RESEARCH ARTICLE

Cardiac specific knock-down of peroxisome proliferator activated receptor α prevents fasting-induced cardiac lipid accumulation and reduces perilipin 2

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Abstract

While fatty acid metabolism is altered under physiological conditions, alterations can also be maladaptive in diseases such as diabetes and heart failure. Peroxisome Proliferator Activated Receptor α (PPARα) is a transcription factor that regulates fat metabolism but its role in regulating lipid storage in the heart is unclear. The aim of this study is to improve our understanding of how cardiac PPARα regulates cardiac health and lipid accumulation. To study the role of cardiac PPARα, tamoxifen inducible cardiac-specific PPARα knockout mouse (cPPARα-/−) were treated for 5 days with tamoxifen and then studied after 1–2 months. Under baseline conditions, cPPARα-/− mice appear healthy with normal body weight and mortality is not altered. Importantly, cardiac hypertrophy or reduced cardiac function was also not observed at baseline. Mice were fasted to elevate circulating fatty acids and induce cardiac lipid accumulation. After fasting, cPPARα-/− mice had dramatically lower cardiac triglyceride levels than control mice. Interestingly, cPPARα-/− hearts also had reduced Plin2, a key protein involved in lipid accumulation and lipid droplet regulation, which may contribute to the reduction in cardiac lipid accumulation. Overall, this suggests that a decline in cardiac PPARα may blunt cardiac lipid accumulation by decreasing Plin2 and that independent of differences in systemic metabolism a decline in cardiac PPARα does not seem to drive pathological changes in the heart.

Introduction

Fatty acid metabolism plays an important physiological role in the heart where fatty acid oxidation typically supplies the majority of ATP [1]. However, dysregulation of fat metabolism can have dramatic deleterious effects. Elevations in fatty acid oxidation have been implicated in driving cardiac insulin resistance and increasing the risk of developing cardiac dysfunction and heart failure [2–8]. On the other hand, in severe heart failure a decline in overall mitochondrial oxidative metabolism (including fatty acid oxidation) has detrimental effects on cardiac function [7, 9–13]. Thus fatty acid metabolism in the heart needs to be carefully regulated.
Free fatty acids enter the heart via fatty acid transporters where they are converted to triacyl-glycerol (TAG) and stored in lipid droplets (LD). In the heart, most fatty acids used for β-oxidation are generated from LD. LD have a core of neutral lipids enveloped within a phospholipid membrane, which contains a number of proteins such as perilipins (Plin). Plin have been shown to shield the LD from lipases as well as regulate the activation of lipases adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) [14–20]. There are 5 members of the Plin family (Plin 1 to 5). Recent studies report an important role for Plin2 and Plin5, which are both highly expressed in heart, in regulating LD turnover in the heart [14–18]. Mice with cardiac specific overexpression of Plin2 [17] exhibit cardiac steatosis suggesting that Plin2 opposes breakdown of LD. Loss of Plin2 has also been reported to reduce lipophagy [18], a process in which LD are trafficked to the lysosome for degradation.

Peroxisome proliferator activated receptor (PPAR)α is a transcription factor that is an important regulator of whole body and cardiac fatty acid metabolism. PPARα is also reported to contribute to the development of cardiovascular diseases including cardiac hypertrophy, heart failure, and diabetic cardiomyopathy through its regulation of fat metabolism [8, 21–36]. Cardiac specific overexpression of PPARα elevates fatty acid oxidation and cardiac lipid accumulation, inducing cardiac dysfunction and increasing susceptibility to cardiac hypertrophy [2, 21, 22, 29, 34, 37]. Changes in binding partners of PPARα and changes in the activity of transcription factors/coactivators have been implicated in this role of PPARα in changes in gene expression and cardiac dysfunction and hypertrophy [8, 33, 38–40]. However, whole body PPARα knockout mice are also more susceptible to cardiac lipid accumulation [41–43] and there are inconsistent reports on the susceptibility of these mice to developing cardiac hypertrophy [8, 29, 30, 44]. The deleterious cardiac effects observed in the whole body PPARα knockout mice could be due to systemic effects of loss of PPARα in other tissues. PPARα elevates fatty acid oxidation by increasing the expression of many fatty acid metabolism enzymes including carnitine palmitoyl transferase (CPT) and CD36 [2, 21, 28, 32, 37, 45, 46]. In addition, PPARα has been shown to be a positive regulator of Plin [47–50], suggesting PPARα also regulates an additional aspect of fatty acid metabolism, lipid droplets.

To gain a better understanding of the role of cardiac PPARα in regulating cardiac metabolism we developed the first cardiac specific PPARα knockout mouse. This mouse is inducible and is deleted by addition of tamoxifen allowing us to study the role of PPARα in the adult heart. The aim of this study was to assess how myocardial PPARα regulates cardiac health and lipid accumulation during physiological stress, in particular increased fatty acid supply. In contrast to studies showing that a global reduction of PPARα can have a deleterious impact, we did not observe any dysfunction or cardiac hypertrophy in cPPARα−/− mice at baseline. Interestingly, Plin2 was reduced in these cPPARα−/− hearts. When we fasted these mice for 16 hours, we observed lower TAG levels in the cPPARα−/− hearts along with a reduction in cardiac Plin2. Taken together, these data suggest that loss of cardiac PPARα decreases levels of Plin2 which may result in the reduced cardiac lipid accumulation.

