Title: Fenofibrate reduces cardiac remodeling and improves cardiac function in a rat model of severe left ventricle volume overload.

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Running head: Fenofibrate and left ventricle remodeling in volume overload.

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Abstract

Aims: Fenofibrate is a peroxisome proliferator associated receptor alpha agonist (PPARα) used clinically for the management of dyslipidemia and is a myocardial fatty acid oxidation stimulator. It has also been shown to have cardiac anti-hypertrophic properties but the effects of fenofibrate on the development of eccentric LVH and ventricular function in chronic left ventricular (LV) volume overload (VO) are unknown. This study was therefore designed to explore the effects of fenofibrate treatment in a VO rat model caused by severe aortic valve regurgitation (AR) with a focus on cardiac remodeling and myocardial metabolism.

Main Methods: Male Wistar rats were divided in four groups (13-15 animals / group): Shams (S) treated with fenofibrate (F; 100 mg/kg/d PO) or not (C) and severe AR receiving or not fenofibrate. Treatment was started one week before surgery and the animals were sacrificed 9 weeks later.

Key findings: AR rats developed severe LVH (increased LV weight) during the course of the protocol. Fenofibrate did not reduce LV weight. However, eccentric LV remodeling was strongly reduced by fenofibrate in AR animals. Fractional shortening was significantly less affected in ARF compared to ARC group. Fenofibrate also increased the myocardial enzymatic activity of enzymes associated with fatty acid oxidation while inhibiting glycolytic enzyme phosphofructokinase.

Significance: Fenofibrate decreased LV eccentric remodeling associated with severe VO and helped maintain systolic function. Studies with a longer follow-up will be needed to assess the long-term effects of fenofibrate in chronic volume overload caused by aortic regurgitation.

Introduction

Chronic left ventricular volume overload (VO) causes severe left ventricular dilatation and eccentric hypertrophy. It is encountered mostly in subjects suffering from chronic heart valve diseases such as mitral (MR) or aortic valve regurgitation (AR). Untreated AR causes severe LV dilatation and hypertrophy (LVH) as well as slowly progressive systolic dysfunction and evolution towards heart failure (Bonow 2000; Bonow et al. 1991). Chronic left ventricular (LV) volume overload diseases such as AR are well tolerated for many years before heart failure occurs. Unfortunately there is currently no treatment yet proven to be effective to decrease morbidity and mortality or to delay the evolution towards heart failure or decrease the need of heart valve surgery in these patients(Bonow et al. 2006). New treatment strategies need to be evaluated.

LVH induces severe alterations in myocardial energy metabolism. The normal heart displays a clear preference for fatty acids as its main substrate for ATP formation. In the hypertrophied heart, an increased reliance on glucose with an overall reduced oxidative metabolism is often observed. This could be explained in part by the down-regulation of the transcriptional cascades promoting gene expression for fatty acid oxidation and mitochondrial oxidative phosphorylation in adult hearts (Allard 2004; Allard et al 2007; Sambandam et al 2002). Impaired myocardial energetics also activate AMP-activated protein kinase (AMPK), leading to increased glucose uptake and glycolysis. Several potential approaches to help the myocardium to maintain adequate energy production are under investigation. Restoring the normal preference and use of fatty acid oxidation (FAO) may be one of them. The peroxisome proliferator-activated receptor-alpha (PPARα) constitutes an interesting therapeutic target to reach such a goal since its

activation can stimulate FAO (Barger and Kelly 2000). Fibrates are a class of hypolipemic drugs acting on PPARα activation. They have been shown in models of concentric hypertrophy due to pressure overload to have some anti-hypertrophic effects (Chen et al. 2007; Diep et al 2004; Harvey et al 2011; Iglarz et al 2003; Lebrasseur et al. 2007; Li et al 2009; Nishida et al 2004; Purushothaman et al. 2011; Rose et al. 2007). Their effects on eccentric LVH caused by chronic VO have never been evaluated. This study was therefore designed to assess the impact of a treatment with PPARα agonist fenofibrate on the development of eccentric LVH and myocardial metabolism in rats with severe LV volume-overload caused by AR.

Methods

Animals:

Adult male Wistar rats purchased from Charles River (Saint-Constant QC, Canada) were divided in 4 groups as follows: 1) Sham-operated control animals (SC; n=14); 2) AR controls (ARC; n=15), 3) Sham treated with fenofibrate (100 mg/kg/day PO in unsweetened fruit gelatin daily; SF; n=14) and AR treated with fenofibrate (ARF n=15). This dose of fenofibrate was similar to the one used by others in concentric LVH models in the rat (Chen et al. 2007; Diep et al 2004; Harvey et al 2011; Iglarz et al 2003; Lebrasseur et al. 2007; Purushothaman et al. 2011). The treatment was started one week before surgery in both SF and ARF groups and continued for 9 weeks until sacrifice. The protocol was approved by the Université Laval's Animal Protection Committee and followed the recommendations of the Canadian Council on Laboratory Animal Care.

Aortic regurgitation: Severe AR was induced by retrograde puncture of the aortic valve leaflets as previously described (Arsenault et al. 2002; Plante et al. 2003). A complete echocardiographic exam was performed two weeks after AR induction and the day before sacrifice 8 weeks later. At the end of the protocol, animals were sacrificed, hearts were quickly dissected and all cardiac chambers were weighed. LV was snap-frozen in liquid nitrogen and kept at -80°C for further analysis. All sacrifices were scheduled at similar times of the day in the fed state to avoid circadian or food-induced variations in myocardial metabolism. Lungs, liver and abdominal fat were rapidly collected and weighed.

