Dietary Fat Supply to Failing Hearts Determines Dynamic Lipid Signaling for Nuclear Receptor Activation and Oxidation of Stored Triglyceride

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Background—Intramyocardial triglyceride (TG) turnover is reduced in pressure-overloaded, failing hearts, limiting the availability of this rich source of long-chain fatty acids for mitochondrial β-oxidation and nuclear receptor activation. This study explored 2 major dietary fats, palmitate and oleate, in supporting endogenous TG dynamics and peroxisome proliferator–activated receptor-α activation in sham-operated (SHAM) and hypertrophied (transverse aortic constriction [TAC]) rat hearts.

Methods and Results—Isolated SHAM and TAC hearts were provided media containing carbohydrate with either 13C-palmitate or 13C-oleate for dynamic 13C nuclear magnetic resonance spectroscopy and end point liquid chromatography/mass spectrometry of TG dynamics. With palmitate, TAC hearts contained 48% less TG versus SHAM (P=0.0003), whereas oleate maintained elevated TG in TAC, similar to SHAM. TG turnover in TAC was greatly reduced with palmitate (TAC, 46.7±12.2 nmol/g dry weight per min; SHAM, 84.3±4.9; P=0.0212), as was β-oxidation of TG. Oleate elevated TG turnover in both TAC (140.4±11.2) and SHAM (143.9±15.6), restoring TG oxidation in TAC. Peroxisome proliferator–activated receptor-α target gene transcripts were reduced by 70% in TAC with palmitate, whereas oleate induced normal transcript levels. Additionally, mRNA levels for peroxisome proliferator–activated receptor-γ-coactivator-1α and peroxisome proliferator–activated receptor-γ-coactivator-1β in TAC hearts were maintained by oleate. With these metabolic effects, oleate also supported a 25% improvement in contractility over palmitate with TAC (P=0.0202).

Conclusions—The findings link reduced intracellular lipid storage dynamics to impaired peroxisome proliferator–activated receptor-α signaling and contractility in diseased hearts, consistent with a rate-dependent lipolytic activation of peroxisome proliferator–activated receptor-α. In decompensated hearts, oleate may serve as a beneficial energy substrate versus palmitate by upregulating TG dynamics and nuclear receptor signaling. (Circulation. 2014;130:1790-1799.)

Key Words: fatty acids • genes • hypotrophy • lipids • metabolism

Long-chain fatty acids (LCFAs) are the preferred and most carbon-efficient substrates for oxidative ATP production in the heart in the absence of impaired oxygen delivery.1–4 The pressure-overloaded failing heart has been well characterized by reduced LCFA oxidation and elevated, albeit impaired and NADH-inefficient, reliance on carbohydrate metabolism.4,6 The intracellular lipid, stored as triglyceride (TG), has recently been identified as a significant source of fuel for mitochondrial oxidative metabolism in the heart7 and has also been implicated as a dynamic source of lipolytic signaling for transcriptional activation of metabolic gene expression.8 In the failing hearts of nonobese human patients, TG content is reduced,9 and, in animal models of pressure overload–induced heart failure, the reduced TG becomes static, no longer supporting oxidative metabolism.4 This study compared TG dynamics and oxidation in failing rat hearts, along with the activation of metabolic gene expression, as supported by the 2 most common dietary fats, palmitate and oleate.

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Among dietary fats that fuel the heart, palmitate (16:0) and oleate (18:1) together comprise ≈60% of the circulating LCFAs in serum from healthy human subjects.10 Following uptake into the cardiomyocyte, LCFAs may be oxidized by mitochondria or incorporated into the endogenous lipid stores as TG.11–14 Interestingly, studies in cell culture suggest that palmitate and oleate may be channeled to distinct metabolic fates.15 Given the reduced lipid content and turnover reported in failing hearts from both human patients9 and animal models,4 these major dietary fats hold potential to affect TG pool size and dynamics in diseased myocardium.

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Commensurate with shifting substrate use, hypertrophied hearts demonstrate decreased signaling through nuclear receptor peroxisome proliferator–activated receptor-α (PPAR-α), a transcription factor for target genes that drive TG turnover and fatty acid use in the cardiomyocyte. Given recent evidence for TG lipolysis to play a major role in supplying ligand for nuclear receptor activation, whether reduced TG turnover in the hypertrophic heart is a cause or a consequence of impaired PPAR-α signaling becomes an important component in determining the etiology of the low-energy state that contributes to progressive decoupling of the pressure-overloaded heart toward overt failure. Therefore, we hypothesized that the turnover of TG within the cardiomyocyte serves as a fundamental process for lipolytic signaling to the nucleus and that, in the normal and failing heart, such lipid dynamics depend on the LCFA chain composition. Changes in LCFA-mediated lipid dynamics may then hold consequences for substrate use, nuclear receptor activation, and cardiac function. To test our hypothesis, we compared the 2 most abundant dietary fats, palmitate and oleate, as sources for lipid storage and oxidation in normal and decompensated rat hearts. Our findings implicate TG turnover as a mediator of deficient PPAR-α activation in failing hearts, and show that, in comparison with palmitate, oleate confers normal nuclear signaling, lipid dynamics, and contractility in decompensated hearts. This study also presents the first evidence for rate-dependent activation of transcriptional regulation and cardiac energy metabolism in diseased hearts to be mediated by common dietary fats.

**Methods**

**Chronic Pressure Overload Cardiac Hypertrophy**

Pressure overload cardiac hypertrophy was produced by transverse aortic constriction (TAC) in 3-week-old male Sprague Dawley rats as previously described. In age-matched male sham-operated rats (SHAM), the aorta was isolated but not constricted. At 12 weeks postsurgery, hearts enter into decompensated, early-stage failure with concentric left ventricular hypertrophy, increased heart weight-to-tibia length ratio, and impaired left ventricular developed pressure and rate-pressure product consistent with previous studies. Rats enter acute end-stage heart failure 4 to 6 months postsurgery. The protocol was approved by the Animal Care Policies and Procedures Committee at the University of Illinois at Chicago (Institutional Animal Care and Use Committee accredited). Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Ex Vivo Isolated Heart Perfusion and Dynamic-Mode 13C Nuclear Magnetic Resonance Spectroscopy**

Animals were heparinized (1000 IU) and anesthetized (100 mg/kg pentobarbital) for cardiectomy. Isolated hearts were Langendorff perfused with modified Krebs-Henseleit buffer (in mmol/L: 116 NaCl, 4 KCl, 1.5 CaCl₂, 1.2 MgSO₄, and 1.2 NaH₂PO₄) equilibrated with 95% O₂/5% CO₂ and containing 0.4 mmol/L palmitate or 0.4 mmol/L oleate complexed to bovine serum albumin (3:1), 5 mmol/L glucose, and 1 mmol/L sodium lactate. Buffer was not recirculated. 13C Spectroscopy

