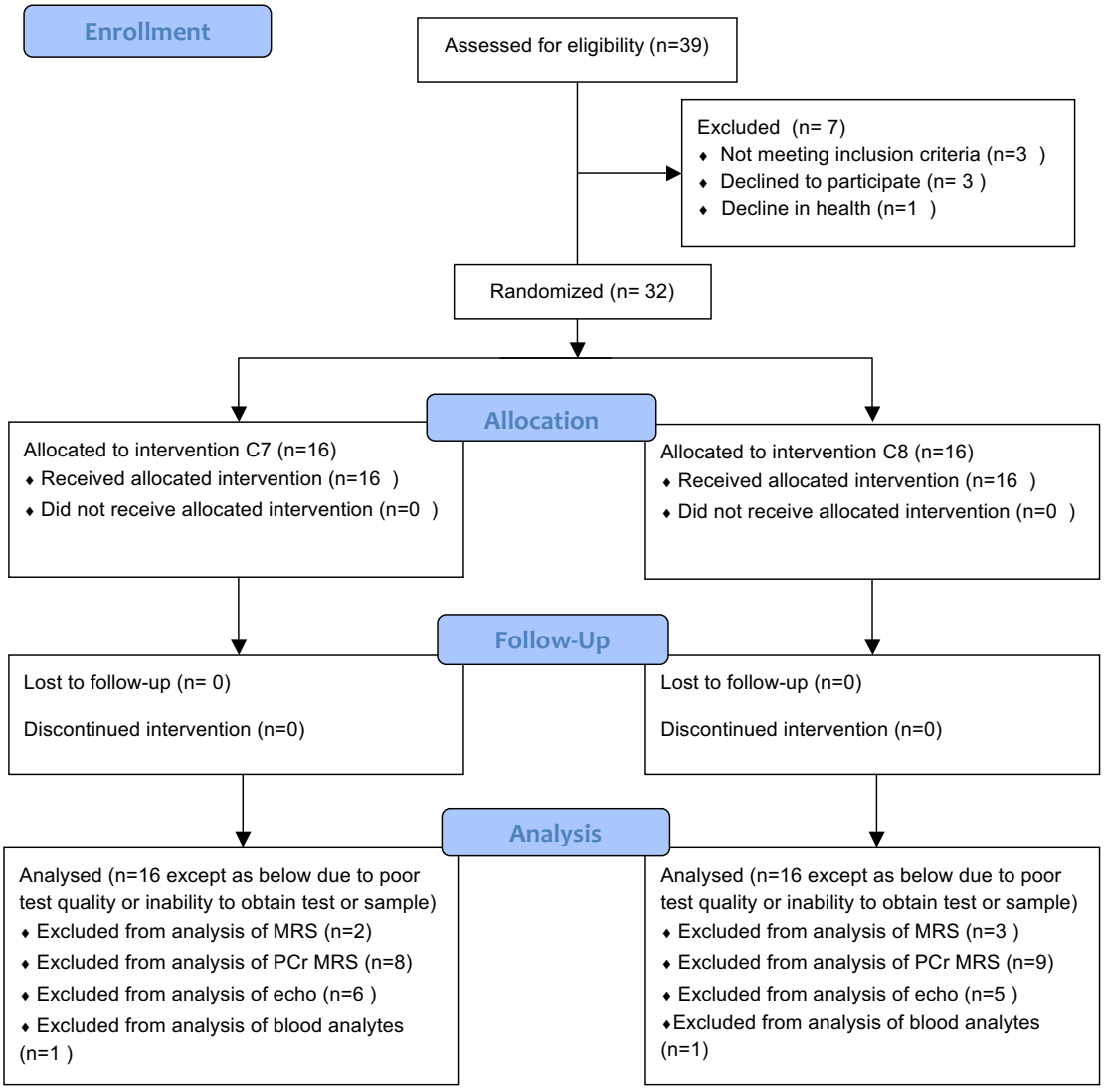
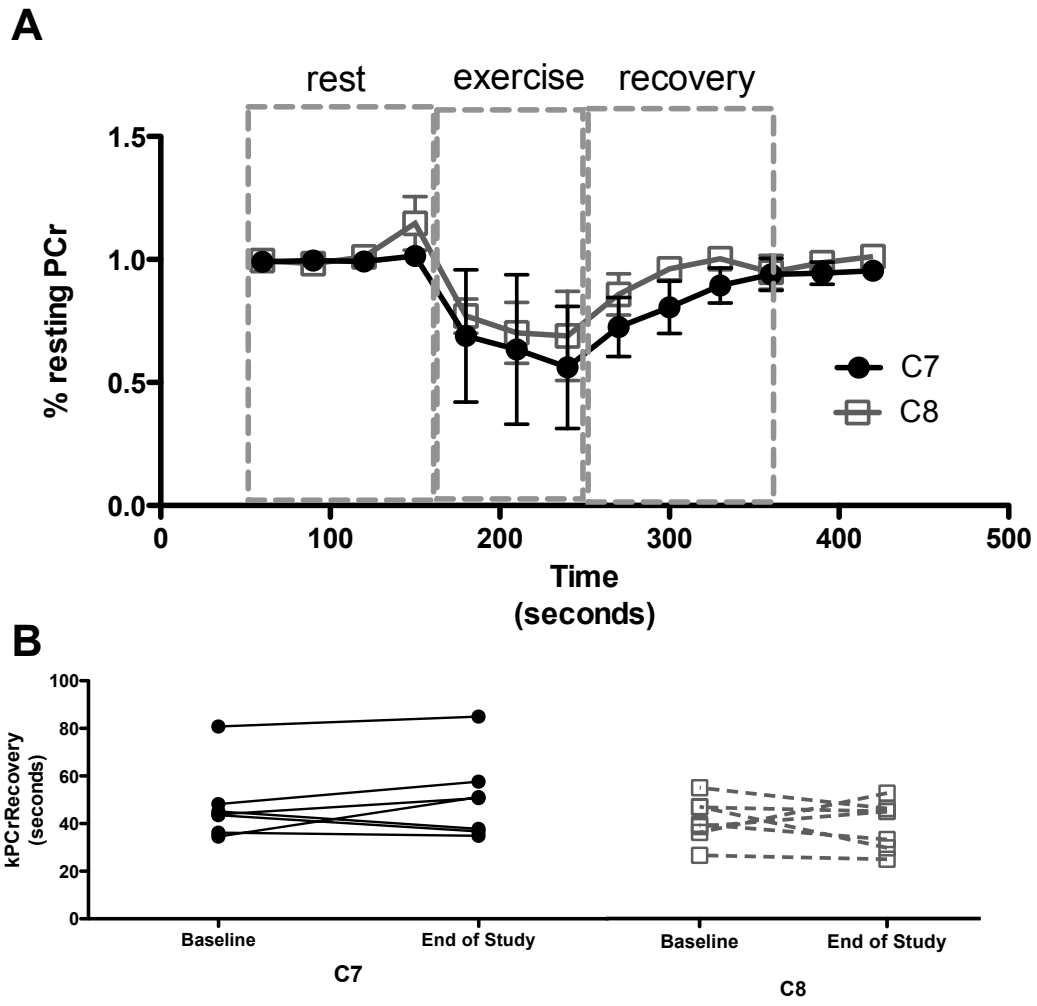
SUPPLEMENTAL MATERIAL

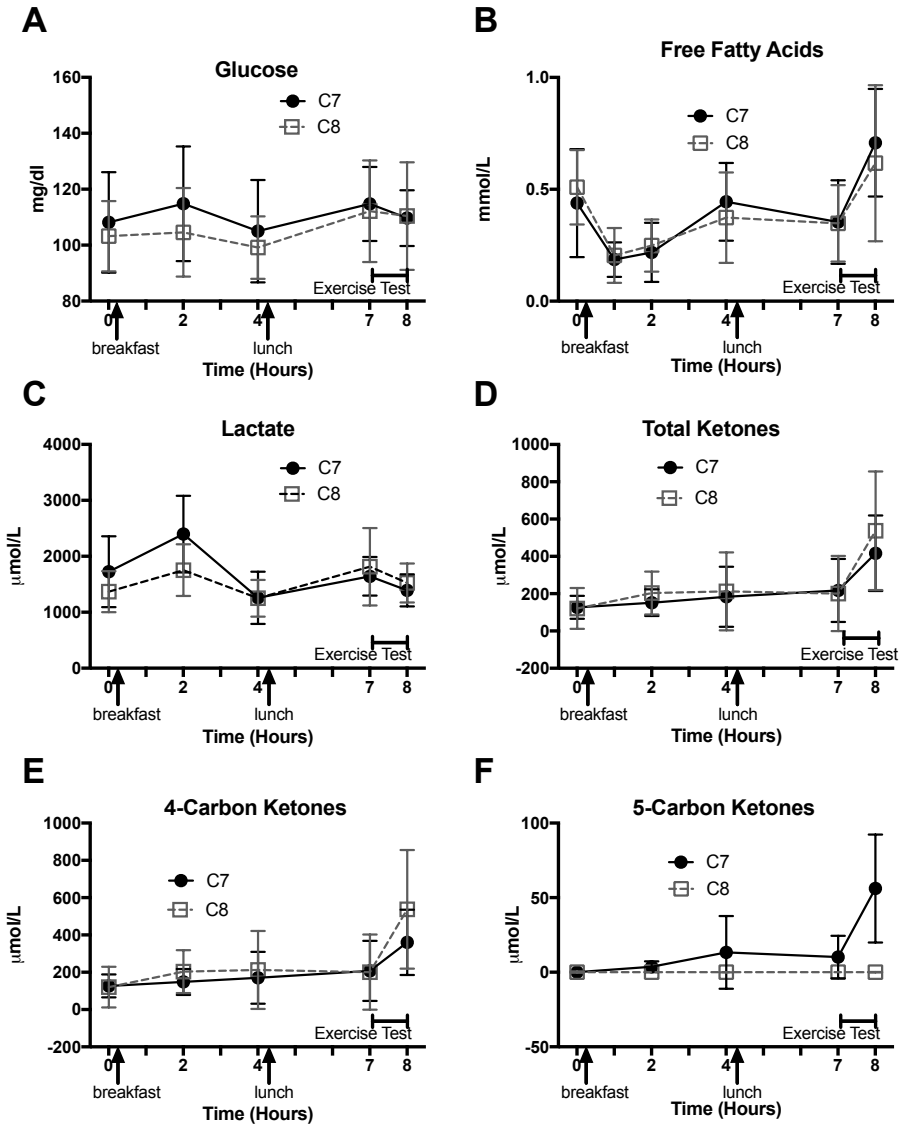
Supplemental Figure 1: Consort diagram of Triheptanoin trial