Echocardiography

A complete M-mode, 2D, and Doppler echocardiogram was performed on the animals

under 1.5% inhaled isoflurane anesthesia using a 12 MHz probe with a Sonos 5500

echograph (Philips Medical Imaging, Andover, Mass). LV dimensions, wall thickness,

ejection fraction, diastolic function, cardiac output (ejection volume in the LV outflow

tract and heart rate) were evaluated as previously reported. Relative wall thickness was

calculated as the ratio of the sum of septal and posterior wall thicknesses to LV internal

cavity. Eccentric remodeling is defined as a decrease of RWT compared to normal vs.

concentric remodeling is defined as an increase of RWT compared to normal (Lachance

et al. 2009a; Plante et al. 2003; Plante et al. 2006; Plante et al. 2008).

Analysis of mRNA accumulation by quantitative RT-PCR

The analysis of LV mRNA levels by quantitative RT-PCR has been described in details

elsewhere (Champetier et al. 2009).

Enzyme activity determinations

Left ventricle samples were kept at -80°C until assayed for maximal (V_{max}) enzyme

activities. Small pieces of LV (20-30mg) were homogenized in a glass-glass

homogenizer with 9 or 39 volumes of ice-cold extracting medium pH7.4 (250mM

sucrose, 10mM Tris-HCl, 1mM EGTA) depending on the enzyme activity assayed.

Enzymatic activities for hydroxyacyl-Coenzyme A dehydrogenase (HADH),

phosphofructokinase (PFK) and citrate synthase (CS) were determined as previously

described (Bouchard-Thomassin et al. 2010). Carnitine palmitoyl transferase (CPT) LV

activity determination was based on a modification of a previously published method (Bieber et Markwell, 1981) and adapted for performing the assays in a 96-well ELISA plate. Briefly, small pieces of LV tissue were homogenized in 40 volumes of a phosphate 0,1M, pH7.2 buffer. The reaction buffer consisted of 0.5 M Tris-HCl pH8, 50 mM EDTA 100 mM L-carnitine and 2.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The assay was started with the addition of 180µl of the reaction buffer, 10µl of the homogenate and 10µl of 0.7mM palmitoyl-CoA. The incubation was performed for 5 min at 25°C in a ELISA plate reader fixed at a 412 nM length wave. A measure of the absorbance was made each 15 s during the incubation. The rate of CoA-SH produced by the reaction of the CPT activity and reacting with DTNB and producing the yellow-colored TNB was then calculated. Medium Chain Acyl-CoA Dehydrogenase (MCAD) enzymatic activity determination was a modification of the previously described method (Djouadi et al. 1997) scaled down to allow the use of 96-well plates. Briefly, small LV tissue pieces were homogenized in 10 volumes of a 100mM Hepes pH 7.6, 0.1mM EDTA. The reaction buffer was as follow: 1M Hepes, 0.5 mM EDTA, pH 7.5, 2mM ferrocenium hexafluorophosphate and 10mM Sodium tetrathionate. The reaction was performed at 37°C in an ELISA microplate reader at 617nM. In each well, 188µl of reaction buffer were pipetted, 12µl of 1mM octanoyl-CoA and then, the reaction was started with adding 20µl of the homogenate. Readings were recorded each 15 seconds for 3 minutes. Malonyl-CoA decarboxylase (MCD) enzymatic activity determination was a modification of the previously described method (Hamilton et Saggerson 2000) scaled down to allow the use of 96-well plates. Briefly, small LV tissue pieces were homogenized in 10 volumes of 100mM Tris-HCl, pH 8 + protease inhibitors buffer. The reaction buffer was as follow: 1M Tris-HCl pH8, 1M sucrose, 10mM NAD, 500mM L-malate, 50mM

dithiothreitol, 170U/ml malate dehydrogenase and 2.5mM rotenone. The reaction was performed at 37°C in an ELISA microplate reader at 340nM. In each well, 170μl of reaction buffer were pipetted, 10μl of 86U/ml citrate synthase, 10μl of homogenate and then, the reaction was started with adding 10μl of 6mM malonyl-CoA. Readings were recorded each 15 seconds for 7 minutes.

Immunoblotting

Crude LV homogenates were separated by SDS-PAGE. Volumes of samples loaded on gel were corrected for the amount of protein. Immunoblotting was performed as described elsewhere (Plante et al 2004a).

Statistical analysis

Results are presented as mean ± SEM unless specified otherwise. Inter-group comparisons were done using two-way ANOVA and using Bonferroni post-test if necessary. Student t-test was used when two groups were compared head to head. Statistical significance was set at a $p<0.05$. Data and statistical analysis were performed using Graph Pad Prism version 5.02 for Windows, Graph Pad Software (San Diego CA).

Results

Clinical data and measured heart weight (Table 1):

All animals were alive at the end of the protocol with the exception of one death in the ARC group. Fenofibrate was well tolerated in all animals. Total heart weight was greatly increased (severe eccentric LVH) in all rats with AR as expected. Fenofibrate treatment did not reduce total heart or LV weight in AR animals. Fenofibrate treatment did not affect LV weight in sham control animals.

