Triheptanoin supplementation in Long-chain fatty acid oxidation disorder patients

Georgia Brown

A Thesis

Presented to the Faculty of Graduate Programs in Human Nutrition
and Oregon Health & Science University
School of Medicine
in partial fulfillment of the requirements for the degree of

Master of Science in Human Nutrition

12/5/19

Table of Contents

Medium-chain Triglyceride (MCT) supplementation for long-chain fatty acid oxidation disorders (LC-FAODs) has been the standard for nutrition therapy. LC-FAODs are inherited disorders of the long-chain fatty acid oxidation pathway that limit the ability to use long-chain fatty acids for energy; MCT bypasses the block in long-chain fatty acid oxidation and provides an alternative energy substrate. However, it has been hypothesized that triheptanoin (C7), a triglyceride comprised of three seven-carbon fatty acids esterified to a glycerol backbone, may provide a therapeutic advantage over MCT by replenishing the tricarboxcylic acid (TCA) intermediates via succinyl-CoA, as opposed to medium chain triglycerides (MCT), which can only supply acetyl-CoA. Previous research is equivocal with some reports demonstrating a benefit of triheptanoin while others have not. Likewise, separate studies have been conducted in specific LC-FAODs including CPT2D and VLCADD with variable results. Patients with LC-FAODs can present with a range of symptoms from milder episodic myalgia or more severe cardiac dysfunction and even death, suggesting patient to patient variability in disease burden is in fact part of the disorders themselves. A recent randomized controlled trial reported significant cardiorespiratory improvements among subjects with three different LC-FAODs consuming triheptanoin compared to subjects consuming trioctanoin, but the initial data analysis included all subjects with a variety of LC-FAODs.[10] It is possible that subjects with more severe symptoms might have a greater benefit from C7 but what factors might be associated with a greater response to C7 among the study participants has not been investigated.

This secondary analysis will include data from 32 study participants[10] enrolled in the RCT with the following LC-FAOD disorders: carnitine palmitoyltransferease 2 (CPT2), very long-chain acetylCoA dehydrogenase (VLCAD), and trifunctional protein/long-chain 3-hydroxy acetylCoA dehydrogenase deficiencies (TFP/LCHAD). While there are many similarities among the disorders, severity and progression of disease can vary across diagnoses and among patients. Infant presentation of VLCAD and TFP/LCHAD deficiencies include hypoketotic hypoglycemia, cardiomyopathy, and cardiac arrhythmia,

metabolic acidosis and hypoglycemia. Adolescent or late-onset patients with VLCADD and CTP2D have a more exercise or stress induced myalgia and rhabdomyolysis phenotype. We propose investigating if the effects of C7 are more pronounced in patients with a more severe phenotype. Another aspect of clinical severity is age at presentation. It is generally thought that patients who present symptomatically early in the neonatal or infant period have less residual enzyme leading to a more severe phenotype than patients who present later in life with exercise intolerance and recurrent rhabdomyolysis. It is also possible that patients who presented very early in life will have a greater response to triheptanoin than patients who presented later in life with a presumably higher residual FAO activity. Finally, the original analysis did not control for compliance with study oil intake. It is possible that subjects who consumed more triheptanoin had a greater response than those who consumed less.

The overall goal of this project is to conduct a sub-analysis of the primary study data to identify factors related to the magnitude of change from baseline with C7 supplementation. I will analyze the data by LC-FAO disorder diagnosis and compare those supplemented with triheptanoin (C7) versus trioctanoin (C8) to determine if any LC-FAOD had a greater response with C7 supplementation compared to C8 supplementation. Age at initial presentation was recorded and will be used as an estimate of disease severity. In the original study, all subjects were counseled to consume 20% of their estimated total energy needs daily from C7 or C8 oil for a 4-month period while following a diet low in long-chain fats. Adherence was assessed by multiple 3-day diet records and measurement of unconsumed daily oil at the end of the study. I hypothesize that participants with a more severe phenotype and those who consumed more of the prescribed C7 will have shown a greater response. I will test this hypothesis with the following aims:

Aims:

1. To determine the change in resting and total energy expenditure, body composition and exercise tolerance between patients given C7 supplementation who were diagnosed with CPT2D, VLCADD, or TFPD/LCHADD.

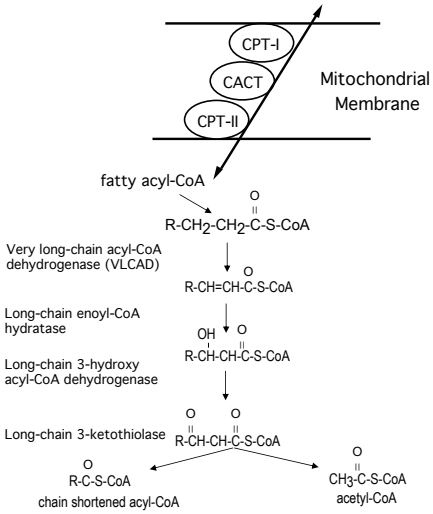
   Hypothesis: LC-FAOD patients with LCHAD will have a greater response to C7 supplementation then those with CPT2D and VLCADD.

2. To determine significant clinical factors that are associated with the greatest response to C7 supplementation compared to C8 among subjects with CPT2D, VLCADD, TFPD/LCHADD such as age at presentation, and compliance with prescribed oil consumption.

   Hypothesis: Subjects with earlier onset of LC-FAOD symptoms, or who consumed more of the prescribed study oil will have a greater change with C7 supplementation.

These results will provide information on the magnitude of the response to C7 that will help tailor treatment of individuals with LC-FAODs. These results will provide greater insight into factors that are associated with a physiologic response to this novel treatment for LC-FAODs and assist clinicians in determining which patients might benefit most from trihpetanoin.

Figure 1:
FAO Pathway



Overview of FAOD

Fatty acid oxidation (FAO) is an essential mitochondrial energy production pathway used during periods of negative energy balance, including fasting, illness, and sub-maximal exercise. In this endocrine-mediated response, glycogen stores are depleted, and triglycerides from adipose tissue are mobilized, and subsequently hydrolyzed into free fatty acids (FFA) through hormone-sensitive lipase (HSL).[1] Non-esterified fatty acids are released into circulation then bind to albumin.[2] Thus, free fatty-acids are mobilized into circulation dispersing to readily available tissues.[3] Free fatty acids are taken up into the cell to be used in the FAO pathway.

Medium-chain fatty acids can readily diffuse into the mitochondria, but long-chain fatty acids cannot, entering the mitochondria via the carnitine shuttle.[3] The carnitine shuttle functions between the outer and inner mitochondrial membrane.[4] Carnitine Palmitoyltransferase-1 (CPT-1) esterifies the long-chain acyl-CoA to form an acylcarnitine through exchange of a CoA moiety for a carnitine molecule.[4] The acylcarnitine is then transported across the inner mitochondrial membrane by carnitine-acylcarnitine translocase (CACT), where carnitine palmitoyltransferase -2 (CPT-2) exchanges the newly attached carnitine molecule for a CoA, restoring the acyl-CoA.[4] In the mitochondrial matrix, the long-chain fatty acyl-CoA undergoes a four-step enzymatic process catalyzed by very long-chain acyl-CoA dehydrogenase (VLCAD) and tri-functional protein (TFP) resulting in two end products: a fatty-acid shortened by two carbons and one acetyl-CoA (Figure 1).[2] Acetyl-CoA enters the TCA cycle and the shortened long-chain

fatty acid undergoes further rounds of FAO. There are separate enzymes that catalyze medium-chain and short-chain FAO in the mitochondrial matrix.

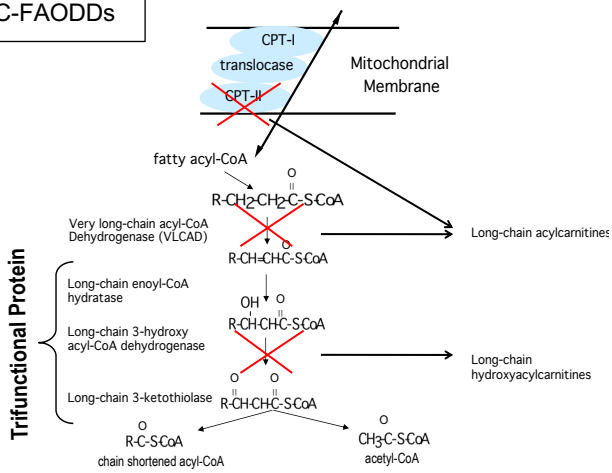
Acetyl-CoA in the liver, and to a lesser extend in the kidney, are used to synthesize ketone bodies, which are released into circulation and serve as an alternative energy source for some tissues such as the brain.[1] Alternatively, in most tissues, acetyl-CoA is oxidized via the TCA Cycle and electron transport chain (ETC) to produce ATP for use as a cellular energy source.[1]

Overview of LCFAODs:

Carnitine Palmitoyltransferase-2 (CPT2)

CPT2 deficiency is caused by a mutation in the *CPT2* gene. Deficiency presents with three distinct phenotypes, a lethal neonatal form, severe infantile hepatocardiomuscular form, and a myopathic form.[7] Symptoms are exacerbated in times of fasting, illness, or bouts of high intensity exercise. The myopathic form is the most prevalent CPT2 deficiency, and the form included in this study.[7,19] Episodes of myalgia, weakness, myoglobinunria, and recurrent rhabdomyolysis typically occur from childhood or early adolescence onward.[7]

Figure 2:
LC-FAODDs



Very-long chain acyl-CoA dehydrogenase (VLCAD)

Very-long chain acyl-CoA dehydrogenase (VLCAD) deficiency is caused by mutations in the *ACADVL* gene, impairing VLCAD activity or stability and impairing the initial step of beta-oxidation of long-chain fatty acids, 14 – 20 carbons in length.[8] Genotype/phenotype correlations have been reported with severity of symptoms reflective of residual enzyme activity. Severe infantile VLCADD is characterized by hypoglycemia, metabolic acidosis, cardiomyopathy and recurrent bouts of

rhabdomyolysis in neonates or infants, often precipitated by illness and fasting.[8] Childhood or adolescent VLCADD presents with symptoms later in life, often triggered through fasting or fevered illness.[8] Hypoketotic hypoglycemia is less common, but hepatomegaly and myopathy may be present. Many infantile and childhood patients develop recurrent rhabdomyolysis as they age.[8] While the occurrence of cardiomyopathy may occur at any age, it is less common in the childhood form than in the infantile form.[8] Finally, late-onset VLCADD is distinct in that symptoms predominantly impact muscular function, with intermittent episodic rhabdomyolysis.[8]

VLCADD is typically identified through newborn screening (NBS) in the US. [8,20] However, milder patients may be missed and still present symptomatically later in life. Failure to initiate treatment in infants identified by newborn screening with the severe form of the disease increases morbidity and mortality, and cardiomyopathy may lead to an early demise but it is difficult to determine the severity of the disorder based on newborn screening results. Many infants diagnosed by newborn screening with presumably mild forms have remained asymtomatic into childhood. Identifying which patients diagnosed by newborn screening may become symptomatic early in life and which patients will remain asymptomatic continues to be one of the major conundrums in the field. Of those infants with a severe phenotype, most typically develop recurrent rhabdomyolysis later in childhood or adolescence, similar to the later onset forms of VLCADD.

Adult onset VLCADD most commonly presents with symptoms including myoglobinuria, muscle pain and rhabdomyolysis. Episodes of exercise intolerance may begin through adolescence or present through an acute event of rhabdomyolysis. Interestingly, plasma acylcarnitines, one of the key biochemical markers used for diagnosis, have been reported to normalize in some patients when well.

Long-chain –hydroxyacyl-CoA dehydrogenase/Trifunctional Protein

Trifunctional protein (TFP) is a mitochondrial inner membrane protein complex composed of 2 alpha-subunits which has long chain enoyl-CoA hydratase and long chain 3-hydroxyacyl-CoA

dehydrogenase (LCHAD) activity, and 2 beta-subunits with long chain 3-ketothiolase activity.[5] The alpha and beta subunits encoded by the HADHA and HADHB genes are located on chromosome 2p23.[5] Mutations can occur in either the alpha subunit *HADHA* gene or beta subunit *HADHB* gene.[5]

LCHAD deficiency (LCHADD) due to a mutation in the *HADHA* gene, c.1528G>C, is the most common mutation reported in this enzyme. This mutation results in substitution of glutamate to glutamine and a decrease of the alpha subunit dehydrogenase activity.[5] The hydratase and ketothiolase activities are relatively preserved. The decrease in this enzyme activity leads to the accumulation of 3-hydroxy fatty acids (3-OH FAs). Symptoms in this disorder are similar to VLCADD and can include hypoketotic hypoglycemia, hepatamegally, cardiomyopathy and recurrent rhabmdomyolysis. However, chorioretinopathy of LCHADD and a peripheral neuropathy are unique to this deficiency.[6] Symptoms are episodic, exacerbated by fasting, stress, and exercise.

