EFFECTS OF MCT SUPPLEMENTATION ON EXERCISE TOLERANCE IN PATIENTS WITH LONG-CHAIN FATTY ACID OXIDATION DISORDERS

By

Annie Behrend

A THESIS

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

September 2010

2009-2010

School of Medicine

Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Annie M Behrend

has been approved

Melanie Gillingham, PhD, RD

Cary Harding, MD

Diane Elliot, MD

TABLE OF CONTENTS

Page

APPENDICES

List of Tables

List of Figures

List of Abbreviations

AI……adequate intake

AKI……acute kidney injury

ATP……..adenosine triphosphate

AUC……area under the curve

A-VO_2……arteriovenous oxygen

BMI…….. body mass index

BP……blood pressure

CACT……carnitine acylcarnitine transferase

CAT I…….. carnitine acyl-transferase I

CAT II…… carnitine acyl-transferase II

CHO……... carbohydrate

CK…… creatine kinase

CM…… chylomicron

CMR…….. chylomicron remnant

CO……cardiac output

CPT I……..carnitine palmitoyl-transferase I

CPT II…….carnitine palmitoyl-transferase II

CTRC…… Clinical & Translational Research Center

DEXA……dual energy x-ray absorpitometry

DP……... double product

ECG…… electrocardiography

EDTA……ethylenediaminetetraacetic acid

EF……ejection fraction

EFA……essential fatty acid

FAO……..fatty acid oxidation

FFA……..…free fatty acid

FFM……..fat free mass

GC/MS……gas chromatography/mass spectrometry

HDL…….. high density lipoprotein

HR…… heart rate

IC……..……indirect calorimetry

IRB……..institutional review board

IV……intravenous

LBM……..lean body mass

LC……long-chain

LCFA…… long-chain fatty acid

LCHAD…… long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency

MCFA…….. medium chain fatty acid

MCT…… medium chain triglyceride

NPRQ……non-protein respiratory quotient

OHSU……..Oregon Health & Science University

PEG……percutaneous endoscopic gastrostomy

PET……positron emission tomography

RDA……...recommended dietary allowance

RER……. ……respiratory exchange ratio

RQ……. respiratory quotient

SCAD……..short-chain acyl-CoA dehydrogenase deficiency

SIDS…… sudden infant death syndrome

SBP……..systolic blood pressure

TAUC……total area under the curve

TF……tube feed

TFP…….. trifunctional protein

VCO_2……..…….rate of elimination of carbon dioxide

VLCAD…….. very long-chain acyl-coenzyme A dehydrogenase

VLDL……. very low density lipoprotein

VO_2……oxygen uptake

Acknowledgements

First and foremost, I would like to thank my thesis committee, Dr. Harding, Dr. Elliot and especially Dr. Gillingham; I could not have asked for a better advisor, teacher & friend to guide me through this process and introduce me to the world of metabolics.

My “clan”, Christy, Sarah and Stephanie will be lifelong friends of mine and I thank for adding so many fond memories to our inseparable years together at OHSU.

I thank my family who has always been in the utmost support of my academic goals and finally, my faithful study partner & husband Travis who I appreciate for the innumerable late night coffee shop dates that kept us, through our first years of grad school together, caffeinated and competent.

ABSTRACT

Background: Inherited disorders of long-chain fatty acid oxidation (FAO) inhibit the ability to oxidize long-chain fatty acids (LCFAs) for energy generation. As a result, bouts of exercise can lead to rhabdomyolysis, impaired cardiac function and, hence, exercise avoidance in these individuals. Medium-chain triglyceride (MCT) supplementation may bypass this defect and reduce the risk of adverse metabolic events.

Objectives: To determine the influence of isocaloric MCT vs carbohydrate (CHO) supplementation prior to exercise on substrate oxidation and cardiac function during exercise in participants with long-chain FAO disorders.

Design & Methods: Two 45-minute, moderate intensity treadmill exercise studies were completed by subjects (n=11) in a randomized crossover design. An isocaloric oral dose of CHO (1 g/kg LBM) or MCT-oil (0.5 g/kg LBM) was administered prior to exercise, hemodynamic and metabolic indices were assessed during exertion and a cardiac echocardiogram was performed following exercise. Energy metabolism and cardiac function during exercise were analyzed using paired T tests.

Results: A statistically significant decrease in respiratory exchange ratio (RER), double product ejection fraction estimation, and steady state heart rate were observed following the exercise test pretreated with MCT.

Conclusions: MCT supplementation prior to exercise in subjects with LCHAD, CPT2 or VLCAD deficiency increased the oxidation of medium chain fats and acutely improved cardiac ejection fraction for the same amount of work performed when compared to CHO supplementation. Results from this study may guide dietary therapies for individuals with long-chain FAO disorders.

Keywords: LCHADD, long-chain FAO disorders, MCT, exercise

CHAPTER 1

INTRODUCTION

Hypothesis & Specific Aims

Individuals with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency, as well as other long-chain metabolic defects such as very long-chain acyl-coenzyme A dehydrogenase (VLCAD) and carnitine palmitoyltranserase II (CPT2) deficiency, often avoid physical activity due to exercise-induced rhabdomyolysis. It is possible that rhabdomyolysis during exercise is the result of depleted energy and/or glycogen stores resulting in muscle breakdown. The accumulation of toxic byproducts, produced by partial fatty acid oxidation, may also be a contributing factor leading to the development of rhabdomyolysis. Previous research has suggested that medium chain triglyceride (MCT) supplementation administered prior to exercise improves exercise tolerance in individuals with LCHAD deficiency. Interestingly, a decrease in steady-state heart rate (HR) has been observed in participants supplemented with MCT oil prior to exercise, suggesting a cardiac benefit [1]. Changes in heart rate are noteworthy due to an increased incidence of cardiomyopathy among individuals with long-chain FAO disorders [2-4]. MCT bypasses the block in long-chain fatty acid (LCFA) oxidation and may provide a usable source of fatty acids for both the heart and exercising skeletal muscle. In a preliminary study of MCT affects, no significant difference in respiratory quotient (RQ) was observed when orange

juice + MCT versus orange juice alone were administered prior to exercise [1]. However, subjects were not given isocaloric supplementation of MCT vs. CHO; the difference in HR may have been due to the difference in total energy and not the difference in substrate oxidation. In addition, only subjects with LCHADD were included in the initial study; however, due to common symptoms, subjects with other long-chain FAO disorders may also benefit from the development of dietary guidelines to enhance exercise tolerance. The specific aims of our study are as follows, and will enable us to examine more precisely the effect of MCT on exercise:

1. To expand the study population to include subjects with VLCAD, CPT2 and LCHADD in the trial.

•We hypothesized that subjects with VLCAD, CPT2, and LCHADD would respond to pre-exercise MCT supplementation in a similar manner.

2. To determine the effect of isocaloric MCT vs. CHO supplementation prior to exercise on substrate oxidation during exercise in subjects with a long-chain fatty acid oxidation disorder.

•We hypothesized that MCT supplementation prior to exercise in subjects with a long-chain fatty acid oxidation disorder will increase the oxidation of medium chain fats and decrease the oxidation of LCFAs when compared with CHO supplementation.

3. To determine the effect of isocaloric MCT vs. CHO supplementation prior to exercise on cardiac output (ejection fraction) in subjects with a long-chain fatty acid oxidation disorder.

•We hypothesized that MCT supplementation prior to exercise in subjects with a long-chain fatty acid oxidation disorder will improve cardiac function for the same oxygen utilization and amount of work performed when compared to CHO supplementation because of the ability of MCT to bypass the long-chain fatty acid oxidation defect and provide fatty acid energy to the heart.

To address these questions, we conducted a randomized crossover clinical trial with subjects who have LCHAD (n=8), CPT2 (n=2), or VLCAD (n=1) deficiency. Participants completed a 45-minute treadmill test at 60-70% estimated max heart rate 20 minutes after consuming an isocaloric oral dose of either CHO or MCT based on body weight. During the treadmill test, respiratory gas exchange, heart rate (via ECG) & blood pressure were monitored. In addition, blood was drawn immediately prior to exercise, immediately post exercise & after 20 minutes recovery to measure levels of lactate, creatine kinase, β-hydroxybutyrate, acetoacetic acid, free fatty acids and acylcarnitines. Heart function was estimated using double product calculation, (systolic BP x HR), an indirect marker of ejection fraction (EF) which enabled us to examine estimated EF while HR was elevated during exercise. Immediately following exercise testing, a subset of participants (n=3) also received an echocardiogram for direct measurement of EF.

Because of our previous work with families who have a child with LCHADD, we were most successful recruiting subjects with LCHAD deficiency. However, we were also able to recruit a small subset of subjects with other long-chain FAO disorders and believe that conclusions drawn from

this study can be expanded and applied to other long-chain fatty acid oxidation disorders. Participants have already benefited first hand by recognizing how, by dietary intervention, they can better tolerate increased levels of activity in their daily living as well as recreational life. Perhaps even more significant, this study has begun to help us understand the relationship between MCT supplementation prior to exercise and changes in heart function.

CHAPTER 2

METABOLIC BIOCHEMISTRY

LCFA β–oxidation

LCFAs are composed of fatty acids greater than 10-12 carbons in length. Specificity of acyl CoA synthetase for LCFAs leads to the activation of these fatty acids after absorption into the enterocyte by coupling with coenzyme A. Intracellular reformation of triacylglycerols, phosphatidylcholine, and cholesterol esters takes place in the intestinal mucosal cell. The endoplasmic reticulum in the enterocyte collects these lipids along with fat-soluble vitamins and these large fat particles are transformed into chylomicrons (CMs) and exocytosed through the cell membrane into lymphatic circulation [5].

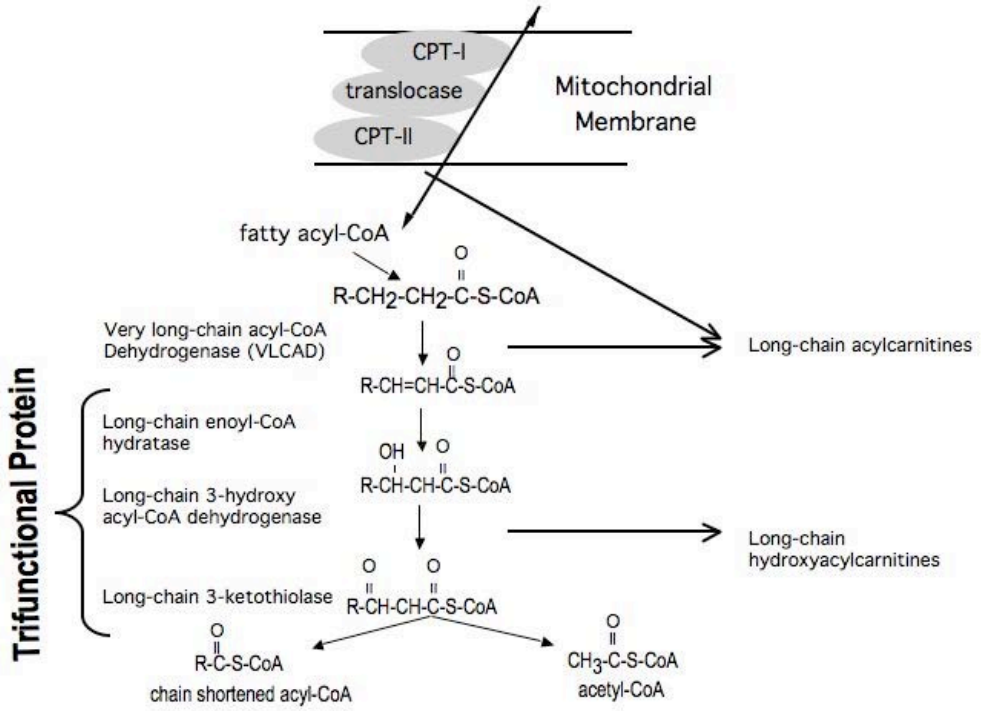
Intravascular conversion of CMs to CM remnants (CMRs) creates relatively smaller lipoproteins containing less triacylglycerol but more cholesterol than the CMs; the large, triacylglycerol-riddled CMs are responsible for the turbidity or “milky” appearance of postprandial plasma. While entry of CMs into the blood from the lymph may continue for up to 14 hours after consumption of a high-fat meal, blood plasma lipid levels peak 30 minutes to 3 hours, (returning to near normal within 5 to 6 hours), postprandially [6].

CMRs are removed from the bloodstream by liver cell endocytosis; once inside the hepatocyte CMR cholesterol and cholesterol esters may be

converted into bile salts, secreted into the bile as neutral sterol, or again repackaged into HDL or VLDL and released into the plasma. In the fed state, lipid metabolism at the adipocyte favors energy storage; in the fasting state, many tissues are capable of oxidizing fatty acids for energy via β-oxidation [7, 8].

Upon entry into the cell of the metabolizing tissue, fatty acids are activated to fatty acyl-CoA; cytoplasmic fatty acyl CoA synthetase catalyzes this initial energy-requiring reaction. The remainder of the oxidation process occurs within the mitochondria of the metabolizing tissue; however, LCFAs and their CoA derivatives cannot cross the inner mitochondrial membrane without the assistance of the carrier molecule carnitine. Joined covalently to carnitine by carnitine palmitoyl-transferase I (CPT I), the LCFA passes through the outer face of the mitochondrial membrane. Carnitine acylcarnitine translocase (CACT) moves the acylcarnitine into the matrix of the mitochondria. Located on the inner face of the inner membrane, a second transferase, carnitine palmitoyl-transferase II (CPT II) releases the fatty acyl CoA and carnitine into the matrix of the mitochondria. Once inside the matrix of the mitochondria, the activated fatty acid undergoes further oxidation via a cyclic degradative pathway. During this process, two-carbon units are cleaved one by one from the carboxyl end in the form of acetyl CoA as depicted in the following figure [9].

Fig 1. Long-Chain Fatty Acid β–oxidation



MCFA β–oxidation

MCTs are composed of glycerol and fatty acids 8-10 carbons in length. They can be oxidized by a separate set of β-oxidation enzymes, bypassing the defect in long-chain FAO in patients with CPT2, VLCAD or LCHAD deficiency. The melting point is lower and molecular size smaller for MCFAs compared to LCFAs; consequently, MCFAs are more soluble in aqueous biological fluids and can be less dependent on lipid transport proteins, like carnitine transport through the mitochondrial membrane. Upon ingestion, MCT's can begin to undergo digestion as early as the stomach by preduodenal lipases, which hydrolyze MCT preferentially [10]. MCFAs are

carried via portal circulation to the liver or to muscle cells for oxidation and can be used to produce ATP within 20 minutes of consumption.

CHAPTER 3

A REVIEW OF LCHADD

LCHADD

Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (LCHADD) is an autosomal recessive genetic defect in mitochondrial β-oxidation of long-chain fatty acids. LCHAD is one of three enzymatic activities in the mitochondrial trifunctional protein (TFP). Both isolated LCHAD deficiency and total TFP deficiency have been described in humans. Although less prevalent, TFP deficiency involves the impairment of all three enzymes comprising mitochondrial TFP and presents with similar symptoms to LCHADD. TFP is required for the 2nd, 3rd and 4th steps in long-chain fatty acid β-oxidation; long-chain fatty acid conversion to acetyl-CoA and ATP is thus impaired when LCHAD function is limited [11, 12]. Alternately, long-chain acyl-CoA esters are formed and due to poor urinary excretion, accumulate within the mitochondria.