**Quantification of TG Dynamics**

With constant TG content confirmed over the course of the protocol (Figure I in the online-only Data Supplement), rates of incorporation to reflect overall turnover (Figure I in the online-only Data Supplement). The resonance signal at 30.5 parts per million (ppm) from 13C-enriched methylene (-CH₂-) groups of TG enabled the detection of LCFA incorporation rates into TG. Lipids were extracted from frozen ventricle as described. The progressive isotopic enrichment of the 4-, then 2- and 3-carbon series, Bruker Inc, Billerica, MA). Sequential, proton-decoupled 13C NMR spectra were acquired in 2-minute time blocks (45° excitation pulse, 2-second interpulse interval). Before introduction of isotopically enriched media, a 13C NMR spectrum of background signal from naturally abundant 13C (1.1%) was collected. The perfusion buffer was then switched to medium containing either 0.4 mmol/L [4,6,8,10,12,14,16-13C] palmitate or [4,6,8,10,12,14,16,18-13C] oleate, 5 mmol/L unlabeled glucose, and 1 mmol/L unlabeled sodium lactate. Dynamic-mode 13C NMR spectra detected the progressive enrichment of metabolites of the 13C-enriched substrate to provide rates and extent of the 13C-enriched LCFA esterification into TG and oxidation via 13C-incorporation rates of glutamate isotopomers for 40 minutes (20 spectra total). At the end of each isotope enrichment experiment, hearts were freeze-clamped in liquid N₂-cooled toluene. The protocol was repeated with 5 mmol/L [1,6,δ-13C]glucose and 1 mmol/L sodium [3-13C]lactate and 0.4 mmol/L unlabeled fatty acid to assess carbohydrate oxidation. Cardiac function was monitored with a saline-filled, intraventricular balloon via Powerlab (ADInstruments, Dunedin, New Zealand) with no significant differences in function over protocol. Experimental groups perfused with 13C-enriched LCFA and unenriched glucose and sodium lactate were as follows: sham-operated hearts (SHAM) supplied oleate (n=6) or palmitate (n=6); hypertrophied hearts (TAC) supplied oleate (n=6) or palmitate (n=6). Experimental groups perfused with unenriched LCFA and 13C-enriched glucose and sodium lactate were as follows: sham-operated hearts (SHAM) supplied oleate (n=3) or palmitate (n=3); hypertrophied hearts (TAC) supplied oleate (n=3) or palmitate (n=3). Additional groups of isolated hearts were perfused with an unenriched substrate media containing a 1:1 mixture of albumin-bound palmitate and oleate (similar total LCFA concentration of 0.4 mmol/L, 5 mmol/L glucose, and 1 mmol/L lactate as follows: shamoperated hearts (SHAM) supplied mix (n=4); hypertrophied hearts (TAC) supplied mix (n=5).

**Diacylglycerol and Ceramide Measurements**

Diacylglycerol was separated from TG and monoacylglycerol and previously described content was determined calorimetrically by using a commercially available free glycerol kit (Abcam). Total ceramides were isolated and quantified by precursor-product scans followed by liquid chromatography-electron spray ionization-tandem mass spectrometry with multiple reaction monitoring.

**Tricarboxylic Acid Cycle Flux and Substrate Oxidation**

The progressive isotopic enrichment of the 4-, then 2- and 3-carbon positions of glutamate (chemical shifts 56, 34.6, and 28.3 ppm, respectively) reflect the oxidation of 13C LCFA by mitochondria. For kinetic analysis to determine tricarboxylic acid cycle flux, α-ketoglutarate, aspartate, citrate, and glutamate content were assayed from frozen perfused hearts fluorometrically and spectrophotometrically. Metabolic flux was determined by using...
tricarboxylic acid cycle metabolite content (Table I in the online-only Data Supplement) and kinetic modeling (Methods in the online-only Data Supplement) applied to progressive enrichment of 2- and 4-carbon positions of glutamate acquired by using dynamic-mode $^{13}$C NMR during perfusion.3,4,12,22,23,27,28

The relative contributions of $^{13}$C exogenous, enriched substrate and endogenous, unenriched substrate to oxidative metabolism was determined with in vitro $^{13}$C NMR as detailed elsewhere.4,6,29,30 Liquid chromatography/mass spectrometry provided fractions of endogenous, unenriched and $^{13}$C-enriched TG for quantification of enrichment rates and contributions to mitochondrial ATP production.14

**Western Blot and Quantitative Reverse Transcription Polymerase Chain Reaction**

Protein content was determined by Western blot and mRNA levels detected by reverse transcription quantitative real-time polymerase chain reaction of frozen perfused heart tissue13,14,15 (Methods and Table II in the online-only Data Supplement).

**Statistical Analysis**

Results are presented as mean±standard error of the mean. Comparisons of 2 means were performed by using the Student unpaired t test, and among >2 means by using analysis of variance and Tukey post hoc test (Prism 4, Graphpad Software Inc). Significant differences between means were determined at 5% probability ($P<0.05$).

**Results**

**Contractility in Decompensated Hearts Is Influenced by LCFA Source**

In comparison with age-matched control rats given a sham surgery (SHAM), rats given TAC showed 30% increase in heart weight (Figure 1A), 39% increase in heart weight-to-body weight ratio (Figure 1B), and 35% increase in heart weight-to-tibia length ratio (Figure 1C; $P<0.0001$). The degree of hyper trophy was similar in TAC hearts perfused with either oleate or palmitate. Postsurgical animals were enrolled randomly for isolated heart perfusions supplied either palmitate or oleate.

Consistent with previous reports,1,4,6 rate-pressure product, an index of cardiac work output, and left ventricular developed pressure were impaired in TAC hearts by 21% ($P=0.0006$) and 26% ($P<0.0001$), respectively, in comparison with SHAM hearts (Figure 1D and 1E). Left ventricular contractility and relaxation, as assessed by +dP/dt and –dP/dt, respectively, were impaired in TAC hearts metabolizing palmitate in comparison with SHAM (Figure 1F and 1G; +dP/dt TAC versus SHAM palmitate, $P=0.0091$; –dP/dt TAC versus SHAM palmitate, $P=0.0028$). In contrast, oleate supported normal contractility in TAC hearts, as determined from both +dP/dt and –dP/dt, indicating that LCFA source impacts left ventricular

![Figure 1](http://circ.ahajournals.org/)

Figure 1. Contractile function of sham-operated (SHAM) and hypertrophic (TAC) isolated perfused hearts. TAC induced an increase in heart weight (A), heart weight-to-body weight ratio (B), and heart weight-to-tibia length ratio (C) 12 weeks after surgery. Left ventricular (LV) function (D through G). Rate-pressure product (RPP; D), an index of cardiac work output, and left ventricular developed pressure (LVDP; E) were similarly reduced in comparison with SHAM in TAC hearts metabolizing oleate or palmitate. Oleate maintained LV contractility and relaxation (+dP/dt and –dP/dt, respectively) in TAC hearts, whereas TAC hearts metabolizing palmitate demonstrated impaired contractility and relaxation vs both SHAM and TAC oleate groups (F and G). White bar indicates SHAM; black bar, TAC (n=13 for all groups). Error bars indicate mean±SEM. *$P<0.05$ vs SHAM, †$P<0.05$ vs TAC oleate. LV indicates left ventricular; SEM, standard error of the mean; and TAC, transverse aortic constriction.
contraction and relaxation rates, despite having no effect on rate-pressure product or left ventricular developed pressure.