Unlike LCHADD, TFP deficiency is caused by mutations in the *HADHA* or *HADHB* genes other than c.1528G>C, resulting in reduced activity of all three TFP enzymes.[2,6,8] While 3-OH FA accumulation is lower than in isolated LCHADD, symptoms may be severe and include cardiomyopathy in infancy.[2,6,8] Development of peripheral neuropathy and retinal changes typically occur; however functional vision appears to be relatively conserved compared to LCHADD.[2,6,8]

Milder forms of these LCHADD and TFPD have been reported, but are uncommon, with most affected individuals experiencing some form of retinal changes by 5 or 6 years of age. Peripheral neuropathy is variable among individuals.[2,6,8]

<u>Similarities and differences between disorders:</u>

Though CPT2D, VLCADD, LCHADD/TFPD have varying degree of severity, it should be noted that these deficiencies are all rare autosomal recessive defects in the mitochondrial beta-oxidation of fatty acids, with abnormal acylcarnitine profiles.[10] Classical presentation of VLCADD, and LCHADD is often observed within infancy, in the form of nonketotic hypoglycemia, and Reye like syndrome.[6,8] Symptoms

may include coma, seizures, cardiomyopathy or death, with potentially toxic accumulation of LCFAs in the liver, muscle and heart.[6,8] Common late-onset disorders such as CPT2D, and some VLCADD patients can experience intermittent rhabdomyolysis from exercise intolerance.[7,8] Biochemically, each disorder is associated with a decrease in acetyl-CoA and ketones, and an increase in disease specific long-chain acylcarnitines during periods of negative energy balance.[6-8]

Fatty acid oxidation disorders were added to expanded newborn screening in the early 2000s and are now screened for in all 50 states. In countries without NBS, the disorders must be suspected clinically. In this setting, initial testing includes acylcarnitine profiling and urine organic acids followed by molecular genetic testing.[6-8,20] Current management of LCFAOD includes dietary modification, including avoidance of fasting, frequent meals, and may include a low-fat diet. Supplementation with medium chain triglycerides (MCT) provides a source of fat that can be metabolized.[6-8,20]

Table 1:

Symptom Similarities and Differences in LC-FAODs			
	Symptoms:		
Disorder	Rhabdomyolysis	Cardiomyopathy	Hypoglycemia
CPT2D			
Early Onset: *lethal neonatal form* or *severe infantile hepatocardiomuscular*		X	X
Late Onset: *myopathic form*	X		
VLCADD			
Early Onset: *severe infantile form*	X	X	X
Mid Onset: *childhood/adolescent form*	X	X	X

Late Onset: *late onset VLCAD*	X		X
LCHADD			
Early Onset: *Infantile or childhood presentation of LCHAD*	X	X	X

Rhabdomyolysis

Rhabdomyolysis is defined as an injury (both internal or external) to skeletal muscle, with release of intracellular contents into the extracellular space.[9] Clinical manifestations include muscle pain, electrolyte imbalances, myoglobinuria, and in more severe cases, acute kidney injury (AKI).[9] While rhabdomyolysis is often caused by traumatic injury, this condition can also be caused by drugs, toxins, infections, muscle ischemia, metabolic disorders, genetic disorders, over-exertion, or prolonged bed rest.[9] Treatment for rhabdomyolysis includes IV fluids, and rest. For patients with FAODs, reversing catabolism and inducing anabolism with IV dextrose and/or adequate oral energy intake is critical.

Diagnostic Criteria

Diagnosis of rhabdomyolysis must be suspected clinically and includes the triad of weakness, myalgia and myoglobinuria.[9] However, myoglobinuria is difficult to identify, as its half-life is approximately 6 hours, and may not be elevated in mild cases. Therefore it is a poor indicator of a rhabdomyolysis event.[9] Elevation of creatine kinase (CK) in blood is a better marker of rhabdomyolysis, and anything over the testing laboratory's normal value is of concern. Levels between 500-1000 IU/L are typically considered clinically significant and higher levels are consistent with greater increases in myoglobinuria risk of AKI.[9] Patients with FAODs can have CK levels that are substaintially higher during episodes of rhabdomyolysis. Renal function should be monitored as CK levels increase.

Cardiomyopathy:

Cardiomyopathy represents a heterogeneous group of diseases of the myocardium, with ventricular dilation and/or hypertrophy.[22] Cardiomyopathy may be primary (genetic, mixed, or acquired) or secondary (inflammatory, infiltrative, toxic) condition.[22] As the heart relies on fatty acid oxidation as its primary energy source, individuals with LC-FAODs are at an increased risk for progressive cardiac dysfunction.[11,22] Cardiomyopathy is more likely to present in early onset LC-FAODs, such as LCHAD or VLCAD but may present later in patients who survive early symptoms. It has not been reported in patients with late onset CPT2D.[11] Dilated cardiomyopathy is most often observed in LC-FAODs. Ventricular dysfunction may progress to congestive heart failure.[11] Initial diagnostic studies include baseline serum chemistries and B-type natriuretic peptide levels; however a 2-dimensional echocardiogram is the primary imaging tool used to establish cardiac function.[11] Conventional treatment includes beta-blockers, diuretics, and angiotensin-converting enzyme (ACE) inhibition.[11,22] In more severe cases respiratory ventilation, inotropic therapy, ventricular assist devices (VAD) and extracorporeal membrane oxygenation (ECMO) may be needed.[11,22] Maintaining anabolism and preventing catabolism is critical for subjects with cardiomyopathy due to LC-FAODs; negative energy balance can exacerbate the cardiac dysfunction.

Diet Management:

The goal of dietary therapy in LC-FAODs is to minimize reliance on fats for energy, and includes restricting intake of long-chain fatty acids (LCFA), adding medium chain triglyceride (MCT) oil supplementation, and avoiding prolonged fasting to minimize metabolic decompensation. MCT oil is a blend of even chain medium length fatty acids including C8, C10 and C12. MCT provides an alternative fuel that can be metabolized by medium chain specific enzymes in patients with LCFAODs. While dietary management decreases episodes of metabolic decompensation, it doesn't eliminate it, and patients are at continued risk for recurrent rhabodmoylysis. The reason for continued symptoms if MCT supplementation provides adequate acetyl-CoA is not clear. One hypothesis is that there is a depletion

of TCA cycle intermediates when only providing acetyl-CoA through MCT supplementation. If correct, then the use of an anaplerotic substrate, such as an odd chain medium length fatty acid that can be converted to succinate, could replenish depleted TCA cycle intermediates, restore complete oxidative phosphorylation of acetyl-CoA and improve symptoms.[18]

Overview of C7:

Triheptanoin (C7) is a medium odd-chain triglyceride, composed of three heptanoate, seven-carbon fatty acids, esterified to a glycerol backbone. While MCT oil only provides acetyl-CoA during oxidation, one heptanoate molecule produces two acetyl-CoAs and one propionyl-CoA when completely metabolized by fatty acid oxidation.[10] Propionyl-CoA is converted to succinyl-CoA thus serving as an anaplerotic agent to replenish TCA cycle substrates.[10]

Initial studies on triheptanoin in LC-FAODs were open-label and examined effects of C7 treatment on patients with VLCADD and CPT2D. Three subjects with VLCAD deficiency, who presented with hypertrophic cardiomyopathy and rhabdomyolysis were treated with C7 at 2-4 g/kg of body weight per day.[12] Patients exhibited rapid improvement in muscle function, and cardiomyopathy.[12] However, substantial weight gain ocurred in some subjects. In a second study, 7 patients with CPT-2 deficiency were treated for 7 to 61 months. Participants followed a fat-restricted diet with supplemental C7 at 30-35% of total daily caloric intake.[13] None of the treatment compliant participants experienced rhabdomyolysis or hospitalization, and returned to normal physical activities, including strenuous exercise.[13] However, in one 13-year-old who was non-compliant for a 'short interval' became symptomatic, with muscle pain, elevated serum CK levels (300-500 IU/L), and a decrease in physical endurance. Subsequently, all symptoms resolved within 24 hrs of re-initiation of the anaplerotic diet. Interestingly, the same patient periodically discontinued treatment, and at 44 months was hospitalized after a sports competition.

In a review of C7 use in 52 confirmed LC-FAOD patients, ages at initiation of treatment ranged from neonatal to 51 years. Serial quantitative measurements of serum carnitine and acylcarnitines, urinary organic acids and routine serum chemistries were reported.[14] Age dependent dosing was used with a target C7 supplementation of 25-35% of total daily calories, while following a low-long-chain fat, high carbohydrate diet. L-carnitine supplementation was used in conjunction with C7 initiation.[14] Subjects were admitted up to 9 days which included dietary instruction and education on the preparation of diet and C7 supplementation.[14] Protein, minerals, and vitamins were added into diets if recommended by a dietitian.[14] The study reported a decrease in rates of hypoglycemic events and creatinine kinase levels along with episodes of rhabdomyolysis requiring hospitalization.[3-4] Rhabdomyolysis rates decreased with C7 supplementation in two pregnant VLCAD patients, with treatment initiated after 20 weeks' gestation. Both women had a history of rhabdomyolysis requiring hospitalization. One had suffered severe episodes during her first two pregnancies and was referred to the trial at 26 weeks gestation. Upon admission it was noted that she had mild hepatomegaly, muscle weakness, and decreased endurance. Initially, with MCT supplementation her CK level was 1172 IU/L However, after starting C7 and carnitine her CK level decreased to 45 IU/L, while reporting increased muscle strength, resolution of hypatomegaly, and improved endurance. She remained in good health for the rest of her pregnancy, and delivered a healthy child. The second woman, referred to the trial at 20 weeks gestation, had no prior history of hypoglycemia or cardiomyopathy, but experienced hospitalizations attributed to rhabdomyolysis. Four days post C7 initiation the patient had resolution of weakness, and decreased C14:1 carnitine in blood from 0.36 to 0.06 μM, increased C3 carnitine from 0.70 to 1.25 μM, and normal CK levels. She did not experience any weakness or rhabdomyolysis for the remainder of her pregnancy and delivered a healthy child. Of 7 participants < 1 year of life, one developed cardiomyopathy and died at 18 months of age.[14]

Two retrospective chart reviews described outcomes among patients who were prescribed C7 on a compassionate use protocol. In the first, a comprehensive, retrospective medical record review was performed for 20 of 24 participants with diagnosed LC-FAODDs who received triheptanoin supplementation for up to 12.5 years. Some of these patients overlapped with the previously described study. Assessment of clinical outcomes included rates of hospitalizations, including length of stay, abnormal laboratory values (for the duration of study, including assessment pre - and post - treatment) and documented events of rhabdomyolysis, hypoglycemia and cardiomyopathy.[15] The disorders represented in this study included carnitine-acylcarnitine translocase deficiency (CACTD), CPT2D, VLCADD, TFPD, and LCHADD with 11 males and 9 females. The median triheptanoin treatment duration was 8.7 years, with 17 of 20 patients receiving supplementation for > 5 years. Dosing was indivisualized, and ranged from 0.17 gm/kg to 5.62 gm/kg (mean (SD) dosing was 69.9 gm/d). Individuals previously receiving MCT oil exchanged MCT with triheptanoin at the same dose, while individuals who had not previously supplemented with MCT received a gradual introduction to triheptanoin. If intolerance occurred, dosing was reduced then titrated up as tolerated, with some patients receiving soluble fiber to decrease gut transit time. The authors concluded that mean hospitalizations per year decreased by 35% following triheptanoin initiation, with an additional decrease in mean length of stay of 67% following treatment. Interestingly, hypoglycemic events, specifically within the LCHAD phenotype dramatically decreased. Pretreatment, 9 patients had documented hypoglycemic events; however, eight out of the nine patients reported no hypogylcemic event during triheptanoin treatment. Hospitalization rates for a rhabdomyolysis event did not significantly differ, with 1.05 events per year pre- treatment and 0.68 events per year post - initiation. Conversely, there was a decrease in mean hospital length of stay post initiation of triheptanoin by 60%. CK was assessed in 7 patients who experienced a rhabdomyolysis event and were hospitalized with mean CK levels pre- treatment at 85,855 U/L, decreasing post-

treatment to 27,597 U/L. There were very few cases of cardiomyopathy in this group of patients, so the authors could not adequately address this outcome.