During times of limited blood glucose supply and low glycogen stores fatty acid metabolism becomes the dominant energy source for many tissues, which can lead to life-threatening episodes of metabolic decompensation in patients with LCHADD. Long-chain FAO disorders often present during the first year of life and can result in death in infants who remain in the fasted state, often during the night, past depletion of glucose and glycogen stores. Severe symptoms also present during periods of stress or illness due to either

poor oral intake and/or increased energy demands. It is estimated that fatty acid oxidation disorders as a whole, including LCHADD, may account for up to 5-10% of sudden infant death syndrome (SIDS) in the US [4]. Recurrent symptoms in survivors include rhabdomyolysis with elevated serum creatine kinase levels, often induced by increased activity, fasting-induced hypoketotic hypoglycemia, and cardiomyopathy [12-14]. Myoglobinuria, lactic acidosis, hepatomegaly and hypoglycemic encephalopathy have also been reported secondary to this condition [12, 14, 15]. In 7 children with LCHADD, Tyni and colleagues observed structural changes at the level of the mitochondria in 4 as well as respiratory chain dysfunction in 5 patients [16]. Long term complications associated with LCHADD include both pigmentary retinopathy and peripheral neuropathy [14, 17]. Although the etiology of both the acute and chronic effects of this disorder are not entirely understood, possible causal factors may involve overall energy deficit or the potentially toxic effects of partial fatty acid oxidation by-products, such as hydroxyacylcarnitines (an LCHADD-specific metabolic byproduct of partial LCFA β-oxidation) [1]. Thus, particularly when diet is not tightly controlled, frequent bouts of metabolic decompensation can result in hospitalization and the progression of long-term complications.

Dietary Therapy

To minimize the use of LCFA for energy, most individuals with LCHADD currently consume frequent meals that have a very low fat, high

carbohydrate macronutrient composition. It is advised that subjects limit dietary (or long-chain) fat intake to less than 10% of total energy, include MCTs as 10-20% of daily energy intake, (or 1.5g/kg), and supplement with essential fatty acids [18]. During infancy, a percutaneous endoscopic gastrostomy (PEG) tube is often placed so LCHADD patients can receive nocturnal, continuous tube feeds (TFs). Body glycogen stores in infancy have been estimated to account for merely 0.5% of total FFM [19]; thus, nightly TFs often persist until around the age of 4 when a FFM capable of housing adequate glycogen stores to sustain blood glucose for multiple hours is achieved. Consumption of uncooked cornstarch before bed, is also practiced by some patients as a method of slow glucose-release while in the fasted state [14]. While controversial, the use of supplemental carnitine is often also prescribed.

The restriction of total LCFA intake in this population can effectively restore plasma acylcarnitines to within normal limits [18]. However, minimal dietary fat intake places this population at increased risk for developing fat-soluble vitamin and essential fatty acid deficiencies. Gillingham et al assessed the vitamin and mineral intake from food and formula of 10 children with LCHADD. Average fat-soluble vitamin consumption was below the RDA/AI for vitamins D, E & K; average vitamin A intake was sufficient. Eight of the 10 participants reported taking a daily multivitamin, an important aspect of LCHADD specific dietary therapy to ensure adequate fat-soluble and overall micronutrient intake [20]. Perhaps of greatest concern, however, was the

prevalence of biochemical essential fatty acid (EFA) deficiency in the participants of this study. Concentrations of EFAs were measured in 9 of the participants in Gillingham's study and all were found to be deficient in at least 1 species of omega-6 or omega-3 fatty acid [20]. While it is important to keep the consumption of dietary fat minimal, it is also advised that vegetable oils and other sources of EFAs subsume an adequate portion of the dietary fat consumed by patients with LCHADD.

While frequent feedings and overall restriction of dietary fat has been effective in preventing LCFA oxidation and, thus, reduction of potentially toxic hydroxyacylcarnitines, partial FAO continues to occur in the fed state in cardiac and skeletal tissue. To minimize the oxidation of LCFAs in these tissues, medium chain triglycerides are widely used in LCHADD-specific dietary therapy and have proven beneficial in both in vitro and in vivo studies [3, 15, 18, 21-23]. In a survey article by Gillingham and colleagues, only 2 of 18 LCHADD patients were reportedly not supplementing with MCTs. Of the two patients not undergoing MCT therapy, one had died in metabolic crisis and the other had experienced an alarming number of episodes of metabolic decompensation [18]. MCTs not only provide a usable source of energy for this population, but also have the potential to generate adequate acetyl-CoA and malonyl-CoA to inhibit the activity of CPT-1 and further accumulation of long-chain metabolites [18, 21]. Upon incubation of LCHAD-deficient fibroblasts with long-chain fatty acids, specifically palmitate (C16) and linoleate (C18), Shen and colleagues observed an increase in hydroxylated long-chain

acylcarnitines and decreased expression of hydroxylated short-chain (<C10) species when compared with controls. Following incubation with MCTs, specifically decanoic (C10) and octanoic acid (C8), both the accumulation of long-chain hydroxyacylcarnitines and the secondary metabolic disturbances were prevented [24]; the use of MCTs as part of an optimal dietary therapy for LCHAD deficient patients was therefore advocated.

For infants, MCTs can be provided via formula; SHS International designed the formula Monogen to minimize the level of LCFAs while providing 21% of energy as MCTs and maintaining an appropriate amount of essential fatty acids. Older children may receive MCTs in home-prepared food or supplements. Lipistart or MCT Procal may be recommended for children over a year of age who struggle to consume an adequate amount of MCT, essential fatty acids, protein or other nutrients in their diet. Most patients consume MCTs at breakfast and/or bedtime, but not necessarily before periods of activity. MCTs supplemented at breakfast may not be available later in the day for fuel as they are preferentially oxidized and not stored. Interestingly, patients experience rhabdomyolysis most frequently in the mid-afternoon, often following a period of activity [1].

Carnitine defiency is commonly observed secondary to inborn errors of metabolism [25]; individuals with LCHADD frequently report the use of carnitine although evidence supporting the benefits of supplementation in this particular population is lacking [20, 25]. As much as 95% of free carnitine in the body resides in muscle tissue [25]. Therefore, while there is a correlation

between plasma and muscle free carnitine, normal plasma carnitine concentrations cannot guarantee adequate muscle stores [26]. Availability of free carnitine is necessary to maintain appropriate ratios of acyl-CoA/free CoA within the mitochondria; however, acylcarnitine accumulation and subsequent urinary excretion promotes the loss of free carnitine in patients with long-chain FAO disorders [27]. Those in favor of carnitine supplementation argue that it selectively facilitates the excretion of potentially toxic acylcarnitines while delivering LCFAs to the mitochondrial matrix [25, 28-30]. Alternatively, it has also been proposed that by facilitating the delivery of LCFAs into the mitochondrial matrix, supplementation endorses further generation and accumulation of long-chain intermediates [25, 31]. Carnitine therapy has been deemed ineffective in VLCAD knockout mice, which demonstrated significant acylcarnitine production without improvement in tissue free carnitine following supplementation [32]. Den Boer and colleagues could not identify a significant treatment effect of carnitine upon evaluation of 50 patients with LCHADD [12]. In an evaluation of 10 patients with LCHADD, Gillingham et al reported no correlation between carnitine supplementation and plasma acylcarnitine levels, suggesting that supplemental carnitine did not alter plasma accumulation of long-chain 3-hydroxycarnitine esters [20]. The current literature does not provide evidence that carnitine supplementation is either helpful or harmful but well controlled studies are lacking.

Rhabdomyolysis

Striated muscle breakdown (rhabdo-myo-lysis) is a frequent complication among patients with LCHADD. Rhabdomyolysis is most commonly the result of infection or inherited disorders in the pediatric population [33]; other common causes, in patients of all ages, include exercise, trauma, infections, medications and a variety of disease states. Conditions resulting in rhabdomyolysis cause cellular membrane damage and release of intracellular contents, including creatine kinase (CK), myoglobin, phosphate and potassium, into the plasma. In addition, patients experience hypoxia, loss of degradative enzymes and ultimately mitochondrial breakdown and depletion of ATP. Short-chain acyl-CoA dehydrogenase deficiency (SCAD), very long chain acyl-CoA dehydrogenase (VLCAD) and other long chain disorders, like LCHADD, are complicated by a lack of cellular energy production and may also present with rhabdomyolysis [34]. The degree of rhabdomyolysis experienced by patients with LCHADD is often expressed symptomatically by severity of pain and clinically by abnormally elevated creatine kinase levels. Aggressive pain medications may be prescribed to help patients better tolerate a bout of rhabdomyolysis; CK levels may well exceed 50,000 U/L during such episodes (normal concentrations generally range from approximately 40-500 U/L) [35]. Aggressive rehydration therapy should be initiated early if rhabdomyolysis is suspected; IV fluids can help prevent the worsening of potential secondary conditions, such as acute kidney injury (AKI).

While AKI may be less common in children than adults, it is deemed the most common complication of rhabdomyolysis, occurring in approximately 24-40% of all cases [33, 36-39]. It has been suggested that the severity of a patient's illness, and in particular, CKs peaking >5000 U/L, may be associated with the development of AKI in approximately 19% of cases. This is more than twice the estimated incidence of approximately 8% when CKs fail to exceed 5000 U/L [33]. Myoglobin toxicity may be largely responsible for the prevalence of AKI resulting from rhabdomyolysis [33]. As excess myoglobin is released into the bloodstream, the capacity of plasma binding proteins for myoglobin is saturated; tubular damage results as myoglobin is filtered through the glomerulus. The dissociation of myoglobin produces toxic byproducts, namely ferrihemate, increasing the level of oxidative free radicals that leads to further damage of the renal parenchyma [40]. When complicated by AKI and CK levels >100,000 U/L, the mortality rate of patients suffering from rhabdomyolysis can reach 80% [40]. Renal presentation (tubulopathy) with transient renal failure was reported in 27% of patients with FAO disorders observed by Saudubray in Hoüpital Necker Enfants-Malades, Paris [4]. In 1999, only 57 of the 107 patients Saudubray followed over a period of 25 years were still living; death of 47 siblings of patients was also reported. [4]. The early reports of mortality in patients with FAO disorders, including LCHADD, echos the staggering estimations of mortality due to rhabdo and AKI reported by Gonzalez et al in 2005 [4, 40].

Hypoketotic Hypoglycemia

LCHADD limits one's hepatic capacity to generate ketones and utilize fatty acids for energy by extra hepatic tissues. Healthy individuals, and particularly children in whom lower glycolytic activity has been observed [41-44], use long-chain fatty acids as a significant source of respiratory fuel for many tissues. The rate of hepatic production of ketone bodies, namely β-OH butyric acid and acetoacetic acid, is markedly increased during periods of fasting and physical activity. Under these circumstances, up to 80% of total energy needs can be met by circulating ketones [45]. The metabolic adaptation of ketone upregulation and utilization enables extra hepatic tissues to spare glucose. Due to the size of cerebral tissue in proportion to body size during infancy, it's not surprising that an especially high utilization of glucose is required during the first year of life [46, 47]. In tandem, glucose stores are limited during infancy, thus making the glucose sparing effects of ketones particularly important. With a highly compromised metabolic capacity to burn FFAs and generate ketones, catabolic stress will drive patients with LCHADD into acute life-threatening episodes of hypoketotic hypoglycemia, often presenting for the first time during infancy.

Cardiomyopathy

Cardiomyopathy can be one of the many deleterious symptoms of a FAO disorder. Saudubray observed cardiac presentations in 51% of the 107 FAO disorder patients he followed in his 1999 study; cardiomyopathy,

primarily hypokinetic hypertrophic, occurred in 67% of those with cardiac complications [4]. Cardiomyopathy presents when the heart muscle is unable to pump and deliver adequate blood to the peripheral tissues of the body. Illness and/or infection, increased physical activity and prolonged fasting in subjects with FAO disorders can all result in secondary cardiomyopathy, due to metabolic decompensation.

It is well understood that in healthy individuals, the majority of energy required by the heart both during rest, but especially during exercise, is supplied by long-chain fatty acids [48-51]. Using positron emission tomography (PET), Bergmann and colleagues studied 11 patients with inherited defects of fatty acid metabolism. They were able to recognize that extraction of palmitate by the myocardium of subjects was comparable to controls, but oxidation was over 75% compromised and appeared to foster increased formation of triglycerides [41]. It has previously been recognized that long-chain acylcarnitines may be arrhythmogenic [52]. In this study, it was postulated that the accumulation of long-chain acylcarnitine intermediates is responsible for the high incidence of fasting-induced sudden death in this population and that the esterification of triglycerides (or other slow turnover pool processes) results from their accrual [41].

It has also been hypothesized that part of the defect in energy production may be attributed to a depletion of catalytic intermediates in the citric acid cycle [53]. Roe suggested that catalytic intermediates could be effectively restored using an odd-chain MCT as part of the treatment therapy

for FAO disorders. While additional research is underway, subjects currently are encouraged to consume even-chain MCTs while restricting dietary LCFAs in order to prevent metabolic crisis.

Overweight & Obesity

The prevalence of overweight and obesity among subjects with TFP, (including LCHAD), and VLCAD deficiency has progressed to 30-40%, double the 12-17% prevalence in the general US pediatric population [54, 55]. Although the physiological basis underlying the high prevalence of overweight and obesity in this population may be multifaceted, limiting physical activity due to rhabdomyolysis can only continue to foster its development. Due to the relatively recent discovery of these disorders, understanding the long-term effects of overweight and obesity is limited to speculation given the lack of good data on long-term morbidity and mortality. Likely, overweight and obesity in subjects with FAO disorders will ultimately contribute to the development of chronic disease (as it does in the general US population) [55].

Preliminary Studies: LCHADD & Exercise Tolerance

Recruitment. An initial study on the effects of dietary intervention on retinal degeneration in children with LCHAD/TFP deficiency recruited subjects through the FAO parent network and physician referral. From 1997 until 2002, 15 subjects were enrolled in this trial. This represents approximately one-tenth of the known living patients with TFP deficiency in the US; recruitment

occurred from within the cohort of 15 for a more intensive exercise study. Nine subjects completed the following protocol.

Methods. Nine LCHADD subjects completed two 45-minute moderate intensity, (60-70% estimated maximum heart rate), treadmill tests [1]. Participants were randomly assigned either MCT (0.5gm MCT/kg lean body mass + 4 oz juice) or carbohydrate (4oz orange juice) supplementation 20 minutes prior to testing in a cross-over design. During testing, a 12-point ECG monitored heart rhythms in the participants. Respiratory gas exchange was also measured during testing, providing respiratory quotient (RQ) readings as an estimate of substrate choice. Blood samples were drawn immediately prior to exercise, immediately following exercise, and 20 minutes post treadmill testing; blood was analyzed for lactate, creatine kinase, β-hydroxybutyrate and acylcarnitines. Following MCT supplementation, there was a decrease in steady-state heart rate among participants. However, RQ was not significantly different when supplemented with 0.5gm MCT/kg lean body mass + 4oz juice vs. 4oz juice alone. Creatine kinase and lactate levels both remained consistent at each blood draw, while cumulative long-chain 3-hydroxyacylcarnitines were 30% lower and β–hydroxybutyrate was three-fold higher following MCT supplementation [1]. MCT with CHO decreased HR & 3-hydroxyaclcarnitines while increasing the production of ketones; however, the pre-exercise supplements were not isocaloric. The observed change in this study could be due to increased calories rather than the calorie source (MCT).