**Methods**

**Mice**

Tamoxifen inducible cardiac-specific PPARα−/− (cPPARα−/−) mice are on the C57BL6N background and are both floxed and express MerCreMer. The floxed PPARα mouse (PPARα<sup>fl</sup>/<sup>fl</sup>) was generated by breeding the PPARα First Knockout mouse (041228-UCD, received from Mutant Mouse Resource Research Center) with the Flp recombinase expressing mouse (036512-UCD, received from Mutant Mouse Resource Research Center). PPARα<sup>fl</sup>/<sup>fl</sup> were then bred with αMHC MerCreMer mice (MerCreMer+/−) to produce an inducible cardiac
specific PPARα Knockout mouse (MerCreMer+/--;PPAR<sup>flox</sup>/<sup>flox</sup>). MerCreMer+/--; and PPAR-<sup>flox</sup>/<sup>flox</sup> results are together indicated as Control in the figures. To induce PPARα knockout, tamoxifen (20 mg/kg body weight each day; tamoxifen is dissolved in 5% ethanol and 95% peanut oil) was administered IP for 5 consecutive days. Echocardiography was performed on iso-flurane anesthetized mice with a 30-MHz linear transducer and the Vevo 2100 system.

Mice were housed in an animal facility with a 12 hour light:12 hour dark cycle. Mice were provided standard chow and water ad libitum and at the start of each experiment were 3–4 months of age. In the fasted studies, mice were fasted overnight for 16 hr. Mouse treatment and handling followed guidelines outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). All animal protocols were approved by the National Heart, Lung and Blood Institute's Institutional Animal Care and Use Committee.

Mouse genotype (presence of MerCreMer and PPARα flox sites) was determined with PCR using these primer sets: MerCreMer, forward primer, 5'-GTCTGACTAGGTGTCCTTCT-3' and reverse primer, 5'-CGTCCTCCTGCTGGTATAG-3'. These primers were used to amplify a 410 bp DNA fragment. Presence of PPARα flox forward primer, 5' -GTTCTTCCTGGGTATAGCCTTGACG-3' and reverse primer, 5'-TGAGCCACAGCCCAGCTCCACC-3'. These primers were used to amplify a 454 bp DNA fragment if PPAR flox sites are present and a 277 bp DNA fragment if Wild Type. Finally, these primers were used to detect whether the Cre cut the flox sites (471 bp DNA fragment): forward primer, 5'-GTTCTTCCTGGGTATAGCCTTGACG-3' and reverse primer, 5'-ATAGATGATTAAAAGGTGATGTTAGGC-3'.

**Western blot**

A standard protocol was followed to assess protein levels via western blot in mouse heart. Hearts that had been crushed in liquid nitrogen were homogenized in RIPA buffer (Thermo Fisher cat no 89900) supplemented with protease/phosphatase inhibitors (Thermo Fisher cat no 78440). SDS PAGE was performed using Bio-Rad Criterion TGX gels. Protein was then transferred from the gel onto a nitrocellulose membrane and then the membrane was blocked for 1 hr in 5% non-fat dry milk (NFDM) in TBST at room temperature, incubated at 4˚C overnight in the appropriate primary antibody diluted in 5% Bovine Serum Albumin (BSA) in TBST, and washed 4x 5 min in TBST. Membranes were then incubated for 1 hr in the appropriate secondary antibody in 1% NFDM in TBST at room temperature and then washed 4x 5 min in TBST. Primary antibodies included Plin2 (Abcam, ab52356), Plin5 (Novus Biologics, NB110-60509), PPARα (Abcam, ab24509), and 4-HNE (Chemicon, AB5605). Secondary antibodies included anti-rabbit (Jackson, AB_2337913) and anti-goat (Santa Cruz, sc-2056). Membranes were then developed with ECL prime (Cytiva Amersham, RPN2232) and results analyzed using Image J. For H-NE and carbonylation antibodies, the entire lane was analyzed.

**Protein carbonylation level**

Heart supernatant homogenized in RIPA buffer was labelled using a Protein Carbonylation Kit (Abcam, cat no ab178020). Levels of protein carbonylation were then assessed via western blot using the antibodies supplied in the Protein Carbonylation Kit. 8 ug protein was loaded for each sample into a 8–16% TGX gel. Western blot was developed and analyzed as described above.

**RT-PCR**

RNA from isolated from mouse heart using the Qiagen miRNeasy kit. RNA concentration was determined using Nanodrop and an equal amount of RNA for each sample was added to the cDNA synthesis reaction performed using the iScript cDNA synthesis kit. Roche FastStart Universal SYBR Green Master (Rox) and respective primers were used to assess expression in
Exercise stress test
Exercise capacity was assessed as described previously [51]. Briefly, mice were acclimated to a mouse treadmill the day before the test. To perform the experiment mice were run on the treadmill at a 10˚ incline with incremental speed increases (10 min at 10 m/min, 5 min at 12 m/min, 3 min at 15 m/min, and then every 3 min belt speed increased by 1.8m/min until each mouse reached exhaustion). Each mouse was removed from the treadmill upon exhaustion (would no longer run).

Lipid extraction
Heart tissue was homogenized and then lipids were extracted using the standard Folch method. Lipids were extracted using 2:1 chloroform methanol. After evaporating the chloroform, lipids were redissolved in a 0.5% Triton X-100 diluted in 2-propanol. Samples were stored at -80˚C.

Lipase activity assay
Cardiac lipase activity was measured using a Lipase Activity Assay kit (Cayman Chemical, cat no 700640). Samples were prepared by homogenizing heart tissue in cold PBS with protease and phosphatase inhibitors (Thermo Fisher, cat no 78440) according to kit instructions. The sample were diluted 50-fold in PBS and a total of 20 ug protein was loaded into each sample well of the white 96 well plate. Fluorescence (excitation: 380nm, emission: 510nm) was measured every 30 seconds for 15 minutes at 37˚C. For analysis, background fluorescence was accounted for using sample background wells in which the lipase substrate was not added. Lipase activity values were calculated using the thioglycerol standard curve.

