Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Mol Genet Metab. Author manuscript; available in PMC 2016 December 01.

Published in final edited form as:

Mol Genet Metab. 2015 December ; 116(4): 260–268. doi:10.1016/j.ymgme.2015.10.005.

Anaplerotic Treatment of Long-Chain Fat Oxidation Disorders with Triheptanoin: Review of 15 years Experience

Charles R. Roe, MD[1,2] and **Henri Brunengraber, MD, PhD**

Departments of Nutrition and Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Abstract

Background—The treatment of long-chain mitochondrial β-oxidation disorders (*LC-FOD*) with a low fat-high carbohydrate diet, a diet rich in medium-even-chain triglycerides (*MCT*), or a combination of both has been associated with high morbidity and mortality for decades. The pathological tableau appears to be caused by energy deficiency resulting from reduced availability of citric acid cycle (*CAC*) intermediates required for optimal oxidation of acetyl-CoA. This hypothesis was investigated by diet therapy with carnitine and anaplerotic triheptanoin (*TH*).

Methods—Fifty-two documented LC-FOD patients were studied in this investigation (age range: birth to 51 years). Safety monitoring included serial quantitative measurements of routine blood chemistries, blood levels of carnitine and acylcarnitines, and urinary organic acids.

Results—The average frequency of serious clinical complications were reduced from ~ 60 % with conventional diet therapy to 10 % with TH and carnitine treatment and mortality decreased from ~ 65 % with conventional diet therapy to 3.8 %. Carnitine supplementation was uncomplicated.

Conclusion—The energy deficiency in LC-FOD patients was corrected safely and more effectively with the triheptanoin diet and carnitine supplement than with conventional diet therapy. Safe intervention in neonates and infants will permit earlier intervention following pre-natal diagnosis or diagnosis by expanded newborn screening.

Keywords

Triheptanoin; Anaplerosis; Fat Oxidation Disorders; Ketogenic Diet Therapy

---

[2]Investigations were performed at the Institute of Metabolic Disease, Baylor University Medical Center, Dallas, Texas

Corresponding Author: Charles R. Roe, MD, 11 Northcrest Circle, Rockwall, Texas 75087, USA, chasroe@earthlink.net, Telephone: 214-236-3923.

[1]Permanent Address: Department of Neurology and Neurotherapeutics, UT Southwestern Medical Center, Dallas, Texas 75390

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

AVAILABILITY OF SUPPORTING DATA:

Supplement File (.xlsx): Metabolic Data from Long-Term Diet Therapy of LC-FOD.

AUTHOR CONTRIBUTIONS:

The authors are equally responsible for experimental design, production of data, its analysis and interpretation. The authors are equally responsible for the writing of this manuscript and approve its submission for publication.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. INTRODUCTION

The long-chain fat oxidation disorders (*LC-FOD*) include inherited deficiencies of (i) the enzymes of the "Carnitine Cycle": carnitine palmitoyltransferase I *(CPT-I), Carnitine acylcarnitine translocase (CACT), and CPT II*), and (ii) enzymes required for β-oxidation: very long-chain acyl-CoA dehydrogenase *(VLCAD), Trifunctional protein (TFP) and* long-chain acyl-CoA dehydrogenase (*LCHAD*). These disorders continue to manifest serious clinical complications including cardiomyopathy, and recurrent episodes of rhabdomyolysis, weakness, hepatic failure, hypoglycemia, and hyperammonemia. These complications have persisted despite decades of diet therapy with low fat-high carbohydrate, a diet rich in medium-even-chain triglycerides (*MCT*), or a combination of both. [1–4]

Previous reviews of patients receiving conventional diet treatment have described the continued high frequency of these clinical complications and extremely high mortality. Increasingly, these complications are considered to be the result of compromised CAC function and reduced ATP availability, in spite of the ample supply of acetyl-CoA derived from dietary carbohydrates or MCT. The most likely explanation of such biochemical pathology is an insufficient supply of CAC intermediates that carry acetyl groups as they are oxidized. There is no evidence of defective anaplerosis in LC-FOD. Thus, we hypothesized that excessive cataplerosis, uncompensated by physiological anaplerosis, results in suboptimal CAC operation. We further hypothesized that replacing medium-even-chain triglycerides with medium-odd-chain triheptanoin would boost anaplerosis and compensate for excessive cataplerosis. The heptanoate component of triheptanoin is oxidized to both acetyl-CoA and anaplerotic propionyl-CoA. Unlike anaplerosis from pyruvate or glutamate/glutamine, anaplerosis from propionyl-CoA is irreversible. In addition to being a direct anaplerotic substrate, heptanoate is indirectly anaplerotic in peripheral tissues via the utilization of C5-ketone bodies (β-hydroxypentanoate + β-ketopentanoate) formed from heptanoate in the liver.