Echocardiographic data:

End-diastolic and end-systolic LV dimensions increased in AR animals as expected in this disease (Table 2). Fenofibrate treatment reduced LV dilatation (both diastolic and systolic diameters) and normalized relative wall thickness (less eccentric remodeling) in the ARF group (Figure 1). Relative wall thickness was also slightly increased in SF animals but measured LV weight remained normal as reported in the previous section. Systolic function evaluated *in vivo* by echocardiography (fractional shortening) was also clearly less affected in ARF compared to ARC. Stroke volume and cardiac output (calculated from the Doppler echocardiography data) were lower and closer to normal values in the ARF compared to ARC (Figure 2).

Markers of elevated filling pressures.

As illustrated in Figure 3, ANP and BNP gene expression was clearly increased in the LVs of AR animals. Fenofibrate treatment had no effect on ANP and BNP expression.

Level of activation of pro-hypertrophic myocardial kinases:

The level of activation of classical pro-hypertrophic MAP kinases was evaluated. Results are shown in Figure 4. A trend for ERK 1/2 activation was observed in AR animals which was not normalized by fenofibrate treatment. p38 activation was reduced by fenofibrate in AR animals as well as in controls . Jnk activation remained stable among the four groups (not shown). Akt phosphorylation was slightly reduced in AR animals and more markedly in those receiving fenofibrate. On the other hand, AR-induced LV hypertrophy was accompanied by a significant increase in the content of the phosphorylated form of AMPKα (Thr172) and this was completely normalized by fenofibrate treatment.

Myocardial energy metabolism

As expected, fenofibrate increased the LV content of PPARα but most notably in AR animals (Figure 5). The mRNA levels of PGC1α were reduced in AR animals and fenofibrate did not restore to normal these levels. The activity levels of enzymes related to fatty acid metabolism in the LV were evaluated. HADH enzymatic activity was reduced in AR animals but partially restored in the fenofibrate group (Figure 6). This was also observed for CPT and MCD enzymatic activities. MCAD activity remained relatively stable but tended to be increased in fenofibrate-treated animals. Gene expression of the FAT/CD36 transporter was decreased in AR animals and fenofibrate treatment did not improve this parameter (Figure 7). Enzymes related to glucose metabolism were also evaluated (Figure 6). Phosphofructokinase (PFK) activity was reduced by fenofibrate treatment in both control and AR rats.

Glut4 and PDHa1 gene expressions remained stable while PDK4 expression was reduced in AR animals (Figure 7). Fenofibrate had no effect on these parameters.

At the mitochondrial level, CS, ANT1 and UCP3 were also evaluated. CS enzymatic
activity was improved by fenofibrate in AR animals while ANT1 and UCP3 mRNA levels,
although decreased in AR animals, were unaffected by the drug.

Discussion

In this study we showed that fenofibrate had significant effects on LV eccentric remodeling and function as well as on myocardial energetic enzyme profile in rats with severe LV volume overload.

Fenofibrate did not prevent left ventricular hypertrophy (no effect on measured LV weight) but it clearly decreased LV dilatation, eccentric remodeling and reduced the loss of systolic function caused by AR. To our knowledge it is the first report of a pharmacological treatment influencing LV remodeling in an animal model but with little influence on LV hypertrophy (Plante et al. 2004a,b; Plante et al 2009; Plante et al. 2008; Zendaoui et al. 2011). However, considering the short duration of our study, it is not totally excluded that a preventive effect on LVH would appear after a longer follow-up. Our study showed that fenofibrate reduced the expected severe eccentric remodeling that is normally seen in severe VO-AR. LV sizes (diastolic and systolic diameters) as well as the ejection fraction are proven predictors of bad outcome in humans with aortic regurgitation and both were significantly improved by fenofibrate in our rats. Fenofibrate also affected LV morphology in normal rats (some concentric remodeling in the SF group) for a reason that remains unclear. It was however reassuring to find that LV weight was not increased in that group. Whether the protective effect of fenofibrate against eccentric remodeling and LV dilatation will translate in clinical benefits in the long term remains unknown. In human clinical studies, subjects with AR with less LV dilatation fare better than those with severely enlarged ventricles (Bonow et al. 2006). Our animals treated with fenofibrate also had a better fractional shortening than those who were untreated. Again in humans, a preserved systolic function usually translates with a better long term prognosis (Bonow et al. 2006). Although we did not measure

intra-cardiac pressures, the elevated ANP and BNP levels suggest that filling pressures were increased and remained so even in animals treated with fenofibrate. This is not surprising considering that the severe volume overload (regurgitant valve lesion) remains severe and unaffected by the drug. Fenofibrate obviously does not reduce the anatomic defect causing AR and is not known to have any significant hemodynamic effects. However, by preventing LV dilatation and eccentric remodeling and improving fractional shortening, fenofibrate probably indirectly helps the LV cope with the same volume overload. We must keep in mind that this protocol was short-termed and was performed in the sub-acute phase of AR. It is possible that the left ventricle needs some capacity to dilate and adjust its compliance to acutely accommodate the sudden volume overload without increasing its filling pressures too much. It is reassuring to note that the ANP and BNP levels were not further increased in the fenofibrate group knowing that they are very sensitive markers of elevated filling pressures and impending heart failure (Plante et al. 2003; Lachance et al. 2009b).