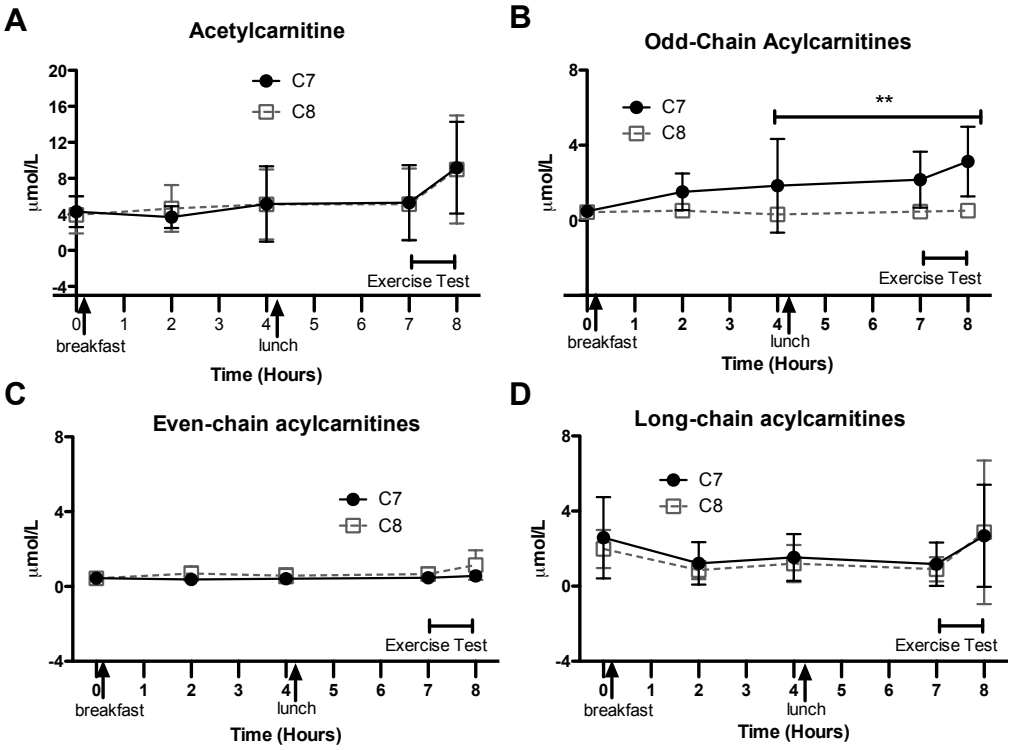
Supplemental Figure 2: Phosphocreatine Recovery after acute exercise

A) The mean ± standard deviation change in percent of resting phosphocreatine (PCr) during acute exercise and recovery was not different between groups. B) The individual recovery constants, kPCr, at baseline and at the end of the study are shown. There is no significant change from baseline in either group.


A
Glucose
C7
C8
mg/dl
160
140
120
100
80
Exercise Test
0
2
4
7
8
breakfast
lunch
Time (Hours)
B
Free Fatty Acids
C7
C8
mmol/L
1.0
0.5
0.0
Exercise Test
breakfast
lunch
Time (Hours)
C
Lactate
C7
C8
µmol/L
4000
3000
2000
1000
0
Exercise Test
breakfast
lunch
Time (Hours)
D
Total Ketones
C7
C8
µmol/L
1000
800
600
400
200
0
-200
Exercise Test
breakfast
lunch
Time (Hours)
E
4-Carbon Ketones
C7
C8
µmol/L
1000
800
600
400
200
0
-200
Exercise Test
breakfast
lunch
Time (Hours)
F
5-Carbon Ketones
C7
C8
µmol/L
100
50
0
-50
Exercise Test
breakfast
lunch
Time (Hours)

Supplemental Figure 3: Glucose, Free Fatty Acids, Lactate and Ketone Concentrations

Data are presented as mean ± standard deviation. Change in metabolite concentration following an overnight fast (time 0), a mixed meal (time 2 and 4) and before and after exercise (time 7 and 8) are shown in each graph. A) Glucose concentrations were similar between groups and varied with the normal range after a mixed meal and after exercise. B) Free fatty acids were increased after an overnight fast, decreased with feeding and rose with exercise. There was no difference between groups. C) Lactate concentrations were highest 2 hours post-prandially and decreased with fasting and exercise. There was no difference between groups. D) The total ketones (sum of acetoacetate, ß-hydroxybutyrate (BOHB), ß-ketopentanoate, ß-hydroxypentanoate) produced were not different between groups. E & F) 4-carbon ketones rose in the Trioctanoate group (acetoacetate, BOHB) and 5-carbon ketones rose in the Triheptanoin group (ß-ketopentanoate, ß-hydroxypentanoate) after exercise.



Supplemental Figure 4 : Acylcarnitine Concentrations.

Data are presented as mean ± standard deviation. Change in metabolite concentration following an overnight fast (time 0), a mixed meal (time 2 and 4) and before and after exercise (time 7 and 8) are shown in each graph. A) Acetylcarnitine was similar between groups and rose after exercise. The change in acetylcarnitine correlated with changes in ketones. B) Odd-chain acylcarnitines including C3, C5, and C7 rose after the meal and rose again after exercise in the participants treated with triheptanoin. C) The even chain acylcarnitines including C4, C6, C8 and C10 were similar between groups. D) The long-chain acylcarnitines include C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2 were similar between groups, decreased after a meal and rose after exercise.

Supplementary Table 1: Primary Outcome Measures

	Change From Baseline				Delta C7 vs Delta C8		
	C7		C8		C7 vs C8		p-value
Primary outcome measures:	**mean**	**95% CI**	**mean**	**95% CI**	**mean**	**95% CI**	
Echocardiogram							
Left ventricular ejection fraction (fold-change)	**1.042**	**0.9907 , 1.0959**	**0.9705**	**0.9249 , 1.0183**	**1.0736**	**1.0013 , 1.1511**	**0.046**
Left ventriclar end systolic volume (fold change)	0.9298	0.7840 , 1.026	1.1182	0.9579 , 1.322	0.8011	.6483 , 1.0468	0.11
Left ventricular wall mass in grams (fold change)	**0.9186**	**0.7853 , 1.0744**	**1.1466**	**0.9945 , 1.322**	**0.8011**	**0.6483 , 0.99**	**0.041**
Total energy Expenditure (kcal/d)	107.4	-83.85 , 299	-72.67	-264 , 119	180	-90 , 451	0.184
Phosphocreatine Recovery constant; kPCR (seconds)	4.2793	-1.8155 , 10.374	-3.575	-10.17 , 3.0197	7.8543	-1.1254 , 16.834	0.081
Treadmill Exercise Test	**Time X Treatment Interaction**						
Heart Rate	F(4, 115) = 0.25 , p=0.92				**-6.983**	**-13.629 , -0.336**	**0.04**
VO2	F(4, 108) = 0.36 , p=0.84				-0.227	-1.661 , 1.207	0.746
Double product	F(3, 76) = 2.0 , p=0.12				-967	-2793 , 859	0.286

Echocardiogram outcomes expressed as fold-change from baseline. Mean and 95% Confidence Interval (CI) for each group and the difference between groups given. Bold values are significantly different. Treadmill exercise testing was a repeated measures over the 45 minute exercise test. Time X treatment interactions for each parameter are given along with the change from baseline (delta) difference between groups.

Supplementary Table 2: Secondary Outcome Measures

	Change From Baseline				Delta C7 vs Delta C8		
	C7		C8		C7 vs C8		p-value
Secondary Outcome Measures:	**mean**	**95% CI**	**mean**	**95% CI**	**mean**	**95% CI**	
Body Weight (kg)	-0.275	-1.4833 , 0.9333	1.3625	0.1542 , 2.5708	-1.6375	-3.3463 , 0.0713	0.06
MRS							
Liver Lipid content (% water peak)	**-1.8614**	**-3.165 , -.5579**	**0.4923**	**-0.7655 , 1.2028**	**-0.3174**	**-1.33 , 0.6952**	**0.013**
Intramyocellular Lipid; IMCL (% water peak)	0.1787	-0.5507 , 0.9081	0.4961	-0.2106 , 1.2028	-0.3174	-1.33 , 0.6952	0.524
Extramyocellular Lipid; EMCL (% of water peak)	-0.1807	-0.8676 , 0.5062	0.1035	-0.5799 , 0.7869	-0.2842	-1.2522 , 0.6838	0.549
% Oleic Acid recovery	1.7791	-0.7766 , 4.3347	1.1597	-1.3104 , 3.6299	0.6193	-2.936 , 4.1747	0.724

Mean and 95% Confidence Interval (CI) for each group and the difference between groups given. Bold values are significantly different.

Supplemental Table 3: Blood Biomarker Secondary Outcome Measures

Marker/Time	C7		C8		C7 vs C8		p-value	Tests			
	mean	95% CI	mean	95% CI	mean	95% CI		trt	time X trt	time (C7)	time (C8)
Total Ketones (µmol/L)								0.381	0.268	<0.0001	<0.0001
Fasting	127	96 , 157	121	66 , 175	6	-57 , 68	0.853				
2 hours	152	116 , 187	204	147 , 261	-52	-119 , 15	0.129				
4 hours	170	102 , 239	213	109 , 317	-42	-167 , 82	0.506				
pre-exercise	218	134 , 302	201	101 , 301	17	-114 , 148	0.8				
post-exercise	417	316 , 517	538	380 , 696	-121	-308 , 67	0.206				
4-carbon Ketones (µmol/L)								0.1592	0.0976	<0.0001	<0.0001
Fasting	127	96 , 157	121	66 , 175	6	-56 , 68	0.851				
2 hours	148	11 , 183	204	147 , 261	-56	-123 , 11	0.102				
4 hours	170	102 , 239	213	109 , 317	-42	-167 , 82	0.506				
pre-exercise	207	128 , 287	201	101 , 301	7	-121 , 135	0.918				
post-exercise	361	273 , 448	538	380 , 696	-177	-358 , 4	0.055				
5-carbon Ketones (µmol/L)											
Fasting	0.017	0.002 , 0.118	0							<0.0001	
2 hours	3.7	2.2 , 6.1	0								
4 hours	13.3	5.2 , 33.6	0								
pre-exercise	10.1	5.0 , 20.7	0								
post-exercise	56.2	40.5 , 77.8	0								
Lactate (mmol/L)								0.0005	0.0003	<0.0001	<0.0001
Fasting	1.7	1.4 , 2.0	1.4	1.2 , 1.6	0.3	-0.008 , 0.8	0.055				
2 hours	2.4	2.1 , 2.7	1.8	1.5 , 2.0	0.6	0.2 , 1.0	0.002				
4 hours	1.3	1.0 , 1.5	1.3	1.1 , 1.4	0.007	-0.2 , 0.2	0.96				
pre-exercise	1.6	1.5 , 1.8	1.8	1.5 , 2.2	-0.2	-0.6 , 0.2	0.384				
post-exercise	1.4	1.2 , 1.5	1.5	1.4 , 1.7	-0.1	-0.4 , 0.09	0.243				
Glucose (mg/dl)								0.5819	0.5354	0.056	0.009
Fasting	108	99 , 117	104	97 , 110	5	-6 , 16	0.39				
2 hours	115	105 , 125	105	97 , 112	10	-3 , 23	0.116				
4 hours	105	96 , 114	99	94 , 105	6	-5 , 16	0.278				
pre-exercise	115	108 , 121	112	103 , 121	2	-9 , 14	0.662				
post-exercise	110	105 , 115	110	101 , 120	-1	-12 , 10	0.887				
Free Fatty Acids (mmol/L)								0.5767	0.433	<0.0001	<0.0001
Fasting	0.47	0.35 , 0.58	0.51	0.43 , 0.59	-0.05	-0.19 , 0.1	0.54				
2 hours	0.22	0.15 , 0.29	0.23	0.19 , 0.31	-0.03	-0.12 , 0.06	0.488				
4 hours	0.44	0.35 , 0.53	0.37	0.27 , 0.47	0.07	-0.07 , 0.2	0.333				
pre-exercise	0.37	0.27 , 0.46	0.36	0.26 , 0.45	0.01	-0.12 , 0.14	0.878				
post-exercise	0.71	0.57 , 0.85	0.62	0.44 , 0.79	0.09	-0.13 , 0.31	0.431				
Acetylcarnitine (µmol/L)								0.3438	0.2579	<0.0001	<0.0001
Fasting	4.3	3.4 , 5.1	4	2.9 , 5.0	0.4	-1 , 1.7	0.62				
2 hours	3.7	3.1 , 4.3	4.7	3.4 , 6.0	-1	-2.4 , 0.5	0.182				
4 hours	5.2	3.1 , 7.2	5.4	3.4 , 7.5	-0.3	-3.2 , 2.6	0.849				
pre-exercise	5.3	3.2 , 7.4	5	3.1 , 7.0	0.3	-2.6 , 3.1	0.858				
post-exercise	9.1	6.5 , 11.7	9	6.0 , 12.0	0.1	-3.8 , 4.0	0.961				
Odd-chain acylcarnitines (µmol/L)								<0.0001	<0.0001	<0.0001	<0.0001
Fasting	0.47	0.38 , 0.56	0.43	0.31 , 0.56	0.03	-0.12 , 0.19	0.671				
2 hours	1.66	1.2 , 2.13	0.51	0.36 , 0.67	1.15	0.66 , 1.65	<0.0001				
4 hours	2.23	0.89 , 3.57	0.31	0.22 , 0.40	1.92	0.58 , 3.26	0.005				
pre-exercise	2.34	1.66 , 3.11	0.46	0.33 , 0.58	1.93	1.2 , 2.67	<0.0001				
post-exercise	3.77	2.77 , 4.77	0.51	0.36 , 0.66	3.26	2.25 , 4.27	<0.0001				
Evan-chain acylcarnitines (µmol/L)								<0.0001	<0.0001	<0.0001	<0.0001
Fasting	0.46	0.38 , 0.54	0.44	0.34 , 0.54	0.02	-0.11 , 0.15	0.754				
2 hours	0.39	0.32 , 0.45	0.69	0.5 , 0.89	-0.3	-0.51 , 0.10	0.003				
4 hours	0.43	0.34 , 0.51	0.6	0.4 , 0.8	-0.18	-0.4 , 0.05	0.12				
pre-exercise	0.47	0.34 , 0.61	0.65	0.46 , 0.83	-0.17	-0.4 , 0.05	0.135				
post-exercise	0.57	0.40 , 0.73	1.15	0.75 , 1.54	-0.58	-1.0 , 0.15	0.008				
Long-chain acylcarnitines (µmol/L)								0.7694	0.8956	<0.0001	<0.0001
Fasting	2.58	1.5 , 3.67	1.98	1.48 , 2.49	0.6	-0.6 , 1.79	0.323				
2 hours	1.22	0.67 , 1.78	0.86	0.62 , 1.09	0.36	-0.25 , 0.97	0.246				
4 hours	1.53	0.92 , 2.15	1.23	0.74 , 1.73	0.3	-0.5 , 1.10	0.458				
pre-exercise	1.17	0.56 , 1.75	0.94	0.63 , 1.24	0.24	-0.41 , 0.89	0.477				
post-exercise	2.74	1.37 , 4.12	2.87	0.97 , 4.78	-0.13	-2.48 , 2.22	0.913				

