Activation of PPARδ signaling improves skeletal muscle oxidative metabolism and endurance function in an animal model of ischemic left ventricular dysfunction

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ABSTRACT (245 words)

**Background** Exercise intolerance in heart failure has been linked to impaired skeletal muscle oxidative capacity. Oxidative metabolism and exercise capacity are regulated by PPARδ signaling. We hypothesized that PPARδ stimulation reverts skeletal muscle oxidative dysfunction.

**Methods** Myocardial infarction (MI) was induced in C57BL/6 mice and the development of ventricular dysfunction was monitored over 8 weeks. Mice were randomized to the PPARδ agonist GW501516 (5 mg/kg body weight per day for 4 weeks) or placebo 8 weeks post-MI. Muscle function was assessed through running tests and grip strength measurements. In muscle, we analyzed muscle fiber cross-sectional area and fiber types, metabolic gene expression, fatty acid (FA) oxidation and ATP content. Signaling pathways were studied in C2C12 myotubes.

**Results** FA oxidation and ATP levels decreased in muscle from MI mice compared to sham-operated mice. GW501516 administration increased oleic acid oxidation levels in skeletal muscle of the treated MI group compared to placebo treatment. This was accompanied by transcriptional changes including increased CPT1 expression. Further, the PPARδ-agonist improved running endurance compared to placebo. Cell culture experiments revealed protective effects of GW501516 against the cytokine-induced decrease of FA oxidation and changes in metabolic gene expression.

**Conclusion** Skeletal muscle dysfunction in HF is associated with impaired PPARδ signaling and treatment with the PPARδ agonist GW501516 corrects...
oxidative capacity and FA metabolism and improves exercise capacity in mice with LV dysfunction. Pharmacologic activation of PPARδ signaling could be an attractive therapeutic intervention to counteract the progressive skeletal muscle dysfunction in HF.

**Key words** Heart failure, skeletal muscle, metabolism, PPARδ
Skeletal muscle weakness and exercise intolerance are hallmarks of advanced heart failure (HF) and have been shown to correlate with a reduction in oxidative metabolism and protein catabolism (5, 37). This metabolic derangement results not only from a decrease in oxygen- and nutrient supply to skeletal muscle but also from intrinsic changes in skeletal muscle gene expression, cytokine activation and mitochondrial dysfunction (24, 33). These changes result in a shift of muscle fibers from oxidative towards more glycolytic fibers and an impaired oxidative capacity of skeletal muscle in HF (5). Effective treatments for skeletal muscle dysfunction in HF are lacking; only aerobic exercise training has been shown to improve skeletal muscle function and exercise intolerance which was linked to an associated improvement in oxidative metabolism and mitochondrial function following exercise (10, 25).

PPARs (Peroxisomal Proliferator Activating Receptors) are a family of nuclear receptors that sense metabolic status and engage the cell in lipid uptake and utilization (28, 31). Among the three members of this family (PPARα, γ and δ), PPAR δ is the predominant isoform in skeletal muscle (1, 3, 16). PPARδ levels are higher in oxidative type I compared to glycolytic type II muscle fibers (34) and PPARδ gene deletion mice have a lower proportion of type I muscle fibers (23). Mice over-expressing constitutively active PPARδ show an increase of type I fibers (34). Wild-type mice receiving a PPARδ agonist elicited a similar type I fiber gene expression profile in their skeletal muscle and showed an increase in expression of genes related to oxidative function of mitochondria and fatty acid
oxidation (34). The specific PPARδ agonist GW501516 up-regulates genes involved in fatty acid transport, beta-oxidation and mitochondrial respiration (29).

Therefore, we hypothesized that in mice with left ventricular dysfunction following myocardial infarction (MI) and signs of skeletal muscle dysfunction, treatment with the PPARδ agonist GW501516 improves oxidative metabolism, skeletal muscle catabolism and endurance function.

METHODS

Animal model

Left ventricular (LV) dysfunction was induced in C57BL/6 mice by ligature of the left coronary artery and confirmed by echocardiography (n=5). Sham-operated animals underwent the same procedure without ligation of the coronary artery (n=5). To test the impact of PPARδ agonism, starting at 8 weeks after surgery, an additional group of mice received the PPARδ agonist GW501516 (5 mg/kg body weight dissolved in DMSO and 0.5% carboxymethylcellulose per day for 4 weeks, n=10) or placebo (DMSO only, n=10) via gavage. Animals were sacrificed 12 weeks post-MI after 4 hours of starvation.

