arbitary units)
20.0
17.5
15.0
5.0
2.5
0.0
*
mcpt1
pdk4
ucp3
ucp2
mte1
cte1
mcd

FIG 2

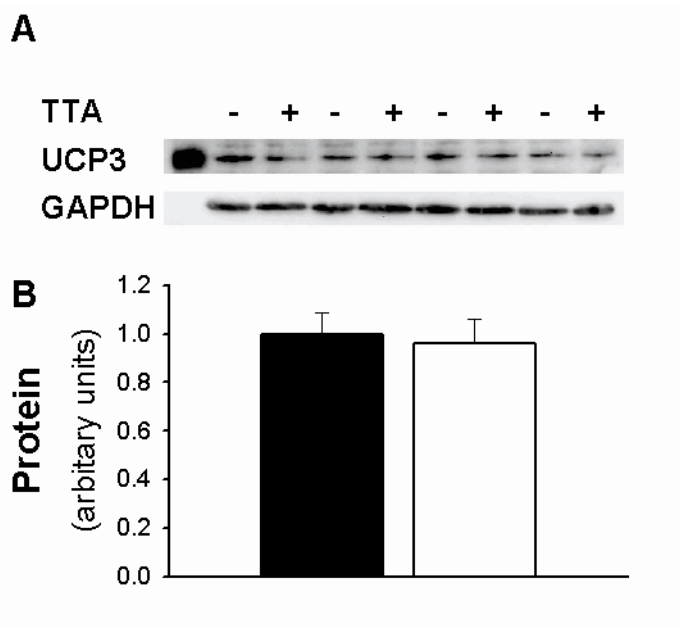

A
TTA
- + - + - + - +
UCP3
GAPDH
B
Protein
(arbitary units)
1.2
1.0
0.8
0.6
0.4
0.2
0.0