Fenofibrate treatment was started 1 week before the surgical induction of AR in our protocol. This clearly does not reflect the clinical reality where the disease cannot be predicted and treated in advance. We intentionally chose to pre-treat the animals in our protocol to assess the role of fenofibrate in the early development of LVH with a mechanistic view in mind. Having obtained significant results with this approach, other more plausible clinical scenarios can now be studied with specific targets to evaluate. Our results suggest that myocardial metabolism may have been modified by fenofibrate in AR animals. The effects of fenofibrate on HADH, CPT and MCD activities in AR rats suggest an adaptation in favor of in fatty acid use by the myocardium while the decrease of PFK activity suggests an adaptation against glucose utilization. Cardiac hypertrophy

is usually associated with a shift towards glucose use as a source of energy. PPARα has been shown to be down regulated in a number of cardiac hypertrophy models and thus the capacity to maintain normal fatty acid oxidation levels seems partly obliterated (Barger et al. 2000). It is still not clear whether blocking the shift toward glucose utilization in cardiac hypertrophy or heart failure is beneficial. Our results suggest that fenofibrate restores PPARα expression and an improvement in fatty acid preference as energetic fuel over glucose in rats with AR. This offers a hypothesis to explore to explain the improved systolic function. Although the metabolic evaluation in this study is far from comprehensive and complete, it did yield interesting results and those results warrant further investigation.

We observed in this study that fenofibrate was able to restore normal levels of a number of metabolic parameters and induced PPARα. PPARα agonist therapy has been evaluated in a number of experimental models of concentric hypertrophy and heart failure. However, those studies have yielded conflicting results, some reporting beneficial effects while others have shown the opposite. For example, fenofibrate has been shown by others to help normalize cardiac metabolism but it was ineffective to slow the onset of decompensation in a heart failure model (Labinskyy et al. 2007). In an acute model of pressure overload, activation of PPARα resulted in severe depression of cardiac power and efficiency in the hypertrophied heart (ex vivo) (Young et al. 2001). Adverse cardiac effects of fenofibrate have also been reported in PPAR$\alpha^{-/-}$ mice. Exacerbated LV dilation and decreased cardiac function were observed after fenofibrate administration with pressure overload in these mice (Duhaney et al. 2007).

On the other hand, other studies have concluded that the effects of fibrate treatment on cardiac hypertrophy were mostly positive. Fenofibrate successfully prevent cardiac hypertrophy in a number of in vivo animal models including: high-fat/high-sucrose diet-induced (Fernandes-Santos et al. 2009), aldosterone- induced (Lebrasseur et al. 2007), partial abdominal aortic constriction induced (Rose et al. 2007) and pressure overload-induced cardiac hypertrophy (Duhaney et al. 2007). Our results suggest at least some beneficial effects by preventing LV dilatation, eccentric remodeling and preserving systolic function despite the absence of a clear anti-hypertrophic effect. Again, the absence of effect on LV mass may be due to the short duration of the study. This will need to be addressed in a longer protocol.

It is not clear how the known metabolic effects of fenofibrate could impact LV remodeling in our model. It is possible that reducing LV dilatation is a major contributing factor in maintaining a more normal myocardial metabolic profile. In addition to their metabolic role in the heart, fibrates such as fenofibrate are believed to exert a number of non-metabolic actions in myocardial tissue on inflammation, extracellular matrix remodeling, oxidative stress, and regulation of hypertrophy (Balakumar et al 2011; Lockyer et al 2010). Some of these actions were shown to be independent of PPARα (Berger and Moller 2002). We observed in our study that fenofibrate significantly reduced the activation of p38 and of AMPK in AR animals. It tended to the same for Erk 1/2, too Fenofibrate has been shown to inhibit stress-activated kinases such as p38 or Jnk in cardiac hypertrophy models in vitro (De Silva et al. 2009; Irukayama-Tomobe et al. 2004). Similar observations were also made for p38 in the kidneys of hypertensive rats treated with fenofibrate (Hou et al. 2010). Nonetheless the role of stress-activated

kinases in cardiac hypertrophy remains controversial. Although their activation seems to lead to a hypertrophic response in cultured isolated cardiac myocytes, some protective (anti-hypertrophic) role has also been suggested for these signaling pathways in the intact adult heart. They may also promote the transition from a compensated state to dilated cardiomyopathy (Liang and Molkentin 2003; Nishida et al. 2004; Sadoshima et al. 2002).

AMPK is a sensor of cellular energy status. It is activated when the AMP/ATP ratio rises resulting in an activation of catabolic energy-generating pathways. An activation of AMPK in various models of cardiac hypertrophy has been reported and its role is still debated (Allard et al. 2007; Dick and Lopaschuk 2006). We observed that fenofibrate treatment strongly reduced the levels of AMPK phosphorylation in AR rats. This may reflect some normalization of the myocardial energy status in the hearts of our animals. But again it is difficult to pinpoint if this normalization is related more to the effects of fenofibrate on LV remodeling or to its effects on myocardial metabolism.

Study limitations: The results of this study have to be viewed in light of some limitations. This was a relatively short study in which fenofibrate was given as a pre-disease treatment. Eight weeks may be too short to assess the impact of fenofibrate treatment on systolic and diastolic functions and clinical evolution on the long term. Rodent heart metabolism may also differ in some aspects from humans. Detailed substrate utilization was not directly assessed *in vivo*.