Marker = blood biomarker; Time = time of sample collection includes fasting, 2 hours = 2 hours post standardized breakfast; 4 hours = 4 hours post-prandially, pre-exercise is prior to treadmill; post-exercise = 30 minutes after exercise completion. 95% CI= 95% confidence interval.

Oregon Health & Science University
University of Pittsburgh
RESEARCH PROTOCOL

Protocol Title: Phase 2 Triheptanoin for Treatment of Long-Chain Fatty Acid Oxidation Disorders

Protocol Date: January 21, 2011
Amendment 1 Date: May 3, 2012
Amendment 2 Date: October 2, 2012
Amendment 3 Date: March 27, 2013
Amendment 4 Date: July 26, 2013

Summary of changes to the Original Protocol:

1) Amendment 1 dated 5-3-2012:
    i) The age range was restricted to 7-45 years because of concern older patients could not complete the treadmill exercise protocol.
    ii) 3-day diet records were increased from 2 to 3 times during the study and the 1st and 3rd records were timed with collection of the DLW urine samples.
    iii) The procedures for blinding and randomization were clarified. The randomization was generated by the statistician and controlled by the primary research coordinator at OHSU. She used the randomization tables to provide group assignment to participants at both Pittsburgh and OHSU. The study coordinator and research pharmacist who dispensed study oils were not blinded. The kitchen staff preparing meals were not blinded. All other study staff conducting assessments were blinded to randomization. The code was not broken until all the data had been collected.
    iv) The DSMB information was updated after the initial meeting and the election of a DSMB chair.
    v) Criteria for early withdrawal and stopping the study were added by request of the FDA IND office

2) Amendment 2 dated 10-2-2012:
    i) The laboratory measure of myoglobin was eliminated from the study. After reviewing data from the initial patients, almost all myoglobin values were listed as below detection and did not provide additional information compared to standard CK measures.
    ii) The timing of breath sample collection for the ^{13}C-oleic acid test was changed based on the analysis of a previous study using the same method to include 2 baseline tubes and eliminating the 30-minute time point.

3) Amendment 3 dated 3-27-2013
    i) The upper age range was eliminated. After discussion with the investigators, it was decided that patients would be evaluated on an individual basis to determine if they could complete the study protocol and that subjects older than 45 should not be excluded based on age alone. No scientific justification for limiting age at 45 was identified.
    ii) The number of subjects recruited at each site was adjusted. There will be 20 at OHSU and 12 at Pittsburgh. Pittsburgh recruitment was slower because they could include only subjects >16 years of age due to institutional limitations of studying children in the adult clinical research center.
    iii) The cardiac MRI was eliminated. After several subjects, including small children under 95 pounds were studied, data from the MRI was highly variable, in some cases not measurable, and did not yield interpretable

functional cardiac parameters. Instead, standard echocardiogram conducted in the cardiac ECHO labs at each institution was used to provide a consistent measure of cardiac function. This measure was more reproducible with less variability than cardiac MRI at our institutions.

iv) ^{31}P spectroscopy to measure PCr depletion and recovery was added. The addition of ^{31}P MRS was designed to measure muscle ATP turnover with acute exercise and thought to better address the hypothesis of the trial.

4) Amendment 4 dated 7-26-2013
   i) The DSMB held a meeting on 6-19-13. The protocol was amended at the request of the DSMB to clarify stopping criteria and how adverse events would be evaluated. The DSMB asked for adjustments to the consent language to remove a statement about gastrointestinal upset that was confusing.
   ii) The DSMB asked to add the exclusion criteria: subjects with history of MI.
   iii) Of note, the DSMB unanimously voted to continue the trial with the above adjustments to the protocol.

Structured Scientific Abstract (one page):

Background: Fatty acid oxidation disorders are a family of rare inherited diseases affecting an estimated 1:9000 infants. The recent addition of the fatty acid oxidation disorders to the expanded newborn screening panel in the United States has led to the early diagnosis and treatment of this group of disorders. Presymptomatic treatment through newborn screening has decreased episodes of metabolic decompensation and nearly eliminated mortality. However, patients with a long-chain fatty acid oxidation disorders experience long-term complications such as recurrent rhabdomyolysis with or without cardiomyopathy. The prevalence of this sub-group of patients with long-chain fatty acid oxidation is unknown but it is estimated to affect approximately 1000 individuals or less in the United States. Novel treatments for these diseases have lagged behind our diagnostic capabilities. We propose to test a novel treatment hypothesized to increase citric acid cycle intermediates and maximize energy production.

Objective: The goal of the project is to determine if triheptanoin therapy (an odd-chain fatty acid triglyceride) has a therapeutic advantage over conventional treatment for long-chain fatty acid oxidation disorders.

Design: This randomized clinical trial will examine the efficacy of triheptanoin supplementation (a heptanoate (C7 triglyceride) to treat humans with long-chain FAO disorders compared to standard of care supplementation with medium chain triglycerides (primarily a C8 triglyceride).

Setting and Subjects: Subjects with a long-chain fatty acid oxidation disorder will be randomly assigned to one of two groups: medium chain triglyceride (MCT) supplementation, the current standard of care, or triheptanoin supplementation for four months.

Intervention: Baseline assessment of total energy expenditure, the response to a moderate intensity treadmill exercise test, cardiac function and long-chain fatty acid oxidation capacity will be completed. Subjects will be randomized to two groups, educated how to consume their supplemental oil and discharged to home. Subjects will consume either MCT or triheptanoin at 20% of their estimated energy requirements for 4 months. At the end of 4 months, baseline assessments will be repeated.

Measurements: Energy expenditure will be measured by indirect calorimetry and doubly labeled water. A 45 minute moderate intensity treadmill exercise study will measure exercise tolerance. A liquid meal containing a stable isotope tracer, ^{13}C-oleic acid, will be used to measure fat oxidation. Body composition will be measured by DEXA. Tissue lipid deposition will be measured by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) in the liver and calf. Cardiac function will be measured by echocardiogram. A separate MRI/ MRS will measure muscle high-energy phosphorous metabolism, to measure how quickly muscles make energy (ATP) after a depleting exercise.

Analysis: The primary analyses to compare change in energy expenditure, cardiac function, and exercise tolerance between Triheptanoate and MCT supplementation will be based on intent to treat and will use linear mixed models, with a separate model for each outcome. The predictors included in these linear models will be treatment group, the randomization factors (diagnostic group and center), and time (baseline or follow-up).

A. Specific Aims

Interaction of the citric acid cycle and fatty acid oxidation is vital to maximize ATP production in human metabolism. Patients with disorders of long chain fatty acid oxidation show signs and symptoms of energy deficiency even when optimally treated with currently available therapies. The overall goal of this application is to examine the efficacy of a novel therapy, triheptanoin supplementation, to prevent or mitigate the symptoms of energy deficiency in patients with inherited defects in the long-chain fatty acid oxidation pathway. We hypothesize that the pathogenesis in these disorders is mediated by a decreased ability to replenish citric acid cycle intermediates during periods of increased energy demand secondary to reduced production of acetyl-CoA by fatty acid oxidation.

Genetic disorders of fatty acid oxidation are a rare family of inherited defects in the mitochondrial fatty acid oxidation pathway. Early disease detection and treatment significantly decreases the frequency and severity of episodes of metabolic decompensation as well as nearly eliminates mortality. Expanded newborn screening has improved our ability to diagnose these disorders prior to the onset of symptoms but patients with the long-chain fatty acid oxidation disorders still suffer recurrent rhabdomyolysis with or without cardiomyopathy, often associated with exercise or intercurrent illness, despite current treatments. The etiology underlying rhabdomyolysis and cardiomyopathy in these disorders is not known but energy deficiency in the form of low ATP generation has been hypothesized. The impairment of ATP production, in turn, has been suggested to be caused by a secondary impairment in citric acid cycle function due to a depletion of citric acid cycle intermediates. Current treatment of long chain fatty acid oxidation disorders consists of frequent high carbohydrate meals, and supplementation with medium chain triglyceride (MCT, predominantly composed of octanoate (C8) triglyceride). MCT bypasses the metabolic block in long-chain fatty acid oxidation and produces acetyl-CoA as substrate for the citric acid cycle, however, it does not replete any other potentially reduced citric acid cycle intermediates. Therefore, anaplerotic therapy (supplementation with compounds that lead to the production of citric acid cycle intermediates) is an attractive possibility to improve energy production and alleviate myopathic symptoms in subjects with these disorders.