**LCFA-Dependent Regulation of TG Content and Turnover**

TG turnover rates and LCFA oxidation rates were detected from sequential $^{13}$C NMR spectra (Figure 2). Because of the potential for confounding kinetic contributions from different substrate affinities for synthase and lipase enzymes, this formative study focused on the individual TG incorporation kinetics of oleate and palmitate in isolation. LCFA incorporation into the TG pool displayed 2 distinct kinetic components: an initial saturable exponential component dependent on carrier mediated LCFA transport across the sarcolemma and a linear component from the rate of TG turnover (Figure 2). Palmitate incorporation into TG stores was much reduced in the hypertrophic heart versus SHAM. In contrast, oleate restored TG enrichment in TAC hearts.

In SHAM hearts, oleate supported a rate of TG turnover that was 71% faster than palmitate (Figure 3A; $P=0.0045$). Consistent with data previously published by O’Donnell et al., palmitate failed to maintain normal lipid storage dynamics in the hypertrophic heart. TG turnover rates in TAC hearts perfused with palmitate were reduced by 47% in comparison with SHAM ($P=0.0212$). However, we found that oleate preserved normal TG turnover rates in the hypertrophic heart. The steady-state condition enables these TG turnover rates to also reflect elevated TG lipolysis in hearts metabolizing oleate. These data suggest that oleate rescues lipid dynamics in the failing heart that are otherwise reduced in the presence of palmitate.

Oleate supported normal TG content in decompensated hearts (Figure 3B). Elevated TG turnover induced by oleate also demonstrates that oleate is more readily incorporated into TG than palmitate, as was evidenced by increased $^{13}$C fractional enrichment of TG with oleate (Figure 3C; SHAM oleate versus palmitate, $P=0.0165$; TAC oleate versus palmitate, $P=0.0039$). TAC hearts perfused with palmitate demonstrated depleted levels of myocardial diacylglycerol in comparison with SHAM (Figure 3D; $P=0.04$), a substrate for diglyceride acyltransferase 1 (DGAT1) for TG formation, and increased content of potentially lipotoxic C16 ceramide in comparison with SHAM (Figure 3E; $P=0.0229$). A potential cardioprotective consequence of oleate is the competitive displacement of TG-derived LCFA away from lipotoxic ceramide species owing to the rapid turnover within the TG pool formation and increased oxidation of TG-derived LCFA, both supported by oleate and consistent with elevated diacylglycerol.

Consistent with the elevated TG turnover rates that were supported by oleate, in comparison with palmitate, the composition of esterified LCFA within the TG pool was also affected (Figure II in the online-only Data Supplement). Hearts displaying more rapid TG turnover in response to oleate held lower esterified stearate and linoleate, as a consequence of the more rapid replacement by oleate.

Despite the evidence for the differential effects on transcriptional activation (see below), the differences in TG content and turnover among experimental groups occurred despite the absence of a detectable difference in protein levels of the enzymes DGAT1 or adipose triglyceride lipase (ATGL; Figure 3F through 3H), suggesting that the rate-limiting TG synthase and lipase in myocardium have a higher affinity for shuttling oleate through TG stores than palmitate.

Time constants of the exponential phase were not significantly different between the experimental groups (Figure 3I), consistent with similar protein content of sarcolemmal fatty acid transporter CD36 (Figure 3J). Although the rates of LCFA uptake were not measured directly, these data indicate that the physical characteristics of the fatty acid transport process were not different for either LCFA and, moreover, were unaffected by the presence of decompensated hypertrophy. The findings are consistent with previous in vitro studies demonstrating that CD36 has similar affinities for palmitate and oleate across various physiological concentrations.$^{12}$

![Figure 2](https://example.com/figure2.png)  
**Figure 2.** Incorporation rates of $^{13}$C-oleate and $^{13}$C-palmitate into triglyceride (TG). A, Representative, selected $^{13}$C NMR spectra (from bottom to top, 2 minutes acquisition each) from a sham-operated (SHAM) heart perfused with $^{13}$C-oleate. Signal at chemical shift 30.5 parts per million (ppm) reflects the $^{13}$C-enriched methylene (-CH$_2$-) groups as the $^{13}$C-enriched long-chain fatty acid (LCFA) is esterified into TG. Signal at chemical shifts 56, 34.6, and 28.3 ppm reflects $^{13}$C enrichment of glutamate at the 2-, 4-, and 3-carbon positions. B, TG enrichment profiles reflect incorporation of $^{13}$C-enriched LCFA throughout perfusion. White circle indicates SHAM oleate; black circle, TAC oleate; white square, SHAM palmitate; black square, TAC palmitate (n=6 for all groups). Error bars indicate means±SEM. NMR indicates nuclear magnetic resonance; SEM, standard error of the mean; and TAC, transverse aortic constriction.
Improved TG Dynamics Restores Expression of PPAR-α Target Genes in Decompensated Hearts

Transcript levels of PPAR-α target genes provided insights into the link between TG turnover and PPAR-α signaling: carnitine palmitoyltransferase 1b (Cpt1b), pyruvate dehydrogenase kinase 4 (Pdhk4), and medium-chain acyl-CoA dehydrogenase (Acadm). Consistent with previous reports, PPAR-α protein was reduced by TAC (Figure 4A and 4B). Transcripts of all 3 target genes were significantly reduced in TAC hearts perfused with palmitate in comparison with SHAM (Cpt1b: –61% [P=0.0096], Pdhk4: –83% [P<0.0001], Acadm: –63% [P=0.0001]). In contrast, improved TG dynamics in TAC hearts conferred by oleate were associated with near-normal levels of PPAR-α target gene mRNA (Figure 4C).