FIG 3

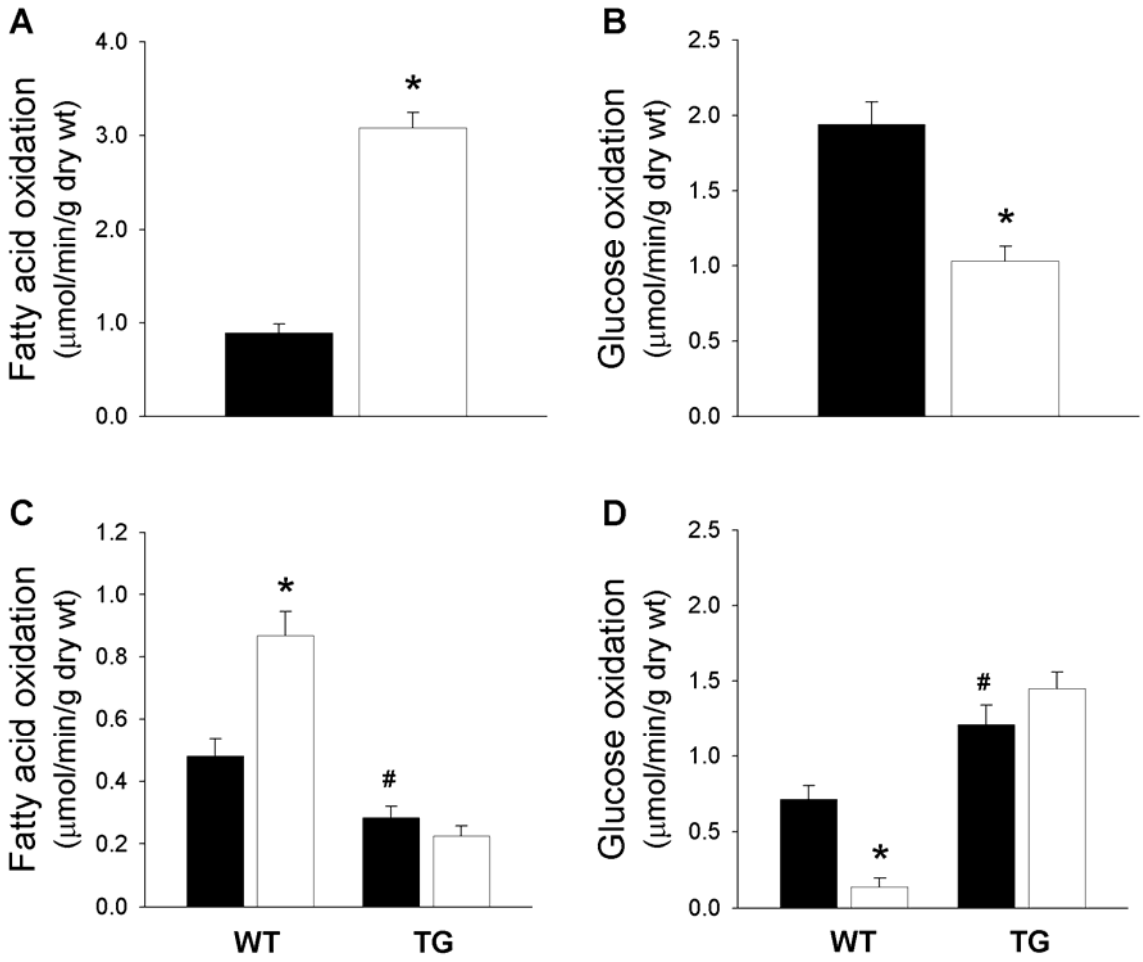

A
Fatty acid oxidation
(μmol/min/g dry wt)
4.0
3.0
2.0
1.0
0.0
*
B
Glucose oxidation
(μmol/min/g dry wt)
2.5
2.0
1.5
1.0
0.5
0.0
*
C
Fatty acid oxidation
(μmol/min/g dry wt)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
#
WT
TG
D
Glucose oxidation
(μmol/min/g dry wt)
2.5
2.0
1.5
1.0
0.5
0.0
*
#
WT
TG

