

Preferential Oxidation of Triacylglyceride-Derived Fatty Acids in Heart Is Augmented by the Nuclear Receptor PPAR α

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Rationale: Long chain fatty acids (LCFAs) are the preferred substrate for energy provision in hearts. However, the contribution of endogenous triacylglyceride (TAG) turnover to LCFA oxidation and the overall dependence of mitochondrial oxidation on endogenous lipid is largely unstudied.

Objective: We sought to determine the role of TAG turnover in supporting LCFA oxidation and the influence of the lipid-activated nuclear receptor, proliferator-activated receptor (PPAR) α , on this balance.

Methods and Results: Palmitoyl turnover within TAG and palmitate oxidation rates were quantified in isolated hearts, from normal mice (nontransgenic) and mice with cardiac-specific overexpression of PPAR α (MHC-PPAR α). Turnover of palmitoyl units within TAG, and thus palmitoyl-coenzyme A recycling, in nontransgenic (4.5 ± 2.3 $\mu\text{mol}/\text{min}$ per gram dry weight) was 3.75-fold faster than palmitate oxidation (1.2 ± 0.4). This high rate of palmitoyl unit turnover indicates preferential oxidation of palmitoyl units derived from TAG in normal hearts. PPAR α overexpression augmented TAG turnover 3-fold over nontransgenic hearts, despite similar fractions of acetyl-coenzyme A synthesis from palmitate and oxygen use at the same workload. Palmitoyl turnover within TAG of MHC-PPAR α hearts (16.2 ± 2.9 , $P < 0.05$) was 12.5-fold faster than oxidation (1.3 ± 0.2). Elevated TAG turnover in MHC-PPAR α correlated with increased mRNA for enzymes involved in both TAG synthesis, Gpm (glycerol-3-phosphate acyltransferase, mitochondrial), Dgat1 (diacylglycerol acyltransferase 1), and Agpat3 (1-acylglycerol-3-phosphate *O*-acyltransferase 3), and lipolysis, Pnliprp1 (pancreatic lipase related protein 1).

Conclusions: The role of endogenous TAG in supporting β -oxidation in the normal heart is much more dynamic than previously thought, and lipolysis provides the bulk of LCFA for oxidation. Accelerated palmitoyl turnover in TAG, attributable to chronic PPAR α activation, results in near requisite oxidation of LCFAs from TAG. (*Circ Res.* 2010; 107:233-241.)

Key Words: PPAR α ■ triacylglyceride ■ fatty acid oxidation

Long chain fatty acids (LCFAs) are well recognized as the preferred substrate for oxidative ATP production by cardiac mitochondria.¹⁻⁶ To date, the exogenous, blood-borne LCFAs have generally been considered as the primary source for fueling oxidative metabolism, with endogenous triacylglyceride (TAG) serving as a biochemically inert lipid store.⁷⁻⁹ The actual involvement of endogenous TAG in supplying LCFAs for oxidation by the heart has largely gone unstudied, particularly in nondestructive studies of intact hearts. In light of the recent findings by Haemmerle et al¹⁰ of increased myocardial TAG in mouse hearts deficient of adipose triglyceride lipase (*ATGL*^{+/-}), it is enticing to speculate that myocardial TAG is an important contributor to cardiac fatty acid oxidation (FAO). This work examines the contributions

of steady-state TAG to LCFA oxidation, through direct comparison of the rates of TAG turnover and LCFA oxidation in the intact, beating heart.

To probe the link between TAG dynamics and FAO rates, we studied hearts of normal mice and a mouse model of a low level of cardiac overexpression of the peroxisome proliferator-activated receptor (PPAR) α following either normal diet or high-fat diet (HFD). This model, which recapitulates many metabolic abnormalities of the diabetic heart, has been previously shown to have elevated TAG content and augmented expression of FAO enzymes that are exacerbated by HFD.^{5,6,11,12} Previous studies have clearly linked PPAR α to altered expression of enzymes for FAO in diseased hearts. However, the mechanistic role of how PPAR α regulates

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Non-standard Abbreviations and Acronyms

Agpat3	1-acylglycerol-3-phosphate <i>O</i> -acyltransferase 3
Agpat5	1-acylglycerolphosphate acyltransferase ϵ
CoA	coenzyme A
FA	fatty acid
FAO	fatty acid oxidation
HFD	high-fat diet
LCFA	long-chain fatty acid
MHC-PPARα	myosin heavy chain–peroxisome proliferator-activated receptor α transgene
NTG	nontransgenic
PPAR	peroxisome proliferator-activated receptor
RCD	regular chow diet
RPP	rate pressure product
TAG	triacylglyceride

fatty acid storage as TAG and turnover of the endogenous TAG pool remains largely unknown. Through a comprehensive analysis of cardiac TAG dynamics and LCFA oxidation rates, the findings elucidate a preferential utilization of a LCFA that cycles LCFAs through the highly dynamic TAG pool as a primary source of FAO.

Methods

Animal Model

Male mice with cardiac-specific overexpression of PPAR α , driven by the α myosin heavy chain promoter (MHC-PPAR α), and nontransgenic (NTG) littermates, weighing \approx 25 g at 12 weeks of age, were used for this study.¹² We chose to study the previously described low level–overexpressing MHC-PPAR α transgenic mouse line (404-4), which does not display cardiac dysfunction at this age, in contrast to mice with higher levels of transgene overexpression.¹² Mice were backcrossed 6 times to C57Bl/6J. Mice were supplied either a regular chow diet (RCD) or HFD (Teklad no. 97268) for 2 weeks before experimentation and fed ad libitum in light and temperature controlled housing. All procedures were approved by the University of Illinois at Chicago Animal Care and Use Committee.