Previous studies involving TH in dietary management have reported beneficial clinical outcomes for VLCAD deficiency [5], late-onset CPT II deficiency [6], adult polyglucosan body disease (*APBD*) [7] and the glucose-1-transport defect [8]. These studies have provided support for the existence of an underlying energy deficiency that benefits from anaplerotic therapy.

The present report evaluates the potential value of diet therapy with triheptanoin + carnitine compared to prior conventional diet therapy in 52 LC-FOD patients. Unlike previous reports [1–4], this analysis distinguishes between the cardiac and mild forms of VLCAD deficiency, and separates TFP from LCHAD patients, thus allowing a more comprehensive evaluation of this therapeutic regimen.

2. METHODS

Triheptanoin Source and Diet Management

TH oil ("*Spezialöl 107*") was provided by SASOL, GmbH, Witten DE and was used exclusively with all patients in this investigation. This oil contained heptanoate esterified to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

glycerol (99.5 %) and TH at > 97.4 % purity. All infants and children with documented LC-FOD also received a daily L-carnitine supplement of 100 mg/kg (*in 4 divided doses*). All adults received a maximum dose of a 330 mg tablet of L-carnitine three times daily. (*Sigma Tau Pharmaceuticals*)

Portagen formula (*Mead-Johnson Nutritionals*) had previously been used as a source of MCT to treat patients in this study. The dose of MCT in Portagen used in other pediatric patients (*30–35% of daily caloric intake*) and in uncomplicated preliminary meal tests with LC-FOD patients [5] was chosen as a safe TH dose prior to initiating this protocol.

Composition of the TH formula: 1. Total daily long-chain fat intake was reduced to < 20% of the daily caloric requirement and essential fatty acids were added to meet the daily requirement. TH was maintained at ~ 30–35% of the daily caloric requirement. 2. Imwitor 375 (*SASOL, GmbH*) was added at 2% as an emulsifier for TH oil prior to completing the final daily formula. 3. The complex carbohydrate, Polycose (*Abbott Nutrition*), was substituted for “simple sugar” intake (*mono- and di-saccharides*) to avoid unnecessary weight gain. Protein, vitamins and minerals were added as recommended by the dietitian. 4. The completed daily TH formula was appropriately divided for infant feedings (*e.g. every 3 hours*). For children, adolescents and adults, a low fat-low carbohydrate diet was maintained along with four “snacks” of TH mixed with low fat-low carbohydrate yogurt at each meal and bedtime. With the gradual decrease in caloric requirement with age, the amount of TH for infants and children was equivalent to ~ 3–4 gm/kg/day and for adolescents and adults it was ~ 1.0 gm/kg/day. All patients and/or parents were given dietary instruction and experience in preparation of diet/formula during their initial hospitalization.

Management of problems with the TH diet: If gastric cramps occurred, the TH dose was transiently decreased until the discomfort resolved (*~ 2 days*) and the previous dose was re-started without recurrence of discomfort.

Following an initial admission for up to 9 days, follow-up clinical and laboratory evaluations occurred up to 12 months. At that time, all patients and parents consented to continue participation in the trial.