For the endurance running test, mice were accustomed to the treadmill by running 5 min per day for 3 days at a 0° incline and a belt speed of 10 m/min. At the time of the test (on the day before sacrifice), mice ran on a treadmill set at a 20° incline with an initial belt speed of 10 m/min. The speed was increased to 14 m/min at minute 10 and to 18 m/min at minute 15. Mice ran until exhaustion at 18 m/min speed. Exhaustion was judged by refusal of the mice to continue running.
on the treadmill belt after being stimulated to run by gently being pushed forward three times.

This study and its protocols were approved by the local IACUC institutional review board of Columbia University.

**Echocardiography**

Cardiac function was assessed by echocardiography at baseline, 8 weeks post-MI and before sacrifice to assess successful MI induction and the possible effect of the treatment on myocardial function and remodeling. Mice were anesthetized with inhalational isoflurane anesthesia. Anesthesia was induced by 1.5-2% isoflurane, and reduced to 0.5-1% once the mouse was asleep. The heart was visualized by using a 30 MHz high frequency ultrasound transducer (Visualsonics Vevo770, Toronto, Canada).

**Muscle fiber histomorphometry**

Quadriceps muscles were excised and fixed in approximately 20 times its volume in 10% buffered Formalin phosphate (Fisher Scientific) and then stored in 70% ethanol with refrigeration until embedding procedure. H and E staining was performed in skeletal muscle sections and used in cross sectional area analysis. Image J (NIH) software was used to measure cross sectional areas in 10x magnification. Pictures were taken using a Leica microscope and Spot camera and software (Diagnostic Instruments) of at least 400 quadriceps fibers per animal.
**Muscle fiber typing**

Quadriceps were excised and fixed in 20 times its volume in 10% buffered Formalin phosphate (Fisher Scientific) and then stored in 70% ethanol with refrigeration until embedding procedure. Paraffin enclosed sections were fixed in 10% buffered Formalin phosphate for 30 min and then deparaffinized in Histo-Clear, histological clearing agent (National Diagnostics) for 5 min, 3 times followed by rehydration from 100% to 75% ethanol. Antigen unmasking was performed by boiling in 1x Antigen retrieval solution (BioGenex, Fremont, CA) for 30 min and then slides were blocked with 6% normal goat serum (Vector) in Phosphate Buffered Solution (PBS) for 30 min. Monoclonal antibody directed against skeletal muscle fast fiber type myosin (Sigma M4276) diluted 1:400 in 6% in normal goat serum (Vector), Phosphate Buffered Solution was used for incubation overnight at 4 °C. After washing in 0.1% Tween PBS and PBS, slides were incubated in 3% Hydrogen peroxide for 10 min and then washed again before incubation with secondary biotinylated Anti-mouse IgG made in goat (Vector) diluted 1:400 in 6% in normal goat serum (Vector), PBS for 30 min at room temperature. Slides were then washed and incubated for 30 min with Vecstatin ABC kit (Vector) at room temperature following manufacturer instructions followed by washing and developed using NovaRED (Vector) diaminobencidine substrate. Reaction was stopped in distilled water and dehydrated in ethanol solutions from 75% to 100 % and cleared with Histo-Clear, histological clearing and finally mounted in Permount (Fisher Scientific). 10x magnification pictures were analyzed for cross sectional area.
Citrate synthase activity assay

Skeletal muscle pieces of about 20 mg were homogenized 20% (WT/V) homogenization buffer (20 mM HEPES, 10 mM EDTA, pH 7.4) on ice. Homogenates were frozen for 1 hour and then diluted 1:10. The reaction was done in 20 mM HEPES, 1 mM EDTA, 220 mM, 40 mM KCl, 0.1 mM 2-nitrobenzonic acid, 0.1 mM acetyl-CoA, pH 7.4) at 25 °C. Reaction was started by adding 0.05 mM oxaloacetate and OD was measured at 412 nm for 3 min.