A second retrospective chart review examined the effects of C7 initiation on echocardiography and ejection fraction (EF) in 10 patients (8 infants) with confirmed cases of LC-FAODs associated cardiomyopathy.[11] Moderate to severe EF, 12-45%, were documented prior to initiation of treatment. Dosing was age dependent, with a target of 25-35% of total calories, roughly 1-4 g/kg/d (dependent on age and tolerability).[11] The most notable adverse side effect was gastrointestinal distress, associated with loose stools and rarely emesis. Often, adverse effects were mitigated by mixing triheptanoin with food, or division of dosing. There were no other issues with tolerability. Treatment with C7 treatment led to long-term stabilization of cardiomyopathy in most patients.[11] Seven of the patients continued treatment, 1 discontinued due to intolerability, and 2 patients died; however cause of death was not attributed directly to the C7 treatment. The first infant, a 3.5 month old affected with VLCAD died from sepsis and necrotizing fasciitis. While the second infant, whose progressive heart failure resulted in metabolic and lactic acidosis requiring ventilation, later developed pericardial effusions which ultimately progressesd to refractory cardiogenic shock with severe pulmonary hemorrhage with unsuccessful resuscitation.[11]

Results of an open-label phase 2 trial of triheptanoin on 29 subjects has recently been reported.[21] The study included primary analysis at 24-week, and a study continuation phase for a total of 78-weeks.[16,21] Entry criteria included a confirmed diagnosis of CPT2, VLCAD, TFP or LCHAD deficiency, age $\geq$6 months, and a history of significant clinical decompensation, including elevated CK levels, muscle dysfunction, severe episodic hypoglycemia or cardiomyopathy, despite conventional therapy. Participants (age 10 months to 58 years old) were monitored for 4 weeks prior to initiation of C7 to establish baseline parameters, particularly laboratory measures of hepatic, skeletal myopathy and cardiac disease.[21] Medication was dosed to provide 25-35% of total caloric intake, titrating as necessary

to ensure tolerance. C7 was administered orally four times daily with food or drink consumption. The majority of patients (72%) were compliant with prescribed dosing, with a daily caloric intake of at least 25% of C7.[21] Most (17; 68%) were too young to complete the planned exercise tests.[21] Seven patients completed cycle ergometry at various times within the 24-weeks, with a mean increase of workload of 60% from baseline.[21] Likewise, 8 patients performed a 12 minute walking test (MWT) during the study period.[21] The mean distance walked at the end of the study was 673.4 m, a 28% increase from baseline.[21] Adverse events were reported in 28 of 29 patients but were considered mild to moderate.

Of the original 29 subjects, 24 continued in the 78 week extension study. The mean C7 dose at 78-weeks was 27.5% of daily calories with 80% of subjects reaching target dose. Increased caloric demands were noted in the study population, felt to be due to growth in the pediatric population. Hospitalizations due to clinically meaningful events (hypoglycemia, cardiomyopathy, or rhabdomyolyisis) reduced from a rate of 81%, prior to C7 treatment, to 74%, with C7 treatment, and overall major clinical events were decreased by 44.3%. Likewise, annual event rates also decreased, with pre - initiation of 1.69 clinical events per year to 0.88 post - C7 initiation. Post C7 initiation, mean hypoglycemic events decreased from a pretreatment rate of 0.32 to 0.02 events per year. Incidence of cardiomyopathy also decreased post treatment. Prior to initiation, three cardiomyopathic events occurred in two LC-FAODD patients, while post treatment only one incident occurred, with the duration of cardiomyopathy events from 0.60 to 0.15 days per year. Hospitalizations from rhabdomyolysis decreased from 1.03 to 0.63 events per year (a 38% reduction)**.** Common side effects reported in participants included diarrhea, vomiting, rhabdomyolysis, abdominal pain, gastroenteritis, upper respiratory tract infection, headache and pyrexia, with 16 of 24 individuals experiencing an event that required hospitalization.

A double blind, randomized control trial compared the therapeutic benefit of C7 to C8 supplementation in 32 patients with LC-FAODs over a 4-month period.[10] The study included patients with a confirmed diagnosis of CPT2D, VLCADD, LCHADD or TFP who were $\geq$ 7 years of age. After baseline

assessments, patients were assigned either C7 or C8 treatment in a 1:1 ratio, stratified by diagnosis (16 participants per treatment arm). All participants were already following a diet low in LCFAs and were supplementing with MCT. Dosing of C7 or C8 was based on 20% of patients estimated total energy needs. Patients were contacted weekly to ensure compliance and report any complications. Adherance was monitored through multiple 3-day diet records and by measuring the amount of unconsumed oil at 4-months. The variables measured in this study included body composition by dual-energy X ray absorptiometry (DEXA), tissue lipid deposition by magnetic resonance spectroscopy (MRS), energy expenditure (both total and resting energy expenditure), resting echocardiogram, metabolic response to a meal, in vivo fatty acid oxidation, and treadmill ergometry. All 32 patients successfully completed the study. Assessment of the 3-day diet records indicated that participants consumed approximately 14% and 16% of total energy intake from C8 and C7 respectively. Oil dispensed versus oil returned was measured, andf participants consumed 74% and 76% of C7 and C8 study oil respectively.[10] Subjects receiving C7 had an increase in left ventricular (LV) ejection fraction of 7.4% compared to the C8 group. LV wall mass decreased by 7.4% in the C7 group on the resting echocardiogram, while subjects receiving C8 participants experienced a 15% increase. During the exercise stress test, C7 patients had on average 7 beats per minute lower in heart rate for equal work during moderate-intensity exercise when compared to their C8 counterparts. There was no difference in total energy expenditure, phosphocreatine recovery, body composition, or incidence of rhabdomyolysis between the two groups.

While C7 supplementation for patients with LC-FAODs appears to be a promising new treatment, there continues to be a gap in understanding of C7 in response to treatment among individual LC-FAODs. With only one double-blind control trial, variability in C7 dosing across studies, discrepancy in study lengths, and a variety of outcome measures in studies to date, further evaluation of C7's efficacy is needed. The goal for this project is to examine differences in the response to C7 among different FAOD diagnoses including CPT2D, VLCADD and LCHADD/TFPD. It is our hypotheses that LC-

FAOD patients with LCHAD will have a greater response to C7 supplementation then those with CPT2D and VLCADD, and subjects with earlier onset of LC-FAOD symptoms, or who consumed more of the prescribed study oil will have a greater change with C7 supplementation.

Table 2:

Study	C7 dosing:	Diagnosis:	Cardiomyopathy:	Rhabdomyolysis:	Positive/ Negative Outcomes:
1.	2-4 g/kg/day	VLCADD; diagnosed hypertrophy cardiomyopathy	Resolution of cardiomyopathy	Resolution of rhabdomyolysis and muscle weakness	Rapid improvement of cardiomyopathy and rhabdomyolysis, however reports of substantial weight gain
2.	30-35% of total daily calories	CPT2D	N/A	Slight improvement	Improvement in CK, decrease hospital readmission rate, decrease rhabdomyolysis
3.	30-35% of total daily calories	CPT1D, CPT2D, CACTD, VLCADD, LCHADD/TFPD	8 infants; only 1 developed and died from cardiomyopathy	Improvement observed	Improvement in hypoglycemic events, CK. 1 patient developed cardiomyopathy and died
4.	25-35% of total daily calories	CPT2D, CACTD, VLCADD, LCHADD/TFPD	Unable to assess	Slight improvement	Improvement in hypoglycemic events, CK, and hospital readmission rate declined
5.	25-35% of total calories, 1-4 g/kg/d – age dependent dosing	VLCADD (n=4) CACTD (n=2) TFPD (n=2) and LCHADD (n=2)	Baseline EF: 12-45%; 7 of 10 patients experienced EF stabilization	N/a	1 patient was removed from study for intolerability; 2 infant deaths, unrelated to C7

6.	25-35% of total daily calories	LC-FAOD	N/a	Increase by 28% in 12MWT at 18-weeks; 60% increase in watts generated at 24-weeks	

Note: all studies used age dependent dosing, infants on average were dosed 3-4 g/kg/d. Children and adolescents 2-3 g/kg/d. Adults averaged 1 g/kg/d.

Methods

Population Description

Study Participants and Recruitment

This is a secondary analysis of a previously conducted randomized double-blind clinical trial. The overall study was published in the Journal of Inherited Metabolic Diseases and used an intent to treat statistical model including 3 different LC-FAODs: CPT2D, VLCADD, LCHADD/TFPD. In this follow up analysis, we examined the effects of C7 by diagnosis. A brief review of the original study methods is provided below. Approval was obtained for this research from OHSU and University of Pittsburgh Institutional Review Boards (IRB). Written informed consent was obtained from each subject and his or her guardian prior to study participation. The study is currently approved for additional analysis by the OHSU IRB (eIRB#7140). This secondary analysis used the primary research data set, all subjects and data were previously collected in the orginal study design.

Study Design: Subjects with a confirmed diagnosis of CPT-2, VLCAD or LCHAD and/or TFP deficiency were recruited to participate in this study at one of two clinical sites: OHSU and the University of Pittsburgh. Subjects were randomly assigned to one of 2 treatment groups C7 or C8. Subjects with CPT-2, VLCAD, and LCHAD/TFP deficiency were stratified such that each experimental group contains subjects with all three diagnoses. Subjects and most of the investigators were blinded to study treatment. The

statistician, the OHSU study coordinator, the OHSU investigational pharmacy and the OHSU bionutritionist were not blinded. Subjects completed all study procedures at enrollment to the study and again after 4 months on study diet. At enrollment, body composition, resting energy expenditure, metabolic response to a test meal and exercise tolerance was measured. Total energy expenditure was measured at home after baseline. Each subject and their family were counseled how to consume their supplement at home. Subjects were then provided the C8 or C7 supplements and were discharged home. After following the diet at home for 4 months, subjects returned to repeat the assessments.

Subjects*:* Subjects with disorders in long-chain fatty acid oxidation were recruited to participate in this study from across the US. Inclusion and exclusion criteria are listed below.

Table 3:

Inclusion criteria	Exclusion criteria
• Confirmed diagnosis	• Hgb < 10 g/dl
• Aged ≥7 years	• Peripheral neuropathy that limits ability to complete treadmill studies
• Ability to travel to CRC to participate	• Inclusion in another research study that alters macronutrient intake
• Ability to follow protocol	• Pregnant or breastfeeding females
• Stable on a diet that includes supplementation with MCT	• History of myocardial infarction
• History of at least one episode of rhabdomyolysis	

C 2.2 Confirming the Diagnosis: The diagnosis of carnitine palmitoyltransferase 2 (CPT2), very long-chain acyl-CoA dehydrogenase (VLCAD), trifunctional protein (TFP) or long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency was confirmed by review of medical records including acylcarnitine profiles, fatty acid oxidation probe studies in cultured fibroblasts and/or mutation analysis (32, 41).

C. 2.3 Outcome Measures: A sample admission schedule is presented in figure 4. Body composition was measured by DEXA scan and tissue lipid content and tissue energetics by magnetic resonance

spectroscopy (MRS). Resting and exercise energy expenditure was measured by indirect calorimetry. Total energy expenditure was measured by the doubly labeled water technique (DLW). Metabolic response to a test meal including change in glucose, insulin, free fatty acids, and acylcarnitines was measured following the meal containing a loading dose of labeled oleic acid (1-^{13}C oleic acid). Using the loading dose of the stable isotope tracer, *in vivo* long-chain fatty acid oxidation was measured. Cardiac function was measured by echocardiogram (ECG). During the exercise treadmill, perceived physical exertion using the Borg perceived exertion scale, heart rate and ventilation at a given work load was measured. Pre- and post-exercise and the following morning blood lactate and CK concentrations was measured as indicators of rhabdomyolysis. Resting and pre- and post-exercise ketones and acylcarnitine profiles was measured as a secondary marker of substrate utilization during exercise.

C. 2.4 Standardized Diets: All research diets had the same total fat (long-chain triglyceride (LCT) + MCT or triheptanoin) content but varied in the source of medium chain fat (MCT vs. triheptanoin; Table 4**).** The MCT diets will contained 20% of total energy from trioctanoin, a C8 triglyceride; the triheptanoin diets contained an equal % of total energy from

Table 4: Macronutrient content of diets.