Isolated Heart Protocols

Mice were heparinized (50 U/10 g, IP) and anesthetized (ketamine, 80 mg/kg, plus xylazine, 12 mg/kg, IP). Hearts were excised and perfused in retrograde fashion with modified Krebs–Henseleit buffer (118.5 mmol/L NaCl, 4.7 mmol/L KCl, 1.5 mmol/L CaCl₂, 1.2 mmol/L MgSO₄, and 1.2 mmol/L KH₂PO₄) maintained at 37°C, equilibrated with 95% O₂/5% CO₂, and containing 0.4 mmol/L unlabeled palmitate complexed to albumin (3:1 molar ratio) and 10 mmol/L glucose. Left ventricular developed pressure and heart rate were continuously recorded from a water-filled balloon in the left ventricle with a pressure transducer for digital recording (Pow-erlab, AD Instruments, Colorado Springs). At the end of each experiment, hearts were frozen in liquid N₂-cooled tongs.

For TAG dynamics, isolated hearts from both NTG and MHC-PPAR α mice were perfused in a 14.1 Tesla NMR magnet at baseline workload (NTG, N=7; MHC-PPAR α , N=12) or with adrenergic challenge (0.1 μ mol/L of isoproterenol) (NTG, N=5; MHC-PPAR α , N=6). After collection of ¹³C-NMR background signals of naturally abundant ¹³C (1.1%), hearts were switched to buffer containing 0.4 mmol/L [2,4,6,8,10,12,14,16-¹³C₈]palmitate (Isotec Inc, Miamisburg, Ohio) plus 10 mmol/L unlabeled glucose.

Perfusion with ¹³C-enriched media continued for 20 minutes at baseline workload for mice fed on RCD (MHC-PPAR α , n=10; NTG n=12) and mice on HFD (MHC-PPAR α , n=7; NTG, n=4), and for 10 minutes during adrenergic challenge (0.1 μ mol/L isoproterenol) (MHC-PPAR α , n=5; NTG n=8). Additional hearts were perfused for 120 minutes to ensure stability of TAG turnover and content over time (n=4).

For palmitate oxidation rates, hearts from MHC-PPAR α and NTG mice on either RCD (MHC-PPAR α , n=6; NTG, n=4) or HFD (MHC-PPAR α , n=7; NTG, n=5) were perfused for 30 minutes with 0.4 or 1.2 mmol/L [4,6,8,10,12,14,16,-¹³C₇]palmitate and 10 mmol/L glucose.

NMR Spectroscopy and Tissue Chemistry

NMR measurements of TAG turnover were performed on perfused hearts with sequential, proton-decoupled carbon-13 (¹³C) NMR spectra (2 minutes each) with ¹³C natural abundance correction, as previously reported.^{13,14}

¹³C enrichment of TAG in the heart was monitored from the NMR signal at 30.5 ppm from the TAG methylene groups. TAG turnover was calculated from total TAG content and enrichment over time.^{14–17} Kinetic analysis of dynamic ¹³C spectra from hearts was performed as previously reported.^{13,14,16,17}

Metabolic flux was determined during [¹³C]palmitate oxidation in the intact mouse heart using a previously described method for kinetic analysis of the progressive ¹³C enrichment of glutamate, as detected via NMR.^{13,18–21}

For kinetic analysis of oxidative rates, glutamate, aspartate, citrate malate, and α -ketoglutarate contents in frozen myocardial samples were assayed spectrophotometrically and fluorometrically.^{18,22} In vitro ¹³C NMR was performed on acid extracts of myocardium to determine fractional enrichment of [2-¹³C]acetyl-coenzyme (CoA).^{19,23}

Lipid extracts were obtained from tissue and TAG quantified by colorimetric assay (Wako Pure Chemical Industries).¹⁴ The fractional ¹³C enrichment of TAG was assessed by liquid chromatography/mass spectrometry. LCFA content in TAG, of carbon lengths 12 to 18, was determined by LCMS as a percentage of total LCFAs. Total TAG turnover (nanomoles of TAG per minute per milligram protein) was quantified from ¹³C enrichment rates and the end point ¹³C enrichment.^{14–17}

Rates of palmitate unit turnover within the TAG pool were determined from TAG turnover rates and the percentage of acyl units represented by palmitate ([¹²C+¹³C]palmitate) present in the TAG pool. LCMS analysis enabled determination of the percentage of each LCFA present in the TAG pool. From the stoichiometry of 3 fatty acyl groups per TAG molecule and the percentage of palmitate present in the TAG pool, TAG turnover rates were converted to rates of palmitate unit turnover within the TAG pool.

RNA Extraction and Quantitative RT-PCR

Total RNA was extracted from hearts from MHC-PPAR α and NTG mice on a RCD (MHC-PPAR α : N=6; NTG: N=7) or HFD (MHC-PPAR α : N=5; NTG: N=7) with TRIzol reagent (Invitrogen) and reverse transcribed with Superscript III Reverse Transcriptase (Invitrogen). Equal amounts of cDNA were subjected to real-time PCR using SYBR Green as a probe as described previously.²⁴ Data were corrected by expressing them relative to the levels of the invariant transcript peptidylprolyl isomerase (Ppia) (also known as cyclophilin) and normalized to wild-type controls on standard chow, which were arbitrarily assigned as 1.0.

An expanded Methods section is available in the Online Data Supplement at <http://circres.ahajournals.org>.

Results

Isolated Mouse Heart Hemodynamics and Oxygen Use

Baseline rate pressure products (RPPs) were similar between hearts of transgenic (MHC-PPAR α) and NTG mice:

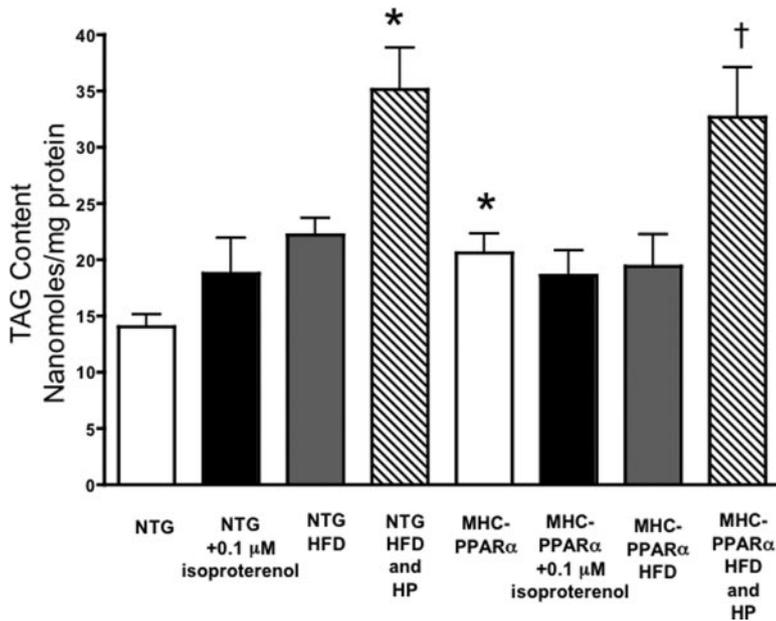


Figure 1. TAG content in hearts of MHC-PPAR α low-overexpressing mice and NTG littermates on a RCD or HFD perfused with 0.4 or 1.2 mmol/L palmitate (high palmitate [HP]). TAG content reported in nmol/mg protein. * $P < 0.05$ vs NTG baseline; † $P < 0.05$ vs NTG HFD and MHC-PPAR α HFD.

MHC-PPAR α : 35 000 \pm 3000 beats \cdot mm Hg per minute; NTG: 35 000 \pm 3000 beats \cdot mm Hg per minute. Baseline oxygen consumption (MVO $_2$) was also similar between MHC-PPAR α and NTG mice (MHC-PPAR α : 42.2 \pm 9.0.1 μ mol/min per gram; NTG: 38.0 \pm 3.8 μ mol/min per gram). With isoproterenol stimulation, RPPs increased by 44 \pm 4.9% and 42 \pm 8.3% in the NTG and MHC-PPAR α mice, respectively ($P < 0.05$), and oxygen use increased as follows: MHC-PPAR α : 74.4 \pm 18.1 μ mol/min per gram; NTG: 65.9 \pm 8.0 μ mol/min per gram. Both NTG and MHC-PPAR α hearts showed similar RPPs under each condition. Thus, the metabolic demand, which is dominated by work performance, was also similar between groups, despite the significant differences in lipid dynamics. A 2-week HFD did not affect MVO $_2$ (MHC-PPAR α : 36.4 \pm 2.2 μ mol/min per gram; NTG: 38.0 \pm 6.9) nor RPPs (MHC-PPAR α : 34 000 \pm 4000

beats \cdot mm Hg per minute; NTG: 36 000 \pm 2000 beats \cdot mm Hg per minute).

TAG Content and Dynamics

MHC-PPAR α hearts displayed elevated TAG content (Figure 1).¹¹ TAG stores remained constant over the duration of each protocol, confirming steady-state conditions for turnover measurements (Figure 2).²⁵ TAG contents at both 20 minutes and 2 hours of perfusion were similar (NTG: 15 \pm 1 nmol/mg protein at 20 minutes of perfusion versus 14 \pm 1 at 120 minutes; MHC-PPAR α : 20 \pm 2 nmol/mg protein at 20 minutes versus 26 \pm 2 at 120 minutes). Liquid chromatographic/mass spectrometric analysis of purified TAG samples revealed that at baseline, [13 C]palmitate comprised a significantly larger percentage of fatty acyl groups within TAG in MHC-PPAR α hearts than NTG (Table). This finding

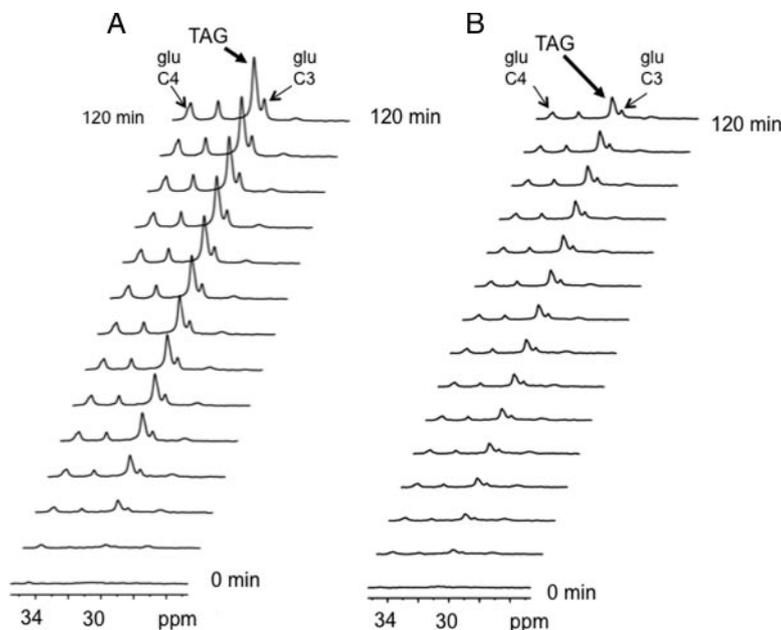


Figure 2. Representative 13 C spectra from isolated mouse hearts displaying progressive 13 C enrichment of TAG and glutamate over 120 minutes from MHC-PPAR α (A) and NTG (B) hearts perfused with 0.4 mmol/L [2,4,6,8,10,12,14,16- 13 C $_8$] palmitate+10 mmol/L unlabeled glucose. The spectral signals from 13 C-enriched methylene carbons of TAG, at 30.5 ppm, resulting from [13 C]palmitate storage and the 4- and 3-carbons of glutamate produced by oxidation of [13 C]palmitate, at 34 and 28 ppm, respectively (glu C-4 and glu C-3).

Table. Total Palmitate (Endogenous [¹²C]Palmitate+ Exogenous [¹³C]Palmitate) As a Percentage of Total Fatty Acyl Units in TAG

	NTG		MHC-PPAR α	
	Baseline	0.1 μ mol/L Isoproterenol	Baseline	0.1 μ mol/L Isoproterenol
Total palmitate (% units of total fatty acyl units of TAG)	23.4 \pm 1.9	14.6 \pm 4.1	32.7 \pm 2.5	35.6 \pm 3.5*
Palmitate turnover in TAG (μ mol/min per gram dry weight)	4.5 \pm 0.4	4.0 \pm 2.3	16.2 \pm 2.9†	36.7 \pm 7.7‡

Rate of palmitate turnover in TAG (μ mol/min per gram dry weight). *Significantly different vs NTG baseline conditions, $P < 0.05$; † $P < 0.005$ vs NTG baseline; ‡ $P < 0.005$ vs baseline MHC-PPAR α .

is consistent with the augmented long-chain fatty acid storage as TAG in MHC-PPAR α , which results in higher TAG content.

