A Comparison of the Digestion and Absorption of Medium-Chain Triglyceride (MCT; C8) and Triheptanoin (C7) in Patients with Long-Chain Fatty Acid Oxidation Disorders

By

Kayla K. Guillory

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This is certifying that the Master's thesis of

Kayla K. Guillory

has been approved

Mentor/ Advisor

Member

Member

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Abbreviations

ACOD	Acyl-CoA Oxidase
AMP	Adenosine Monophosphate
ASC	Acyl-CoA Synthase
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BHB	β-Hydroxybutyrate
BMI	Body Mass Index
C7	Heptanoate
C8	Octanoate
C16	Palmitate
CAC	Citric Acid Cycle
CHO	Carbohydrates
CNS	Central Nervous System
CPT-1	Carnitine Palmitoltransferase I
CPT-2	Carnitine Palmitoltransferase II
DEXA	Dual-Energy X-Ray Absorptiometry
ECG	Electrocardiogram
FA	Fatty Acid(s)
FAO	Fatty Acid Oxidation
FFA	Free Fatty Acid(s)
FOD	Fatty-Acid Oxidation Disorder

GC-MS	Gas Chromatography Mass Spectrometry
HCL	Hydrogen Chloride
HGB	Hemoglobin
IMTG	Intramuscular Triglyceride
KCAL	Kilocalories
LCFA	Long-Chain Fatty Acid(s)
LCHAD	Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase
LCT	Long-Chain Triglyceride
MCAD	Medium-Chain Acyl-CoA Dehydrogenase
MCFA	Medium-Chain Fatty Acid(s)
MCT	Medium-Chain Triglyceride(s)
MEHA	3-Methyl-N-Ethyl-N-A-Hydroxyethyl-Alanine
MTFP	Mitochondrial Trifunctional Protein
N	Number
NEFA	Non-Esterified Fatty Acid(s)
OAA	Oxaloacetate
OHSU	Oregon Health Science University
OMMBID	Online Metabolic & Molecular Bases of Inherited Disease
PFB	Plentaflurobenzyl
POD	Peroxidase
PPi	Pyrophosphate
PRO	Protein
PSI	Per Square Inch

RER	Resting Energy Requirements
SD	Standard Deviation
TEE	Total Energy Expenditure
TFP	Trifunctional Protein
TYG	Triglycerides
UbFA	Unbound Fatty Acid
UP	University of Pittsburg
TCA	Tricarboxylic Acid
VLCAD	Very-Long-Chain Acyl-CoA Dehydrogenase
VLCADD	Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency

Units of Measure

C°	Celsius
dL	Deciliter
g	Gram
kg	Kilograms
g/kg	Grams per Kilograms
mg	Milligram
Ht	Height
hrs	Hours
L	Liter
mL	Milliliter
mol	Mole
mmol	Millimole
mmol/L	Millimole per Liter
mmol/mg	Millimole per Milligram
nmol	Nanomole
nmol/L	Nanomole per Liter
nmol/L/min	Nanomole per Liter per Minute
μmol	Micromole
μmol/L	Micromole per Liter
min	Minute
nm	Nanometer

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Abstract

***Objective**:* To determine if there is a difference in first pass metabolism between triheptanoin (an odd-chain fatty acid triglyceride) and medium chain triglyceride (MCT).

***Methods*:** Subjects with Carnitine Palmitoltransferase II(CPT2), Very-Long-Chain Acyl-CoA Dehydrogenase (VLCAD), or Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD)/ Trifunctional Protein (TFP) deficiencies were randomly assigned to consume MCT or triheptanoin in a mixed breakfast meal and an oral dose before exercise. Blood was drawn fasting, 1,2, and 4 hours post-prandial and pre and post-exercise. Plasma samples were analyzed for free fatty acids, quantitative total fatty acid profiles, ketones and acylcarnitines.

***Results*:** A total of 32 subjects completed this study (n=16 MCT; n=16 triheptanoin). Free fatty acids were higher at fasting and decreased with feeding as expected. After a MCT or triheptanoin mixed meal, we observed a gradual rise in total C8 and C7 in blood that was highest at 4 hours. However, there was less C7 in plasma than C8 after comparable intakes. Percent recovery of C8 was also greater than recover of C7. There were no differences in 4-carbon ketone production in either group. However, significantly greater 5-carbon ketone and odd-chain acylcarntine production was found in the triheptanoin group.

Discussion: We expected MCT and triheptanoin to be rapidly absorbed through portal circulation and peak after 1 hour. However, levels of both MCT and triheptanoin peaked at 4 hours, suggesting they may be incorporated into chylomicrons after a meal containing long-chain fat. The greater rise in C8 plasma levels was also unexpected which suggests there is a difference in the digestion or absorption of C8 and C7. Lack of ketone production and increased concentrations of odd-chain acylcarnitines further supports this hypothesis.

Conclusions**:** Plasma levels of both C8 and C7 peaked at 4 hours instead of 1 hour as expected. A significantly greater rise was seen in plasma C8 than C7 after consuming a mixed meal. Lower levels of C7 in blood suggests a difference in first pass metabolism. Our results challenge the dogma of MCT digestion and absorption because our findings support that intake of MCT/LCT together in a mixed meal affects the proportion of MCFA transported in chylomicrons.

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Chapter 1

Specific Aims

Medium chain triglycerides (MCT) are widely used as an important component in medical nutrition therapy and are thought to have many therapeutic benefits. However, the general digestion, absorption, transport and metabolism of MCT from the diet is not fully understood in humans. Further research on the utilization of MCT is needed in order to understand their metabolic processes and their key roles in nutrition therapy.

Medium chain triglycerides (MCT) undergo a very unique digestion, absorption and metabolic process. MCT consist of 3 fatty acids, 6 to 12 carbons in chain length (C6:0 to C12:0) bound to a glycerol backbone. They differ from long-chain triglycerides (LCT), the form of lipids predominately found in our diet, because LCT contain fatty acids of 14-18 carbons in length. It is proposed that MCT are digested to free fatty acids and glycerol in the intestine, absorbed across the intestinal mucosa and enter into the portal venous system without being incorporated into chylomicrons. After entering portal circulation, medium chain fatty acids (MCFA) reach the liver and some are taken up into hepatocytes while others continue on into systemic circulation. Those that enter the hepatocyte will enter the mitochondrial matrix to undergo beta-oxidation, without using carnitine palmitoyltransferase transport system used by LCT. The rapid digestion, absorption and transport into the hepatocyte mitochondria leads to rapid oxidation of these fatty acids. Increased beta-oxidation in the liver increases the amount of acetyl-CoA, resulting in greater ATP synthesis and production of ketone bodies via the Ketogenesis pathway. There is minimal research regarding the proportion of fatty acids from MCT that are

metabolized by hepatocytes compared to what proportion makes it past the liver into peripheral circulation to be utilized for energy by peripheral tissues.

The large majority of medium-chain triglycerides contain fatty acids of even carbon lengths that are sequentially cleaved into 2 carbon metabolites, acetyl-CoA by the β-oxidation enzymes. Odd-chain MCT metabolism has been proposed to have a therapeutic advantage over even chain MCT but the metabolism of odd-chain MCT has not been extensively researched. Odd-chain fatty acids are anaplerotic, or form intermediates of the citric acid cycle (CAC), through β-oxidation. The odd-chain fatty acid can be oxidized to acetyl-CoA units and one propionyl-CoA. Propionyl-CoA can be converted into succinate through a series of enzymatic reactions, increasing the pool of CAC intermediates. Studies done in animals suggest that the oxidation of odd-chain MCT is significantly greater than even-chain MCT. The goal of this research project is to increase our understanding of the general metabolism of MCT in humans. In addition, this project aims to determine if odd-chain MCT, in comparison to even-chain MCT, are utilized differently during first pass metabolism.

For this research project, we hypothesize that the majority of even-chain medium chain triglycerides will be metabolized by the liver via β-oxidation, and only a portion will be released into peripheral circulation to be utilized for energy by peripheral tissues. Further, we hypothesize that a greater portion of odd-chain fatty acids will be found in peripheral circulation in comparison to even-chain fatty acids. To test these hypotheses, we will:

1. ***Specific Aim #1***: Measure peripheral concentrations of fatty acid species, total triglycerides and free fatty acids in blood samples before and after both a mixed meal

and bolus dose of MCT and calculate the amount and timing of the appearance of octanoate (C8:0) in peripheral circulation.

   a. *Hypothesis:* We expect that the majority of MCT will be metabolized in the liver and only a small proportion (15-30%) will make it into peripheral circulation to be utilized for energy.

2. ***Specific Aim #2:*** Determine if there is a difference in peripheral concentrations of total fatty acid species, triglycerides and free fatty acids in blood samples before and after both a mixed meal and bolus dose of either trioctanoate, C8:0 or triheptanoin C7:0

   a. *Hypothesis:* We expect that the majority of even-chain MCT (Octanoate, C8:0) will be oxidized by the liver and more odd-chain fatty acids (Triheptanoin, C7:0) will make it past the liver into peripheral circulation to be utilized by peripheral tissues for energy.

Chapter 2

Background

Introduction

Medium-chain triglycerides (MCT) are mixtures of triacylglycerols comprised of 3 saturated fatty acids, 6 to 12 carbons in chain length (C6:0-Hexanoic acid, C8:0-Octanoic acid, C10:0-Decanoic acid and C12:0-Lauric acid) bound to a glycerol backbone (1). They differ from long-chain triglycerides (LCT), in that LCT contain fatty acids of 14-18 carbons in length. Fats that naturally occur in our diet consist primarily of long-chain fatty acids with a chain length of 14 carbons or more. However, high amounts of MCT can be found in coconut and palm kern oils (1).

Since the 1950's, MCT have been used as an important component in medical nutrition therapy within clinical settings (1). Due to their physiochemical properties, MCT were initially used for their unique digestion and absorption. Research done in animals, specifically in piglets and rats, opened the door in exploring the digestion, absorption, transport and oxidation of MCT (2, 3, 4, 5). Medium-chain fatty acids are preferentially oxidized, decreasing the breakdown of body proteins and improving glucose and energy status (4). However, the proportion of MCT that bypass the liver into peripheral circulation is unclear. It is uncertain whether MCT pass the liver as free fatty acids. Or if they are incorporated into TGY and stored as MCT in adipose and TGY droplets in humans. As uses expand in MNT, it is important to further explore and understand the general metabolism of MCT in humans.

MCT vs. LCT Metabolism

The physical and chemical properties of MCT differ significantly from LCT (6). These properties contribute to their unique digestion, absorption and metabolism. MCT are small in molecular size, have short hydrocarbon chain lengths, low melting points, are liquid at room temperature and are highly ionized at neutral pH increasing water solubility (3, 6, 7). Studies have shown that piglets fed MCT in comparison to LCT, observed less weight gain, increased hepatic lipogenesis, greater ketone body production, and increased thermogenesis (4, 5, 6, 8). The majority of MCT are absorbed as free fatty acids, which are hydrolyzed at a higher rate compared to LCT (7, 9). Long-chain fatty acids (LCFA) enter the lymphatic system via chylomicrons. The LCFAs are re-esterified into LCT in the enterocyte after conversion to an acyl-CoA in the presence of acyl-CoA synthase. This enzyme is specific for fatty acids containing 12 carbon atoms or more. The reformed LCT are packaged with lipoproteins and cholesterol into chylomicrons and secreted into the lymph system. Medium-chain fatty acids (MCFA) are rarely incorporated into chylomicrons because they are not readily re-esterified into the triacylglycerol (5). However, the proportion of MCT that are packaged into chylomicrons increases with increasing chain length, chronic MCT therapy and simultaneous ingestion with LCT (1, 10). In a study done in MCT fed piglets, greater than 90% of esterified C10:0 was measured in the blood, most likely due to a lipoprotein-lymphatic route of absorption (5).

MCFA are absorbed across the intestinal mucosa and enter into the portal venous system (3, 6). Nutrients absorbed via the portal vein, including MCFAs, pass the liver in minutes rather than entering circulation at the thoracic duct hours after a meal like

chylomicrons (5). The liver is the first major organ encountered by MCFA after portal vein transport (5), but there is a lack of research regarding the proportion of fatty acids from MCT that are metabolized by hepatocytes, compared to what proportion that make it past the liver into peripheral tissues. Animal studies suggest that some portion of the MCFA are absorbed into the hepatocyte and converted to ketone bodies (2, 3, 5). However, some portion of the fatty acids are transported past the liver into peripheral circulation to be utilized for energy production by peripheral tissues. These studies have demonstrated that following MCT supplementation, MCFA are rapidly absorbed into the circulatory system, elevating levels of plasma free fatty acids, are oxidized preferentially by muscle tissues and can be deposited in subcutaneous fat (5, 9, 11, 12).

The first step in fatty acid utilization is fatty acid uptake across the plasma membrane. Fatty acids are utilized for numerous biological functions. Therefore, successful transport across the plasma membrane is essential. Plasma membrane transport of LCFA involves two components, passive diffusion through the lipid bilayer and a protein facilitated transfer process (13). Determinants of LCFA uptake are dependent on two factors: molar ratio of fatty acids to albumin in circulation and cellular fatty acid metabolism (13, 14). Passive diffusion across the plasma membrane occurs when serum concentration of unbound fatty acids (ubFA) are high. Conversely, protein-mediated transport involving membrane proteins and FA-biding proteins, is indicated when ubFA concentrations are low (14). In MCFA transport, FA are passively diffused across both plasma and mitochondrial membranes independent of ubFA concentrations (14).

LCFA and their CoA derivatives are incapable of entering the mitochondrial matrix and must undergo a complex transfer system. This transfer system is referred to as a

"carnitine shuttle", where carnitine is the carrier molecule necessary for LCFA transport. Carnitine is found in vast amounts in muscle tissue and can be synthesized in humans from the amino acids, lysine and methionine (14). Acyl-CoA derived from LCFA, covalently bind to carnitine outside of the mitochondrial matrix and carnitine palmitoyl transferase I (CPTI) converts LCFAs into a fatty-acylcarnitine. The acylcarnitine is transported into the mitochondrial by carnitine acylcarnitine translocase and the fatty-acyl-CoA is regenerated by Carnitine palmitoyl transferase II (CPTII) (11, 14). Conversely, MCFA may cross the mitochondrial membranes, and do not necessarily require a transport system. Acyl-CoA derived from any chain length will enter the beta-oxidation cycle to produce acetyl-CoA.

Beta Oxidation

Beta-oxidation is the oxidation of activated fatty acids within the mitochondrial matrix, resulting in energy production. It occurs when two carbons are cleaved to acetyl-CoA from the carboxyl end. There are four enzymatic steps within one cycle, and there are three chain length specific cycles for long, medium and short-chain fatty acids. The products of this metabolic pathway are acetyl-CoA and an activated fatty acid with two fewer carbons. Activated fatty acids will continue through the cyclic degradative pathway, losing two carbons each turn. Acetyl-CoA produced during beta-oxidation, enter the tricarboxylic acid (TCA) cycle for further oxidation.