	Protein	CHO*	LCFA*	MCT*	Triheptanoin

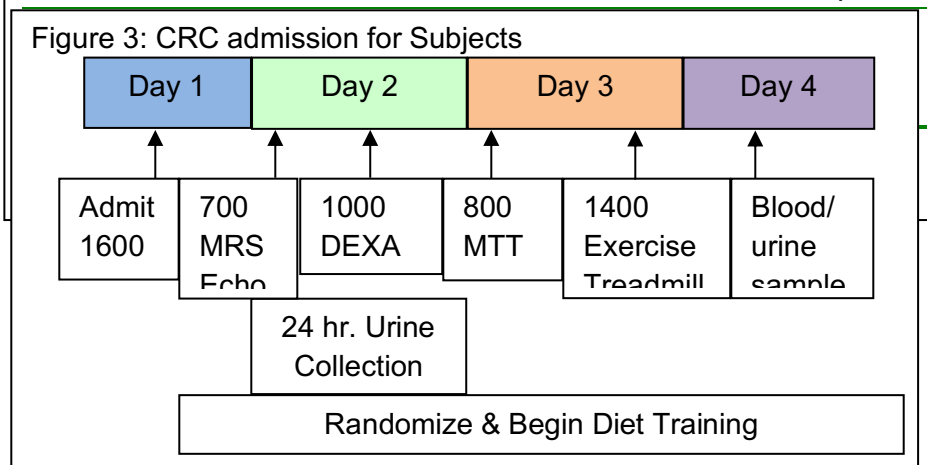


Figure 4: CRC admission for subjects. Day 2 subjects will complete a MRS to measure ectopic fat stores and tissue energetics, Echo for cardiac function, DEXA for body composition and 24 hr urine collection. Day 3 metabolic response to a test meal and exercise tolerance will be measured. Day 4 a final blood sample and spot urine are collected prior to discharge. During days 2 & 3, subjects will be fed the diet they were randomly assigned to by CRC bionutrition.

DEXA = dual energy x-ray absorptiometry, MTT = meal tolerance test; MRS = magnetic resonance spectroscopy; MRI = magnetic resonance imaging

triheptanoin. Each subject and his or her guardian was instructed on how to follow the prescribed experimental diet by the study coordinator. A detailed diet was developed based on the measured resting energy needs of that subject plus 40% for activity and growth. Subjects were not required to eat all the calories given in their diet plan but they were required to follow general types of foods included in the plan. They were encouraged to eat foods from their prescribed diet until they are satisfied. Diet instructions included lists of allowed foods and quantities, recipes for preparing meals and suggested menus to follow. Subjects were provided with food intake forms on which to record 3-day diet records. Subjects completed a 3-day diet record three times during the study; once for each measurement of total energy expenditure using doubly-labeled water at the beginning and end of the study, and mid-study at the end of 8 weeks of study participation.

C. 2.5 Compliance Monitoring: Subject compliance with the prescribed experimental diet was monitored by weekly communication with the study coordinator, the analysis of 3-day diet records and the amount of supplemental oil consumed. Completed diet records were mailed to the principal investigator. Dietary macronutrient content was determined using –Food Processor (Esha Research, Inc) software. The amount of oil provided to the subject during the trial was tracked as a third method of compliance monitoring. Depending on the daily dose prescribed of supplemental oil (MCT or triheptanoin) the study staff predicted how many bottles of oil was used during the 4 months by each subject. The subject was asked to return any unsed oil. The returned amount was measured and used to estimate compliance as amount of oil provided – amount of oil returned at the end of the trial.

Secondary Data Analysis: *For Current Project Summary*

Mean, standard deviation and 95% confidence intervals was used to summarize all of the variables by diagnosis: (CPT2D, VLCADD, TFPD, or LCHADD). Other key medical history variables were age at initial diagnosis, and dietary compliance. Key dependent variables included: resting energy expenditure (REE), estimates of substrate oxidation, total energy expenditure (TEE), body composition,

and exercise tolerance (specifically RER and heart rate). The software used was Graphpad Prism 8. The study was initially powered for a treatment effect analysis of all diagnoses combined together. Consequently, this sub-analysis is underpowered and conducted predominantly for additional hypothesis generation. In this analysis we were looking for trends, using a P value <0.1.

For the first aim, an ANOVA was performed to assess the magnitude of change (4 month - baseline) of our dependent variables by diagnosis with C7 supplementation. It was our prediction that LCHADD subjects will have a greater response to these variables with C7 supplementation than the CPT2D or VLCADD subject groups. We anticipated an increase in total energy expenditure, a decrease in LV EF and heart rate with treadmill ergometry, and a decrease in hepatic lipid deposition.

For the second aim, we performed a multiple linear regression to determine if group randomization (C8 or C7) and other independent factors, such as compliance or age at diagnosis, predict change in our dependent variables of interest, such as TEE. From a group of 32 subjects, sixteen were randomized to receive C8 supplementation, while the remaining sixteen received C7. Division of diagnosis was nearly even, with 5 CPT2D subjects, 4 VLCADD, and 7 LCHADD/TFPD receiving C7. Additionally, 6 CPT2D subjects, 5 VLCADD, and 5 LCHADD/TFPD received C8.

Table 5: Statistical Analysis Summary

Specific Aim	Hypothesis	Statistical Test
Specific Aim 1: To determine the change in resting and total energy expenditure, body composition, and exercise tolerance between subjects given C7 supplementation who were diagnosed with CPT2D, VLCADD, or TFP/LCHADD.	**Hypothesis 1:** LC-FAOD subjects with LCHADD will have a greater response with C7 supplementation than those with CPT2D and VLCADD.	An ANOVA was used to compare change between resting and total energy expenditure (REE and TEE), body composition (FFM% and LBM%) and exercise tolerance (treadmill ergonometry) between each FAO group from baseline to 4 months of treatment.

Specific Aim 2: To determine significant clinical factors that are associated with the greatest response to C7 supplementation compared to C8 among subjects with CPT2D, VLCADD, TFPD/LCHADD such as age at presentation, compliance with prescribed oil consumption and/or diagnosis	**Hypothesis 2:** Subjects with earlier onset of LC-FAOD symptoms, who consumed more of the prescribed study oil, and who are diagnosed with TFP/LCHAD deficiency will have a greater change with C7 supplementation.	A multiple linear regression was used to examine the relationship between predictors and our dependent variables. Predictors include age at diagnosis, diagnosis, diet compliance, and history of rhabdomyolysis episodes, requiring hospitalization. Our dependent variable includes resting and total energy expenditure, exercise tolerance and body composition.

Abbreviations: *% FFM, percent-fat free mass; % FM, percent fat mass; RQ, respiratory quotenient; REE, resting energy expenditure; TEE, total energy expenditure; AEE, activity energy expenditure; LV EF, left ventricular ejection fraction; LV WM, left ventricular wall mass; Liver, hepatic lipid deposition; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; IMCL, lipid deposition of soleus intra-myocellular; EMCL, lipid deposition of soleus extra-myocellular; HR, heart rate.*

Results:

Comparison between LCFAOD diagnostic groups and their responses to treatment

Baseline characteristics of the study participants are outlined in table 6. There were no differences between subjects diagnosed with CPT2D, VLCADD or LCHADD/TFPD at baseline, described in table 7. Body composition, energy expenditure, and cardiac function parameters are similar across diagnostic groups suggesting groups are indeed comparable prior to randomization to C7 or C8. The variables analyzed in this sub-study included: % FFM, %FM, RQ, REE, TEE, AEE, left ventricular (LV) ejection fraction, LV wall mass, hepatic lipid deposition, VAT, SAT, IMCL, EMCL, and Heart Rate.

The change of each outcome variable (4 months – baseline) was tested for normality using the D'Agostino & Pearson and Shapiro-Wilk tests on Graphpad Prism 8. Variables that were considered non-normal were log-transformed. Log-transformed variables include %FFM, IMCL, EMCL, hepatic lipid deposition, RQ, REE, TEE, AEE, LV ejection fraction, LV wall mass. One-way ANOVA tests were conducted comparing outcome variables for the subjects diagnosed with CPT2D, VLCADD, and LCHADD/TFPD randomized to C7. Separate ANOVAs were conducted comparing outcomes for the subjects diagnosed with CPT2D, VLCADD, and LCHADD/TFPD randomized to C8. Results appear in the box and whisker plots

below. The results of this secondary study are compared to the original intent to treat analysis[10]. The goal of this analysis was to determine if one diagnostic group is driving overall population differences and thus the overall response to treatment with C7 versus C8.

Table 6:

Baseline Characteristics for CPT2

Diagnosis:		**CPT2 (n=11)**	
	C7 (n=5)		**C8 (n=6)**
Age, *mean ± SD*	30 ± 16.7		
Sex, n (%)			
Male	3 (27)		
Female	8 (73)		
Age at Diagnosis, *mean ± SD*	25.5 ± 17.3		
Anthropometrics, *mean ± SD*			
Weight (kg)	72.6 ± 21.4		
Height (cm)	163.0 ± 11.3		

Baseline Characteristics for LCHAD/TFP

Diagnosis:		**LCHAD (n=12)**	
	C7 (n=7)		**C8 (n=5)**
Age, *mean ± SD*	14.6 ± 7.3		
Sex, n (%)			
Male	5 (41)		
Female	7 (58)		
Age at Diagnosis, mean ± SD	1.9 ± 4.2		
Anthropometrics, *mean ± SD*			
Weight (kg)	48.9 ± 20.3		
Height (cm)	150.3 ± 22.7		

Baseline Characteristics for VLCAD

Diagnosis:		**VLCAD (n=9)**	
	C7 (n=4)		**C8 (n=5)**
Age, *mean ± SD*	31.7 ± 11.4		
Sex, n (%)			
Male	4 (44)		
Female	5 (55)		
Age at Diagnosis, mean ± SD	23.4 ± 14.8		
Anthropometrics, *mean ± SD*			
Weight (kg)	75.5 ± 25.9		
Height (cm)	168.1 ± 18.8		

SD, standard deviation; n, number; kg, kilogram; cm, centimeter

Table 7:Descriptive Statistics at Baseline:

CPT2 Baseline:	%FFM	%FM	IC/RQ	IC/REE	DLW/TEE	AEE	LV EF	LV WM	Liver	VAT	SAT	IMCL	EMCL	RQAUC	VO2AUC	HRAUC	SBPAUC	DPAUC
Mean	64.5	35.4	0.86	1400	2449	629.8	61	96.2	3.2	6.7	49.8	1	2.1	36.7	538.9	4836	3514	429367
Std. Deviation	6.3	6.3	0.05	305.2	675	224.8	5.3	35.3	3.2	4.8	24.6	0.58	1.3	1.3	85.4	468.2	557.8	85776
Std. Error of Mean	1.9	1.9	0.02	101.7	213.4	79.5	2.2	14.4	1.0	1.6	8.2	0.19	0.43	0.4	28.5	148.1	176.4	27125
Lower 95% CI of mean	60.3	31.2	0.82	1165	1966	441.8	55.4	59.2	0.73	2.9	30.9	0.55	1.1	35.7	473.3	4501	3114	368007
Upper 95% CI of mean	68.8	39.7	0.89	1634	2931	817.7	66.6	133.2	5.6	10.4	68.7	1.5	3.1	37.7	604.5	5171	3913	490727

LCHAD Baseline:	%FFM	%FM	IC/RQ	IC/REE	DLW/TEE	AEE	LV EF	LV WM	Liver	VAT	SAT	Soleus/IMCL	Soleus/EMCL	RQAUC	VO2AUC	HRAUC	SBPAUC	DPAUC
Mean	67.1	32.6	0.89	1268	2217	727.4	62.1	100.8	2.5	4.8	26.3	0.58	2.5	36.4	619.4	5076	3292	410714
Std. Deviation	7.5	7.2	0.06	351.9	484.5	247.8	4.9	39.9	1.9	2.6	15.2	0.54	1.4	2.6	123.5	337.2	625.4	69229
Std. Error of Mean	2.2	2.1	0.02	101.6	139.9	71.5	1.6	13.3	0.6	0.8	4.8	0.17	0.45	0.8	35.6	97.3	180.5	19985
Lower 95% CI of mean	62.3	28.0	0.85	1044	1909	570	58.3	70.1	1.2	2.9	15.4	0.19	1.5	34.7	540.9	4862	2895	366727
Upper 95% CI of mean	71.8	37.2	0.93	1491	2525	884.8	65.9	131.5	3.9	6.7	37.2	0.97	3.5	38.1	697.8	5290	3689	454700