CHAPTER 4

SUBSTRATE CHOICE DURING EXERCISE

Moderate Intensity Exercise

During moderate intensity exercise, substrate to fuel aerobic activity comes partly from muscle glycogen and partly from circulating blood glucose and fatty acids. Moderate-low intensity exercise is fueled solely by the aerobic pathway, allowing the greatest proportion of fat to be used. Fat utilization also increases with duration of activity. Interestingly, it has been recognized in some research studies that women may be better suited for endurance exercise due to an increased ability to oxidize fatty acids for energy during exercise [56, 57]. Votruba et al. studied the effects of exercise on subsequent use of dietary fat in seven healthy females [58]. Each participant completed three randomized exercise sessions, 3-4 weeks apart, modeling rest, light (120 min at 25% VO_{2peak}) and heavy (3 bouts, 10 min apart, 10-12 min intervals at 85% VO_{2peak}) exercise. The amount of energy expended during both light (~375 kcals) and heavy (~336 kcals) exercise sessions (measured by indirect calorimetry in a respiratory chamber) were significantly greater than rest ($p < 0.0001$) but not significantly different from each other. However, substrate utilization during light and heavy exercise was considerably different. Non-protein respiratory quotient (NPRQ) during rest (0.89 +/- 0.01) and heavy (0.89 +/- 0.01) exercise exceeded that of the light (0.85 +/- 0.01) exercise trial ($p < 0.001$ and $p < 0.01$, respectively). It was estimated that 27%

(20 g) more fat was utilized for fuel during light versus heavy exercise ($p < 0.01$). Although the greatest effect on CHO oxidation during exercise was observed during the heavy exercise trial, Votruba et al found that the light exercise protocol required 33 g more CHO for fuel than rest ($p<0.05$) [58]. Subjects were consuming an approximate 1880 kcal diet composed of 54% carbohydrate (CHO), 32% fat, and 15% protein during all 3 exercise periods to keep diet controlled in this study. Similarly, Romijn et al compared lactate concentration, glucose metabolism and free fatty acid metabolism in eight healthy women at light, moderate and heavy exercise intensity [59]. The light exercise regimen involved 60 minutes, at 25% $V0_{2MAX}$ on a stationary ergometer; moderate exercise consisted of 60 minutes at 65% $V0_{2MAX}$, and heavy exercise required 30 minutes of exercise at 85% $V0_{2MAX}$. As expected, lactate concentrations and glucose utilization significantly increased with increased exercise intensity while fat utilization significantly decreased with increased exercise intensity [59]. While typical fat oxidation rates are estimated to be between 0.2 and 0.5 g/min [60, 61], Achten and Jeukendrup reported that fat oxidation peaks at ~0.5 g/min around 60-65% $V0_{2MAX}$ [60, 61]. Rates of fat oxidation achieved approximately 0.71 g/min in a study performed by Carey and colleagues [62] who investigated the effects of dietary fat “loading” on performance in endurance athletes. Strong evidence suggests that a fatty acid substrate is preferred during low-moderate intensity, long duration exercise. However, the necessity of relatively minor amounts of muscle glycogen and blood glucose during fat oxidation cannot be discounted

because of the anaplerotic role carbohydrates play in the Kreb's cycle. "Hitting the wall", or failure in the ability to continue exercising, occurs when all body carbohydrate stores are depleted.

Increased intensity during exercise drives greater utilization of carbohydrate for fuel. At high intensity, ATP requirements exceed the ability of the body to supply oxygen, forcing the body into anaerobic metabolic pathways which use glucose and glycogen, (or in the case of the myocardium, creatine phosphate), for fuel. In the study performed by Votruba et al, carbohydrate use was 17% greater over the course of a participant's chamber stay when randomized to the heavy versus light exercise admission ($p < 0.05$); no difference was observed in protein utilization across trials [58].

The metabolic advantages of physical activity have not only been observed during a bout of exercise, but data has also confirmed that for hours following exercise, substrate oxidation continues to be upregulated. The influence of exercise on trafficking dietary fat has been dissected by various researchers who seek solutions to issues related to weight control and postprandial lipemia. Votruba et al observed that light and heavy trials had the same post-exercise effect on whole-body fat oxidation, which was 12 grams greater when compared to rest [58]. In addition to measuring lipid oxidation in the respiratory chamber, immediately post exercise subjects were given a meal containing 10 mg/kg [1-^{13}C] oleic acid and 15 mg/kg [d_{31}] palmitic acid. Subsequent breath tests were collected to measure $^{13}CO_2$; urine samples were collected to measure $^{2}H_20$. While both heavy and light exercise tended

to enhance oleic acid oxidation in the hours following exercise, heavy exercise resulted in significantly greater ($p<0.05$) $^{13}CO_2$ recovery when compared to rest. At 11.5 hours postdose, total cumulative recovery of [1-^{13}C] oleic acid was estimated to be approximately 49%, 40% and 34% following heavy, light and resting protocols, respectively. While the postdose pattern of oleate oxidation in the resting individual reached a plateau at 4 hours, postexercise oleate oxidation peaked at 4 hours postdose following heavy exercise and 6 hours postdose following the light exercise. These results were not consistent with the patterns of post-exercise oxidation of palmitate. The cumulative recovery of d_{13}palmitate following heavy exercise, light exercise and rest ranged from 10-12%, 11.5 hours postdose and did not significantly differ from one another. This study suggests that while overall fatty acid oxidation is upregulated postexercise, dietary monounsaturated fatty acids (versus saturated fatty acids) account for the majority of the observed increase.

As the prevalence of obesity and chronic disease has increased in America's youth, studies examining the benefits of exercise and its effects on the metabolism of dietary fat have begun to emerge in pediatric populations. A recent review investigating the relationship between health, physical fitness, and exercise in children and adolescents considered a number of health indicators in generating an updated recommendation, specific to youth, for daily physical activity type and duration [63]. High blood cholesterol, high blood pressure, the metabolic syndrome, obesity, low bone density, depression, and

injuries in children were all considered as indicators of health in this population. Upon review of the literature, Janssen concluded, "*the more physical activity, the greater the health benefit*", and recommended that children and adolescents ages 5-17 accumulate at least 60 minutes of, primarily aerobic, activity every day [63]. A similar review, based on the relationship between physical activity and all cause mortality, incidence of chronic disease and the dose-response of health outcomes in adults suggested that individuals ages 19-65 accumulate 20-60 minutes (duration dependent on level of effort) to maximize primary prevention of disease [64]. While there does not appear to be a significant difference in muscle composition between prepubertal youth and adults, a difference in anaerobic capacity has been observed; a lower RER is frequently observed in children [65]. Prepubertal youth, who have less anaerobic capacity than adults, rely more heavily on aerobic metabolism during periods of activity [66]. The exercise intensity that causes maximal fat oxidation decreases during development. When compared to adults, prepubertal adolescents have greater reliance on fat as a fuel source during heavy exercise.

Fueling the Heart

Although the degree of fat and carbohydrate oxidation is primarily regulated by intensity and duration of exercise, the heart, brain, skeletal and other muscle systems may independently prefer one fuel to another. The heart requires a constant supply of energy; in the presence of both

carbohydrate and lipid, the heart will utilize fatty acid in preference to glucose [67, 68]. Furthermore, ketone bodies are oxidized in preference to fatty acids by cardiac muscle and may inhibit mobilization of fatty acids from adipose [69-72]. While fasting, up to 90% of myocardial oxygen consumption in the normal myocardium comes from the oxidation of free fatty acids [48-50]. In the resting state, it has also been recognized that the majority of myocardial energy supply comes from FFA oxidation [51]. Myocardial uptake is regulated by arterial substrate concentration and the utilization of substrate by the myocardium may influence energy efficiency [51]. Glucose metabolism alone cannot produce enough energy to maintain optimal contractile function of the myocardium. If the heart shifts to exclusive glucose metabolism, only the minimum amount of energy to survive will be supplied to the heart [48]. Neely and Morgan suggest that the rate of fatty acid uptake and oxidation by aerobic heart muscle is the product of:

1. the availability of exogenous fatty acid
2. (at low rates of energy utilization), the rate of acetyl-CoA oxidation by the citric acid cycle
3. (at high rates of energy utilization), the rate of fatty acid transport into the mitochondria via the carnitine palmitoyl transferase system [67].

Fat Supplementation & Exercise Performance in Healthy Athletes

Historically, maximizing CHO availability has been the primary focus of optimizing endurance performance via nutrition intervention. Endogenous

glucose and glycogen stores are sufficient to fuel submaximal exercise for just a few hours before depletion. In contrast, endogenous fat stores can fuel physical activity for days, providing approximately 60 times the energy storage of glycogen [73]. Therefore, additional fat supplementation during exercise is not generally warranted. Those who argue in favor of fat supplementation, however, claim it may improve exercise performance by sparing endogenous glycogen stores [74]. In the world of ultra-endurance sports, the energy density of lipids and glycogen sparing effects are very appealing as energy expenditure can range from 5,000-18,000 kcals/day [73].

Long-chain (>12C) or medium-chain (8-10C) fatty acids can be ingested, although long-chain fat supplementation has been deemed ineffective for fueling during exercise. Upon ingestion, long-chain fats inhibit gastric emptying and as a result, are slow to reach circulation [75]. Circulating long-chain TGs, captured in CMs, fail to efficiently provide fuel during exercise; however, they may serve to replenish intramuscular TG stores post-exercise [76, 77].

Alternately, medium-chain fats may be a useful exogenous aid during exercise. In contrast to long-chain fats, MCTs rapidly reach systemic circulation following consumption. It has been suggested that by elevating plasma fatty acid levels, MCTs may have a glycogen sparing effect [78] and thus improve exercise performance by delaying the onset of exhaustion [79]. Massicotte et al. reported that MCTs (1.4 kcal/min; 0.16 g/min) may have an "energetic contribution rate" just slightly lower than that of CHO (1.6 kcal/min;

0.4 g/min) during submaximal, prolonged exercise [80]. Jeukendrup et al explored the effects of MCT co-ingested with CHO vs MCT alone on a population of eight trained athletes during moderate intensity (57% VO_{2MAX}), endurance (180 min) exercise [81]. Athletes completed 4 exercise tests in a randomized double-blind fashion, receiving either CHO alone, CHO + MCT, MCT alone, or a high-CHO-MCT suspension. Participants consumed a bolus of supplement prior to exercise and half the original dose every 20 minutes during the exercise protocol. Interestingly, when compared to MCT supplementation alone, it was observed that the rate of MCT oxidation significantly increased when MCTs were coingested with CHO; 71-76% of MCTs consumed were oxidized following CHO-MCT supplementation versus only 33% following MCT supplementation alone. In addition, endogenous fat utilization and FFA concentrations were significantly greater following MCT versus CHO supplementation alone.

Despite evidence that MCTs may compliment CHO-supplementation and provide a readily available source of exogenous energy during exercise, the contribution of MCT toward total energy expenditure is limited. Merely 3-7% of total energy expenditure, or about 30 grams of MCT in 3 hours, can be tolerated before gastrointestinal symptoms arise [81, 82].

Methods of Measurement

Indirect calorimetry. IC can be used to measure energy expenditure during an acute bout of exercise on a treadmill or exercise bike in the lab.

Fuel utilization can also be determined via IC by measuring the volume of carbon dioxide produced (VCO_2) and oxygen consumed (VO_2). Respiratory exchange ratio (RER), or respiratory quotient (RQ), is determined by taking VCO_2/VO_2. RER can be used as an integral part of study designs that target specific carbohydrate, protein or fat oxidation during exercise. A recent collaboration study between three different institutions assessed the validity and reliability of six different gas analysis systems. The Vmax Encore 29 and TrueOne 2400 systems were found to be the most valid instruments when compared to the Deltatrac Metabolic Monitor (DTC), which has historically been regarded as the gold standard in IC but is no longer manufactured [83, 84]. The standard for reliability in these systems was defined as having a within-person coefficient of variance (CV) of ≤ 3%. Independent of the DTC CV, the Vmax and TrueOne systems achieved a within-subject CV of 2.9% and 3.6%, respectively, for RER [83].

Double Product. The product of systolic blood pressure (SBP) and heart rate (HR) can be used as a reliable estimate of myocardial oxygen consumption and workload [85]. In an effort to establish reference data for a pediatric population during exercise, Riopel and colleagues measured HR, BP, double product (DP), and electrocardiographic changes in 288 healthy children and young adults exercised to exhaustion [86]. Participants, ages 4-21, were divided into 4 groups based on body size; the maximum exercise time recorded was 14 minutes for the two groups of smaller size and 13 minutes for the remaining two groups. Changes in double product were similar,

varying a maximum of about 5000 beats*mm Hg^{-1} at 10 minutes into the course of exercise. As could be expected, the largest divergence in DP occurred between the groups with the smallest and largest body surface areas; this divergence was maximized at the last time point collected before termination of exercise when a difference of approximately 10,000 beats*mm Hg^{-1} was observed [86]. According to the data from this study, average DP (including all groups) at rest, 3, 6, 9, and 12 minutes could be estimated to be approximately 10,000, 18,500, 24,000, 29,000, and 32000 beats*mm Hg^{-1}, respectively. Similarly, Christos et al reported double products averaging roughly 11,000 and 27,700 beats*mmHg^{-1} at rest and peak exercise in an adolescent group of healthy controls used for a study assessing heart function in patients post cardiac transplant [87]. Upon cessation of exercise, heart rate resumes its pre-exercise rate within a matter of minutes to hours depending on the nature of the exercise; the most marked reduction, however, occurs within the first few minutes [88]. Assessing post-exercise heart function via echocardiogram could potentially be challenged by the fact that heart rate can be expected to slow by 18 beats/minute following just 1 minute of rest in the supine position [88]. The double product calculation, however, enables researchers to indirectly gauge changes in ejection fraction during exercise upon collection of ambulatory blood pressure and heart rate [89].

Echocardiogram. Echocardiography uses ultrasound techniques to image two or three-dimensional slices of the heart. Non-invasive assessment of cardiac valve areas and function, valvular regurgitation, abnormal

communication between the right and left side of the heart and calculation of cardiac output (CO) and ejection fraction can all be accomplished via the echocardiogram using Doppler ultrasound. Applegate and colleagues used echocardiography pre and post exercise while studying the heart function of patients who had experienced acute myocardial infarction [90]. It was concluded that the sensitivity of 2-D echocardiographic images in detecting exercise-induced ischemia were not compromised, due to slight delay, upon recovery from exercise. Researchers in this study, however, reported initiating the sonograph 30 seconds following cessation of a modified Naughton treadmill test[1] [90].

[1] While the Naughton protocol may be abbreviated in comparison to other methods of exercise stress testing, it does facilitate participants in reaching "peak exercise" via incremental increases in grade; Kapoor and colleagues reported reaching a grade of 17.5% during the last stage of exercise in 9 year old (average age) children [92].

CHAPTER 5

IMPROVED HEART FUNCTION & EXERCISE TOLERANCE IN LCFAODs

Behrend AM[1], Harding C[2], Shoemaker J[3], Matern D[4], Sahn D[5], Elliot D[6], Gillingham MB[1,2]

Oregon Health & Science University

[1]Graduate Programs in Human Nutrition, OHSU

[2]Molecular & Medical Genetics, OHSU

[3]Biochemistry & Molecular Biology, St. Louis University

[4] Biochemical Genetics, Mayo Clinic

[5]Pediatric Cardiology, OHSU

[6]Health Promotion & Sports Medicine, OHSU

Manuscript for the Annals of Pediatric Cardiology

ABSTRACT

Background: Inherited disorders of long-chain fatty acid oxidation (FAO) inhibit the ability to oxidize long-chain fatty acids (LCFAs) for energy generation. As a result, bouts of exercise can lead to rhabdomyolysis, impaired cardiac function, and hence, exercise avoidance in individuals with long-chain FAO disorders. Medium-chain triglyceride (MCT) supplementation may bypass this defect and reduce the risk of adverse metabolic events.

Objectives: To determine the influence of isocaloric MCT vs carbohydrate (CHO) supplementation prior to exercise on substrate oxidation and cardiac function during exercise in participants with long-chain FAO disorders.

Design & Methods: Two 45-minute, moderate intensity treadmill exercise studies were completed by subjects (n=11) in a randomized cross-over design. An isocaloric oral dose of CHO (1 g/kg LBM) or MCT-oil (0.5 g/kg LBM) was administered prior to exercise, hemodynamic and metabolic indices were assessed during exertion and a cardiac echocardiogram was performed following exercise. Exercise tolerance and cardiac function were analyzed using paired T tests.

Results: A statistically significant decrease in respiratory exchange ratio (RER), double product ejection fraction estimation, and steady state heart rate was observed following the exercise test pretreated with MCT.

Conclusions: MCT supplementation prior to exercise in subjects with LCHAD, CPT2 or VLCAD deficiency increased the oxidation of medium chain fats and acutely improved the double product estimate of cardiac ejection fraction for the same amount of work performed when compared to CHO supplementation. Results from this study may guide dietary therapies for individuals with long-chain FAO disorders.