β -Adrenergic challenge did not alter myocardial TAG in either group. β -Adrenergic stimulation did not affect the [¹³C]palmitate incorporation into TAG, despite a potentiated increase in TAG turnover (Table; Figure 3). TAG content was maintained in both groups, despite the higher energetic demands of increased workload.

HFD did not affect TAG content in hearts perfused with 0.4 mmol/L palmitate. Elevated concentrations of palmitate in the buffer (1.2 mmol/L) did increase TAG content in hearts of mice fed a HFD (Figure 1). This different response between 0.4 and 1.2 mmol/L palmitate could be attributable to net use of TAG stores with lower exogenous fatty acid (FA) concentration, partially depleting the otherwise elevated TAG content. However, TAG turnover significantly increased in both NTG and MHC-PPAR α hearts perfused with 1.2 mmol/L palmitate. HFD did not alter expression of genes that regulate TAG turnover, thus the increases in TAG

turnover appear related to the increase in exogenous FA supply.

Overall, TAG dynamics were significantly elevated in the hearts of MHC-PPAR α mice, yet the ¹³C enrichment of acetyl CoA from palmitate was similar in both groups, irrespective of diet (Figure 4). The combined results of TAG turnover and palmitate oxidation indicate that both groups rely to a great extent on LCFAs that initially esterified to TAG before oxidation.

Palmitoyl Unit Turnover Within TAG and Oxidation Rates

The Table shows palmitate content (¹²C and ¹³C) in TAG, as a percentage of total fatty acyl groups, and palmitate turnover rates within the TAG pool for each group. Palmitate turnover within the TAG pool of MHC-PPAR α hearts was 4-fold higher than that of NTG hearts. Unlike NTG, MHC-PPAR α hearts responded to isoproterenol challenge by doubling the turnover rate of palmitate within TAG.

Palmitate oxidation rates (Figure 4B) were 1.2 \pm 0.4 and 1.3 \pm 0.2 μ mol/min per gram dry weight in NTG and MHC-PPAR α hearts, respectively (Figure 4B), consistent with previous reports.^{5,11,26–29} Palmitate oxidation rates at baseline work from mice on a RCD or a HFD were similar between the MHC-PPAR α and NTG groups. In contrast, TAG turnover was much greater in the MHC-PPAR α hearts than NTG. Comparison of these palmitate unit turnover rates within TAG at baseline (RCD: NTG=4.5 \pm 0.4 to PPAR α =16.2 \pm 2.9 μ mol/min per gram dry weight; HFD: NTG=22.9 \pm 0.5 to PPAR α =63.12 \pm 26 μ mol/min per gram dry weight) to the respective rates of palmitate oxidation (RCD: NTG=1.2 \pm 0.4 and PPAR α =1.3 \pm 0.2 μ mol/min per gram dry weight; HFD: NTG=0.9 \pm 0.23 and PPAR α =1.1 \pm 0.1 μ mol/min per gram dry weight) indicates a much more rapid rate of palmitate turnover within TAG compared to that of palmitate oxidation (Figure 4D).

From the measured palmitate turnover within the TAG pool and previously published acyl-CoA pool size data, the

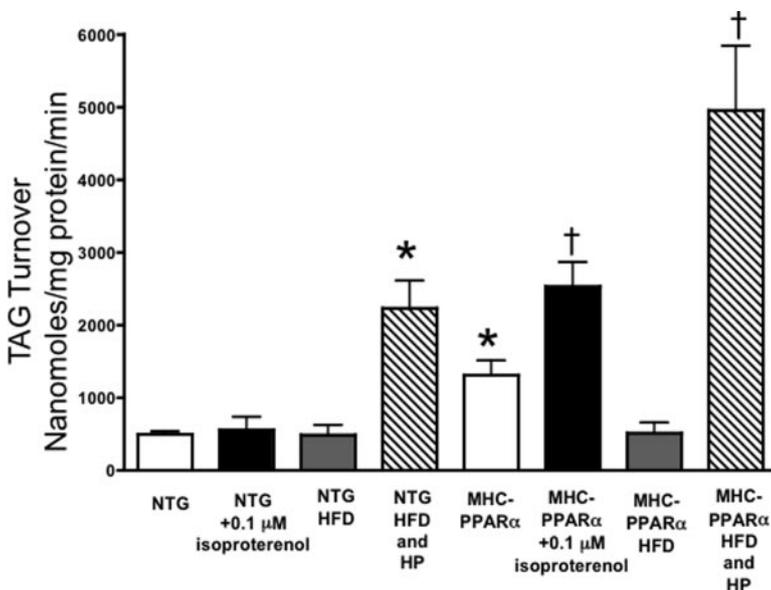


Figure 3. TAG turnover in MHC-PPAR α low-overexpressing mice and NTG littermates. * $P < 0.05$ vs NTG baseline TAG; † $P < 0.05$ vs NTG+isoproterenol and PPAR α baseline.