Triglyceride and free fatty acid measurement
TAG was measured in hearts with lipids extracted as described above. Infinity Triglyceride Reagent (Thermo Fisher Scientific cat no TR22421) was used in accordance with manufacturer’s instructions. Serum fatty acid levels were measured in serum using a free fatty acid kit (Wako NEFA-HR(2)). Manufacturer’s instructions were followed. To prepare the serum, the collected blood was left at room temperature for 30 min and then centrifuged at 6000xg for 2 min at 4˚C. The serum (supernatant) was stored at -80˚C.

Statistics
T-test or two-way ANOVA with Bonferroni posthoc test were performed as appropriate (p<0.05). Graphpad prism software was used to perform the statistical analyses. Data are presented as Mean±SEM.

Results
Cardiac PPARα knockdown does not induce cardiac hypertrophy
PPARα is a key transcriptional regulator of fatty acid metabolism and plays a role in the development of heart disease [2, 8, 21, 22, 30, 32, 44]. Overexpressing PPARα in the heart can
induce cardiac dysfunction and cardiac hypertrophy [21–23]. On the other hand, studies have been inconsistent on the effect of whole body PPARα knockout on cardiac function and hypertrophy [24, 30, 42, 44, 52]. To better understand the impact of myocardial PPARα, we generated an inducible cardiac-specific PPARα knock-out (cPPAR-/-) mouse. We observed no evidence of cardiac hypertrophy in cPPAR-/- vs control mice (Fig 1A, 1B and 1D) at baseline. Heart weight/tibia length was also similar in hearts from fasted Control and cPPAR-/- mice (Fig 1B). These measurements were made in 3–4 month old mice after waiting 1–2 months after tamoxifen injection. Further, cPPAR-/- mice have a similar cardiac function and exercise capacity to control mice (Fig 1C and 1E). After the 16 hr fast, exercise capacity was similar between cPPAR-/- and control mice (Fig 1F). Genotyping, mRNA, and protein all indicated a reduction in PPARα in hearts after tamoxifen treatment (Fig 1G–1I).

Cardiac PPARα knockdown reduces lipid accumulation during fasting despite similar fatty acid supply

One of the ways PPARα has been linked to cardiac dysfunction and hypertrophy is through regulation of lipid accumulation. However, since both whole body PPARα knockout and cardiac PPARα overexpression increases induction of lipid accumulation in the heart [2, 41–43, 53], it is unclear whether a reduction in cardiac PPARα drives or protects against lipid accumulation in the heart. As mentioned in the whole body PPARα knockout mouse there are systemic effects that can complicate the interpretation. We utilized the cPPAR-/- mice to test whether a reduction in cardiac PPARα impacts the response of the heart to an acute elevation in fat supply. Circulating fatty acids and cardiac TAG levels become elevated in response to a short term fast. As expected, the level of circulating fatty acids in response to a 16 hr fast was similar between cPPAR-/- and control mice (Fig 2A). Thus, it was perhaps surprising that in response to fasting, and an elevation in circulating fatty acid levels, cPPAR-/- hearts had dramatically lower cardiac TAG levels than control mice (Fig 2B). This indicates that a reduction in cardiac PPARα attenuates cardiac lipid accumulation, at least under a short term elevation in fatty acid supply to the heart.

Cardiac specific PPARα knockout mouse hearts have reduced PLIN2

We therefore were interested in understanding how a reduction in cardiac PPARα reduces accumulation of TAGs in the heart. We hypothesized that the mechanism might involve alterations in cardiac accumulation or breakdown of TAG stores. Since PPARα has been linked to regulating SREBP [54], a transcriptional regulator of fatty acid synthesis, we first examined whether there might be decreased expression of genes regulated by SREBP1c. Interestingly, we did not observe lower fatty acid synthase (FASN) mRNA under baseline or fasting conditions between cPPAR-/- and control hearts (Fig 3A). The elevation in FASN mRNA in fasted cPPAR-/- vs Control hearts may be a compensatory increase in response to overall reduced TAG synthesis in the fasted cPPAR-/- hearts.

Recently, it has become recognized that Plin also regulates the accumulation of TAG [19, 55], and that a loss of Plin2 can lead to reduced lipid accumulation. Plin2, in particular, is believed to play an important role in controlling lipid droplet and triglyceride accumulation [17–20, 56]. We, therefore, examined whether Plin2, a Plin that is highly expressed in the heart, was altered in the cPPAR-/- hearts. Interestingly, cardiac Plin2 was lower at baseline cPPAR-/- vs control hearts. Expression was also lower under fasting conditions in cPPAR-/- hearts (Fig 4A). This difference in Plin was Plin2 specific since Plin5 levels were not different between cPPAR-/- and control hearts (Fig 4B).
Fig 1. Reduction in cardiac PPARα does not induce cardiac hypertrophy or reduce cardiac function. A. Hearts were weighed at baseline to assess cardiac hypertrophy. n = 12–19. B. Hearts from mice fasted for 16 hrs were weighed. n = 6–10. Heart weights were normalized to tibia length. C. Ejection Fraction and D. LV Diameter in Diastole were measured via echocardiography. n = 6. E. Exercise capacity was assessed via treadmill. n = 5–10. This was done in a single session in one day. F. Exercise capacity was assessed via treadmill in fasted mice. n = 5–6. This was done in a single session in one day. G. Genotyping of mouse tissues using genomic DNA. ~ 450 bp band indicates Mer-Cre-Mer (MCM) cut the loxp flanked sequence in the PPARα gene. H. PPARα protein levels were measured in hearts. n = 6–8. Representative blots are from the same membrane. I. PPARα mRNA levels were measured in hearts. n = 4–5 Values are mean ± SEM. * p<0.05 compared to Control.

