# Lipid Dynamics in a Mouse Model of Cardiac-Specific Overexpression of the Fatty Acid Transporter, FATP1

BY

ELLEN SHAMANSKY
B.A., Mount Holyoke College, 2012

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Science in Physiology and Biophysics
in the Graduate College of the
University of Illinois at Chicago, 2016

Chicago, Illinois

Defense Committee:

R. John Solaro, Chair
E. Douglas Lewandowski, Advisor
Jesus Garcia-Martinez, Physiology and Biophysics

Acknowledgments

I would like to thank the members of my thesis committee – Douglas Lewandowski, John Solaro, and Jesus Garcia-Martinez— for their support of this project. I also would like to thank the members of the Lewandowski laboratory for sharing their research expertise and friendship with me during my time in the program.

ES

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ATP | Adenosine triphosphate |
| CD36 | Cluster of differentiation 36 |
| CoA | Coenzyme A |
| FATP1 | Fatty acid transport protein 1 |
| FATP6 | Fatty acid transport protein 6 |
| Fc | Fractional contribution of acetyl CoA to the TCA cycle |
| PGC1 | PPAR gamma coactivator 1 |
| PPARα | Peroxisome proliferator-activated receptor alpha |
| TCA cycle | Tricarboxylic acid cycle |

## SUMMARY

The uptake and level of long-chain fatty acids in the myocardium of human subjects are not influenced by estrogen, although estrogen has been shown to correlate with higher rates of myocardial fatty acid oxidation in women (Herrero et al. 2005). This contributed to the hypothesis that fatty acid uptake and intracellular lipid handling occur differently in male and female mice with cardiac-specific overexpression of the fatty acid transport protein, FATP1. Rates of long-chain fatty acid uptake, esterification, and turnover from the triglyceride pool were examined in the hearts of male and female mice with cardiac-specific overexpression of FATP1 to identify potential points of sexual dimorphism in intramyocardial lipid handling. Dynamic $^{13}C$ NMR experiments and liquid chromatography/mass spectrometry were used to quantify the time constant for long-chain fatty acid uptake, the level of $^{13}C$ enrichment in the triglyceride pool, and triglyceride turnover. Results showed a trend towards faster uptake, greater triglyceride enrichment, and higher triglyceride turnover in FATP1 males compared to non-transgenic males. In females, there was a trend towards equal uptake and triglyceride enrichment, but FATP1 females displayed faster triglyceride turnover relative to non-transgenic females. Further investigation is needed to confirm these findings and determine if FATP1 mediates a sex-dependent mechanism that results in altered lipid metabolism.

# CHAPTER I: INTRODUCTION

## A. Importance of Lipid Metabolism in Heart Disease

Lipids are the primary fuel source for the heart, which generates approximately 70% of its ATP from the oxidation of fatty acids (Neely and Morgan 1974). Perturbations in lipid metabolism can lead to inefficient ATP synthesis, accumulation of toxic intermediates thought to impair contractility, and disruptions to lipolytic signaling (Carley et al. 2014).

The production of ATP is especially important in the heart, where the cellular processes that enable contractile function, such as crossbridge cycling at the sarcomeres and sequestration of calcium into the sarcoplasmic reticulum, are ATP-dependent. The cell also relies on ATP to maintain active transport and enzymatic reactions. The ATP pool in the heart turns over rapidly, and the heart will use 80-90% of its ATP at high workloads (Mootha et al. 1997). Because there is very little ATP reserve, it is essential that the heart be able to produce ATP efficiently and continuously. In addition, the diffusion of ATP through the cell is limited (Carley et al. 2014). The creatine phosphate system plays a role in facilitating the rapid transfer of phosphoryl groups from ATP to phosphocreatine, which diffuses more readily through the cytoplasm and donates phosphoryl groups to re-form ATP at peripheral sites in the cell (Carley et al. 2014). During the progression to heart failure, the phosphocreatine pool is depleted, and the heart must rely on anaerobic energy pathways or aerobic mechanisms to produce ATP (Carley et al. 2014).

The oxidation of fatty acids is the most efficient way to supply the cell with ATP per mole of substrate under conditions of abundant oxygen (Kessler and Friedman 1998).

The heart also uses circulating glucose, lactate, and ketones when available, including endogenous glucose from glycogen and fatty acids from the stored triglyceride pool. Fatty acid oxidation predominates in the healthy adult heart, but substrate selection switches to favor glucose use in hearts showing hypertrophy (Lehman and Kelly 2002). Furthermore, the changes in lipid metabolism that characterize cardiac hypertrophy occur differently depending on the source of long-chain fatty acids. In the failing heart, the percentage of ATP generated from glucose increases, while the percentage of ATP synthesized from the oxidation of long-chain fatty acids decreases for those released from the endogenous triglyceride pool, but not the long-chain fatty acids that are exogenously supplied and brought into the cell (O'Donnell et al. 2008). Consequently, there is a decrease in the storage of exogenously supplied long-chain fatty acids, as well as decreased turnover of long-chain fatty acids from the endogenous triglyceride pool in cardiac hypertrophy (O'Donnell et al. 2008).

The increased reliance on glucose that occurs in cardiac hypertrophy is not matched by increased activity of the pyruvate dehydrogenase complex, which produces acetyl CoA for the TCA cycle. In addition, cardiac hypertrophy is marked by decreased flux of fatty acids into the mitochondria through carnitine palmitoyltransferase 1, which also limits the availability of acetyl CoA for oxidative metabolism (Sorokina et al. 2007). The hypertrophied heart makes up for the deficit in acetyl CoA by using the pyruvate produced from glycolysis to supply the TCA cycle with intermediates other than acetyl CoA, which is a process known as anaplerosis. Rather than being directed primarily through pyruvate dehydrogenase, pyruvate in the hypertrophied heart is used to form the TCA cycle intermediate malate through upregulated malic enzyme 1 (Sorokina et al.

2007). One consequence of increased anaplerosis through malic enzyme 1 is that 11 fewer ATP molecules are made for every pyruvate molecule that enters the TCA cycle as malate instead of acetyl CoA (Carley et al. 2014). Combined with the decreased fatty acid oxidation that occurs in hypertrophy, anaplerosis causes the failing heart to generate less of the ATP it needs to support contractile function.

In addition to its impact on ATP synthesis, lipid metabolism is important in heart disease because it can lead to the accumulation of lipotoxic intermediates, such as diacylglycerol and ceramide. Diacylglycerol is produced in the glycerol phosphate pathway of triglyceride synthesis (Takeuchi and Reue 2009). The enzyme diacylglycerol acyltransferase combines an acyl CoA molecule to diacylglycerol to produce triacylglycerol, otherwise known as triglyceride, in the final step of the glycerol phosphate pathway (Takeuchi and Reue 2009). Studies have shown that the lipotoxicity observed in a mouse model of cardiac-specific overexpression of acyl CoA synthetase can be improved by crossing the mice to those with cardiac-specific overexpression of diacylglycerol transferase 1, which results in decreased levels of diacylglycerol and improved cardiac function (Liu et al. 2009). In addition, this cross results in reduced levels of ceramide, another fatty acid compound thought to contribute to lipotoxicity (Liu et al. 2009). The effects of ceramide alone were studied in a mouse model with cardiac-specific overexpression of glycosylphosphatidylinositol-anchored human lipoprotein lipase, which develops dilated cardiomyopathy and shows high ceramide levels (Park et al. 2008). Experiments demonstrated that inhibition of *de novo* ceramide synthesis through pharmacological or genetic means resulted in reduced ceramide levels, decreased oxidation of fatty acids, increased glucose oxidation, as well as better systolic function

and a longer lifespan (Park et al. 2008). Additional evidence shows that a reduction in C16 ceramide, in particular, is associated with the improved contractility seen in hypertrophied rat hearts supplied with the dietary fat oleate rather than palmitate (Lahey et al. 2014). Alterations in lipid metabolism are of interest because they may influence the accumulation of toxic intermediates, such as diacylglycerol or ceramide, that contribute to cardiomyopathies.