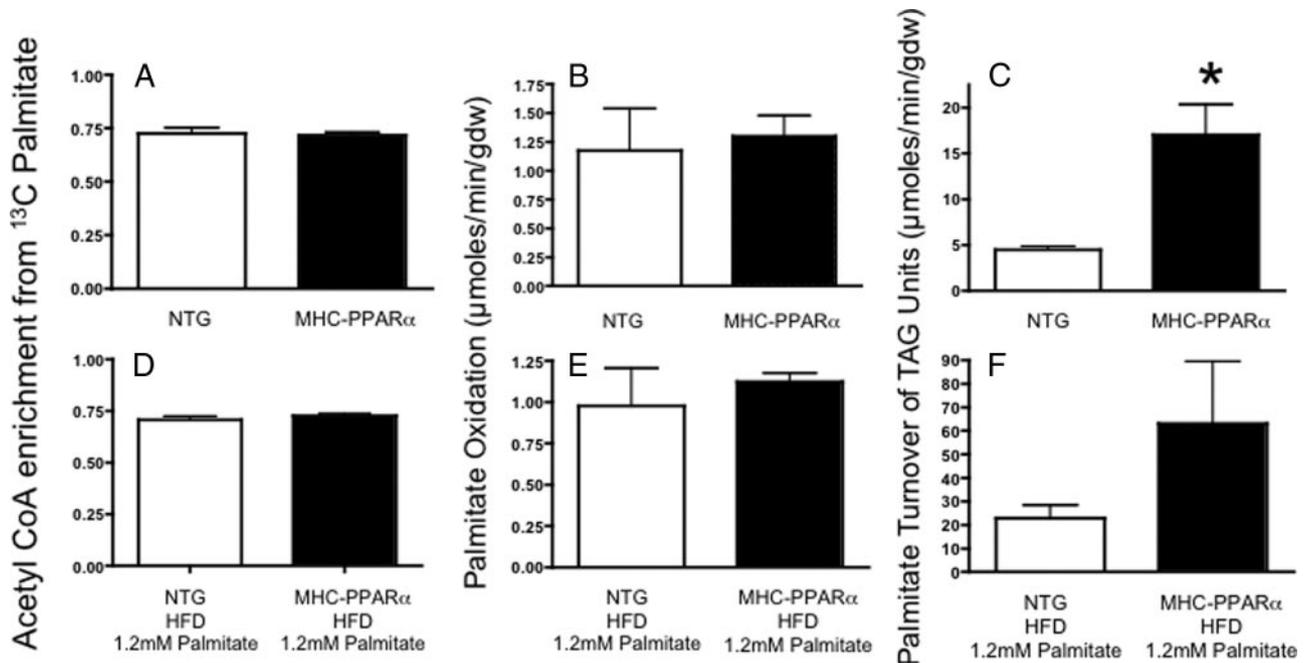


Figure 4. Metabolic fate of palmitate. **A**, Acetyl CoA enrichment from [^{13}C]palmitate in MHC-PPAR α low-overexpressing mice (PPAR α) and NTG littermates. **B**, Rates of palmitate oxidation and MHC-PPAR α low-overexpressing mice (PPAR α) and NTG littermates ($\mu\text{mol/min}$ per gram dry weight). **C**, Palmitate turnover of TAG units in NTG and MHC-PPAR α low-overexpressing mice (MHC-PPAR α) ($\mu\text{mol/min}$ per gram dry weight). **D**, Acetyl CoA enrichment from [^{13}C]palmitate in MHC-PPAR α low-overexpressing mice (PPAR α) and NTG littermates fed a HFD for 2 weeks and perfused with 1.2 mmol/L Palmitate. **E**, Palmitate oxidation rates from MHC-PPAR α low-overexpressing mice (PPAR α) and NTG littermates ($\mu\text{mol/min}$ per gram dry weight) fed a HFD and perfused with 1.2 mmol/L Palmitate. **F**, Palmitate turnover of TAG units in NTG and MHC-PPAR α low-overexpressing mice (MHC-PPAR α) fed a HFD and perfused with 1.2 mmol/L palmitate ($\mu\text{mol/min}$ per gram dry weight). * $P < 0.05$ vs NTG.

time for complete turnover of the available acyl-CoA pool is 16 and 4 seconds in NTG and MHC-PPAR α hearts, respectively.¹¹ Thus, the turnover of the palmitoyl pool that is available for oxidation is 4 times faster in MHC-PPAR α hearts versus NTG hearts. Accounting for palmitate alone, complete exchange of the cytosolic palmitoyl-CoA pool with palmitoyl units from lipolysis of TAG would require 0.33 and 0.09 seconds, for NTG and MHC-PPAR α hearts, respectively.

These relative rates indicate a large contribution from total TAG lipolysis to palmitate oxidation in the intact heart. Specifically, the difference between the rates of palmitate turnover in TAG and palmitate oxidation in MHC-PPAR α hearts, which is an order of magnitude, indicates a high rate of mixing between exogenous palmitate and TAG-derived palmitate within the pool of fatty acids available for oxidative metabolism. These data provide evidence that the preferential oxidation of TAG-derived fatty acid units is augmented by PPAR α activation.

Gene Expression

Transcription levels for enzymes involved in TAG trafficking and turnover was determined (Figure 5). In all MHC-PPAR α hearts tested, PPAR α expression was induced 9- to 13-fold (not shown). mRNAs for Gpm (glycerol-3-phosphate acyltransferase, mitochondrial), Agpat3 and -5, and Dgat1 (diacylglycerol acetyltransferase 1), all key enzymes involved in TAG synthesis, were higher in MHC-PPAR α mice than NTG (Figure 5). Increased Agpat3 is consistent with findings of PPAR α regulation of Agpat3.³⁰ The results for Gpm and

Dgat confirm previous findings in MHC-PPAR α hearts by Finck et al.³¹ Pnliprpl (pancreatic lipase related protein 1) was also increased in MHC-PPAR α hearts, which is consistent with increased lipolysis (Figure 5). These changes suggest that PPAR α activation determines TAG turnover by regulating the expression of genes involved in both TAG synthesis and lipolysis.

Discussion

The present study provides evidence that, in normal hearts, fatty acyl units from a comparatively dynamic TAG pool are preferentially oxidized because of the relative rates of TAG turnover and palmitate oxidation. In otherwise normal, NTG mouse hearts, the high turnover rate of palmitoyl units within TAG, relative to the slower palmitate oxidation rate, indicates a large contribution from TAG to β -oxidation. Chronic activation of PPAR α in MHC-PPAR α mouse hearts drives oxidation of TAG-derived LCFAs to near complete levels, through greatly accelerated TAG turnover rates. Whereas total palmitate oxidation in both the NTG and the MHC-PPAR α hearts were relatively similar, the rate of palmitate turnover of TAG units was 4 times greater in the transgenic than NTG hearts at baseline. The augmented TAG dynamics in MHC-PPAR α hearts, and thus increased contribution of TAG to β -oxidation, correlates with elevated transcript levels of enzymes involved in both TAG synthesis and degradation. Thus, this mechanism of preferential oxidation of TAG versus exogenous free fatty acids is driven, at least in part, by PPAR α via regulation of the expression of enzymes that determine the rates of TAG synthesis and lipolysis.

