Cardiac peroxisome proliferator-activated receptor-α activation causes increased fatty acid oxidation, reducing efficiency and post-ischaemic functional loss

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Aims Myocardial fatty acid (FA) oxidation is regulated acutely by the FA supply and chronically at the transcriptional level owing to FA activation of peroxisome proliferator-activated receptor-α (PPARα). However, in vivo administration of PPARα ligands has not been shown to increase cardiac FA oxidation. In this study we have examined the cardiac response to in vivo administration of tetradecylthioacetic acid (TTA, 0.5% w/w added to the diet for 8 days), a PPAR agonist with primarily PPARα activity.

Methods and results Despite the fact that TTA treatment decreased plasma concentrations of lipids [FA and triacylglycerols (TG)], hearts from TTA-treated mice showed increased mRNA expression of PPARα target genes. Cardiac substrate utilization, ventricular function, cardiac efficiency, and susceptibility to ischaemia-reperfusion were examined in isolated perfused hearts. In accordance with the mRNA changes, 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 owing to a direct stimulatory effect on cardiac PPARα. Hearts from TTA-treated mice also showed a marked reduction in cardiac efficiency (because of a two-fold increase in unloaded myocardial oxygen consumption) and decreased recovery of ventricular contractile function following low-flow ischaemia.

Conclusion This study for the first time observed that in vivo administration of a synthetic PPARα ligand elevated FA oxidation, an effect that was also associated with decreased cardiac efficiency and reduced post-ischaemic functional recovery.

KEYWORDS Cardiac metabolism; Glucose oxidation; Fatty acid oxidation; Ischaemia-reperfusion; Cardiac function; Cardiac efficiency; PPARα target genes

1. 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.1 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.2,3 Fatty acids (FA) are endogenous activators of PPARs and accordingly, FA activation of PPARα in isolated cardiomyocytes upregulates expression of key proteins that regulate nearly every step in the cellular FA utilization pathway, including sarcolemmal FA transport (cellular uptake), FA esterification to triacylglycerol (TG), mitochondrial transport, and β-oxidation.4,5

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 oxidation6,7 and reduced expression of several genes involved in FA oxidation,8 whereas cardiac-specific PPARα over-expressing (MHC-PPARα) mice showed increased FA oxidation and upregulation of genes involved in FA uptake.7,9 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 activation leads to upregulation of key
enzymes in the FA-metabolizing pathway.\textsuperscript{4,5} experiments using \textit{in vivo} administration of PPAR\textsubscript{\alpha} ligands are inconsistent. Some reports show upregulation\textsuperscript{10–12} while others report minor changes in PPAR\textsubscript{\alpha} target genes in the heart when compared with much more pronounced increases in liver.\textsuperscript{12–14} The effect of \textit{in vivo} PPAR\textsubscript{\alpha} agonist administration on cardiac substrate metabolism has not been well studied. In our own laboratory, we found that \textit{in vivo} treatment with the PPAR\textsubscript{\alpha} ligand K-111 (previously called BM 17.0744) did not alter FA oxidation in normal mice.\textsuperscript{15} In hyperlipidaemic animals, this treatment actually reduced cardiac FA utilization,\textsuperscript{15,16} probably a secondary response to reduced plasma FA supply to the heart.

Tetradecylthioacetic acid (TTA) is a non-\textbeta-oxidizable FA analogue. Although TTA is a pan PPAR agonist,\textsuperscript{17,18} it regulates lipid metabolism mainly through activation of PPAR\textsubscript{\alpha}.\textsuperscript{17,18} TTA has been shown to increase peroxisomal proliferation and FA oxidation in the liver,\textsuperscript{17} lower the concentration of plasma lipids, improve insulin sensitivity, and prevent feeding-induced obesity.\textsuperscript{18} In this study, oral administration of TTA to mice markedly increased cardiac expression of PPAR\textsubscript{\alpha} target genes and increased FA oxidation, in spite of decreased circulating lipids. In accordance with other studies where myocardial FA oxidation was increased,\textsuperscript{19,20} these TTA-induced metabolic changes were associated with reduced cardiac efficiency and decreased tolerance to ischaemia.