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Cardiac specific PPARα knockout mouse hearts do not show evidence of increased protein oxidation

Lipid accumulation can have detrimental effects through increasing oxidative injury. We considered the possibility that the attenuated TAG accumulation in the cPPAR−/− hearts might be associated with accumulation of other lipid species that might lead to lipid peroxidation and protein oxidation which can have detrimental effects in the heart. To check this, we first measured 4-HNE as an indicator of lipid peroxidation. Importantly, there was no evidence of 4-HNE levels being elevated in cPPAR−/− hearts (Fig 5A). Further, protein carbonylation was also not elevated in cPPAR−/− hearts (Fig 5B). This was the case under both control and fasted conditions. This indicates that oxidation and peroxidation were not elevated in cPPAR−/− hearts.

**Discussion**

A role for the transcription factor PPARα in fatty acid metabolism, cardiac function, and hypertrophy was reported more than two decades ago [2, 8, 21, 22, 30, 31, 33, 37, 38, 44, 57]. In
this study, we demonstrate for the first time the effect of a cardiac specific decrease in PPARα in vivo. While it is clear that PPARα regulates fatty acid metabolism it is unclear whether PPARα reduces or exacerbates cardiac lipid accumulation.

Our aim in the current study was to examine the role of myocardial PPARα in regulating TAG accumulation. We fasted the mice to increase adipocyte lipolysis, thereby increasing the supply of fat to the heart; this normally results in a dramatic increase in the amount of TAG accumulation in the heart. Because whole body PPARα knockout mice showed a much larger increase in cardiac lipid levels under conditions that stimulate cardiac lipid accumulation [42, 43], we expected that fasting would also induce an increase in lipid accumulation in the

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**Fig 3.** FASN was not altered in fasting in mice with reduced myocardial PPARα. Mice were fasted for 16 hrs and then A. FASN mRNA and B. SCD1 was measured in the hearts. Values are mean ± SEM. n = 5–11; *p*<0.05 compared to Control Fasted.

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cPPAR-/- compared to control hearts. In contrast to our hypothesis, cPPAR-/- hearts exhibited less cardiac lipid accumulation (Fig 2). TAG levels were ~3 fold lower in fasted cPPAR-/- hearts vs fasted control hearts. This occurred even though there was a similar level of circulating fatty acids in fasted cPPAR-/- and control mice (Fig 2).

One possible explanation for the reduction in TAG accumulation could be less fatty acid synthesis. In particular, we examined whether targets of SREBP (which participate in fatty acid synthesis) might be reduced. SREBP1c was examined because it regulates the expression of proteins involved in fatty acid synthesis and triglyceride accumulation [58]. While the literature did not cause us to expect FASN to increase in response to fasting in Control mouse hearts, a
lower expression of FASN in cPPAR\(-/-\) vs Control hearts might help explain the difference in TG accumulation we observed [59]. However, the expression of FASN was not lower in cPPAR\(-/-\) compared to control hearts (Fig 3). It is still possible, however, that there was a change in the expression and/or activity of proteins involved in TAG synthesis. We also investigated a role for Plin2, as it has been reported to regulate TAG levels. We did not observe a difference in Plin2 levels in fed vs fasted hearts which agrees with Varghese et al. who do not see a total change in Plin2 in heart homogenate but instead an increase in Plin2 localization to lipid droplets [14]. Plin2 protein was lower in cPPAR\(-/-\) vs control in fasted hearts (Fig 4A) suggesting it could be involved in the reduced lipid levels in cPPAR\(-/-\) fasted hearts. These data are consistent with the hypothesis that reduced levels of cardiac PPAR\(\alpha\) during short term fasting

**Fig 5.** Control or fasted mice with reduced myocardial PPAR\(\alpha\) do not have elevated levels of protein oxidation. Mice were fasted were 16 hrs and then A. 4-HNE and B. protein carbonylation levels were measured in heart. C. Representative western blot images. Values are mean ± SEM. n = 5–12.
Plin2 is a member of a family of proteins that bind to the outside of LD. LD store neutral lipids including TAG within a phospholipid membrane. Plin have been shown to regulate LD turnover through multiple mechanisms. Plin can both shield LD from lipases and regulate the activity of lipases ATGL and HSL on lipid droplets [14–20]. Mice with cardiac specific overexpression of Plin2 [17] developed cardiac steatosis suggesting that Plin2 opposes breakdown of LD. In this study, a moderate reduction in cardiac Plin2 is associated with a reduction in cardiac lipid levels. An important question for understanding fatty acid metabolism and its role in disease is how Plin and lipid droplets are regulated. Our study and others suggest that PPAR is an important regulator of Plin [47–50, 60, 61]. In particular, the data in our study links a reduction in cardiac PPARα to a reduction in cardiac Plin2, indicating that cardiac PPARα is an important regulator of cardiac Plin2 expression.