Additional reductions in mRNA for DGAT1, ATGL, PPARγ-coactivator-1α (PGC-1α) and PGC-1β occurred in TAC hearts supplied palmitate (Figure 4C). The data indicate that dramatically reduced rates of lipolysis in TAC hearts supplied palmitate were not sufficient to maintain the transcription of genes involved in LCFA metabolism. In contrast, oleate restored normal PPAR-α signaling via enhanced rates of ATGL-mediated TG lipolysis. Additional hearts perfused with a physiological 1:1 mixture of oleate and palmitate demonstrated intermediate transcript levels of PPAR-α targets, that were generally midrange of those observed in TAC hearts supplied either of the individual fatty acids (Figure 5A). Similarly, the response to the palmitate:oleate mixture resulted in a mean TG content that was also midrange of values observed in the TAC palmitate and TAC oleate groups (Figure 5B).
Importantly, despite evidence for activation of PPAR-α during this acute protocol in the intact, perfused heart, these changes occurred in response to the exogenous LCFA source rather than a change in DGAT1 or ATGL protein levels. The significance of this distinction is that TG turnover was then determined by the different affinities of the synthase and lipase enzymes for LCFA substrate in the diseased hearts, rather than the intracellular content of these enzyme proteins. Thus, the chemical composition of the dietary, exogenous LCFA that fuels cardiac energy metabolism regulates activation of nuclear transcription through the apparent affinities of the enzyme–substrate interactions that determine the rates of TG lipolysis and synthesis. These data demonstrate for the first time the dynamic activation of PPAR-α target gene transcription, including PGC-1α and PGC-1β, through LCFA-dependent processes and the transcriptional consequences of LCFA affinity for TG turnover in the diseased heart.

**Lipolytic Rate Determines Mitochondrial Oxidation of TG**

Oleate induced greater use of endogenous TG for mitochondrial β-oxidation and ATP production in normal hearts and attenuated the decline in lipolytic support of mitochondrial oxidation in TAC hearts metabolizing palmitate. Tricarboxylic acid cycle flux rate was not different between SHAM groups and was similarly reduced with either palmitate or oleate in TAC (Figure 6A),...
correlating with work output as determined by rate-pressure product (Figure 1D) without influence from a LCFA source.

Substrate use in both TAC groups reflected an increased contribution of carbohydrate to oxidative ATP production and a drop in fatty acid oxidation versus SHAM hearts (Figure 6B). This change coincided with increased expression of the liver isoform of carnitine palmitoyl transferase 1 (CPT1a), and unchanged expression of the muscle isoform (CPT1b; Figure 6C through 6E). Although the fractional contribution of carbohydrate and fat (either exogenous or endogenous) to oxidative ATP production was not influenced by exogenous LCFA, the increased TG turnover driven by oleate increased TG oxidation in both SHAM and TAC groups. Notably, lipolytic support of β-oxidation in TAC hearts metabolizing palmitate was nearly undetectable, consistent with the dramatically reduced rates of TG turnover supported by palmitate. In contrast, increased lipolysis of TG driven by oleate in TAC hearts resulted in significantly more TG oxidation than TAC palmitate (P<0.001). These data imply that TG turnover directly determines the extent to which LCFA stored in TG contribute to mitochondrial β-oxidation.

**Discussion**

This study is the first to demonstrate in the intact heart that the intracellular fate of exogenous LCFA, including storage dynamics, mitochondrial β-oxidation, and nuclear receptor activation, depends on chain composition. By comparing the 2 major dietary fats oleate and palmitate, we found that oleate is more readily esterified into intramyocardial TG than palmitate. In the normal heart, oleate drove elevated rates of TG turnover in comparison with palmitate. In the diseased heart, the previously observed drop in TG content and turnover with provision of palmitate was attenuated by oleate. Indeed, oleate supported greater contributions of TG to mitochondrial β-oxidation and aided cardiac contractility in decompensated hearts in comparison with palmitate.

Although it has long been appreciated that dietary LCFA can regulate gene transcription in cardiomyocytes by serving as ligands for activating the various PPARs, recent work by Haemmerle et al demonstrated that ATGL-mediated TG lipolysis was requisite for nuclear signaling via PPAR-α, suggesting that LCFA must first traverse the TG pool to activate transcription. The evidence presented here indicates that lipolytic support of nuclear signaling via PPAR-α and
mitochondrial biogenesis via PGC-1 complex is a rate-dependent process that can be rescued by inducing TG turnover with oleate as the source of esterification. In the decompensated, failing heart, the dramatically impaired rate of TG turnover supported by palmitate provided insufficient lipolytic flux through ATGL to support PPAR-α activation. This drop in activation occurred despite the absence of a detectable change in ATGL protein content. The importance of myocardial lipid dynamics supporting transcriptional regulation of fatty acid metabolism is underscored by recent studies in nonobese human patients with heart failure. Reduced TG content in tissue from failing human hearts was linked with decreased transcription of Cpt1b, Pdhk4, and Ppargc1a, consistent with impaired lipolytic signaling to the nucleus that we observed here and in previous studies with rodent models of chronic pressure overload hypertrophy.

Interestingly, TAC hearts supplied with a 1:1 mixture of palmitate and oleate demonstrated transcript levels that were higher than supplying palmitate alone, yet generally lower than supplying oleate alone. These intermediate mRNA levels suggest that the concentration of oleate may be a determining factor in attenuating the reduction in nuclear signaling. Alternatively, palmitate may have an inhibitory effect on the transcription of certain PPAR-α target genes in pressure-overload hypertrophy. These data indicate that dietary studies aimed at increasing oleate in the blood might hold promise for rescuing the otherwise impaired lipid dynamics and PPAR-α activation in decompensated hearts. However, examination of the kinetic features of the TG pool in the presence of a mixed supply of exogenous LCFA actually introduces additional complexities owing to the potential differences in the affinity of the intracellular lipases, namely ATGL, for each of the specific LCFA species. Therefore, further investigation into the affinities of TG synthase and lipase for individual LCFA species is necessary to fully elucidate the precise mechanisms of transcriptional regulation.

Importantly, although LCFA species influenced metabolic gene transcription, the kinetics of LCFA storage over the relatively short protocol were greatly affected by the presence of oleate versus palmitate before changes in protein levels were realized. Therefore, in addition to influencing message levels for PGC-1α and PGC-1β and PPAR-α target genes, these 2 LCFA species hold different affinities for the key rate-limiting TG synthase and lipase activities. Indeed, in TG extracted from both SHAM and TAC hearts, exogenous oleate constituted a significantly greater proportion of the acyl chains esterified to glycerol backbone than palmitate, indicating that oleate is more readily esterified than palmitate. Listenberger et al demonstrated differential fates of palmitate and oleate in cultured Chinese hamster ovary cells. We show here, for the first time, that such distinctions occur in the intact heart. In the absence of any difference in protein content of DGAT1, known to be the rate-limiting enzyme of TG formation, the data are consistent with a higher affinity of DGAT1 for esterifying oleate.