One potential anaplerotic supplement for use in these disorders is oral triheptanoin (a C7 triacylglyceride). Complete mitochondrial β-oxidation of heptanoate yields two acetyl-CoA molecules and one propionyl-CoA. Propionyl-CoA can subsequently be converted to succinate, a key citric acid cycle intermediate. However, while triheptanoin is anaplerotic and MCT oil is not, a comparison of supplementation with isocaloric amounts of triheptanoin and MCT in preventing symptoms in fatty acid oxidation disorders has not been performed. We hypothesize that oral consumption of triheptanoin will increase tissue citric acid cycle intermediates, and maximize ATP generating capacity in human patients improving their outcome.

Specific aim: To determine the efficacy of triheptanoin supplementation using a randomized study design in humans with long-chain fatty acid oxidation defects. We hypothesize that a diet supplemented with triheptanoin will improve energy production, exercise tolerance and cardiac function in these patients. Subjects with a long-chain fatty acid oxidation disorder including carnitine palmitoyltransferase 2 (CPT2), very long-chain acyl CoA dehydrogenase (VLCAD), trifunctional protein (TFP) and long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiencies will be randomly assigned to one of two groups: 1) medium chain triglyceride (MCT; current standard of care), 2) triheptanoin (C7) for four months. After 4 months we will test the effects of diet treatment on total energy expenditure, the response to a moderate intensity treadmill exercise test, and cardiac function. Long-chain fatty acid oxidation will be measured with an *in vivo* assay using stable isotopes.

B. Background

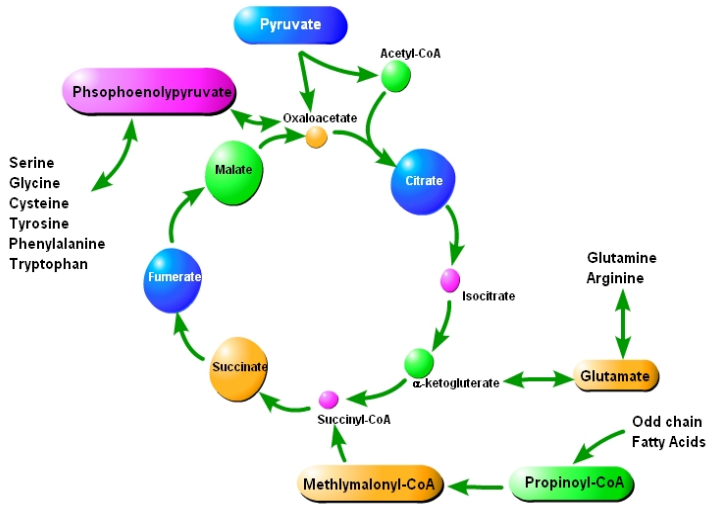
B.1. Prevalence: Long-chain fatty acid oxidation disorders are a family of very rare defects with an estimated prevalence of < 1000 patients in the United States. There are slightly more than 200 cases of CPT2 reported in the literature with an expected incidence of less than 1:100,000 live births or less than 40 infants with CPT2 born in the US per year (1). Likewise, VLCAD deficiency has an estimated incidence of 1:100,000 live births. Exact prevalence is unknown but can be estimated based on data from newborn screening programs and family support networks. Less than 500 subjects with VLCAD deficiency are believed to be identified living in the US. Currently, there is an estimated prevalence of 250 patients living with LCHAD or TFP deficiency (personal communication with Deb Gould, director of the Fatty Acid

Oxidation Parent Support Network **www.fod.org**). In sum, the long-chain fatty acid oxidation disorders represent a truly rare disease population.

B.2. Significance: Analysis of newborn blood spots via tandem mass spectroscopy (MS/MS) has become a standard component of newborn screening in the United States. The American College of Medical Genetics Newborn Screening Expert Group recommends most of the fatty acid oxidation disorders be included in the newborn panel (1, 2). Screening has led to a dramatic increase in identification of presymptomatic infants with fatty acid oxidation disorders, including the long-chain fatty acid oxidation disorders in this proposal (41). However, development and evaluation of existing and new treatment options have significantly lagged behind our ability to diagnose affected infants. Depletion of citric acid cycle intermediates has been suggested as one possible mechanism for pathogenesis in fatty acid oxidation defects (8, 48).

B.3. The Citric Acid Cycle: Mitochondrial pools of citric acid cycle intermediates are relatively small (1-2 µmol/g tissue for all 8 intermediates) with a very rapid turnover (5-100 times per minute) (8). Loss of intermediates (cataplerosis) is a normal part of mitochondrial metabolism as intermediates enter alternative anabolic pathways. In cardiac tissue, 1-2% of the citric acid cycle intermediate pool is estimated to be lost per minute (11, 70). Continued energy production by the citric acid cycle and the electron transport chain is thus dependent upon maintaining mitochondrial pools of citric acid cycle intermediates with loss of intermediates balanced by the addition of new ones (45). Substrates that increase the citric acid cycle intermediate pools are termed anaplerotic (Figure 1) and include pyruvate, amino acids, and propionate (8). The pool size of citric acid cycle intermediates is also dynamic and changes with alterations in metabolic state. Citric acid cycle intermediates increase in skeletal muscle during the first 15 minutes of moderate-intensity exercise; liver citric acid cycle intermediates increase during the transition from feeding to the fasting state; and cardiac citric acid cycle intermediates increase during reperfusion after ischemia (7, 21, 22, 42). Under normal physiologic conditions, changes to citric acid cycle intermediate pools are exquisitely regulated, insuring maintenance of normal ATP production. Individuals with long-chain fatty acid oxidation disorders may face a limited capacity to replete citric acid cycle intermediate pools. Current treatment for long chain fatty acid oxidation defects traditionally seeks to supply adequate acetyl-CoA for vital cellular reactions from the oxidation of glucose and medium-chain triglycerides, thus bypassing the block in long-chain fatty acid oxidation. However, despite MCT supplementation and adequate caloric intake, patients often still experience episodic myopathic events including rhabdomyolysis and cardiomyopathy.

Figure 1: Balance of Citric Acid Cycle intermediates



Adapted from Brunengraber H, Roe, C JIMD 2006. Size of the circles represents relative pool sizes of citric acid cycle intermediates.

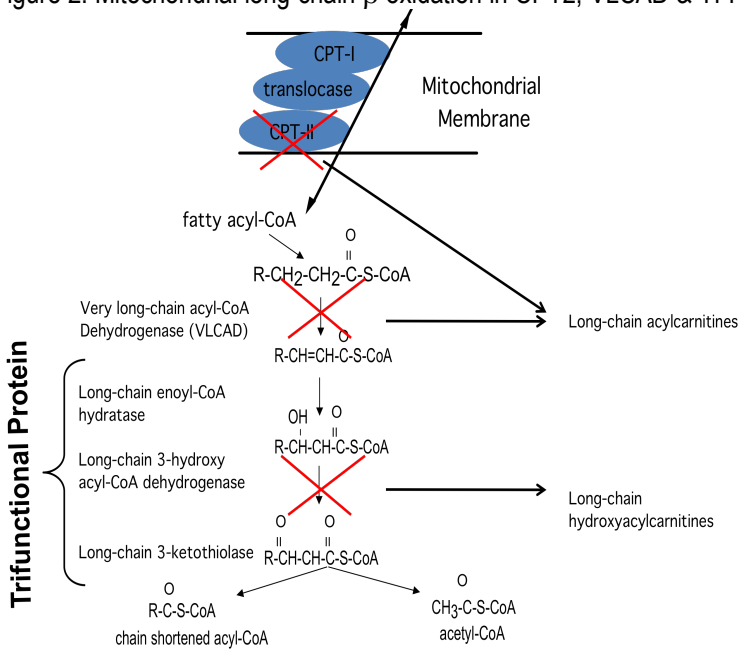
B.4. CPT2, VLCAD LCHAD/TFP Deficiency: Carnitine Palmitoyltransferase-2 (CPT2), very-long chain acyl-CoA dehydrogenase (VLCAD), long-chain 3-hydroxyacyl-Co A dehydrogenase (LCHAD), and trifunctional protein (TFP) deficiency are autosomal recessive defects in mitochondrial ß-oxidation of fatty acids (3, 16, 68). Severe deficiencies of CPT2, VLCAD and LCHAD/TFP in infants present with fasting induced hypoketotic hypoglycemia, lactic acidosis, hepatomegaly and severe dilated cardiomyopathy (5, 19, 39). Milder phenotypes in adolescents present with exercise intolerance and muscle pain, and can be exacerbated by episodes of exercise or stress. Symptoms of metabolic decompensation include rhabdomyolysis, recurrent or chronic cardiomyopathy, hypoglycemia with or without hyperammonemia, and decreased muscle strength. In addition, patients with LCHAD/TFP deficiency can develop peripheral neuropathy and chorioretinopathy with potential loss of vision (14, 52, 69). Current treatment for all of these

disorders includes avoiding fasting and eating a high carbohydrate, low-fat diet, resulting in significant improvement in survival and outcome (24, 56).

Prevalence of myopathic symptoms: Among 107 patients with fatty acid oxidation disorders, 61% had cardiomyopathy, and 41% had cardiac arrhythmias at presentation and/or during recurrent metabolic decompensation (52). The cardiac dysfunction was characterized by hypertrophic dilated cardiomyopathy, conduction abnormalities and cardiac arrhythmias. 41% of patients had significant muscular symptoms including myoglobinuria, muscular weakness, myalgia and elevated creatine kinase (CK). Specific fatty acid oxidation disorders observed to have a high incidence of cardiac and muscular symptoms include patients with CPT-2, Carnitine Acylcarnitine Translocase (CACT), VLCAD, LCHAD and TFP deficiency. Cardiac and muscular symptoms were <u>not</u> observed among patients with carnitine palmitoyltransferase 1A (CPT1A) deficiency, the hepatic isoform of CPT1, and patients with medium chain acyl-CoA dehydrogenase deficiency (MCAD). In our survey of US physicians, we found 30-40% of subjects with LCHAD deficiency had repeated episodes of myoglobinuria and/or cardiomyopathy even after specific dietary treatment had been initiated (23). Most subjects have repeated myopathic symptoms despite current dietary treatment.

Long-chain fatty acid β-oxidation. Transport of long-chain fatty acids into the mitochondria uses the carnitine palmitoyl transferase (CPT) system (Figure 2). CPT-1 forms a fatty acylcarnitine that is transported into the mitochondria by carnitine acylcarnitine translocase. Inside the mitochondria, the conjugation reaction is reversed by CPT-2 yielding free carnitine and a long chain fatty acyl-CoA. ß-oxidation occurs in a repeating four-enzyme cycle, catalyzed by very long-chain acyl-CoA dehydrogenase (step 1) and TFP (steps 2-4). Inherited deficiencies of CPT-2, VLCAD or TFP lead to partial or complete loss of function (30, 31, 68). The most common mutation in the TFP gene is c.1528G>C accounting for 80% of the mutant alleles identified in patients. Individuals who are homozygous for c.1528G>C have a 90% reduction in the 2nd enzymatic function of TFP, long-chain 3-hydroxyacylCoA dehydrogenase (LCHAD). Activities of the hydratase and ketothiolase are reduced by about 50%. Mutations in the TFP gene can result in severe LCHAD deficiency with partial hydratase and ketothiolase deficiency (referred to clinically as LCHAD deficiency) or to severe loss of all three enzymatic functions (referred to as TFP deficiency). Deficiencies of CPT2, VLCAD or TFP lead to accumulation of long-chain acyl-CoA (VLCAD, & CPT2) or long-chain hydroxyacyl-CoA moieties (LCHAD/TFP) in the mitochondria. These fatty acids exit the mitochondria via the reversal of the CPT system and appear in plasma as long-chain acylcarnitines or hydroxy-acylcarnitines and their free fatty acids. Periods of fasting and exercise increase fatty acid oxidation and increase circulating acylcarnitines in subjects with long-chain fatty acid oxidation disorders.