MCT are readily available and rapidly oxidized within hepatocytes to acetyl-CoA. They are capable of producing increased amounts of acetyl-CoA very rapidly, within 30 minutes of ingestion (9). β -oxidation is assumed to be the primary metabolic pathway of

MCT degradation (5). Multiple studies have observed increased fatty acid oxidation after MCT supplementation (3, 4, 15, 16).

Studies done in newborn piglets showed that force-feeding MCT significantly spared glycogen stores and MCT supplementation increased plasma glucose concentrations and decreased nitrogen excretion (15, 16). In a study done in preterm infants, glucose oxidation was decreased in infants fed MCT containing formulas in comparison to those fed LCT (16). It is hypothesized that due to preferential oxidation of medium-chain fatty acids, MCT supplementation decreases the breakdown of body proteins and improves glucose and energy status (4, 15).

Figure 1: β-Oxidation Cycle

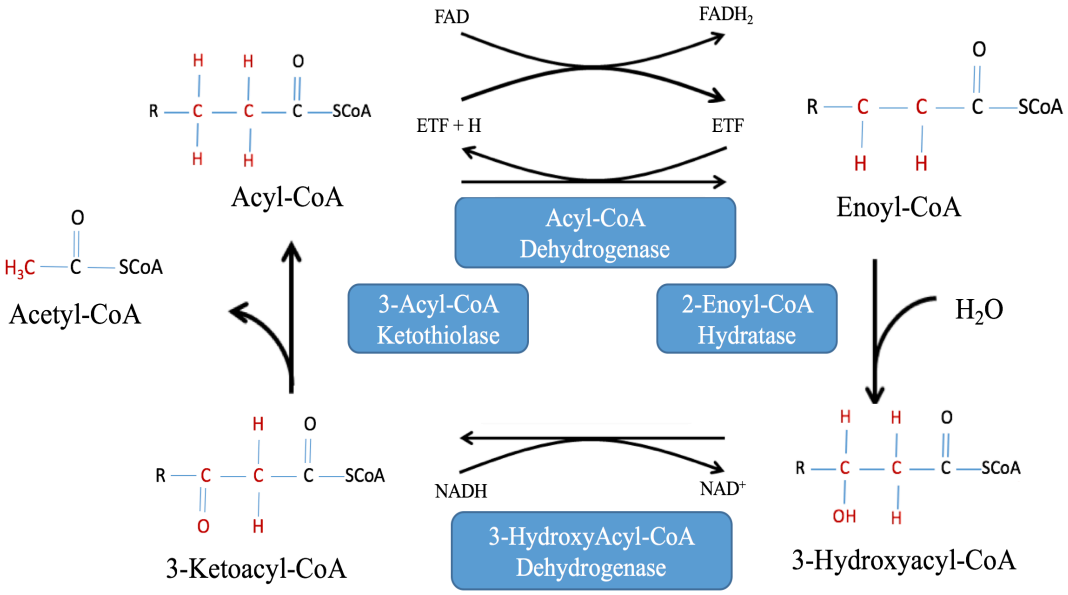

Figure 1: Displays the intramitochondrial steps of fatty acid β-oxidation. Once inside the mitochondrial matrix, acyl-CoA undergoes a repetitive four-step, enzymatic cycle that releases one molecule of acetyl-CoA and an acyl-CoA that is two-carbons shorter. The

carbons involved in one cycle of fatty acid oxidation are shown in red. Chain length-specific enzymes catalyze each step *Figure adapted from the Online Metabolic & Molecular Bases of Inherited Disease (OMMBID)* (17).

Tricarboxylic Acid Cycle

The tricarboxylic acid (aka Kreb's or citric acid) cycle converts acetyl-CoA to reducing equivalents, CO_2 and water in the presence of oxygen. This aerobic pathway is located within the mitochondrial matrix of all mitochondrial containing cells in the body. ATP production is heavily dependent on the TCA cycle and it is estimated that 90% of energy produced by this pathway is from the breakdown of dietary macronutrients such as acetyl-coA from fatty acid, amino acid and carbohydrate oxidation.

To begin the pathway, acetyl-CoA couples with oxaloacetate (OAA) by adding two carbons. There are eight intermediates within the TCA cycle, four are responsible for formation of OAA: citrate, succinate, fumarate and malate. All intermediates can be formed by amino acids. Continuous replenishment of OAA and the cycle's intermediates keep the TCA cycle functioning. Increased amounts of acetyl-CoA resulting from fatty acid oxidation, amino acid catabolism or pyruvate production via glycolysis without a similar increase in OAA will result in an imbalance between acetyl-CoA and OAA. In this setting, acetyl-CoA allosterically binds to pyruvate carboxylase to increase the conversion of pyruvate to OAA. In addition, anaplerotic mechanisms enhance the production of TCA cycle intermediates, increasing the formation of OAA. Under these conditions, anaplerosis increases TCA cycle intermediates to meet energy demands (18).

Ketogenesis

In the liver, increased fatty acid oxidation produces increased concentrations of acetyl-CoA in conjunction with normal amounts of OAA. Under these conditions, ketones are produced and excreted into circulation. Two molecules of acetyl-CoA condense to form a ketone body acetoacetate, which is further converted to β-hydroxybutyrate (BHB). Acetoacetate and BHB enter peripheral circulation as an alternative energy source for the periphery, particularly the central nervous system (CNS).

Several studies have found that ingestion of MCT are associated with ketone body production. Because MCFA are rapidly absorbed and readily available to be metabolized by the liver, increased rates of beta-oxidation occur — resulting in elevated amounts of acetyl-CoA. Rats fed 10ml/kg body weight of MCT oil had increased plasma levels of total ketone bodies 30 minutes after feeding and reached maximum ketosis 1.5 hours later (19). Piglets fed odd-chain or even-chain MCT had elevated levels of β-hydroxybutyrate 1 hour after feeding (5, 9).

Anaplerotic Mechanisms

Catalytic intermediates refer to the eight intermediates of the TCA cycle that are responsible for carrying acetyl-CoA as it is oxidized. Mitochondrial pools of TCA cycle intermediates are relatively small (1-2 umol/g for all intermediates) with a rapid turnover (5-200 times per minute) (20). Anaplerotic and cataplerotic reactions play an important role in the TCA cycle. In normal mitochondrial metabolism, cataplerosis occurs when catalytic intermediates are lost through mitochondrial and cell membranes to be used in other metabolic pathways. Without balancing these losses, the TCA cycle cannot be

sustained. Anaplerotic substrates such as pyruvate, glutamine/glutamate and propionyl-CoA replenish catalytic intermediate pools in all tissues.

Odd-chain fatty acids are precursors to propionyl-CoA; and therefore have anaplerotic properties that are not observed in even-chain fatty acids. Oxidation of odd-chain fatty acids provides anaplerotic substrates to the TCA cycle and may increase energy metabolism. Triheptanoin is a MCT composed of three seven-carbon fatty acids, bound to a glycerol backbone. It is broken down into heptanoate (C7:0) and glycerol, which are absorbed across the intestinal mucosa and enter into the portal venous system. Heptanoate reaches the liver and some of the fatty acids are taken up by the hepatocytes and are oxidized to 1 propionyl-CoA and 2 acetyl-CoAs via beta-oxidation. Propionyl-CoA can be converted to succinyl-CoA by carboxylation of propionyl-CoA carboxylase and methyl-malonyl-CoA mutase, resulting in anaplerosis (11, 21). Rapid oxidation of heptanoate stimulates ketone production of C5 ketones: β-hydroxypentanoate and β-ketopentanoate, which are released from the liver into peripheral circulation to be utilized by peripheral tissues. Research suggests that the route of absorption is similar for all MCFAs, however the rate of digestion/absorption and their effect on energy metabolism may differ significantly (3).

Several studies observing the metabolic effects of dietary supplementation with odd-chain MCT on energy metabolism in comparison to even-chain MCT have been performed (3, 5, 22). In one study using hepatocytes isolated from newborn piglets, a 45 percent greater flux in oxidation was observed in odd-chain fatty acids, C7:0 and C9:0. The flux was a result of propionyl-CoA supplying an anaplerotic carbon to the TCA cycle, resulting in greater CO_2 and ketone production in comparison to even-chain fatty

acids (5). In a subject with very long-chain fatty acid deficiency (VLCAD), profound improvement in cardiovascular and muscular status occurred when dietary treatment with octantoic acid (C8:0), was changed to treatment with triheptanoin (C7:0) (21). The authors hypothesized that anaplerotic properties of triheptanoin would be beneficial in this population.

Figure 2: Anaplerotic Mechanism of Triheptanoin (C7)

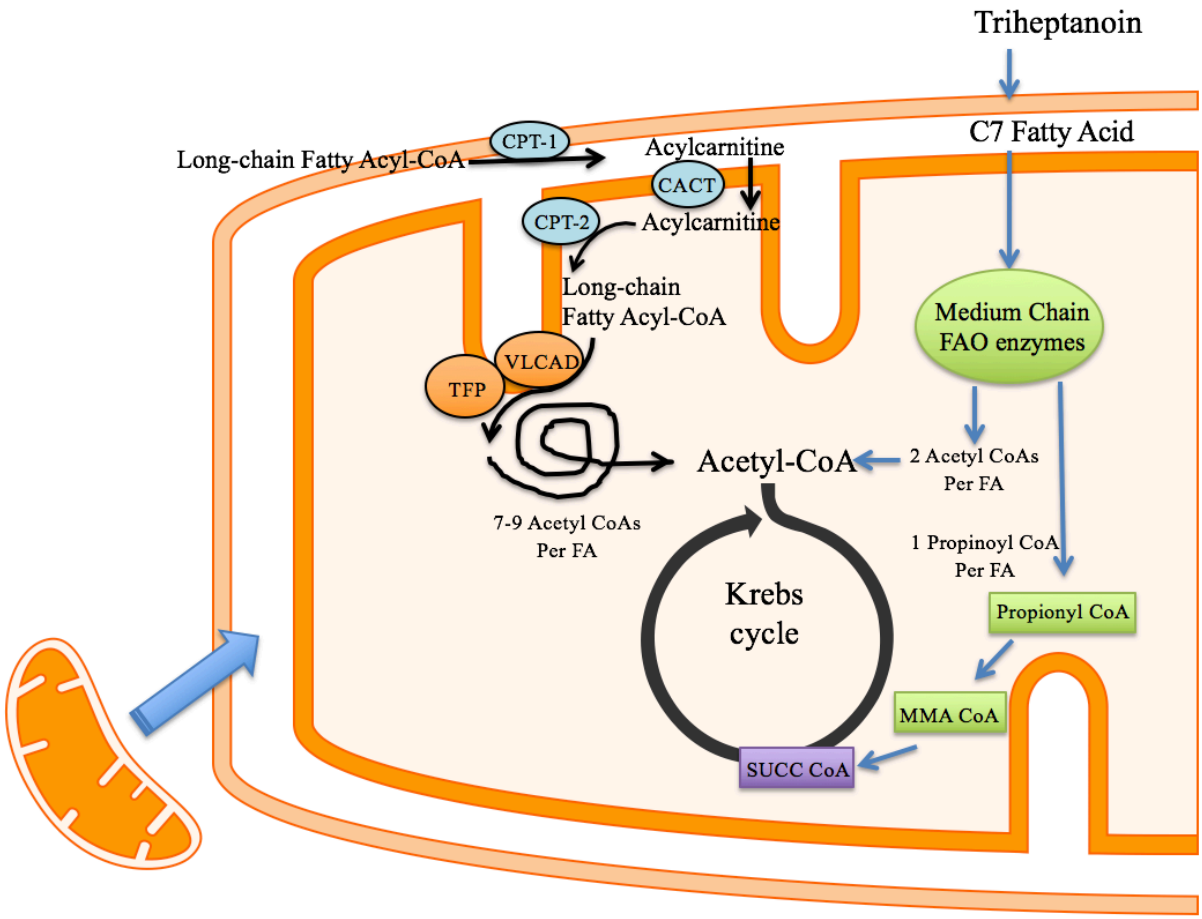

Figure 2: Depletion of TCA cycle intermediates occurs in the presence of long-chain fatty acid oxidation defects. Loss or imbalance of TCA cycle intermediates leads to depletion of OAA, therefore limiting the incorporation into the TCA cycle of acetyl-CoA

derived from MCT. Supplementation with odd chain carbon sources generates propionyl-CoA in the last cycle of fatty acid oxidation, which can anapleurotically replete carbon substrates in the TCA (17).

Effects of MCT on Exercise Metabolism

Fatty acids in the form of free fatty acids (FFA), intramuscular triacylglycerols (IMTG) and lipoprotein bound plasma triacylglycerols are capable of supplying muscle tissues with up to 80 percent of energy during periods of physical activity (18). Muscle glycogen stores are the primary source of energy during the first 20 minutes of exercise, where declining levels of FFA in the blood stimulate glycogenolysis. Equal amounts of glucose and fatty acids supply the body with energy in prolonged exercise exceeding 20 minutes. However, the ratio of energy from glucose and fatty acids is dependent on intensity and duration. Utilization of FFA increase simultaneously as glycogen stores become depleted and will account for the majority of energy requirements by the end of prolonged intense-exercise. In a study evaluating the effects of endurance training on fatty acid turnover, IMTG were the preferred source of post-training fuel (18). Another study found that endurance training alters the relative proportion of plasma and non-plasma sources of fatty acids oxidized by human subjects performing 90-120 minutes of strenuous, submaximal exercise (23). Many studies conclude that fatty acids play an important role in energy metabolism during and post-exercise.

Human studies have demonstrated that oral supplementation of MCT are rapidly absorbed into the circulatory system in less than 20 minutes and are oxidized by liver within minutes of ingestion (11). Numerous studies in humans have documented that

MCT supplementation has performance enhancing benefits on energy metabolism. Trained adult athletes who received MCT containing beverages before exercise oxidized 72 percent of their given dose (24). MCT co-ingested with carbohydrates before exercise resulted in the oxidation of exogenous MCT during that bout of exercise, suggesting that MCT was beneficial to athletic performance (11, 24). In a study done in patients with long-chain fatty acid oxidation disorders, MCT supplementation prior to exercise increased the oxidation of medium-chain fats, decreased the oxidation of glucose and acutely lowered cardiac workload during exercise (28).

Indicated Use of Medium-Chain Triglycerides

Medium-chain triglycerides have played a beneficial role in medical nutrition therapy since the 1950's, due to their unique metabolism. Although the general metabolism of MCT is not completely understood, there are various clinical presentations indicating the use of MCT as an effective medical treatment.