Another area of interest in the study of lipid metabolism and heart disease is the role of fatty acids in transcriptional activation of metabolic gene programs. Peroxisome proliferator-activated receptor alpha (PPARα) is considered a global regulator of cardiac metabolism, and free fatty acids are a ligand for its activation (Stanley 2005). Activated PPARα complexes with retinoid X receptors to bind PPAR response elements, which leads to upregulation of genes involved in fatty acid oxidation and downregulation of genes involved in glucose oxidation (Stanley 2005). PPAR gamma coactivator 1 (PGC1) is a binding partner for PPARα, and further enhances the ability of PPARα to induce transcriptional activation of genes involved in metabolism (Stanley 2005). Examples of genes with altered expression as a result of PPARα activation are pyruvate dehydrogenase kinase 4, glucose transporter 4, acyl CoA synthetase, and long-chain acyl CoA dehydrogenase (Stanley 2005 and Gilde 2003). PPARα mediates decreased glucose oxidation by activating the expression of pyruvate dehydrogenase kinase 4, which phosphorylates pyruvate dehydrogenase on the E1 subunit to cause its inactivation, leading to less flux of pyruvate through pyruvate dehydrogenase (Stanley 2005). In addition, PPARα activation leads to decreased expression of glucose transporter 4, further reducing glucose metabolism. To enhance fatty acid metabolism, PPARα

upregulates the gene involved in intracellular fatty acid esterification, acyl CoA synthase, as well as the gene for long-chain acyl CoA dehydrogenase, which is the enzyme isoform responsible for catalyzing the first step of beta-oxidation of long-chain acyl CoA molecules (Gilde 2003). It has been shown that fatty acids released from the intracellular triglyceride pool through adipose triglyceride lipase are required to activate PPARα (Haemmerle et al. 2011), which highlights the importance of triglyceride turnover for lipolytic signaling in the heart.

B. Fatty Acid Transport into the Cardiomyocyte

Fatty acid transport into the cardiomyocyte is primarily a protein-mediated process, though some free fatty acids also diffuse across the membrane in a concentration dependent manner (Kazantiz and Stahl 2012). Three proteins thought to play a role in transport of fatty acids across the plasma membrane of the cardiomyocyte include CD36, FATP1, and FATP6 (Kazantiz and Stahl 2012). CD36 is a glycosylated membrane protein that is ubiquitous in muscle, adipose, and cardiac tissue, and studies have shown that the hearts of CD36 null mice have a 50-80% reduction in the uptake of fatty acid analogs (Coburn et al. 2000). The FATP proteins have AMP-binding and lipocalin motifs, and based on their amino acid sequences, it is thought that these proteins pass once across the cell membrane so that the N-terminus is exposed to the extracellular side and the C-terminus faces the cytosol (Kazantiz and Stahl 2012). The FATP proteins may facilitate transfer of free fatty acids by forming oligomers, which is supported by studies showing that co-expression of wild type and mutant FATP1 results in dominant negative inhibition of fatty acid import (Kazantiz and Stahl 2012). In addition, FATP1 has been

shown to have synthetase activity, so it may play a role in esterifying long-chain fatty acids into acyl CoA, which could further support its activity as a transporter (Jia et al. 2007). Finally, FATP1 is the insulin-sensitive isoform of the FATP proteins, meaning that its subcellular localization is dynamically regulated (Kazantiz and Stahl 2012). While FATP6 is found in the sarcolemma at regions close to blood vessels, FATP1 has been shown to localize to the perinuclear space in the absence of insulin (Kazantiz and Stahl 2012).

C. Physiological Implications of Fatty Acid Transport Protein 1

Previous studies have shown that cardiac-specific overexpression of FATP1 leads to a 4-fold increase in fatty acid uptake, along with 2-fold increased fatty acid content and metabolism, with functional changes resembling those that occur in dilated cardiomyopathy (Chiu et al. 2005). Additional work has shown that the cardiac-specific overexpression of FATP1 leads to increased levels of diacylglycerol, a lipotoxic metabolite of triglyceride synthesis, and decreased expression of PGC1 and PPARα, which promote triglyceride turnover and metabolism in the heart (Elezaby et al. 2014).

D. Evidence for Sex-Dependent Differences in Lipid Metabolism

Myocardial lipid metabolism has been shown to differ between the sexes in specific ways, but the mechanisms accounting for these differences have yet to be identified. A study of PPARα homozygous knockout mice showed that males and females are affected differently by inhibition of carnitine palmitoyltransferase 1, the protein responsible for

importing fatty acids into the mitochondria (Djouadi et al. 1998). All of the male PPARα knockout mice showed fatal lipotoxicity in the heart and liver, while only 25% of female PPARα knockout mice died from the etomoxir treatment, which inhibits carnitine palmitoyltransferase 1 (Djouadi et al. 1998). Furthermore, male PPARα knockout mice were rescued from lipotoxicity if they were treated with beta-estradiol for two weeks prior to the etomoxir treatment (Djouadi et al. 1998). These findings suggest that females have a greater ability to metabolize fatty acids through an estrogen-dependent mechanism, but the details of how this occurs remain elusive. Studies in humans show that postmenopausal women receiving estrogen have higher myocardial fatty acid utilization, though not uptake or content, relative to men and, to a lesser extent, postmenopausal women not receiving estrogen (Herrero et al. 2005).

## E. Study Aims

The aim of this study was to address whether sexual dimorphism exists in intramyocardial lipid handling for FATP1 mice, and if so, at what points along the process of fatty acid import and metabolism. It was hypothesized that lipid dynamics in the hearts of male and female FATP1 mice will display sex-dependent differences. The rate and extent of triglyceride enrichment was examined, as well as the rate of triglyceride turnover and the fractional contribution of acetyl CoA to the TCA cycle. An overview of fatty acid uptake, esterification, and turnover from the triglyceride pool is shown in Figure 1.

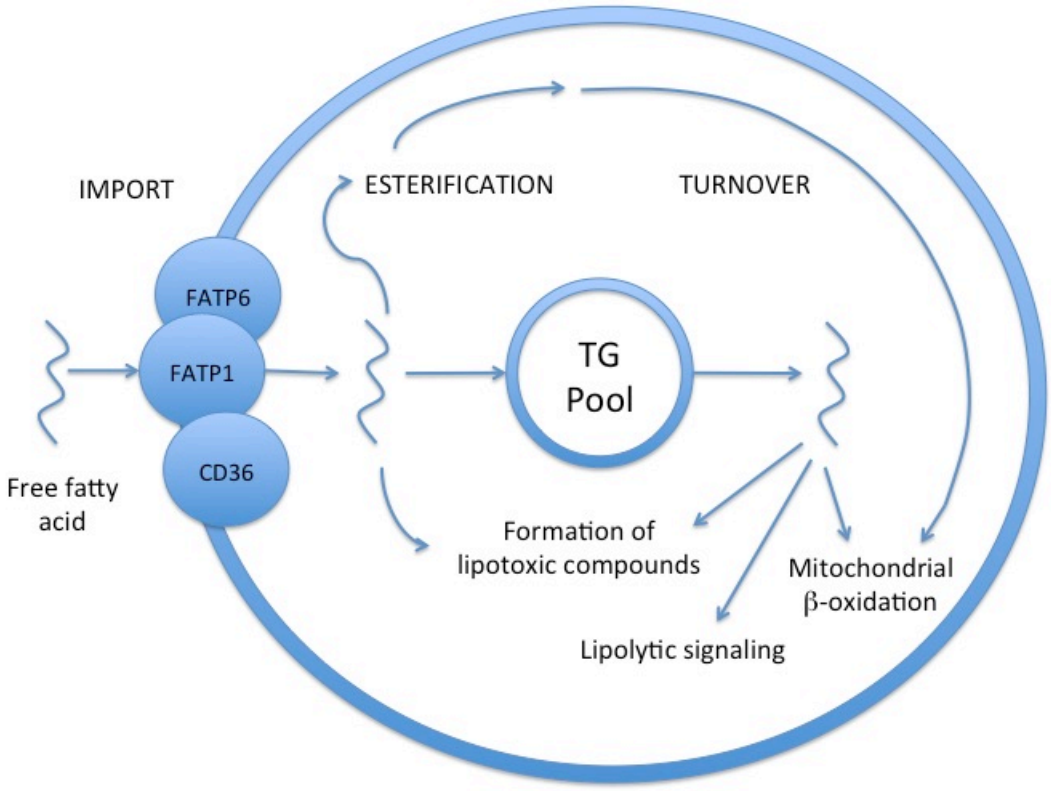


Figure 1: A General Overview of Fatty Acid Metabolism. The protein transporters CD36, FATP1, and FATP6 are thought to be responsible for free fatty acid import into the cardiomyocyte. Once inside the cell, free fatty acids are esterified, which allows for oxidation in the mitochondria, storage in the triglyceride pool, or formation of lipotoxic molecules. Free fatty acids released from the triglyceride pool can be directed to β-oxidation, lipolytic signaling, or the formation of lipotoxic compounds.