Keywords: LCHADD, long-chain FAO disorders, MCT, exercise

INTRODUCTION

Previous research has suggested that medium chain triglyceride (MCT) supplementation administered prior to exercise improves exercise tolerance in individuals with LCHAD deficiency. Interestingly, a decrease in steady-state heart rate (HR) has also been observed in participants supplemented with MCT oil prior to exercise, suggesting a cardiac benefit [1]. Changes in heart rate are noteworthy due to an increased incidence of cardiomyopathy among patients with long-chain fatty acid oxidation disorders [2-4]. MCT bypasses the block in long-chain fatty acid (LCFA) oxidation and may provide a usable source of fatty acids for both the heart and exercising skeletal muscle. In a preliminary study of MCT affects, no significant difference in respiratory exchange ratio (RER) was observed when orange juice + MCT versus orange juice alone were administered prior to exercise [1]. However, subjects were not given isocaloric supplementation of MCT vs. CHO. In addition, only subjects with LCHADD were included in the initial study; however, due to common symptoms, subjects with other long-chain FAO disorders may also benefit from the development of dietary guidelines to enhance exercise tolerance.

Specific Aims

1. To expand the study population to include subjects with VLCAD, CPT2 and LCHADD in the trial.

•We hypothesized that subjects with VLCAD, CPT2, and LCHADD would respond to pre-exercise MCT supplementation in a similar manner.

2. To determine the effect of isocaloric MCT vs. CHO supplementation prior to exercise on substrate oxidation during exercise in subjects with a long-chain fatty acid oxidation disorder.

• We hypothesized that MCT supplementation prior to exercise in subjects with a long-chain fatty acid oxidation disorder will increase the oxidation of medium chain fats and decrease the oxidation of LCFAs when compared with CHO supplementation.

3. To determine the effect of isocaloric MCT vs. CHO supplementation prior to exercise on cardiac function in subjects with a long-chain fatty acid oxidation disorder.

• We hypothesized that MCT supplementation prior to exercise in subjects with a long-chain fatty acid oxidation disorder will improve cardiac function for the same VO_2 and amount of work performed when compared to CHO supplementation because of the ability of MCT to bypass the long-chain fatty acid oxidation defect and provide fatty acid energy to the heart.

METHODS

Recruitment. Subjects were recruited from previous studies, via announcements on the FAO family support network website or by physician referral. The inclusion criteria involved a confirmed diagnosis of a long-chain fatty acid oxidation disorder including very long-chain acyl-CoA dehydrogenase (VLCAD), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), mitochondrial trifunctional protein (TFP) or carnitine palmitoyltransferase-2 (CPT2) deficiency, seven years of age or older and the ability to comply with the study protocol. Subjects participating in ongoing research projects that altered macronutrient content of the diet were excluded. A member of the study team screened all potential subjects over the phone. Subjects with any endocrine or metabolic disorders that were known to alter body composition (such as diabetes) were excluded. The study was approved by the OHSU Institutional Review Board (OHSU eIRB 817, NCT00654004). Subjects over 18 years of age gave written informed consent to participate. Each participant under 18 years of age was accompanied by a legal guardian who gave written informed consent along with the assent of the young participant.

Study design. Each subject completed the treadmill exercise test two times in a randomized crossover design. The exercise studies were conducted 4 months apart. Subjects were admitted to the inpatient research unit at OHSU for approximately 3 nights for each test. The order in which the pre-exercise supplement was administered was randomized.

Pre-Exercise Supplement & DEXA. Lean body mass (LBM) in kilograms was determined by DEXA prior to treadmill testing. MCT, (8 kcals/gm), was administered prior to exercise testing relative to the amount of LBM measured at 0.5g MCT/kg LBM, and was then added to approximately 12 ounces of a calorie-free beverage. For the CHO supplemented exercise test, subjects consumed a full calorie juice or soda beverage at 1 gm/kg LBM to achieve isocaloric supplementation prior to exercise.

Exercise Testing. Patients began treadmill ergometry two hours after lunch in a postabsorptive state; randomized supplementation was administered 20 minutes prior to exercise. Four months later, subjects repeated treadmill ergometry at the same grade and speed as the first test to keep work performed constant between trials and received the beverage supplement not consumed during the 1st exercise study.

Treadmill testing was performed on an SMC 2000 treadmill (Sensor Medics, Yorba Linda, CA) with continuous ECG monitoring using a Sensor Medics Cardiosoft digital system. Respiratory gases were collected using a mouthpiece or Hans-Rudolph mask and gas exchange was measured using a Sensor Medics VMAX 29 metabolic cart.

After the pre-exercise blood draw, the exercise protocol was performed as follows: 3 minute warm up phase with a slow walk at 1.5 miles per hour at 0% grade followed by increases in rate and incline every two minutes until the subject's heart rate achieved 60-70% of his/her predicted maximum heart rate. Subjects were asked to continue exercising at 60-70% of their predicted

maximum heart rate for an additional 40 minutes after the warm up phase. Predicted maximum heart rate was calculated using the formula: 220 (beats per minute) – age in years. A 12-lead ECG was placed prior to exercise for the continual monitoring of the electrical activity and rate (bpm) of the heart during exercise. Using a blood pressure cuff, BP data was also collected during exercise at least one time during the first 20 minutes and last 20 minutes of the test. Gas exchange was measured continuously; however, in some cases was collected just during the first 15 minutes and the last 15 minutes of exercise, giving the subject a period of rest from wearing the collection apparatus. Heart rate, VO_2 (mL/kg/min) & RER were recorded at one-minute intervals. Heart function during exercise was estimated using double product calculation, (systolic BP x HR), an indirect marker of ejection fraction (EF).

Following exercise and the post-exercise blood draw, a subset of our participants (n=3) underwent echocardiography to directly measure EF.

Subject monitoring for the development of any adverse or unexpected symptoms continued for the remainder of the evening following treadmill ergometry; the protocol also included premature stop criteria if the subject developed any symptoms such as dizziness, severe respiratory distress, chest pain or palpitations, or muscle pain during exercise.

Blood Samples. An indwelling catheter was placed for repeated phlebotomy. Eight ml blood samples were drawn at baseline (time 0), after exercise, and after 20 minutes recovery time. Tetrahydrolipostatin was added

to plasma collected in EDTA to inhibit lipase hydrolysis of free fatty acids ex-vivo. Free fatty acid levels were measured by a commercially available enzymatic colorimetric kit (NEFA-2hr, Wako Chemicals USA, Inc, Richmond, VA). Plasma was collected in sodium heparin and acylcarnitines were measured by electrospray tandem mass spectrometry by the Biochemical Genetics Laboratory at Mayo Clinic [5]. Serum was frozen and lactate, pyruvate, β-hydroxybutyrate and acetoacetic acid were measured by GC/MS in the Laboratory of James Shoemaker, MD, PhD, at St. Louis University. The principal investigator held the code to link data to patient information. Results from the outside labs were reported back to the principal investigator for data analysis.

Statistical Analysis. Blood data, (at pre, post and recovery time-points), and measures of exercise performance were collected during two separate exercise studies with and without MCT oil. Total area under the curve (AUC) was calculated using the trapezoidal method for all parameters. The difference between the total AUC from the MCT and CHO supplemented tests were analyzed by paired t-tests with $p < 0.05$ being considered significant. The treatment effect and change over time during exercise testing was analyzed with a repeated measures (RM) ANOVA. Post-hoc paired t-tests were used to determine individual point differences if the treatment effect was significant. Participants in whom accurate HR and/or RER data was not collected during a given time interval were excluded from the average and

statistical analysis of that time point. All analysis was conducted using Prism Software (Version 5.0, Graphpad, La Jolla, CA).

RESULTS

Demographics. All subjects completed both arms of the trial and contributed to the results reported. Eight patients, (average age = 12 yrs), had a diagnosis (dx) of LCHADD; seven of these subjects were from the continental US, 1 traveled from Canada. Three local participants, (average age = 25 yrs), with CPT2 (n=2) and VLCAD (n=1) also participated in this study. Overall, more males (n=7) than females (n=4) were included; all but 1 of the LCHADD participants were male.

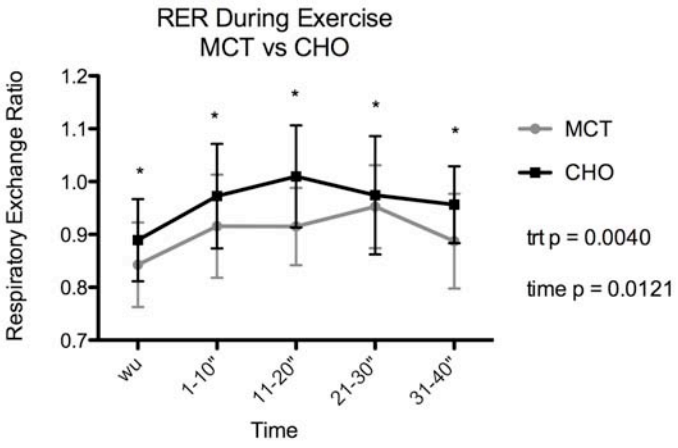
Table 1. Participant Characteristics

Subject	Dx	Age (yrs)	Sex	MCT @ 1st admit	CHO @ 1st admit	Location
1	VLCAD	20	F		√	Oregon
2	LCHAD	17	M		√	California
3	LCHAD	14	M	√		Utah
4	LCHAD	16	M		√	California
5	LCHAD	7	M	√		Conneticut
6	CPT2	37	F		√	Oregon
7	LCHAD	7	M	√		Washington
8	LCHAD	16	F	√		Wisconsin
9	LCHAD	9	M	√		New York
10	CPT2	17	F	√		Oregon
11	LCHAD	8	M		√	Canada

Substrate Oxidation Data

Respiratory Exchange Ratio. Total area under the curve (TAUC) for the MCT supplemented trial (3.648) was significantly lower than TAUC for the CHO supplemented trial (3.879). There was a significant MCT effect at baseline that increased over the course of the exercise trial [Fig 1 RM ANOVA: treatment (trt) p = 0.012, time p = 0.004]. The lower RER following MCT supplementation suggests subjects oxidized more fat during that bout of exercise than following CHO supplementation. An average difference of 0.046, 0.057, 0.0948, 0.022, and 0.07 in RER between exercise tests was observed during warm up, minutes 1-10, 11-20, 21-30 and 31-40 respectively.

Fig 2. Change in respiratory exchange ratio with and without MCT

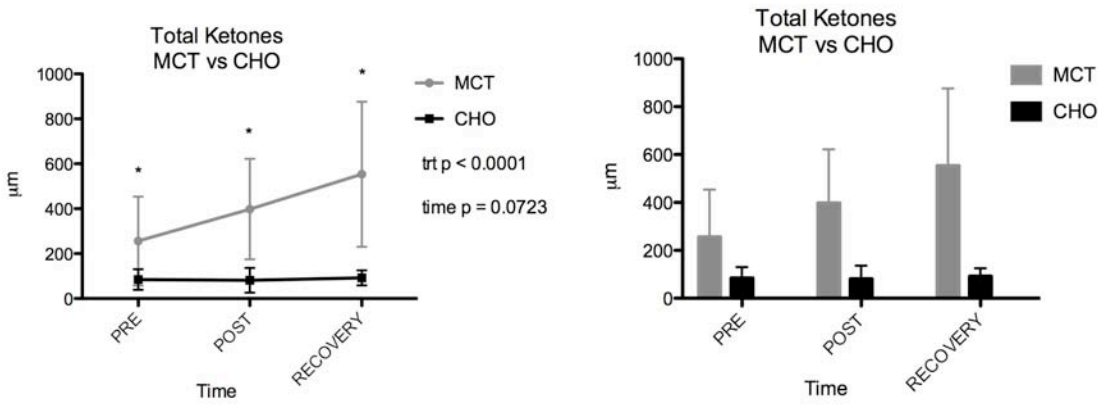


Oxygen Utilization. Because work performed was kept constant between trials, $V0_2$ in mL/kg/min did not significantly differ following MCT

(TAUC = 56.72) vs CHO (TAUC = 48.34) supplementation. While treatment did not have a significant affect on $V0_2$, duration of exercise did cause a significant increase in $V0_2$ over time (RM ANOVA: trt p = 0.0805 , time p < 0.0001).

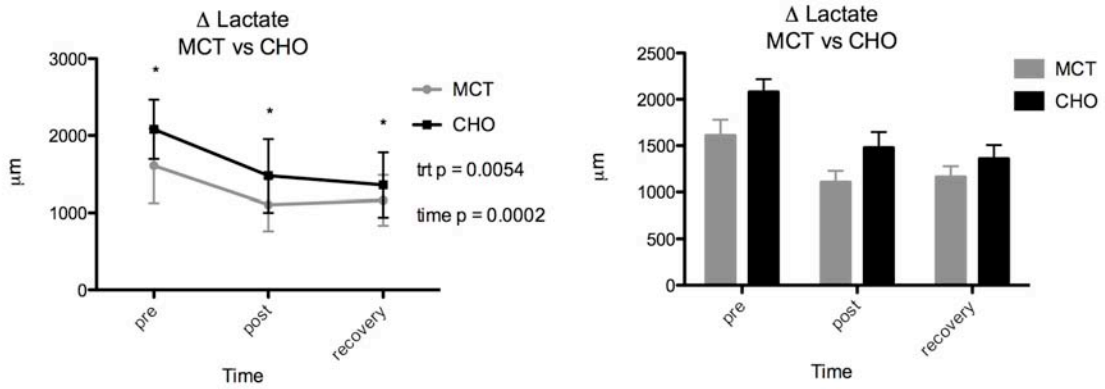
Ketones. TAUC for total serum ketone bodies (β-OH butyric acid and acetoacetic acid) was significantly higher when subjects received MCT supplementation (TAUC = 802.8) prior to exercise versus pre-supplementation with CHO alone (TAUC = 169.8). The significant treatment effect was observed even prior to exercise (RM ANOVA: trt p < 0.0001), and there was no significant effect of time (RM ANOVA: time p= 0.0723). The average difference between interventions, measured in micromoles, at pre, post and recovery time points was 171.2, 316.8 and 461.1 respectively.

Fig 3. Change in total serum ketones during exercise with and without MCT



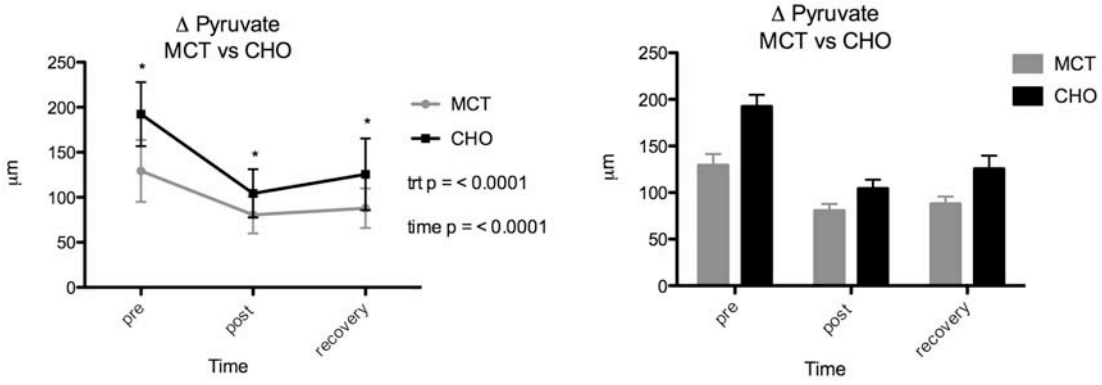
Lactate. TAUC was significantly lower when subjects received MCT supplementation (TAUC = 2491) prior to exercise versus CHO alone (TAUC = 3198). The magnitude of the treatment effect decreased as duration of exercise increased (RM ANOVA: trt p = 0.0054, time p = 0.0002); plasma lactate dropped significantly during exercise, and plateaued upon recovery. The average difference, measured in micromoles, at pre, post and recovery time points was 471.92, 372.46 and 196.90 respectively.