Conclusions: In conclusion, an 8-week fenofibrate treatment was associated with less LV dilatation, less eccentric remodeling and improved systolic function in this

experimental model of LV volume overload caused by severe AR. These beneficial effects may have been related to myocardial metabolic effects of the drug on the LV. The effects of fenofibrate on left ventricular hypertrophy, systolic function, myocardial metabolism and eventually occurrence of heart failure and overall survival should be evaluated in long-term protocols. Considering the current lack of pharmacological treatment for AR, the results of this study opens a new therapeutic avenue to be explored in the treatment of this disease.

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Figure legends:

Figure 1: Typical macroscopic examples of left ventricular hypertrophy and remodeling in specific groups. Representative midventricular sections of the left ventricle stained with trichrome-Masson are shown. SC: control sham group, SF: fenofibrate-treated sham group, ARC: untreated AR group and ARF: fenofibrate-treated AR group.

Figure 2: Fenofibrate treatment effects on stroke volume (A) and cardiac output (B) as evaluated by echocardiography. Results are reported in % of change relative to the untreated control sham group (SC) as mean ± SEM (n = 13–15/group). ¶¶¶: P<0.001 vs. sham animals. *: p<0.05 and **: p<0.01 vs. untreated corresponding group from Bonferroni post-test.

Figure 3: Up-regulation of hypertrophy markers in AR animals. LV mRNA levels of (A) atrial natriuretic peptide (ANP), (B) brain natriuretic peptide (BNP) were evaluated as described in the Material and methods section. Results are reported in arbitrary units as mean ± SEM (n = 13–15/group). Untreated sham (sham operated animals) group mRNA levels were normalized to 1. ¶¶¶: P<0.001 vs. sham animals.

Figure 4: MAP kinases and AMP kinase activation in AR rats treated or not with Fenofibrate. Evaluation of the phosphorylated and the total protein contents for each kinase were performed by immunoblotting as described in the Materials and methods section. Results are reported in arbitrary units as mean ± SEM (n = 10–12/group) of the ratio of the phosphorylated content on the total protein content. Untreated sham (sham

operated animals) group protein contents are expressed in arbitrary units (AU). Two-way ANOVA statistical analysis results are displayed below each graph. ¶: P<0.05 vs. untreated animals. *: p<0.05 and **: p<0.01 vs. untreated corresponding group from Bonferroni post-test.

Figure 5: Fenofibrate treatment effect on cardiac protein content of PPARα and PGC1α gene expression. Evaluation of PPARα protein content was performed by immunoblotting as described in the Materials and methods section while PGC1α gene expression was evaluated by quantitative RT-PCR. Results are reported in arbitrary units as mean ± SEM (n = 10–12/group). Protein content and mRNA levels are expressed in arbitrary units (AU). Two-way ANOVA statistical analysis results are displayed below each graph. **: p<0.01 vs. untreated corresponding group from Bonferroni post-test.

Figure 6: Enzymatic activity of enzymes implicated in myocardial energy metabolism. (A) HADH (hydroxyacyl-Coenzyme A dehydrogenase), (B) medium chain acyl-CoA dehydrogenase (MCAD), (C) CPT (carnitine palmytoyltransferase), (D) PFK (phosphofructokinase), (E) CS (citrate synthase), and (F) MCD (malonyl-CoA decarboxylase) enzymatic activities were measured in LV homogenates from at least 10 animals in each group as described in the Materials and Methods. Results are reported as mean ± SEM (n=10-15/gr). Two-way ANOVA analyses are displayed below each panel. ¶: P<0.05 and ¶¶¶: P<0.05 vs. sham animals. *: p<0.05 and **: p<0.01 vs. untreated corresponding group from Bonferroni post-test.

Figure 7: Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of genes

related to cardiac metabolism. (A) Fatty acid transporter (FAT/CD36), (B) glucose

transporter 4 (Glut4), (C) PDHa1 (pyruvate dehydrogenase alpha1), D) PDK4 (pyruvate

dehydrogenase kinase 4), (E) Ant1 (adenine nucleotide transferase 1) and (F) UCP3

(uncoupling protein 3). Results are reported in arbitrary units as mean ± SEM (n =

10/group). Untreated sham (sham operated animals) group mRNA levels were

normalized to 1. Two-way ANOVA statistical analysis results are displayed below each

graph.). Two-way ANOVA analyses are displayed below each panel. ¶: P<0.05 and ¶¶¶:

P<0.05 vs. sham animals.

Table 1. Sacrifice data.

Parameters	SC (14)	SF (14)	ARC (14)	ARF (15)
Body Weight, g	562 ± 7.8	550 ± 8.1	548 ± 5.3	546 ± 7.1
Total heart weight, mg	1192 ± 41.5	1199 ± 27.5	1990 ± 58.0***	1958 ± 51.2***
LV weight, mg	867 ± 27.8	866 ± 14.8	1480 ± 51.7***	1478 ± 33.9***

LV: left ventricle. SC: sham controls; SF: sham treated with fenofibrate; ARC: AR controls; ARF: AR treated with fenofibrate. Values are expressed as mean ± SEM. Number of animals per group indicated in parenthesis. ***: $p < 0.001$ vs. sham animals.

Table 2. Echocardiography data.