Succinate dehydrogenase activity assay

Skeletal muscle tissue was homogenized in 250 mM saccharose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.2, centrifuged for 10 min and 4°C at 600 xg. The supernatant was centrifuged at 12000 xg for 10 min, 4 °C. The pellet was resuspended in incubation medium (5 mM KPO, pH 7.2, 220 mM saccharose, 20 mM, KCl, 10 mM Hepes, pH 7.2). Reaction was assayed in 100 mM triethanolamine (HCl), pH 8.3, 0.5 mM EDTA, 2mM KCN, 2 mM iodonitrotetrazolium chloride (INT), 2 g/l Cremophor EL, 20 mM succinate, previously adjusted to pH 7.4. The reduction of INT to a formazan was followed at 500 nm at 30 °C for 6 min.

Calculations were performed according to the respective assay protocol (Sigma Aldrich, Citrate Synthase Assay Kit, Catalog Number CS0720; Sigma Aldrich, Succinate Dehydrogenase Activity Colorimetric Assay Kit, Catalog
Number MAK197) with an abundant substrate concentration. All analyses are based on endpoint measurements. The kinetic parameter $K_m$ was not measured.

**Mitochondrial DNA copy number**

The number of mitochondria was assessed by the ratio of cytochrome c oxidase subunit 2 (a mitochondrion-encoded gene) mRNA expression levels corrected for cyclophilin A (nuclear-encoded gene) mRNA expression levels and compared between groups.

**ATP content analysis**

Skeletal muscle was excised and immediately frozen in liquid nitrogen. Tissue was cut into 20 mg pieces an immediately homogenized in 10 volumes of 0.25% trichloroacetic acid, 2 mM EDTA using 1.0 mm Zirconia/Silica beads (BioSpec Products). Homogenates were spun at 4500 rpm for 10 min at 4 °C. The supernatant was diluted 1:10 with 250 mM Tricine buffer, pH 7.8, 50 mM MgSO$_4$, 1 mM EDTA and 1 mM sodium azide. ATP concentration was determined in the diluted supernatant using a firefly luciferase bioluminescence-based ATP determination kit (Invitrogen) following manufacturer instructions. Protein concentration was determined by BCA method.

**Fatty acid oxidation measurements**

30 mg of quadriceps muscles was assayed for fatty acid oxidation activity as previously described (12) with modifications as follows: Tissue pieces were placed in flask containing 1 ml low glucose DMEM cell culture medium (Cellgro),
1.5% delipidated bovine serum albumin fraction V (Sigma), 0.2 mM oleic acid, 1 µCi/ml [14C] oleic acid, cup with rubber stoppers that had been inserted a cell holding a rolled filter paper and incubated at 37 °C for 2 hours. The filter papers were soaked with 1N KOH and the reaction was stopped injecting 200 µl of 70% perchloric acid and incubating for 1 hour with shaking. At the end of the incubation, the filter papers were transferred to vials containing scintillation liquid and counts per minute (cpm) were measured using a scintillation liquid counter. The total oleic acid oxidation reaction rate was evaluated as $^{14}$CO$_2$ released and analyzed from cpm in the filter papers.

RT-PCR

Gene expression was analyzed by real time PCR (iCycler, Biorad) using specific oligonucleotides: PDK4: 5'-'AACGCAACACAAAAACCAAGC-3'(f), 5''-
CATTGCCAAAGGAGAAGCAG-3'(r), CD36: 5'-'CAGCCTCCTTTCCACCTTTT-3'(f), 5''-GCATTGGCTGGAAGAACAA-3'(r), CPT1: 5''-
CCTGCAAGATGGCCTGT-3'(f), 5''-GCTCAACCACACAGTGTCCT-3'(r),
PDC1: 5''-
PGC1a: 5''-CAGCCCTTCCTATTCA-3'(f), 5''-GTCGTTAGGTGAAACAGAAA-3'(r), LPL:
5''-GCTGCGAATGATGTG-3'(f), 5''-TGGACGTGCTAGGGGTA-3'(r),
ANGPTL4: 5''-GGAAAAGATGCCCTCAA-3'(f), 5''-TGCTGGATCTTGCTGTTTTG-3'(r), ATG1: 5''-
TGCTGGATTGGAATGATGTG-3'(f), 5''-GTCGTTAGGTGAAACAGAAA-3'(r), LPL:
5''-GCTGCGAATGATGTG-3'(f), 5''-TGGACGTGCTAGGGGTA-3'(r),
UCP2: 5''-
ACGACCATTGACGGAG-3'(f), 5''-CATGGTCAGGGCAGAGTGGC-3'(r),
COXII: 5''-CAGGCGACTAAATCAAGCAAC-3'(f), 5''-
CTAGGACAATGGGCATAAAGCT -3’ (r), Cyclophilin A: 5’-
TTCCTCCTTTCACAGAATTATTCCA-3’(f), 5’-
TTCCTCCTTTCACAGAATTATTCCA-3’(f), 5’- CCGCCAGTGGCCATTATGG-3’(r),
FABP3: 5’-CATGACCAAGCCTACCACAAT-3’(f), 5’-
CCCAAACCTTAAGCTGATCTCTG-3’ (r).