Fig 4. Change in lactate during exercise with and without MCT



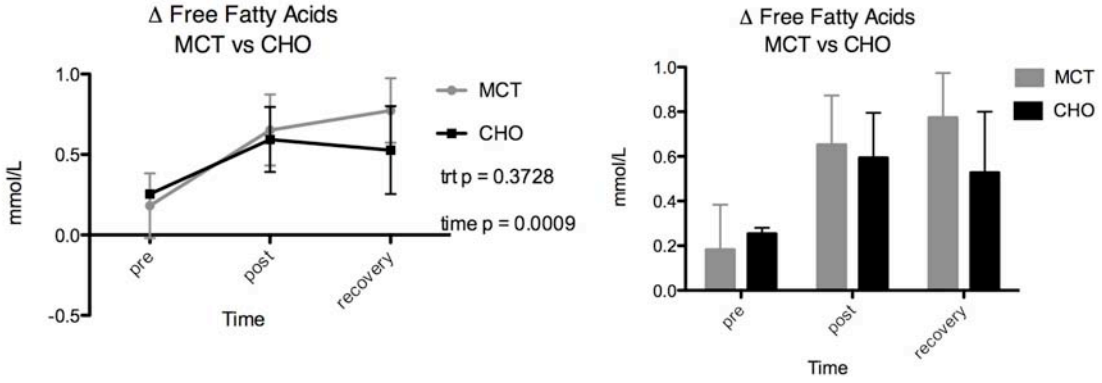
Pyruvate. TAUC was significantly lower when subjects received MCT supplementation (TAUC = 189.1) prior to exercise versus CHO alone (TAUC = 263.3). The magnitude of the treatment effect decreased as duration of exercise increased (RM ANOVA: trt p < 0.0001, time p < 0.0001); plasma pyruvate dropped significantly during exercise, and rose again slightly upon recovery. The average difference, measured in micromoles, at pre, post and recovery time points was 63.11, 23.79 and 37.62 respectively.

Fig 5. Change in pyruvate during exercise with and without MCT



Free Fatty Acids. TAUC did not differ significantly with respect to plasma FFAs in response to MCT (TAUC = 1.130) versus CHO (TAUC = 0.9841) pre-supplementation. While treatment did not significantly affect fatty acid mobilization, duration of exercise did cause a significant rise in FFAs during both exercise trials (RM ANOVA: trt p = 0.3728, time p = 0.0009).

Fig 6. Change in FFA during exercise with and without MCT



Hydroxyacylcarnitines. TAUC for sum hydroxyacylcarnitines following pre-exercise supplementation with MCT (TAUC = 1.929) was not significantly different than TAUC following pre-supplementation with CHO alone (TAUC = 2.169). Neither treatment nor time significantly affected sum hydroxyacylcarnitines in our subjects with LCHADD (RM ANOVA: trt p = 0.493, time p = 0.802). Despite significant changes in RER, the data suggests that in the presence of MCT, overall β-oxidation of LCFAs and the formation of hydroxyacylcarnitines remains unchanged (table 2).

Figure 7. Change in hydroxyacylcarnitines during exercise with and without MCT

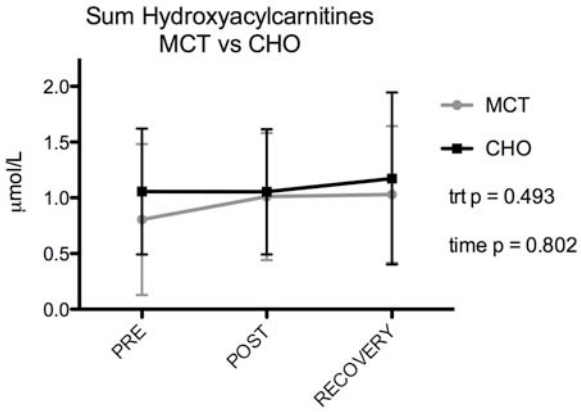


Table 2. Mean changes in hydroxyacylcarnitines during exercise

	Sum Hydroxyacylcarnitines* (μmol/L)				
	MCT		CHO		MCT vs CHO
	Mean	+/- SD	Mean	+/- SD	paired t test
Pre	0.805	0.677	1.057	0.565	p = 0.202
Post	1.011	0.570	1.054	0.561	p = 0.382
Recovery	1.030	0.613	1.173	0.771	p = 0.236

*C12:1-0H+C12-0H+C14:1-0H+C14-0H+C16:1-0H+C16-0H+C18:2-0H+C18:1-0H+C18-0H

Creatine Kinase. All subjects fell within normal limits (40-500 U/L) with the exception of 1 subject who exceeded this range during one of the two admits. Furthermore, there was not a significant difference in serum CK levels between interventions (TAUC MCT = 358.7, CHO = 530.6; RM ANOVA trt p = 0.3366, time p = 0.9785).

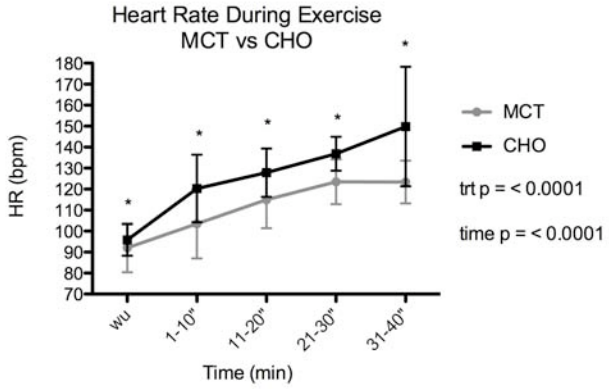
Table 3. Change in creatine kinase levels during exercise with and without MCT

	Pre	Post	Recovery
MCT	165.2 +/- 235.1	181.9 +/- 242.5	188.3 +/- 249.0
CHO	254.1 +/- 356.8	265.4 +/- 369.8	276.2 +/- 392.5
Paired t-test: p	0.254	0.287	0.258

Heart Function Data

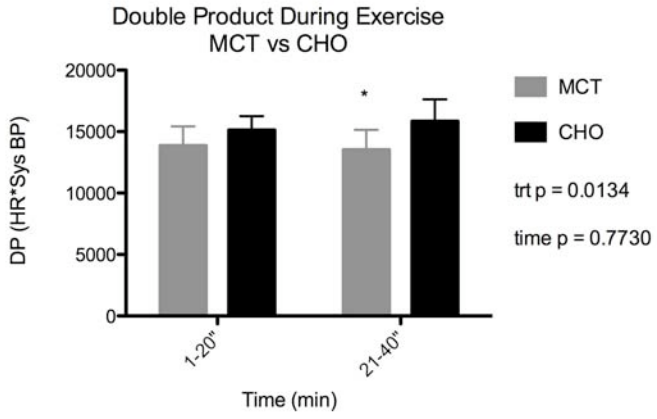
Heart Rate. Heart rate was significantly lower during the exercise regimen preceded by MCT supplementation (TAUC = 449.7) when compared to CHO alone (TAUC = 507.9; RM ANOVA: trt p < 0.0001). A statistically significant difference was also observed between time-points (RM ANOVA: time p < 0.0001); as depicted in Fig 8, the magnitude of the difference in HR increased as duration of exercise increased.

Fig 8. Change in steady-state HR with and without MCT



Double Product. A significant difference in systolic BP was not observed between trials; therefore, as a result of the drop in HR, a significantly lower DP resulted when subjects were supplemented with MCT versus CHO alone (RM ANOVA trt p = 0.0134). Duration of exercise did not significantly affect DP (RM ANOVA time p = 0.7730). An average difference of 1265.67 in DP was observed during the first half of exercise; during the second half of exercise DP differed by 2311 between exercise protocols.

Fig 9. Change in double product estimate with and without MCT



Echocardiogram. Results from echocardiography were limited by quality and sample size (n=3); however, in the small subset of patients tested, there was no difference in EF post exercise. Heart rates in all participants had returned to resting upon initiation of the echo.

DISCUSSION

Subjects with long-chain FAO disorders have recurrent symptoms that limit activities of daily living, such as rhabdomyolysis with elevated creatine kinase levels, often induced by exercise, and cardiomyopathy [6-8]. As a result, many of these patients avoid exercise and adopt a relatively sedentary lifestyle. The current exercise study was designed to explore energy metabolism during exercise and heart function in response to pre-exercise supplementation with MCT versus CHO alone.

As expected, plasma FFA concentrations rose during both exercise tests. While defects in long-chain β-oxidation diminish the use of long-chain fatty acids for energy, the data suggests that lypolysis and mobilization of fatty acids occur normally in this population independent of exercise pre-supplementation. The significant decrease in RER following MCT supplementation suggests a change in substrate oxidation with an increase in FAO and decrease in CHO oxidation. While the RER suggests a whole body increase in FAO, it does not differentiate between medium and long-chain FAO. The significant increase in ketone production, in subjects who do not usually produce ketones, however, suggests that the liver is able to oxidize MCT and produce ketones in the presence of MCT. The decrease in glycolytic intermediates, namely pyruvate and lactate, suggests that MCT decreases the oxidation of CHO and may lower the risk of lactic acidosis in these patients. With an alternate FA substrate, such as octanoic (C8) and decanoic (C10) acid, total oxidation of fat increased, but did not affect the

rate of partial LCFA β-oxidation and production of acyl and hydroxyacylcarnitines in our study. These findings differ from the previous trial, which demonstrated a reduction in plasma OH-acylcarnitines upon recovery from exercise in participants with LCHADD, suggesting that total LCFA oxidation was suppressed following MCT supplementation [1]. A difference, however, between our current study and the preliminary research was that the metabolic control of the participants in our study was significantly better than those included in the previous study. In the current study, the sum of the hydroxyacylcarnitines (LCHADD population only) averaged 0.95 and 1.1 following MCT and CHO presupplementation, respectively. In contrast, during the preliminary trial, sum hydroxyacylcarnitines were significantly higher (paired T-tests: $p < 0.01$), averaging approximately 1.8 μmol/L and 2.4 μmol/L following MCT and CHO pre-supplementation, respectively. It is possible that because our subjects were already in excellent metabolic control, the sum of the hydroxyacylcarnitines could not be further improved upon. Both the preliminary and present study, however, demonstrate the ability of MCTs to change substrate oxidation during exercise by bypassing the defect in LCFA oxidation and increasing the oxidation of medium-chain fatty acids.

Findings suggest that the change in substrate oxidation may be associated with an increase in cardiac output during submaximal exercise. The significant decrease in heart rate and double product for the same VO_2

and work performed suggests that EF during exercise improves when patients receive pre-exercise MCT supplementation.

The heart requires a constant supply of energy; in the presence of both carbohydrate and lipid, the heart will utilize fatty acid in preference to glucose [67, 68]. Furthermore, ketone bodies are oxidized in preference to fatty acids by cardiac muscle [69-72]. We propose that MCT may have facilitated optimal contractile function by increasing the preferred substrate of the myocardium, circulating ketone bodies. By altering substrate oxidation and decreasing heart rate in subjects with long-chain FAO disorders, an active and healthy lifestyle can be facilitated. The implications of improved cardiac function are invaluable in this population and may have the potential to decrease future risks of cardiomyopathy.

A similar change in HR has not been reported in healthy athletes given MCT prior to exercise [10, 11]. We suspect this finding is unique to subjects with a long-chain FAO disorder. We did not observe a change in EF by echocardiogram after exercise; however, HR had already returned to pre-exercise resting rate before the echocardiograms were performed. Echocardiography may be insensitive to the changes that occurred, or this may imply that increased workload exceeds the oxidative capacity of the heart despite adequate substrate oxidation during rest. We propose that MCT may expand the usable energy supply, such as ketone bodies, and thus improve the oxidative capacity of the heart, primarily during a period of increased workload.

Alternatively, the lower HR with MCT may represent decreased O_2 demand and blood flow with increased FAO because less O_2 is required to burn fat than CHO, however, we did not measure AVO_2 difference to directly test this hypothesis. While the data supports increased EF during exercise, decreased O_2 extraction with increased FAO may have also contributed to decreased HR.

The changes in substrate oxidation were observed even prior to the initiation of exercise. Thus, MCT given at rest or prior to exercise can rapidly alter energy metabolism in patients with long-chain FAO disorders. We propose that the benefit of MCT supplementation on heart rate will be greatest when timed with periods of activity rather than during periods of rest.

In conclusion, MCT supplementation prior to exercise altered substrate oxidation and decreased heart rate during that bout of exercise. We believe this effect is unique to subjects with an inherited defect in long-chain FAO and would not be observed in otherwise healthy individuals. In practice, MCT supplementation of approximately 0.3-0.4 gm/kg total body weight mixed with a CHO containing beverage will allow subjects with a long-chain FAO disorder to safely exercise at a moderate intensity for up to an hour.

References

1. Gillingham MB, Scott B, Elliott D, Harding CO. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2006;89:58-63.

2. Bonnet D, Martin D, Pascale De L, et al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation.* 1999;100:2248-2253.

3. Moore R, Glasgow JF, Bingham MA, et al. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency--diagnosis, plasma carnitine fractions and management in a further patient. *Eur J Pediatr.* 1993;152:433-436.

4. Saudubray JM, Martin D, de Lonlay P, et al. Recognition and management of fatty acid oxidation defects: A series of 107 patients. *J Inherit Metab Dis.* 1999;22:488-502.

5. Gillingham MB, Connor WE, Matern D, et al. Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Mol Genet Metab.* 2003;79:114-123.

6. Schaefer J, Jackson S, Dick DJ, Turnbull DM. Trifunctional enzyme deficiency: Adult presentation of a usually fatal beta-oxidation defect. *Ann Neurol.* 1996;40:597-602.

7. Vici CD, Burlina AB, Bertini E, et al. Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Pediatr.* 1991;118:744-746.

8. den Boer ME, Wanders RJ, Morris AA, IJlst L, Heymans HS, Wijburg FA. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Clinical presentation and follow-up of 50 patients. *Pediatrics.* 2002;109:99-104.

9. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol.* 1974;36:413-459.

10. Jeukendrup AE, Thielen JJ, Wagenmakers AJ, Brouns F, Saris WH. Effect of medium-chain triacylglycerol and carbohydrate ingestion during exercise on substrate utilization and subsequent cycling performance. *Am J Clin Nutr.* 1998;67:397-404.

11. Jeukendrup AE, Saris WH, Schrauwen P, Brouns F, Wagenmakers AJ. Metabolic availability of medium-chain triglycerides coingested with carbohydrates during prolonged exercise. *J Appl Physiol.* 1995;79:756-762.

CHAPTER 6: CONCLUSIONS

We expected to observe normal lipid mobilization following both MCT and CHO supplementation. FFAs were therefore not expected to differ significantly between exercise trials. A decrease was expected in hydroxyacylcarnitines among LCHADD participants in response to MCT supplementation versus supplementation with CHO alone. We confirmed that lipid mobilization occurs normally in this population; therefore this hypothesis was accepted. However hydroxyacylcarnitines remained unchanged between the CHO and MCT trials suggesting that there was no difference in long-chain FAO; thus, this hypothesis was rejected.

We expected lactate and pyruvate production post-exercise to be suppressed by MCT supplementation when compared to CHO alone. Alternately, β-hydroxybutyrate and acetoacetic acid concentrations were expected to increase in response to MCT versus CHO supplementation. The significant decrease in glycolytic intermediates, increase in ketone production and decrease in RER following MCT pre-supplementation suggests that MCT was preferentially oxidized over CHO during moderate intensity exercise and this hypothesis was accepted.

While VO_2/kg was kept constant for a given workload, RER and heart rate were expected to decrease in response to MCT supplementation compared to the alternate exercise trial with CHO alone. This hypothesis was

also accepted, suggesting that overall exercise performance improves following MCT supplementation in this population.

While an observed decrease in heart rate during MCT-supplemented exercise testing may be indicative of an increased ejection fraction, change in heart rate is also related to efficiency of oxygen delivery. Rate of blood flow due to its degree of oxygenation and the arteriovenous oxygen, (A-V 0_2), difference (rate of oxygen extraction by tissue) may also affect oxygen delivery [91]. We anticipated ejection fraction would improve in the presence of a fatty acid substrate; thus, double product estimation, a measure of the workload of the heart, was expected to decrease following MCT versus CHO supplementation. This hypothesis was accepted upon observation of significantly lower double product in response to MCT treatment.