Metabolic Monitoring

Blood chemistries included glucose, potassium, CO_2, anion gap, BUN, creatinine, albumin, AST (*SGOT*), ALT (*SGPT*), ammonia, GGT, creatine kinase (*CPK*), cholesterol, triglycerides, HDL, and LDL. Blood levels of 3-OH-butyrate (*BHB*), acetoacetate (*AcAc*), 3-OH-pentanoate (*BHP*), 3-ketopentanoate (*BKP*), heptanoate (*C7*) and octanoate (*C8*) were obtained as previously described. [9,10, 12]

Blood levels of free carnitine and the following acylcarnitines were measured by electrospray mass spectrometry [5]: acetyl- (*C2AC*), propionyl- (*C3AC*), heptanoyl- (*C7AC*), octanoyl- (*C8AC*), 5-cis-myristenoyl- (*C14:1AC*), palmitoyl- (*C16AC*), 3-OH-palmitoyl- (*C16-OHAC*), oleoyl- (*C18:1AC*) and long-chain odd-carbon acylcarnitines.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Plasma levels (*nmol/L*) of s-adenosyl-methionine (*SAM*), and s-adenosyl-homocysteine (*SAH*) were measured by a modification of the stable-isotope dilution liquid chromatography-electrospray injection tandem mass spectrometry (*LC-ESI-MS/MS*) previously described. [11]

Quantitative urinary organics acids by GC-MS (*mmol/mol creatinine*) included 3-OH-propionate, heptanoate, octanoate, methylmalonate (*MMA*), 3-OH-pentanoate (*BHP*), 3-keto-pentanoate (*BKP*), pimelate, methylcitrate, pyruvate, acetoacetate (*AcAc*), lactate, 3-OH-butyrate (*BHB*), 2-oxoglutarate, succinate, fumarate, malate, aconitate, isocitrate, citrate adipate, suberate, and sebacate. [12]

Statistical Analyses

were performed with Graph Pad Prism (*GraphPad Software, Inc. version 6*) and were tested (non-parametric) with Welch's Correction except when geometric means were required for data sets with abnormal distributions.

Ethical Approval and Consent

Informed consent was obtained from all patients or their parents as approved by the Baylor Research Institute's Institutional Research Board (*IRB protocol 099-135*). The investigation was performed under FDA Sponsor-Investigator IND 59,303.

3. RESULTS

3.1 Patient Description

Between 1999 and 2009, 52 patients with proven long-chain fat oxidation disorders along with prior clinical and dietary history were referred to this clinical trial. Except for CPT-I patients, the remaining 50 patients received supplemental liquid carnitine. These patients included 8 infants, 28 children, 7 adolescents, and 9 adults representing the following disorders: CPT-I, CACT, CPT II, VLCAD, TFP, and LCHAD. The number of patients with each disorder, their age at entry into the protocol, their pre-protocol diet, and duration of their TH diet are described in Table 1.

Prior to the trial with TH, 41 of the patients were receiving the MCT diet. Of these, the daily dose of MCT was available in 32 patients and ranged from 0.2 to 6.8 gm/kg/day (mean= 2.5) compared to the subsequent TH dose range of 1.0 to 4.5 gm/kg/day (mean= 2.6) in all 52 patients. Although the doses appear equivalent, the daily dose of TH used with infants and children < 12 years old was ~ 3.0 – 4.0 gm/kg/day and for adolescents and adults the dose was ~ 1.0 gm/kg/day. These doses corresponded to ~ 30–35% of the daily caloric requirement that decreases with age and ideal body weight. These dosages permitted detection and measurements of intermediates reflecting oxidation of heptanoate to propionyl-CoA (*as C3AC*). [5, 6]

The Supplement File also describes the age at entry, duration of participation, diets, daily oil doses, results of routine blood chemistries, lipid panels, carnitine, acylcarnitines, and urinary organic acids for each patient.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The cardiac and non-cardiac phenotypes of VLCAD deficiency were differentiated by the ratio of [$^2H_3$]C16AC :[$^2H_3$]12AC following incubation of fibroblasts with [–$^2H_3$]palmitate. [13] LCHAD deficiency was distinguished from TFP deficiency by direct enzyme assays of long-chain L-3-hydroxy acyl-CoA dehydrogenase (*LC-HAD*) and long-chain L-3-ketoacyl-CoA thiolase (*LKAT*) from isolated inner mitochondrial membranes from fibroblasts. The C1528 mutation was analyzed in all TFP and LCHAD patients, and was positive only in LCHAD cell lines. [14]

3.2 Metabolic Observations

3.2.1: Safety Monitoring of High Risk Patients—Nine of the 52 patients were considered to be potentially at high risk: those under 6 months of age: (*CACT (2), VLCAD (3) and LCHAD (2)*) and two affected VLCAD women when pregnant at > 20 weeks gestation. Recognizing the severity of the disorders in these infants, and knowing that early intervention with MCT was a routine clinical practice, TH was started during their initial hospitalization. Frequent metabolic monitoring was used to detect and respond to any unanticipated adverse effects. The pregnant women were admitted to the High Risk Obstetrical Facility for frequent metabolic monitoring and twice daily sonograms.