Cell Culture
Mouse C2C12 myoblasts were cultured in Dulbecco’s modified essential medium HighGlucose (Gibco) with 10% fetal calf serum, 1% Penicillin-Streptomycin at 37 ºC, 5% CO₂. After reaching 80-90% confluence cells were washed with PBS removing the media and incubated with DMEM containing 3% Horse Serum (Sigma) to induce differentiation into myotubes for 2-5 days.

Statistical analysis
Statistical analysis was performed using Student’s t-test for comparison between two groups and ANOVA with subsequent post-hoc analysis for more than 2 groups. A p-value <0.05 was considered statistically significant. Analysis was performed using GraphPad Prism (version 5). All values are presented as means ± SEM.

RESULTS
Animal model
MI by ligation of the left coronary artery was confirmed by echocardiography (8 weeks post-surgery: fractional shortening 31.6 ± 2.9 in the sham group vs. 18.5 ± 4.2% in the MI group, p=0.041, LVEDD 4 ± 0.2 vs. 5 ± 0.3 mm, p=0.033). Skeletal muscle histomorphometry showed no significant changes in oxidative fiber proportion in the MI group, also, cross-sectional area analysis revealed no changes (Fig. 1F, G). Exercise endurance function was reduced (Fig. 1H, running to exhaustion: 31 ± 2.7 min vs. 25 ± 0.7, p=0.046) without changes in grip strength (4.8 ± 0.4 vs. 5.5 ± 0.5 g/g BW; p=0.25).

Characterization of abnormal skeletal muscle oxidative capacity post-MI

Ex vivo oleic acid oxidation measurements in quadriceps muscle showed a decrease in fatty acid oxidation rates in HF animals compared with control (-57%, p=0.005, Fig. 1A). Expression of pro-inflammatory TNFα was increased in skeletal muscle of animals following MI (fold change 1 ± 0.27 n=3 vs 3.94 ± 0.66 n=3, p=0.015) (Fig. 1E). Analysis of skeletal muscle ATP content revealed significant differences in ATP content in skeletal muscle of animals following MI compared to sham surgery (-54%, p=0.009, Fig. 1B).

To address whether mitochondrial function was abnormal at the level of Krebs' cycle enzymatic activity, we assessed the activity of citrate synthase (CS), a rate-limiting enzyme of the Krebs cycle, and succinate dehydrogenase (SDH), which is pivotal for electron chain transfer, in skeletal muscle. No difference between sham-operated mice and the MI group was found (Fig. 1C, D). Of note,
citrate synthase has been established as a marker of skeletal muscle mitochondrial density (9, 17).

**Analysis of PPAR signaling in C2C12 cells**

We next assessed the role of PPAR signaling on cytokine-induced changes of oxidative capacity in C2C12 myotubes. TNF$_{\alpha}$ treatment (25 ng/ml, 16h) reduced oleate oxidation rates in vitro by 28.9% (p<0.001 versus unstimulated cells, **Fig. 2A**). Co-stimulation with the PPAR$\delta$ agonist GW501516 (5 $\mu$M, (2)) at baseline and in the presence of TNF$_{\alpha}$, increased oleate oxidation rates by 65% (p<0.001 versus unstimulated cells) and 40.8% (p<0.01 versus TNF$_{\alpha}$-stimulated cells). Co-stimulation with the PPAR$\alpha$ agonist WY14643 (80 $\mu$M, (17)) had no effects at baseline and did not correct the TNF$_{\alpha}$-induced decrease in FA oxidation rates (**Fig. 2A**). Further, expression of the marker gene and rate limiting enzyme of FA oxidation, carnitine palmitoyltransferase-1 (CPT1), decreased in the presence of TNF$_{\alpha}$ (-43%, p=0.045) and increased upon co-stimulation with the PPAR$\delta$ agonist GW501516 (TNF$_{\alpha}$ vs. TNF$_{\alpha}$+GW501516: +196%, p=0.017) (**Fig. 2B**).