2. Methods

2.1 Animals

Twelve-week-old male BALB/cA Bom mice were purchased from M&B Taconic (Ry, Denmark). In addition, male SVEV129 wild-type (WT) and PPAR\textsubscript{\alpha}-null mice (TG) were received as a gift from Dr Frank J. Gonzalez (National Cancer Institute, Bethesda, MD, USA). The latter groups were bred and housed in the animal facilities at the University of Oxford, UK, and the perfusion of the hearts of these mice were performed in UK. The rest of the work was carried out in Norway. 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. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Norwegian National Animal Research Committee or the University of Oxford Animal Ethics Review Committees and the Home Office (London, UK). The treated mice received food mixed with 0.5% (w/w) TTA for a period of 8 days. Age-matched untreated SVEV129 mice served as controls for the PPAR\textsubscript{\alpha} KO mice. BALB/cA Bom mice were used for all other experiments.

2.2 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 Boehhringer Mannheim (Mannheim, Germany), Wako Chemicals (Neuss, Germany), and ABX Diagnostics (Montpellier, France), respectively.

2.3 Real-time quantitative RT–PCR

Fresh samples from unperfused liver and heart (left ventricle) 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, USA). The primer/probe sequences for the genes studied (see Supplementary material online) were obtained from Eurogentec Ltd (Seraing, Belgium) or Roche Diagnostics GmbH (Roche Universal ProbeLibrary, Mannheim, Germany). Primers 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. House-keeping genes were selected on the basis of the average expression stability values determined with geNorm Normalisation kit\textsuperscript{21} out of a selected pool of five to six candidate genes. The expression of target gene mRNAs was normalized to the geometric mean of the three best house-keeping genes. For quantification of cardiac mRNA content, we used the geometric mean of cyclo (cyclophilin), gapdh (glyceraldehyde-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.

2.4 Heart perfusion

Mice were given an intraperitoneal injection of heparin (100 U) and anaesthetized 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 mmHg), 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 ischaemia-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 of perfusion, baseline (preschaemic) functional parameters were recorded, and thereafter hearts were subjected to 40 min low-flow ischaemia (1.68 mL/g dry weight/min) followed by 35 min reperfusion (5 min in Langendorff and 30 min in working mode). Post-ischaemic recovery of ventricular function was calculated based on values obtained at the end of reperfusion relative to pre-ischaemic baseline values. Glucose and palmitate oxidation were determined in the pre-ischaemic perfusion period by collection of \textsuperscript{14}CO\textsubscript{2} and \textsuperscript{3}H\textsubscript{2}O released by the
oxidation of [U-\(^{14}\text{C}\)]-glucose and [9,10-\(^{3}\text{H}\)]-palmitate, respectively.\(^{22,23}\)

In a separate series of hearts, cardiac performance was measured using a 1.4 F micromanometer-conductance catheter (Millar Instruments, Houston, TX, USA) inserted into the left ventricle through the apex. Partial oxygen pressure in the buffer entering (PO\(_{2}\)-buffer) and leaving (PO\(_{2}\)-effluent) the heart was measured using fibre-optic oxygen probes (FOXY-AL300, Ocean Optics Ltd., Duiven, The Netherlands) placed in the preload line and in the pulmonary trunk, respectively. Myocardial oxygen consumption (MVO\(_{2}\)) was calculated by the following equation: MVO\(_{2}\) = (PO\(_{2}\)-buffer – PO\(_{2}\)-effluent) \(\times\) Bunsen solubility coefficient of O\(_{2}\) \(\times\) coronary flow. Electrodes were connected to the right atrium for electrical pacing of the heart. In order to determine cardiac efficiency, hearts were exposed to different workloads by trical pacing of the heart. In order to determine cardiac efficiency, 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 pressure–volume loops created by a temporary effluent (PO\(_{2}\)-effluent) were the same as in controls (\(\Delta\)TTA) and normal SVEV129 (WT) mice were used in these experiments. In line with earlier studies,\(^{6,7}\) PPAR\(_{\alpha}\)-null mice exhibited diminished rates of cardiac FA oxidation compared with WT mice (Figure 2C). 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 PPAR\(_{\alpha}\)-null mice (Figure 2C and D).

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (g)</th>
<th>TTA (g)</th>
</tr>
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<tbody>
<tr>
<td>Body weight</td>
<td>29.8 ± 0.4</td>
<td>26.4 ± 0.6*</td>
</tr>
<tr>
<td>Body weight change (%)</td>
<td>4.4 ± 0.8</td>
<td>(-8.0 ± 1.0^*)</td>
</tr>
<tr>
<td>Heart weight (dry, mg)</td>
<td>143 ± 3</td>
<td>145 ± 3</td>
</tr>
<tr>
<td>Liver weight (wet, g)</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>16.1 ± 0.8</td>
<td>12.2 ± 0.7*</td>
</tr>
<tr>
<td>Plasma FA (mM)</td>
<td>0.98 ± 0.08</td>
<td>0.63 ± 0.08*</td>
</tr>
<tr>
<td>Plasma TG (mM)</td>
<td>0.64 ± 0.06</td>
<td>0.39 ± 0.04*</td>
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Body weight changes are given in % of the body weights prior to treatment. Results are from 22–25 mice in each group. *\(P < 0.05\) vs. untreated mice.