3.2.1.1 TH Interventions before one month of age

Intervention at Birth: This family had already lost two infants with CACT deficiency during the first week of life. This third pregnancy was also diagnosed with CACT deficiency by amniocentesis. Gastrostomy was performed following delivery, and MCT feeds with carnitine were provided for the first 18 hours after which TH began and carnitine supplement was continued. At birth, all laboratory values were normal except for the serum CPK = 659 (*Nl 38-174 IU/L*) that became normal after 2 days on TH. Blood levels of C16AC decreased from ~ 7.5 to 4.2 μM (*normal for neonates*), and C3AC increased from 1.9 to 3.1 μM. The creatinine level was normal at birth but decreased progressively from 0.8 to 0.3 mg/dl (*normal: 0.6-1.2 mg/dl*). Urinary organic acid analyses were unremarkable except for transient elevations of heptanoate, pimelate and methylcitrate. (Fig 1, Left Panel) However, extreme and simultaneous elevations of both succinate and lactate occurred that had not been observed in any other patients. Cardiomyopathy, arrhythmia, rhabdomyolysis, acidosis, hepatomegaly and hypoglycemia did not occur during the first month of treatment or during the ensuing 7 months of treatment. (Fig 1, Right Panel)

TH Interventions in Infants: Early in the trial, patient VLCAD-C-9 (*22 days old*) whose affected sibling died from hypertrophic cardiomyopathy, and patient LCHAD-5 (*15 days old*) entered the TH trial. For the first 2 days, both infants received Portagen formula (*~ 3.0 gm MCT/kg/day*) and carnitine. Then, the TH dose was increased step-wise from 2.0 to 3.5 gm/kg/day and carnitine without complications. On admission, patient VLCAD-C-9 had a normal physical exam, echocardiogram, and liver ultrasound. While receiving MCT, glucose and CPK levels were normal, but creatinine was low (*0.3 mg/dl, NL 0,6-1.2*). C3AC levels were also decreased. Although C14:1AC was increased, C16AC was normal. With TH treatment, the level of C3AC increased from 0.3 to 4.9 μM (*normal for age < 5.38 μM*). C16AC remained normal but C14:1AC remained unchanged. (Fig 2, Left Panel) Serial blood chemistries remained normal except for creatinine that remained low. After 14 years

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

on TH and carnitine, he remains asymptomatic without cardiomyopathy with normal growth, development and physical activity.

LCHAD-5 was 15 days old and had been treated with MCT and carnitine supplement when she was admitted. After two days, TH was gradually increased from 2.0 to 3.5 gm/kg/day and carnitine supplement was continued. Her physical exam, blood levels of glucose, liver enzymes, CPK, and C16AC were normal but creatinine was also decreased (*0.3 mg/dl*). Initially, C16-OHAC was elevated at 0.19 (*NL < 0.05* μ*M*) and C3AC was low at 1.2 μM. C3AC increased to 6.0 μM and both C16AC and C16-OHAC decreased as the TH dose was increased. (Fig 2, Right Panel) At 15 years of age, she continues the TH diet with carnitine and has not developed hypoglycemia, cardiomyopathy, hepatomegaly, or retinopathy but has occasional rhabdomyolysis with illness.

CACT Infant during Metabolic Crisis: Patient CACT-2 developed ventricular tachycardia, ventricular fibrillation with concentric ventricular hypertrophy, hyperammonemia, hepatomegaly and hypotonia with respiratory insufficiency requiring tracheostomy. He was initially hospitalized for five months and treated with MCT and protein restriction via gastrostomy, supplemental carnitine, phenylbutyrate and intermittent peritoneal dialysis.

At 6 months of age, he was referred to the TH protocol in a near terminal state. MCT formula was maintained initially via gastrostomy and TH was substituted at 2.5 gm/kg/day during the first week, and increased stepwise to 4.0 gm/kg/day thereafter with continuous carnitine supplement.