The rate-limiting step of LCFA oxidation is catalyzed by CPT1, which transports acylcarnitines across the outer mitochondrial membrane. In the normal adult heart, the muscle isoform CPT1b is predominately expressed with a minor contribution of liver isoform CPT1a. In the development of heart failure, the heart reverts to a fetal metabolic profile with reduced fatty acid oxidation and greater reliance on carbohydrate metabolism. Our laboratory was the first to report a shift in CPT1 isoform expression in the hypertrophic heart, which demonstrates a marked increase in CPT1a protein content, and to link the isoform shift with reduced rates of LCFA oxidation. In the current study, we observed increased expression of CPT1a in TAC hearts, which occurred alongside decreased fractional contribution of LCFA (both endogenous and exogenous) and increased contribution of carbohydrate to mitochondrial ATP production. Consistent with previous reports, TG oxidation in TAC hearts supplied palmitate was nearly undetectable, whereas TAC hearts supplied oleate demonstrated significantly greater TG oxidation. Although an isoform shift may determine the fractional contributions of fat and carbohydrate to oxidative ATP synthesis, accelerated TG turnover, as supported by oleate, enabled a supply of energy-rich endogenous lipid to the mitochondria.

The observed beneficial effect of oleate on contractility in TAC hearts may be partially attributed to reducing C16 ceramide (Figure 3E). Although total ceramide content was not affected and holds little specificity to impaired heart function, of much greater relevance is the increased content of the ceramide species most closely associated with lipotoxicity and apoptotic signaling, C16 derived from palmitate. As a
consequence of reduced TG turnover and LCFA oxidation in TAC hearts, palmitate is then increasingly available as a substrate for C16 ceramide production. Thus, the benefits of oleate on contractility shown here on the intact heart may stem from elevated dynamics of both LCFA storage and oxidation, both related to the long-term affects of lipolytic signaling through PPAR-α, plus the lack of substrate for C16 ceramide.

Additional data on total and individual ceramides are shown in Figure III in the online-only Data Supplement.

Our study provides evidence of a regulatory role for dietary fats on TG turnover to dynamically balance the reciprocal processes of energy production by mitochondrial β-oxidation, supplying substrate via lipolysis, and target gene expression via PPAR-α activation (Figure 7, and Figures I, II, and IV in the online-only Data Supplement). Although the relatively brief protocols did not accommodate immediate differences in protein content, additional implications for the responses of the endogenous TG content in TAC hearts include potential LCFA effects on the function of the perilipin proteins. Additionally, because it is known that micro-RNAs located on the PGC-1α gene act to regulate adiposity, data of oleate normalizing PGC-1α message levels in TAC hearts hold implications for mechanisms by which dietary fats affect the cardiac-adipose axis. Prolonged increase in PPAR-α signaling supported by oleate in TAC, beyond the time course of our protocol, may be sufficient to restore protein levels of target genes involved in fatty acid oxidation and attenuate the maladaptive shift in substrate use away from LCFA oxidation. These findings provide the impetus for future studies supplying diets artificially enriched with either palmitate or oleate following TAC procedure, to assess the long-term effects of dietary fats on cardiac function and metabolism. Thus, this study provides the necessary, direct comparison of each LCFA in isolation from the other.

In summary, chronic hypertension, as a consequence of poor management or lack of treatment, is one of the leading causes of heart failure. Although the benefits of diets rich in unsaturated fats and low in saturated fats are well-documented and the risk of developing congestive heart failure.

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Disclosures

None.

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Pathogenic cardiac hypertrophy has long been associated with a low myocardial energy state, and, more recently, defects in metabolic pathways providing ATP. Recently, nondiabetic patients with heart failure were found to have reduced intramyocellular lipid in the form of triglyceride. That clinical finding recapitulated previous findings in animal models and established a link between reduced myocardial triglyceride and elevated lipotoxic intermediates. This current study, on a rat model of chronic pressure overload, enabled stable isotope measurements of the dynamics of triglyceride turnover and contribution to oxidative energy metabolism in the beating heart. Experiments elucidated differences in triglyceride content, turnover, and oxidation between hearts supplied with either of 2 major circulating and dietary long-chain fats, palmitate and oleate. Rat heart expresses two forms of mitochondrial carnitine palmitoyltransferase I. The minor component is identical to the liver enzyme. J Biol Chem. 1994;269:18712–18715.


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SUPPLEMENTAL MATERIAL

DETAILED METHODS

Pressure-overload hypertrophy animal model. Cardiac hypertrophy by chronic pressure-overload was induced by constricting the transverse aorta (hemoclip) of three-week-old male Sprague Dawley rats, as previously described (1-4). This banding procedure relies on the natural growth of the animal to produce a gradually increasing degree of aortic constriction. The rats develop a concentric hypertrophy and increased heart weight, heart weight-to-body weight ratio, and heart weight-to-tibia length ratio (see Figure 1A-C) which is associated, in the short term, with improvement in the systolic function of the heart (2,5-7). At 12 weeks post-banding, the animals enter a decompensated stage with depressed left ventricular developed pressure (LVDP) and dP/dt. Mean body weights of rats allocated to each experimental group 12 weeks post-surgery, measured immediately prior to heart excision, are as follows: SHAM oleate: 427.3 ± 9.7 g; SHAM palmitate: 430.7 ± 5.7 g; TAC oleate: 400.8 ± 6.8 g; TAC palmitate: 400.2 ± 9.6 g (n=10-15 for each group). Mean body weights were significantly lower in TAC groups compared to SHAM for both oleate (P=0.03) and palmitate (P=0.01), consistent with previously published reports on this animal model (3). In this model of left ventricular hypertrophy, no systemic activation of the sympathetic nervous system or of the renin-angiotensin-aldosterone system occurs (7). Consequently, there are no signs of cardiac lesions, peripheral arteritis, myocardial necrosis, or extensive fibrosis. The rats progress to a dilated cardiac hypertrophy with acute end-stage heart failure at 4-6 months post-banding. The sham groups underwent similar surgery without placement of the aortic-band. Rats had free access to food and water while being housed under controlled temperature and lighting. All experimental procedures were approved by the University of Illinois at Chicago Animal Care and Use Committee.