**Analysis of in vivo effects of PPAR$\delta$ stimulation post-MI**

To investigate whether GW501516 improves skeletal muscle oxidative capacity in an animal model with left ventricular dysfunction following MI, 8 weeks old C57BL/6 mice were subjected to ligation of the left coronary artery leading to MI. 8 weeks after the surgery, animals with echocardiographic
evidence of left ventricular dysfunction were randomized to GW501516 or placebo via daily gavage for a total of 4 weeks. Echocardiography revealed that 4 weeks of treatment with the PPARδ agonist GW501516 had no significant effect on fractional shortening 12 weeks following MI (FS: 15.5±3.5 before vs. 19.4±3.7% after treatment, p=0.46; LVEDD: 5.6±0.3 vs. 5.2±0.5 mm, p=0.323).

Oleic acid oxidation rates increased in skeletal muscle of animals treated with GW501516 compared to placebo (+47.6%, p=0.048, Fig. 3A). This was accompanied by changes in skeletal muscle gene expression showing increased CPT1 and Angptl4 levels in the treatment group (CPT1: +101%, p<0.01; Angptl4: +78.2%, p<0.01, Fig. 3B) and a non-significant increase of ATP normalized for protein content in skeletal muscle (Fig. 3C). Analysis of skeletal muscle histomorphometry demonstrated no differences in muscle CSA (Fig. 3D) or fiber type composition. No statistical differences in the number of mitochondria as assessed by DNA copy number (ratio mitochondrial DNA copy number/genomic DNA copy number: 0.51±0.11 vs. 0.74±0.16, p=0.27) were found between groups while a numerical trend towards higher numbers in response to GW501516 treatment was noted. We finally evaluated exercise endurance function by performing a treadmill running test. As expected, mice with left ventricular dysfunction following MI had shorter running times compared to sham-operated animals (Fig. 1H). 4 weeks of treatment with GW501516 resulted in an increased running time of PPARδ- agonist treated animals compared to the untreated MI-
group, indicating improved endurance exercise function (distance and time)
(25.0±0.7 vs. 33.7±3.8 min, p=0.05; distance: 271.8±21.3 vs. 399.5±58.5 m, 
p=0.067; Fig. 3E). Grip strength did not differ between the two groups (5.5±0.48 vs. 4.9±0.31 g/g BW, p=0.25).
Analysis of plasma TAG concentrations showed a significant decrease of
triglyceride concentrations in the GW501516-treated group compared to placebo 
(5 ± 0.35 vs. 3 ± 0.62 µg/µl, p=0.017 (Table 1).
No other significant differences were found in plasma FFA concentrations 
(FFA: 0.8±0.1 vs. 0.58±0.1 pmol/µl) or other metabolic parameters between
placebo-treated and PPARδ-agonist treated groups of the murine MI model
except for a trend (p= 0.14) towards an increase of TAG in skeletal muscle in the
GW501516 treated group compared to placebo (100.2 ± 14.9 µg/mg vs. 72.6 ±
5.4 µg/mg) Treatment with the PPARδ agonist GW501516 did not result in
differences in baseline fasting glucose levels compared to placebo treatment
(Table 1).