Figure 2: Mitochondrial long-chain β-oxidation in CPT2, VLCAD & TFP deficiency



B.5. Anaplerotic Substrates: Heptanoate (C7:0) is oxidized by the medium-chain enzymes of the fatty acid oxidation pathway to 2 acetyl-CoA molecules and one propionyl-CoA. Propionate can be converted to succinate by the combined actions of propionyl-CoA carboxylase and methyl-malonylCoA mutase (figure 1). Several studies using dietary triheptanoin supplements (a triacylglycerol of C7) in metabolic disorders have been published (38, 49, 50). In one study, subjects reported significant improvement in cardiomyopathy and decreased frequency and severity of rhabdomyolysis when supplemented with triheptanoin (49). Additionally, seven subjects with CPT2 deficiency decreased the frequency of

episodes of rhabdomyolysis and were able to return to normal life activities with triheptanoin supplementation (50). However, both studies were open-label observational trials of a high triheptanoin diet, and did not directly compare supplementation with equivalent amounts of MCT and triheptanoin. Rather, subjects received significantly greater amounts of triheptanoin than the amount of MCT consumed prior to entry into the study. Thus, it is not clear if the reported results were due to increased total energy intake or were specific to the potential anaplerotic effects of triheptanoin.

B.6 Innovation: Patients with long-chain fatty acid oxidation disorders frequently exhibit myopathic symptoms in spite of currently accepted treatment (59, 60). These symptoms occur or worsen during periods of increased energy demands such as illness or exercise. The block in fatty acid oxidation may limit the ability of muscle tissue to increase citric acid cycle function to meet increased energy demands and lead to decreased total energy production. Several observational reports have suggested that substrates able to increase citric acid cycle intermediates via anaplerosis may have therapeutic advantages over traditional dietary therapy but controlled trials have not been reported (25, 49, 50). This proposal will compare traditional dietary therapy to triheptanoin therapy in human subjects with long-chain fatty acid oxidation disorders in a randomized clinical trial. We hypothesize that this approach of triheptanoin supplementation will prevent or decrease the frequency and severity of myopathic symptoms in this patient population.

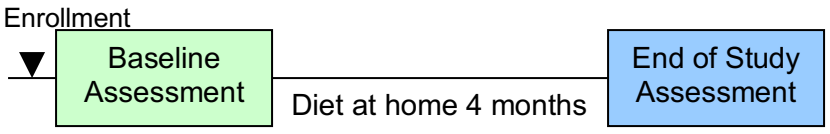
C. Research Design & Methods

C2.0 Study Design: Subjects with a confirmed diagnosis of CPT-2, VLCAD or LCHAD and/or TFP deficiency will be recruited to participate in this study at one of two clinical sites: OHSU and the University of Pittsburgh. Subjects will be randomly assigned to one of 2 treatment groups (Table 1). Treatment groups will include the control based on current dietary recommendations (supplemental MCT), and triheptanoin supplementation. Subjects with CPT-2, VLCAD, and LCHAD/TFP deficiency will be stratified such that each experimental group contains subjects with all three diagnoses. Subjects will be blinded to study treatment. The principal investigator, co-investigators and all other sub-investigators will be blinded to treatment with the exception of the statistician, the OHSU study coordinator, the OHSU investigational pharmacy and the OHSU bionutritionist. Subjects will complete all study procedures at OHSU or UP CTSA's on enrollment to the study and again after 4 months on study diet (Figure 3). At enrollment, baseline assessments will be completed for each subject including: body composition, cardiac function, and metabolic response to a test meal and exercise tolerance. Each subject and their family will be counseled how to consume their supplement at home. Subjects will be provided the MCT and triheptanoin supplements. They will be discharged to home. After following the diet at home for 4 months, subjects will return to repeat assessments. Change from baseline (Δ) will be used in the analysis to determine efficacy of triheptanoin among subjects.

Table 1: Overall Study Design:

	MCT			Triheptanoin			Total
DX:	CPT2	LCHAD/TFP	VLCAD	CPT2	LCHAD/TFP	VLCAD	
OHSU	3	4	3	3	4	3	20
U of Pittsburgh	3	1	2	3	1	2	12
Total	6	5	5	6	5	5	32

Figure 3: Study Protocol for Subjects



Subjects complete a baseline assessment at enrollment and are then randomized to one of 2 diet treatment groups. Subjects follow their assigned diet for 4 months and are reassessed at the end of the trial for efficacy of diet therapy.

C 2.1 Subject Recruitment*:* Subjects with disorders in long-chain fatty acid oxidation will be recruited to participate in this study through announcements on the fatty acid oxidation family support network website, through participating site clinical populations, and through letters sent to metabolic specialist across the United States. Inclusion and exclusion criteria are given in Table 2. Subjects must be 7 years of age or older, and be able to complete and comply with the protocol. Subjects may not be participating in another research project that alters macronutrient content of the diet or

includes a drug that may alter fatty acid oxidation.

Table 2:

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:
Each subject must have a confirmed 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. Diagnosis of a disorder in long-chain fatty acid oxidation will be confirmed by obtaining medical record results of acylcarnitine profiles, fatty acid oxidation probe studies in cultured fibroblasts and/or mutation analysis (32, 41). Table 3 outlines the medical records to be reviewed prior to enrollment to confirm the diagnosis for each disorder. Establishing the diagnosis of these disorders is a complex process. Not all potential subjects will have had a skin biopsy and FAO probe studies in cultured fibroblasts. In many cases mutation analysis can identify only one recognized mutation in the sequenced exons of the gene. Thus, a combination of methods is most often used to establish the diagnosis. If two of the three diagnostic tests suggest a diagnosis of CPT2, VLCAD, or LCHAD/TFP, the subject will be eligible to participate in the trial. In addition, if there are 2 known mutations identified and a history of at least one episode of rhabdomyolysis, the subject will be eligible to participate in the trial.

Table 3: Medical Records to Confirm the Diagnosis

Disorder	Acylcarnitine Profiles	Fatty acid oxidation Probe studies	Mutation analysis
CPT-2	↑ C16:0 or C18:0	↓ FAO flux with	1 or 2 known
VLCAD	↑ C12:1 or C14:1	↑ disease specific	mutations in
LCHAD/TFP	↑ OH-acylcarnitines	acylcarnitines	specific gene

C. 2.3 Outcome Measures: A sample admission schedule is presented in figure 4. Body composition will be measured by DEXA scan and tissue lipid content and tissue energetics by magnetic resonance spectroscopy (MRS). Some outcome measures such as energy expenditure and fat oxidation will be normalized to lean mass measured by DEXA. Resting and exercise energy expenditure will be measured by indirect calorimetry. Total energy expenditure will be measured by the doubly labeled water technique (DLW). Metabolic response to a test meal including change in glucose, insulin, free fatty acids, and acylcarnitines will be measured following the meal containing a loading dose of labeled oleic acid (1-^{13}C oleic acid). Using a stable isotope tracer, we will measure *in vivo* long-chain fatty acid oxidation. Cardiac function will be measured by echocardiogram (ECG). During the exercise treadmill, we will measure perceived physical exertion using the Borg perceived exertion scale, heart rate and ventilation at a given work load. Lactic acidosis and significantly elevated creatine phosphokinase (CK) typically accompanies exercise-induced rhabdomyolysis. Pre- and post-exercise and the

following morning blood lactate and CK concentrations will be measured as indicators of rhabdomyolysis. A rise in post exercise ketones and a decrease in post exercise long-chain acylcarnitines would suggest the subject is utilizing an alternative fuel (MCT or triheptanoin) in preference to long-chain fatty acids. Resting and pre- and post-exercise ketones and acylcarnitine profiles will be measured as a secondary marker of substrate utilization during exercise. Urine organic acids will be analyzed for excretion of citric acid cycle intermediates as an indirect marker of anaplerosis.

Figure 4: CRC admission for Subjects

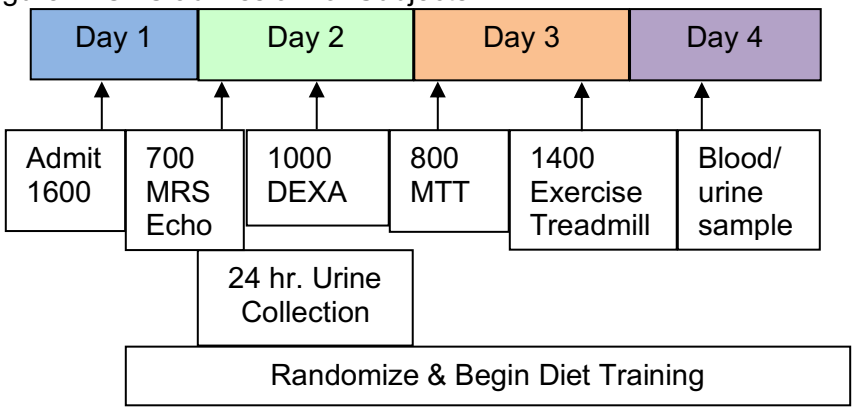


Figure 4: CRC admission for subjects. Day 2 subjects will complete a MRS to measure ectopic fat stores and tissue energetics, Echo for cardiac function, DEXA for body composition and 24 hr urine collection. Day 3 metabolic response to a test meal and exercise tolerance will be measured. Day 4 a final 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

Group:			% total energy		
MCT	15	55	10	20	0
Triheptanoin	15	55	10	0	20

*CHO = carbohydrate, LCFA = long-chain fatty acids,
MCT = medium-chain triglyceride, triheptanoin = C7 triacylglyceride

C. 2.4 Standardized Diets: All research diets will have the same total fat (long-chain triglyceride (LCT) + MCT or triheptanoin) content but vary in the source of medium chain fat (MCT vs. triheptanoin; Table 4**).** The MCT diets will contain 20% of total energy from medium chain triglyceride (MCT); the triheptanoin diets will contain an equal % of total energy from triheptanoin. Each subject and his or her guardian will be instructed on how to follow the prescribed experimental diet by the study coordinator. A detailed diet will be developed based on the measured resting energy needs of that subject plus 40% for activity and growth. Subjects will not be required to eat all the calories given in their diet plan but they must follow the general types of foods included in the plan. They will be encouraged to eat foods from their prescribed diet until they are satisfied. Diet instruction will include lists of allowed foods and quantities, recipes for preparing meals and suggested menus to follow. Subjects will be provided with food intake forms on which to record 3-day diet records. Subjects will complete 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 will be monitored by weekly communication with the study coordinator, the analysis of 3-day diet records and the amount of supplemental oil consumed. During the at home diet period the study coordinator will talk with each subject and/or a parent once per week. The purpose of the phone call is to assess adverse events, to discuss any problems or difficulties with the experimental diet and assess compliance to the protocol. Study staff will ask about potential adverse effects of the supplemental oil including presence of gastrointestinal upset, steatorrhea or frequent loose stools, weight gain, muscle pain and symptoms of lethargy. Staff will inquire about how much of the supplemental oil the subject is taking, and how much of the quart bottle is remaining. They will discuss strategies to minimize GI upset and provide suggestions, new recipes, and encouragement to subjects and families to adhere to the dietary protocol and to consume the supplement.