Isolated heart perfusion protocol. 12 weeks post-surgery, animals were heparinized (1000 IU, intraperitoneal injection) and anesthetized (100 mg/kg pentobarbital, intraperitoneal injection). Hearts were excised and retrogradely perfused with modified Krebs-Henseleit buffer (in mmol/L: 116 NaCl, 4 KCl, 1.5 CaCl₂, 1.2 MgSO₄ and 1.2 NaH₂PO₄) equilibrated with 95% O₂/5% CO₂ and containing 0.4 mmol/L ¹³C palmitate or 0.4 mmol/L ¹³C oleate complexed to bovine serum albumin in a 3:1 molar ratio, 5 mmol/L ¹³C glucose and 1 mmol/L ¹³C sodium lactate. Temperature was maintained at 37°C. A water filled latex balloon, connected to a force transducer, was fitted into the left ventricle (LV) and set to a diastolic pressure of 5 mmHg. LV developed pressure (LVDP) data was continuously acquired during perfusion with Powerlab (ADInstruments, Dunedin, New Zealand). Rate-pressure product (RPP) was calculated as heart rate (HR)×LVDP, and mean peak + and -dP/dt were calculated from the first derivative of the LVDP trace. Functional data are reported at midpoint of perfusion and were determined to be not significantly different over the entire protocol using repeated measures ANOVA.

Hearts were situated in a 20 mm broadband NMR probe within a 9.4-T, vertical bore (89 mm) NMR magnet interfaced to a spectroscopy console (Avance series, Bruker Inc., Billerica, MA). Magnetic field homogeneity was optimized by shimming to a proton line width of 20-30 Hz. The bioenergetic status of perfused hearts was determined by the relative content of phosphocreatine to ATP (PCr:ATP, Online Figure 4) from ³¹P NMR as per methods extensively described elsewhere (1-3). Prior to introduction of isotope-enriched media, a ¹³C NMR spectrum of background signal from naturally abundant ¹³C (1.1%) was collected. Isotopic enrichment was initiated by switching the perfusate supply to buffer containing either 0.4 mmol/L [4,6,8,10,12,14,16-¹³C₇] palmitate or 0.4 mmol/L [4,6,8,10,12,14,16,18-¹³C₈] oleate, 5 mmol/L unlabeled glucose, and 1 mmol/L unlabeled sodium lactate. Enrichment continued for 40 minutes while sequential proton decoupled ¹³C NMR spectra (2 second interpulse interval, averaged over 2 minutes, 20 spectra total) were acquired to detect the progressive isotopic
enrichment of myocardial triglyceride (TG) and glutamate isotopomers (1,3,4,8). At endpoint of the enrichment protocol, hearts were removed from the magnet and oxygen consumption was determined from pulmonary artery effluent with a blood gas analyzer (GEM Premier 300, Instrumentation Laboratory) prior to freeze clamping hearts with liquid N₂-cooled tongs for subsequent in vitro biochemical analysis.

Additional hearts were perfused under similar conditions with unlabeled 0.4 mmol/L palmitate or oleate and 5 mmol/L [1,6-¹³C₂] glucose and 1 mmol/L sodium [3,¹³C] lactate to assess carbohydrate oxidation (3,9) or unlabeled 0.2 mmol/L palmitate, 0.2 mmol/L oleate, 5 mmol/L glucose and 1 mmol/L sodium lactate to assess gene expression in hearts supplied an equimolar mix of albumin bound LCFA.

**Western Blot and Quantitative RT-qPCR.** Diacylglycerol acyltransferase 1 (DGAT1, Abcam), adipose triglyceride lipase (ATGL, Cell Signalling), carnitine palmitoyltransferase 1b (CTP1b, Alpha Diagnostic), and peroxisome proliferator-activated receptor-α (PPAR-α, Abcam) expression were measured in whole-tissue lysates, with calsequestrin (CALSEQ, Thermal Scientific) as a loading control. CPT1a (primary antibody generously provided by Dr. Charles Hoppel, Case Western Reserve University, Cleveland, Ohio) and CD36 (Cascade Bioscience) were assayed from cardiac membranes isolated as previously described (1,8) with the α1 subunit of the Na⁺/K⁺ ATPase as a loading control. Western band intensity, normalized to loading control, was analyzed by NIH Image software.

Total RNA was extracted from frozen heart tissue by using an RNeasy Lipid Tissue kit (Qiagen), according to the manufacturer's instructions. RNA quantity was determined at 260 nm (NanoDrop 1000 Spectrometer, Thermal Scientific). Single-stranded cDNA was synthesized from the prepared RNA by using High Capacity cDNA Reverse Transcription kit (Applied Biosystems), and gene products were determined by quantitative real-time polymerase chain reaction, using Fast SYBR Green Master Mix (Applied Biosystems) with an ABI ViiA7 instrument. The cycle profile was: 1 cycle at 95°C for 20 sec, 40 cycles of 95°C for 1 sec, 60°C for 20 sec. The mRNA levels were determined by a comparative C_T method, normalized to ribosomal protein S29. Primer sequences are provided in Online Table 2.

**TG content and ¹³C fractional enrichment.** Lipid extracts were obtained from frozen perfused heart samples and quantified by calorimetric assay (Wako Pure Chemical Industries) as previously described (3,8,10). TG content was normalized to tissue dry weight (dw). TG was isolated and saponified for analysis by liquid chromatography/mass spectrometry (LC/MS, Waters X-Terra MS C18 3.5 µm 2.1×100 mm column; MS: scan m/z 100-600 Fragmentor 75 V Negative ESI) to determine the ¹³C fractional enrichment of the myocardial TG pool (3,8,10).

**Diacylglycerol (DAG) content.** DAG was separated from TG and monoacylglycerol from frozen perfused heart samples as previously described (8) and saponified to release free glycerol. Free glycerol concentration was determined with a commercially available kit (Abcam) and DAG content was normalized to tissue dw.

**Ceramide content.** Individual ceramides (C16, C18, C20, C22, C24, C24:1) were isolated from frozen perfused hearts and quantified by precursor-product scans followed by LC-ESI-MS/MS with multiple reaction monitoring as described previously (8,11,12) with some modifications. Extracted ceramides were dissolved in 300 ul of mobile phase A (60:40:0.2 isopropanol:acetonitrile:formic acid with 1 mM ammonium formate), as were all ceramide standards. Extracted samples (10 ul) and standards were injected into a 125/2 Nucleodur 100-3 C8sec column (Macherey-Nagel). Ceramides were separated via reverse phase chromatography starting from a gradient of 75% mobile phase A:25% mobile phase B (8:1:1:0.2 H₂O:isopropanol:acetonitrile:formic acid with 2 mM ammonium formate) held for 2 min, 2 to 15
min a linear gradient to 90% mobile phase A, 15 to 18 min a linear gradient to 100% mobile phase A, 18 to 20 a linear gradient to 75% mobile phase A, then held from 20 to 23 min. The flow rate was 0.3 ml/min.