VLCAD Baseline:	%FFM	%FM	IC/RQ	IC/REE	DLW/TEE	AEE	LV EF	LV WM	Liver	VAT	SAT	Soleus/IMCL	Soleus/EMCL	RQAUC	VO2AUC	HRAUC	SBPAUC	DPAUC
Mean	67.4	32.6	0.90	1513	2576	774	55.3	119.4	6.2	9.9	36.8	1.8	1.8	36.5	574.8	4802	3952	492178
Std. Deviation	4.4	4.4	0.07	312.5	626.8	355.5	11.1	51.3	11.2	8.9	20.7	1.5	1.5	1.2	63.6	281.3	711.7	90122
Std. Error of Mean	1.5	1.5	0.02	104.2	221.6	125.7	4.2	19.4	3.9	3.1	7.1	0.61	0.62	0.38	21.2	93.8	237.2	30041
Lower 95% CI of mean	63.9	29.3	0.85	1273	2052	476.8	45.1	71.9	-3.1	2.5	19.9	0.19	0.17	35.6	525.9	4585	3405	422904
Upper 95% CI of mean	70.8	36.0	0.95	1754	3100	1071	65.5	166.9	15.6	17.4	53.7	3.34	3.4	37.4	623.7	5018	4499	561452

Table 7 *%FFM, percent fat-free mass; %FM, percent fat mass; IC, indirect calorimetry; RQ, respiratory quotient; REE, resting energy expenditure; DLW, doubly labeled water; TEE, total energy expenditure; AEE, activity energy expenditure; LV EF, left ventricular ejection fraction; LV WM, left ventricular wall mass; Liver, hepatic lipid deposition; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; IMCL, lipid deposition of soleus intra-myocellular; EMCL, lipid deposition of soleus extra-myocellular; AUC, area under the curve; VO2, treadmill VO2 interval average; HR, heart rate; SBP, systolic blood pressure; DP, double product.*

Heart Rate

In the original study, subjects heart rate was measured throughout two moderate intensity exercise trials, performed at baseline and again following four months of treatment. Heart rate was measured over a 40-minute period and averaged in intervals of 10 mins. In the larger study analysis, a mixed-effect model analyzed change in heart rate, with the models treating particpants as a random effect, with treatment group and study length as fixed effects. For this sub-study, the area under the curve (AUC) was calculated for each participant. From there, the difference (4 month AUC – baseline AUC) was calculated and ANOVA tests were performed. In the original intent to treat analysis, there was an observable decrease in heart rate from baseline in the C7 group but not in the C8 group. In this secondary study, the original observation was not driven by one diagnostic group. Change in HR area under the curve was lower in all three diagnoses, CPT2D, LCHADD and VLCADD randomized to C7 compared to the change in HR area under the curve for subjects randomized to C8. Therefore, the change observed was attributed to the overall treatment effect.

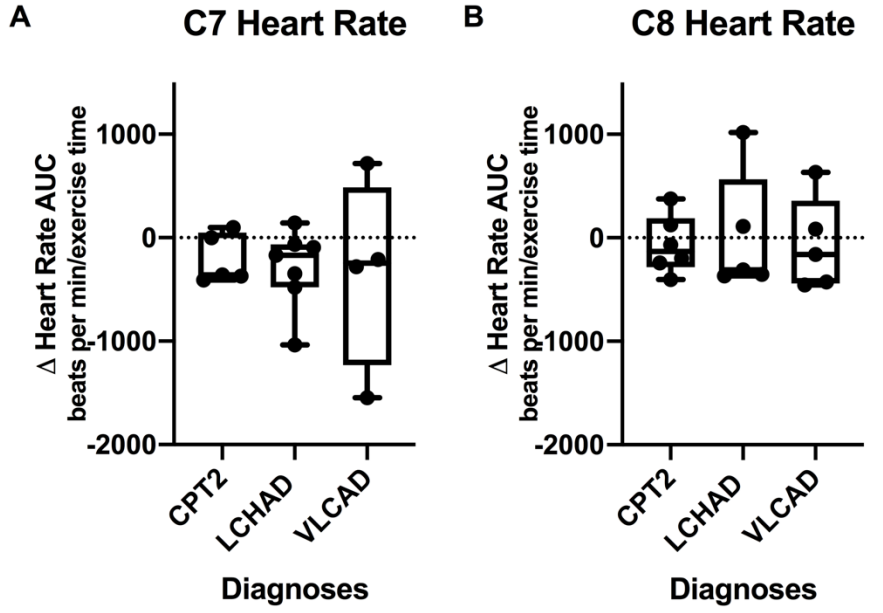


Fig. 4 Change in heart rate (HR) during moderate treadmill ergometry. A one-way ANOVA was used to analyze all data. Data is change in HR (4 months minus baseline) among subjects supplemented with triheptanoin (C7) and subjects supplemented with trioctanoin (C8). Area under the curve (AUC) was calculated for each treadmill test. The box plot represents the 25^{th} and 75^{th} interquartile range. The horizontal line indicates the median and the whiskers represents the minimum and maximum values. The y-axis on both graphs represents the change in HR AUC in beats per minute over a 40-minute exercise period. **A)** There was an overall decrease in HR in the C7 treatment groups compared to C8 treatment groups. However, there was no difference in HR between CPT2D, LCHADD, VLCADD groups receiving C7. **B)** There was no difference in HR between the three treatment groups receiving C8.

Echocardiograms

Echocardiographic data was limited, and only available in 2 CPT2D subjects receiving C7 and 4 subjects receiving C8. Likewise, data was available in only 3 LCHADD subjects receiving C7 and 4 receiving C8, 4 VLCADD subjects receiving C7 and 3 receiving C8. The change from 4-months to baseline was taken for ejection fraction and left ventricular wall mass per diagnostic group. The data was then sorted according to treatment assignment (C7 or C8) and ANOVA tests were performed. While left ventricular ejection fraction increased and left ventricular wall mass decreased among subjects randomized to C7 in the original analysis, there was no significant difference between the three diagnostic groups within subjects randomized to C7. Similarly, there was no difference between diagnostic groups within subjects randomized to C8. Consequently, the change from baseline reported in the intent to treat analysis was attributed to the overall treatment effect of C7.

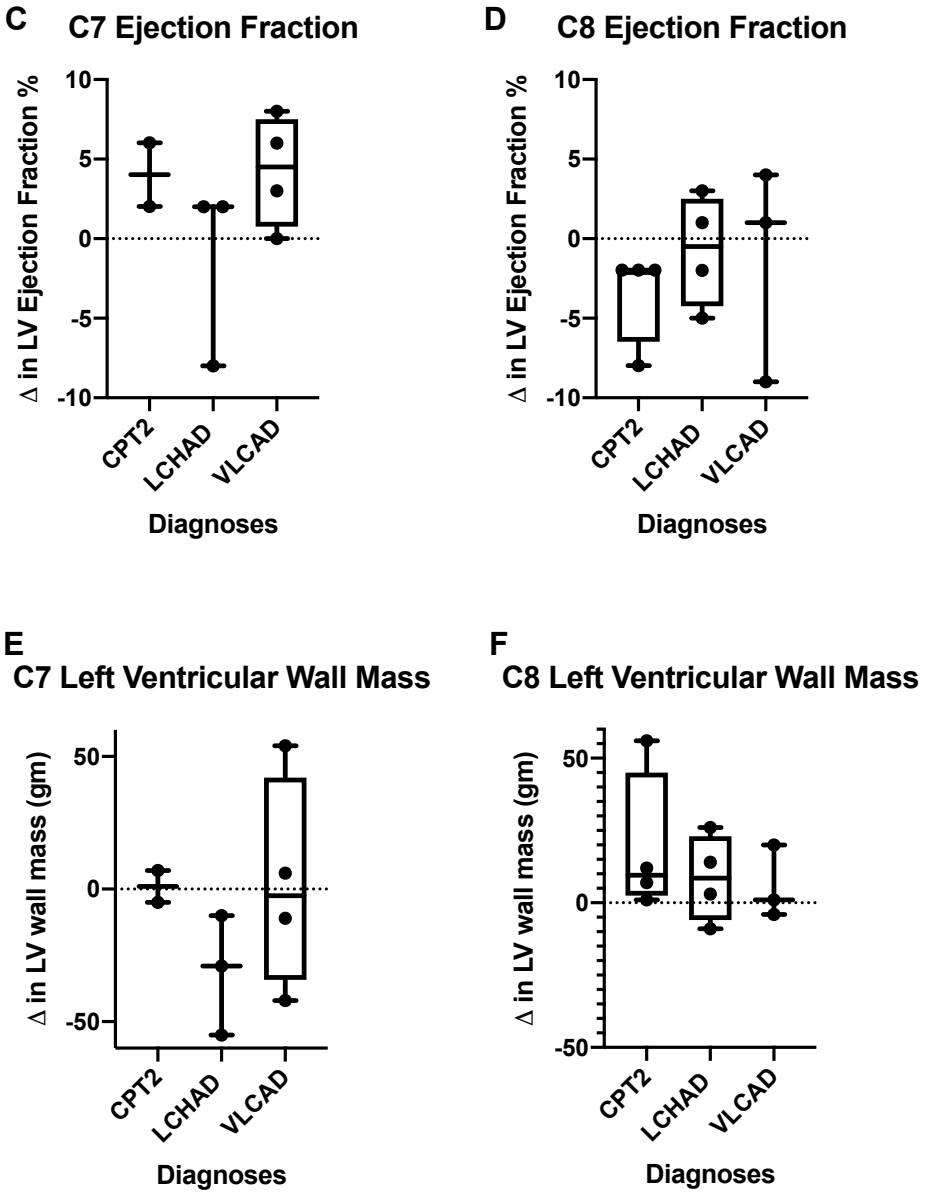


Fig. 5 Echocardiogram results. A one-way ANOVA was used to analyze the data. Data is presented as change in left ventricular (LV) ejection fraction and LV wall mass from 4 months to baseline. The box plot represents the 25th and 75th interquartile range. The horizontal line indicates the median and the whiskers represents the minimum and maximum values. In graphs **C)** and **D)** the y-axis represents the percent change in LV ejection fraction, while in graphs **E)** and **F)** the y-axis describes change in LV wall mass in grams. The x-axis provides the data from each of the three diagnostic groups tested. There was an increase in LV ejection fraction in the C7 treated group **(C)** compared to the C8 group **(D)**. **C)** There was no difference in LV ejection fraction between the three treatment groups receiving C7. **D)** There was

no difference in LV ejection fraction between the three treatment groups receiving C8. In LV wall mass there was a decrease in the C7 treated group but no change in the C8 treated group. **E)** There was no difference in LV wall mass between the groups receiving C7 supplementation. F) There was no difference in LV wall mass between the groups receiving C8 supplementation.

Hepatic Lipid Deposition

Soleus muscle and liver lipid deposition was studied through H magnetic resonance spectroscopy (MRS) in all study participants both at baseline and 4 months. In the original analysis, there was an observable decrease in hepatic lipid deposition among subjects randomized to C7 but not among subjects randomized to C8. However, while there was a significant decrease in lipid deposition, this sub-analysis indicated there was no difference in hepatic lipid change between the diagnostic groups. We conclude the change observed was attributed to the overall treatment effect of C7.