Measured ejection fraction via echocardiogram was expected to increase in the MCT-supplemented intervention; however, the results from echocardiography in our small subset of participants were not different. Echos were completed post-exercise, but after HR had returned to baseline. We suspect the reason we saw no change in echo could be related to 1) the quality of the images and/or 2) a return to resting HR. Therefore, we cannot confirm or reject this hypothesis at this time.

References

1. Gillingham MB, Scott B, Elliott D, Harding CO. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2006;89:58-63.

2. Bonnet D, Martin D, Pascale De L, et al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation.* 1999;100:2248-2253.

3. Moore R, Glasgow JF, Bingham MA, et al. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency--diagnosis, plasma carnitine fractions and management in a further patient. *Eur J Pediatr.* 1993;152:433-436.

4. Saudubray JM, Martin D, de Lonlay P, et al. Recognition and management of fatty acid oxidation defects: A series of 107 patients. *J Inherit Metab Dis.* 1999;22:488-502.

5. Mahan LK. Nutrition for exercise and sports performance. In: Escott-Stump S., ed. *Krause's Food, Nutrition & Diet Therapy.* USA: Saunders; 2004:616-620.

6. Hudgins LC, Seidman CE, Diakun J, Hirsch J. Human fatty acid synthesis is reduced after the substitution of dietary starch for sugar. *Am J Clin Nutr.* 1998;67:631-639.

7. Borensztajn J, Getz GS, Kotlar TJ. Uptake of chylomicron remnants by the liver: Further evidence for the modulating role of phospholipids. *J Lipid Res.* 1988;29:1087-1096.

8. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 1986;232:34-47.

9. Holloway GP, Bezaire V, Heigenhauser GJ, et al. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol.* 2006;571:201-210.

10. Borg G, Linderholm H. Exercise performance and perceived exertion in patients with coronary insufficiency, arterial hypertension and vasoregulatory asthenia. *Acta Med Scand.* 1970;187:17-26.

11. Vianey-Saban C, Divry P, Brivet M, et al. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: Clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta.* 1998;269:43-62.

12. den Boer ME, Wanders RJ, Morris AA, IJlst L, Heymans HS, Wijburg FA. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Clinical presentation and follow-up of 50 patients. *Pediatrics.* 2002;109:99-104.

13. Schaefer J, Jackson S, Dick DJ, Turnbull DM. Trifunctional enzyme deficiency: Adult presentation of a usually fatal beta-oxidation defect. *Ann Neurol.* 1996;40:597-602.

14. Vici CD, Burlina AB, Bertini E, et al. Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Pediatr.* 1991;118:744-746.

15. Glasgow AM, Engel AG, Bier DM, et al. Hypoglycemia, hepatic dysfunction, muscle weakness, cardiomyopathy, free carnitine deficiency and

long-chain acylcarnitine excess responsive to medium chain triglyceride diet. *Pediatr Res.* 1983;17:319-326.

16. Tyni T, Majander A, Kalimo H, Rapola J, Pihko H. Pathology of skeletal muscle and impaired respiratory chain function in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency with the G1528C mutation. *Neuromuscul Disord.* 1996;6:327-337.

17. Tyni T, Kivela T, Lappi M, Summanen P, Nikoskelainen E, Pihko H. Ophthalmologic findings in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation: A new type of hereditary metabolic chorioretinopathy. *Ophthalmology.* 1998;105:810-824.

18. Gillingham M, Van Calcar S, Ney D, Wolff J, Harding C. Dietary management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). A case report and survey. *J Inherit Metab Dis.* 1999;22:123-131.

19. Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC. Body-composition assessment in infancy: Air-displacement plethysmography compared with a reference 4-compartment model. *Am J Clin Nutr.* 2007;85:90-95.

20. Gillingham MB, Connor WE, Matern D, et al. Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Mol Genet Metab.* 2003;79:114-123.

21. Duran M, Wanders RJ, de Jager JP, et al. 3-hydroxydicarboxylic aciduria due to long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency

associated with sudden neonatal death: Protective effect of medium-chain triglyceride treatment. *Eur J Pediatr.* 1991;150:190-195.

22. Jackson S, Bartlett K, Land J, et al. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Res.* 1991;29:406-411.

23. Pons R, Roig M, Riudor E, et al. The clinical spectrum of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatr Neurol.* 1996;14:236-243.

24. Shen JJ, Matern D, Millington DS, et al. Acylcarnitines in fibroblasts of patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and other fatty acid oxidation disorders. *J Inherit Metab Dis.* 2000;23:27-44.

25. Winter SC. Treatment of carnitine deficiency. *J Inherit Metab Dis.* 2003;26:171-180.

26. Shapira Y, Gutman A. Muscle carnitine deficiency in patients using valproic acid. *J Pediatr.* 1991;118:646-649.

27. Pons R, De Vivo DC. Primary and secondary carnitine deficiency syndromes. *J Child Neurol.* 1995;10 Suppl 2:S8-24.

28. Colonna P, Iliceto S. Myocardial infarction and left ventricular remodeling: Results of the CEDIM trial. carnitine ecocardiografia digitalizzata infarto miocardico. *Am Heart J.* 2000;139:S124-30.

29. Helton E, Darragh R, Francis P, et al. Metabolic aspects of myocardial disease and a role for L-carnitine in the treatment of childhood cardiomyopathy. *Pediatrics.* 2000;105:1260-1270.

30. Lango R, Smolenski RT, Narkiewicz M, Suchorzewska J, Lysiak-Szydlowska W. Influence of L-carnitine and its derivatives on myocardial metabolism and function in ischemic heart disease and during cardiopulmonary bypass. *Cardiovasc Res.* 2001;51:21-29.

31. Spiekerkoetter U, Lindner M, Santer R, et al. Treatment recommendations in long-chain fatty acid oxidation defects: Consensus from a workshop. *J Inherit Metab Dis.* 2009;32:498-505.

32. Primassin S, Ter Veld F, Mayatepek E, Spiekerkoetter U. Carnitine supplementation induces acylcarnitine production in tissues of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. *Pediatr Res.* 2008;63:632-637.

33. Elsayed EF, Reilly RF. Rhabdomyolysis: A review, with emphasis on the pediatric population. *Pediatr Nephrol.* 2009.

34. Kim JJ, Miura R. Acyl-CoA dehydrogenases and acyl-CoA oxidases. structural basis for mechanistic similarities and differences. *Eur J Biochem.* 2004;271:483-493.

35. Burtis CA AE, ed. *Tietz Textbook of Clinical Chemistry.* Philadelphia: WB Saunders Company; 1999.

36. Mannix R, Tan ML, Wright R, Baskin M. Acute pediatric rhabdomyolysis: Causes and rates of renal failure. *Pediatrics.* 2006;118:2119-2125.

37. Ward MM. Factors predictive of acute renal failure in rhabdomyolysis. *Arch Intern Med.* 1988;148:1553-1557.

38. Holt S, Moore K. Pathogenesis of renal failure in rhabdomyolysis: The role of myoglobin. *Exp Nephrol.* 2000;8:72-76.

39. Watanabe T. Rhabdomyolysis and acute renal failure in children. *Pediatr Nephrol.* 2001;16:1072-1075.

40. Gonzalez D. Crush syndrome. *Crit Care Med.* 2005;33:S34-41.

41. Bergmann SR, Herrero P, Sciacca R, et al. Characterization of altered myocardial fatty acid metabolism in patients with inherited cardiomyopathy. *J Inherit Metab Dis.* 2001;24:657-674.

42. Eriksson BO, Karlsson J, Saltin B. Muscle metabolites during exercise in pubertal boys. *Acta Paediatr Scand Suppl.* 1971;217:154-157.

43. Berg A, Kim SS, Keul J. Skeletal muscle enzyme activities in healthy young subjects. *Int J Sports Med.* 1986;7:236-239.

44. Kaczor JJ, Ziolkowski W, Popinigis J, Tarnopolsky MA. Anaerobic and aerobic enzyme activities in human skeletal muscle from children and adults. *Pediatr Res.* 2005;57:331-335.

45. Olpin SE. Implications of impaired ketogenesis in fatty acid oxidation disorders. *Prostaglandins Leukot Essent Fatty Acids.* 2004;70:293-308.

46. Rozance PJ, Hay WW,Jr. Describing hypoglycemia--definition or operational threshold? *Early Hum Dev.* 2010;86:275-280.

47. Bier DM, Leake RD, Haymond MW, et al. Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes.* 1977;26:1016-1023.

48. Yoshinaga K, Tamaki N. Imaging myocardial metabolism. *Curr Opin Biotechnol.* 2007;18:52-59.

49. Tamaki N, Morita K, Kuge Y, Tsukamoto E. The role of fatty acids in cardiac imaging. *J Nucl Med.* 2000;41:1525-1534.

50. Wisneski JA, Gertz EW, Neese RA, Mayr M. Myocardial metabolism of free fatty acids. studies with 14C-labeled substrates in humans. *J Clin Invest.* 1987;79:359-366.

51. Bergman BC, Tsvetkova T, Lowes B, Wolfel EE. Myocardial FFA metabolism during rest and atrial pacing in humans. *Am J Physiol Endocrinol Metab.* 2009;296:E358-66.

52. Corr PB, Yamada KA. Selected metabolic alterations in the ischemic heart and their contributions to arrhythmogenesis. *Herz.* 1995;20:156-168.

53. Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. *J Clin Invest.* 2002;110:259-269.

54. Gillingham MB, Purnell JQ, Jordan J, Stadler D, Haqq AM, Harding CO. Effects of higher dietary protein intake on energy balance and metabolic control in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2007;90:64-69.

55. Overweight, obesity, and health risk. national task force on the prevention and treatment of obesity. *Arch Intern Med.* 2000;160:898-904.

56. Froberg K, Pedersen PK. Sex differences in endurance capacity and metabolic response to prolonged, heavy exercise. *Eur J Appl Physiol Occup Physiol.* 1984;52:446-450.

57. Tarnopolsky MA. Gender differences in substrate metabolism during endurance exercise. *Can J Appl Physiol.* 2000;25:312-327.

58. Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA. Prior exercise increases subsequent utilization of dietary fat. *Med Sci Sports Exerc.* 2002;34:1757-1765.

59. Romijn JA, Coyle EF, Sidossis LS, Rosenblatt J, Wolfe RR. Substrate metabolism during different exercise intensities in endurance-trained women. *J Appl Physiol.* 2000;88:1707-1714.

60. Achten J, Jeukendrup AE. Maximal fat oxidation during exercise in trained men. *Int J Sports Med.* 2003;24:603-608.

61. Jeukendrup AE. Modulation of carbohydrate and fat utilization by diet, exercise and environment. *Biochem Soc Trans.* 2003;31:1270-1273.

62. Carey AL, Staudacher HM, Cummings NK, et al. Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise. *J Appl Physiol.* 2001;91:115-122.

63. Janssen I, Leblanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. *Int J Behav Nutr Phys Act.* 2010;7:40.

64. Kesaniemi A, Riddoch CJ, Reeder B, Blair SN, Sorensen TI. Advancing the future of physical activity guidelines in canada: An independent expert panel interpretation of the evidence. *Int J Behav Nutr Phys Act.* 2010;7:41.

65. Montoye HJ. Age and oxygen utilization during submaximal treadmill exercise in males. *J Gerontol.* 1982;37:396-402.

66. Bell RD, MacDougall JD, Billeter R, Howald H. Muscle fiber types and morphometric analysis of skeletal msucle in six-year-old children. *Med Sci Sports Exerc.* 1980;12:28-31.

67. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol.* 1974;36:413-459.

68. Oey NA, den Boer ME, Wijburg FA, et al. Long-chain fatty acid oxidation during early human development. *Pediatr Res.* 2005;57:755-759.

69. Forsey RG, Reid K, Brosnan JT. Competition between fatty acids and carbohydrate or ketone bodies as metabolic fuels for the isolated perfused heart. *Can J Physiol Pharmacol.* 1987;65:401-406.

70. Green A, Newsholme EA. Sensitivity of glucose uptake and lipolysis of white adipocytes of the rat to insulin and effects of some metabolites. *Biochem J.* 1979;180:365-370.

71. Little JR, Goto M, Spitzer JJ. Effect of ketones on metabolism of FFA by dog myocardium and skeletal muscle in vivo. *Am J Physiol.* 1970;219:1458-1463.

72. Olson RE. Effect of pyruvate and acetoacetate on the metabolism of fatty acids by the perfused rat heart. *Nature.* 1962;195:597-599.

73. Ferreira A, Barbosa P, Ceddia R. The influence of medium-chain triglycerides supplementation in ultra-endurance exercise performance. *Rev Bras Med Esporte.* 2003;9:420-425.

74. Jeukendrup AE, Saris WH, Wagenmakers AJ. Fat metabolism during exercise: A review--part III: Effects of nutritional interventions. *Int J Sports Med.* 1998;19:371-379.

75. Hunt JN, Knox MT. Regulation of gastric emptying. In: *Handbook of Physiology.* American Physiology Society; 1968:1917-1935.

76. Kiens B, Lithell H. Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. *J Clin Invest.* 1989;83:558-564.

77. Oscai LB, Essig DA, Palmer WK. Lipase regulation of muscle triglyceride hydrolysis. *J Appl Physiol.* 1990;69:1571-1577.

78. Van Zyl CG, Lambert EV, Hawley JA, Noakes TD, Dennis SC. Effects of medium-chain triglyceride ingestion on fuel metabolism and cycling performance. *J Appl Physiol.* 1996;80:2217-2225.

79. Vukovich MD, Costill DL, Hickey MS, Trappe SW, Cole KJ, Fink WJ. Effect of fat emulsion infusion and fat feeding on muscle glycogen utilization during cycle exercise. *J Appl Physiol.* 1993;75:1513-1518.

80. Massicotte D, Peronnet F, Brisson GR, Hillaire-Marcel C. Oxidation of exogenous medium-chain free fatty acids during prolonged exercise: Comparison with glucose. *J Appl Physiol.* 1992;73:1334-1339.

81. Jeukendrup AE, Saris WH, Schrauwen P, Brouns F, Wagenmakers AJ. Metabolic availability of medium-chain triglycerides coingested with carbohydrates during prolonged exercise. *J Appl Physiol.* 1995;79:756-762.

82. Goedecke JH, Clark VR, Noakes TD, Lambert EV. The effects of medium-chain triacylglycerol and carbohydrate ingestion on ultra-endurance exercise performance. *Int J Sport Nutr Exerc Metab.* 2005;15:15-27.

83. Cooper JA, Watras AC, O'Brien MJ, et al. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J Am Diet Assoc.* 2009;109:128-132.

84. Alam DS, Hulshof PJ, Roordink D, et al. Validity and reproducibility of resting metabolic rate measurements in rural bangladeshi women: Comparison of measurements obtained by medgem and by deltatrac device. *Eur J Clin Nutr.* 2005;59:651-657.

85. Hermida RC, Fernandez JR, Ayala DE, Mojon A, Alonso I, Smolensky M. Circadian rhythm of double (rate-pressure) product in healthy normotensive young subjects. *Chronobiol Int.* 2001;18:475-489.

86. Riopel DA, Taylor AB, Hohn AR. Blood pressure, heart rate, pressure-rate product and electrocardiographic changes in healthy children during treadmill exercise. *Am J Cardiol.* 1979;44:697-704.

87. Christos SC, Katch V, Crowley DC, Eakin BL, Lindauer AL, Beekman RH. Hemodynamic responses to upright exercise of adolescent cardiac transplant recipients. *J Pediatr.* 1992;121:312-316.

88. Miller TD. Exercise treadmill test: Estimating cardiovascular prognosis. *Cleve Clin J Med.* 2008;75:424-430.

89. Balu V, Szmedra L, Dean D, Bhayana J. Stress test evaluation of patients with poor left ventricular function before and after coronary artery bypass surgery. *Tex Heart Inst J.* 1986;13:109-112.

90. Applegate RJ, Dell'Italia LJ, Crawford MH. Usefulness of two-dimensional echocardiography during low-level exercise testing early after uncomplicated acute myocardial infarction. *Am J Cardiol.* 1987;60:10-14.