# CHAPTER II: MATERIALS AND METHODS

## A. Animal Model

Mice with cardiac-specific overexpression of the fatty acid transport protein 1 (FATP1) were used for this study. The mice were a gift from the Schaffer Lab at the Washington University School of Medicine in St. Louis, where they were genetically engineered from a FVB/N background strain to overexpress FATP1 using an α-MHC promoter (Chiu et al. 2005). Four groups of mice were examined, including non-transgenic males, transgenic males, non-transgenic females, and transgenic females.

## B. Langendorff Heart Perfusions and Dynamic NMR Spectroscopy

Mice were anesthetized with 134 mg/kg ketamine and 20 mg/kg xylazine administered by an intraperitoneal injection prior to heart excision. The excised heart was submerged in ice-cold modified Krebs-Henseleit buffer (116 mmol/L NaCl, 4 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, and 1.2 mmol/L $NaH_2PO_4$) and positioned under a microscope to allow for isolation and cannulation of the aorta. The cannulated heart was moved to the perfusion apparatus, where it was supplied with modified Krebs-Henseleit buffer kept at a temperature of 37 °C, equilibrated with 95% $O_2$/5% $CO_2$, and containing 10 mmol/L glucose and 0.4 mmol/L palmitate complexed to albumin in a 3:1 ratio. The left ventricle was fitted with a balloon, and changes in pressure were recorded through a pressure transducer to monitor heart function throughout the protocol (Powerlab, AD Instruments, Colorado Springs).

The intact, beating heart was placed in a broadband 10-mm NMR probe within a 14.1 T NMR spectrometer. The water signal was shimmed to a proton line width of less than 20 Hz to ensure homogeneity of the magnetic field. $^{31}P$ spectra were collected at a frequency of 242.95 MHz at the beginning and end of the experiment to measure the phosphocreatine to ATP ratio (Figure 2). Sequential, proton-decoupled $^{13}C$ spectra were acquired in two-minute intervals using a 45-degree excitation angle and a 2-second pulse interval (Figure 3). The first $^{13}C$ spectra were collected while the heart was being perfused with unlabeled palmitate, with which it was supplied for the first 25 minutes of the protocol to allow the heart to reach steady state and to enable background detection of the endogenous $^{13}C$ signal. The perfusion medium was switched to buffer containing uniformly labeled $^{13}C$ palmitate, and the experiment was allowed to continue for 40 minutes while $^{13}C$ spectra were acquired, as well as a final $^{31}P$ spectrum. At the end of the experiment, the heart was freeze-clamped between tongs cooled with liquid $N_2$.

Sequential $^{13}C$ spectra showed growth of the triglyceride signal at 30.5 ppm and that of the C4, C3, and C2 carbons of glutamate at 34.6, 28.0, and 56.0 ppm, respectively (Figure 3). The time constant associated with the exponential phase of triglyceride enrichment was found by plotting the integral of the triglyceride peak over time, subtracting out the linear phase at each time point, and analyzing the resulting exponential curve by non-linear fit with one-phase association.

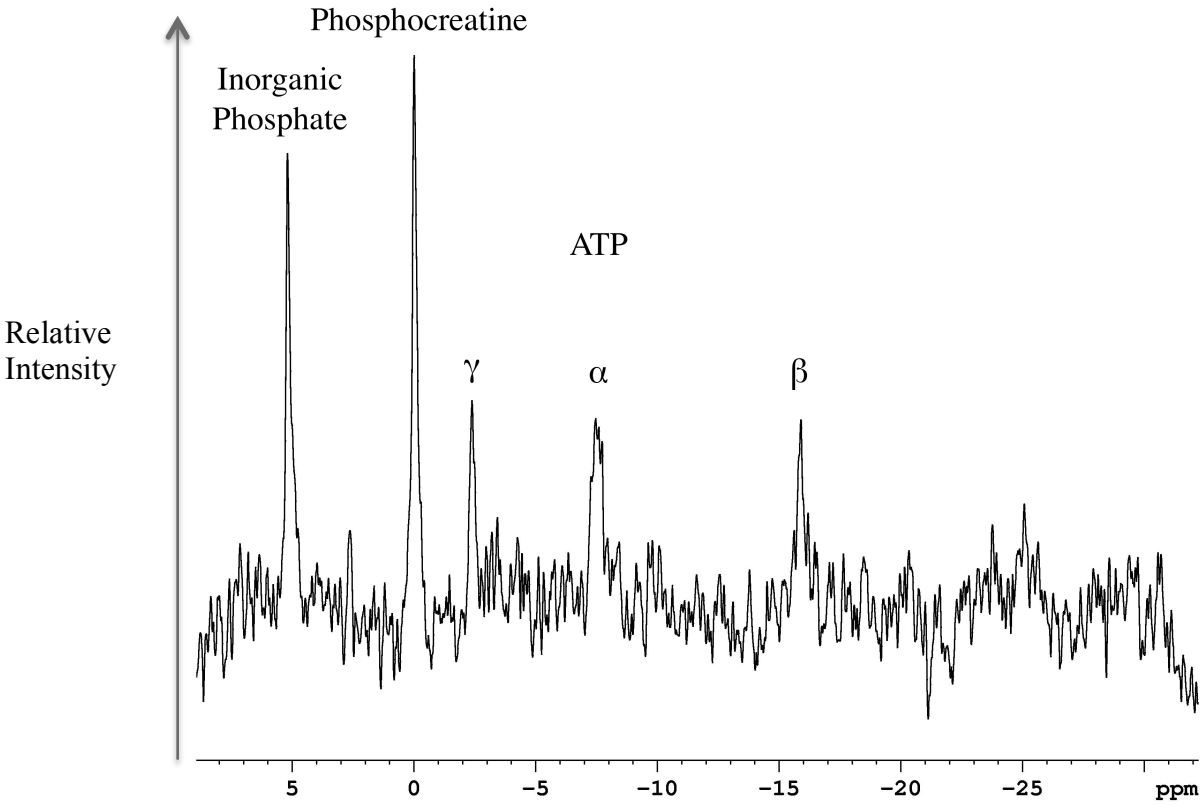


Figure 2: A Sample Phosphorous-31 Spectrum with Phosphocreatine and ATP

A. Quantification of Triglyceride Content and Enrichment

Triglyceride content was assayed using a calorimetric kit (Wako Pure Chemical Industries) and a visible light spectrophotometer, and it was normalized to tissue dry weight. The assay monitors the blue pigment generated from two sets of enzymatic reactions, which produce glycerol-3 phosphate from free glycerol and triglyceride.

The successive incorporation of $^{13}C$ into the triglyceride pool was measured from the growth of the integral at 30.5 ppm, which represents the methylene carbons ($–CH_2–$) of triglyceride, during dynamic NMR experiments (Figure 3). The enrichment of $^{13}C$ triglyceride was found from liquid chromatography/mass spectrometry. Triglyceride turnover was calculated as the product of the percent of triglyceride enrichment determined from LC/MS, the total triglyceride content found by calorimetric assay, and the slope of triglyceride enrichment during the linear phase.

B. Calculation of the Fractional Contribution of Acetyl CoA to the TCA Cycle

Frozen heart tissue was ground and extracted in 7% perchloric acid, neutralized with KOH, and dried overnight in a lyophilizer to prepare samples for *in vitro* NMR experiments. Dried samples were reconstituted in 0.5 mL of $D_2O$ and placed in a 5-mm NMR probe for high-resolution, proton-decoupled *in vitro* $^{13}C$ NMR detection of the C1, C2, C3, C4, and C5 glutamate peaks. Spectra were deconvoluted to allow for clearer visualization of the glutamate signal. Analysis of the glutamate peaks was performed using the program TCAcalc to determine the fractional contribution of acetyl CoA to the TCA cycle.

C. Statistical Analysis

Comparisons were made using two-way ANOVA with the Tukey post-test. Statistical significance was determined at p-values $< 0.05$. Data are reported as means +/- SEM.

# CHAPTER III: RESULTS

## A. Time Constant for the Exponential Phase of Triglyceride Enrichment

### i. Progressive Enrichment of Triglyceride and Glutamate

Sequential $^{13}C$ spectra showed progressive enrichment of triglyceride and glutamate, indicating that the heart preparations were taking up fatty acids from the buffer and metabolizing them to fuel contractile activity. The signal at 30.5 ppm indicates incorporation of the exogenously supplied $^{13}C$ palmitate into the methylene carbons (-$CH_2$-) of triglyceride, while the signal at 56.0 ppm, 34.6 ppm, and 28.0 ppm show the successive labeling of glutamate at the C2, C4, and C3 carbons, respectively, as the $^{13}C$ palmitate is oxidized in the TCA cycle (Figure 3).