Fat Maldigestion/Malabsorption: For many years, MCT have been successfully used to treat steatorrhea in infants, children and adults with the following disorders: major resection of esophagus or stomach, biliary cirrhosis, obstructive jaundice, blind-loop syndrome, cystic fibrosis, cerebral palsy, celiac disease, resection of small bowel, Crohn's disease, tropical sprues, deficiency of chylomicron synthesis, lymphatic disorders, and chylothorax (6). These disorders effect lipid digestion and absorption resulting in fat maldigestion or malabsorption. MCT have a greater advantage in treating maldigestion/malabsorption in comparison to low-fat diets due primarily to their unique physiochemical properties; they bypass the lymph, are easily absorbed and are a

concentrated source of calories (8.3g/kcal) in comparison to carbohydrates and proteins (4g/kcal) (6).

Ketogenic Properties & Epilepsy: Many studies done in humans have shown that the Ketogenic Diet is remarkably beneficial and one of the most effective therapies for children with epilepsy (25). Ketone bodies have narcotic and anticonvulsive properties that are used as a viable treatment method when an individual is resistant to drug therapy, intolerant to anticonvulsant medications, or both. In a MCT-based Ketogenic Diet, it is recommended that 70% of calories come from MCT, allowing for more carbohydrate and protein rich foods. This is in comparison to a LCT-based Ketogenic Diet, where 90% of calories come from LCT and therefore less carbohydrate and protein are included in the diet (25).

Parenteral Nutrition: The caloric demand of a stressed individual is difficult to meet without administering lipids into their parenteral nutrition regimen. Lipid emulsions containing primarily LCT are not capable of supplying the body with quick, abundant energy. Unlike LCT, the metabolism of MCT is significantly increased when supplied intravenously. The majority of MCT are rapidly oxidized by surrounding tissues or incorporated into lipids. In critically ill patients, MCT are capable of easily meeting the individual's high-energy needs and reducing catabolism by contributing to a sparing action for lowered muscular carnitine levels (6, 26). Unfortunately, parental nutrition containing MCT is not available in the United States but is widely used in Europe.

Metabolic Disorders that affect Fatty Acid Oxidation: Medical nutrition therapies for long-chain fatty acid (LCFA) oxidation disorders are designed to provide at up to one-third of calories from MCT. Oral MCT supplementation with or without carbohydrates

immediately prior to exercise may bypass the metabolic block and improve exercise tolerance (11, 27, 28). Research is currently exploring the effectiveness of odd-chain MCT supplementation, specifically triheptanoin (C7), in patients with LCFA disorders.

Fatty Acid Oxidation Disorders

Fatty acid oxidation disorders (FOD) are inherited defects in the mitochondrial fatty acid β-oxidation pathway. These autosomal recessive disorders have serious clinical consequences, which include pediatric and maternal morbidity and mortality. Over twenty different types of FOD have been identified since the first documentation in 1973. It is estimated that approximately 1 in 10,000 individuals are affected (27). Due to the extensive number of enzymes affected by these disorders, a variety of clinical presentations are observed these include: fasting hypoketoic hypoglycemia, rhabdomyolysis, muscle weakness or myalgia, cardiomyopathy, peripheral neuropathy, non-fasting hypoglycemia or hyperinsulinism and maternal pregnancy complications (27, 29).

Fatty acid oxidation disorders are broadly categorized by the enzyme system affected including disorders of the carnitine transport cycle, long-chain, medium or short-chain fatty acid oxidation disorders. In long chain fatty acid metabolism, acylcarnitine requires a carnitine shuttle to enter the mitochondrial matrix to be oxidized and utilized for energy. There are three enzymes that are crucial for this process to occur; carnitine palmitoyltranferase I (CPT-1), carnitine/acylcarnitine translocase (CACT) and carnitine palmitoyltranferase II (CPT-2). Deficiencies of these enzymes are often life threatening. CPT-2 deficiency was the first FOD to be identified. CPT-2 is one of the most common

long-chain fatty acid oxidation disorders. CPT-2 deficiency includes a variety of clinical presentations, where exercise-induced rhabdomyolysis, occurring in either adolescence or adulthood, is the most common.

The first step in the β-oxidation cycle is the dehydrogenation of the acyl-CoA by chain length specific enzymes. Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency results in impaired oxidation of long chain fatty acids, C:12 to C:18. Defects in VLCAD disrupt the oxidation cycle by not allowing fatty acids to be metabolized and used as energy. Neonatal onset is the most severe form of VLCADD where in times of fasting or illness the neonate experiences cardiomyopathy, hypoketoic hypoglycemia, metabolic acidosis, hepatomegaly and death. Later onset is a much milder form of VLCADD and can occur during adolescence or adulthood, presenting with exercise induced rhabdomyolysis, muscle soreness, weakness and myoglobinuria.

Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) and Mitochondrial Trifunctional Protein (MTFP) deficiencies are defects of the enzymes located in the mitochondrial trifunctional protein complex. In addition to growth retardation, premature birth and intrauterine death, these disorders typically have very similar presentations to VLCADD. Additional clinical complications of LCHAD and MTFP deficiencies include a unique pigmentary retinopathy resulting in vision loss and a peripheral neuropathy that may impair mobility.

Medical nutrition therapies for long-chain fatty acid (LCFA) oxidation disorders are designed to provide a diet high in carbohydrates to reduce body fat utilization and prevent hypoglycemia, reduce the amount of long-chain fatty acids consumed in the diet, provide small and frequent meals to avoid periods of fasting, and provide about 10-20% of

calories from medium-chain triglycerides (MCT) (21).

Future Research

There are several aspects of MCT metabolism that are not well described. The amount of both a mixed meal or bolus dose containing MCT that make it pass the liver into peripheral circulation is not documented in humans. It is not well known if MCT pass the liver as free fatty acids or if they are incorporated into TGY. It is not fully known if MCT are stored as MCT in adipose and TGY droplets in humans. We hypothesize that the liver will metabolize the majority of medium chain triglycerides and only a minor portion will be released into peripheral circulation to be utilized for energy by peripheral tissues. This thesis project will address these aims using blood assays to measure the amount of fatty acid profiles, free fatty acids and triacylglycerols in the blood.

Chapter 3

Research Design

General Design

This study is a secondary analysis using blood samples collected from a double-blinded randomized controlled trial. “Phase 2 Triheptanoin for Treatment of Long-Chain Fatty Acid Oxidation Disorders”. The study included 32 individuals with a long-chain fatty acid oxidation disorder and is the largest study within this unique population. Subjects who participated in this study had a confirmed diagnosis of one of the following long-chain fatty acid oxidation disorders: carnitine palmitoyltransferase 2 (CPT-2), very long-chain acyl-coA dehydrogenase (VLCAD) or long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) and/or trifunctional protein (TFP) deficiency. Subjects were recruited to participate at one of two clinical sites: Oregon Health & Science University (OHSU) or the University of Pittsburgh (UP), and were randomly assigned to one of two groups: the control group based on the current standard of care, medium-chain triglyceride (MCT) supplementation or triheptanoin supplementation (table 1). Each experimental group was arranged to contain subjects with all three diagnoses CPT-2, VLCAD and LCHAD/TFP deficiency. Subjects were blinded to the study treatment. Furthermore, the principle investigator, co-investigators and all other sub-investigators were blinded with the exception of the statistician, the OHSU study coordinator, the OHSU investigational pharmacy and the OHSU bionutritionist. All study procedures were completed at either OHSU or the University of Pittsburgh Clinical and Translational Science Centers on enrollment and again after 4 months on the study diet. Subjects

completed baseline assessments at the time of enrollment consisting of: body composition, cardiac function, metabolic response to a test meal and exercise tolerance. Subjects were provided with the control or triheptanoin supplements during enrollment, and with their families, were counseled on how to properly consume their supplement at home. After following the diet at home for 4 months, subjects returned to repeat assessments.

Table 1: Overall Study Design

Treatment Group	***MCT; C8 (Control)***			***Triheptanoin; C7***			***Total***
DX:	**CPT2**	**LCHAD/ TFP**	**VLCAD**	**CPT2**	**LCHAD/ TFP**	**VLCAD**	**_____**
OHSU	3	4	3	3	4	3	24
UP	3	1	2	3	1	2	8
Total	6	5	5	6	5	5	32

Study Population & Recruitment

Subjects with long-chain fatty acid oxidation disorders were recruited to participate in the initial study using announcements posted on the fatty acid oxidation family support network website, through participating clinical populations and by sending letters to metabolic specialists across the United States. Inclusion and exclusion criteria are shown in Table 2. Subjects were to be over 7 years of age and have a confirmed diagnosis of CPT-2, VLCAD or LCHAD and/or TFP. Subjects had to be able to complete and comply with the study protocol and could not be participating in another research project that

1. Could alter macronutrient content of their diet (or)
2. Included a drug that may alter fatty acid oxidation.

Medical records were reviewed prior to enrollment to confirm the diagnosis for each disorder by evaluating acylcarnitine profiles, fatty acid oxidation (FAO) probe studies in cultured fibroblasts and/or mutation analysis. Not all potential subjects had skin biopsies and FAO probe studies in cultured fibroblasts. Therefore, establishing diagnosis of these disorders was a complex process. If two of the three diagnostic tests suggested a diagnosis of CPT-2, VLCAD, or LCHAD/TFP, the subject was deemed eligible to participate in the study. In addition, if 2 mutations were identified and a history of at least one episode of rhabdomyolysis was experienced, the subject was eligible to participate in the study.

Table 2: Inclusion and Exclusion Criteria

Inclusion	*Exclusion*
Confirmed diagnosis of CPT2, VLCAD or LCHAD/TFP	Hgb < 10 g/dL
Aged ≥ 7 years old	Peripheral neuropathy that limits ability to complete treadmill studies
Ability to follow and comply with protocol	Inclusion in another study that alters macronutrient intake or on drug that alters fatty acid metabolism
Ability to travel to CRC to participate in study	History of myocardial infraction
Stable on a diet that includes supplementation with MCT	
History of at least 1 episode of rhabdomyolysis	

Chapter 4

Methods and Procedures

Standardized Diets

All research diets had the same total fat content (LCT+MCT or triheptanoin) but varied in the source of medium-chain fat (MCT vs. triheptanoin). The MCT diets contained 20% of total energy from MCT or an equal % of total energy from triheptanoin. The dietitian instructed subjects and their families how to properly follow their prescribed diet. The detailed diet was developed based on the subject's measured resting energy requirements plus 30% for activity and growth. Subjects had to follow the general types of foods provided in their prescribed diet plan but were not required to meet their recommended caloric needs. Diet instructions included a list of allowed foods and quantities, recipes for preparing the meals and suggested menus to follow. Food intake forms were provided to the subjects, where they were instructed to record 3-day diet records. Subjects completed a 3-day diet record three times over the duration of the study: at the beginning of the study, mid-study at 8 weeks' participation and at the end of the study.

Compliance Monitoring

Subject compliance with their prescribed experimental diet was monitored by weekly communication between the study coordinator and subjects and/or guardians, 3-day diet record analyses and monitoring the amount of supplemental oil consumed. Weekly communication with subjects was used to assess adverse events, discuss problems or

difficulties and to monitor compliance. Subjects and their families were encouraged to adhere to their prescribed diet plan and to properly consume the supplement. Completed diet records were mailed to the principle investigator and a Registered Dietitian at OHSU analyzed dietary macronutrient content using Food Processor (ESHA Research, Inc) to monitor compliance. For the last measure of compliance, subjects were asked to return all supplemental oil. Percent compliance was calculated as (oil dispensed – oil returned)/oil dispensed X 100.

Meal Tolerance Test

Meal tolerance test entailed a controlled overnight fast, where subjects fasted for a total of 10 hours before they consumed a standardized breakfast. All standardized breakfasts contained the same total fat (long-chain triglyceride (LCT) + MCT or triheptanoin) content but vary in the source of medium chain fat. Subjects were not required to eat all the calories given in their diet plan but they must follow the general types of foods included in the plan. All subjects were encouraged to eat foods from their prescribed diet until they were satisfied. Remaining food was taken back to the research kitchen at the Hatfield Research Center and weighed for accuracy. Four blood samples were drawn over the course of the MTT at defined time points: fasting, and 1, 2, 4 hours post-prandial.

Treadmill Ergometry

Treadmill ergometry was performed 2 hours following a lunch meal while the patient remained in a post absorptive state. Subjects were given a carbohydrate and protein free

beverages containing MCT or triheptanoin oil 20 minutes prior to starting exercise. Supplemental dose of oil was measured based on the subject's lean body mass which was measured by a DEXA scan. A pre-exercise blood draw was taken before starting the exercise protocol. The exercise protocol was performed as follows: 3 minute warm up phase with a slow walk at 1.5 miles per hour at 0% grade followed by increases in rate and incline every two minutes until the subject's heart rate is 50-60% of his/her predicted maximum heart rate. Predicted maximum heart rate was calculated using the formula: 220 (beats per minute) – age in years. Subjects were asked to continue exercising at 50-60% of their predicted maximum heart rate for 40 minutes, followed by a 2-minute cool down period. During recovery, blood pressure and ECG were recorded and respiratory rate and heart rate were monitored until close to baseline. Blood was drawn after 20 minutes of recovery time. If subjects experienced adverse symptoms such dizziness, severe respiratory distress, chest pain or palpations, or muscle pain, the exercise test was terminated. The same grade and speed completed at baseline was repeated for exercise tests at follow-up to keep workload constant.

Blood Samples

A total of 7 blood samples were collected from each subject during study admissions. Blood was drawn before and 1,2, and 4 hours post meal tolerance test. Blood samples were drawn for the treadmill ergometry test at 20 minutes after consuming C8/C7 dose (baseline, time 0), and after 20 minutes' recovery time. A final blood sample was drawn the morning after exercise. Blood samples are frozen and stored at -80C in our laboratory and were thawed before use in this secondary analysis. Laboratory studies performed on

these samples include, free fatty acid and, triglyceride concentrations and fatty acid profiles.

Fatty Acid Profiles

The Mayo Clinic's Biochemical Genetics Laboratory performed a Comprehensive (C7-C26) Fatty Acid Profile assay for the quantitative determination of fatty acids in plasma. Samples were prepared and analyzed as follows: as described by Langerstedt et al., internal standard mixture was added to 100µl of plasma. Fatty acids were hydrolyzed from triglycerides, phospholipids and cholesterol esters in two steps: 1. Addition of 2 mL of acetonitrile:6 N hydrochloric acid followed by mixing and baking at 104°C for 45 minutes 2. Addition of 2 mL of methanol:10 N sodium hydroxide (90:10, v:v) followed by mixing and baking at 104°C for 45 min. Samples were re-acidified with 350 µL of 6 N HCl. Total fatty acids were extracted in hexane and evaporated to dryness under nitrogen. Derivatization to the pentafluorobenzyl ester was accomplished by the addition of 10 µL of triethylamine and 50 µL of 10% pentafluorobenzyl bromide in acetonitrile, mixing, and allowing the solution to react 15-30 minutes at room temperature. The resulting fatty acid pentafluorobenzyl (PFB) esters were dissolved in hexane and analyzed on a GC-MS.