DISCUSSION

In the current study, we show positive effects of PPARδ agonism on
skeletal muscle oxidative capacity and endurance function. Our data reveal a
potential therapeutic role of PPARδ signaling for the control of skeletal muscle
homeostasis and functional improvement in the setting of HF, which is known to
be associated with a profound skeletal muscle myopathy (5, 37). We show that
the oral PPARδ agonist GW501516 is capable of significantly improving impaired
oxidative metabolism in skeletal muscle. Skeletal muscle dysfunction is a central impairment in advanced HF (5, 24, 33, 37). This has been linked to intrinsic skeletal muscle abnormalities that include abnormal metabolic gene expression, reduced oxidative capacity and overall mitochondrial dysfunction, increased cytokine levels, muscle fiber atrophy and type switching. Type I muscle fibers, with preferential utilization of fatty acids as substrate for oxidative metabolism, are known to have better endurance function compared to type II glycolytic fibers (30) and have been shown to be decreased in HF (5). Further, functional assessments demonstrate the development of a specific HF-related myopathy with reduced endurance function contributing to exercise intolerance in patients with HF and animal models of HF (33, 37). Unfortunately, therapeutic interventions are lacking and the majority of pharmacologic and non-pharmacologic interventions in HF are focused on improving cardiac function or remodeling (36). The only intervention proven to affect skeletal muscle function, metabolism and structure is aerobic exercise training which has been shown to improve (but not correct) mitochondrial dysfunction, skeletal muscle metabolism and structure (7, 25). Only recently, several studies have implicated PPARδ signaling in favorable changes in skeletal muscle structure and metabolism that were considered beneficial in the setting of HF.

PPARδ agonists mediate a shift of glucose oxidation towards fatty acid oxidation in muscle tissue (11). *In vitro* experiments in L6 cells using GW501516 showed increased expression of genes involved in fatty acid transport, beta-oxidation and mitochondrial respiration (29). Wang et al. showed an increase in
mRNA of CPT1, troponin I and cytochrome c oxidase IV in gastrocnemius muscle of mice treated with GW501516 (34). CPT1 is a mitochondrial enzyme crucial for fatty acid transportation into the mitochondrion and an essential, rate limiting step for fatty acid oxidation overall. Troponin I is a muscle marker of oxidative fiber types while cytochrome c oxidase IV is involved in mitochondrial electron transport, suggesting that PPARδ agonists increase oxidative metabolism in skeletal muscle. In contrast, neither PPARγ nor PPARα agonists are able to modify fatty acid oxidation capacity of skeletal muscles (29).

PPARδ expression is regulated by exercise. Exercise training and brief bouts of exercise increase PPARδ expression in skeletal muscle (6, 15, 22, 35). Further, PPARβ/δ and PGC-1α mRNA increase early after one session of high intensity exercise training (20). Similarly, a 12 week exercise training protocol in rats increased PPARδ in plantaris muscle (26). PPARδ expression is positively correlated with the proportion of type I fibers in skeletal muscle and is essential for maintaining the oxidative fiber type composition that defines muscle endurance function (13).

In our current study, we defined PPARδ signaling-specific effects on skeletal muscle homeostasis. Using the PPARα-specific agonist WY14643 in C2C12 cells, we did not observe any beneficial effects on cellular oxidative function when compared to the strong effects of the PPARδ-specific agonist GW501516. These findings are well in line with prior studies by other groups. Tanaka et al. reported that in L6 cell myotubes, fenofibrate, another PPARα
agonist, does not increase palmitate oxidation rates in contrast to GW501516, which produced an increase in fatty acid oxidation rates (29). In another study, the PPARδ-specific agonist GWD0742 was able to stimulate fatty acid oxidation in human skeletal muscle tissue with high potency compared to PPARα stimulation (5). Our results demonstrate that the PPARδ agonist GW501516 induces an increase in free fatty acid oxidation rates in skeletal muscle and an improvement in endurance exercise performance of animals with ischemic LV dysfunction in response to PPARδ agonist treatment compared to placebo. The analysis of fiber type composition failed to reveal significant differences. Other authors described similar results in normal and diabetic animals (18, 29).

Increased fatty acid oxidation rates in skeletal muscle correlated with increased expression of CPT1 mRNA indicating an increase of fatty acid transport capacity into mitochondria. CPT1 has a pivotal role in the transport of fatty acids in form of acylcarnitines into the mitochondria and functions as the rate-limiting step. Hence, changes in CTP1-expression are a major indicator for alterations of fatty acid utilization on the mitochondrial level.

As it is known from changes in cardiac metabolism in HF, a switch from oxidative towards more glycolytic metabolism occurs in skeletal muscle in HF. It is, however, unclear whether this is driven by changes in oxygen supply since arterio-venous oxygen gradients are normal while under exercise the venous oxygen saturation decreases suggesting either decreased peripheral flow or increased utilization. On the other hand, local inflammation as highlighted by increased expression of TNFα in our current study might contribute to changes in
oxidative capacity. It has been demonstrated that metabolism under inflammation is driven by glycolysis for ATP production. It is, however, unclear whether skeletal muscle substrate utilization of fatty acids, glucose, amino acids and lactate is deranged in vivo.