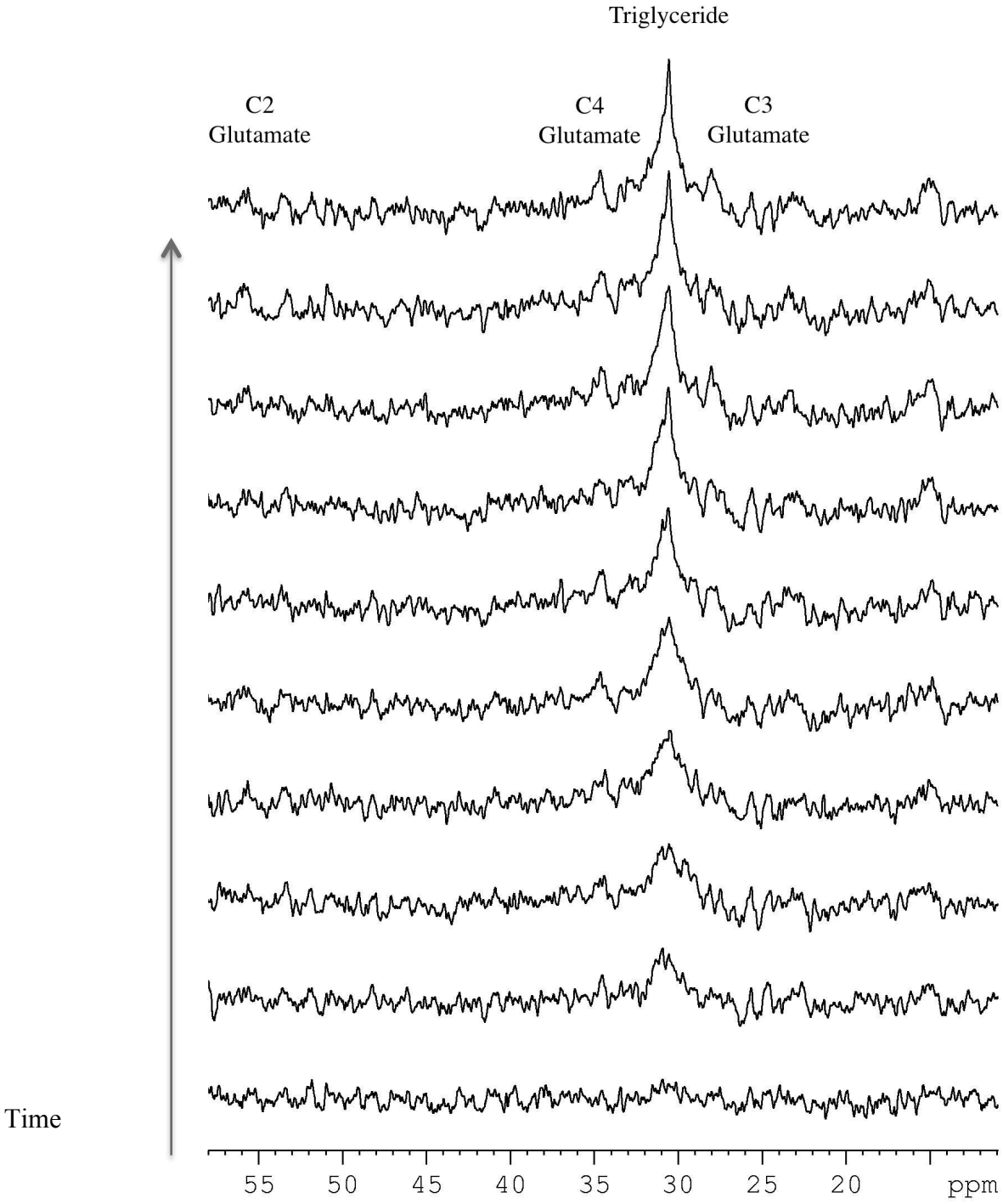


Figure 3: Successive Incorporation of Carbon-13 into the Triglyceride and Glutamate Pools

i. Kinetic Phases of Triglyceride Enrichment

Triglyceride enrichment occurs through distinct kinetic phases, which can be separated into two components (Carley et al. 2013). The first component is a saturable, exponential phase representative of $^{13}C$ palmitate uptake and esterification (Carley et al. 2013). The second component is a linear phase associated with triglyceride turnover (Carley et al. 2013). Triglyceride enrichment in FATP1 hearts displayed these kinetic phases, which can be seen more clearly when the linear phase is subtracted from the raw triglyceride enrichment to reveal the exponential phase (Figure 4). The example shown in the figure below is from a female FATP1 mouse heart.

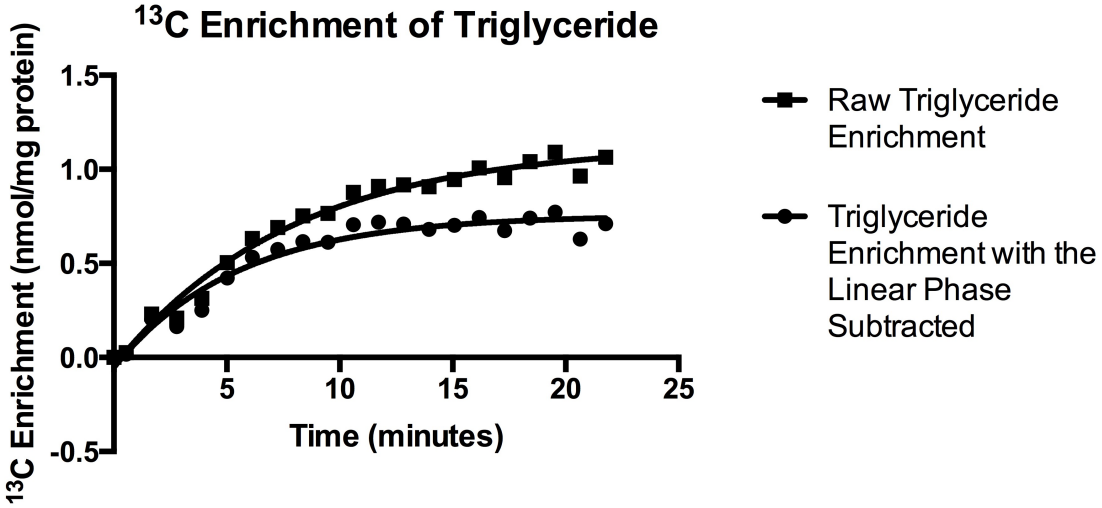


Figure 4: The Exponential Phase of Triglyceride Enrichment

ii. Time Constants of the Exponential Phase

The exponential phase of triglyceride enrichment has a time constant, which indicates how quickly the heart is taking up and esterifying the $^{13}C$ palmitate supplied in the perfusion buffer into the cell's endogenous triglyceride pool. The FATP1 males displayed a trend towards a lower time constant than non-transgenic males (2.74 minutes and 8.16 minutes, respectively), although this result was not statistically significant due to limited sample size. The FATP1 females and non-transgenic females had similar time constants (4.59 minutes and 3.37 minutes, respectively), but this result did not reach statistical significance for the same reason. Non-transgenic females showed a trend towards a lower time constant (3.37 minutes) relative to non-transgenic males (8.16 minutes), which recapitulates findings from the Lewandowski lab that females display faster uptake and esterification of long-chain fatty acids than males at baseline (Figure 5). For comparison, the non-transgenic males studied in another project from the Lewandowski lab showed a time constant of 2.89 minutes, while non-transgenic females had a time constant of 1.54 minutes.