Changes in Plin have been linked to changes in oxidative stress and cardiac dysfunction [18, 56, 62, 63]. In particular, a rise in the lipid species diacylglycerol (DAG) and ceramides have been linked to cardiac lipotoxicity [64–67]. Cardiac DAG increases along with TAG in mice with cardiac Plin2 overexpression [17]. Interestingly, studies have shown that increasing flux toward TAG and away from DAG and ceramides seems to reduce this lipotoxicity [56, 65, 66]. We therefore wanted to make sure the reduced TAG in fasted cPPARα-/- hearts was not associated with increased oxidative stress. Notably, levels of oxidative stress were similar between fasted cPPARα-/- hearts and control hearts. This also indicates that the change in Plin2 does not appear to be associated with the deleterious results that have been reported in some models where lipid storage is dysregulated [18, 56, 62, 63]. It will be interesting in the future to investigate how specific lipids (including DAGs and ceramides) are different between cPPARα-/- hearts and control hearts. Further studies will provide novel insight into the regulation of lipid species and how specific lipid species change in lipid droplets. This indicates that at least under physiological conditions, a decline in PPARα decreases cardiac Plin2 without inducing protein oxidation or cardiac hypertrophy. Further studies will be needed to investigate the direct link between this protection against cardiac triglyceride accumulation and the development of cardiac hypertrophy using disease models.

Conclusions
In summary, this study finds that a reduction in cardiac PPARα has a dramatic impact on cardiac lipid accumulation. A 16 hr fast resulted in less cardiac lipid accumulation in cPPARα-/- compared to control hearts. This was accompanied by lower levels of cardiac Plin2 protein. Furthermore, cPPARα-/- hearts did not have higher markers of protein oxidation or cardiac hypertrophy when compared to control. Overall, these data suggest that decreased cardiac PPARα reduces cardiac lipid accumulation in the heart, and could potentially protect against diseases with dysregulated lipid storage such as diabetic cardiomyopathy.

Supporting information
S1 Raw images.
(PDF)

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References

1. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schonekess BO. Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochimica et biophysica acta. 1994; 1213(3):263–76. https://doi.org/10.1016/0005-2760(94)00082-4 PMID: 8049240

2. Finck BN, Leeman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, et al. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. The Journal of clinical investigation. 2002; 109(1):121–30. https://doi.org/10.1172/JCI14080 PMID: 11781357

3. Carley AN, Severson DL. Fatty acid metabolism is enhanced in type 2 diabetic hearts. Biochimica Biophys Acta. 2005; 1734(2):112–26. https://doi.org/10.1016/j.bbalip.2005.03.005 PMID: 15904868

4. Keung W, Ussher JR, Jaswal JS, Raubenheimer M, Lam VH, Wagg CS, et al. Inhibition of carnitine palmitoyltransferase-1 activity alleviates insulin resistance in diet-induced obese mice. Diabetes. 2013; 62(3):711–20. https://doi.org/10.2337/db12-0259 PMID: 23139350

5. Robins SJ, Lyass A, Zachariah JP, Massaro JM, Vasan RS. Insulin resistance and the relationship of a dyslipidemia to coronary heart disease: the Framingham Heart Study. Arterioscler Thromb Vasc Biol. 2011; 31(5):1208–14. https://doi.org/10.1161/ATVBAHA.111.219055 PMID: 21311041

6. Zlobine I, Gopal K, Ussher JR. Lipotoxicity in obesity and diabetes-related cardiac dysfunction. Biochimica et biophysica acta. 2016; 1861(10):1555–68. https://doi.org/10.1016/j.bbalip.2016.02.011 PMID: 26899197

7. Fillmore N, Mori J, Lopaschuk GD. Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. British journal of pharmacology. 2014; 171(8):2080–90. https://doi.org/10.1111/bph.12475 PMID: 24147975

8. Oka S, Alcendor R, Zhai P, Park JY, Shao D, Cho J, et al. PPARalpha-Sirt1 complex mediates cardiac hypertrophy and failure through suppression of the ERR transcriptional pathway. Cell metabolism. 2011; 14(5):598–611. https://doi.org/10.1016/j.cmet.2011.01.001 PMID: 22055503

9. Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD. Contribution of Oxidative Metabolism and Glycolysis to ATP Production in Hypertrophied Hearts. American Journal of Physiology—Heart and Circulatory Physiology. 1994; 267(2):H742–H50. https://doi.org/10.1152/ajpheart.1994.267.2.H742 PMID: 8067430

10. Beer M, Seyfarth T, Sandstedt J, Landschutz W, Lipke C, Kostler H, et al. Absolute concentrations of high-energy phosphate metabolites in normal, hypertrophied, and failing human myocardium measured noninvasively with (31)P-SLOOP magnetic resonance spectroscopy. Journal of the American College of Cardiology. 2002; 40(7):1267–74. https://doi.org/10.1016/s0735-1097(02)02160-5 PMID: 12383574

11. Conway MA, Allis J, Ouwerkerk R, Nioka T, Rajagopalan B, Radda GK. Detection of low phosphocholine to ATP ratio in failing hypertrophied human myocardium by 31P magnetic resonance spectroscopy. Lancet. 1991; 338(8773):973–6. https://doi.org/10.1016/s0140-6736(91)91838-4 PMID: 1681342

12. Nascimben L, Friedrich J, Liao R, Pialetto P, Pessina AC, Ingwall JS. Enalapril treatment increases cardiac performance and energy reserve via the creatine kinase reaction in myocardium of Syrian myopathic hamsters with advanced heart failure. Circulation. 1995; 91(6):1824–33. https://doi.org/10.1161/01.cir.91.6.1824 PMID: 7882493

13. Neglia D, De Caterina A, Marraccini P, Natali A, Ciardetti M, Vecoli C, et al. Impaired myocardial metabolic reserve and substrate selection flexibility during stress in patients with idiopathic dilated cardiomyopathy. American journal of physiology Heart and circulatory physiology. 2007; 293(6):H3270–8. https://doi.org/10.1152/ajpheart.00887.2007 PMID: 17921325
14. Varghese M, Kimler VA, Ghazi FR, Rathore GK, Perkins GA, Ellisman MH, et al. Adipocyte lipolysis affects Perilipin 5 and cristae organization at the cardiac lipid droplet-mitochondrial interface. Sci Rep. 2019; 9(1):4734. https://doi.org/10.1038/s41598-019-4329-4 PMID: 30894648