91. Buchheit M, Laursen PB, Ahmaidi S. Effect of prior exercise on pulmonary O2 uptake and estimated muscle capillary blood flow kinetics during moderate-intensity field running in men. *J Appl Physiol.* 2009;107:460-470.

92. Kapoor RK, Kumar A, Chandra M, Misra PK, Sharma B, Awasthi S. Cardiovascular responses to treadmill exercise testing in anemia. *Indian Pediatr.* 1997;34:607-612.

APPENDIX A: SAFETY & ADVERSE EVENTS

The risk of exercise-induced muscle cramping and rhabdomyolysis is very prevalent among patients with LCHADD; therefore, the intensity of the exercise protocol was limited to minimize this risk. Subjects were monitored closely to prevent the development of muscle cramps and testing was to be terminated if symptoms appeared. Pre- and post-exercise, degree of rhabdomyolysis was evaluated using the serum creatine kinase marker. The study subject was to be treated with bed rest, oral hydration and high CHO-containing electrolyte solutions or intravenous fluids should severe cramping or evidence of rhabdomyolysis (CK > 50,000 U/L) develop. Study subjects completed a total of eighteen exercise protocols during preliminary studies without developing any symptoms of rhabdomyolysis. Two participants, each during one admit, did report some muscle discomfort following exercise testing. However, no changes in vitals or CKs were observed in these individuals. One subject had CKs that reached 1357 U/L upon recovery from exercise; however, this participant did not report any unusual muscle discomfort nor were CKs in this range unusual for this particular individual who was a competitive athlete.

IV placement is unfortunately particularly difficult in this population. The nursing staff used a numbing cream for the skin site prior to IV placement to minimize or completely eliminate discomfort to the participant. The total number of IV attempts was limited in our study protocol; the CTRC RN would

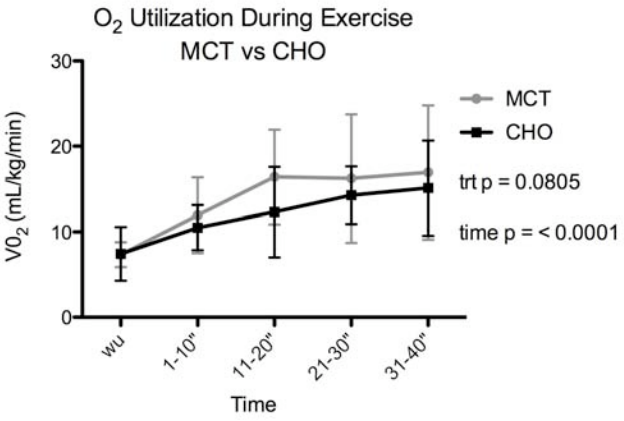
attempt to place the IV catheter twice, at most. If he or she was unable to establish the line, an IV therapy nurse was then called. The IV nurse then had, at most, two attempts to place the IV and if still unsuccessful, the subject would be exempt from further blood draws during the study. Although this was the maximal number of attempts we allowed, it was also our practice to restrict the number of attempts further at the participant or parent's discretion. This was indicated in one case when an IV stopped functioning properly and the participant refused having another one placed in order to collect the remaining blood data.

Development of sensorimotor peripheral neuropathy occurs in some subjects with TFP deficiency. This is predominantly manifested as areflexia and foot drop initially. Subjects with significant peripheral neuropathy would have difficulty completing the exercise protocol due to gait disturbances. Subjects who could not complete a treadmill study were excluded. We can validate that all participants successfully completed the protocol without medical complications.

APPENDIX B: ADDITIONAL FIGURES

Ventilation. Work performed was kept constant between trials; thus, VO_2 in mL/kg/min did not significantly differ following MCT vs CHO supplementation.

Fig 10. Change in VO_2 during exercise with and without MCT

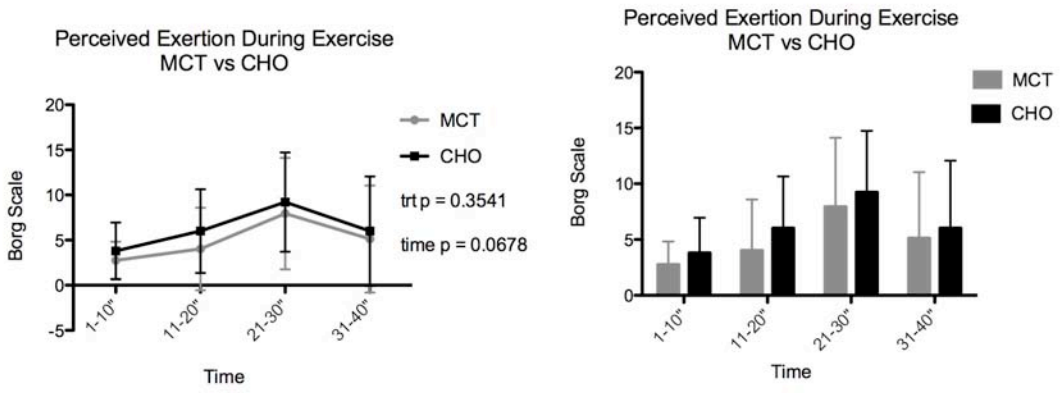


Perceived level of physical exertion. The Borg scale (a scale of 1 to 20 of increasing exertion) was employed to measure each participant's perceived level of physical exertion [1]. Borg levels of 10-16 correlate with moderate intensity aerobic exercise.

TAUC for perceived physical exertion following MCT supplementation (TAUC = 15.91) was not significantly different from that of the exercise trial pre-treated with CHO alone (TAUC = 20.12). While the treatment effect did not achieve statistical significance, as could be expected, an increase in perceived physical exertion was observed with increased duration of exercise and this pattern trended toward significance (RM ANOVA: trt p = 0.3541, time

p = 0.0678). Perhaps the biggest limitation in this data was that it was not consistently collected in all participants at all time points. It is probable that statistical significance may have been achieved had all time points been collected on each participant. For some, the Borg scale has served as a reliable gauge of rate of perceived exertion during exercise studies [2], others have countered that it may not be as reliable as previously thought [3, 4]. Certainly the reliability of this tool, taking into account the population and nature of our investigation, could be a potential limiting factor to the validity of the data as well.

Fig 11. Borg scale with and without MCT



Echocardiogram. The left ventricle end-diastolic volume (LVEDV) and left ventricle end-systolic volume (LVESV) were calculated from either the 2 or 4 chamber view (A2C or A4C) and used to calculate ejection fraction with the formula (LVEDV-LVESV)/LVEDV. TAUC for EF following MCT pre-supplemented exercise (TAUC = 118.5) was not statistically different (paired

t-test: p = 0.171) than TAUC for EF following CHO pre-supplemented exercise (TAUC = 126.5).

Table 4. Echocardiogram Results

	MCT				CHO			
Subject	LVEDV A2C	LVESV A2C	EF (%)	HR bpm	LVEDV A2C	LVESV A2C	EF (%)	HR bpm
1	59*	24*	59	85	60*	26*	57	84
2	118	50	57	83	118	58	62	92
3	48	21	64	73	72	13	72	87

*A4C

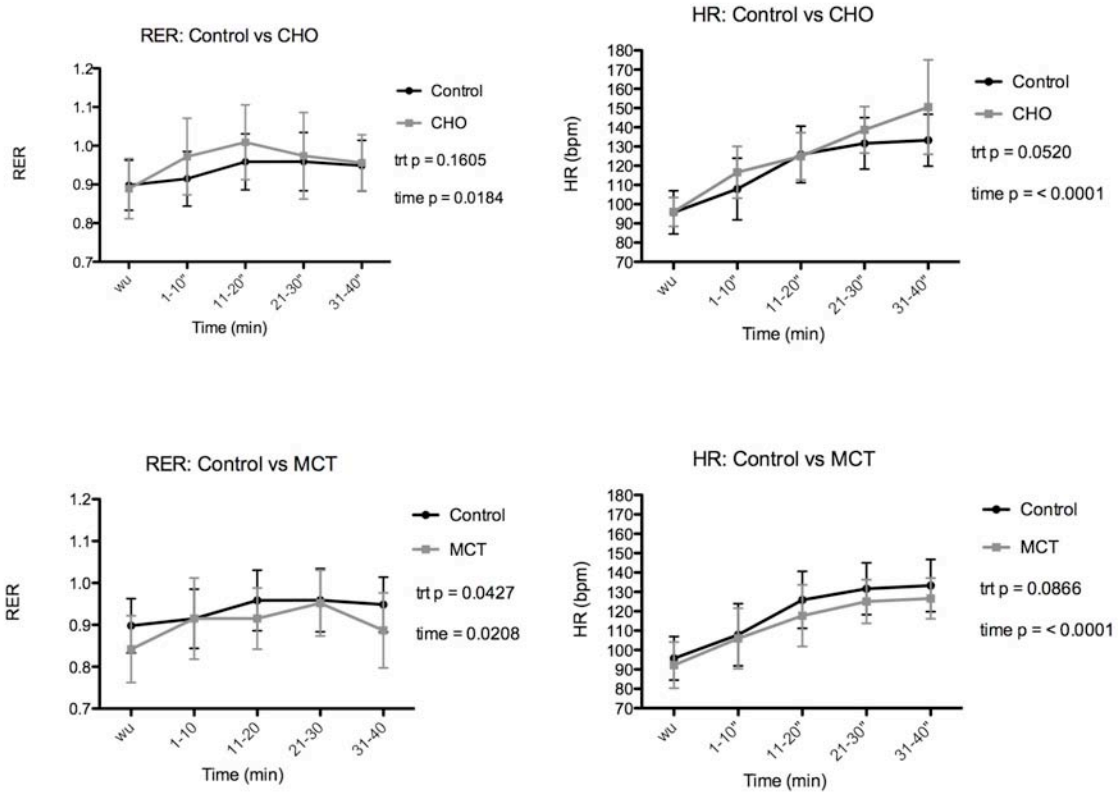
Subjects vs. controls.

Availability of exercise physiology data in children and adolescents is limited. Although there is significant variability in exercise performance among individuals, nine age, sex, and BMI-matched controls were evaluated in this study in order to generate comparison data. We did observe high variability among the control subjects which compromised the statistical significance when comparing workload achieved and VO_2/kg. However, some comparisons between control subjects and subjects with a long-chain FAO disorder are presented here.

Respiratory exchange ratio is similar between controls and subjects pre-supplemented with CHO (RM ANOVA: trt p = 0.161, time p = 0.018);

however, following CHO pre-supplementation, HR is higher in subjects compared to controls (RM ANOVA: trt p = 0.052, time < 0.0001). In contrast, RER is lower in subjects compared to controls when subjects are pre-supplemented with MCT (RM ANOVA: trt p = 0.043, time p = 0.021), HR is also lower and this difference is trending towards significance (ANOVA: trt p = 0.0866, time p < 0.0001).

Fig 12. Average RER & HR during exercise: subjects vs controls



APPENDIX C: ADDITIONAL TABLES

Exercise Data

Table 5. Change in exercise measures: MCT vs CHO

P	warm-up	1-10"	11-20"	21-30"	31-40"
RER *	0.0375	0.0109	0.0157	0.0527	0.0017
HR *	0.0297	0.0155	0.0285	0.0005	0.0243
DP *	-	0.0690		0.0098	
VO_2	-	0.4392		0.5114	
Borg	-	0.075	0.094	0.144	0.066

Blood Data

Table 6. Change in blood measures: MCT vs CHO

P	Pre	Post	Recovery
β-OH butyric acid *	0.028	0.002	0.002
acetoacetic acid *	0.015	0.013	0.001
lactate *	0.016	0.005	0.068
pyruvate *	< 0.001	0.031	0.003
FFA	0.62	0.74	0.28
OH-acylcarnitines	0.202	0.382	0.236
creatine kinase	0.254	0.287	0.258

* denotes statistical significance

References

1. Borg G, Linderholm H. Exercise performance and perceived exertion in patients with coronary insufficiency, arterial hypertension and vasoregulatory asthenia. *Acta Med Scand.* 1970;187:17-26.

2. Lagally KM, Amorose AJ. The validity of using prior ratings of perceive exertion to regulate resistance exercise intensity. *Percept Mot Skills.* 2007;104:534-542.

3. Chen MJ, Fan X, Moe ST. Criterion-related validity of the borg ratings of perceived exertion scale in healthy individuals: A meta-analysis. *J Sports Sci.* 2002;20:873-899.

4. Lamb KL, Eston RG, Corns D. Reliability of ratings of perceived exertion during progressive treadmill exercise. *Br J Sports Med.* 1999;33:336-339.

OREGON HEALTH & SCIENCE UNIVERSITY
Consent Form
FOR SUBJECTS WITH A FATTY ACID OXIDATION DISORDER

TITLE: **Fatty Acid Oxidation Disorders and Body Weight Regulation**

PRINCIPAL INVESTIGATOR: Melanie B. Gillingham, PhD, RD (503) 494-1682

CO-INVESTIGATORS: Cary O. Harding, MD (503) 494-2783
Jonathan Q. Purnell, MD (503) 494-1056
Diane Elliott, MD (503) 494-1056
Diane Stadler, PhD, RD (503) 494-0168

SPONSOR: American Diabetes Association & the National Institutes of Health

PURPOSE:

"You" refers to you or your child in this consent form. You have been invited to be in this research study because you have one of the following fatty acid oxidation disorders:

- trifunctional protein deficiency (TFP deficiency),
- long-chain 3-hydroxyacyl CoA dehyrogenase deficiency (LCHADD),
- very-long chain acyl-CoA dehydrogenase deficiency (VLCADD),
- carnitine palmitoyltransferase type 2 deficiency (CPT2).

The purpose of this study is to determine if having a fatty acid oxidation disorder changes how your body controls your weight. It is also to determine if eating more protein will change the amount of muscle you have. Foods that have a lot of protein include skim milk, yogurt or chicken. The last purpose is to determine if taking a medium-chain triglyceride (MCT) supplement before exercising will improve your ability to exercise without muscle pain. (MCT is the oil that can be used for energy by people with fatty acid oxidation disorders.) This study requires 2 admissions to the Oregon Clinical and Translational Research Institute (OCTRI) and will take about 4 months to complete. We will collect blood samples to use in this study and store for use in future research studies.

There will be a total of 24 subjects with fatty acid oxidation disorders enrolled in this research study at OHSU. There will also be 24 biological parents of subjects with a problem burning fat and 24 subjects that do not have a problem burning fat or a child with a problem burning fat enrolled.

PROCEDURES:

Study Protocol: If you agree to be in this study, you will stay at the Oregon Clinical and Translational Research Institute (OCTRI) on two different occasions. Your first stay at the OCTRI will be for 4 days. You will be admitted to the OCTRI in the afternoon. You will sleep in a hospital room in the research center. If you are a child a parent or guardian will stay with you in the same room. Your meals and the meals for your parent or guardian will be provided.

If you are a female who can get pregnant, we will test a urine sample to see if you are pregnant. Pregnant females can not be in this study. The test results will be given to your parents if you are younger than 15 years old. If you are 15 or older, you will be able to choose to receive the results alone without your parents.

If you can continue in the study, a series of tests will be completed. Each test is explained below. All of the tests listed will be completed while you are admitted to the Oregon Clinical and Translational Research Institute (OCTRI) but the order of the tests may be different than listed in this consent form.

After the tests, you will be told to follow your regular diet or a diet higher in protein at home for 4 months. The diet you will follow is randomly assigned. This means that neither you nor the investigator get to pick which diet you follow. Half of the subjects will be told to follow their regular diet. The other half will be told to follow a diet high in protein. You will be taught how to follow the diet while you are at the OCTRI for the first time. You will be sent home to follow the diet for 4 months. At the end of the 4 months, you will come back to the OCTRI and the tests will be repeated.