Gas chromatography-mass spectrometry was performed using a Hewlett Packard-6890/5973, operating in negative chemical ionization mode using ammonia as reagent. Separation of fatty acid-PFB esters was achieved in two 15 minute analyses using HP-5 capillary column. Helium was the carrier gas used and column pressure was set to 20 psi. The temperatures of the injector, transfer line, and source was respectively 350, 280, and

280°C. The initial oven temperature was set to 100°C, with a ramp rate of 20°C/min, final temperature reached was 325°C and held for 3.75 min. Fragmentation of fatty acid-PFB esters by negative chemical ionization resulted in a reproducible loss of the PFB-moiety giving a stable carboxyate anion. Each analyte was matched to the labeled internal standard of closest chain length, retention time, and concentration. (30) The following fatty acids were quantified and expressed as nmol/mL for each sample:

Table 3: Fatty Acid Profiles

FATTY ACID PROFILE	*CHAIN LENGTH*
Heptanoic	C7:0
Octanoic	C8:0
Decenoic	C10:1
Decanoic	C10:0
Lauroleic	C12:1
Lauric	C12:0
Tetradecadienoic	C14:2
Myristoleic	C14:1
Myristic	C14:0
Hexadecadienoic	C16:2
7-Hexadecenoic	C16:1w9
Palmitoleic	C16:1w7
Palmitic	C16:0
y-Linolenic	C18:3w6
a-Linolenic	C18:3w3
Linoleic	C18:2w6
Oleic	C18:1w9
Vaccenic	C18:1w7
Stearic	C18:0
Eicosapentaenoic	C20:5w3
Arachidonic	C20:4w6
Mead	C20:3w9
Homo-y-linolenic	C20:3w6
Arachidic	C20:0
Docosahexaenoic	C22:6w3
Docosapentaenoic	C22:5w6
Docosapentaenoic	C22:4w6
Docosatetraenoic	C22:1
Docosenaoic	C22:0
Tetracosenoic	C24:1w9
Tetracosenoic	C24:0
Hexacosenoic	C26:1
Hexacosenoic	C26:0

Free Fatty Acid Concentrations

An in vitro enzymatic colorimetric method assay, HR Series NEFA-HR(2), was used for the quantitative determination of non-esterified fatty acids in plasma. Plasma samples with lipase inhibitor were prepared and analyzed using the following procedure.

Reagent preparation: Reagents were prepared by adding one bottle of Solvent A (50 mmol/L phosphate buffer, pH 7.0 0.05% Sodium azide) to one vial of Color Reagent A (0.53 U/mL Acyl-coenzyme A synthetase, 0.31 mmol/L Coenzyme A , 4.3 mmol/L Adenosine triphosphate,1.5 mmol/L 4-aminoanitipyrine, 2.6 U/mL Ascorbate oxidase, 0.062% Sodium azide) and gently mixed by inverting the vial until the contents were completely dissolved. One bottle of Solvent B (2.4 mmol/L 3-methyl-N-ethyl-N-(À-hydroxyethyl)-aniline) was added to one vial of Color Reagent B (12 U/mL Acyl-coenzyme A oxidase, 14 U/mL Peroxidase) and gently mixed by inverting the vial until the contents were completely dissolved.

Assay principal: NEFA in serum, when treated with acyl-CoA synthetase (ACS) in the presence of adenosine triphosphate (ATP) and CoA, will form the thiol esters of CoA known as acyl-CoA along with the adenosine monophosphate (AMP) and pyrophosphate (PPi). In the second portion of this procedure, the acyl-CoA was oxidized by adding acyl-CoA oxidase (ACOD) producing hydrogen peroxide which in the presence of added peroxidase (POD) allowing for the oxidative condensation of 3-methyl-N- ethyl- N- (À-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine, forming a purple colored end product with an absorption maximum at 550 nm. The amount of NEFA in the sample was determined from the optical density measured at 550 nm. (31)

Assay Procedure: 2.5 µl of sample and standard was added to each well of a 96 well

microtiter plate. 100 µl of reagent A was added to each well, mixed and incubated for 5 minutes at 37C. Then addition of 50 µl of reagent B to all the wells. The absorbance was read at 550 nm. The final absorbance of standards was plotted on the standard curve and NEFA concentrations of samples were calculated and presented in mmol/L. No fasting samples needed to be diluted to fall on the standard curve. Expected values for NEFA ranged between 1-0.4 mmol/L fasting to near zero post-prandial.

Triglyceride Concentrations:

A Serum Triglyceride Determination Kit was used for the quantitative enzymatic measurement of triglycerides in plasma. Reagents were prepared according to the kit instructions and a serial dilution of the glycerol standard was used to generate a standard curve. The Triglyceride Reagent is linear up to 10 mg/ml. The triglyceride reagent uses lipase hydrolyze triglycerides in the sample to free fatty acids and glycerol, converting the glycerol to a color product and measuring with a spectrophotometer. The spectrophotometer wavelength was set to 570 nm and the absorbance reading to zero with water as the reference. Ten µl (0.01 ml) of water, Glycerol Standard, and sample was added to the wells of a 96 well microplate. A standard working assay solution was mixed and 100 µl was added to each well. The microplate was mixed and intubated for 5 minutes at 37 °C. Absorbance (FA) of Standard and Sample at 570 nm was read and recorded. Unknowns were plotted on the standard curve and the concentrations of total triglycerides in the sample was calculated (32).

Ketone Concentrations:

Glucose, lactate, pyruvate, β-hydroxybutyrate, acetoacetic acid and the 5 carbon ketones, 3-OH valeric and 3-keto valeric acids were measured in serum by stable isotope dilution gas chromatography-mass spectrometry (GC/MS) (33).

Acylcarnitine Concentrations:

Plasma was analyzed for acylcarnitines by electrospray tandem mass spectrometry at the Biochemical Genetics Laboratory, Mayo Clinic (34).

Calculations of % MCT/C7 in peripheral circulation:

An estimated amount of medium-chain supplement observed in plasma was calculated for the meal tolerance test samples 1, 2 and 4 and for the pre and post exercise samples. The procedure for estimating amount of dose in peripheral circulation is as follows:

1. Estimate the subjects total blood volume using the Nadler equation.
2. Determine the mmol of supplement provided in the breakfast and lunch meals and in the pre-exercise supplement
3. Multiply the subjects blood volume in liters by the concentration of C8 or C7 measured in the fatty acid profile to determine total mmol in circulation
4. Divide total mmol in circulation by mmol in meal or supplement X 100; the percent of dose in peripheral circulation was plotted over time and compared between MCT and triheptanoin.

Calculations of % cumulative recovery of MCT/C7 in peripheral circulation:

It is presumed that oral MCT and triheptanoin will pass the liver and enter peripheral circulation as a free fatty acid rather than be incorporated into a triglyceride. In an effort to determine if this appears to be true, we calculated the % of MCT/C7 from an oral dose recovered in blood after the meal tolerance test. We also described the change over time of these 2 different lipid compounds. The procedure for estimating the % of dose in peripheral circulation is as follows:

1. Calculate the % of MCT/C7 in peripheral circulation at fasting, 1, 2, 4 hours post-prandial and pre and post-exercise.
2. Add % at fasting to % at 1 hour post-prandial.
3. Add % at 1 hour to % at 2 hour
4. Add % at 2 hours to % at 4 hour
5. Add % at 4 hours to % before exercise

Chapter 5

Statistical Analysis

Descriptive Statistics

The statistical analysis was run on blood samples drawn during the final study visit, after 4 months of treatment with either MCT or triheptanoin oil. The results were calculated and expressed as the mean +/- SD and 95% confidence interval for each variable. The variables that were analyzed in individuals who were supplemented with either MCT or triheptanoin oil are listed below. Each variable was plotted over time to determine the pattern of change with feeding and exercise. Change over time was summarized as total area under the curve, and % change of peak to nadir.

For specific aim #1: We used an ANOVA to determine the amount of an oral dose of MCT that makes it past the liver into peripheral circulation to be utilized by peripheral tissues for energy.

For specific aim #2*:* We used a t-test to determine if there is a difference in the metabolism and utilization of medium chain triglycerides; Trioctanoate, C8:0 and Triheptanoin, C7:0. A repeated measure of variance was performed to compare the statistical outcomes between each of the variables. $P<0.05$ was considered statistically significant for all outcomes.

Measured Variables:

1. NEFA 6 time points: fasting, 1,2, 4 hours post-prandial, pre and post exercise
2. TGY 6 time points: fasting, 1,2, 4 hours post-prandial, pre and post exercise

3. Fatty acid profiles, specifically C8 and C7 6 time points: fasting, 1,2, 4 hours post-prandial, pre and post-exercise

Calculated Variables:

1. % C7 or C8 in peripheral circulation at 1,2,4 hours post-prandial, and pre and post-exercise
2. Cumulative recovery of C7/C8 in peripheral circulation at 1, 2, 4 post-prandial and, pre and post-exercise
3. Change in C7/C8 over time from fasting, and before and after exercise

Chapter 6

Results

A total of 32 subjects completed this study (n=16 MCT; n=16 triheptanoin). Subjects who were diagnosed with CPT2, VLCAD, or LCHAD/TFP deficiencies were randomly assigned to consume MCT or triheptanoin in a defined mixed breakfast meal and before exercise. Measured macronutrient distribution of the mixed breakfast consumed was similar between study groups. Detailed diets were developed based on the subject's measured resting energy requirements (RER) plus 30% for activity and growth (estimated TEE). Supplemental oil consisted of approximately 20% of subject's estimated TEE. Blood was drawn at fasting, 1, 2, and 4 hours after eating, and before and after moderate intensity exercise. Plasma samples were analyzed for free fatty acids, quantitative total fatty acid profiles, ketones, and acylcarntines. Blood samples could not be obtained during the four-month assessment from one subject from each group (n=15 MCT; n=15 triheptanoin). All statistical analyses were performed using the statistic software Prism 6.0 (Graphpad, La Jolla, CA).

Table 4: Subject Characteristics

TRIHEPTANOIN (N=16)						*MCT (N=16)*					
Age (Yrs)	**Sex**	**Diagnosis**	**Mutations**	**Weight (kg)**	**BMI (kg/m^2)**	**Age (Yrs)**	**Sex**	**Diagnosis**	**Mutations**	**Weight (kg)**	**BMI (kg/**
7	F	LCHAD/TFP	c.1528G>C/ c.1528G>C	24.2	16.97	8	F	LCHAD/TFP	c.1528G>C/ ?	21.9	14.91
7	M	LCHAD/TFP	c.1528G>C/c.703C>T	18.6	14.21	9	M	LCHAD/TFP	c.1528G>C/ c.1528G>C	34.3	18.68
7	F	VLCAD	c.1619T>C/c.1707_1716dupGACGGGGCC	26.4	17.42	9	M	CPT2	c.338C>T/c.340+3A>T	29.8	16.57
11	M	LCHAD/TFP	c.1528G>C/ c.1528G>C	64.5	23.86	11	F	LCHAD/TFP	c.1528G>C/ c.1528G>C	49.0	23.18
16	M	LCHAD/TFP	c.1150-1G>T/ c.208T>C	80.7	24.36	15	M	CPT2	c.338C>T / c.1239_1240delGA /c.1342T>C	72.8	26.42
21	F	CPT2	Common mutations not detected	63.2	23.70	16	F	CPT2	c.338C>T/c.1666_1667delTT	69.3	24.70
23	M	LCHAD/TFP	c.1528G>C/ ?	65.5	22.09	17	F	LCHAD/TFP	c.1528G>C/ c.1528G>C	65.5	23.07
24	F	LCHAD/TFP	c.1528G>C/c.479-482T AGC>AATA	55.2	21.56	17	F	LCHAD/TFP	c.901G>A/?	44.7	18.49
29	F	LCHAD/TFP	c.1528G>C/ c.1528G>C	63.8	23.55	19	M	CPT2	c.338C>T and c.1666_1667delTT	91.7	29.60
33	M	VLCAD	c.1322G>A/ c.1837C>T	92.1	31.31	24	F	CPT2	c.1500_1502delCTT and c.1500_1502delCTT	54.4	21.65
36	F	VLCAD	No DNA analysis available	68.1	24.21	24	F	CPT2	c.338C>T/?	91.4	32.23
39	F	CPT2	No DNA analysis available	93.8	32.65	27	F	VLCAD	c.898A>C/c.1097G>A	82.1	27.43
39	M	VLCAD	c.343delG/c.1244C>T	88.0	27.50	39	F	VLCAD	c.637G>A/ c.1065_1067delCAT	61.1	20.37
41	F	CPT2	c.338C>U/c.1238_1239delAG	63.5	26.50	42	M	VLCAD	c.637G>A/ c.1065_1067delCAT	113.0	31.47
41	F	CPT2	c.338C>T/c.1511C>T	81.4	28.17	43	M	VLCAD	c.694G>A/c.1388G>A	94.8	30.23
64	F	CPT2	c.338C>T/ c.338C>T	45.1	18.53	43	F	CPT2	c.338C>T/c.1239>1240delGA	97.1	34.53
27.38				62.13	23.54	22.69				67.06	24.60
15.93				23.51	5.09	12.64				26.80	5.96

Table 4: Individual subject characteristics for each participant include age in years, gender (Sex; F= female; M= male), long-chain FAO diagnosis (LCHAD/TFP = long-chain hydroxyacylCoA dehydrogenase/trifunctional protein, VLCAD = very long-chain acylCoA dehydrogenase, CPT2 = carnitine palmitoyltransferase 2 deficiencies), mutations note the change in the respective cDNA of the gene corresponding to the protein listed under diagnosis, body weight in kg, and body mass index (BMI). Mutations: ? = no 2nd mutation was identified after sequencing all the exons of the gene; for CPT2, common mutations not detected indicates sequencing for the 6 common mutations in CPT2 was completed but complete sequencing of the gene was not performed. Mean for age, body weight, BMI and % of kcals from study oil is provided below the respective column for each group and the standard deviation is listed below the mean.