### 3.3 Myocardial metabolism

Isolated perfused hearts from TTA-treated BALB/c mice showed a 2.5-fold increase in FA oxidation (Figure 2A) with a concomitant decrease (\(-47\%\)) in glucose oxidation (Figure 2B). Despite the decrease in body weight in the TTA-treated mice, there was no increase in the plasma concentration of FA, indicating that the TTA-induced increase in FA oxidation was independent of the weight reduction. To find out whether the cardiometabolic effect of TTA was because of PPAR\(_{\alpha}\) activation, FA and glucose oxidation were measured in hearts from TTA-treated WT and PPAR\(_{\alpha}\)-null mice. PPAR\(_{\alpha}\) KO mice are on a SVEV129 background and, accordingly, both TTA-treated and untreated PPAR\(_{\alpha}\) KO (TG) and normal SVEV129 (WT) mice were used in these experiments. In line with earlier studies,\(^{6,7}\) PPAR\(_{\alpha}\)-null mice exhibited diminished rates of cardiac FA oxidation compared with WT mice (Figure 2C). 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 PPAR\(_{\alpha}\)-null mice (Figure 2C and D).

![Figure 1](image_url)  
Cardiac mRNA content of muscle type carnitine palmitoyl transferase (mcpt1), pyruvate dehydrogenase kinase 4 (pdk4), uncoupling protein 3 and 2 (ucp3, ucp2), mitochondrial and cytosolic thioesterase (mte1, cte1), and 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 eight hearts in each group. The geometric mean of cyclo, gapdh, and hprt was used as house-keeping genes. *\(P < 0.05\) vs. hearts from untreated mice.
3.4 Ventricular function and tolerance to ischaemia

Assessment of mechanical function by pressure–volume analysis (Table 2) revealed that TTA treatment did not alter heart rate, ventricular pressure, or cardiac output. We did find, however, that TTA treatment significantly increased \( \frac{dP}{dt_{\text{max}}} \), end-systolic elastance (\( E_{\text{es}} \), i.e. the slope of the regression line of the end-systolic pressure–volume relationship) and the preload-recruitable stroke work (PRSW), suggesting that TTA treatment increased myocardial contractility. The relaxation constant \( \tau \), \( \frac{dP}{dt_{\text{min}}} \), and the slope of the end-diastolic pressure–volume relationship were, however, not changed.

As increased FA oxidation has been associated with reduced myocardial tolerance to ischaemia-reperfusion, hearts were exposed to 40 min low-flow ischaemia followed by 35 min reperfusion. Again, we found no differences with respect to pre-ischaemic cardiac function (Figure 3). TTA treatment, however, resulted in reduced functional recovery, as indicated by a significant decrease in post-ischaemic recovery of aortic flow, cardiac output, and rate–pressure product.

3.5 Cardiac efficiency

The decreased tolerance to ischaemia-reperfusion in hearts with elevated FA oxidation has been associated with, and may be caused by, decreased cardiac efficiency.\textsuperscript{19} In accordance with this notion we found that TTA treatment significantly reduced cardiac efficiency (Figure 4). 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.

![Figure 2](image_url)

**Figure 2** Rates of fatty acid (palmitate) oxidation (A) and glucose oxidation (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 (C) and glucose oxidation (D) in isolated perfused hearts from untreated (black bars) and TTA-treated (white bars) wild-type (WT) and PPAR\( \alpha \)-null mice (TG). Results are mean of six to seven 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. Note that (A,B) were obtained from untreated and TTA-treated BALB/cA Bom mice. Untreated and TTA-treated WT and TG mice in (C,D) are on a different background (SVEV129).