Phenylbutyrate and protein restriction for his mild hyperammonemia were discontinued on admission and 10 % IV glucose was reduced to 5%. On the TH diet, the highest blood level of C3AC was 4.7 μM (*Nl <2.98* μM). C16AC decreased from 6.8 to 2.5 μM. Oleoylcarnitine (*C18:1AC*) also decreased from 4.3 to 1.6 μM. (Fig 3, Left Panel) All liver enzymes and ammonia levels progressively decreased. (Fig 3, Right Panel) He tolerated TH well and by the end of the fifth week, there was no evidence for hypertrophic cardiomyopathy, hepatomegaly, hyperammonemia, hypotonia and respiratory insufficiency. His growth, physical, and social responses were normal and appropriate over the ensuing 5 months.

3.2.1.2. TH Intervention in Pregnant Affected VLCAD Mothers: The TH diet was initiated after 20 weeks gestation during pregnancies of VLCAD-3 age 36 and VLCAD-4 age 34. Both women had major complaints of muscle pain and weakness. During her first 2 pregnancies, VLCAD-3 had suffered multiple severe episodes of rhabdomyolysis during the third trimesters. At 26 weeks during this third pregnancy she was referred to the TH trial. On admission mild hepatomegaly, muscle weakness, and decreased endurance were noted. Initial abnormal labs, with MCT intake, included CPK of 1172 IU/L, C14:1AC of 1.68 (*NL < 0.05* μM), and reduced C3AC of 0.46 (*NL < 2.64* μM). While receiving TH (*1.0 gm/kg/day*) and carnitine, her muscle strength and endurance increased and hepatomegaly resolved. CPK decreased from 1172 IU/L to 45 IU/L, C14:1AC decreased from 1.68 to 0.12 μM and C3AC increased from 0.46 to 1.93 μM. All twice-daily fetal sonograms were normal. She had no muscle pain, weakness, or episodes of rhabdomyolysis and serum CPK levels

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

remained normal for the remainder of the pregnancy. She delivered a normal boy who is unaffected at 14 years of age.

VLCAD-4 (*Non -Cardiac*): This 34 year old woman had no history of hypoglycemia or cardiomyopathy but, after puberty, she had multiple hospitalizations due to profound weakness and rhabdomyolysis. She was referred at 20 weeks gestation for the TH trial. After four days on the TH diet (*1.0 gm/kg/day*) weakness was absent, C14:1AC decreased from 0.36 to 0.06 μM (*Nl <0.05* μ*M*), C3AC increased from 0.70 to 1.25 μM and CPK levels and all twice-daily fetal sonograms were normal. She did not experience any muscle pain, weakness or rhabdomyolysis during the remainder of the pregnancy. She delivered a normal unaffected girl who is normal at 15 years of age.

3.2.2. Metabolic Observations during the Long-Term TH Trial: (Supplement File)—Most routine blood chemistries were normal in these patients. Hypoglycemia, and metabolic acidosis did not occur and except for CACT-2, described above in crisis when entering the trial, hyperammonemia was also not observed. Serum enzyme levels (*CPK, AST, ALT, GGT*) varied but were also not consistently abnormal. However, creatinine levels were significantly decreased below the normal range in all FOD patients on either the MCT or TH diets. ($P < 0.0001$). In contrast, the levels for those on a low fat diet were at the lower end of the normal range but significantly higher than those observed with patients receiving the MCT or TH diets. ($P < 0.0002$) Comparison of the VLCAD phenotypes revealed that creatinine levels for the more severe cardiac phenotype were significantly lower than the clinically milder form ($P = 0.0006$).

Blood levels for free carnitine, C2AC, and C3AC were obtained from 35 patients initially treated with MCT and compared with their subsequent levels when treated with TH and carnitine supplement. Free carnitine levels were significantly lower with the MCT diet than with the TH diet plus carnitine. ($P < 0.006$) C2AC levels were below the normal range but not significantly different between diets. The levels of C3AC with the MCT diet were much lower than with the TH diet ($P < 0.0001$). As expected, free carnitine, C2AC, and C3AC were all extremely elevated in CPT-1 patients.

Although C16AC was not consistently elevated in the late-onset CPT-II, VLCAD, TFP, or LCHAD deficiencies, it was elevated with both CACT patients and the neonatal CPT II patient. However, C14:1AC was consistently above normal in all VLCAD patients as C16-OHAC levels were for all TFP and LCHAD patients but neither was increased by supplemental carnitine intake.