Quantification of TG turnover. $^{13}$C enrichment of TG in intact hearts was detected from the NMR signal at 30.5 parts per million (ppm), corresponding to the methylene groups within TG (3,8). The integral of the peak at 30.5 ppm plotted over time yielded an enrichment curve with biphasic kinetics: an early exponential phase, previously shown to be dependent on LCFA uptake (8), and a linear phase whose slope reflects the rate of incorporation of exogenous $^{13}$C-enriched LCFA into TG. Exponential phase time constants and linear phase slopes were determined by regression analysis. Rate of LCFA incorporation into TG was calculated as: (linear phase slope)×(myocardial TG content)×($^{13}$C fractional enrichment of TG)(8). Because myocardial TG content remained constant during the enrichment protocol (Online Figure 1), influx of LCFA into TG was balanced by efflux of LCFA from TG, allowing the rate of incorporation to reflect the overall rate of TG turnover.

Tissue chemistry and in vitro NMR spectroscopy. Tissue metabolites were extracted from frozen heart tissue using 7% perchloric acid and neutralized with KOH. Tissue extracts were analyzed spectrophotometrically and flurometrically for quantification (13). Glutamate concentration was determined with glutamate dehydrogenase and diaphorase (Roche L-Glutamic acid colorimetric kit). α-Ketoglutarate content was measured by coupling glutamate-oxaloacetate transaminase (GOT, Roche) with malate dehydrogenase (MDH, Roche) in the presence of excess L-aspartate. Aspartate concentration was measured by coupling GOT with MDH similar to α-ketoglutarate with the exception of excess α-ketoglutarate. Citrate content was determined with citrate lyase (Roche) and MDH.

In vitro high-resolution $^{13}$C NMR spectra of tissue extracts reconstituted with 0.5 mL of D$_2$O were collected with a 5 mm $^{13}$C probe (Bruker Instruments, Billerica, MA). Analysis was performed to determine fractional enrichment of [2-$^{13}$C] acetyl CoA (14,15). The fractional contribution of $^{13}$C-enriched LCFA, $^{13}$C-enriched glucose, $^{13}$C-enriched lactate, and endogenous unenriched glycogen to mitochondrial acetyl-CoA formation was determined by analyzing the isotopomer distribution of glutamate, as was the fractional enrichment of glutamate, as previously described (3,9,14,16). Fractional contribution of endogenous, unenriched TG to mitochondrial ATP production was calculated as 100% minus the fractional contributions from carbohydrate and exogenous LCFA. Fractional contribution of TG was corrected to account for oxidation of $^{13}$C LCFA that had entered the TG pool and originated from lipolysis of TG prior to β-oxidation, using the fractional enrichment of TG determined by LC/MS.

$^{13}$C enrichment kinetics and tricarboxylic acid (TCA) cycle flux. A set of nine differential equations describing the concentration history of the $^{13}$C in each metabolite and developed in our laboratory was modified to include the additional, rate-determining components of long chain fatty acid uptake into the mitochondria. With a single 9x1 vector $q$ to represent the fractional enrichment of each compartment as a function of time, the model is described in matrix form as

$$\frac{d}{dt} q = M_{TCA} \cdot q + U_{Acetyl-CoA}$$

where $M_{TCA}$ is a 9x9 matrix characteristic of the TCA cycle, its elements are determined by the TCA cycle flux ($V_{TCA}$), the interconversion rates between the TCA cycle intermediate and glutamate or aspartate ($F_1$ and $F_2$), the level of anaplerosis ($y$), and the concentrations of each
metabolite. The input vector, $U_{\text{Acetyl-CoA}}$, is governed by the fraction of $^{13}$C enriched acetyl-CoA entering the TCA cycle through citrate synthase ($F_c$). The only non-zero element in $U_{\text{Acetyl-CoA}}$ corresponds to the labeling of the 4-carbon position of citrate since $[2,^{13}C]$ acetyl-CoA enters the TCA cycle through citrate synthase to enrich the 4-carbon position of citrate ($16$-$18$). The nine differential equation in series are:

\[
\begin{align*}
\frac{d}{dt} \text{CIT}_4 &= \frac{V_{TCA}}{[\text{CIT}]} \cdot (F_c - \text{CIT}_4) \\
\frac{d}{dt} \alpha\text{KG}_4 &= \frac{V_{TCA}}{[\alpha\text{KG}]} \cdot \text{CIT}_4 - \frac{V_{TCA} + F_1}{[\alpha\text{KG}]} \cdot \alpha\text{KG}_4 + \frac{F_1}{[\alpha\text{KG}]} \cdot \text{GLU}_4 \\
\frac{d}{dt} \text{GLU}_4 &= \frac{F_1}{[\text{GLU}]} \cdot (\alpha\text{KG}_4 - \text{GLU}_4) \\
\frac{d}{dt} \text{CIT}_2 &= \frac{V_{TCA}}{[\text{CIT}]} \cdot (\text{OAA}_2 - \text{CIT}_2) \\
\frac{d}{dt} \alpha\text{KG}_2 &= \frac{V_{TCA}}{[\alpha\text{KG}]} \cdot \text{CIT}_2 - \frac{V_{TCA} + F_1}{[\alpha\text{KG}]} \cdot \alpha\text{KG}_2 + \frac{F_1}{[\alpha\text{KG}]} \cdot \text{GLU}_2 \\
\frac{d}{dt} \text{GLU}_2 &= \frac{F_1}{[\text{GLU}]} \cdot (\alpha\text{KG}_2 - \text{GLU}_2) \\
\frac{d}{dt} \text{MAL}_2 &= \frac{V_{TCA}}{[\text{MAL}]} \cdot \left[ \frac{1}{2} \cdot \alpha\text{KG}_2 + \frac{1}{2} \cdot \alpha\text{KG}_4 - (1 + y) \cdot \text{MAL}_2 \right] \\
\frac{d}{dt} \text{OAA}_2 &= \frac{V_{TCA}}{[\text{OAA}]} \cdot \text{MAL}_2 - \frac{V_{TCA} + F_2}{[\text{OAA}]} \cdot \text{OAA}_2 + \frac{F_2}{[\text{OAA}]} \cdot \text{ASP}_2 \\
\frac{d}{dt} \text{ASP}_2 &= \frac{F_2}{[\text{ASP}]} \cdot (\text{OAA}_2 - \text{ASP}_2)
\end{align*}
\]

where CIT, $\alpha$KG, GLU, MAL, OAA, and ASP denote the metabolites citrate, $\alpha$-ketoglutarate, glutamate, malate, oxaloacetate, and aspartate, respectively, with the corresponding number of the $^{13}$C-enriched carbon position indicated. Where CIT4 is the fractional enrichment level of $^{13}$C at the 4-carbon position of citrate (i.e., CIT4=[(4-$^{13}$C)CIT]/[CIT]). The equation describing malate enrichment includes anaplerotic and cataplerotic effects ($4,13,16,17$). $F_1$ and $F_2$ are fluxes for interconversion via both transamination and membrane transport, between $\alpha$-ketoglutarate and glutamate, and between aspartate and oxaloacetate, respectively. Under the current experimental conditions of limited aspartate and alanine, $F_1=F_2$ ($15$-$18$).