Parameters	SC (14)	SF (14)	ARC (14)	ARF (15)
EDD, mm	7.6 ± 0.09	7.3 ± 0.15	10.6 ± 0.17***	8.4 ± 0.19***,c
ESD, mm	3.5 ± 0.11	3.6 ± 0.12	6.6 ± 0.24***	4.4 ± 0.15***,c
SW, mm	1.2 ± 0.02	1.3 ± 0.03	1.2 ± 0.03	1.4 ± 0.04*
PW, mm	1.5 ± 0.04	1.6 ± 0.05*	1.4 ± 0.06	1.6 ± 0.04*
RWT	0.32 ± 0.008	0.35 ± 0.008b	0.23 ± 0.006***	0.31 ± 0.008***,c
FS, %	54.0 ± 1.29	52.0 ± 0.99	38.5 ± 1.86***	47.2 ± 1.45***,c

EDD: end-diastolic diameter, ESD: end-systolic diameter, SW: septal wall thickness, PW: posterior wall thickness, RWT: relative wall thickness, FS: fractional shortening. Measurements obtained under 1.5% isoflurane anesthesia. Values are expressed as mean ± SEM. The number of animals per group is indicated in parenth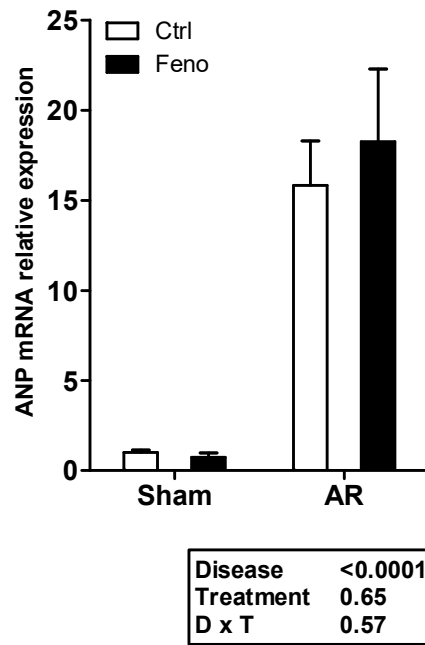esis. ***: $p<0.001$ vs. sham animals. b: $p<0.01$ and c: $p<0.001$ vs. untreated corresponding group from Bonferroni post-test.
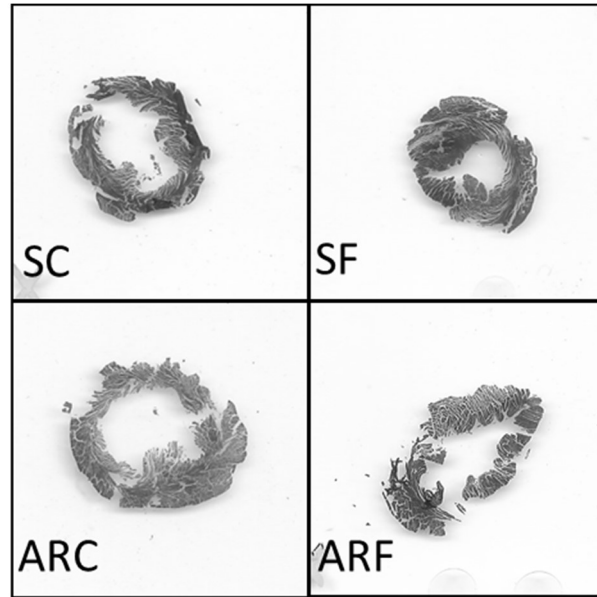
SC   SF   ARC   ARF