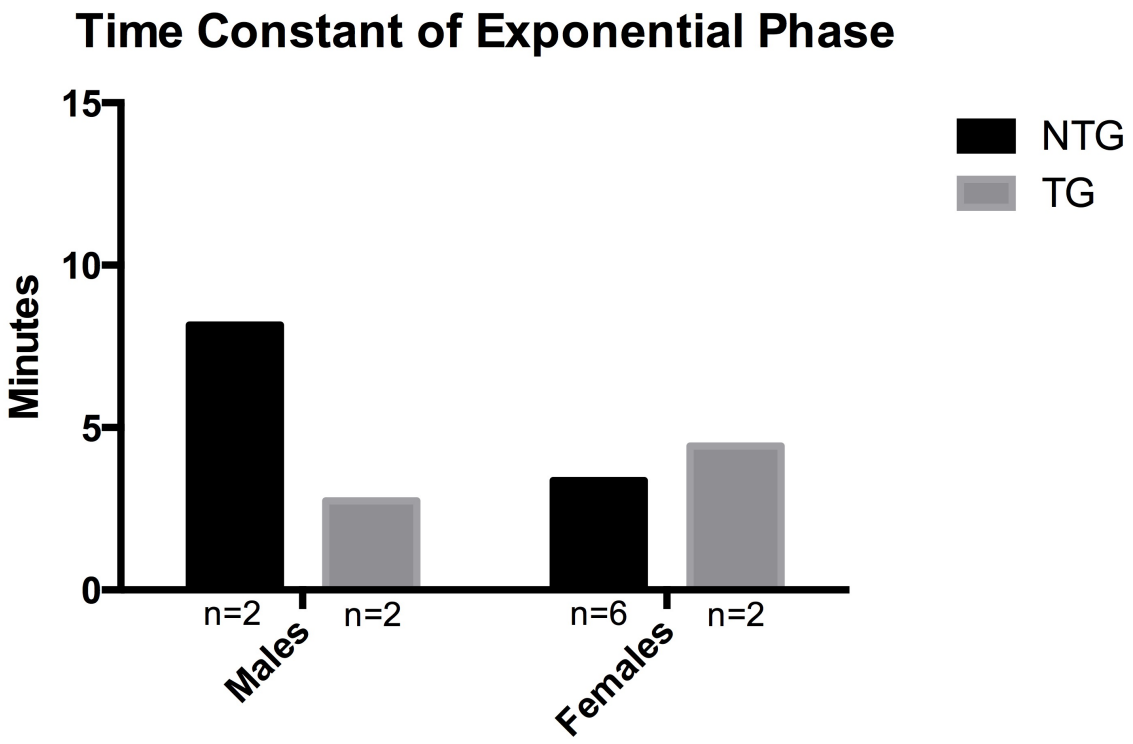


Figure 5: Time Constant of the Exponential Phase of Triglyceride Enrichment

B. Triglyceride Enrichment and Turnover

i. Triglyceride Enrichment

Triglyceride enrichment in FATP1 males (6.30 nmol/mg protein) tended to be higher than in non-transgenic males (2.62 nmol/mg protein), while triglyceride enrichment in females occurred at similar levels (5.74 nmol/mg protein for FATP1

females and 5.90 nmol/mg protein for non-transgenic females). Due to the limited sample size, the results were not found to be statistically significant (Figure 6).

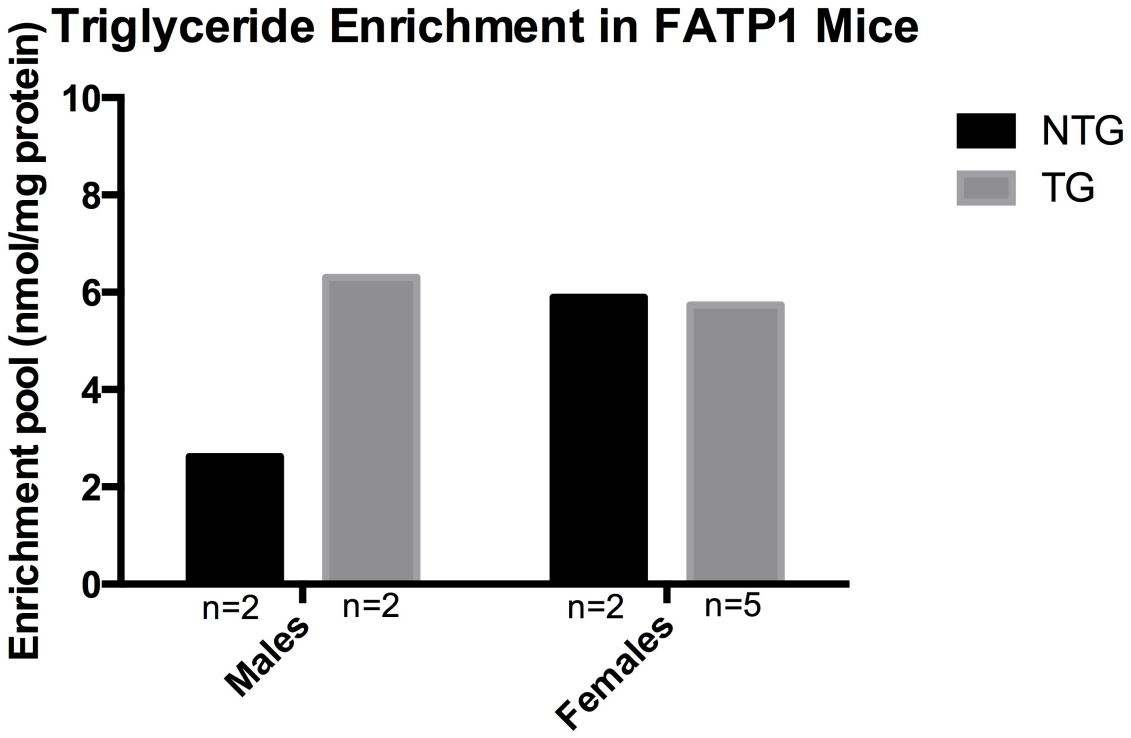


Figure 6: Triglyceride Enrichment in Male and Female FATP1 Mice

ii. Triglyceride Turnover

Triglyceride turnover tended to be higher in FATP1 males (158.22 pmoles/min/mg protein) and females (152.63 pmoles/min/mg protein) compared to non-transgenic males (65.23 pmoles/min/mg protein) and females (98.23 pmoles/min/mg protein), but the results are not statistically significant due to the small cohort of mice studied (Figure 7).

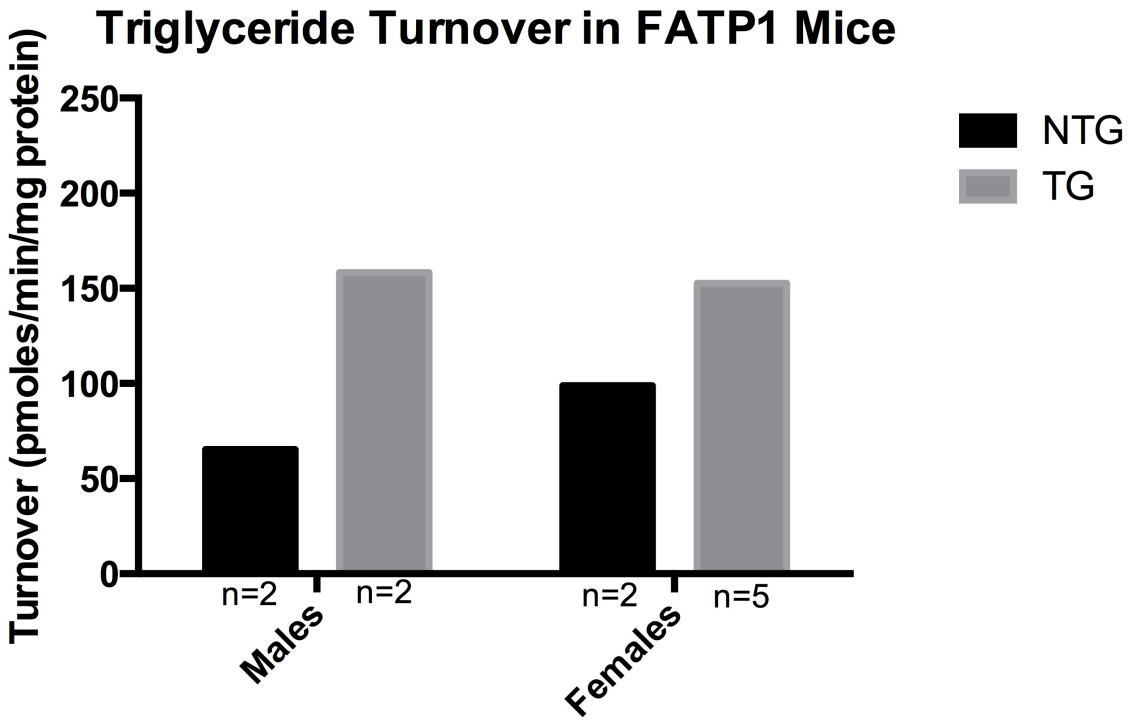


Figure 7: Triglyceride Turnover in Male and Female FATP1 Mice

C. Fractional Contribution of Acetyl CoA to the TCA Cycle

*In vitro* NMR spectra of tissue samples extracted from hearts perfused with uniformly labeled $^{13}C$ palmitate show isotopic enrichment at each of the five carbons of glutamate. The splitting of the C3 and C4 glutamate peaks at 28.3 ppm and 34.6 ppm, respectively, is shown in Figure 8. The splitting of C3 and C4 glutamate carbons makes the greatest impact on the calculation of the fractional contribution (Fc) of acetyl CoA to the TCA