15. Pollak NM, Jaeger D, Kolleritsch S, Zimmermann R, Zechner R, Lass A, et al. The interplay of protein kinase A and perilipin 5 regulates cardiac lipolysis. The Journal of biological chemistry. 2015; 290(3):1295–306. https://doi.org/10.1074/jbc.M114.604744 PMID: 25418045

16. Kolleritsch S, Kien B, Schoiswohl G, Diwoky C, Schreiber R, Heier C, et al. Low cardiac lipolysis reduces mitochondrial fission and prevents lipotoxic heart dysfunction in Perilipin 5 mutant mice. Cardiovascular research. 2020; 116(2):339–52. https://doi.org/10.1093/cvrv/czv119 PMID: 31166588

17. Ueno M, Suzuki J, Hirose M, Sato S, Imagawa M, Zenimaru Y, et al. Cardiac overexpression of perilipin 2 induces dynamic steatosis: prevention by hormone-sensitive lipase. American journal of physiology Endocrinology and metabolism. 2017; 313(6):E699–E709. https://doi.org/10.1152/ajpendo.00098.2017 PMID: 28581734

18. Mardani I, Tomas Dalen K, Drevinge C, Miljanovic A, Stahlman M, Klevstig M, et al. Plin2-deficiency reduces lipolysis and results in increased lipid accumulation in the heart. Sci Rep. 2019; 9(1):6909. https://doi.org/10.1038/s41598-019-43335-y PMID: 31061399

19. Sztalryd C, Brassaeule ML. The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. Biochim Biophys Acta Mol Cell Biol Lipids. 2017; 1862(10 Pt B):1221–32. https://doi.org/10.1016/j.bbalip.2017.07.009 PMID: 28754637

20. Itabe H, Yamaguchi T, Nimura S, Sasabe N. Perilipins: a diversity of intracellular lipid droplet proteins. Lipids Health Dis. 2017; 16(1):83. https://doi.org/10.1186/s12944-017-0473-y PMID: 28454542

21. Hafstad AD, Khalid AM, Hagve M, Lund T, Larsen TS, Severson DL, et al. Cardiac peroxisome proliferator-activated receptor-alpha activation causes increased fatty acid oxidation, reducing efficiency and post-isaehaemic functional loss. Cardiovascular research. 2009; 83(3):519–26. https://doi.org/10.1093/cvr/cvp132 PMID: 19398469

22. Park SY, Cho YR, Finck BN, Kim HJ, Higashimori T, Hong EG, et al. Cardiac-specific overexpression of perilipin proliferator-activated receptor-alpha causes insulin resistance in heart and liver. Diabetes. 2005; 54(9):2514–24. https://doi.org/10.2337/diabetes.54.9.2514 PMID: 16123338

23. Sambandam N, Morabito D, Wagg C, Finck BN, Kelly DP, Lopaschuk GD. Chronic activation of PPAR-alpha is detrimental to cardiac recovery after ischemia. American journal of physiology Heart and circulatory physiology. 2006; 290(1):H87–95. https://doi.org/10.1152/ajpheart.00285.2005 PMID: 16155108

24. Loichot C, Jesel L, Tesse A, Tabernero A, Schoonjans K, Roul G, et al. Deletion of peroxisome proliferator-activated receptor-alpha induces an alteration of cardiac functions. American journal of physiology Heart and circulatory physiology. 2006; 291(1):H161–6. https://doi.org/10.1152/ajpheart.01065.2004 PMID: 16461373

25. Karbowska J, Kochan Z, Smolenski RT. Peroxisome proliferator-activated receptor alpha is downregulated in the failing human heart. Cell Mol Biol Lett. 2003; 8(1):49–53. PMID: 12653556

26. Duhaney TA, Cui L, Rude MK, Lebrasseur NK, Ngoy S, De Silva DS, et al. Peroxisome proliferator-activated receptor-alpha-independent actions of fenofibrate exacerbates left ventricular dilation and fibrosis in chronic pressure overload. Hypertension. 2007; 49(5):1084–94. https://doi.org/10.1161/HYPERTENSIONAHA.107.086926 PMID: 17353090

27. Young ME, Laws FA, Goodwin GW, Taegtmeyer H. Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. The Journal of biological chemistry. 2001; 276(48):44390–5. https://doi.org/10.1074/jbc.M103826200 PMID: 11574533

28. Li S, Yang B, Du Y, Lin Y, Liu J, Huang S, et al. Targeting PPARalpha for the Treatment and Understanding of Cardiovascular Diseases. Cell Physiol Biochem. 2018; 51(6):2760–75. https://doi.org/10.1007/s00018-019-4333-5 PMID: 30962729

29. Finck BN, Han X, Courtois M, Almond F, Nerbomme JM, Kovacs A, et al. A critical role for PPARalpham ediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. Proc Natl Acad Sci U S A. 2003; 100(3):1226–31. https://doi.org/10.1073/pnas.0336724100 PMID: 12552126

30. Sleets PJ, Teunissen BE, Willemsen PH, van Nieuwenhoven FA, Brouns AE, Janssen BJ, et al. Cardiac hypertrophy is enhanced in PPAR alpha/- mice in response to chronic pressure overload. Cardiovascular research. 2008; 78(1):79–89. https://doi.org/10.1093/cvrv/cvn001 PMID: 18187461