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Figure 1
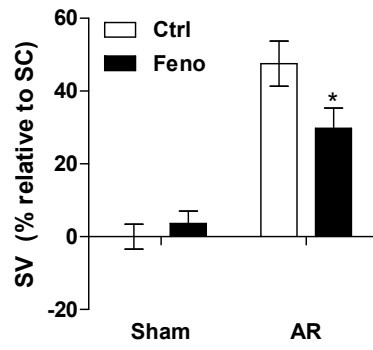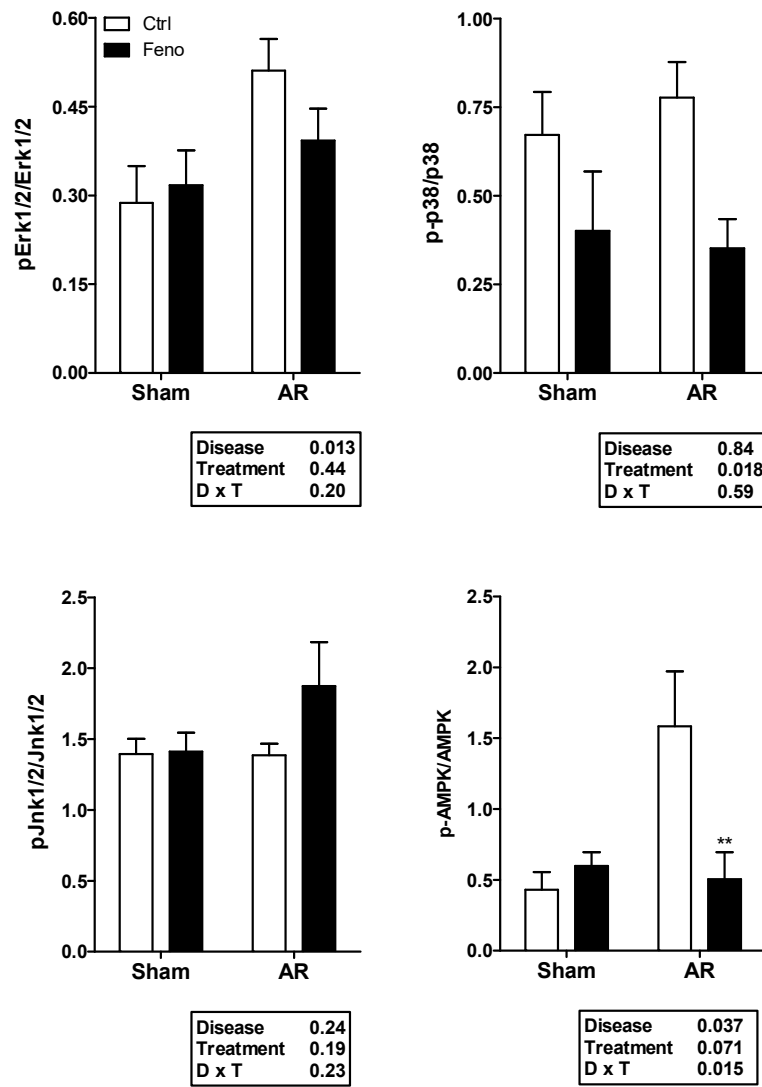
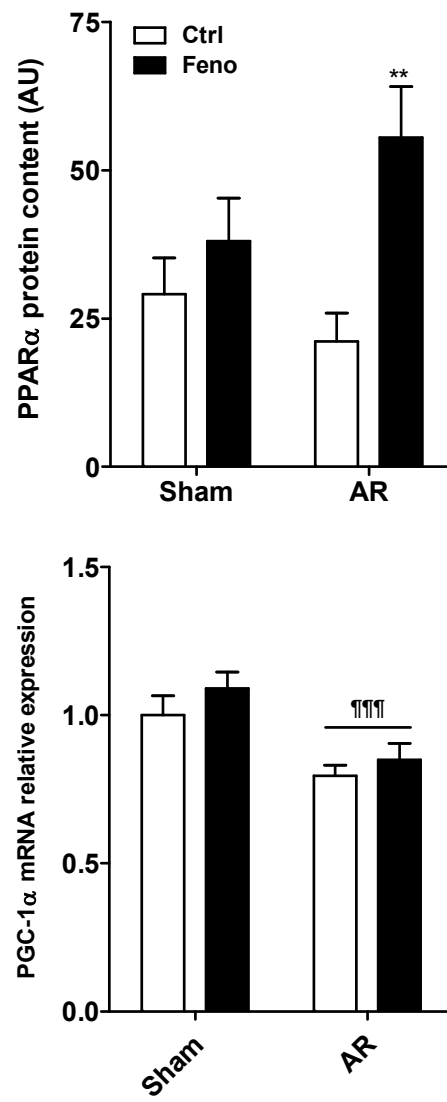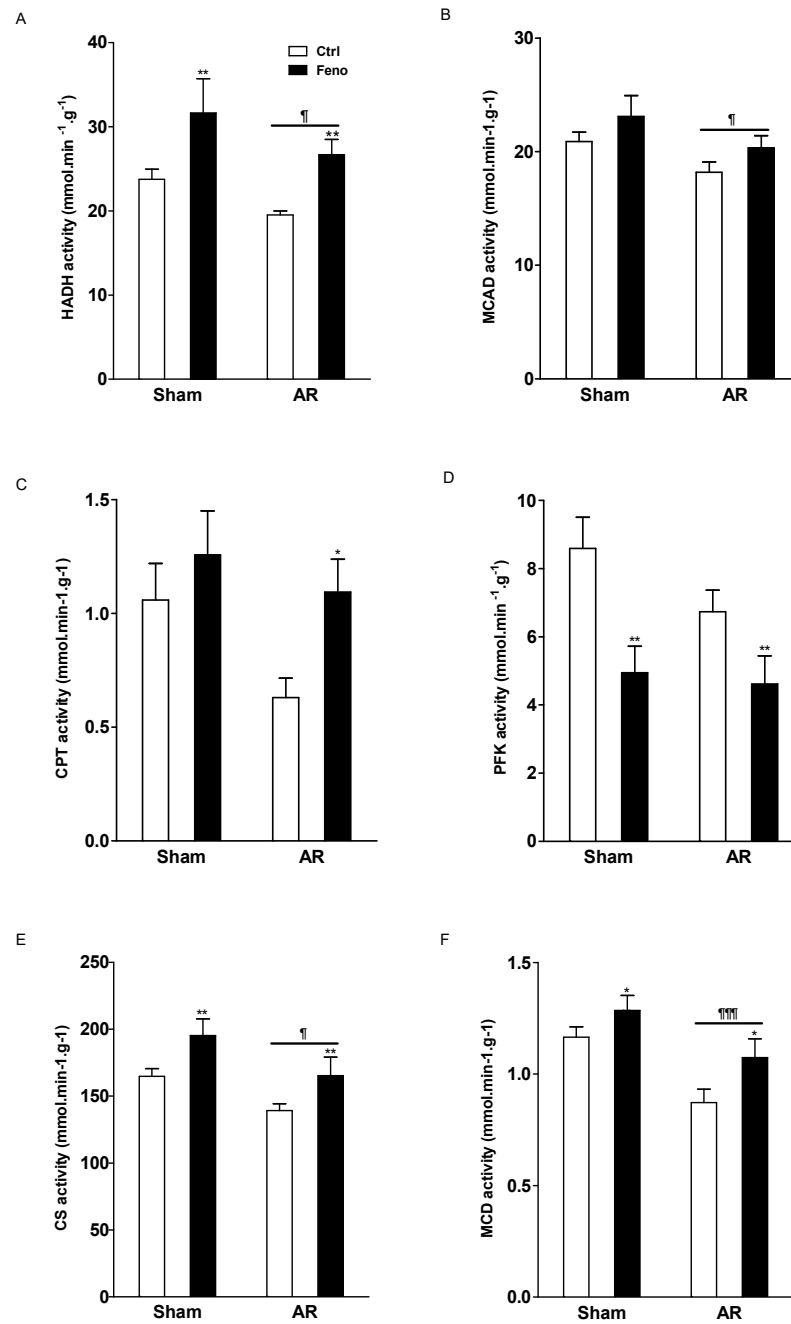Dhahri et al.
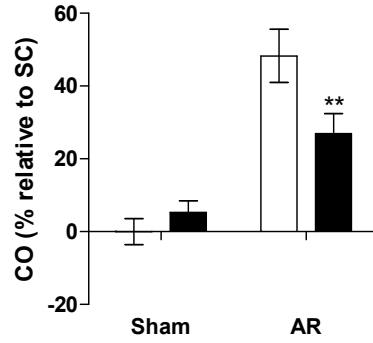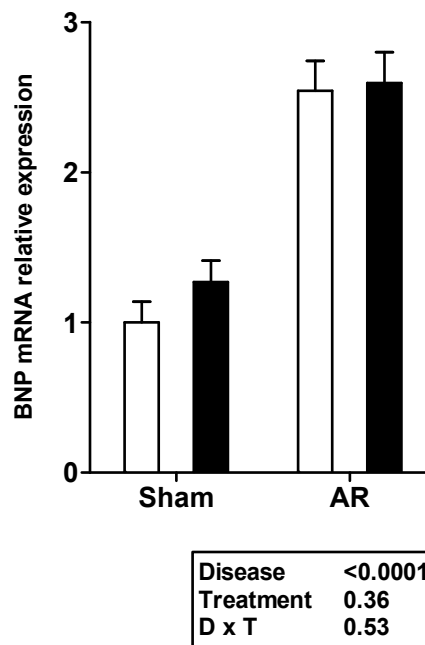
Figure 2