Figure 3: Macronutrient Distribution of Standardized Meal

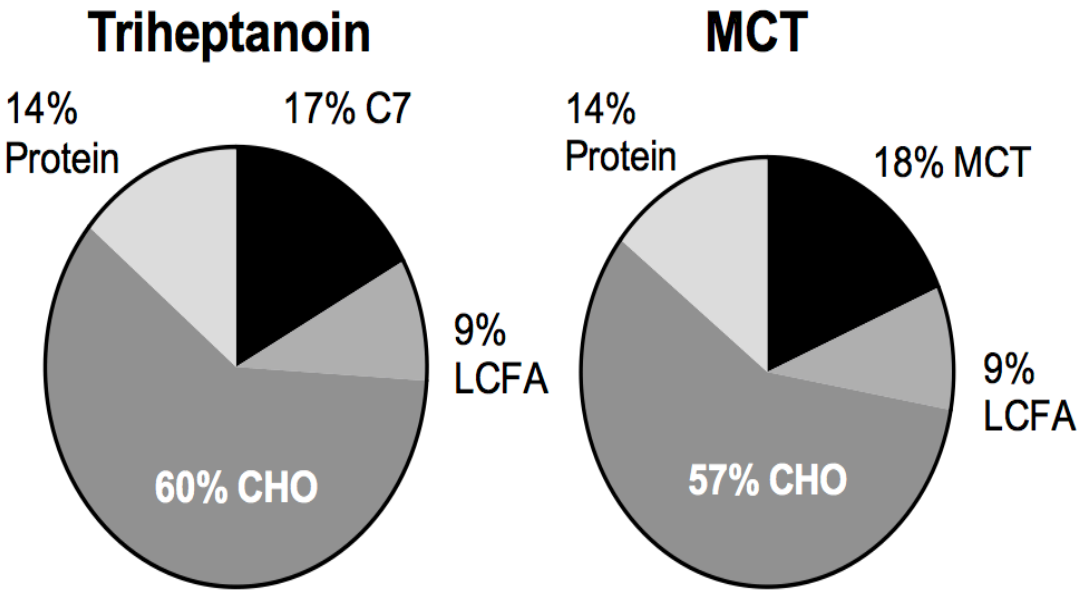

Figure 3: Consumed macronutrient distribution of the defined mixed meals subjects were fed on day 2 of the 4-month assessment. Meals were weighed before and after consumption and nutrient intake calculated with Pronutra software. Distribution of macronutrient content is displayed in %. Triheptanoin/MCT was fed at 20% of subject's total estimated energy needs. Detailed diets were developed based on the subject's measured RER plus 40% for activity and growth. Meal composition and amount of supplemental oil given were similar between the two groups.

Table 5: Amount of Supplement Consumed

MCT			*TRIHEPTANOIN*		
Subject #	**Standardized Meal (g)**	**Oral Dose (g)**	**Subject #**	**Standardized Meal (g)**	**Oral Dose (g)**
3	6.9	17.1	1	6.2	26.2
4	5.8	26.6	2	5.1	19.6
7	6.0	15.3	5	7.0	9.4
8	5.1	25	6	9.0	7.4
11	6.7	20.8	10	5.66	19.9
14	2.6	4.6	12	7.2	10.5
17	9.8	7.6	13	7.1	15.9
18	6.7	16.1	15	7.7	18.9
19	8.2	13.6	16	7.5	14.9
21	9.2	20.3	22	6.4	12.6
24	8.2	14.8	23	8.6	10.4
51	7.5	28.6	55	12.3	27.3
52	5.8	22.4	56	4.01	8.92
53	9.8	14.1	57	4.3	10.36
54	5.1	17.48	58	7.5	29.1

Table 5: Amount of supplemental oil given prior to exercise displayed in g. Subjects were given a carbohydrate and protein free beverages containing MCT or triheptanoin oil 20 minutes prior to starting exercise. Supplemental dose of oil was measured based on the subject's lean body mass which was measured by a DEXA scan. Dosing started at 0.5g/kg at the beginning of the study but was decreased to 0.3g/kg to decrease incidences of gastrointestinal side effects.

Free Fatty Acids

Plasma FFA were analyzed to determine the proportion of FFA present in peripheral circulation before and after an oral dose of supplemental oil. Severely hemolyzed blood samples from one subject in the triheptanoin group could not be analyzed and therefore was removed (n=14). During fasting or exercise, lipid stores from adipose tissue are the major source of FFA in plasma. Under normal conditions, it is expected that circulating concentrations of FFA are highest at these time points and decrease with feeding.

Plasma FFA followed a similar pattern in both MCT and triheptanoin groups. FFA were high at fasting, declined with feeding and gradually started to rise again over time. In both groups, FFA were highest post-exercise. As expected, there were no significant differences in the rise or decline of plasma FFA at any time point between the two groups.

Figure 4: Plasma Free Fatty Acids

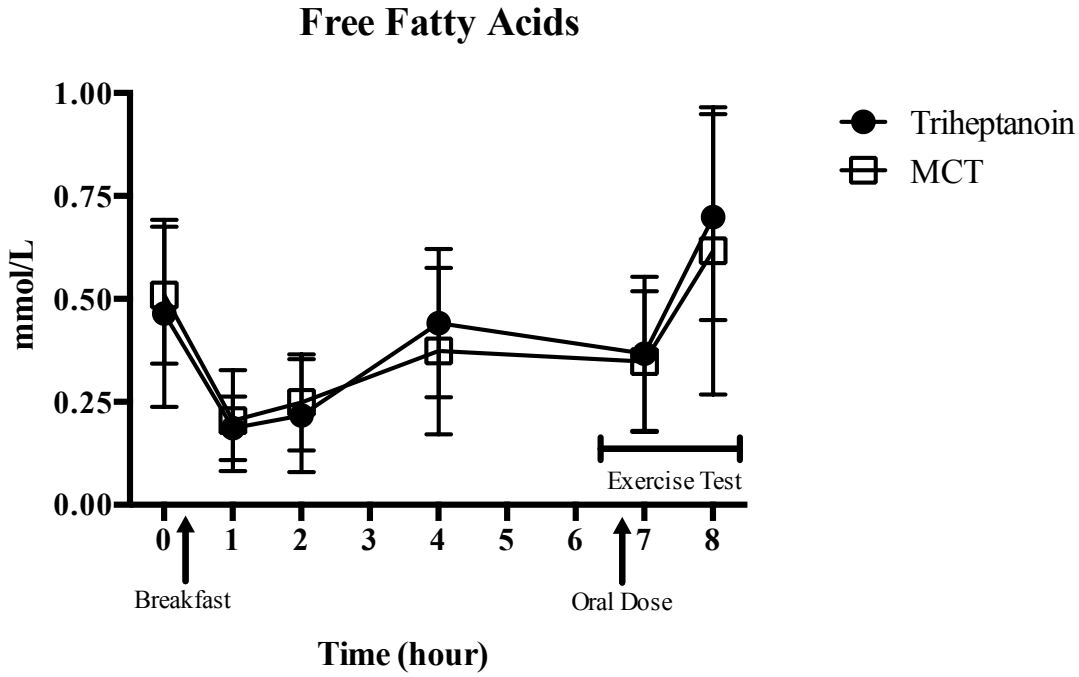

Figure 4: Mean ± SD plasma free fatty acids at different time points: fasting (hr 0), 1, 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 & 8) in thirty subjects randomized to either triheptanoin or MCT. The pattern and rate of changes in plasma FFA were similar between the two groups. FFA were high at fasting, declined with feeding and gradually started to rise again over time. In both groups, FFA were highest post-exercise. There were no significant differences at any of the time points.

Fatty Acid Profiles

Quantitative fatty acid profiles measure the total concentration of individual fatty acid species and do not differentiate between fatty acids that are bound or free. Plasma concentrations of heptanoate (C7) and octanoate (C8) were analyzed to determine if there is a difference in the metabolism and utilization of MCT (C8) and triheptanoin (C7). In

addition, plasma concentrations of palmitate (C16), a long-chain fatty acid was analyzed as a method of comparison when evaluating absorption patterns.

D'Agostino and Pearson omnibus test was used to test for normality. The data distribution for both C7 and C8 data sets were not normal, and therefore were log transformed. Statistical comparisons were made on log transformed data. Outliers were identified using the ROUT method. A total of 5 outliers were removed from the MCT group and 8 from the triheptanoin group. Data distribution for C16 was normally distributed and no outliers were identified.

Statistical tests were run with and without outliers for both C7 and C8. The statistical differences observed were similar with and without outliers. Because they did not alter the conclusions, the data including outliers are presented in the results.

After a mixed breakfast meal containing either MCT or triheptanoin, there was a gradual rise in total C8 and C7, which peaked at 4 hours. The rise in plasma C8 levels in the MCT group were significantly greater than the rise in C7 levels in the triheptanoin group. Area under the curve (AUC) was calculated, which indicated that the total change in plasma concentrations of C8 was significantly greater than C7 after a meal ($P \leq 0.0015$).

Subjects were given an oral dose of MCT or triheptanoin in a carbohydrate-protein free beverage 20 minutes prior to starting exercise. Supplemental dose of oil was measured based on the subject's lean body mass. Significant rises in both plasma C8 levels in the MCT group and plasma C7 levels in the triheptanoin group were observed from 4 hours post-prandial to pre-exercise, and from pre to post exercise.

Post-prandial long-chain C16 concentrations followed a similar pattern in comparison to C7 and C8. Plasma C16 levels did not increase with exercise, but rather declined in the triheptanoin group. However, this finding was not significant, and there where no significant differences in the rise or decline of plasma C16 levels at any time points between the two groups.

Figure 5: Medium-Chain Fatty Acid Profiles

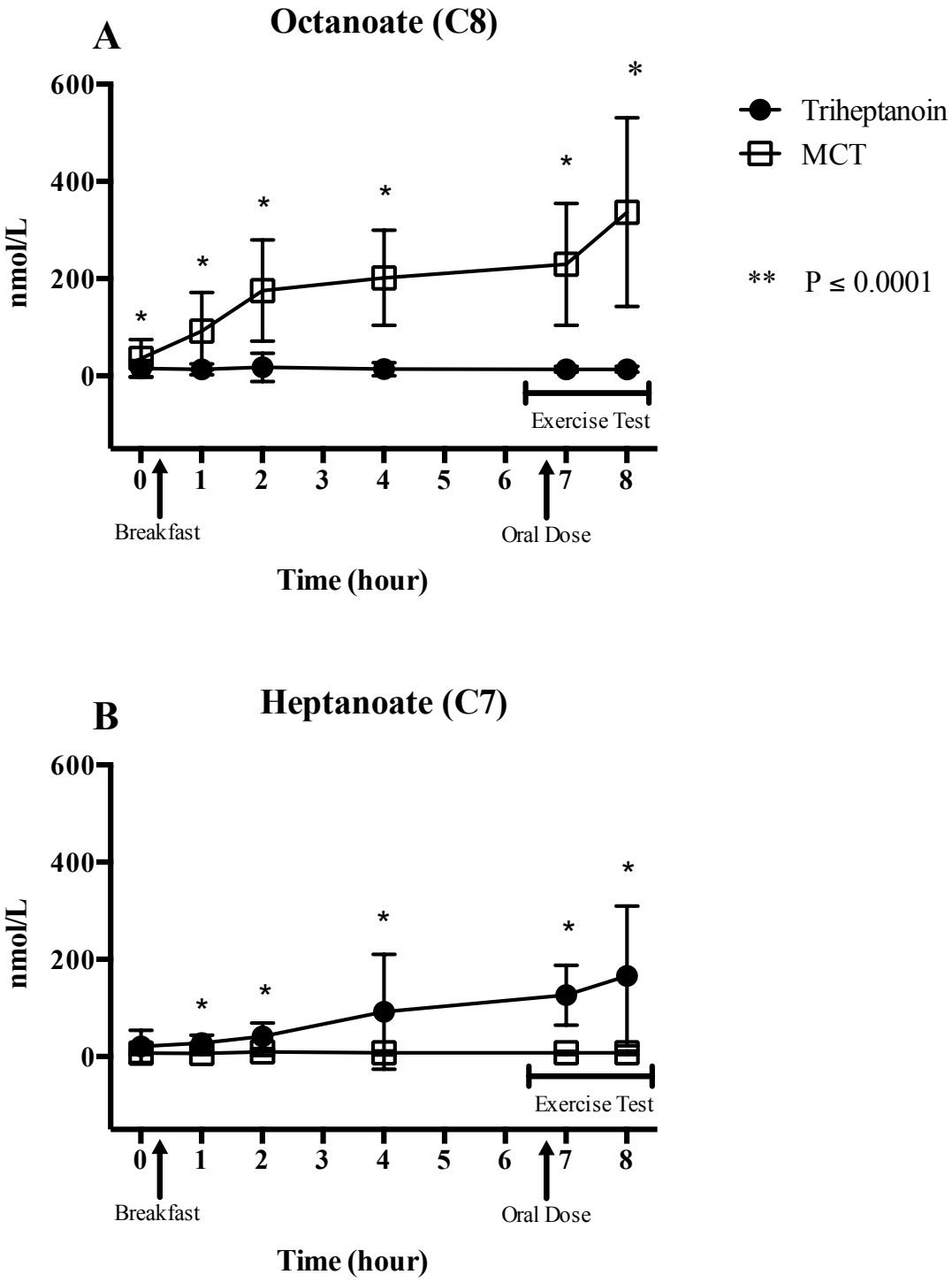

Figure 5: Mean ± SD quantitative medium-chain fatty acid profiles C7 and C8 in plasma at different time points: fasting (hr 0), 1, 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 & 8) in thirty subjects randomized to either triheptanoin or MCT. **(A)** Gradual rise from fasting in plasma C8, peaking at 4 hours after mixed meal in MCT group. Significant rise in C8 from pre to post-exercise was also observed in MCT group. No change from fasting in plasma C8 concentrations at any time points in triheptanoin group. **(B)** Gradual rise from fasting of plasma C7, peaking at 4 hours after mixed meal in triheptanoin group. Significant rise in C7 from pre to post-exercise was also observed. No change from fasting in plasma C7 concentrations in MCT group at any time points. The rise in C8 levels observed in MCT group was greater than the rise in C7 levels in triheptanoin group. Significantly lower levels of C7 in the blood than levels of C8 after comparable intakes.

Figure 6: Total Change in Plasma C8 and C7 Post-Prandial

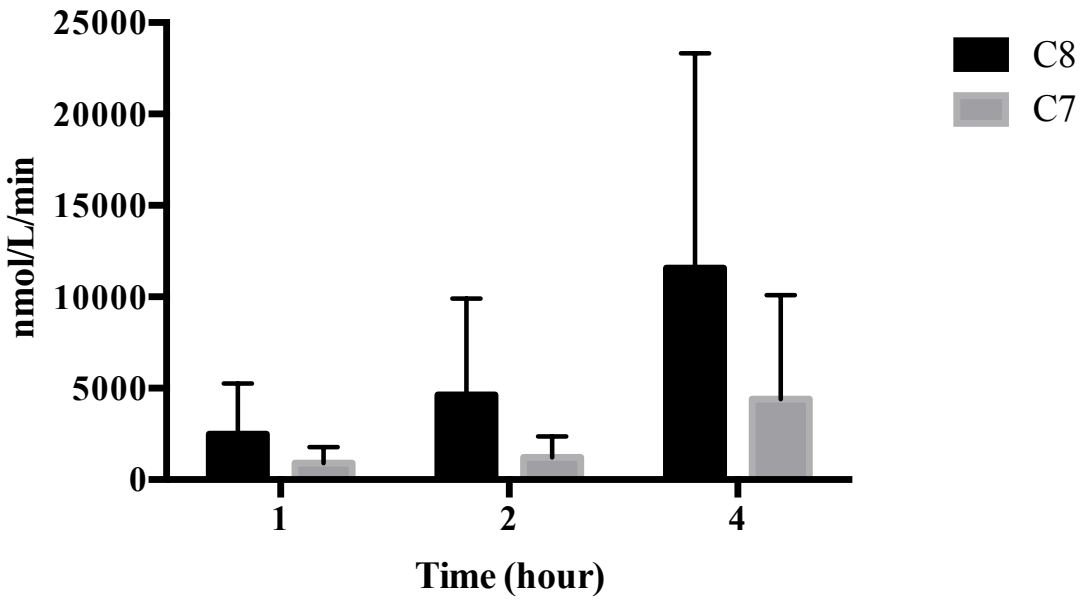

Figure 6: Mean ± SD total change in plasma quantitative fatty acid profiles C7 and C8 at 1, 2 and 4 hours after a defined breakfast meal. Gradual rise from fasting in both C8 and C7 levels, peaking at 4 hours. Significantly greater total change in plasma concentrations of C8 in comparison to C7 at $P \leq 0.0015$.