A penalty function was applied, using MVO$_2$ as an external measured parameter, to constrain optimization of fitting data to the kinetic model within the known physiological limits ($14,16$):
\[ f(p) = \sum_{i=1}^{n} \left( \frac{d(t_i, p) - s(t_i)}{\sigma_i} \right)^2 + \left( \frac{V_{TCA} - V_{MVO2}}{\sigma_{MVO2}} \right)^2 \]

where \( t_i \) are the data-sampling times, \( d(t_i, p) \) are glutamate enrichment predicted by the model, \( s(t_i) \) are the NMR measurements of glutamate enrichment, and \( \sigma_i \) are the error associate with NMR measurements. \( V_{TCA} \) and \( V_{MVO2} \) are measured from oxygen consumption rate and \( \sigma_{MVO2} \) is the error associate with the measurement of oxygen consumption (15,17).
Supplemental Figures and Figure Legends

Online Figure 1. Steady-state triglyceride (TG) content was established prior to supplying hearts with $^{13}$C-enriched long-chain fatty acids (LCFAs) and did not change significantly over the course of perfusion. Unchanged TG content in each experimental group ($P>0.05$) demonstrates that incorporation of exogenous LCFA into TG was balanced by an equivalent efflux of LCFA from TG. Endpoint TG content of TAC hearts supplied palmitate was significantly lower than all other groups. White bars indicate TG content of perfused hearts prior to initiation of $^{13}$C enrichment protocol; black bars indicate TG content of hearts at the endpoint of the enrichment protocol. Values are reported as µmol TG / g dry weight; *$P<0.05$ versus endpoint SHAM ($n=7-9$ in each group; error bars indicate mean ± SEM).
Online Figure 2. Constituent long-chain fatty acids (LCFAs) in the intramyocardial triglyceride (TG) pool as determined by LC/MS. Consistent with the elevated TG turnover rates that were supported by oleate, hearts held lower esterified stearate and linoleate, as a consequence of the more rapid replacement by oleate. Values are reported as % of LCFAs in TG; *P<0.05 (n=6-8 in each group; error bars indicate mean ± SEM).
Online Figure 3. Ceramide content of perfused hearts. (A) Total ceramide content was not different between groups (n=5 in each group; error bars indicated mean ± SEM). (B) Content of individual ceramide species; *$P<0.05$ versus corresponding SHAM, †$P<0.05$ versus all other groups (n=5 in each group; error bars indicate mean ± SEM).
Online Figure 4. Phosphocreatine:ATP ratios of perfused hearts as determined by $^{31}$P NMR. Hypertrophied hearts (TAC) demonstrated impaired bioenergetic state consistent with previous reports on this model (1,3) that was unaffected by fatty acid supply. *$P<0.05$ versus corresponding SHAM ($n=6-8$ in each group; error bars indicate mean ± SEM).
### Online Table 1. Steady-state metabolite content in intact perfused hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Metabolite</th>
<th>Glutamate</th>
<th>α-Ketoglutarate</th>
<th>Aspartate</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>Oleate</td>
<td>19.32 ± 1.06</td>
<td>0.22 ± 0.02</td>
<td>3.48 ± 0.18</td>
<td>1.66 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Palmitate</td>
<td>16.22 ± 1.70</td>
<td>0.24 ± 0.02</td>
<td>2.81 ± 0.27</td>
<td>1.07 ± 0.19</td>
</tr>
<tr>
<td>TAC</td>
<td>Oleate</td>
<td>17.69 ± 0.90</td>
<td>0.22 ± 0.03</td>
<td>4.81 ± 0.40</td>
<td>1.57 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Palmitate</td>
<td>17.58 ± 0.78</td>
<td>0.21 ± 0.03</td>
<td>4.09 ± 0.64</td>
<td>1.33 ± 0.15</td>
</tr>
</tbody>
</table>

Values are reported as µmol / g dry weight (n=5 for each group; error bars indicate mean ± SEM).
**Online Table 2.** Primer sequences used in quantification of mRNA levels by quantitative real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene symbol</th>
<th>Primer sequences (5' to 3')</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase</td>
<td>Acadm</td>
<td>Sense: GTATTGACCGCGATCTAACC CG  Antisense: TAGAGGCAAAGTACGTGTCCCG</td>
<td>19</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase 1</td>
<td>Cpt1b</td>
<td>Sense: GCGGAAGCACACCAGGCAGTA  Antisense: ATGTTTGAAGCTATAGAGCA</td>
<td>20</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase kinase 4</td>
<td>Pdhk4</td>
<td>Sense: CCTTTGGCTGTTTGGTTGA  Antisense: CACCATCATCAGCCTCAGA</td>
<td>21</td>
</tr>
<tr>
<td>Adipose triglyceride lipase</td>
<td>Atgl</td>
<td>Sense: AGTTCAACCTTCGCAATCTC  Antisense: GTCACAAAATTTCTCTCAGA</td>
<td>22</td>
</tr>
<tr>
<td>Diglyceride acyltransferase 1</td>
<td>Dgat1</td>
<td>Sense: CACAGGATGCATCATCAGAG  Antisense: ACTCTTGTTCAGCTCAGACAG</td>
<td>*</td>
</tr>
<tr>
<td>Peroxisome proliferator activated receptor, gamma, coactivator 1 alpha</td>
<td>Ppargc1a</td>
<td>Sense: ACCCAGAGAAGACAGAAGACAG  Antisense: GGTACAGAGAGAAGATTTGTTG</td>
<td>*</td>
</tr>
<tr>
<td>Peroxisome proliferator activated receptor, gamma, coactivator 1 beta</td>
<td>Ppargc1b</td>
<td>Sense: AACCTTACGCTGAGCAGACAG  Antisense: GTATACACGGACCTTCACCC</td>
<td>*</td>
</tr>
<tr>
<td>Ribosomal protein S29</td>
<td>S29</td>
<td>Sense: TCTGATCCGTAATACGGGC  Antisense: CTGTGTGCGCAAAGACTAGC</td>
<td>23</td>
</tr>
</tbody>
</table>

* : Primer sequences were custom designed for this study by Integrated DNA Technologies (Coralville, IA).
SUPPLEMENTAL REFERENCES