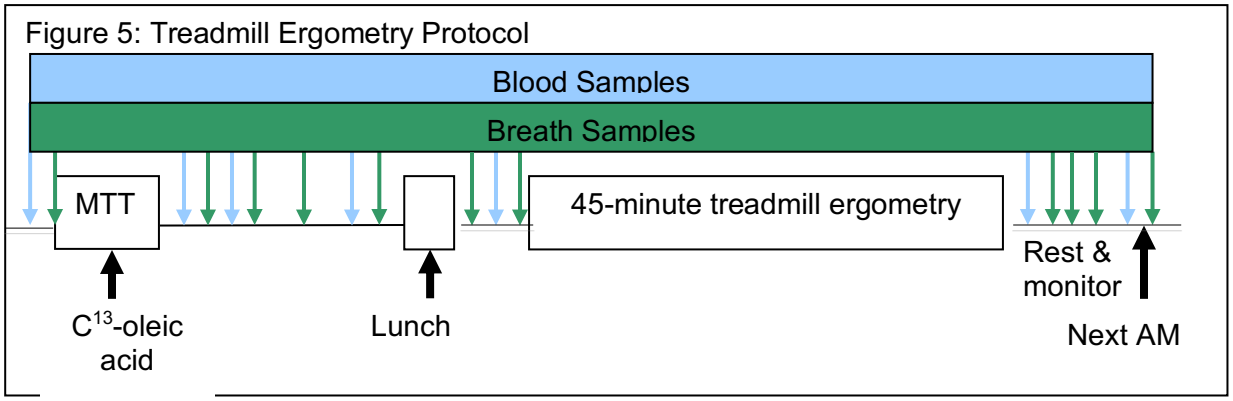
Completed diet records will be mailed to the principal investigator. Dietary macronutrient content will be determined using –Food Processor (Esha Research, Inc) software to monitor compliance with the protocol. The 3-day diet records from both sites will be analyzed by a registered dietitian at OHSU to minimize variability in the analysis. The amount of

oil provided to the subject during the trial will be tracked as a third method of compliance monitoring. Depending on the daily dose prescribed of supplemental oil (MCT or triheptanoin) the study staff will predict how many bottles of oil will be used during the 4 months by each subject. The subject will be asked to report how much is remaining in their quart jar to estimate compliance as amount of oil provided – amount of oil remaining at the end of the trial.

Noncompliance: If the study coordinator estimates the subject is consuming less than ½ of the prescribed amount of supplemental oil, if the subject reports significant GI upset or excessive weight gain (>10% body weight), or the 3-day diet record indicates noncompliance to the protocol, the PI will be notified. The PI will call the subject and attempt to address any issues with noncompliance. If the problem is weight gain or significant GI distress, the initial supplement dosing will be reevaluated. A decrease in the dose of 5% and spacing the dose throughout the day may improve the symptoms. Phone calls to subjects who are noncompliant will increase to twice per week. It is our experience that children and adolescents are able to tolerate 20% of energy from MCT without significant GI distress or weight gain when working closely with the study staff and metabolic dietitian. **Subjects will not be removed from the study due to noncompliance**. However, noncompliance will be scored as % of supplement prescription consumed and used as a covariant in the statistical analysis at the end of the study.

C. 2.6 Treadmill Ergometry: Exercise testing will be performed on a treadmill with continuous ECG monitoring and collection of respiratory gases by metabolic cart at each of the participating sites. Treadmill ergometry will be performed 2 hours after the patient finishes lunch in a post absorptive state (Figure 5). Each subject will be given a carbohydrate and protein free beverage which contains MCT or triheptanoin oil 20 minutes before starting to exercise. The dose will be calculated based on each individuals lean body mass as measured by DEXA .After the pre-exercise blood draw, the exercise protocol will be 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 will be calculated using the formula: 220 (beats per minute) – age in years. The Borg perceived exertion scale (a scale of 1 to 20 of increasing exertion) will be employed to allow each subject to express their perceived level of exertion (6). ECG and gas exchange will be monitored continuously during the exercise test. Blood pressure will be measured every 10 minutes. Subjects will be asked to continue exercising at 50-60% of their predicted maximum heart rate for 40 minutes, followed by a 2 minute cool down period. At the end of the exercise testing, blood pressure and ECG will be recorded during recovery. Monitoring of the subject will continue until respiratory rate and heart rate are close to baseline. The exercise test will be terminated prematurely if the subject develops any adverse, unexpected symptoms such as dizziness, severe respiratory distress, chest pain or palpitations, or muscle pain. The same grade and speed maintained at baseline will be repeated for exercise tests at follow-up to keep workload constant.

Figure 5: Treadmill Ergometry Protocol



C 2.7 Blood samples: A total of 7 blood samples will be collected at each admission. Blood will be drawn before and 1, 2 and 4 hours after the meal tolerance test (MTT). Blood samples for the exercise study will be drawn at baseline (time 0), and after 20 minutes recovery time. A final blood sample will be drawn the morning after exercise to measure the effects of exercise on biochemical markers of metabolic control. Laboratory studies to be performed on these samples include glucose, insulin, CK, free fatty acids, lactate, pyruvate, ketones, and plasma acylcarnitine profiles.

C. 2.8 In vivo Fatty Acid Oxidation We will be using a stable isotope method to measure in vivo residual fatty acid oxidation capacity. Enrichment of $^{13}CO_2$ only occurs by one complete round of fatty acid oxidation. At breakfast prior to

treadmill ergometry, subjects will be given a meal containing 17-mg/kg ^{13}C -oleic acid. Breath samples will be collected prior to (time 0) and then hourly for 12 hours following the ^{13}C -oleic administration and before and after exercise. ^{13}C in breath samples will be measured as a ratio of ^{13}C /^{12}C using the Delta Plus IRMS (Finnigan MAT, Bremen, Germany). Recovery will be calculated as ^{13}C divided by the dose of ^{13}C administered. (The amount of excess ^{13}C in breath is a measure of residual fatty acid oxidation capacity in subjects with disorders of long-chain fatty acid oxidation.)

C. 2.9 Cardiac Function Ultrasound Cardiac function will be assessed at baseline and after treatment using 2 Dimensional and 3 Dimensional echocardiography. Subjects will complete the echo at rest. RV and LV chamber volumes and ejection fraction will be evaluated on 3 D cardiac images. Speckle tracking cardiac muscle strain rates will be obtained from processing of short axis and apical 2 and 4 chamber images obtained with a General Electric Vivid E9 ultrasound system.

C. 3.0 Phosphorus Spectroscopy: Magnetic resonance spectroscopy (MRS) techniques can be used for the non-invasive measurement of tissue energetics. The purpose of this scan is to evaluate tibialis muscle high-energy phosphate metabolism at rest and after exercise. To accomplish this, 3-D ^{31}P magnetic resonance spectroscopic imaging (MRSI) will be collected using a 7T MR instrument and absolute concentrations of phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (Pi), and other phosphorus metabolites in the tibialis will be measured. MRI data will be collected using 7T MRI instrument. Prior to all imaging studies the magnetic field homogeneity will be optimized using first and second order room-temperature shims with automated optimization protocols supplied by the manufacturer. Anatomical images will be attained to permit assessment of calf muscle volume and mass. Proton coil will be switched to phosphorus coil and the subject repositioned in the same location in the magnet MRS data will be acquired with a 3D nominal resolution of about 3.4 cc (FOV: 24x24x24 cm^3, data matrix: 16x16x16).

The MRS will take approximately 1 hour. A surface coil will be placed over the tibialis muscle of the lower calf. The subject lies on a padded table that slides into the bore of the magnet. Data are collected from the muscle at rest and then before, during, and after voluntary muscle contractions (exercise) against a foot peddle. The subject will wear earplugs during the procedure. The voluntary muscle contractions performed are similar to pushing on a peddle in which the subject attempts to push his or her foot against the resistance of a band placed across the lower leg. This movement will be practiced before the actual test begins. Subjects will then be instructed to push their foot repeatedly as fast and hard as they can for 30 seconds when they are given the signal. Subjects may be asked to repeat the exercise 1 or 2 more times for slightly more or less time (18-36 sec) if the researchers determine that the exercise bout did not produce the desired change in energetic state.

C. 3.1 Statistical Considerations.
Randomization: Subjects will be randomized separately within each diagnostic group (CPT-2, VLCAD, TFP/LCHAD deficiency) at each center. We are targeting between 5 and 6 subjects per diagnostic group at each center to achieve 32 subjects (see Table 1). We will use a permuted block randomization scheme to randomize within the center/diagnostic group strata. The study coordinator at OHSU will be responsible for completing the randomization process for both sites, using a table prepared by the statistician. This information will then be communicated to OHSU investigational pharmacy. The study oil will then be prepared by the OHSU investigational pharmacy and shipped directly to the study subjects.

Analyses: The primary analyses to compare change in energy expenditure, cardiac function, and exercise tolerance between Triheptanoate and MCT supplementation will be based on intent to treat and will use linear mixed models, with a separate model for each outcome. The predictors included in these linear models will be treatment group, the randomization factors (diagnostic group and center), and time (baseline or follow-up). Subjects will be incorporated as random factors in the analysis, inducing correlation between baseline and follow-up observations on each subject. The formal test for comparing change between groups will be based on an interaction between time (baseline versus follow-up) and treatment group. In addition to formal tests, we will construct 95% confidence intervals for the mean outcome for each treatment group at each time point, for the difference in mean outcomes between the treatment groups at each time point, and for the difference in mean change between the treatment groups.

In exploratory analyses, we will use simple descriptive statistics to examine mean differences between treatment groups within each of the diagnostic groups (CPT2, VLCAD and LCHAD/TFP). 95% confidence intervals for the mean change

and differences in mean change will be provided for each diagnostic group. Although the sample sizes will be small within each diagnostic group, limiting power, the information will be useful for describing possible differential affects of treatment among the diagnostic groups.

Secondary analyses will examine whether individuals who comply with treatment respond differently from individuals who do not comply. We will conduct separate analyses based on summary information from dietary questionnaires and from estimates of remaining supplemental oil as reported by subjects. Dietary records will be analyzed to determine macronutrient content and the estimated fat consumed. MCT and triheptanoin intake expressed as a % of the prescribed intake will be used in the analysis. We will also estimate the percent of the total prescribed oil consumed from estimates of remaining oil at the end of the trial. We will examine the relationship between compliance and change in outcomes within each treatment group and whether the difference in outcome between treatment groups appears to depend on compliance.

Power calculations: Sample size assessments were conducted for comparing change between groups in adjusted total energy expenditure. In our preliminary study, 6 subjects were randomized to a standard high carbohydrate diet and 6 were randomized to high protein diet. The adjusted total energy expenditure (TEE) outcome used here is a z-score for TEE, measured via doubly labeled water, that is normed based on age, height, weight, and sex. Preliminary data on 12 subjects found a mean (SD) adjusted total energy expenditure z-score of -1.12 (3.12) for individuals on a high protein diet and -5.22 (1.82) for individuals on a standard diet. The mean difference we observed in TEE z-score between the diets was 4.1. We anticipate a similar difference in change from baseline between triheptanoin and MCT. The SD for change depends on the SD's at baseline and follow-up and the correlation between the baseline and follow-up measures. Using the SD's above and assuming that within each treatment group the SD's at baseline and follow-up are the same, we calculated the sample sizes per group needed to detect a 4.1 mean difference in z-score change for a range of correlations between 0 and 0.75. These calculations were performed using a simple two-sample t-test for change. Table 5 presents the required sample sizes corresponding to a minimum of 80% power at significance level 0.05. If we conservatively assume zero correlation between baseline and follow-up we would need 14 subjects per group to detect a mean difference in change of 4.1. Our targeted sample size will be 16 subjects per group.

Table 5. Sample sizes per group needed to detect a mean difference of 4.1 in TEE z-score with 80% power at significance level 0.05.