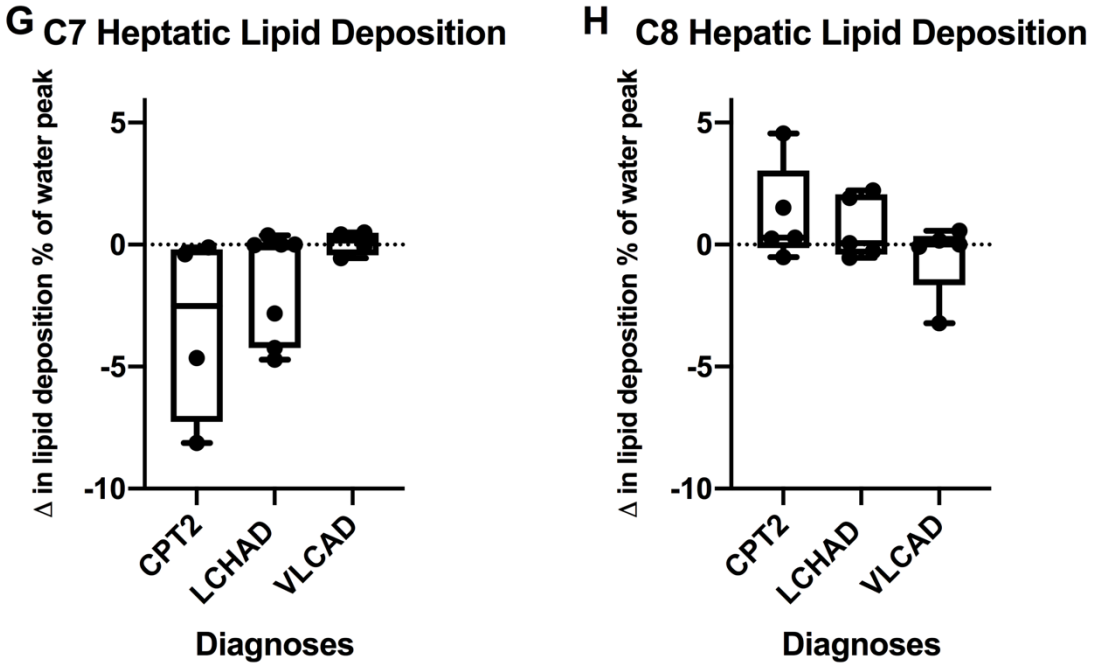
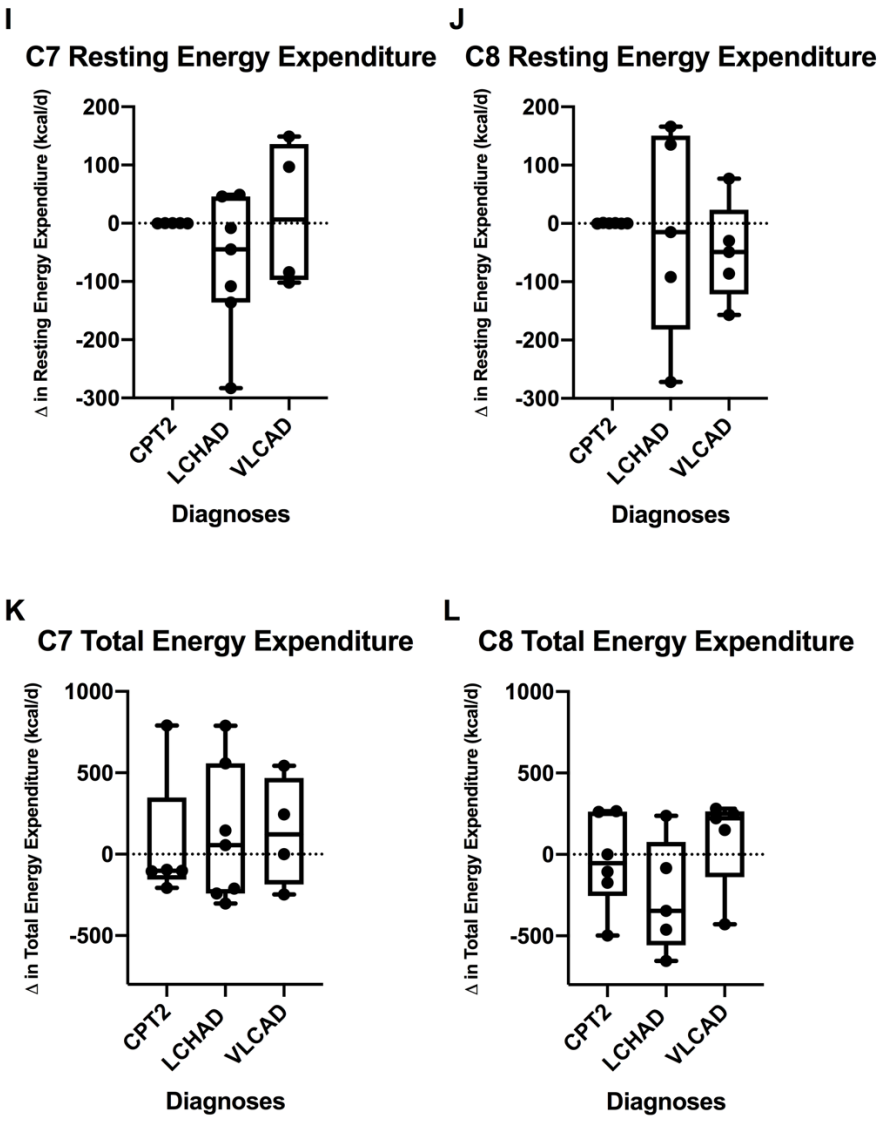


Fig. 6 Hepatic lipid deposition results. A one-way ANOVA was used to analyze the data. Percent of the water peak in hepatic lipid deposition decreased significantly in the C7 treated group compared to the C8 group. The box plot represents the 25th and 75th interquartile range. The horizontal line indicates the median and the whiskers represents the minimum and maximum values. The y-axis in both graphs is the

change in percent in water peak while the x-axis describes the three diagnostic groups. **G)** There was no difference in liver lipid deposition between the three treatment groups receiving C7. **H)** There was no difference in liver lipid deposition between the three treatment groups receiving C8.

Energy Expenditure

Total energy expenditure (TEE) was measured by doubly labeled water (DLW) and, resting energy expenditure (REE) was studied after overnight fasting using an indirect calorimetry. While there was no observable difference in TEE and REE reported in the intent to treat analysis, TEE and REE were lower within the LCHAD diagnostic group regardless of treatment assignment (C7 or C8). However, subjects in the LCHADD diagnostic group were considerably younger than subjects diagnosed with CPT2 and VLCAD deficiency. Therefore, the difference is likely due to participants' ages and not driven by a treatment effect as younger subjects have lower REE and TEE.


I
C7 Resting Energy Expenditure
Δ in Resting Energy Expendiure (kcal/d)
200
100
0
-100
-200
-300
CPT2
LCHAD
VLCAD
Diagnoses
J
C8 Resting Energy Expenditure
Δ in Resting Energy Expendiure (kcal/d)
200
100
0
-100
-200
-300
CPT2
LCHAD
VLCAD
Diagnoses
K
C7 Total Energy Expenditure
Δ in Total Energy Expenditure (kcal/d)
1000
500
0
-500
CPT2
LCHAD
VLCAD
Diagnoses
L
C8 Total Energy Expenditure
Δ in Total Energy Expenditure (kcal/d)
1000
500
0
-500
CPT2
LCHAD
VLCAD
Diagnoses

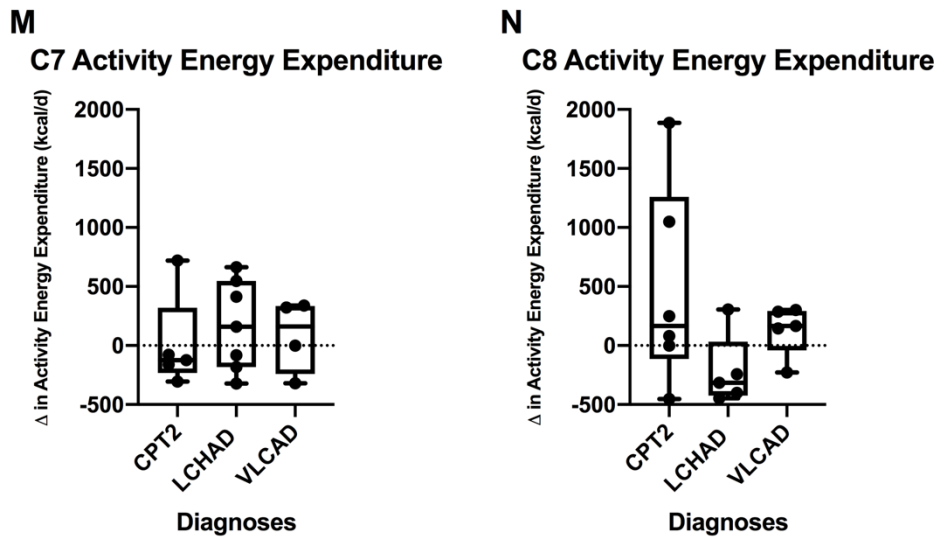


Fig. 7 Energy expenditure results. A one-way ANOVA was used to analyze the data. Over 4-month period participants were encouraged to consume 20% of estimated energy needs in their prescribed oil. The box plot represents the 25th and 75th interquartile range. The horizontal line indicates the median and the whiskers represents the minimum and maximum values. In graphs **I)** and **J)** the y-axis represents the change in resting energy expenditure (REE) in kcals per day, while the x-axis describes the three diagnostic groups. In graph **I)** the change in REE was lower in the LCHAD group receiving C7. However, LCAHD subjects were on average much younger than in the CPT2D and VLCADD groups. Younger subjects have lower REE thus the smaller change. In graph **J)** There was no difference in REE between the three treatment groups receiving C8. Graphs **K)** There was no difference in TEE between the three treatment groups receiving C7. *In graph **L)** there was slight decrease in the LCHAD group receiving C8. However, subjects with LCHAD on average were much younger than in the CPT2D and VLCADD groups. Younger subjects have lower TEE thus the lower change in TEE. **M)** There was no difference in AEE between the three treatment groups receiving C7. In graph **N)** there was no difference in AEE between the three treatment groups.

In this sub-study we completed a similar analysis by diagnostic group for the following variables: %FFM, %FM, RQ, VAT, SAT, IMCL, EMCL, RQ AUC, VO2 AUC, SB AUC, DP AUC (table 2). These variables were not different between subjects randomized to C7 versus C8 in the original analysis. The variables that did have a significant treatment effect in the original analysis were EF, HR, hepatic lipid deposition, and no change in TEE. Similar to the initial report, we did not find any differences in the other variables tested when we analyzed these variables by diagnostic group (CPT2D, VLCADD, and LCHADD/TFPD).

Compliance

At baseline, participants were randomly assigned C7 or C8 supplementation in a 1:1 ratio. Compliance of prescribed study oil was assessed through two methods, multiple 3-day diet records and by measuring the final unconsumed oil at 4 months. In the original study[10], 3-day diet records indicated that approximately 14% and 16% of total energy intake was consumed from C8 and C7 supplementation. Likewise, according to oil dispensed versus oil returned, participants averaged 76% and 74% of C8 and C7 after 4 months of treatment. Compliance data expressed as a percent of the total oil dispensed – oil returned was available for 30 of the 32 subjects; two subjects did not return their study oil and complicance could not be measured. We initially assessed the relationship of compliance expressed as a percent of oil consumed with outcomes by a simple linear regression. Subjects randomized to C7 supplementation had statistically higher LV efection fraction, but this was not linearly related to compliance or amount of study oil consumed; ie. subjects who consumed more C7 did not have a greater change in ejection fraction than those who consumed less of the study oil. Likewise, hepatic lipid deposition decreased in the C7 group, but was also not linearly related to compliance. These results suggest that changes observed in these variables were not linearly related to participant compliance, but rather the treatment effect was observed across a range of C7 intake.

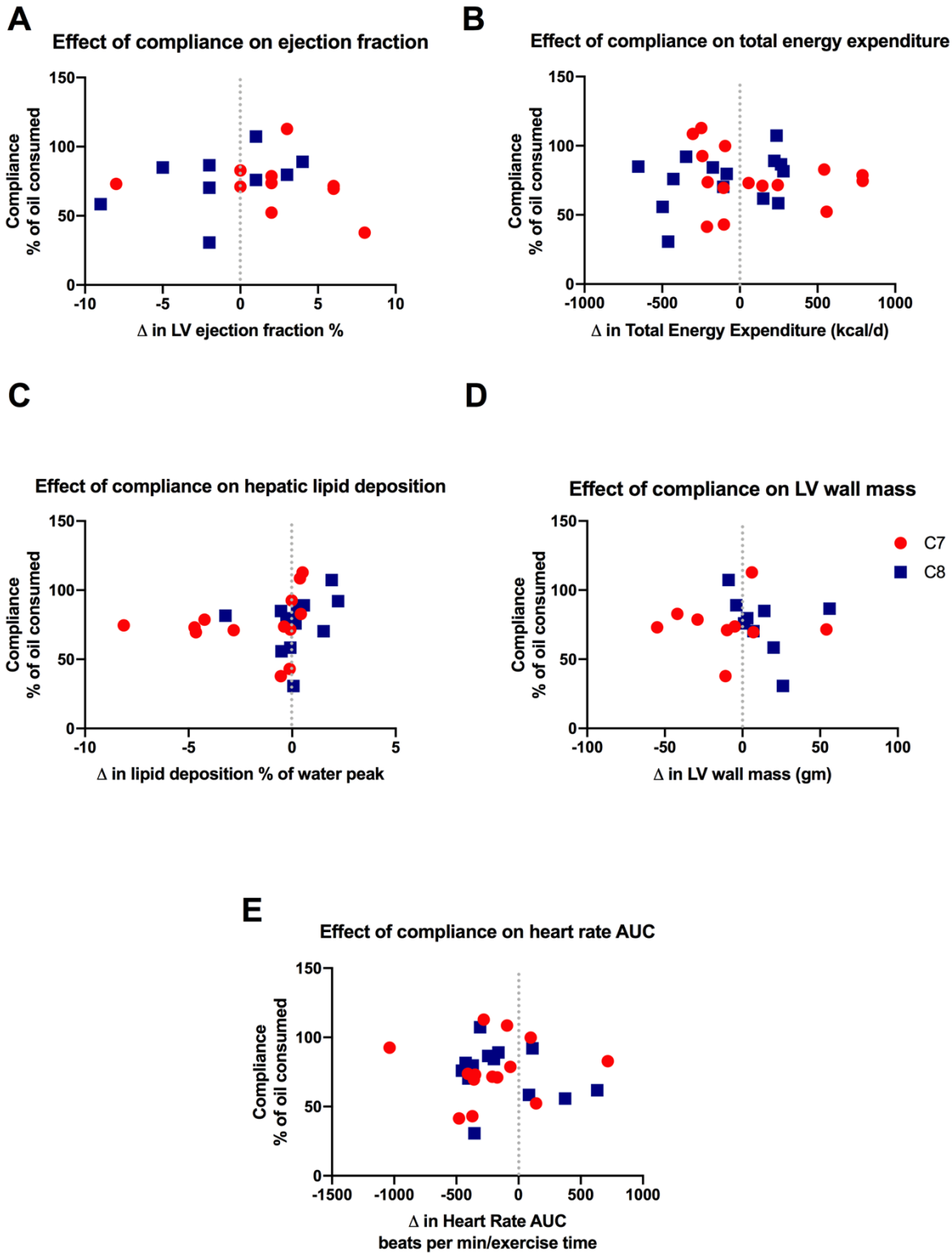


Fig. 8 Effect of compliance (% of study oil consumed). A simple regression was used to analyze all data. Over a 4-month period participants were encouraged to consume 20% of estimated energy needs in