The following table outlines your first stay at the OCTRI:

Test:	Day 0:	Day 1:	Day 2:	Day 3:	Day 4:
Come to OCTRI 4:00 PM	X				
48-hour urine collection	X	X	X		
Energy expenditure		X			
DEXA		X			
IV placed		X			
OGTT		X			
Exercise test			X		
IV removed			X		
MRI/MRS				X	
Diet training			X	X	
Urine sample					X
Go home at 9:00 AM					X

OCTRI stay #1:

Energy Expenditure Test: In the morning, your energy expenditure will be measured. Energy expenditure is how many calories you use during the day. This test will be measured at 7:00 a.m. A clear, colorless, Plexiglass canopy (bubble) will be placed over your head and chest while you rest on a bed. Samples of the air that you breathe out will be collected for about 45 minutes. A trained research assistant will perform this test in a private room to make you feel comfortable and relaxed.

Dual Energy X-Ray Absorptionometry (DEXA): We will measure how much muscle and fat you have by giving you a DEXA scan. DEXA is a low power x-ray machine. This test is painless and involves a very small amount of radiation exposure. To perform this test, you must lie still on a bed while the DEXA machine scans your body. This procedure takes up to 10 minutes.

IV Catheter: An intravenous (IV) catheter or tube will be placed in your arm to draw blood. (The IV catheter will stay in place approximately 36 hours until the end of the Oral Glucose Tolerance Test and the exercise test.) You may request a numbing cream to be placed on your arm before the IV catheter is inserted. The cream will numb the skin where the nurse will insert the IV catheter. Two teaspoons of blood will be drawn through the IV when it is placed.

Oral Glucose Tolerance and Fat Oxidation Test: Two teaspoons of blood will be drawn from your IV catheter. After this blood is drawn, you will be asked to drink a bottle of orange soda within the next 10 minutes. Two teaspoons of blood will be drawn through the IV after 30, 60, 90, and 120 minutes. A total of 6 blood samples and 3 tablespoons of blood will be drawn during this test. This test measures how much sugar is in your blood.

Meals: The research kitchen will provide all of your meals for you. The food will be low in fat and contain the recommended amount of MCT. You will be asked to eat only food provided to you by the research kitchen and not to consume food from restaurants or from home. Three meals and one snack will be provided for you. You do not have to eat all the food that is given to you but you must return any uneaten food and their containers to the kitchen.

Exercise Test: You will exercise on a treadmill for 40 minutes. Your blood pressure and heart activity will be measured in both a standing and lying down position. Blood pressure and heart activity will also be measured during the exercise test. To measure your heart activity, small wires are taped to your chest and both arms and legs. The electrocardiogram instrument will record the electrical signals produced by your beating heart. The equipment does not give an electrical shock. Right before starting the exercise, you will be asked to drink a beverage. It may or may not have an MCT supplement added to it. The possibility that the juice will have MCT added to it is 50%. (It is like tossing a coin; heads could mean you get no MCT and tails would then mean you get MCT added to the juice).

Echocardiogram: After you finish the treadmill test, you will lie down on a bed and an ultrasound probe will be held to your chest. The probe will take pictures of your

heart. There is no radiation (like x-rays) exposure from an ultrasound machine. This procedure takes up to 20 minutes.

Drs. Harding and Gillingham will monitor you during the exercise session. You will begin walking slowly on the treadmill and the pace of walking will gradually increase to a brisk walk. One teaspoon of blood will be drawn from the IV catheter right before you begin the exercise, at the end of the exercise and after you have rested for 20 minutes. A total of 3 blood samples and about one tablespoon of blood will be drawn during the exercise test. After the last blood sample is drawn, the IV catheter will be taken out. You will be asked to breathe into a mask during the exercise so that samples of the air you breathe out can be collected. If your legs begin to hurt or you feel bad during the test you may ask to stop and the test will be stopped immediately.

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) of the abdomen and thigh: The purpose of this test is to measure the amount of fat in your abdomen, liver, and muscle. During this procedure you will lie still on a table inside a powerful magnet. There is no radiation (like x-rays) exposure from an MRI machine. If you have any metal inside of your body (shrapnel, plates) you will not have this procedure. This procedure takes up to 60 minutes.

Heart MRI: The purpose of this test is to measure the function of your heart. After the MRS described above, you will get out of the magnet and stretch. Then you will lie still on a table back inside the same magnet while the technicians takes pictures of your heart. There is no radiation (like x-rays) exposure from an MRI machine. If you have any metal inside of your body (shrapnel, plates) you will not have this procedure. This procedure takes up to 60 minutes.

Diet Assignment and Training: You will be told to follow your regular diet or a diet higher in protein at home for 4 months. The diet you are told to follow is randomly assigned. This means that neither you nor the investigator get to pick which diet you follow. Half of the subjects will be told to follow their regular diet. The other half will be told to follow a diet high in protein. The investigator and the nutritionist will teach you how to follow your particular diet. They will teach you how to plan your meals and what things to look for at the grocery store. They will give you ideas about how to eat at restaurants and recipes to cook at home. They will also mail to you diet record forms and urine collection cups. You will be asked to write down everything you eat for 3 days at the end of each month. You will be asked to collect a morning urine sample. You will need to mail the urine sample and the diet record back to the investigator each month. At the end of the training, we will collect a morning urine sample and you may go home.

Monitoring your progress at home: After you go home, the nutritionist will call you each week to find out how you are doing on your diet. She will be able to help you with problems and encourage you to follow your diet. You will also receive diet tips in the mail or by email. She will remind you to complete your diet records each

month. She will also remind you to send your urine sample and diet records back to OHSU. At the end of 4 months, you will return to the OCTRI for 3 days.

Total Energy Expenditure: One week after you go home, we will use a special water to measure how much energy you burn in 7 days doing your normal activities. This special water has a high amount of heavy hydrogen and heavy oxygen. Heavy hydrogen and oxygen are naturally occurring substances that are more concentrated in the special water. We will mail you a bottle of special water, 6 urine cups, urine storage vials and boxes to return the urine samples. The morning you start the test, you will collect your first urine into a collection cup and transfer to a urine storage vial. Then you will drink the special water. Fill the water bottle to the black line and drink the water in the bottle again. The water will have no taste. Two hours after you drink the special water, collect another urine sample. Collect your urine again 3 hours and 4 hours after you drank the special water. Store the urine vials in the refrigerator. 7 days after you drank the special water, collect a urine sample when you wake up. Collect another urine sample 1 hour later. Mail all 6 urine samples to Dr. Gillingham in the shipping box provided. The test for measuring total energy expenditure is now complete.

3-Day Diet Record: You will record all the food and beverages you eat for 2 weekdays and 1 weekend day. A form to record the food you eat will be provided to you. The researcher will explain how to complete the form during your 1st OCTRI stay. You will mail the records and a urine sample to the researchers each month. Mailing boxes and labels will be provided.

OCTRI stay #2: Most of the tests completed during your first stay will be repeated. These include oral glucose tolerance test (OGTT), energy expenditure, DEXA, MRI/MRS, and the exercise test.

These tests were described above. In addition, you will complete a test called the meal tolerance test (described below). All of the tests listed will be completed while you are admitted to the Oregon Clinical and Translational Research Institute (OCTRI) but the order of the tests may be different than listed in this consent form.

The following table outlines your second stay at the OCTRI:

Test:	Day 0:	Day 1:	Day 2:	Day 3:	Day 4:
Come to OCTRI 4:00 PM	X				
Total body water	X				
48 hour urine collection	X	X	X		
Energy expenditure		X			
DEXA		X			
IV placed		X			
OGTT		X			
Meal and fat oxidation test		X	X		

Exercise test	X	X	
IV removed	X	X	
MRI/MRS	X	X	
Go home at 9:00 AM		X	X

Meal and Fat oxidation Test: Your ability to use a meal for energy will be measured on Day 2 of your second OCTRI stay. An intravenous (IV) catheter or tube will be placed in your arm to draw blood before the meal test. (The IV catheter will stay in place approximately 36 hours during the exercise test and the meal test). About two teaspoons of blood will be drawn from your arm. You will be given a liquid shake with a labeled fat and asked to drink it within the next 10 minutes. The labeled fat is fat with a heavy form of carbon. Heavy carbon is a natural compound that is concentrated in the fat given to you. Two teaspoons of blood will be drawn 1 hour, 2 hours, and 4 hours after you drink the shake. A total of 4 blood samples and 2.6 tablespoons of blood will be drawn following the test meal. You will be allowed to drink water but not consume any other drinks or food for 4 hours. During that time, you may watch TV, read, or do other quiet activities. After the 4 hour blood sample is taken, you will be given lunch.

Exercise and MCT oxidation Test: Right before starting the second exercise, you will be asked to drink a beverage. If you received the drink with added MCT before your 1st exercise test, you will receive the juice alone before the second exercise test. If you received the juice alone before your 1st exercise test, you will receive the beverage with added MCT. You will be asked to give a breath sample every 15 minutes for several hours and every hour for several more hours. To give a breath sample, you blow into a straw connected to a test tube. The rest of the exercise test will be the same as the time before.

Blood Samples: About 5 tablespoons or about 2.5 ounces of blood will be drawn during you first OCTRI stay. About 7.6 tablespoons or about 3.8 ounces of blood will be drawn during your second OCTRI stay. Your blood samples will be stored in a secure freezer indefinitely for future analysis. The blood samples will be coded with a number and only the investigators will be able to identify the samples. We may analyze the samples for a new compound that will help in caring for children with problems burning fat. You may choose not to have your blood sample stored for future use. If you choose not to have your blood stored, the samples will be destroyed at the conclusion of the study.

If you have any questions regarding this study now or in the future, contact Melanie Gillingham, PhD, (503) 494-1682 or Cary Harding, MD, (503) 494-2783.

RISKS AND DISCOMFORTS:

Participation in this study involves some risks, discomforts, and inconveniences. These include:

Participation requires two stays at the OHSU OCTRI in a 5-month period.

Change to a High Protein Diet: There is a chance you will be asked to follow a high protein diet at home during the study. If you do not eat enough calories on your diet plan or go for too long without food, there is a small risk you may become sick. Not enough calories can be a problem for people with fatty acid oxidation disorders. It can cause episodes of low blood sugar, acidosis, or muscle breakdown and pain. Treatment of low blood sugar or muscle pain may require hospitalization for IV fluids, sugar (dextrose) and rest.

Estimation of Energy Expenditure (calorie use): There are no risks associated with having energy expenditure measured. Some individuals with claustrophobia (fear of closed spaces) may find the canopy equipment too confining. The procedure takes about an hour to complete and will be performed in a private room.

DEXA scans: During the body scan you will be exposed to a small dose of radiation (x-rays). This test will measure the total amount of fat, muscle, and bone content in your body. While no amount of radiation has been proven to be safe, there is no evidence that the amount of radiation to which you will be exposed in this study is harmful.

MRI/MRS: The magnetic resonance imaging (MRI) machine is a powerful magnet, this magnet may cause any metal in your body to move. If you know of any metal in your body, you will need to tell the investigator right away. (Dental fillings are an exception to the metal-free requirement for this test.) Otherwise, there are no known risks of MRI. Some individuals with claustrophobia (fear of closed spaces) may find the MRI equipment too confining. In that case, you can request to be removed from the scanner and this will be done immediately. The MRI scanner makes a loud thumping sound. You may be asked to wear protective earplugs during scanning.

Blood Samples/IV Catheter: Blood drawing will cause some pain and carries a small risk of bleeding, bruising or infection at the puncture site. There is a small risk of developing anemia from the blood sampling in this study. Anemia is when you do not have enough red blood cells. Anemia can make you feel tired or weak. You will have a catheter (tube) in your vein for more than 24 hours. You may get an infection where the tube is placed. This would cause swelling, redness, and pain. You may bleed or get a bruise. There is a small chance your blood stream or heart valves might get a serious infection. You may get a blood clot that could go to your lungs. These problems are very rare. If you have these problems, you will need hospital care. You will have an IV catheter in your arm for about 36 hours twice during the study.

Oral glucose tolerance, meal tolerance and fat oxidation tests: There is no known risk from consuming orange soda, the liquid meal or the labeled fat in these tests. Not enough calories can be a problem for people with fatty acid oxidation disorders. It can cause episodes of low blood sugar, acidosis, or muscle breakdown and pain. The soda

and the liquid meal are designed to provide plenty of calories for the entire morning. The risk of complications from consuming these drinks is very small.

Total energy expenditure: There are no known risks from consuming the special labeled water or collecting urine. Fasting for too long can be a problem for people with fatty acid oxidation disorders. It can cause episodes of low blood sugar. You must wait one hour before eating breakfast after you drink the special water. The risk of low blood sugar from waiting for one hour to eat breakfast is very small.

Exercise Tests: Exercise can be associated with muscle pain, dark urine, and acidosis. These are symptoms of rhabdomyolysis or muscle breakdown. There is a moderate risk of developing rhabdomyolysis during the exercise tests. Treatment of rhabdomyolysis will require hospitalization for IV fluids, sugar (dextrose) and rest. You will be carefully monitored during the exercise to determine if you have any signs of rhabdomyolysis. If your blood sugar drops too low, the exercise test will be stopped. If you tell the doctor your legs hurt or you feel bad while exercising, the exercise test will be stopped.

Echocardiogram: There are no risks associated with an echocardiogram.

Under Oregon law, suspected child abuse must be reported to the appropriate authorities.

BENEFITS:

Results from this study may improve dietary and exercise guidelines for people with fatty acid oxidation disorders. You may or may not personally benefit from participating in this study. However, by serving as a subject, you may contribute new information that may benefit patients in the future.

ALTERNATIVES:

1. You may choose not to be in this study. If you chose not to participate, you should continue to follow your low-fat diet and your physician's instructions to treat your disorder.
2. You may choose to participate. If you choose to participate:

    a. You may choose to participate and allow your blood samples to be stored for future research.

    b. You may choose to participate and request your blood samples be destroyed at the end of the study.

CONFIDENTIALITY:

We will not use your name or your identity for publication or publicity purposes.

A code number will be assigned to you and your blood samples sent outside of OHSU. A code number will also be given to information about you or your family. Only the investigators named on this consent form will be authorized to link the code number to you. Other investigators will be given only the code number that will not identify you or any of your relatives. These investigators are the researchers at the Mayo Clinic, the University of Wisconsin-Madison, and St. Louis University who will measure some of the compounds in your blood or urine.

Research records may be reviewed and copied by the sponsors, the OHSU Institutional Review Board, the Oregon Clinical and Translational Research Institute, the Office for Human Research Protections, and the National Center for Research Resources.

COSTS:

There is no cost to you for the tests described in this study.

The costs of transportation to Portland, Oregon, are not covered by the study.

You will stay in an OHSU Oregon Clinical and Translational Research Institute ward during your visit. There will be no charge for lodging. Meals will be provided for you. Meals will also be provided for one parent accompanying a child to Portland. There may be incidental expenses associated with travel that are not covered by the study. If you wish, you may discuss these expenses with Dr. Gillingham.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Dr. Melanie Gillingham at 503-319-2404 or ask to have Dr. Cary Harding paged at (503) 494-9000.

It is not the policy of the U.S. Department of Health and Human Services, or any federal agency funding the research project in which you are participating, to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

The Oregon Health & Science University is subject to the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you suffer any injury and damage from this research project through the fault of the University, its officers or employees, you have the right to bring legal action against the University to recover the damage done to you subject to the limitations and conditions of the Oregon Tort Claims Act. You have not waived your legal rights by signing this form. For clarification on this subject, or if you have further questions, please call the OHSU Research Integrity Office at (503) 494-7887.

PARTICIPATION:

If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

You may be removed from the study prior to its conclusion if the study is stopped by the sponsor or if you do not follow study instructions.

We will give you a copy of this consent form.

SIGNATURES:

Your signature below indicates that you have read this entire form and that you agree to be in this study.

- I give my consent for my blood/tissue samples to be stored and used for this study only.

- I give my consent for my blood/tissue samples to be used for this study and stored for possible use in future studies, but I wish to be contacted for permission prior to any future use.

- I give my consent for my blood/tissue samples to be used for this and future studies and do not need to be contacted for permission in the future.

OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

PHONE NUMBER (503) 494-7887

CONSENT/AUTHORIZATION FORM APPROVAL DATE

Dec. 4, 2009

Do not sign this form after the

Expiration date of: 11/18/2010

_____

Printed name of Subject

_____ _____

Signature of Legal Guardian Date

_____ _____

Signature of Person Obtaining Consent Date