Correlation between baseline and follow-up TEE z-score	Estimated SD of TEE z-score difference from baseline for MCT	Estimated SD of TEE z-score difference from baseline for triheptanoin	Mean difference in TEE z-score detected	Sample size per treatment group	Power
0.00	2.57	4.41	4.1	14	82%
0.25	2.23	3.82	4.1	11	83%
0.50	1.82	3.12	4.1	8	84%
0.75	1.29	2.21	4.1	5	86%

D. Protection of Human Subjects

1. Risks to Human Subjects

a) Human Subjects Involvement and Characteristics

1) Involvement of human subjects:

The proposed research involves the use of human subjects age 7 years or older.

2) Subject population:

Inclusion Criteria: 32 subjects with a confirmed diagnosis of CPT-2, VLCAD TFP, or LCHAD deficiency will be recruited for this study. Subjects must be ≥7 years of age, stable on a diet that includes supplementation with MCT, have a history of at least one episode of rhabdomyolysis, and be willing to commit to the changes to their diet and complete 2 CRC admissions.

Exclusion Criteria: Subjects with CPT-2, VLCAD, TFP, or LCHAD deficiency may not be actively participating in another research project that alters the macronutrient content of the diet. All subjects will be screened for anemia prior to study participation. Female subject of childbearing potential will be screened for pregnancy. If female subjects are not pregnant and agree to participate in the study, subjects will need to agree to use adequate birth control during the 4-month dietary intervention. Anemic (Hgb < 10 g/dl), pregnant or breastfeeding subjects will be excluded from the study. Subjects with a past history of myocardial infarction or peripheral neuropathy that limits the ability to walk on a treadmill will also be excluded.

3) Rationale for inclusion of vulnerable populations:

Fatty acid oxidation disorders including CPT-2, VLCAD TFP, or LCHAD deficiency are recently described diseases. Thus, the majority of patient with these disorders are children or young adults. We will include children and young adults in this study because that is the usual age of the population with these rare diseases, long-chain fatty acid oxidation disorders. This population has expanded to include all adults because older adults are now being diagnosed with CPT-2 and are capable of participating in this research study.

4) Collaborating sites:

Subjects will be enrolled at Oregon Health & Science University (OHSU) and at the University of Pittsburgh. The principal investigator and study coordinator at each site will be responsible for enrolling subjects, completing the protocol and ensuring the safety and protection of the subject.

b) Sources of Materials:

Data will be obtained from the subjects while they are housed at the CRC and will include dietary intake, urine, breath and blood samples, and respiratory gases. Urine samples and dietary intake data will be obtained from subjects while they are consuming their assigned diet at home. Previous medical records of subjects CPT-2, VLCAD TFP, or LCHAD deficiency will be reviewed to confirm the diagnosis and document the history of disease.

Blood and urine samples will be stored with a unique identifier in locked laboratory facilities of participating centers. Long-term storage of blood and urine samples will be stored at -80 and -20 °C respectively in the laboratory of the principal investigator. The laboratory is a locked facility in the Richard T. Jones building, room 4549 at OHSU, Portland, OR.

Participating site principal investigators, and study coordinators will have access to the identifiable health information of subjects. Samples will be coded with a unique identifier to be sent to outside laboratories for analysis and for long-term storage.

c) Potential Risks:

Risks from the procedures in this study include: pain, bleeding, bruising and infection from venipuncture, and exposure to a small amount of radiation (1.5 – 3.0 mrem). There is a small risk from radiation exposure during the DEXA scan for fetal abnormalities. Nonradioactive stable isotopes of ^{13}C, ^{2}H, and ^{18}O will be given to subjects. The safety of stable isotopes is well documented and does not pose an added risk to the subjects (34). Alterations in dietary intake associated with poor intake or prolonged fasting in children with CPT-2, VLCAD, LCHAD or TFP deficiency carry the risk of associated hypoglycemia, lactic acidosis, or rhabdomyolysis. Exercise tests in subjects with CPT-2, VLCAD, LCHAD or TFP deficiency also carry the risk of hypoglycemia, lactic acidosis, or rhabdomyolysis. The feeding study is designed to provide adequate total energy and poses little risk to subjects. Signs of hypoglycemia, lactic acidosis and/or rhabdomyolysis will be closely monitored during and following the exercise tests. Preliminary studies did not pose any increased risk to children or adolescents with VLCAD, LCHAD or TFP deficiency. At very high intakes, both MCT and triheptanoin have been reported to cause gastrointestinal upset, cramping and steatorrhea. In the event of GI distress (diarrhea or cramping), the dose of triheptanoin or MCT will be decreased in increments of 5% until the symptoms resolve. High intakes of MCT or triheptanoin are also associated with excessive weight gain. Triheptanoin

supplementation may also be associated with elevated propionic acid. The development of propionic academia will be monitored through measurement of the acylcarnitine profile. If significant levels accumulate (greater than the upper limit of normal of the testing laboratory) the dose will be decreased incrementally by 5% until the level returns to an acceptable range. We do not anticipate these side effects because the dose proposed in this trial (20% of total energy) is considerably lower than previously reported trials (51, 52).

2. Adequacy of Protection Against Risks

a) Recruitment and Informed Consent:

Subject Recruitment: Subjects with CPT-2, VLCAD, LCHAD or TFP deficiency will be recruited to participate in this study. Recruitment techniques will include announcements on Fatty Acid Oxidation Support group web pages and chat boards and through our participating center's clinic population. Subjects must meet the inclusion and exclusion criteria detailed in Table 2. Diagnosis of CPT-2, VLCAD, LCHAD or TFP deficiency will be confirmed by obtaining medical record results of acylcarnitine profiles, fatty acid oxidation probes in cultured fibroblasts or by mutation analysis (32, 41).

Informed Consent: The site principal investigator or study coordinator will explain and discuss all procedures and tests to be completed during the study and will be available to answer questions for the subjects, their families or their primary care physicians. Families will be encouraged to discuss the trial with their physician. Informed consent will be obtained from each subject's legal guardian and each child will complete a child assent form prior to beginning the study.

b) Protection against risk:

Studies will be reviewed and approved by OHSU and University of Pittsburgh Institutional Review Boards (IRB). All subjects will be admitted to the inpatient unit of a Clinical Research Center for outcome measurements. Nursing staff and the investigative team will monitor subjects and care for wound or bleeding complications as a result of venipuncture. To prevent radiation exposure to a fetus, a urine pregnancy test will be completed for females of child bearing potential prior to each DEXA Scan. Subjects who are pregnant will be excluded. ^{13}C-oleic acid and $^{2}H_{2}{}^{18}O$ will be given to subjects. As noted above, the safety of stable isotopes is well documented and does not pose an added risk to the subjects (34). Special care will be taken to explain the use of stable isotopes to the subjects and their families. The feeding trial will be designed to provide frequent feedings and adequate energy to prevent complications of a fatty acid oxidation disorder. Plasma CK levels and glucose will be monitored prior to and following exercise. If the subject experiences any muscle pain during the test, the test will be stopped. If the subject experiences muscle pain and/or dark urine following the exercise test, they will be treated with bed rest and fluids.

To ensure confidentiality, all data and specimens collected will be kept in locked laboratory facilities. Computer files of collected data will be password protected and stored on a drive behind the OHSU and University of Pittsburgh firewall. The study coordinators will code blood and urine samples with a number to remove any identifiable information on the sample prior to sending the samples to other laboratories for analysis or storage. Research data transmitted between the two research sites will be encrypted. Subject's names or other identifiable information will not be published or shared with outside research facilities.

3. Potential Benefits of the Proposed Research to the Subjects and Others:

The proposed studies may provide alternative dietary supplements that will allow patient with CPT-2, VLCAD, LCHAD or TFP deficiency to exercise safely. Improving exercise tolerance and decreasing episodes of rhabdomyolysis will improve quality of life for patients with long-chain fatty acid oxidation disorders. Decreased hospitalization and treatment of acute rhabdomyolysis will also lower medical care costs associated with long-term care of fatty acid oxidation disorders. Thus, subjects may directly benefit from this research.

4. Importance of Knowledge to be Gained:

Recurrent rhabdomyolysis and cardiomyopathy are common complications of long-chain fatty acid oxidation disorders.

The proposed studies will explore dietary supplements that could potentially allow patients with CPT-2, VLCAD, LCHAD or TFP deficiency to exercise safely and decrease the incidence of rhabdomyolysis and cardiomyopathy. The results of this study could improve the quality of life for patients and decrease the physical, emotional and financial costs of the major complications of long-chain fatty acid oxidation disorders.

5. **Data and Safety Monitoring Plan:** see attached DSMP

6.. Safety Review Plan and Monitoring

a). Justification of sample size. The primary power outcome for these studies is to detect a difference in total energy expenditure. Power calculations from our preliminary studies suggest a sample size of 32 for both sites will be able to detect significant differences at $p < 0.05$ with 80% power (See Power Calculations in Methods section). We will recruit 32 subjects each with CPT-2, VLCAD, and LCHAD/TFP deficiency, 20 at OHSU and 12 at the site in Pittsburgh.

b). Adverse Event Definitions and Grading: An adverse event is defined as any unfavorable or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. Adverse events in this study are evaluated for relationship to study intervention and graded by the physician sub-investigator using the Common Terminology Criteria for Adverse Events version 4.0. This grading criterion is listed as follows:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization indicated; disabling; limiting self care activities of daily living.
Grade 4: Life-threatening consequences; urgent intervention needed.
Grade 5: Death related to AE.

A Serious Adverse Event (SAE) is defined as an event that results in death, is life threatening, requires in-patient hospitalization, results in persistent or significant disability or is an important medical event that does not meet the above criteria but may require medical or surgical intervention. SAE's that are unexpected and possibly related will be reported to the DSMB chair within 24 hours and will be reported to the IRB as indicated in the OHSU Research Integrity Office policy on Reporting Unanticipated Problems and Adverse Events.

c). Safety Monitoring: Safety and efficacy of this study will be monitored by a data safety and monitoring board (DSMB) established for this clinical trial. The charter of the DSMB will be as follows:

DSMB Charter

Introduction:

This charter is for the Data and Safety Monitoring Board for Phase 2 Trial of Triheptanion for treatment of long-chain fatty acid oxidation disorders.

The charter will define the primary responsibilities of the DSMB, its membership, and the purpose and timing of its meetings. The Charter will also provide the procedures for ensuring confidentiality and proper communication, the statistical monitoring guidelines to be implemented by the DSMB and an outline of the content of the Open and Closed Reports that will be provided to the DSMB.

Primary Responsibilities of the DSMB:

The DSMB will be responsible for safeguarding the interests of trial participants, assessing the safety and efficacy on the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DSMB will provide recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection/recruitment/retention of participants, their management, improving adherence to protocol specified regimens and retention of participants, and the procedures for data

management and quality control.

The DSMB will be advisory to the coordinating center Principal Investigator (PI). The Coordinating Center PI will be responsible for promptly reviewing the DSMB recommendations, to decide whether to continue or terminate the trial, and to decide whether amendments to the protocol or changes in study conduct are required.

Membership of the DSMB:

The DSMB is an independent multidisciplinary group consisting of a Biostatistician, a Gastroenterologist and a Pediatrician and a Metabolic Geneticist that have experience in the management of pediatric patients and in the conduct of monitoring of randomized clinical trials.

Conflicts of interest:

The DSMB membership has been restricted to individuals free of apparent significant conflicts of interest. The source of these conflicts may be financial, scientific or regulatory in nature. Thus, neither study investigators nor individuals employed by the sponsor, nor individuals who might have regulatory responsibilities for the trial products, will be members of the DSMB.