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated (+TTA)</th>
<th>TTA-treated (+TTA)</th>
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<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>442 ± 29</td>
<td>443 ± 29</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>6.5 ± 2.2</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>LVESP (mmHg)</td>
<td>63.5 ± 2.4</td>
<td>67.6 ± 6.0</td>
</tr>
<tr>
<td>( \frac{dP}{dt_{\text{max}}} ) (mmHg/s)</td>
<td>4934 ± 429</td>
<td>6074 ± 694*</td>
</tr>
<tr>
<td>( \frac{dP}{dt_{\text{min}}} ) (mmHg/s)</td>
<td>4579 ± 561</td>
<td>4900 ± 287</td>
</tr>
<tr>
<td>( \tau ) (Glantz method) (ms)</td>
<td>13.0 ± 2.0</td>
<td>11.3 ± 1.4</td>
</tr>
<tr>
<td>Cardiac output (mL/min)</td>
<td>13.6 ± 1.8</td>
<td>13.6 ± 0.7</td>
</tr>
<tr>
<td>Stroke work (mmHg × μL)</td>
<td>1809 ± 341</td>
<td>1840 ± 193</td>
</tr>
<tr>
<td>( E_{\text{es}} ) (mmHg × μL)</td>
<td>0.93 ± 0.18</td>
<td>1.54 ± 0.47*</td>
</tr>
<tr>
<td>EDPVRslope (mmHg/μL)</td>
<td>0.22 ± 0.06</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>PRSW (mmHg)</td>
<td>36.3 ± 2.4</td>
<td>46.4 ± 7.5*</td>
</tr>
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</table>

Steady-state parameters were obtained at pre- and afterloads of 10 and 50 mmHg, respectively. LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure. \( \tau \) is the isovolumic relaxation constant analysed using Glantz method. The slope of the end-systolic pressure–volume relationship (\( E_{\text{es}} \)) and the end-diastolic pressure–volume relationship (EDPVR), as well as the preload-recruitable stroke work (PRSW) were assessed from a family of pressure–volume loops created by a temporary reduction of the preload. Results are mean of five hearts in each group.

*\( P < 0.05 \) vs. hearts from untreated mice.
4. 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 TTA leads to upregulation of PPARα target genes and increased myocardial fatty acid (FA) oxidation, despite decreased circulating levels of lipids. The finding that TTA treatment had no effect in PPARα-null 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- and loss-of-function approaches, i.e. MHC-PPARα mice and PPARα-null mice.7–9,24

Figure 3  Pre- and post-ischaemic 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-ischaemic values relative to the mean of the last three pre-ischaemic values). Results are mean of eight to nine hearts in each group. *P < 0.05 vs. hearts from untreated mice.

Figure 4  (A) Pooled scatter plot showing the linear relationship between oxygen consumption (MVO₂) and cardiac work (PVA), both expressed as Joules/beat/g dry weight, at increasing workloads in hearts from untreated (filled circles, dotted line) and TTA-treated (open circles, solid line) mice. (B) The table gives individual values and group means of the y-intercept and slope of the MVO₂–PVA relationships. The y-intercept represents unloaded MVO₂ (expressed as Joules/beat/g dry weight). The slope indicates contractile efficiency (dimensionless) and r² is the square of the regression coefficient. *P < 0.05 vs. hearts from untreated mice.
TTA is a saturated FA which is modified by insertion of a single sulphur atom at the third position in the carbon backbone. The presence of the sulphur atom makes TTA coenzyme A resistant to β-oxidation. Although studies have revealed that TTA is a pan PPAR agonist and the degree of activation of the different PPARs depends on the cell type studied, the ranking order of activation is: PPARα > PPARδ > PPARγ. It is well documented that TTA has a hypolipidaemic 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.17,18,25,26

Myocardial FA oxidation rate is largely determined by lipid supply to the heart. Thus, an acute increase in the plasma FA level in intact animals,27 or increased perfusate FA in isolated heart perfusions,19 will accelerate myocardial FA oxidation and reduce glucose utilization (Randle cycle). It is also known that chronic elevation of plasma lipids will increase the reliance on FA as energy substrate in the heart, because of transcriptional changes in metabolic enzymes (PPARα target genes).2,2 Therefore, increased myocardial FA oxidation in type 2 diabetes/obesity is because of increased FA supply, as well as an FA-induced activation of cardiac PPARα. The regulatory role of PPARα in the heart has also been demonstrated by increased FA oxidation in MHC–PPARα mice and decreased FA oxidation in PPARα-null mice.7–9,24 In accordance with this, studies on isolated cardiomyocytes have shown that PPARα agonists lead to upregulation of PPAR target genes, coincident with increased FA oxidation rates.4,5 However, 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, and transcriptional changes in the heart were minor when compared with much more pronounced increases in the liver.9,12–14 In the current study, the PPAR ligand TTA produced 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 palmitoyl CoA in cardiac tissue from rats treated with TTA.28 The fact that treatment with TTA increased myocardial FA oxidation, despite its hypolipidaemic effect indicates that the effect on cardiac metabolism must be direct owing to changes in gene expression rather than an altered FA supply. Although TTA is a pan-PPAR agonist, there is no evidence for direct PPARγ regulation of heart metabolism. TTA could activate PPARδ with stimulatory effects on FA oxidation. Our observation that TTA had no stimulatory effect on FA oxidation in perfused hearts from PPARα-null mice, however, argues strongly that the metabolic effects of TTA on the heart are because of PPARα activation.