their prescribed oil. The y-axis represents each participants compliance as a % of study oil consumed, while the x-axis represents change (4-month measure – baseline) in the five variables tested. Blue squares are participants randomized to C8 and red circles are participants randomized to C7. In graph **A)** more of the participants prescribed C7 had an increase in LV ejection fraction than the C8 participants, however there was no linear relationship between the percent of study oil consumed and the magnitude of the response. In graph **B)** there was no linear relationship between LV wall mass and compliance in either C7 or C8 groups. **C)** Subjects randomized to C7 had lower hepatic lipid deposition than subjects randomized to C8 , however this was not linearly related to compliance. **D)** Change in TEE was not related to compliance. **E)** Subject randomized to C7 had a lower HR AUC, however this was not linearly related to compliance.

Age at diagnosis

Age at diagnosis was used as a surrogate for severity of the LC-FAOD because it is generally assumed participants who present with symptoms early in life have more severe forms of the disorder than those who present with symptoms in adolescence or adulthood. The age at diagnosis was assessed by review of medical records and defined as the age at which the subject was officially diagnosed with a LC-FAOD. The data is complicated by the fact that NBS for these disorders was initiated in the early 2000's and subjects born during the NBS era did not present symptomatically. We again assessed if there was the relationship between disease severity and outcomes with simple linear regression. Because NBS diagnosed subjects prior to the onset of symptoms, it is impossible to predict severity of disease in these subjects. We removed all individuals whose medical records indicated they were diagnosed via NBS. In total, 7 participants were removed. Including 6 LCHADD and 1 VLCADD participant. Therefore, our simple regression only included subjects who presented symptomatically and were not diagnosed with their LC-FAOD through NBS.

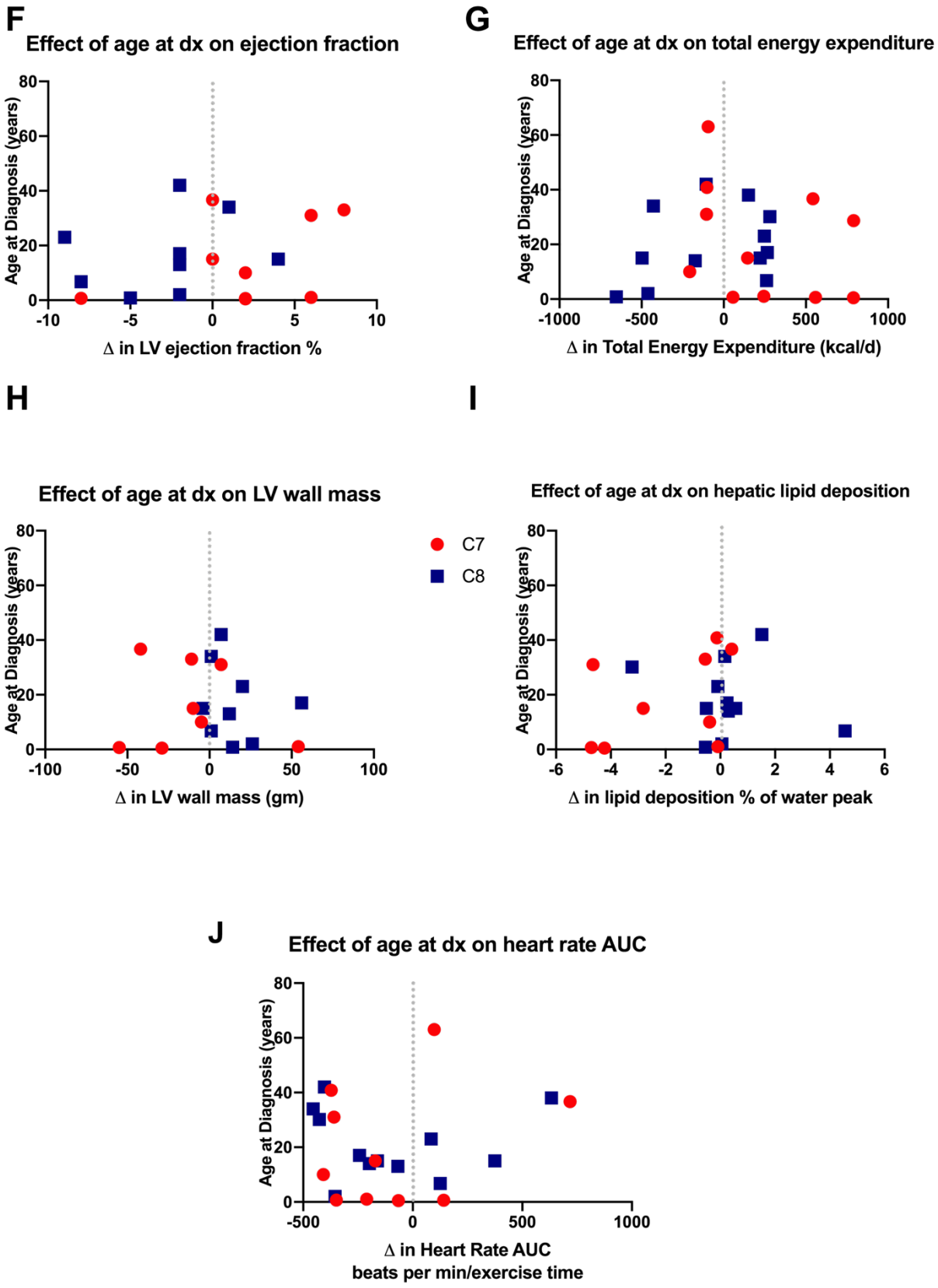

F
Effect of age at dx on ejection fraction
Age at Diagnosis (years)
Δ in LV ejection fraction %
G
Effect of age at dx on total energy expenditure
Age at Diagnosis (years)
Δ in Total Energy Expenditure (kcal/d)
H
Effect of age at dx on LV wall mass
Age at Diagnosis (years)
Δ in LV wall mass (gm)
C7
C8
I
Effect of age at dx on hepatic lipid deposition
Age at Diagnosis (years)
Δ in lipid deposition % of water peak
J
Effect of age at dx on heart rate AUC
Age at Diagnosis (years)
Δ in Heart Rate AUC
beats per min/exercise time

Fig. 9 Age at participant diagnosis. A simple regression was used to analyze all data. In all four plots the y-axis represents participants age at initial diagnosis of their LC-FAOD, respectively. Blue squares are participants randomized to C8 and red circles are participants randomized to C7. **F)** Subjects randomized to C7 had an increased in LC EF compared to subjects randomized to C8, however this was not linearly related to participant age at initial diagnosis. In graph **G)** there was linear relationship in LV wall mass and participant age at initial diagnosis. **H)** subjects randomized to C7 had lower hepatic lipid deposition than subjects randomized to C8 , however this was not correlated to age at initial diagnosis. In graph **I)** there was no relationship between in TEE and age at initial diagnosis. **J)** Change in HR AUC during moderate intensity exercise was lower in subjects randomized to C7 compared to subjects randomized to C8, however this was not correlated to age at initial diagnosis.

Multiple Linear Regression

We analyzed the relationship between 4 independent co-variants, diagnosis, treatment assignment, compliance and age at diagnosis with our dependent variables, LV EF, LV wall mass, TEE, HR AUC and liver lipid deposition. Consistent with the simple linear regression, our multiple linear regression (MLR) indicated that there was no significant relationship between all four independent variables and our outcome variables, with the exception of hepatic lipid deposition. Initially, we ran a multiple linear regression using the whole model and including all 4 co-variates. Next, we performed a manual step-wise and removed participant diagnosis, then we removed participant age at diagnosis and finally compliance, leaving us with our dependent variables and treatment assignment. Interestingly, the only significant variable was in the final step for the MLR, when all other dependent variables were removed. The P value for treatment group in EF was 0.04, which is considered significant. While LV wall mass was not, it was trending towards significance with a P value of 0.08.

Interestingly, change in heptic lipid deposition and heart rate AUC were not normally distributed when a D'Agostino & Pearson and Shapiro-Wilk test were performed. Outliers were then removed, in total 2 for heart rate AUC and 1 for hepatic lipid deposition. The cleaned data indicated that heart rate AUC and hepatic lipid deposition were now normally distributed. A MLR was performed, with the same manual step-wise routine as previously described. The only indepdent variable that showed significance was hepatic lipid deposition. This P value of 0.04 was consistent for the initial two steps in the MLR, and trended lower for the final two with a P value of 0.03 and 0.02, respectively. Heart rate AUC was considered insignificant for all independent variables in all the MLRs.

Table 8:

Independent Variables	Outcome Variables (P value)			
	Ejection fraction	LV wall mass	HR AUC	Hepatic lipid deposition
Diagnosis	0.70	0.72	0.24	0.82
Age at diagnosis	0.64	0.62	0.12	0.78
% of participant oil consumed, compliance	0.77	0.57	0.75	0.30
Treatment (C7 or C8)	0.13	0.13	0.82	*0.04

Independent Variables	Outcome Variables (P value)			
	Ejection fraction	LV wall mass	HR AUC	Hepatic lipid deposition
Age at diagnosis	0.62	0.61	0.17	0.77
% of participant oil consumed, compliance	0.75	0.55	0.86	0.28
Treatment (C7 or C8)	0.12	0.11	0.71	*0.04

Independent Variables	Outcome Variables (P value)			
	Ejection fraction	LV wall mass	HR AUC	Hepatic lipid deposition
% of participant oil consumed, compliance	0.83	0.63	0.79	0.28
Treatment (C7 or C8)	0.12	0.10	0.63	*0.03

Independent Variables	Outcome Variables (P value)			
	Ejection fraction	LV wall mass	HR AUC	Hepatic lipid deposition

Treatment (C7 or C8)	*0.04	0.08	0.48	*0.02

Discussion:

In the initial report[10], subjects randomized to treatment with Triheptanoin (C7) had a significant increase in LV ejection fraction, a trend toward smaller LV wall mass, a decrease in heart rate during moderate intensity exericse, and a decrease in hepatic lipid deposition, but no change in TEE. The study population included subjects diagnosed with CPT2D, VLCADD, and LCHADD/TFPD, which, although similar, have some distinct phenotypic differences. In this sub-study we aimed to distinguish if the overall response reported in the entire mixed population was driven by one diagnostic group with a greater response to C7 treatment than the other diagnoses. We separated each randomized treatment group, C7 and C8, by diagnosis and looked at the change with treatment. There was no differences between the three diagnostic groups, CPT2D, LCHADD, VLCADD, in the magnitude of change with C7 treatment. Likewise, the normal variability of change within the C8 group was not different between the diagnoses. Therefore, we conclude the statistically significant changes reported in the initial study were not driven by one diagnosis, but rather a result of the overall treatment effect of C7, across all subjects with a long-chain fatty acid oxidation disorder.