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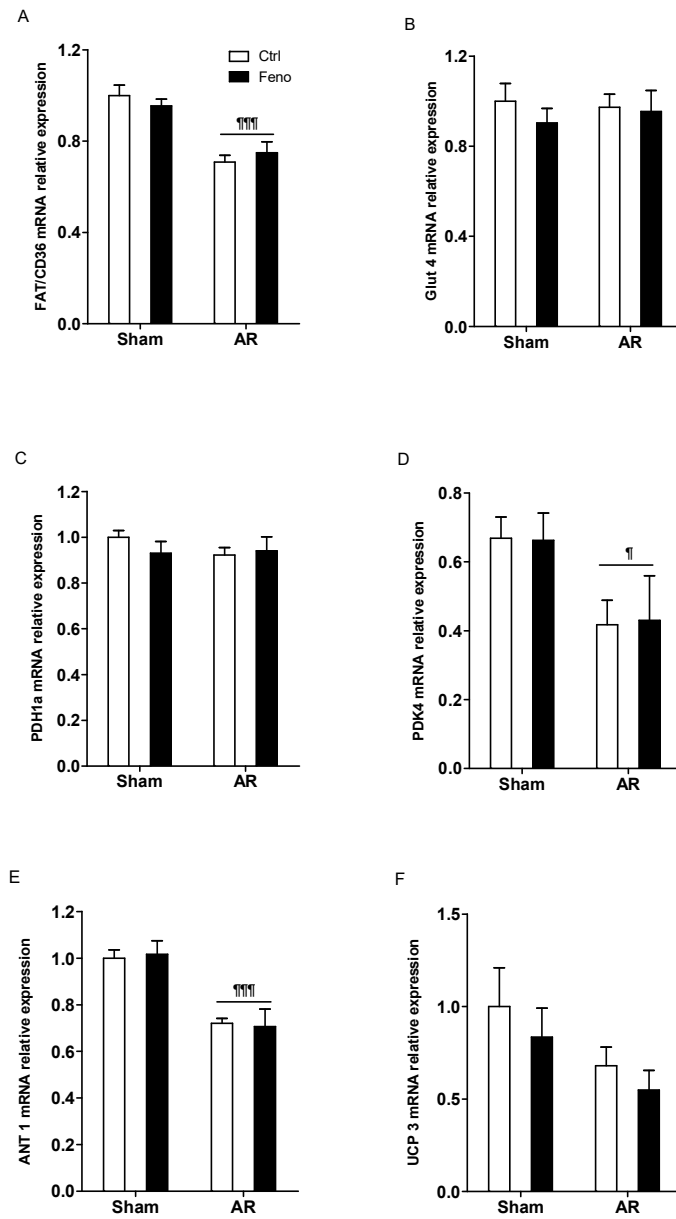
Figure 3

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Figure 4

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Figure 5

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Figure 6

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Figure 7