It must be acknowledged, however, that elevated rates of cardiac FA oxidation after TTA treatment were observed with ex vivo hearts perfused with the same FA (palmitate) concentration as was used with untreated control heart perfusions. The net in vivo effect of TTA administration will have to take into consideration the opposing effects of TTA: to directly upregulate PPARα target genes in the heart that can result in increased rates of FA oxidation measured ex vivo vs. the indirect FA-lowering effect. Therefore, in vivo experimentation with a tracer FA analogue will be required to establish the net effect of TTA on myocardial FA oxidation. Notwithstanding this caveat, it is still notable that TTA treatment was able to show evidence for cardiac PPARα activation, and that this 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,15 whereas both K-11116 and fenofibrate13 reduced myocardial FA oxidation in hyperlipidaemic mice, an effect which was most likely secondary to the lipid-lowering effect of these PPARα ligands. Our working hypothesis prior to the present study, therefore, was that PPARα ligands primarily affect cardiac metabolism indirectly through changes in the lipid supply. As far as we know, this study is the first to report that a synthetic PPAR ligand, following in vivo administration on a normal animal, promotes a switch in myocardial substrate preference towards FA.

The discrepancy between the cardiac metabolic effects of TTA compared with K-11115,16 and fenofibrate13 may be related to differences in tissue uptake of the synthetic ligands and/or affinity for 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.29 reported higher concentrations of TTA in the heart than in the liver of TTA-treated rats.

Both in vivo and ex vivo studies have shown that increased myocardial FA oxidation is associated with increased myocardial oxygen consumption (MVO2) and decreased cardiac efficiency,19,27 similarly to what has also been reported in diabetic hearts.19,20 In accordance with this, TTA treatment reduced cardiac efficiency because of a significant increase in unloaded MVO2. As increased MVO2 may have deleterious consequences, particularly under conditions of limited oxygen supply by narrowing the window of reversible damage; it most likely caused the decreased post-ischaemic function in hearts from TTA-treated mice.

Elevated unloaded MVO2 (work-independent MVO2) reflects increased oxygen cost for non-mechanical purposes such as basal metabolism and excitation–contraction (E–C) coupling. The mechanism explaining this elevation in unloaded MVO2 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),31 (ii) induction of futile cycles, including FA–TG cycling,32 and/or increased H+ transport owing to uncoupling of glucose oxidation from glycolysis,13 and/or (iii) mitochondrial uncoupling.34 UCP3 has been proposed to play an important role in permitting high rates of FA oxidation,35 and to contribute to reducing the mitochondrial membrane potential,36 which could lead to increased MVO2. The lack of functional studies, however, makes it difficult to assign a role for UCP3 in the TTA-induced increase in MVO2. Finally, oxygen consumption in the unloaded heart also includes oxygen cost for E–C coupling, and it is well known that inotropic agents will increase unloaded MVO2.27 We found that TTA treatment induced a clear increase in myocardial contractility, as indicated by both an increase in the dp/dtmax, PRSW, and end-systolic elastance. Although the mechanism and physiological implication of this require
further studies, it is likely that altered cardiac Ca²⁺ handling may at least partly account for the TTA-induced increase in MVO₂.

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. TTA treatment was also associated with decreased cardiac efficiency and reduced post-ischaemic functional recovery. The TTA-induced increase in FA oxidation occurred in the face of decreased circulating lipid levels, demonstrating that an exogenous ligand can override the impact of endogenous (circulating) FAs on PPARα-mediated control of cardiac metabolism. It illustrates the potential of PPAR ligands as regulators of myocardial metabolism via both direct and indirect routes, and that further studies are required to clarify the cardiometabolic effects of these compounds.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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Conflict of interest: Thia Medica AS holds patents regarding effects of TTA. R.K.B., the University of Bergen, and Haukeland University Hospital are shareholders of Thia Medica AS.

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