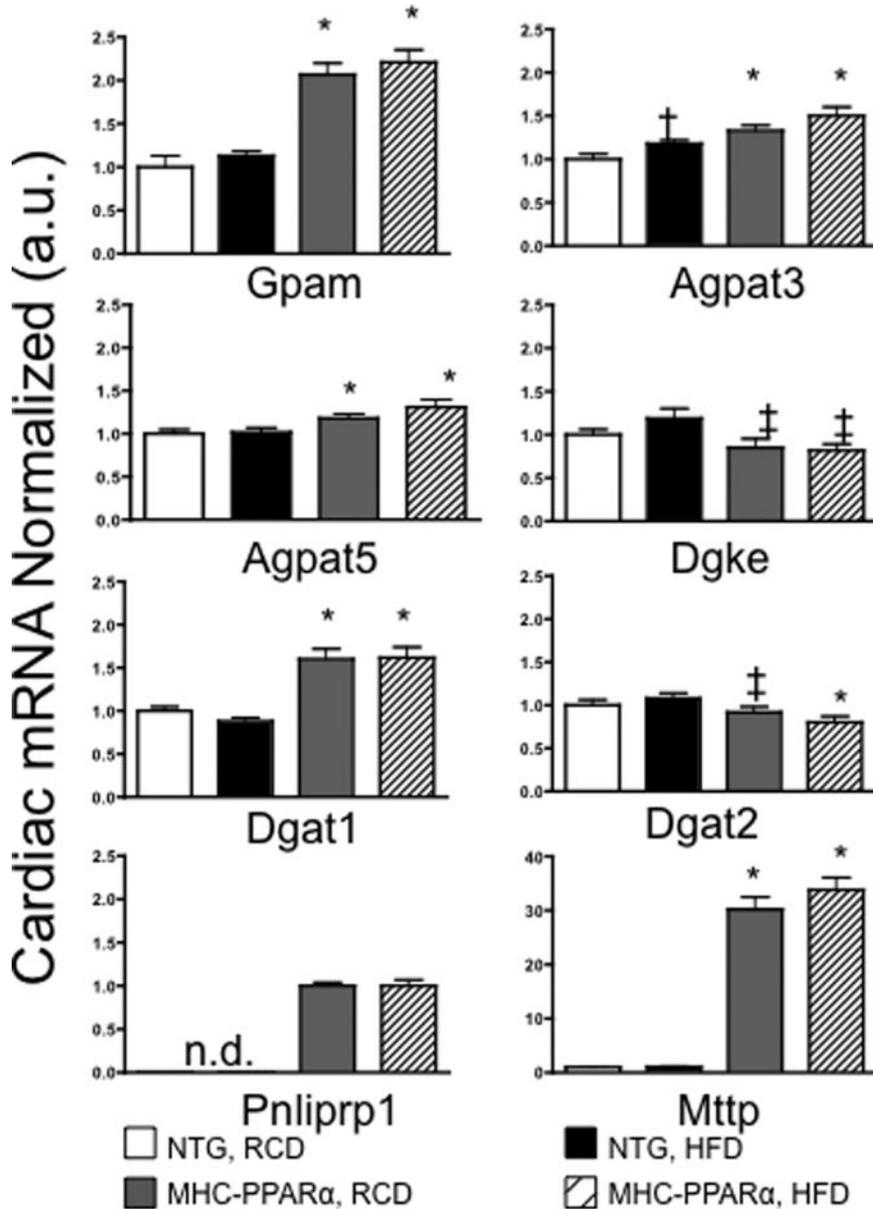


Figure 5. mRNA levels of genes encoding enzymes that regulate TAG synthesis and lipolysis in heart tissue from NTG and MHC-PPAR α mice fed a RCD or a HFD. * $P < 0.03$ vs NTG; †Significantly different compared to NTG-RCD; ‡ $P < 0.05$ vs NTG-HFD. Dgke indicates diacylglycerol kinase ϵ ; Mttp, mitochondrial triglyceride transfer protein; n.d., not detectable.

Both exogenous palmitate and palmitoyl units derived from lipolysis of the intracellular steady-state TAG pool contribute to palmitoyl-CoA in the cytosol, as a “commonly accessible” pool for either reincorporation into TAG or oxidation within the mitochondria (Figure 6). From previously reported measures of the acyl pool sizes, the total fatty acyl pool is 140 and 100 nmol/g in NTG and MHC-PPAR α hearts, respectively.¹¹ The palmitoyl-CoA pool sizes were reported at 25 nmol/g for both NTG and MHC-PPAR α .¹¹ Because the rate of turnover of palmitate TAG units is significantly greater than the palmitate oxidation rate, there is significant cycling of palmitoyl units through the TAG pool before β -oxidation. Hearts of PPAR α transgenic mice undergo significantly higher rates of TAG turnover compared to NTG, indicating an elevated recruitment of the TAG pool, which is consistent with the upregulation of known lipid metabolism and LCFA oxidative enzymes in this model (Figure 7). In light of work showing rescue of metabolism

when hearts with high PPAR α overexpression are crossed with mice deficient in the LCFA transporter CD36, the observed group differences in TAG turnover relative to oxidation suggest a mechanism whereby hearts respond to imbalances between LCFA uptake and oxidation.⁶

Differences in the time required for complete turnover of the palmitoyl content in the TAG pool mean that 26% of the cytosolic palmitoyl-CoA pool is oxidized in NTG hearts, whereas 8% of palmitoyl-CoA in this pool is oxidized in MHC-PPAR α hearts. Nearly 4 times more of the cytosolic palmitoyl-CoA pool in NTG hearts than MHC-PPAR α is oxidized during the time required for complete exchange of the palmitoyl-CoA pool with TAG. Thus, faster TAG turnover, and palmitoyl-CoA exchange with TAG, corresponds to a lower percentage of the palmitoyl-CoA pool being oxidized in the same time period. The distinction between the influence of relative rates of palmitate turnover within TAG and palmitate oxidation and the source of palmitoyl-CoA indi-