During treatment with TH and carnitine, C16AC levels actually remained normal or decreased. (Fig 2 and 3) In particular, the 21 VLCAD patients maintained normal levels without any complications despite carnitine supplementation.

3.3 Clinical Observations during the Long-Term TH Trial

3.3.1 Severe Adverse Events (*SAE*)—There were 47 SAE reported to the FDA for patients receiving TH for up to 62 months. They were not associated with hypoglycemia, recurrent or sustained hepatomegaly, excessive weight gain, or persistent gastric

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

disturbance. The adverse events involved intermitternt episodes of illness associated mainly with intercurrent infection (*respiratory, gastroenteritis, rotavirus*) or, rarely, following elective surgical procedures. These were not considered to be related to TH. All reported SAE's were associated only with mild to moderate rhabdomyolysis and a single case of death due to severe cardiomyopathy. Are more detail needed for this case? Twenty-four of the 52 patients had one or more SAE that accounted for 44 of the 47 reports. They all resolved promptly with treatment. The cause of the SAE in 3 patients with only a single SAE report were also associated with infection and mild rhabdomyolysis as documented by the parents and their physicians. None of these were attributed to triheptanoin.

3.3.2 Comparison of Clinical Symptoms and Complications with both Diets— The clinical complications recorded with prior conventional diet therapy were compared with those observed during the long-term trial in the same patients. These included Cardiac (*cardiomyopathy*), rhabdomyolysis, persistent weakness, hypoglycemia, and hepatomegaly. (Table 2) Each complication was markedly reduced when treated with TH and carnitine compared to those associated with conventional diet therapy. ($P < 0.0001$)

Cardiomyopathy occurred during the first year of life prior to entering the TH protocol in 18 of the 52 patients. VLCAD patients accounted for 14 of these cases. There were 7 patients that entered the TH trial when less than one year of age. One of these (*CACT-2*) had cardiomyopathy on entry that resolved completely with TH and carnitine. (Fig 3) Of the remaining six patients, only one developed cardiomyopathy at 18 months of age and died at a local hospital. (*VLCAD-14*).

There were fewer rhabdomyolytic episodes requiring hospitalization (*reduced from 85% to 31%*) and fewer complaints of weakness (*reduced from 92% to 12%*). Hypoglycemia was frequent with patients on conventional diets (*42%*) but did not occur in any patients during the TH trial. Hepatomegaly and elevated liver enzymes were present in 24 patients (*46%*) prior to the trial compared to 3 patients (*6%*) during inter-current illness that resolved rapidly with treatment. Retinopathy developed in only 3 of the 10 LCHAD patients and was not observed in any of the TFP patients.

3.3.3 Mortality: (Table 3)—Six of the 52 patients (11.5%) died during this study. These deaths were due to: ***Parental Withdrawal of all Therapy:*** CACT-1 died from Rotavirus infection after discontinuing TH at 7 months of the trial. CACT-2 decompensated with a severe methylation disorder (*SAM = 25 nmol/L (Nl 59-84 nmol/L) and extreme elevation of SAH at 58 nmol/L (Nl 16-25 nmol/L*) and died after 11 months of TH therapy when the parents withdrew all therapy; ***Surgical and Medical Malpractice:*** VLCAD-3 exsanguinated due to a tear in the superior vena cava during a mediport replacement. TFP-5 died after five uneventful years on the TH diet due to extreme delay of treatment of diarrhea and dehydration in a local hospital emergency room; and ***Metabolic Failure on the TH Diet:*** VLCAD-C-14 died while in the TH protocol from intractable cardiomyopathy that did not respond to repeated emergency treatments. TFP-2 died suddenly from acute respiratory failure after nearly 9 months on the TH diet. None of these deaths could be attributed to TH toxicity. There were no deaths with CPT-1, late-onset CPT II, or LCHAD patients.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The overall mortality in the TH study was 11.5% compared to 65.1% in a recent study [3,4] of the same FOD treated conventionally. ($P < 0.0001$) These earlier reports contained more patients with CACT and neonatal CPT II than in the TH study. When mortality was compared with only VLCAD, LCHAD, and TFP deficiency patients treated with MCT (N=74) with the same groups treated with TH (N=37) the difference was equally significant. ($P < 0.0001$) Taking into account the causes of death, the mortality rate due to metabolic failure while being treated with TH, was only 3.8%.