Analysis of TNFα mRNA expression levels in skeletal muscle of our treatment groups revealed a significant increase in TNFα expression in the MI group compared to sham but no effects of GW501516 on TNFα expression levels were observed. This suggests that the effects on skeletal muscle oxidative capacity and structure are either independent or downstream of TNFα signaling in the murine model.

In our study we could not observe significant changes in the number of mitochondria as assessed by DNA copy number nor in activities of enzymes of the Krebs cycle. This is in accordance with previous findings describing that PPARδ agonists do not stimulate mitochondrial gene expression but stimulate gene expression controlling fatty acid oxidation (10, 18).

An intriguing finding of our study is the increased expression of angiopoietin-like 4 (Angptl4) in response to PPARδ stimulation. Angptl4 is a multifunctional protein and known to be a target of PPARδ (21). Angptl4 increases levels of hormone-sensitive lipase, a triglyceride lipase that breaks down intracellular triglyceride levels and increases fatty acid release through enhanced triglyceride turnover. Therefore, an increase in Angptl4 might indicate
increased lipid utilization as substrate for oxidative phosphorylation in skeletal muscle (27).

Further qPCR analysis of key metabolic genes revealed no significant effect of PPARδ agonism on these gene expression levels (Figure 3B). Unchanged levels of PDK4 mRNA expression go well in line with the observed unchanged ATP concentrations in skeletal muscle. PGC1α is a crucial regulator of mitochondrial biogenesis and muscle fiber type determination. Fiber type analysis showed no changes in muscle fiber type composition. We hypothesize that treatment for 4 weeks with the PPARδ agonist is either not powerful enough or might have been too short to induce significant structural alterations in muscle fiber composition. Hence, considering the duration of treatment over 4 weeks, our study is limited. Atrogin-1 has been linked to skeletal muscle atrophy. Consistent with preserved muscle fiber cross sectional areas, atrogin-1 expression levels remained unchanged. UCP2, COX, FABP3 and LPL expression levels remained unchanged. UCP2 and COX are linked to mitochondrial oxidation and ROS production, whereas FABP3 is involved in cellular lipid storage. LPL is essential for lipoprotein metabolism. CD36 is pivotal for cellular fatty acid uptake.

The method used to analyze muscle ATP content is limited in the sense that ATP content could be underestimated due to rapid ATP metabolization. However, due to the consistency in our tissue harvesting protocol, we assume this to be a systematic bias still allowing a comparative analysis between our groups.
In conclusion, we here show beneficial effects of PPARδ stimulation on skeletal muscle oxidative capacity and endurance function in an animal model with left ventricular dysfunction following myocardial infarction. Our data suggest that PPARδ agonism is a novel and unique strategy to enhance skeletal muscle oxidative function counteracting exercise intolerance in HF.

AKNOWLEDGEMENT

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DISCLOSURES

None.
REFERENCES


Figure 1. Skeletal muscle dysfunction in an animal model with left ventricular dysfunction following myocardial infarction. (A) Reduced oleic acid oxidation rate in skeletal muscle of animals 8 weeks post-MI (B) Decreased ATP content in skeletal muscle tissue in MI mice compared to the sham-operated group. (C, D) No differences in SDH or CS activity were noted between groups. (E) Increased TNFα mRNA expression levels in skeletal muscle of MI mice. (F, G) Muscle fiber CSA analysis, no significant changes in CSA were observed between sham and MI mice. (H) Decreased running time in the MI group compared to sham operated mice (*p<0.05, **p<0.01, n=5 per group).

Figure 2. In vitro analysis of effects of PPARδ stimulation. (A) Reduced fatty acid oxidation rates in response to TNFα stimulation is corrected by PPARδ-agonist (GW501516) co-incubation but not in response to PPARα-agonism (WY14643) (B) Expression of CPT1 decreases under TNFα treatment and increases with co-incubation using the PPARδ agonist (C2C12 skeletal muscle myotubes, n=3 independent experiments per condition. *p<0.05, **p<0.01, ***p< 0.001).