FIG 4

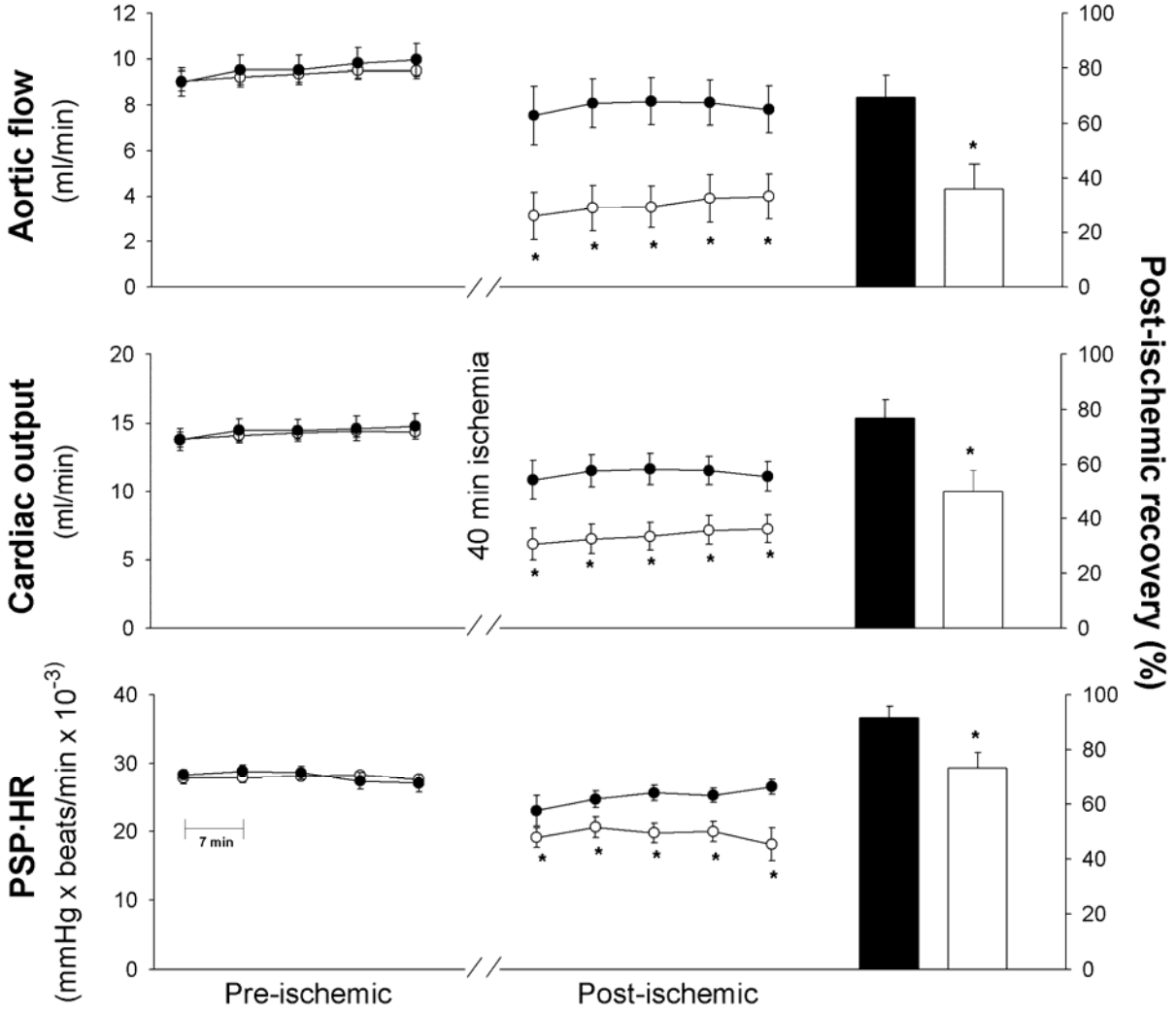
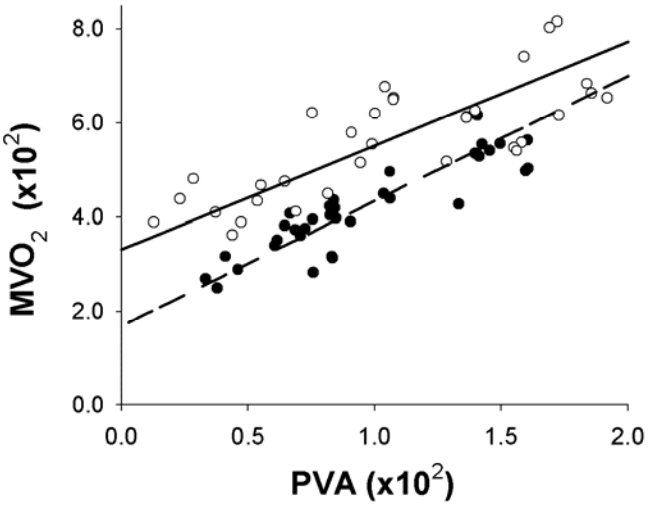

Aortic flow
(ml/min)
Cardiac output
(ml/min)
PSP·HR
(mmHg x beats/min x 10-3)
40 min ischemia
7 min
Pre-ischemic
Post-ischemic
Post-ischemic recovery (%)

FIG 5

A



B

	y-intercept (10^2)	Slope	r^2
#1	2.10	2.28	0.97
#2	2.14	2.28	0.95
#3	1.15	3.60	1.00
#4	1.96	2.66	0.93
#5	1.04	2.46	1.00
	1.68±0.24	2.66±0.25	0.97±0.01
+TTA			
#1	3.22	2.81	0.99
#2	3.32	2.11	0.99
#3	3.16	1.86	0.96
#4	3.82	2.70	0.96
#5	3.04	1.59	0.98
	3.31±0.13*	2.21±0.24	0.98±0.01