cycle, and it is due to spin-spin coupling of the glutamate isotopomers (Malloy et al. 1988). Glutamate is a five-carbon molecule, and the splitting of the signal for the C3 and C4 carbons is due to resonance from glutamate molecules enriched at more than one position (Malloy et al. 1988). For the C3 carbon observed at 28.0 ppm, the signal is a combination of a singlet arising from glutamate molecules labeled at C3 only, two doublets from glutamate molecules labeled at the C2 and C3 or the C3 and C4 positions, and a triplet due to glutamate molecules enriched at the C2, C3, and C4 positions (Malloy et al. 1988). The singlet, doublets, and triplet peaks for the C3 carbon of glutamate overlap and show up as a peak split into five components at 28.0 ppm (Malloy et al. 1988). Similarly, the signal from the C4 carbon of glutamate at 34.6 ppm is split into six peaks, which can be resolved into individual signals arising from a singlet for C4 labeled glutamate, a doublet for C3 and C4 or C4 and C5 labeled glutamate, and a doublet of doublets, or a quartet, for C3, C4, and C5 labeled glutamate (Malloy et al. 1988). Though the theoretical number of peaks from the C4 carbon of glutamate is nine, only six are visible in the spectrum due to background noise and the relative distribution of the isotopomers. The peaks attributed to labeling of glutamate at the C4 and C3 positions are shown in Figure 8, and occur at 34.6 and 28.0 ppm, respectively.

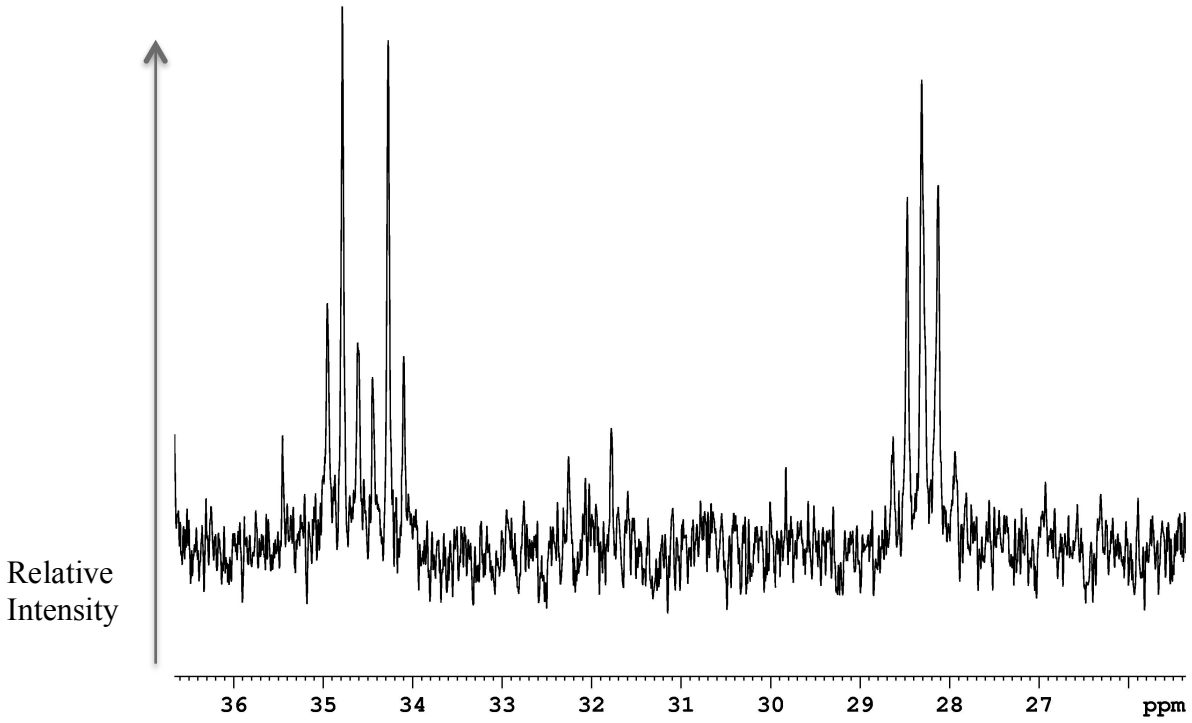


Figure 8: Splitting of the C4 and C3 Glutamate Peaks

The fractional contribution of acetyl CoA to the TCA cycle was found not to differ between groups. The Fc values for non-transgenic males, FATP1 males, non-transgenic females, and FATP1 females were 0.495, 0.482, 0.524, and 0.541, respectively (Table I).

Table I. Values for the Fractional Contribution of Acetyl CoA to the TCA Cycle

| | NTG Males | TG Males | NTG Females | TG Females |
|---|---|---|---|---|
| | 0.529 | 0.477 | 0.631 | 0.571 |
| | 0.512 | 0.516 | 0.491 | 0.616 |
| | 0.446 | 0.495 | 0.475 | 0.575 |
| | | 0.456 | 0.593 | 0.478 |
| | | 0.513 | 0.429 | 0.466 |
| | | 0.437 | | |
| Mean +/- SEM | 0.495 +/- 0.025 | 0.482 +/- 0.013 | 0.524 +/- 0.038 | 0.541 +/- 0.029 |

# CHAPTER IV: DISCUSSION

The aim of this study was to identify how cardiac-specific overexpression of FATP1 affects lipid dynamics and metabolic flexibility in male and female mice. Trends in the data indicate that male FATP1 mice display faster uptake and esterification of $^{13}C$ palmitate, greater triglyceride enrichment, and higher rates of triglyceride turnover than non-transgenic males. Female FATP1 mice, in contrast, show a trend towards equal uptake and esterification of $^{13}C$ palmitate and equal enrichment of triglyceride compared to non-transgenic females, but higher rates of triglyceride turnover. FATP1 overexpression does not affect the fractional contribution of acetyl CoA to the TCA cycle in male or female mice.

The time constant of the exponential phase of triglyceride enrichment is an indication of how quickly the heart takes up $^{13}C$ labeled long-chain fatty acids, such as palmitate, and esterifies them into the cell's endogenous triglyceride pool. The mechanism by which long-chain fatty acids traverse the cell membrane of the cardiomyocyte is thought to be transporter dependent, relying on CD36, FATP6, and FATP1 proteins (Carley et al. 2014). Once inside the cell, long-chain fatty acids are esterified to a coenzyme A thioester through the actions of acyl-CoA synthetase (Carley et al. 2014). These acyl CoA molecules are able to enter the mitochondria through the carnitine shuttle, so they may be used for oxidative metabolism, or be synthesized into triglyceride through reactions with glycerol phosphate acyltransferases and lipins of the glycerol phosphate pathway (Carley et al. 2014 and Takeuchi and Reue 2009). The important products of these reactions are diacylglycerol and triacylglycerol, otherwise known as triglyceride (Takeuchi and Reue 2009). These compounds and how they are

processed are of interest in the study of cardiac metabolism and physiology because diacylglycerol is thought to be a toxic intermediate that contributes to cardiomyopathies (Liu et al. 2009), and triglyceride is an important fuel source, as well as the origin of long-chain fatty acids that are capable of transcriptionally activating PPARα (Haemmerle et al. 2011). Consequently, the rate at which long-chain fatty acids are brought into the triglyceride pool and how rapidly they turnover is an interesting component to consider when examining lipid dynamics in the cardiomyocyte.

Dynamic NMR experiments with FATP1 mice showed progressive enrichment of triglyceride and glutamate (Figure 3). The growth of the area under the triglyceride peak was analyzed to find the time constant for the exponential phase of triglyceride enrichment. There was a trend towards a lower time constant in FATP1 males compared to non-transgenic males (Figure 5). The most likely explanation for this result, if it is indeed valid, is that the FATP1 males have faster uptake and esterification of long-chain fatty acids due to having overexpression of FATP1, one of the transporters responsible for bringing fatty acids into the cell. It is also possible that overexpression of FATP1 contributes to a lower time constant in male mice due to its synthetase activity, which would accelerate the process of triglyceride formation through the glycerol phosphate pathway. FATPs are known to have synthetase activity (Jia et al. 2007), so it could be that overexpression of FATP1 is lowering the time constant through synthetase activity as well as transporter activity. An effect on the time constant from FATP1 overexpression is not seen in female mice (Figure 5). This may be due to limited sample size, or there may be sex differences in intracellular lipid handling. If the lack of a difference in the time constant of FATP1 females is real, there are three plausible explanations for why

this may be so. The first is that the female FATP1 mice do not transport long-chain fatty acids into the cell at increased rates. The second is that the female FATP1 mice do not esterify long-chain fatty acids into the triglyceride pool as quickly. The third is that the female FATP1 mice direct the long-chain fatty acids to oxidative metabolism rather than storage. It is possible that any one of these scenarios or a combination of them accounts for the normalized time constant for triglyceride enrichment in female FATP1 mice.