The DSMB members should not own stock in the companies having products being evaluated by the clinical trial. The DSMB members will disclose to fellow members before the initial DSMB meeting and if any changes occur during the course of the trial. The DSMB will be responsible for deciding whether these consulting agreements for financial interests materially impact their objectivity.

DSMB membership is to be for the duration of the clinical trial. If any members leave the DSMB during the course of the trial, the Coordinating Center PI will promptly appoint their replacements.

Timing and purpose of the DSMB meetings:

The initial meeting of the DSMB occurred 4-18-2012. During this organizational meeting, the DSMB discussed the standard operating procedure for the DSMB, and the format and content of the Open and Closed Reports that will be used to present trial results at future DSMB meetings.

The DSMB elected Dr. David Koeller as the DSMB chair. The meetings will be held at OHSU and those in attendance will be the 4 members of the board and an outside staff member to keep minutes.

The DSMB will meet again after half of the subjects have completed their 4-month follow-up. We anticipate this will occur at the end of year 2. The DSMB will meet again when ¾ of the subjects have completed their 4-month follow –up. The DSMB will review the study data and make recommendations to the PI.

The Coordinating Center PI will report Unexpected and Serious Adverse Events within 24 hours of notification to the DSMB Chair. The Chair will determine if an additional DSMB meeting should be convened to review the study data.

Procedures to Ensure Confidentiality & Proper Communication

To enhance the integrity and credibility of the trial, procedures will be implemented to ensure the DSMB has access to evolving information from the clinical trial regarding comparative result of efficacy and safety data, aggregated by the treatment arm. The study's Medical monitors will be provided immediate access on an ongoing basis to patient-specific information on serious adverse events (AEs) to satisfy the standard requirement for prompt reporting to the regulatory authorities.

At the same time, procedure will be implemented to ensure proper communication is achieved between the DSMB and the trial investigators and sponsor. To provide a forum for exchange of information among various parties who share responsibility for the successful conduct of the trial, a format for Open Sessions and Closed Sessions will be implemented. The intent of this format is to enable the DSMB to have unbiased discussions of the comparative efficacy results while at the same time providing opportunities for interaction between the DSMB and others who have valuable insights into trial-related issues.

Closed Sessions
Sessions involving only DSMB membership will be held to allow discussion of confidential data from the clinical trial, including information about the relative efficacy and safety of interventions. The primary mission of the DSMB is to safeguard the interest of the study subjects, and the DSMB will be responsible for the assessment of safety and efficacy data. During the closed sessions the DSMB will develop a consensus on its list of recommendations, including whether the trial should continue.

Open Session
In order to allow the DSMB to have adequate access to information provided by study investigators a joint session between these individuals and the DSMB members (called an open session) will be held between the Closed Sessions. This session gives the DSMB an opportunity to query these individuals about issues that have arisen during their review in the initial closed session. With this format, the important interactions are facilitated through which problems affecting trial integrity can be identified and resolved. These individuals will either be present at the DMSB meeting or be provided a telephone link.

Open and Closed Reports
For each DSMB meeting, Open and Closed Reports will be prepared by the coordinating center staff. Open reports will be available to all who attend the DSMB meeting and will include the following:

- Summary of the study design
- Summary of major protocol changes
- Information on patient screening and study accrual by month and institution
- Protocol deviations/violations including missed visits, procedures and, medication noncompliance
- Adverse Events
- Subject characteristics (as appropriate)
    - Demographics (sex, age, race)
    - Lab values and other measurements
    - Previous treatment and other similar information
    - Length of follow-up data available (min, max, and median)
    - Participant treatment and study status (pooled by treatment regimen)

Closed reports, available only to those attending the closed session of the DSMB meeting will include the following:

- Analyses of primary and secondary efficacy endpoints
- Protocol deviations with treatment status (if applicable)
- Analysis of adverse events and overall safety data
- Analysis of lab values, including basic summaries and longitudinal analysis
- DSMB member vote for study continuation/discontinuation

The open and closed reports will provide information that is complete within two months of the data of the DSMB meeting. The reports should be provided to DSMB members approximately two weeks prior to the meeting.

Minutes of the DSMB Meeting:
The DSMB will prepare minutes of their meetings. Two sets will be prepared: the Open Minutes and the Closed Minutes. Open minutes will describe the proceedings of the Open Session of the DSMB meeting and will summarize all recommendations of the DSMB. The closed minutes will describe the proceedings from closed session of the DSMB meetings, including the listing of recommendations by the Committee.

Statistical Monitoring Guidelines

As required by the FDA IND office, stopping criteria have been defined as below. These criteria are also included in IND 113386.003

Exercise Protocol: Criteria for inducing rhabdomyolysis with the exercise protocol is a CPK rise of >25% from baseline and significant muscle pain (>7 on 1-10 pain scale). If more than 3 subjects develop rhabdomyolysis as a result of the exercise protocol, we will modify the exercise protocol. If three patients develop Grade 3 or higher adverse events that are determined by the PI and a physician sub-investigator to be related to the exercise protocol, we will stop the study.

Subject Completion and Early Withdrawal

1. **Stopping criteria.** Individual patients who experience a Grade 3 or higher adverse event, that is determined by the PI and a physician sub-investigator to be unexpected and related to the study treatment, will be withdrawn from the study. The study will be stopped if 2 patients develop the same Grade 3 Adverse Event, or if any patient develops a Grade 4 Adverse Event that is determined by the PI and a physician sub-investigator to be unexpected and related to the study treatment. Any patient reporting a pregnancy will be immediately removed from the study and returned to a standard low fat diet containing MCT oil. The exercise protocol stopping criteria are listed above.
2. **Early Withdrawal:** Subjects may withdraw their consent to participate in the study at any time without prejudice. The investigator will withdraw any subject from the study who requests to be withdrawn.

E. Informed Consent

Written informed consent will be obtained from each subject and his or her guardian at entry into the study. Informed consent is obtained by the following process:

1. A potential subject or his/her guardian will contact the PI.
2. A consent will be mailed to their address. The subject will review the study consent form.
3. The PI or co-investigators will review the consent on the phone, confirm subject and guardian's understanding, and answer any questions.
4. Once the investigator is convinced that the subject verbally demonstrates understanding and agrees to the process, the consent and medical release documents will be signed and mailed or faxed to the investigator.
5. When the investigator receives the consent and medical release forms, study staff will obtain the necessary records to verify the diagnosis of a long-chain fatty acid oxidation disorder.
6. If the subject is eligible to participate, travel arrangements will be made.
7. Individuals authorized to obtain written consent are the principal investigator, co-investigators, and assigned medical staff specifically designated by the principal investigator to work on this project.

1. Subject will review the study consent form.
2. PI or co-investigators meet with the subject to review the consent, confirm subject and guardian's understanding, and answer any questions.
3. Once the investigator is convinced that the subject verbally demonstrates understanding and agrees to the process, the consent is signed. Individuals authorized to obtain written consent are the principal investigator, co-investigators, and assigned medical staff specifically designated by the principal investigator to work on this project.

F. Confidentiality

- Protection of subject privacy. Medical history and physical examination are performed. Blood is drawn, urine and breath collected and body composition scans obtained. Other data include dietary intake and respiratory gases analyzed. All of these materials are obtained for research purposes only; the data are kept in strict confidence. No information will be given to anyone without permission from the subject or subject's guardian. Confidentiality is assured by use of coded patient identification numbers, which will be used for all databases and samples sent outside the OHSU or Univeristy of Pittsburgh system. Only the principal investigators will have access to the key code for the identification numbers.
- Database protection. The database is secured with password protection and is stored on a drive behind the OHSU or University of Pittsburgh firewall. Written or electronic communication with outside collaborators will involve password protection and encryption of information.
- Confidentiality during adverse event reporting. Adverse event reports and annual summaries will not include subject-identifiable material. Each will include a coded identification number only.

G. Inclusion of Women and Minorities

1. Targeted distribution of subjects by gender & racial/ethnic groups:

See Targeted/Planned Enrollment table

2. Rationale for selection of gender& racial/ethnic groups:

Both genders will be included in this research project. Long-chain fatty acid oxidation disorders appear to occur equally in boys and girls. We expect to have equal numbers of males and females in this study.

Both Hispanic and Non Hispanic ethnic groups will be included in this study.

All minorities will be included. Recruitment should result in study demographics similar to that of the national disease demographics. Caucasians will comprise the majority of subjects in this study. To date, patients with long-chain fatty acid oxidation disorders have been of Caucasian ancestry. We would welcome subjects from other racial backgrounds but anticipate that most, if not all, subjects will be white.

H. Inclusion of Children: Children will be included**.**

1) Rationale for selection of a specific age range:

Children able to complete the protocol as described will be eligible to participate. Very young children (< 7 years) will probably not be able to comply with the protocol such as completing a 45-minute exercise treadmill test and thus will be excluded. Older individuals with fatty acid oxidation disorders may also have difficulty completing the exercise treadmill test, so we will evaluate each adult subject interested in the study to determine if the subject is capable of completing the tests. We have successfully enrolled and completed studies in three 7 year olds in our preliminary trial. The majority of subjects with CPT-2, TFP, LCAHD or VLCAD deficiency are less than 30 years of age because the disease was first described in the late 1980's. We will recruit subjects ≥7years of age.

2) Description of expertise of investigative team for dealing with this age of children:

Dr. Gillingham is a registered dietitian and has been working with children with long-chain fatty acid oxidation disorders and their families for the past 15 years. She specializes in the nutritional management of fatty acid oxidation disorders.

Dr. Harding is a board certified pediatrician and geneticist with extensive experience in fatty acid oxidation disorders diagnosis and treatment. He maintains board certifications in clinical biochemical genetics, and routinely provides medical care for both stable and critically ill children with rare disorders of metabolism. Dr. Harding provides medical care for approximately 180 patients in the clinic setting and 50 patients in the acute care setting annually.

Dr. Vockley is a board certified pediatrician and geneticist and chief of Medical Genetics at the University of Pittsburgh. He has a large clinical practice of patients with fatty acid oxidation disorders and an active laboratory conducting basic and clinical studies in this group of disorders. Dr. Vockley holds an IND from the FDA to use triheptanoin in human patients. Dr. Charles Roe who recently retired from practice formerly held a similar IND. Dr. Roe transferred the care of over 50 patients with fatty acid oxidation disorders taking triheptanoin to Dr. Vockley. This group of patients would be eligible participants in this study and will be traveling to Pittsburgh for their medical care and to obtain the triheptanoin supplement.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Title page
	1b	Structured summary of trial design, methods, results, and conclusions	Abstract page
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	1-2, supp 11-13
	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	supp 8-9
Participants	4a	Eligibility criteria for participants	3 supp 15
	4b	Settings and locations where the data were collected	3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4-6, supp 14-17
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	supp 8-9
Sample size	7a	How sample size was determined	4, supp 19
	7b	When applicable, explanation of any interim analyses and stopping guidelines	supp DSMB charter 23-25
Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	3, supp 11
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	17, supp 18
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	3-4, supp 14
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	3-4, supp 14, 18
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	3-4

	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	22
	13b	For each group, losses and exclusions after randomisation, together with reasons	22
Recruitment	14a	Dates defining the periods of recruitment and follow-up	3
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	29
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	22
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Supp tables 1,2, 3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	na
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11, 31
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-14
Other information			
Registration	23	Registration number and name of trial registry	Abstract, 3
Protocol	24	Where the full trial protocol can be accessed, if available	3, Supp 8-27
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.