There was a difference in energy expenditure between LCHADD subjects and subjects diagnosed with VLCAD and CPT2 deficiencies. Both REE and TEE for participants diagnosed with LCHADD were lower regardless of treatment randomization. We also observed that study participants diagnosed with LCHADD were younger in comparison to CPT2D or VLCADD subjects. The mean age for LCHADD participants was 14.6 $\pm$ 7.3, while the mean age for subjects with CPT2D was 30 $\pm$ 16.7 and the mean age for subjects with VLCADD was 31.7 $\pm$ 11.4. As a result of younger age, and smaller body size, the energy expenditure was lower in the LCHADD subjects. This finding was not due to a different response to C7 or C8 among participants diagnosed with LCHADD but most likely a reflection of the younger age of the participants in this diagnostic group.

A previous publication also reported increases in ejection fraction from baseline in LCFAOD participants, including subjects with CPT2D, LCHADD, and VLCADD that received target dosing of 25-35% of total energy intake.[11] However, the majority of subjects enrolled in the study were infants, with LCFAOD- associated cardiomyopathy. Participates in the current study were $\geq$ 7 years in age, and stable following a diet including MCT oil supplementation prior to enrollment with normal cardiac function at enrollment except for one participant.[10] When looking at previous literature and this sub-study, the

cardiac effects of C7 are remarkably consistent. The data suggests treatment with C7 improves cardiac function at rest and during exercise in both young and older subjects with FAODs, with impaired or normal cardiac function and irrespective of the specific enzyme deficiency, CPT2, VLCAD or LCHAD deficiencies.

Another open-label study followed LCFAOD subjects for a 24-week period, measuring change in cycle ergometry and 12-minute walking test (MWT) to observe differences from baseline after starting C7 treatment.[21] Cycle ergometry workload increased by 60% from baseline, and there was a mean increase of 673.4 m, or 28% from baseline with the 12 MWT.[21] In the overall population for this study, heart rate during exercise was 6.98 beats per minute lower in the C7 treated group for a given workload but there was no change in heart rate in the C8 group. The results of this secondary analysis suggests the change in exercise heart rate was similar across the diagnostic groups indicating an overall treatment effect of C7 to improve exercise capacity among subjects with CPT2 VLCAD and LCHAD deficiencies.

In a previous report, some CPT2D and VLCADD participants experienced weight gain while receiving C7 treatment.[13] In those reports treatment length ranged from 7 to 61 months, and individuals were instructed to consume 30-35% of total daily caloric intake in study oil, a substantially higher dose compared to our goal of 20% of total caloric intake. In both the primary analysis and in this sub-study, we observed no major differences in body composition, or substantial change in body weight between subjects randomized to C7 or to C8 and no difference between the three diagnostic groups over a 4 treatment period. Treatment with C7 or C8 does not necessarily result in weight gain or in changes in overall body composition although dose of study oil as a % of total energy most likely plays an important role in this observation.

For our second aim, we wanted to determine what significant clinical factors, if any, were associated with the greatest response to C7 supplementation. Specifically we wanted to determine if increased compliance with consuming study oil was associated with a greater response to C7. Level of compliance or study oil consumption is a co-variate that was not controlled for in the initial statistical analysis. Based on amount of oil returned at the end of the study, subjects consumed on average 74% and 76% of dispensed study oil from C7 and C8, respectively but this ranged from 37.8-112.8% in the C7 group and from 30.7-107.3% in the C8 group. Compliance was not associated with the primary outcomes LV EF, wall mass, HR AUC , liver lipid deposition and TEE by either simple or multiple linear regression.

Interestingly, there was no evidence of a dose response relationship within our range of study oil consumption. Perhaps smaller doses of C7 treatment oil could be as effective as larger doses, thus increasing treatment tolerability. However, this study used a treatment range of 20% of total energy intake, a considerably lower range then in previous studies of C7 which prescribe as high as 30-35% of energy from C7. Therefore, our findings were only relavent to our treatment range. There is some controversy surrounding triheptanoin dosing. A C7 intake of 35% of energy with 10% long-chain fat for essential fatty acids will provide a 45% total energy from fat and this is above the Institute of Medicine's acceptable macronutrient distribution range of 20-35%. Diets higher in fat than the AMDR may be limiting in micronutrients and fiber found in grains and vegetables and increase the risk of chronic illness. Additional studies, perhaps including a dose response study are needed to establish an optimal dose, that also maintains good overall nutritional status, including the balance between macro and micronutrients.

Age at diagnosis was used as a predictor of severity of the disorder, with most cases indicating the younger the disorder the more severe. Participant age at diagnosis was measured in years, and derived from medical records indicating the age at which a subject presented symptomatically and was subsequently diagnosed with a FAOD. The original analysis did not control for disease severity in the statistical analysis. Age at diagnosis was not associated with any of our independent variables including LV EF, wall mass, HR AUC, liver lipid desposition and TEE in either the simple or multiple linear regression. Therefore, we conclude that the statistically significant changes observed in the initial analysis did not differ across the wide range of age at diagnosis, rather they were a result of overall treatment effect of C7. It is possible that age a diagnosis is not a good surrogate for disease severity and that this analysis did not adequately test the relationship between response to treatment and disease burden. It is also possible there is no relationship and the changes observed in the population are truly a treatment effect regardless of the large variability in disease severity reported among subjects with LC-FAOD

One of the major limitations of this sub-analysis was the small sample size. A total of 32 subjects participated in this study, and they were further sub-divided between the three diagnostic groups, CPT2D, LCHADD/TFPD, VLCADD. This resulted in our sub-study being woefully underpowered. Therefore we cannot draw a strong conclusion on whether there was or was not a true correlation between the four variables and participant compliance of study oil or participant age at diagnosis. A larger sample size would be necessary to test these relationships but given the rarity of the disorders, it is unlikely that such a study will be conducted. Likewise, the majority of the echocardiogram data was missing, and this

significantly limited population size for those variables. Furthermore, as mentioned above, using age at diagnosis may be poor surrogate for disease severity, particularily as all participants were considered well-controlled and on a stable diet of MCT oil prior to study enrollment, a signficantly different population than infants presenting with severe cardiomyopathy or individuals admitted with rhabdomyolysis. Similarly, there was quite a discrepency in age variablilty, as seen in the LCHADD ANOVAs for energy expenditure. Mean LCHADD age was 14.6 $\pm$ 7.3, while CPT2D mean age was 30 $\pm$ 16.7 and VLCADD mean age was 31.7 $\pm$ 11.4. The variability in age lowering energy expenditure results for subjects with LCHAD and may have decreased the ability to detect change in the whole population. Another limitation includes the overall short treatment length. The study was only 4 months in length, a realitively short treatment period. Additional studies that follow subjects for a longer treatment period would be ideal, but such studies are difficult and expensive to conduct.

Strenghts of the study included measurements of dietary intake and compliance with study oil perscription. The measurements of oil dispensed versus oil returned allowed us to estimate compliance as a percent of oil consumed. This was further validated by the completion of 3-day diet records, and this detailed diet data is unique compared to previous C7 studies. Likewise, the measurement of change from baseline was able to reduce variablity with such a large age range, such that change with treatment was used in the statistical analysis accounting for baseline measures and allowed for a comparision across the wide age range. This approach worked for some outcomes such as the HR with exercise. Younger subjects have a higher resting and exercise heart rate associated with a younger age but the change in HR with treatment accounted for the higher baseline values. It may not have been as effective in controlling for differences in energy expenditure related to age. Younger subjects have lower TEE associated with younger age and smaller body size. The proportional change (%TEE) may also be smaller with smaller TEE values at both baseline and end of study.

Conclusion

The results of this sub-analysis conclude that the response seen in the original study was attributed to overall treatment effect, and was not driven by one diagnostic group. Subjects randomized to triheptanoin responded similarly to treatment regardless of the specific LC-FAOD diagnosis. Disease severity estimated by the subject's age at diagnosis, and compliance measured as percent of oil consumed were not correlated to any of our outcome variables. There was no relationship between higher doses and a higher treatment response. Perhaps smaller doses of C7 treatment oil could be as

effective as larger doses, and smaller doses could increase oil tollerability and decrease the likelihood of nutrient deficiencies. Additional studies with larger sample size would be ideal but given the rarity of the disorders and complications with conducting such a study, these studies are unlikely to be completed. This secondary analysis does appear to indicate the significant results reported in our original population analysis was due to a true treatment effect of C7.

References:

1. Bennett MJ. Pathophysiology of fatty acid oxidation disorders. *J Inherit Metab Dis.* 2010;33(5):533-537.
2. De Biase I, Viau KS, Liu A, et al. Diagnosis, Treatment, and Clinical Outcome of Patients with Mitochondrial Trifunctional Protein/Long-Chain 3-Hydroxy Acyl-CoA Dehydrogenase Deficiency. *JIMD reports.* 2017;31:63-71.
3. Knottnerus SJG, Bleeker JC, Wust RCI, et al. Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. *Rev Endocr Metab Disord.* 2018.
4. and PR, Matern D, Bennett MJ. Fatty Acid Oxidation Disorders. *Annual Review of Physiology.* 2002;64(1):477-502.
5. Joost K, Ounap K, Zordania R, et al. Prevalence of Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency in Estonia. *JIMD reports.* 2012;2:79-85.
6. Raval DB, Cusmano-Ozog KP, Ayyub O, et al. Diagnosis of LCHAD/TFP deficiency in an at risk newborn using umbilical cord blood acylcarnitine analysis. *Mol Genet Metab Rep.* 2017;10:8-10.
7. Wieser T. *Carnitine Palmitoyltransferase II Deficiency.* University of Washington, Seattle, Seattle (WA); 1993.
8. Leslie ND, Valencia CA, Strauss AW, Zhang K. Very long-chain acyl-coenzyme A dehydrogenase deficiency. In: *GeneReviews®[Internet].* University of Washington, Seattle; 2018.
9. Zimmerman JL, Shen MC. Rhabdomyolysis. *Chest.* 2013;144(3):1058-1065.
10. Gillingham MB, Heitner SB, Martin J, et al. Triheptanoin versus trioctanoin for long-chain fatty acid oxidation disorders: a double blinded, randomized controlled trial. *J Inherit Metab Dis.* 2017;40(6):831-843.
11. Vockley J, Charrow J, Ganesh J, et al. Triheptanoin treatment in patients with pediatric cardiomyopathy associated with long chain-fatty acid oxidation disorders. *Mol Genet Metab.* 2016;119(3):223-231.
12. 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(2):259-269.
13. Roe CR, Yang BZ, Brunengraber H, Roe DS, Wallace M, Garritson BK. Carnitine palmitoyltransferase II deficiency: successful anaplerotic diet therapy. *Neurology.* 2008;71(4):260-264.
14. Roe CR, Brunengraber H. Anaplerotic treatment of long-chain fat oxidation disorders with triheptanoin: Review of 15 years Experience. *Mol Genet Metab.* 2015;116(4):260-268.
15. Vockley J, Marsden D, McCracken E, et al. Long-term major clinical outcomes in patients with long chain fatty acid oxidation disorders before and after transition to triheptanoin treatment--A retrospective chart review. *Mol Genet Metab.* 2015;116(1-2):53-60.
16. Vockley J, Burton B, Berry GT, et al. Results from a 78-week, single-arm, open-label Phase 2 study to evaluate UX007 in pediatric and adult patients with severe long-chain fatty acid oxidation disorders (LC-FAOD). *J Inherit Metab Dis.* 2018.
17. Pascual JM, Liu P, Mao D, et al. Triheptanoin for glucose transporter type I deficiency (G1D): modulation of human ictogenesis, cerebral metabolic rate, and cognitive indices by a food supplement. *JAMA Neurol.* 2014;71(10):1255-1265.
18. Roe, C.R. and F. Mochel, Anaplerotic diet therapy in inherited metabolic disease: therapeutic potential. J Inherit Metab Dis, 2006. 29(2-3): p. 332-40. And Brunengraber, H. and C.R. Roe, Anaplerotic molecules: current and future. J Inherit Metab Dis, 2006. 29(2-3): p. 327-31.
19. National Institute of Health (2019, April 16). Carnitine palmitoyltransferase ii deficiency. from https://ghr.nlm.nih.gov/condition/carnitine-palmitoyltransferase-ii-deficiency#statistics.

20. Southeast regional genetics network (2019, Febuary). Vlcad nutrition management guidelines. from https://southeastgenetics.org/ngp/guidelines.php/106/bg/0/0/VLCAD%20Nutrition%20Guidelines/Version%201.0/Background.
21. Vockley, J., et al. (2017). UX007 for the treatment of long chain-fatty acid oxidation disorders: Safety and efficacy in children and adults following 24weeks of treatment. *Mol Genet Metab* **120**(4): 370-377.
22. Wexler, R, Elton T, Pleister A, et al. Cardiomyopathy: An Overview. *Am Fam Physician.* 2009;79(9):7780784.