Figure 7: Long-Chain Fatty Acid Profiles

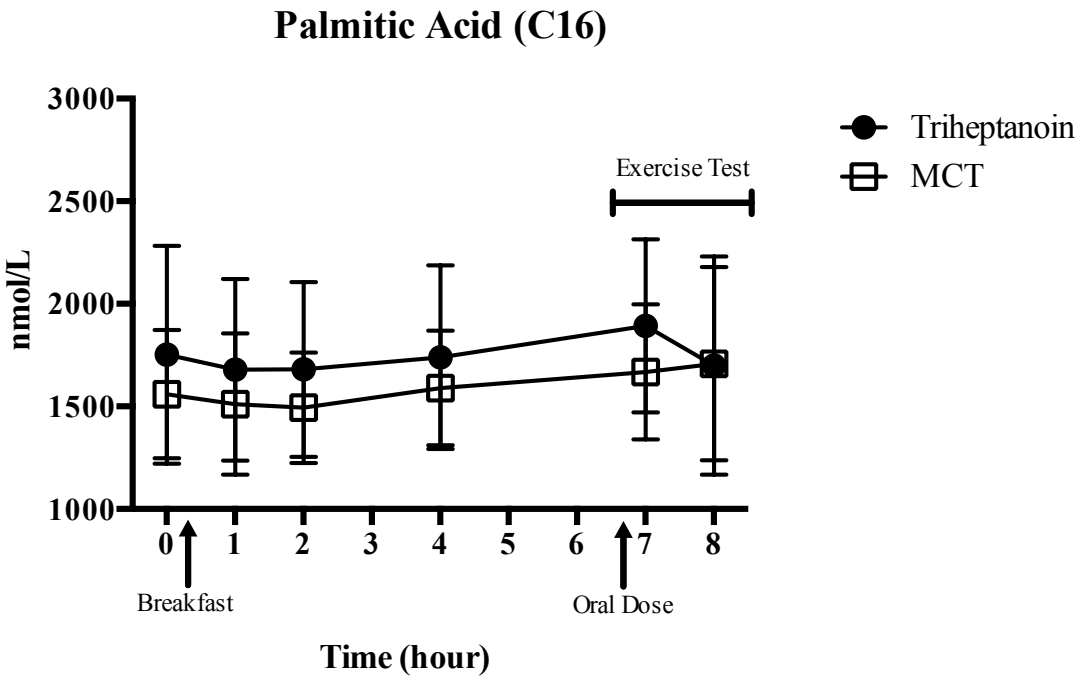

Figure 7: Mean ± SD quantitative long-chain fatty acid profile C16 in plasma at different time points: fasting (hr 0), 1, 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 & 8) in thirty subjects randomized to either triheptanoin or MCT. A decline after fasting, followed by a gradual rise in plasma C16 observed 1 hour after a defined mixed meal, which peaked at 4 hours. Plasma C16 concentrations were highest pre-exercise and declined post-exercise in the triheptanoin group alone. A slight rise in plasma C16 from pre to post-exercise was observed in the MCT group. No significant differences at any of the time points between the two groups, suggesting changes in plasma C8/C7 are due to supplement alone.

Cumulative Recovery

Cumulative recovery was calculated to assess the differences in the percent of C8/C7 recovered in plasma after both a mixed meal containing either MCT or triheptanoin. The percent recovered between the two groups were analyzed to determine if there is a difference in the metabolism and utilization of MCT (C8) and triheptanoin (C7).

D'Agostino and Pearson omnibus test was used to test for normality. The data distribution for both data sets were not normal, and therefore were log transformed. Outliers were identified using the ROUT method. A total of 2 outliers were removed from the MCT group and no outliers were removed from the triheptanoin group when analyzing cumulative recovery of C8. A total of 2 outliers were removed in the triheptanoin group and no outliers removed from the MCT group when analyzing cumulative recovery of C7. Statistical tests were run with and without outliers. The statistical differences observed were similar with and without outliers. Because they did not alter the conclusions, the data with outliers included are presented in the results.

The percent of both C7 and C8 recovered in the blood after a mixed meal and an oral dose before exercise resembled the pattern of plasma fatty acid profiles. There was a gradual rise in the percent of C8 and C7 recovered in peripheral circulation, in which both peaked at 4 hours. The percent of C8 recovered from the MCT group was significantly greater than the percent of C7 recovered in the triheptanoin group after comparable doses.

Figure 8: Cumulative Recovery

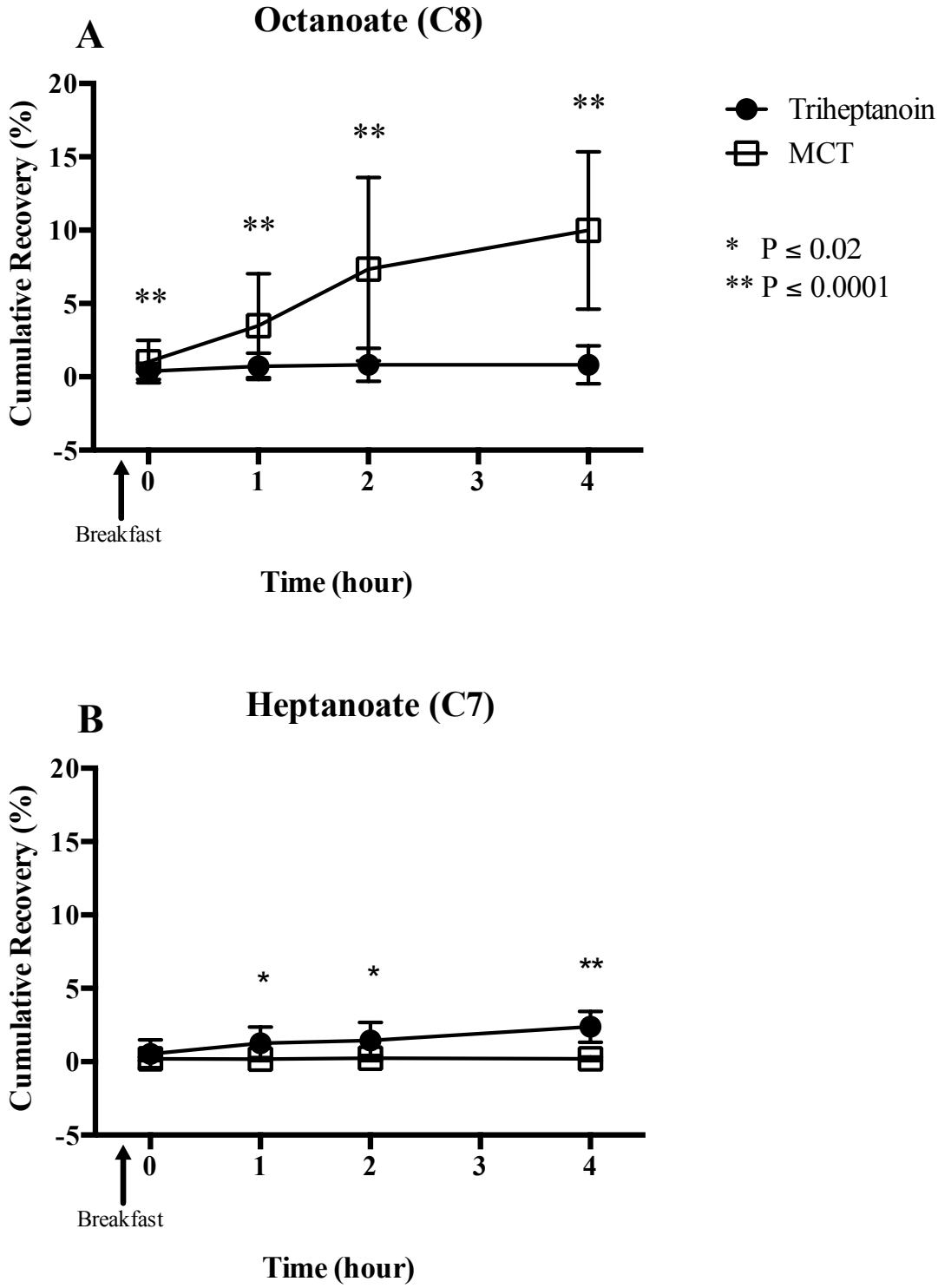

Figure 8: Mean ± SD percent cumulative recovery of C7 and C8 in plasma at different time points: fasting (hr 0), 1, 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 & 8) in thirty subjects randomized to either triheptanoin or MCT. **(A)** Gradual rise from fasting in the % of C8 recovered after a mixed meal, peaking at 4 hours in MCT group. No change at any of the time points in the % of C8 recovered in the triheptanoin group. **(B)** Gradual rise from fasting in the % of C8 recovered after a mixed meal, peaking at 4 hours in MCT group. No change at any of the time points in the % of C7 recovered in MCT group. The % of C8 recovered in MCT group compared to % of C7 recovered in triheptanoin group is significantly greater after comparable intakes.

Ketones

With increased fat intake, free fatty acids taken up by the liver are metabolized into ketones which can supply the brain and other peripheral tissues with an alternative source of energy. The majority of ketones produced by the liver from fatty acids are typically four carbons in length, referred to as 4-carbon ketones (ß-OH-butyrate and acetoacetate). However, odd-chain fatty acids such as heptanoate (C7) can produce ketones that are 5 carbons in length (3-OH-valeric and 3-keto-valeric) due to their extra carbon. Ketones were analyzed to determine the portion of MCT and triheptanoin metabolized by the liver.

D'Agostino and Pearson omnibus test was used to test for normality. The data distribution for both 4 and 5-carbon ketones were not normal, and therefore were log transformed. Statistical comparisons were made using log transformed data. The ROUT

method was used to identify outliers. No outliers were identified and therefore, none were removed.

The pattern and rate of 4-carbon ketone production was similar between the two groups, with no significant differences at any of the time points. Four-carbon ketone production was greatest post-exercise in subjects randomized to both triheptanoin and MCT.

A gradual rise from fasting in 5-carbon ketone production, which peaked at 4 hours after a mixed meal and was highest after exercise, was observed in the triheptanoin group alone. As expected, there was no change from fasting in 5-carbon ketone concentrations at any time points in subjects randomized to MCT. Significant differences between the two groups were observed at 2 and 4 hours post-prandial, and pre and post-exercise ($P \leq 0.0001$).

Figure 9: Ketones

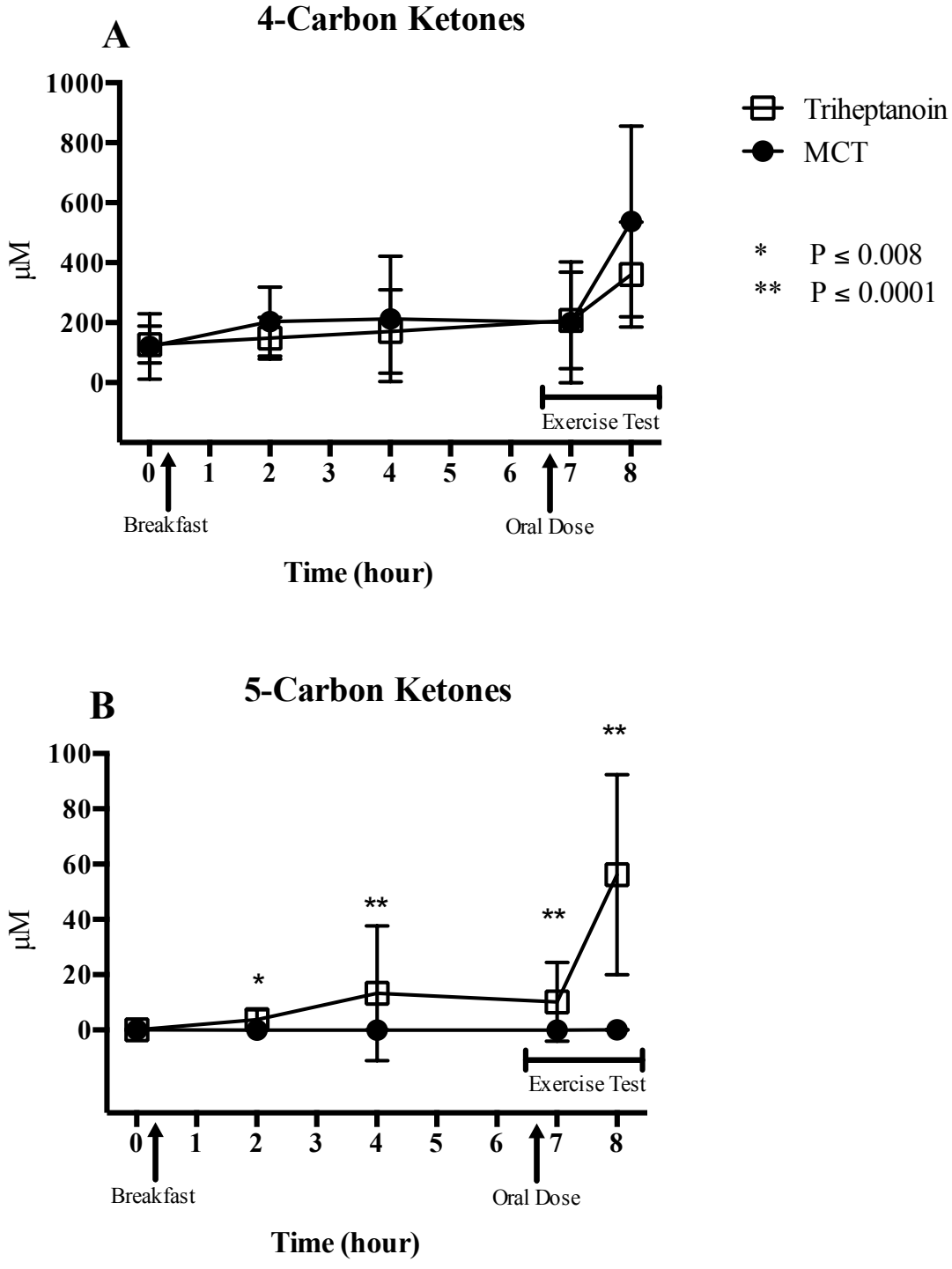

Figure 9: Mean ± SD 4-carbon and 5-carbon ketone bodies in plasma at different time points: fasting (hr 0), 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 &

8) in thirty subjects randomized to either triheptanoin or MCT. **(A)** The pattern and rate of changes in 4-carbon ketone production were similar between the two groups. **(B)** Significant rise in production of 5-carbon ketones in the triheptanoin group. Five-carbon ketones peaked at 4 hours post-prandial and were highest post-exercise. No change from fasting in 5-carbon ketone production in MCT group.