31. Zungu M, Young ME, Stanley WC, Essop MF. Chronic treatment with the peroxisome proliferator-activated receptor alpha agonist Wy-14,643 attenuates myocardial respiratory capacity and contractile function. Mol Cell Biochem. 2009; 330(1–2):55–62. https://doi.org/10.1007/s11010-009-0100-y PMID: 19360380

32. Gilde AJ, van der Lee KA, Willemsen PH, Chinetti G, van der Leij FR, van der Vusse GJ, et al. Peroxisome proliferator-activated receptor (PPAR) alpha and PPARbeta/delta, but not PPARgamma,
modulate the expression of genes involved in cardiac lipid metabolism. Circulation research. 2003; 92 (5):518–24. https://doi.org/10.1161/01.RES.0000060700.55247.7C PMID: 12600885

33. Oka S, Zhai P, Yamamoto T, Ikeda Y, Byun J, Hsu CP, et al. Peroxisome Proliferator Activated Receptor-alpha Association With Silent Information Regulator 1 Suppresses Cardiac Fatty Acid Metabolism in the Failing Heart. Circulation Heart failure. 2015; 8(6):1123–32. https://doi.org/10.1161/CIRCHEARTFAILURE.115.002216 PMID: 26443578

34. Karam CN, Warren CM, Henze M, Banke NH, Lewandowski ED, Solaro RJ. Peroxisome proliferator-activated receptor-alpha expression induces alterations in cardiac myofilaments in a pressure-overload model of hypertrophy. American journal of physiology Heart and circulatory physiology. 2017; 312(4): H681–H90. https://doi.org/10.1152/ajpheart.00469.2016 PMID: 28130336

35. Wang L, Cai Y, Jian L, Cheung CW, Zhang L, Xia Z. Impact of peroxisome proliferator-activated receptor-alpha on diabetic cardiomyopathy. Cardiovascular diabetology. 2021; 20(1):2. https://doi.org/10.1186/s12933-020-01188-0 PMID: 33397369

36. Nakamura M, Liu T, Husain S, Zhai P, Warren JS, Hsu CP, et al. Glycogen Synthase Kinase-3alpha Promotes Fatty Acid Uptake and Lipotoxic Cardiac Contractility. Cell metabolism. 2019; 29(5):1119–34 e12. https://doi.org/10.1016/j.cmet.2019.01.005 PMID: 30745182

37. Banke NH, Wende AR, Leone TC, O’Donnell JM, Abel ED, Kelly DP, et al. Preferential oxidation of triacylglyceride-derived fatty acids in heart is augmented by the nuclear receptor PPARalpha. Circulation research. 2010; 107(2):233–41. https://doi.org/10.1161/CIRCRESAHA.110.221713 PMID: 20522803

38. Oka S, Zhai P, Alcendor R, Park JY, Tian B, Sadoshima J. Suppression of ERR targets by a PPARalpha/Sirt1 complex in the failing heart. Cell Cycle. 2012; 11(5):856–64. https://doi.org/10.4161/cc.11.5.19210 PMID: 22335351

39. Kalliora C, Kyriazis ID, Oka SI, Lieu MJ, Yue Y, Area-Gomez E, et al. Dual peroxisome-proliferator-activated-receptor-alpha/gamma activation inhibits SIRT1-PGC1alpha axis and causes cardiac dysfunction. JCI Insight. 2019; 5. https://doi.org/10.1172/jci.insight.129556 PMID: 31393858

40. Warren JS, Oka SI, Zabolaki D, Sadoshima J. Metabolic reprogramming via PPARalpha signaling in cardiac hypertrophy and failure: From metabolomics to epigenetics. American journal of physiology Heart and circulatory physiology. 2017; 313(3):H584–H96. https://doi.org/10.1152/ajpheart.00103.2017 PMID: 28646024

41. Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci U S A. 1999; 96(13):7473–8. https://doi.org/10.1073/pnas.96.13.7473 PMID: 10377439

42. Djouadi F, Weinheimer CJ, Saffitz JE, Pitchford C, Bastin J, Gonzalez FJ, et al. A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice. The Journal of clinical investigation. 1998; 102(6):1983–91. https://doi.org/10.1172/JCI9349 PMID: 979327

43. Djouadi F, Brandt JM, Weinheimer CJ, Leone TC, Gonzalez FJ, Kelly DP. The role of the peroxisome proliferator-activated receptor alpha (PPAR alpha) in the control of cardiac lipid metabolism. Prostaglandins Leukot Essent Fatty Acids. 1999; 60(5–6):339–43. https://doi.org/10.1016/S0952-3278(99)80009-x PMID: 10471118

44. Guerre-Millo M, Rouault C, Pouliain P, Andre J, Poitout V, Peters JM, et al. PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes. 2001; 50(12):2809–14. https://doi.org/10.2337/diabetes.50.12.2809 PMID: 11723064

45. Pol CJ, Lieu M, Drosatos K. PPARs: Protectors or Opponents of Myocardial Function? PPAR research. 2010; 2010:233–41. https://doi.org/10.1161/CIRCRESAHA.110.221713 PMID: 20522803

46. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiological reviews. 2010; 90(1):207–58. https://doi.org/10.1152/physrev.00015.2009 PMID: 20086077

47. Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Croce MA, Gropler MC, et al. OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization. Diabetes. 2006; 55(12):3418–28. https://doi.org/10.2337/db06-0399 PMID: 17130488

48. Shimizu M, Akter MH, Emi Y, Sato R, Yamaguchi T, Hirose F, et al. Peroxisome proliferator-activated receptor subtypes differentially cooperate with other transcription factors in selective transactivation of the perilipin/PEX11 alpha gene pair. J Biochem. 2006; 139(3):563–73. https://doi.org/10.1093/jb/mvl053 PMID: 16567422