Consistent with a lower time constant, FATP1 males have greater triglyceride enrichment relative to non-transgenic males (Figure 6). Similarly, FATP1 females have triglyceride enrichment that is indistinguishable from non-transgenic females, which follows from the two groups of females having nearly equal time constants (Figure 6).

There was a trend towards higher triglyceride turnover in FATP1 mice of both sexes (Figure 7). For the males, the higher rate of triglyceride turnover is in agreement with the data showing faster uptake and esterification of triglyceride, as well as greater triglyceride enrichment. The male FATP1 mice are taking up long-chain fatty acids more quickly, esterifying them more readily, and drawing on them from the endogenous TAG pool to support oxidative metabolism or lipolytic signaling. In the females, the picture is less clear. Based on the trends in the data, it would appear that female FATP1 mice do not take up or esterify long-chain fatty acids more quickly than non-transgenic female mice, and so they do not show greater triglyceride enrichment; however, the female FATP1 mice do show greater rates of triglyceride turnover. It is not obvious why the female FATP1 mice would have elevated triglyceride turnover in the absence of elevated triglyceride uptake, esterification, and enrichment. In mouse skeletal muscle, FATP1 is localized to the mitochondria, mostly in the outer membrane and intermembrane fractions

(Guitart et al. 2014). The subcellular localization of FATP1 to the mitochondria in mouse skeletal muscle was associated with enhanced fatty acid oxidation, possibly through a mechanism of metabolic trapping (Guitart et al. 2014). Metabolic trapping refers to an effect whereby the activity of a transporter or enzyme increases metabolism by providing more substrate to the cellular compartment where the reaction takes place. By Le Châtelier's principle, an increase in the concentration of available substrates will shift an equilibrium reaction towards increased usage of those substrates through the law of mass action. If FATP1 is localized to the mitochondrial membrane, it may fuel oxidative metabolism of long-chain fatty acids that have been taken up into the cell through synthetase activity at the mitochondria, converting them to acyl CoA and allowing their import into the mitochondria through the carnitine shuttle. In this way, mitochondrial FATP1 would direct long-chain fatty acids to oxidative metabolism rather than storage in the triglyceride pool. It is conceivable that a similar distribution pattern of FATP1 occurs in the heart, and a sex-dependent mechanism is in play for the mitochondrial FATP1, resulting in increased triglyceride turnover and oxidation though metabolic trapping, but not at the plasma membrane where free fatty acid uptake takes place. It has been shown in human studies that postmenopausal women have similar myocardial fatty acid uptake and content compared to men, regardless of hormone therapy; however, postmenopausal women taking estrogen show increased fatty acid oxidation (Herrero et al. 2005). This would suggest that estrogen plays a role in enhancing the metabolism of fatty acids without affecting their import.

The fractional contribution of acetyl CoA to the TCA cycle was found not to differ among groups. The significance of the fractional contribution of acetyl CoA is that

it can be used to measure the extent of anaplerosis, which occurs when pyruvate is converted to the TCA cycle intermediates oxaloacetate or malate instead of acetyl CoA. Directing pyruvate towards the formation of malate instead of acetyl CoA results in the generation of 11 fewer ATP per molecule of pyruvate, bypasses reactions that generate NADH, and consumes NADPH via malic enzyme (Carley et al. 2014). The consequence is less efficient oxidative metabolism and imbalances in the availability of cytosolic NADH, as well as NADPH. NADPH is used in one of the first reactions of triglyceride synthesis, so increases in anaplerosis are linked to a reduction in the triglyceride pool (Pound et al. 2009). The fractional contribution of acetyl CoA to the TCA cycle was examined in FATP1 mice to see if overexpression of FATP1 led to an increase or decrease in anaplerosis, but no difference was found.

A limitation of this study is that there is a small sample size of mice in each of the four groups, and the data is underpowered due to these constraints. More experiments are needed in each group to confirm a statistically significant difference in the time constant, enrichment, and turnover of triglyceride with respect to FATP1 overexpression and sex.

An additional limitation of this study, which may not be easily resolved, is the high sensitivity of the FATP1 mouse to anesthesia. Higher doses of anesthesia than are usually used for heart excisions in our lab are needed to fully anesthetize the FATP1 mouse. As a result, FATP1 mice show depressed respiratory function prior to heart excision, and the heart preparations used in the Langendorff setup do not beat as robustly as would be expected, and likely not at physiological rates. The consequence of this is that fat utilization may be underestimated, which could affect the results for the time constant, enrichment, and turnover of triglyceride, as well as the absolute value of Fc. It

is possible to still make comparisons across groups, but the values may not accurately reflect the *in vivo* situation due to the high dose of anesthesia that must be used in FATP1 mice.

Future studies could expand on this work by doing additional perfusions in FATP1 mice and assessing for changes in triglyceride uptake, enrichment, and turnover. If the findings presented in this work are confirmed and there is sexual dimorphism in the lipid handling of FATP1 overexpressing mice, further experiments could determine if the effect is estrogen-dependent by including a cohort of mice with ovariectomies. In addition, fractionation studies could shed light into whether cardiac tissue also contains FATP1 in mitochondria, which may contribute to a metabolic trapping effect. Finally, as FATP1 is the insulin-dependent isoform of the transporter, it would be interesting to perfuse hearts with buffer containing insulin to see if that has an effect on the transporter's activity and the overall lipid dynamics of the FATP1 mouse heart.

# CHAPTER V: CONCLUSION

Mouse hearts with cardiac-specific overexpression of FATP1 were perfused with uniformly labeled $^{13}C$ palmitate for dynamic NMR experiments, which examined long-chain fatty acid uptake and esterification, as well as enrichment and turnover of the triglyceride pool. The data show a trend towards increased long-fatty acid uptake and esterification in FATP1 males relative to non-transgenic males, as well as increased triglyceride enrichment and turnover. The FATP1 females showed a trend towards equal uptake and esterification of long-chain fatty acids relative to non-transgenic females, as well as similar levels of triglyceride enrichment, but showed higher rates of triglyceride turnover. These results need to be validated by additional experiments, and it may be useful if they are considered in light of the synthetase activity and possible intracellular localization of FATP1 in order to determine possible points of dimorphism in the lipid handling of male and female FATP1 mice. Experiments to assess the fractional contribution of acetyl CoA to the TCA cycle showed no difference between transgenic and non-transgenic mice of either sex.

# APPENDIX

## Triglyceride Content in Male and Female FATP1 Mice

| | NTG Males (nmol/mg protein) | TG Males (nmol/mg protein) | NTG Females (nmol/mg protein) | TG Females (nmol/mg protein) |
|---|---|---|---|---|
| | 41.68 | 33.86 | 45.56 | 576.9 |
| | 78.64 | 1309 | 39.54 | 27.56 |
| | | | | 20.43 |
| | | | | 37.72 |
| | | | | 32.24 |
| | | | | 46.62 |
| Mean | 60.16 | 671.4 | 42.55 | 123.6 |

CITED LITERATURE


1. Takeuchi, K., and K. Reue. "Biochemistry, Physiology, and Genetics of GPAT, AGPAT, and Lipin Enzymes in Triglyceride Synthesis." *AJP: Endocrinology and Metabolism* 296, no. 6 (June 1, 2009): E1195–1209. doi:10.1152/ajpendo.90958.2008.

2. Guitart, Maria, Óscar Osorio-Conles, Thais Pentinat, Judith Cebrià, Judit García-Villoria, David Sala, David Sebastián, et al. "Fatty Acid Transport Protein 1 (FATP1) Localizes in Mitochondria in Mouse Skeletal Muscle and Regulates Lipid and Ketone Body Disposal." Edited by Cedric Moro. *PLoS ONE* 9, no. 5 (May 23, 2014): e98109. doi:10.1371/journal.pone.0098109.

3. Kazantzis, Melissa, and Andreas Stahl. "Fatty Acid Transport Proteins, Implications in Physiology and Disease." *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1821, no. 5 (May 2012): 852–57. doi:10.1016/j.bbalip.2011.09.010.