Acylcarnitines

Acylcarnitines are key intermediates in fatty acid metabolism and are the transport form for fatty acids crossing into the mitochondria. Acylcarnitines can be used as biochemical markers to assess fatty acid oxidation and therefore, were analyzed to determine what portion of MCT and triheptanoin that were oxidized by peripheral tissues.

D'Agostino and Pearson omnibus test was used to test for normality. The data distribution for both even and odd-chain acylcarnitine data sets were not normal, and therefore were log transformed. Statistical comparisons were made using log transformed data. The ROUT method was used to identify outliers. No outliers were identified and therefore, none were removed.

A minimal change from fasting in plasma even-chain acylcarnitine concentrations were observed in the MCT group. No change was observed in the triheptanoin group. Significant differences between groups were observed at 2 hours post-prandial ($P \leq 0.03$) and after exercise ($P \leq 0.009$).

A gradual rise from fasting in plasma concentrations of odd-chain acylcarnitines were observed in the triheptanoin group alone. Odd-chain acylcarnitine concentrations were highest at 4 hours after a mixed meal and post-exercise. Statistically significant

differences between the two groups were observed 2 and 4 hours post prandial, and before and after exercise ($P \leq 0.0001$)

Figure 10: Acylcarnitines

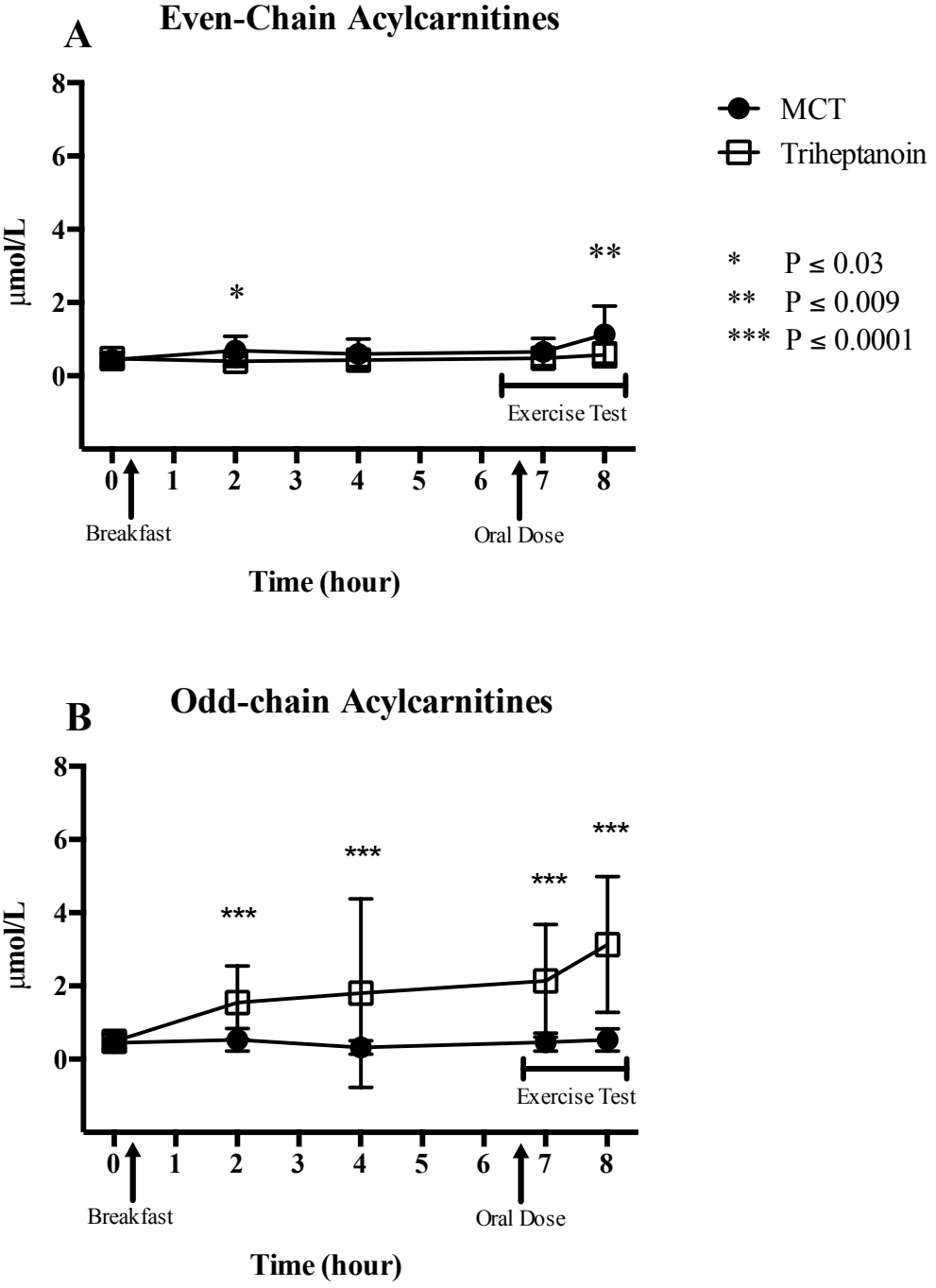

Figure 10: Mean ± SD even and odd-chain acylcarnitines in plasma at different time points: fasting (hr 0), 2 and 4 hrs after a mixed meal, and pre and post-exercise (hrs 7 &

8) in thirty subjects randomized to either triheptanoin or MCT. **(A)** Small change from fasting in even-chain acylcarnitines concentrations in MCT group. No change from fasting in triheptanoin group. **(B)** Significant change from fasting in plasma concentrations of odd-chain acylcarnitines in triheptanoin group. Odd-chain acylcarnitine concentrations were greatest at 4-hours post-prandial and post-exercise. No change from fasting in MCT group.

Triglycerides

Triglycerides were analyzed to determine the portion of TGY present in peripheral circulation after an oral dose of supplemental oil. After analyzing assay results, we determined that our TGY levels obtained for both groups suggested in-accurate values measured in the laboratory that were outside the range of physiologically expected values. To confirm our suspicion that the results were inaccurate, our lab results were compared to fasting TGY panels taken at the 4-month study admission completed in the hospital core laboratory. Our assay results were significantly lower than panel TGY levels. Due to these unforeseen circumstances, TGY data will no longer be included in this study.

Chapter 7

Discussion

The most interesting significant finding of this study was that quantitative fatty acid profiles C7 and C8 peaked at 4 hours after a defined mixed breakfast meal. We initially hypothesized that both MCT and triheptanoin would be rapidly absorbed via the portal vein and peak in peripheral circulation at 1 hour post-prandial. The peak at 4 hours in peripheral circulation suggests that both MCT and triheptanoin were not rapidly absorbed across the intestinal mucosa into the portal venous system. Instead, our results suggest that the absorption pattern of C7 and C8 were similar to LCT, in that some of the MCFA were incorporated into chylomicrons and transported from the gut into the lymphatic system.

We predicted that the majority of even-chain MCT would be metabolized by the liver, increasing the rate of ketone production. However, this was not observed other than post exercise when an oral dose of MCT was given alone. The pattern and rate of changes in 4-carbon ketone production from fasting were statistically similar between the two groups. We observed a slightly greater production in 5-carbon ketones in comparison to four, however overall ketone production was still far less than what we hypothesized. The small fraction of both MCT and triheptanoin metabolized by the liver into ketones, supports our theory that neither MCT or triheptanoin were rapidly absorbed through the portal vein like expected. Fatty acids that are absorbed from the gut into the portal vein, rapidly appear in peripheral circulation. It is predicted that rapid absorption of fatty acids into portal circulation results in increased ketone production by the liver. Fatty acids

reach the liver first in great abundance – stimulating the Ketogenesis pathway. However, this was not observed in our study. We believe MCFA from both MCT and triheptanoin were instead incorporated into chylomicrons, exited the gut into the lymphatic system, where they were hydrolyzed, taken up and potentially oxidized by peripheral tissues first and reached the liver last.

In support of our chylomicron theory, a study done in 1968 by Lee et.al, which assessed the effects that LCT had on the digestion and absorption of MCT. Rats were fed through thoracic cannulas, either odd-chain MCT tripelargonin (C9) alone or simultaneously with LCT in the form of safflower oil. Results concluded that when rats were fed MCT alone, less than one percent of C9 was transported via chylomicrons (10). Whereas, when rats were fed MCT and LCT simultaneously, there was a 3-fold increase in the amount of C9 present in chylous transport (10). Their findings support that intake of LCT/MCT together in a mixed meal affects the proportion of MCFA transported in chylomicrons. This explains the decreased production of ketones and peak in fatty acid profiles observed in both groups of this study.

Interestingly, there was a greater concentration of plasma C8 in comparison to C7 in the blood after comparable intakes. In addition, the percent of C8 recovered in plasma was significantly higher than the percent recovery of C7. There were no significant differences in plasma concentrations of long-chain C16 over time, demonstrating that differences observed in plasma C8 and C7 levels are primarily due to supplementation. We believe that significantly lower levels of C7 in the blood could have been due to one of the following reasons: decreased absorption of C7 at the level of the gut, decreased sensitivity to lab measures when analyzing quantitative fatty acid profiles for C7,

increased removal of C7 from circulation or C7 is being converted into a different substrate such as acylcarnitines or glucose. There was no difference between groups in the reported incidence of diarrhea or gastrointestinal complaints suggesting similar absorption of the two oils making the first possibility unlikely, but balance studies were not conducted.

We observed a significantly greater production of odd-chain acylcarnitines in comparison to even-chain, suggesting that heptanoate is being partially oxidized by peripheral tissues. These findings also suggest that cellular uptake and clearance of C7 occurs at a faster rate in comparison to C8. This may explain the low levels of circulating C7 and high odd-chain acylcarnitine concentrations observed in blood. This study did not measure the rate at which C7 entered and exited tissues. Flux studies need to be performed to assess the pattern and rate in which C7 is partially oxidized to acylcarnitines to determine the true pattern of absorption and utilization of triheptanoin.

The dogma of the digestion and absorption of MCT is that MCFA are rapidly absorbed across the intestinal mucosa into the portal venous system. Animal studies have shown that MCFA appear in circulation within minutes of absorption and they do so in great abundance (5). Once in circulation, they reach the liver first and are readily available to be metabolized, increasing the rate of ketone production. MCFA are rarely incorporated into chylomicrons because they are not readily re-esterified into triacylglycerols.

MCT supplementation plays a key role in the medical nutrition therapy of individuals with long-chain fatty acid oxidation disorders. Therapeutic doses of MCT are often mixed into meals that contain LCT. The majority of studies done in both animals

and humans administer MCT doses either alone or in conjunction with carbohydrates (3, 4, 5, 11, 24). The amount of literature assessing the effects of LCT on MCT metabolism when fed simultaneously is minimal. Our results challenge the dogma of MCT digestion and absorption because our findings support that intake of MCT/LCT together in a mixed meal affects the proportion of MCFA transported in chylomicrons. In addition, our results demonstrate that the digestion and absorption of MCT is still not well understood. To better understand the general metabolism of MCT, more studies exploring the effects of LCT on MCT digestion and absorption are necessary.

There were many strengths to our study. First, our study was very well controlled. The study's sample size of thirty-two participants is the largest study completed in this patient population. The diets subjects were fed during both study admissions were very specific, therefore we knew the exact amount and content of what they consumed. Lastly, study diets were defined and blood samples were drawn at defined time points.

Limitations of our study include the following: our measurements were taken at one point in time, not controlling for the flux of fatty acids when assessing what portion of C7/C8 were oxidized by tissues. Future studies should consider analyzing flux data to achieve a better understanding of the utilization of MCT by tissues. In addition, we did not measure chylomicron transport. We recommend that future studies investigating the effects of LCT on MCT metabolism, should directly measure the amount of MCT transported in chylomicrons after a mixed meal containing LCT. Lastly, we did not measure the amount of C7 excreted in feces. Therefore, we cannot definitively rule out that lower levels of C7 in the blood are due to malabsorption of C7 at the level of the gut.

In conclusion, the unexpected peak in plasma levels at 4 hours rather than 1 hour, observed in both MCT and triheptanoin groups, suggest that MCFA were not rapidly absorbed through the portal vein, but instead incorporated into chylomicrons. Only a small fraction of both MCT and triheptanoin were metabolized into ketones, further supporting chylomicron transport of MCFA. Lastly, acylcarnitine data suggests partial oxidation of heptanoate by peripheral tissues, contributing to lower levels of C7 compared to C8 in the blood.

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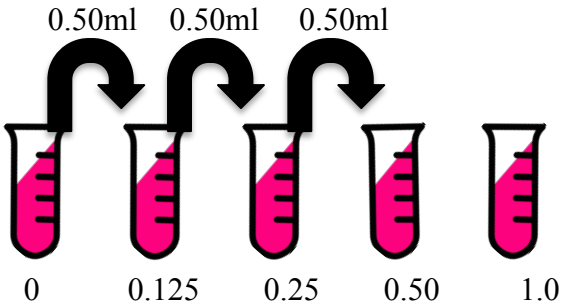
Appendix 1: Free Fatty Acid Assay Procedure

Materials:

1. 96 well plate
2. NEFA Assay Kit

Methods:

1. Label 5 test tubes as follows:
    a. 0 mmol/L
    b. 0.125 mmol/L
    c. 0.25 mmol/L
    d. 0.50 mmol/L
    e. 1.0 mmol/L
2. Start with a serial dilution by adding 0.5ml of H2O to all test tubes except test tube labeled 1.0.
    a. Add 1ml of standard to test tube labeled 1.0.
    b. Add 0.50ml of 1.0 to test tube labeled 0.50 and vortex.
    c. Add 0.50ml of 0.50 to test tube labeled 0.25 and vortex.
    d. Add 0.50ml of 0.25 to test tube labeled 0.125 and vortex.