Figure 3. Effects of PPARδ treatment in an animal model of chronic left ventricular dysfunction following MI. (A) Increased fatty acid oxidation rate in response to PPARδ stimulation, (B) changes in skeletal muscle gene expression with PPARδ stimulation, (C) skeletal muscle ATP level changes, p=0.38, (D) no differences in skeletal muscle CSA. (E) prolonged running time in animals
following MI treated with a PPARδ agonist (*p<0.05, **p<0.01; placebo n=5-9 and GW501516 n=6 per analysis).

Table 1. Baseline characteristics and echocardiographic parameters in animals following myocardial infarction treated with GW501516 or placebo. (FS=fractional shortening in %; PWT=posterior wall thickness in mm; AWT= anterior wall thickness in mm; LVDd=left ventricular end-diastolic diameter in mm); LVDs= left ventricular systolic diameter in mm
Figure 1

A. Oleic acid oxidation (mg per minute) in Sham and MI groups.

B. ATP/protein (nM/mg) in Sham and MI groups.

C. Sarcoplasmic reticulum calcium ATPase activity (nmol/mg/min) in Sham and MI groups.

D. Citrate synthase activity (nmol/mg/min) in Sham and MI groups.

E. TNF-α mRNA expression fold change in Sham and MI groups.

F. % of fibers in Sham and MI groups.

G. CSA average (μm²) in Sham and MI groups.

H. Time running (sec) in Sham and MI groups.
Figure 2

A

B

(A) Cytosolic oxidation (relative changes) and (B) CPT1 (relative mRNA expression) in cells treated with different concentrations of GW961516 and W14421.
Table 1

Body morphometry, laboratory data and echocardiographic assessment of animals with left ventricular dysfunction following MI (treated vs. placebo)

<table>
<thead>
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<th>Placebo (n=10)</th>
<th>GW501516 (n=10)</th>
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<tr>
<td>Body weight (BW) (g)</td>
<td>29.3 ± 0.6</td>
<td>28 ± 1.1</td>
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<td>Heart weight/ BW (g)</td>
<td>5.9 ± 0.4</td>
<td>6.1 ± 0.5</td>
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<td>Quadriceps weight/ BW (g)</td>
<td>13.5 ± 0.8</td>
<td>12.9 ± 1.3</td>
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<td>Glucose (mg/ dl)</td>
<td>167.4 ± 20.7</td>
<td>142.6 ± 8.7</td>
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<td>Plasma FFA (pmol/µl)</td>
<td>0.8 ± 0.1</td>
<td>0.58 ± 0.1</td>
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<tr>
<td>Plasma TAG (µg/µl)</td>
<td>5.0 ± 0.4</td>
<td>3.02 ± 0.6</td>
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<tr>
<td>Quadriceps TAG (µg/mg)</td>
<td>72.6 ± 5.4</td>
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**Echocardiographic Analysis**

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<td>FS% before intervention</td>
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<td>15.5 ± 3.5</td>
</tr>
<tr>
<td>FS% after intervention</td>
<td>20.6 ± 3.3</td>
<td>19.4 ± 3.7</td>
</tr>
<tr>
<td>HR (bpm) before intervention</td>
<td>431.4 ± 13</td>
<td>444.5 ± 12.2</td>
</tr>
<tr>
<td>HR (bpm) after intervention</td>
<td>423.7 ± 10</td>
<td>451.8 ± 11.3</td>
</tr>
<tr>
<td>AWT (mm) before intervention</td>
<td>0.61 ± 0.04</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>AWT (mm) after intervention</td>
<td>0.72 ± 0.02</td>
<td>0.75 ± 0.03</td>
</tr>
<tr>
<td>PWT (mm) before intervention</td>
<td>0.83 ± 0.06</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>PWT (mm) after intervention</td>
<td>0.76 ± 0.05</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td>LVDs (mm) before intervention</td>
<td>4.17 ± 0.48</td>
<td>4.75 ± 0.45</td>
</tr>
<tr>
<td>LVDs (mm) after intervention</td>
<td>4.22 ± 0.47</td>
<td>4.22 ± 0.48</td>
</tr>
<tr>
<td>LVDD (mm) before intervention</td>
<td>5.02 ± 0.34</td>
<td>5.56 ± 0.34</td>
</tr>
<tr>
<td>LVDD (mm) after intervention</td>
<td>5.27 ± 0.43</td>
<td>5.16 ± 0.38</td>
</tr>
</tbody>
</table>