4. Herrero, P, P Soto, C Dence, Z Kisrievaware, D Delano, L Peterson, and R Gropler. "Impact of Hormone Replacement on Myocardial Fatty Acid Metabolism: Potential Role of Estrogen." *Journal of Nuclear Cardiology* 12, no. 5 (September 2005): 574–81. doi:10.1016/j.nuclcard.2005.05.009.

5. Carley, A. N., H. Taegtmeyer, and E. D. Lewandowski. "Matrix Revisited: Mechanisms Linking Energy Substrate Metabolism to the Function of the Heart." *Circulation Research* 114, no. 4 (February 14, 2014): 717–29. doi:10.1161/CIRCRESAHA.114.301863.

6. Elezaby, Aly, Aaron L. Sverdlov, Vivian H. Tu, Kanupriya Soni, Ivan Luptak, Fuzhong Qin, Marc Liesa, et al. "Mitochondrial Remodeling in Mice with Cardiomyocyte-Specific Lipid Overload." *Journal of Molecular and Cellular Cardiology* 79 (February 2015): 275–83. doi:10.1016/j.yjmcc.2014.12.001.

7. Carley, A. N., J. Bi, X. Wang, N. H. Banke, J. R. B. Dyck, J. M. O'Donnell, and E. D. Lewandowski. "Multiphasic Triacylglycerol Dynamics in the Intact Heart during Acute in Vivo Overexpression of CD36." *The Journal of Lipid Research* 54, no. 1 (January 1, 2013): 97–106. doi:10.1194/jlr.M029991.

8. Neely, J R, and H E Morgan. "Relationship Between Carbohydrate and Lipid Metabolism and the Energy Balance of Heart Muscle." *Annual Review of Physiology* 36, no. 1 (March 1974): 413–59. doi:10.1146/annurev.ph.36.030174.002213.

9. Banke, Natasha H., Lin Yan, Kayla M. Pound, Sunil Dhar, Heather Reinhardt, Mariana S. De Lorenzo, Stephen F. Vatner, and E. Douglas Lewandowski. "Sexual Dimorphism in Cardiac Triacylglyceride Dynamics in Mice on Long Term Caloric Restriction." *Journal of Molecular and Cellular Cardiology* 52, no. 3 (March 2012): 733–40. doi:10.1016/j.yjmcc.2011.11.014.

10. Pound, K. M., N. Sorokina, K. Ballal, D. A. Berkich, M. Fasano, K. F. LaNoue, H. Taegtmeyer, J. M. O'Donnell, and E. D. Lewandowski. "Substrate-Enzyme Competition Attenuates Upregulated Anaplerotic Flux Through Malic Enzyme in Hypertrophied Rat Heart and Restores Triacylglyceride Content: Attenuating Upregulated Anaplerosis in Hypertrophy." *Circulation Research* 104, no. 6 (March 27, 2009): 805–12. doi:10.1161/CIRCRESAHA.108.189951.

11. O'Donnell, J. Michael, Aaron D. Fields, Natalia Sorokina, and E. Douglas Lewandowski. "The Absence of Endogenous Lipid Oxidation in Early Stage Heart Failure Exposes Limits in Lipid Storage and Turnover." *Journal of Molecular and Cellular Cardiology* 44, no. 2 (February 2008): 315–22. doi:10.1016/j.yjmcc.2007.11.006.

12. Jia, Zhenzhen, Zhengtong Pei, Dony Maiguel, Cicely J. Toomer, and Paul A. Watkins. "The Fatty Acid Transport Protein (FATP) Family: Very Long Chain Acyl-CoA Synthetases or Solute Carriers?" *Journal of Molecular Neuroscience: MN* 33, no. 1 (September 2007): 25–31.

13. Chiu, H.-C. "Transgenic Expression of Fatty Acid Transport Protein 1 in the Heart Causes Lipotoxic Cardiomyopathy." *Circulation Research* 96, no. 2 (February 4, 2005): 225–33. doi:10.1161/01.RES.0000154079.20681.B9.

14. Mootha, V. K., A. E. Arai, and R. S. Balaban. "Maximum Oxidative Phosphorylation Capacity of the Mammalian Heart." *The American Journal of Physiology* 272, no. 2 Pt 2 (February 1997): H769–75.

15. Lehman, John J., and Daniel P. Kelly. "Gene Regulatory Mechanisms Governing Energy Metabolism during Cardiac Hypertrophic Growth." *Heart Failure Reviews* 7, no. 2 (April 2002): 175–85.

16. Sorokina, Natalia, J. Michael O'Donnell, Ronald D. McKinney, Kayla M. Pound, Gebre Woldegiorgis, Kathryn F. LaNoue, Kalpana Ballal, Heinrich Taegtmeyer, Peter M. Buttrick, and E. Douglas Lewandowski. "Recruitment of Compensatory Pathways to Sustain Oxidative Flux with Reduced Carnitine Palmitoyltransferase I Activity Characterizes Inefficiency in Energy Metabolism in Hypertrophied Hearts." *Circulation* 115, no. 15 (April 17, 2007): 2033–41. doi:10.1161/CIRCULATIONAHA.106.668665.

17. Liu, Li, Xiaojing Shi, Kalyani G. Bharadwaj, Shota Ikeda, Haruyo Yamashita, Hiroaki Yagyu, Jean E. Schaffer, Yi-Hao Yu, and Ira J. Goldberg. "DGAT1 Expression Increases Heart Triglyceride Content but Ameliorates Lipotoxicity." *The Journal of Biological Chemistry* 284, no. 52 (December 25, 2009): 36312–23. doi:10.1074/jbc.M109.049817.

18. Stanley, W. C. “Myocardial Substrate Metabolism in the Normal and Failing Heart.” *Physiological Reviews* 85, no. 3 (July 1, 2005): 1093–1129. doi:10.1152/physrev.00006.2004.

19. Gilde, A. J. “Peroxisome Proliferator-Activated Receptor (PPAR) Alpha and PPARbeta/delta, but Not PPARgamma, Modulate the Expression of Genes Involved in Cardiac Lipid Metabolism.” *Circulation Research* 92, no. 5 (March 21, 2003): 518–24. doi:10.1161/01.RES.0000060700.55247.7C.

20. Haemmerle, Guenter, Tarek Moustafa, Gerald Woelkart, Sabrina Büttner, Albrecht Schmidt, Tineke van de Weijer, Matthijs Hesselink, et al. “ATGL-Mediated Fat Catabolism Regulates Cardiac Mitochondrial Function via PPAR-A and PGC-1.” *Nature Medicine* 17, no. 9 (August 21, 2011): 1076–85. doi:10.1038/nm.2439.

21. Djouadi, F, C J Weinheimer, J E Saffitz, C Pitchford, J Bastin, F J Gonzalez, and D P Kelly. “A Gender-Related Defect in Lipid Metabolism and Glucose Homeostasis in Peroxisome Proliferator- Activated Receptor Alpha- Deficient Mice.” *Journal of Clinical Investigation* 102, no. 6 (September 15, 1998): 1083–91. doi:10.1172/JCI3949.

22. Kessler, Gerald, and Jacob Friedman. “Metabolism of Fatty Acids and Glucose.” *Circulation* 98 (1998): 1350a – 1353.

23. Malloy, C. R., A. D. Sherry, and F. M. Jeffrey. “Evaluation of Carbon Flux and Substrate Selection through Alternate Pathways Involving the Citric Acid Cycle of the Heart by 13C NMR Spectroscopy.” *The Journal of Biological Chemistry* 263, no. 15 (May 25, 1988): 6964–71.

24. Coburn, C. T., F. F. Knapp, M. Febbraio, A. L. Beets, R. L. Silverstein, and N. A. Abumrad. “Defective Uptake and Utilization of Long Chain Fatty Acids in Muscle and Adipose Tissues of CD36 Knockout Mice.” *Journal of Biological Chemistry* 275, no. 42 (October 20, 2000): 32523–29. doi:10.1074/jbc.M003826200.

25. Park, T.-S., Y. Hu, H.-L. Noh, K. Drosatos, K. Okajima, J. Buchanan, J. Tuinei, et al. “Ceramide Is a Cardiotoxin in Lipotoxic Cardiomyopathy.” *The Journal of Lipid Research* 49, no. 10 (July 24, 2008): 2101–12. doi:10.1194/jlr.M800147-JLR200.

26. Lahey, R., X. Wang, A. N. Carley, and E. D. Lewandowski. “Dietary Fat Supply to Failing Hearts Determines Dynamic Lipid Signaling for Nuclear Receptor Activation and Oxidation of Stored Triglyceride.” *Circulation* 130, no. 20 (November 11, 2014): 1790–99. doi:10.1161/CIRCULATIONAHA.114.011687.