4. DISCUSSION

Conventional diet therapy has not improved the clinical course or reduced mortality for LC-FOD patients for nearly two decades. [1–4] It is now generally thought that the clinical complications with these patients are largely due to an energy deficiency resulting from insufficient availability of CAC intermediates that compromises ATP production. This clinical trial with TH and carnitine supplement was undertaken to determine if heptanoate oxidation could replenish CAC intermediates, increase ATP production and reduce clinical complications including mortality.

4.1 The Biochemical Rationale for this Clinical Trial

Fig 4 compares our view of the biochemical consequences of conventional diet therapy (*Upper Panel*) with those of TH and carnitine during illness (*Lower Panel*). The following basic concepts for this rationale are: 1. Availability of propionyl-CoA (*C3-CoA*) is necessary to replenish CAC intermediates from succinyl-CoA to OAA; 2. OAA must be available continuously for the following functions: as co-substrate with acetyl-CoA for the citrate synthase reaction, as an intermediate of gluconeogenesis, and to enable uninterrupted urea cycle function by providing aspartate 3. Intra-mitochondrial carnitine is required for conversion of excessive amounts of acyl-CoA intermediates into acylcarnitines for export while maintaining adequate amounts of CoA to enable other catabolic reactions (*e.g. amino acid oxidation*); and 4. AMPK is activated when the ratio of ATP:AMP is reduced during energy deficiency. The net result is stimulation of catabolic pathways to provide intermediates for the CAC and inhibition of synthetic pathways that require ATP. [15]

The combination of TH and carnitine would be expected to replenish CAC intermediates, including OAA, and stimulate the CAC and ATP production. AMPK would be inactivated by increased ATP availability and facilitate synthetic pathways while reducing catabolic pathways such as lipid mobilization and β-oxidation that is already severely compromised. (Fig 4, *lower panel*)

The acute management of the CACT patient referred during metabolic crisis as proposed in Fig 4 reflects the abnormalities and their correction by TH and carnitine. Evaluation on admission revealed concentric ventricular hypertrophy with congestive heart failure, tracheostomy for respiratory insufficiency, severe hypotonia, hepatomegaly (*6 cm below RCM*) extremely elevated level of C16AC, and no response to pain. Prior treatment included the MCT diet, IV glucose, dietary protein restriction, phenylbutyrate, and intermittent peritoneal dialysis for hyperammonemia. Reversal of the urinary BHB:AcAc ratio reflected intra-mitochondrial acidosis that can accompany a reduced NADH:NAD ratio with

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

decreased ATP production. The need for anaplerosis was indicated by very low levels of C3AC. Following initiation of the TH diet with carnitine supplementation and elimination of phenylbutyrate and protein restriction, all of the above abnormalities were corrected by the fifth week and growth and development had returned to normal thereafter. (Fig 3, Supplement File)

4.2 Interventions with Infants and During Pregnancy

Although early interventions with conventional diets have been employed safely with infants, results of treatment with TH in infants or during pregnancies of affected mothers have not been previously reported. Three infants were treated with TH and carnitine at birth, and at 15 and 22 days old. CACT-1 was given MCT at birth via gastrostomy for 2 days and changed to TH and carnitine 18 hours later. Increased levels of CPK and C16AC, and low levels of C3AC (*C3-CoA*) were observed at birth and were corrected within 2 days with TH intake. Unlike any other patient in this study, there was intermittent extremely elevated excretion of both succinate and lactate without clinical consequence. (Fig 1) This may have been the result of excessive OAA production inhibiting succinic dehydrogenase and Complex II in this immature neonate. [18,19] Her growth and social development over the next five months were normal in contrast with her two siblings that died during the first week of life. Unfortunately, she also died due to rotavirus infection at six months.

Treatment of the other neonates was also uncomplicated. VLCAD-C-5 had a sibling who died with cardiomyopathy and was admitted when 15 days old. He is now an adolescent and has not developed cardiomyopathy. LCHAD-5 was admitted at 22 days and is also an adolescent now who has occasional episodes of rhabdomyolysis. (Fig 2) These 3 infants did not develop cardiomyopathy, arrhythmia, hypoglycemia, acidosis, hepatomegaly, hyperammonemia or gastric intolerance. These results support early intervention with TH and carnitine supplement following diagnosis by expanded newborn screening.