3. In duplicate, add 2.5 µl of standards (row A) and samples (row B-H) into wells
4. Add 100 µl of Reagent A to all wells
5. Cover and vortex.
6. Incubate well plate at 37°C for 5 minutes
7. Add 50µl of color reagent B to all wells
8. Read absorbance at 550nm

Appendix 2: Evidence Table

#	*STUDY ID*	*STUDY DESIGN*	*SUMMARY/ ABSTRACT*
14	Abumrad N, Harmon C, Ibrahimi A. Membrane transport of long-chain fatty acids: evidence for a facilitated process. J Lipid Res 1998; 39:2309-2318	Review	Summarizes research relating to the mechanism of fatty acid transport. Includes facilitated uptake processes in relation to the different cell types or membrane systems. Discusses recent knowledge related to membrane proteins thought to be implicated in the uptake processes and factors that may modulate uptake or alter the relative contribution of passive versus facilitated components. In addition, reviews molar ratio of fatty acid to its physiological carrier, plasma albumin and the metabolic or hormonal milieu.
6	Bach AC, Babayan VK. Medium-chain triglycerides: an update. Am J Clin Nutr 1982;36:950-62.	Review	A review of the literature on the medical and nutritional use of medium-chain triglycerides since the 1970. Discusses medical nutrition therapies, and the role of MCTs in the synthesis of certain structured lipids. MCT metabolism is discussed, which includes extrahepatic tissues and the liver. Recent applications of MCTs and modified MCTs in hyperalimentation, deficiency in the carnitine system, epilepsy, obesity, and other special areas of application are discussed.
28	Behrend AM, Harding CO, Shoemaker JD. Substrate oxidation and cardiac performance during exercise in disorders of long chain fatty acid oxidation. Mol Genet Metab. 2012;105:110-115.	Experimental	Experimental study in evaluating the influence of isocaloric MCT v.s CHO supplementation prior of exercise on substrate oxidation and cardiac workload in participants with fatty acid oxidation disorders. MCT supplementation increased the oxidation of medium-chain fats and decreased the oxidation of glucose during exercise in comparison to CHO supplementation
26	Border JR, Burns GP, Rump C, Schenk WG JR. Carnitine levels in severe infection and starvation: a possible key to the prolonged catabolic state. Surgery 1970; 68:175-179	Experimental	Experimental study that explores the effects of starvation and infection on carnitine levels in human and dog. Skeletal muscle carnitine levels rise in the dog with starvation to roughly twice the normal level. An equal degree of starvation plus peritonitis is associated with unchanged skeletal

			muscle carnitine levels. Normal human skeletal muscle levels are essentially the same as in the dog. Suggested that these changes are in the direction expected for a limitation of fat catabolism and, in the presence of a limited exogenous source of glucose, that this would result secondarily in a protein catabolic state to supply glucose for the body's energy needs.
20	Brunengraber H, Roe CR. Anaplerotic molecules: current and future. J Inherit Metab Dis 2006;29:327-31.	Review	Presents the concepts of anaplerosis and cataplerosis in relation to the regulation of citric acid cycle operation. Anaplerosis is the re-filling of the catalytic intermediates of the cycle that carry acetyl-CoA as it is oxidized. Cataplerosis balances anaplerosis by removing excess intermediates from the citric acid cycle. The properties of the main anaplerotic substrates are reviewed from the point of view of potential clinical applications to the treatment of some inherited and acquired conditions.
2	Crozier G, Bois-Joyeux B, Chanez M, Girard J, Peret J. Metabolic effects induced by long-term feeding of medium-chain triglycerides in the rat. Metabolism 1987;36:807-14.	Experimental	Energy intake, weight gain, carcass composition, plasma hormones and fuels, hepatic metabolites and the activities of phosphoenolpyruvate carboxykinase (PEPCK), malic enzyme, and glucose 6-phosphate dehydrogenase (G6P-DH) were examined in adult rats during a 44-day period of low fat, high carbohydrate (LF) feeding or of consumption of one or two high fat diets composed of LCT or MCT. Energy intake was similar in the LCT and MCT groups but was less than that of LF group. Blood ketone body concentrations in rats fed the high fat diets were extremely elevated, particularly in the MCT group.

11	Gillingham MB, Scott B, Elliott D, Harding CO. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. Mol Genet Metab 2006;89:58-63.	Experimental	Nine subjects completed two 45 min moderate intensity treadmill exercise tests. Subjects were given 4oz of orange juice alone or orange juice and 0.5 g MCT per kg LBM, 20 min prior to exercise in a randomized cross-over design. Blood levels of acylcarnitines, creatine kinase, lactate, and beta-hydroxybutyrate were measured prior to and immediately after exercise, and after 20 min rest. Cumulative long-chain 3-hydroxyacylcarnitines were 30% lower and beta-hydroxybutyrate was three-fold higher after the MCT-pretreated exercise test compared to the test with orange juice alone. Coordinating MCT supplementation with periods of increased activity may improve the metabolic control of children with LCHAD and TFP deficiency following exercise.
13	Hajri, T, Abumrad NA. Fatty Acid Transport Across Membranes: Relevance to nutrition and metabolic pathology. Annu Rev Nutr, 22:383-415	Review	Discusses long-chain fatty acid transport, and it's relevance to nutrition therapy and metabolic pathology
24	Jeukendrup AE, Saris WH, Schrauwen P, Brouns F, Wagenmakers AJ. Metabolic availability of medium-chain triglycerides coingested with carbohydrates during prolonged exercise. J Appl Physiol 1995;79:756-62.	Experimental	Examined the metabolic response to MCT ingestion with or without carbohydrates. The rate of MCT oxidation increased more rapidly during the HCHO+MCT and CHO+MCT trials compared with the MCT trial, yet in all three cases the oxidation rate stabilized at 0.12 g/min during 120-180 min of exercise. It is concluded that more MCTs are oxidized when ingested in combination with CHOs. MCTs might serve as an energy source in addition to glucose during exercise because the metabolic availability of MCTs was high during the last hour of exercise, with oxidation rates being approximately 70% of the ingestion rate.
19	Kompare M, Rizzo W. Mitochondrial fatty-acid oxidation disorders. Seminars in Pediatric Neurology 2008; 107(9091) 140-149	Review	Discusses the different FAO disorders and therapeutic approaches are generally effective in preventing severe symptomatic episodes, including sudden death. Newborn

			screening for fatty-acid oxidation disorders promises to identify many affected patients before the onset of symptoms.
15	Lepine AJ, Boyd RD, Welch JA, Roneker KR. Effect of colostrum or medium-chain triglyceride supplementation on the pattern of plasma glucose, non-esterified fatty acids and survival of neonatal pigs. J Anim Sci 1989; 67(4):983-890	Experimental	Explored the effects of MCT on plasma glucose levels. MCT supplementation was given to less competitive pigs to determine if MCT supplementation would improve glucose status and survival. MCT supplementation reduced survival, possibly related to an indirect effect of excessive doses of FA, lower plasma glucose.
10	Lee SD, Hashim SA, Van Itallie TB. Effect of long chain triglyceride on chylous transport of medium chain fatty acids. Am J Phys 1968; 214:294-297	Experimental	Fed rats MCT alone or together with LCT. MCT was an odd-chain MCT (C9). <1% of MCT was transported in chylomicrons when fed alone. Three fold increase in MCT chylo-transport when fed simultaneously with LCT
25	Liu, YM, Wang HS. Medium-Chain Triglycerides Ketogenic Diet, An effective treatment for drug-resistant Epilepsy and a comparison with other Ketogenic diets. Biomed J 2013; 36:9-15	Review	Discusses the beneficial effects that MCT may have when used therapeutically in the ketogenic diet. MCT oils are more ketogenic than LCT.
1	Marten B, Pfeuffer M, Schrezenmeir J. Medium-chain triglycerides. Int Dairy J 2006;1374-1382	Review	Discusses the digestion, absorption and overall metabolism of MCT. Reviews the effects of MCT on plasma TYG, cholesterol, and glucose levels.
23	Martin WH,3rd, Dalsky GP, Hurley BF, Matthews DE, Bier DM, Hagberg JM, Rogers MA, King DS, Holloszy JO. Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. Am J Physiol 1993;265:E708-14.	Experimental	Training increased total fat oxidation during prolonged exercise. Endurance exercise training results in decreased plasma FFA turnover and oxidation during a 90- to 120-min bout of submaximal exercise because of a slower rate of FFA release from adipose tissue.
3	Odle J. New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. J Nutr 1997;127:1061-7.	Review	Review of their research done in neonatal piglets. MCT utilization improves rapidly with postnatal age (within 24 h), which is likely due to the ontogeny of pancreatic lipase. Additional data delineate the dramatic effects of emulsification and fatty acid chain length on utilization. Isolated hepatocytes have shown greater oxidation rates of odd-chain fatty acids compared with even-chain, in part as a result of the anaplerotic potential of propionyl-CoA arising from odd-carbon fatty

			acid oxidation. Improved octanoate oxidation to CO2, with a concomitant reduction in urinary dicarboxylic acid excretion when colostrum-deprived piglets were supplemented with L-carnitine.
4	Odle J, Benevenga NK, Crenshaw TD. Utilization of medium-chain triglycerides by neonatal piglets: II. Effects if even- and odd-chain triglyceride consumption over the first 2 days of life on blood metabolites and urinary excretion. J Anim Sci 1989; 67:3340-3351	Experimental	Neonatal pigs were force-fed either odd or even chain MCT and LCT. Pigs were individually caged for measurement of urinary N excretion and/or blood metabolites over 24 h. Study suggests that MCT may be better utilized than LCT and that there may be a differences in the utilization of even-MCT vs odd-MCT.
5	Odle J, Benevenga NJ, Crenshaw TD. Utilization of medium-chain triglycerides by neonatal piglets: chain length of even- and odd-carbon fatty acids and apparent digestion/absorption and hepatic metabolism. J Nutr 1991;121:605-14.	Experimental	Jugular plasma concentrations of medium-chain fatty acids (MCFA) and whole blood concentrations of ketones were measured in newborn piglets at 0, 1, 2, 4 and 8 h after force feeding C7, C8, C9 or C10. Both BHBA and MCFA were highest at 1 hour post prandial. Ketones were not different in pigs given tri-7:0, 8:0 or 9:0 but was lower for pigs given tri-10:0. Oxidative flux was 45% higher in odd-chain MCT and acylcarnitine production was low in all groups.
8	Papamandjaris AA, MacDougall DE, Jones PJ. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. Life Sci 1998;62:1203-15.	Review	Examined the metabolic handling of medium chain fatty acids (MCFA) with specific reference to intermediary metabolism and postprandial and total energy expenditure.
29	Rector RS, Ibdah JA. Fatty acid oxidation disorders: Maternal health and neonatal outcomes. Seminars in Fetal & Neonatal Medicine 2010; 15:122-128.	Review	Reviews maternal and neonatal outcomes in regards to fatty acid oxidation disorders.
22	Roe CR, Mochel F. Anaplerotic diet therapy in inherited metabolic disease: therapeutic potential. J Inherit Metab Dis 2006;29:332-40.	Review	Focuses on inherited diseases of mitochondrial fat oxidation, glycogen storage, and pyruvate metabolism using the anaplerotic compound triheptanoin. Discusses the inter-organ requirements for more normal metabolic function during crisis and how anaplerotic therapy using triheptanoin, as a direct source of substrate to the CAC for energy production. Triheptanoin appears to be a more successful approach to an improved quality of life for these patients.

21	Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. J Clin Invest 2002;110:259-269.	Experimental	In three patients with VLCAD, this treatment led rapidly to clinical improvement that included the permanent disappearance of chronic cardiomyopathy, rhabdo and muscle weakness varied in observed patients. The treatment was well tolerated for up to 26 months.
18	Romijn J.A., Coyle F, Disossis L.S., Gastaldelli A, Horowitz JF., Endert E., Wolfe R.R. Regulation of endogenous fat and carbohydrate metabolism in regulation to exercise intensity and duration. Am J Physiol 1993;265:380-391	Experimental	Five trained subjects were studied during different exercise intensities. Plasma glucose tissue uptake and muscle glycogen oxidation increased in relation to exercise intensity. In contrast, peripheral lipolysis was stimulated maximally at the lowest exercise intensity, and fatty acid release into plasma decreased with increasing exercise intensity. Muscle triglyceride lipolysis was stimulated only at higher intensities. In recovery from high-intensity exercise, the rate of release of fatty acids into plasma increased, indicating release of fatty acids from previously hydrolyzed triglycerides.
12	Sarda P, Lepage G, Roy CC, Chessex P. Storage of medium-chain triglycerides in adipose tissue of orally fed infants. Am J Clin Nutr 1987;45:399-405.	Experimental	The effect of the fatty acid content of the diet on that of adipose tissue was studied in 5 newborn infants studied prior to feeding and 30 infants fed either human milk or a commercial formula as the sole nutrient. MCFAs are not used solely as a source of energy. They can be re-esterified or serve for chain elongation, before being deposited in fat stores.
16	Sulkers EJ, Lafeber HN, Van Goudoever JB, Beaufrere B, Sauer PJ. Decreased glucose oxidation in preterm infants fed a formula containing medium-chain triglycerides. Pediatr Res 1993; 33:101-105	Experimental	Researchers hypothesized that when a portion of the fat content in preterm formula is substituted by MCT oil, a different metabolic pattern may be observed. The preferential oxidation of MCT, increases lipogenesis from glucose, leading to an increase in metabolic rate. To study the impact of MCT on glucose metabolism, 18 preterm infants were randomized to receive either an MCT or an LCT formula.
27	Vockley, J, & Whiteman DA. Defects of mitochondrial beta-oxidation: A growing group of disorders. Neuromuscular Disorders: 2002; 12:235-246.	Review	Recurrent rhabdo and hypoglycemia are frequent clinical problems. Reviews appropriate tests and screenings to help identify mitochondrial fatty acid oxidation disorders. Also discusses clinical presentations and treatments.
9	Yeh YY, Zee P. Relation of	Experimental	Rats were fed either MCT or LCT in

	ketosis to metabolic changes induced by acute medium-chain triglyceride feeding in rats. J Nutr 1976;106:58-6		the form of corn oil. Ketosis was measured by evaluating changes in concentrations of selected metabolites in plasma and synthetic and oxidative capacities of the liver. By 1 hour after MCT feeding, plasma levels of total ketone bodies had increased 18-fold, with a maximum value reached 1 hour later. Hepatic concentrations of ketone bodies also increased after MCT or corn oil feeding. MCT-induced ketosis was decreased with glucose administration. Ketogenesis from C8 was 10-fold higher than from C16, and C8 was oxidized more rapidly than C16. Results support that ketosis induced by MCT stems from rapid oxidation of MCFA.
7	Zentek J, Buchheit-Renko S, Ferrara F, Vahjen W, Van Kessel AG, Pieper R. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim Health Res Rev 2011;12:83-93.	Review	Discussed the nutritional benefits that MCT and MCFA have on the neonatal piglet.