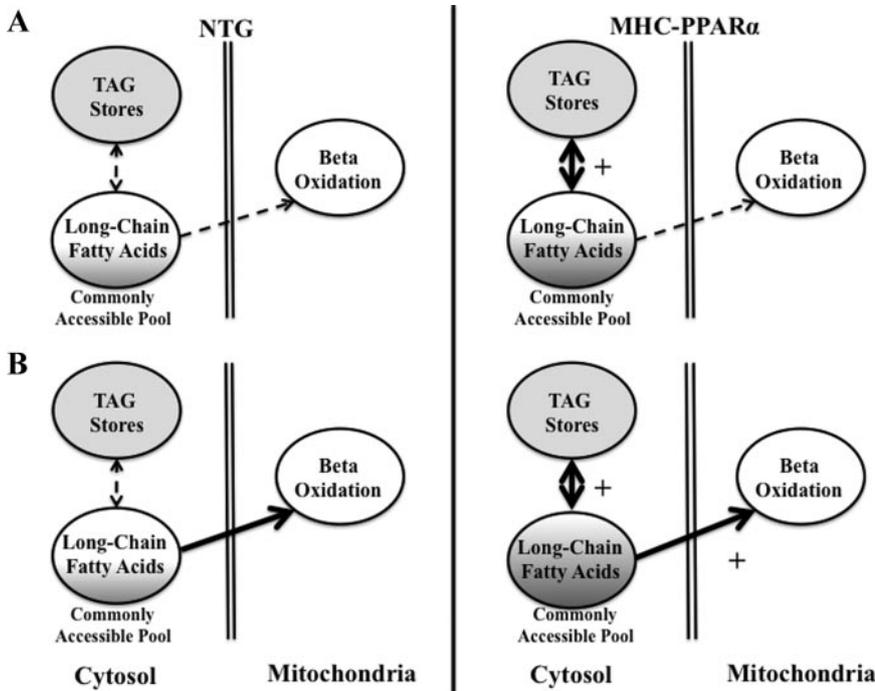


Figure 6. Schematic of LCFA sources fueling beta oxidation. **A**, Fate of LCFAs in the myocardium at baseline. Both MHC-PPAR α and NTG hearts exhibit cytosolic mixing of endogenous LCFAs (shown in the **solid gray**) with exogenous LCFAs. **B**, Fate of LCFAs during β -adrenergic stimulation. MHC-PPAR α hearts exhibit increased mixing to meet energy demands, shown in **darker shade**. **Thickness of arrow** indicates pathway preference. **Shaded gray areas** represent increased mixing of exogenous and endogenous LCFAs. Both figures show bidirectional pathway of LCFAs entering and exiting the TAG pool.

indicates that palmitate preferentially enters the TAG pool before lipolysis and, ultimately, β -oxidation (Figure 6A) in the MHC-PPAR α heart. With such rapid turnover, LCFAs enter the cell, are stored, and then, following lipolysis, are finally oxidized. These findings are also consistent with the hypothesis that preference for TAG-derived LCFAs drives elevated TAG turnover in PPAR α hearts.

During adrenergic challenge, myocardial TAG content remained constant, but TAG turnover in MHC-PPAR α hearts was significantly faster than in NTG. Surprisingly, adrenergic stress potentiated the elevated TAG dynamics in the MHC-PPAR α hearts, indicating homeostatic mechanisms exist that maintain this enlarged endogenous lipid pool. Consequently, β -adrenergic stimulation accelerates exchange of palmitate through the TAG pool before oxidation. The greater rate of

palmitate turnover in TAG, during β -adrenergic stimulation of the MHC-PPAR α hearts, indicates a further increase in the contribution of stored fatty acyl units (ie, palmitate) from TAG to the common acyl-CoA pool that supplies β -oxidation (Figure 6B). Rather than primarily shifting toward glucose oxidation to meet immediate energy demands, there is a remarkable increase in the shuttling of LCFAs through the TAG pool in the MHC-PPAR α . Importantly, not only are LCFAs being recruited from the TAG pool to meet energy demands, but they are also being cycled through it, which in itself is an energy-consuming process. The constant TAG pool size during elevated turnover reflects the impact of increased activity of the enzymes that regulate catabolic and anabolic TAG turnover pathways in response to stress in MHC-PPAR α hearts. This increase in TAG synthesis and

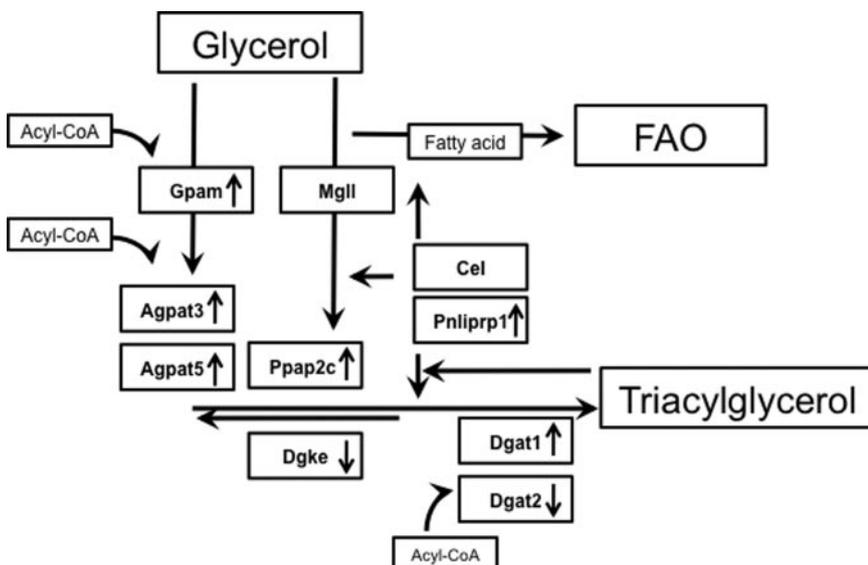


Figure 7. Schematic of enzymatic pathways for the synthesis of triacylglycerol. **Up arrow** indicates an increased expression in the transgenic model. **Down arrow** indicates a decreased enzyme expression. Cel indicates carboxyl ester lipase; Mgll, mono-glyceride lipase.

lipolysis supports β -oxidation during β -adrenergic challenge (Figure 6B).