49. Dalen KT, Ulven SM, Arntsen BM, Solaas K, Nebb HI. PPARalpha activators and fasting induce the expression of adipose differentiation-related protein in liver. J Lipid Res. 2006; 47(5):931–43. https://doi.org/10.1194/jlr.M500459-JLR200 PMID: 16489205
50. Shimizu M, Takeshita A, Tsukamoto T, Gonzalez FJ, Osumi T. Tissue-selective, bidirectional regulation of PEX11 alpha and perilipin genes through a common peroxisome proliferator response element. Mol Cell Biol. 2004; 24(3):1313–23. https://doi.org/10.1128/MCB.24.3.1313-1323.2004 PMID: 14729975

51. Levin MD, Bianconi S, Smith A, Cawley NX, Do AD, Hammond D, et al. X-linked creatine transporter deficiency results in prolonged QTc and increased sudden death risk in humans and disease model. Genet Med. 2021; 23(10):1864–72. https://doi.org/10.1038/s41436-021-1224-8 PMID: 34050321

52. Luptak I, Balschi JA, Xing Y, Leone TC, Kelly DP, Tian R. Decreased contractile and metabolic reserve in peroxisome proliferator-activated receptor-alpha-null hearts can be rescued by increasing glucose transport and utilization. Circulation. 2005; 112(15):2339–46. https://doi.org/10.1161/CIRCULATIONAHA.105.534594 PMID: 16203912

53. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. The Journal of clinical investigation. 1999; 103(11):1489–98. https://doi.org/10.1172/JCI6223 PMID: 10359558

54. Knight BL, Hebbachi A, Hauton D, Brown AM, Wiggins D, Patel DD, et al. A role for PPARalpha in the control of SREBP activity and lipid synthesis in the liver. Biochem J. 2005; 389(Pt 2):413–21. https://doi.org/10.1042/BJ20041896 PMID: 15777286

55. Tsai TH, Chen E, Li L, Saha P, Lee HJ, Huang LS, et al. The constitutive lipid droplet protein PLIN2 regulates autophagy in liver. Autophagy. 2017; 13(7):1130–44. https://doi.org/10.1080/15548627.2017.1319544 PMID: 28548876

56. Akoumi A, Haffar T, Mousterji M, Kiss RS, Bousette N. Palmitate mediated diacylglycerol accumulation causes endoplasmic reticulum stress, Plin2 degradation, and cell death in H9C2 cardiomyoblasts. Experimental cell research. 2017; 354(2):85–94. https://doi.org/10.1016/j.yexcr.2017.03.032 PMID: 28336294

57. Labinskyy V, Bellomo M, Chandler MP, Young ME, Lionetti V, Qanud K, et al. Chronic activation of peroxisome proliferator-activated receptor-alpha with fenofibrate prevents alterations in cardiac metabolic phenotype without changing the onset of decompensation in pacing-induced heart failure. The Journal of pharmacology and experimental therapeutics. 2007; 321(1):165–71. https://doi.org/10.1124/jpet.106.116871 PMID: 17215446

58. Wang Y, Viscarra J, Kim SJ, Sul HS. Transcriptional regulation of hepatic lipogenesis. Nat Rev Mol Cell Biol. 2015; 16(11):678–89. https://doi.org/10.1038/nrm4074 PMID: 26490400

59. Cotter DG, Schugar RC, Crawford PA. Ketone body metabolism and cardiovascular disease. American journal of physiology Heart and circulatory physiology. 2013; 304(8):H1060–76. https://doi.org/10.1152/ajpheart.00646.2012 PMID: 23396451

60. Muoio DM, MacLean PS, Lang DB, Li S, Houmard JA, Way JM, et al. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. The Journal of biological chemistry. 2002; 277(29):26089–97. https://doi.org/10.1074/jbc.M203997200 PMID: 12118038

61. Yamaguchi T, Matsushita S, Motojima K, Hirose F, Osumi T. MLDP, a novel PAT family protein localized to lipid droplets and enriched in the heart, is regulated by peroxisome proliferator-activated receptor alpha. The Journal of biological chemistry. 2006; 281(20):14232–40. https://doi.org/10.1074/jbc.M601682200 PMID: 16717212

62. Lin J, Chen A. Perilipin 5 restores the formation of lipid droplets in activated hepatic stellate cells and inhibits their activation. Lab Invest. 2016; 96(7):791–806. https://doi.org/10.1038/labinvest.2016.53 PMID: 27135793

63. Amen T, Kaganovich D. Stress granules inhibit fatty acid oxidation by modulating mitochondrial permeability. Cell Rep. 2021; 35(11):109237. https://doi.org/10.1016/j.celrep.2021.109237 PMID: 34133922

64. Schulze PC, Drosatos K, Goldberg IJ. Lipid Use and Misuse by the Heart. Circulation research. 2016; 118(11):1736–51. https://doi.org/10.1161/CIRCRESAHA.116.306842 PMID: 27230639

65. Listenberger LL, Han X, Lewis SE, Cases S, Farese RV Jr., Ory DS, et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proc Natl Acad Sci U S A. 2003; 100(6):3077–82. https://doi.org/10.1073/pnas.0630588100 PMID: 12629214

66. Liu L, Shi X, Bharadwaj KG, Ikeda S, Yamashita H, Haygu H, et al. DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. The Journal of biological chemistry. 2009; 284(52):36312–23. https://doi.org/10.1074/jbc.M109.049817 PMID: 19778901

67. Nakamura M, Sadoshima J. Cardiomyopathy in obesity, insulin resistance and diabetes. The Journal of physiology. 2020; 598(14):2977–93. https://doi.org/10.1113/JP276747 PMID: 30869158