Our findings are consistent with, and are further supported by, longstanding findings that exogenous TAG-derived acyl esters contribute to β -oxidation as opposed to free fatty acids.^{32,33} We now identify a similar role for FAs derived from endogenous TAG, and underscore that FA-acyl CoA that enter the heart after hydrolysis of exogenous TAG are largely recycled through an endogenous TAG pool. Although TAG stores are known to contribute to LCFA oxidation, the extent of that contribution has not previously been comprehensively analyzed, in part, because a lack of steady-state TAG content, known isotopic enrichment levels, and/or serial measurements of actual TAG dynamics in the same hearts.⁹

The present protocol enabled quantitative analysis at steady state without perturbing TAG dynamics and content. These steady-state measures allowed direct measurement of the dynamics of stored and oxidized LCFAs. Importantly, the highly regulated processes of lipid storage and oxidation preclude any conclusive predictions from observing increased protein levels alone. Indeed, the observed rates of turnover in the TAG pool of the intact, beating heart could not otherwise be predicted (Figure 7). Therefore, the near-requisite oxidation of LCFAs from TAG in MHC-PPAR α could only have been discerned from the relative rates of palmitoyl turnover within TAG and palmitate oxidation by mitochondria in the intact heart.

Changes in substrate oxidation and lipid handling have previously been implicated in various cardiomyopathies.^{9,16,34} Increased PPAR α expression leads to an increase in myocardial LCFA uptake with augmented FA oxidation, which mimics the metabolic phenotype of the diabetic heart.⁹ Conversely, PPAR α expression is reduced in the hypertrophic heart where FAO is limited and turnover of TAG is slowed.^{16,34} Whereas the previous studies revealed FAO defects that contributed to cardiomyopathy and LCFA storage as TAG, this present study provides a direct link between PPAR α expression and TAG turnover, where an increase in PPAR α expression resulted in an increase in TAG turnover dynamics. Thus, MHC-PPAR α hearts exhibit a more dynamic TAG pool than do NTG. Active recruitment of LCFAs from TAG indicates that the TAG pool is integral in maintaining elevated energy demands in the PPAR α overexpressing heart. Because lipid dysregulation is at the center of metabolic disorders such as diabetes, the ability to efficiently access the TAG pool to maintain energy demands and changes in the TAG pool dynamics may be a key limitation of metabolic syndromes.

Although transgenic mouse models are powerful tools, caution should be taken not to overinterpret the findings for human disease states. However, rodent models can mimic the physiology of human cardiomyopathies,³⁵ and the mechanisms elucidated in this study contribute to a new understanding of the dynamic balance of lipid utilization/storage in the heart.

In summary, this study reveals a mechanism for handling LCFAs in cardiomyocytes within intact beating hearts, whereby the TAG pool is an integral, dynamic source of LCFAs for oxidation. The kinetic data indicate continuous

mixing and rapid cycling of extracellular and TAG-derived LCFAs in the cytosol, by which the TAG pool is a preferred source of LCFA oxidation. Increased TAG turnover and increased transcription of genes encoding enzymes for both TAG synthesis and degradation, induced by chronic PPAR α activation, augmented the contribution of lipolysis to LCFA oxidation in MHC-PPAR α transgenic mouse hearts. In addition to elucidating the dynamic nature of myocardial TAG stores relative to LCFA oxidation rates, the present findings demonstrate the action of PPAR α on the relative contribution of lipid stores to oxidative energy metabolism.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- The heart relies on long chain fatty acids (LCFAs) as the primary fuel for energy metabolism, oxidizing both circulating lipid and free fatty acids, as well as stored fats from the endogenous TAG pool.
- Altered lipid dynamics in the heart are linked to the development of cardiomyopathy.
- The lipid-activated nuclear receptor peroxisome proliferator-activated receptor (PPAR) α induces expression of enzymes for fatty acid metabolism.

What New Information Does This Article Contribute?

- The application of ^{13}C NMR for serial measures of TAG turnover in intact mouse hearts demonstrates that the TAG pool is more dynamic than previously thought.
- Rates of TAG turnover and LCFA oxidation indicate that LCFAs from lipolysis of endogenous TAG is preferentially oxidized over exogenous LCFAs for energy metabolism.
- PPAR α activation induces the expression of enzymes involved in the turnover of the TAG pool, augmenting the contribution of TAG to β -oxidation.

Human and animal studies link altered lipid storage to cardiomyopathy. The present study used ^{13}C NMR for comparing the storage kinetics and oxidation rates of ^{13}C -enriched palmitate from sequential measurements in individual mouse hearts. We found that the turnover of palmitoyl units within TAG was almost 4-fold faster than palmitate oxidation. This rate difference indicates preferential oxidation of LCFAs from the lipolysis of TAG versus oxidation of exogenous LCFAs. Hearts of transgenic mice overexpressing the nuclear receptor PPAR α showed palmitoyl turnover in TAG to be 13-fold faster than oxidation, indicating near requisite oxidation of LCFAs from TAG. Increased TAG turnover in PPAR α transgenic hearts correlated to elevated transcription levels for enzymes involved in TAG synthesis and lipolysis. Although PPAR α is known to induce expression of enzymes for LCFA metabolism, this is the first demonstration that PPAR α drives the dynamics of TAG synthesis/lipolysis. The findings elucidate the role of intramyocardial TAG in providing the majority of LCFAs to fuel β -oxidation and the influence of PPAR α on this process. Thus, altered cardiac lipid storage dynamics determine the contribution of stored lipids to oxidative energy metabolism, which may be a factor in linking imbalances in lipid metabolism to cardiomyopathy.