TH intervention with mothers affected with VLCAD deficiency, only during the third trimester of pregnancy, was equally uncomplicated. The previous symptoms of daily muscle pain, weakness, and fatigue that existed were eliminated during the remainder of their pregnancies. Both offspring are heterozygotes and now normal adolescents.

4.3 The Issue of Carnitine Supplementation

This is the first study to evaluate the risk of carnitine supplementation in FOD patients. Carnitine has been largely avoided in these patients mainly due to animal studies suggesting that carnitine might elevate long-chain acylcarnitine levels (*C16AC*) and cause arrhythmias or exacerbate cardiomyopathy. [1, 20] These animal studies investigated the effect of extreme levels of palmitoylcarnitine *without* carnitine supplement. [21–25] The presumed danger of long-chain acylcarnitines for LC-FOD patients does not reflect their actual fate in mitochondria. After crossing into mitochondria, long-chain acylcarnitines are re-activated to long-chain acyl-CoA intermediates by CPT II. Carnitine levels were already decreased in these patients. Extreme elevations, due to the β-oxidation defects, of long-chain acyl-CoA compounds like palmitoyl-CoA, deplete mitochondrial CoA during fasting or illness. A recent study with the LCAD$^{-/-}$ mouse model that develops hypertrophic cardiomyopathy

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

with lipid deposition and hypoglycemia when fasted demonstrated the benefit of carnitine supplementation with TH: Myocardial triglyceride content was reduced, cardiac performance was preserved without arrhythmia by exporting toxic acyl-CoA intermediates as acylcarnitines and there was no increased accumulation of long-chain acylcarnitines. [26] The energy deficiency based on reduced CAC intermediates and low levels of C3AC (*C3-CoA*) also responded to anaplerosis in this mouse model [27] as was observed with the CACT patient in crisis. (Fig 3)

In this patient study, free carnitine levels from 35 patients originally receiving the MCT diet were significantly lower compared to their normal levels on the TH diet with carnitine. ($P < 0.006$) C3AC levels were also reduced in those receiving MCT compared to those with TH and carnitine reflecting the need for more C3-CoA for anaplerosis. ($P < 0.0001$) Arrhythmia or exacerbation of cardiomyopathy did not occur in any of the patients in this clinical trial. The level of C16AC did not increase in infant VLCAD-C-9 and actually decreased in infant LCHAD-5 and both CACT patients while receiving carnitine. (Fig 2, 3) Long-chain acylcarnitines were not persistently elevated in any of the other patients including the 21 VLCAD patients. (Supplement File)

We conclude that carnitine supplement does not elevate long-chain acylcarnitines in LC-FOD patients on the TH diet. Instead, carnitine supplement facilitates export of excess toxic long-chain acyl-CoA intermediates as acylcarnitines and preserves levels of free CoA.

4.4 Serum Creatinine Levels: A Methylation Problem?

Decreased creatinine levels have lead to identification of many defects affecting creatine biosynthesis and transport in humans. [28] However, a methylation problem has not been suspected or demonstrated in association with LC-FOD patients. In this study, creatinine levels were significantly decreased in FOD patients on the MCT and TH diets ($P < 0.0001$). Creatine biosynthesis and therefore creatinine production, utilizes ~ 70% of the total labile methyl groups in the body from S-adenosyl-L-methionine (*SAM*) as methyl donor. Synthesis of SAM, creatine, and creatine-PO_4 require large amounts of ATP. [28] In an energy deficit, as proposed for LC-FOD, it seems reasonable to consider that the requirement for ATP and SAM may not be sufficient for efficient creatine biosynthesis during severe illness. The possibility may exist that the availability of creatine-PO4 would also be reduced and exacerbate the energy deficit in both skeletal and cardiac muscle and contribute to rhabdomyolysis and/or cardiomyopathy during illness. The TH diet with carnitine had no effect on this abnormality in any of the patients. Unfortunately, since a methylation problem was not suspected, routine measurements of creatine, SAM, SAH, etc. were not included in this study.

4.5 Comparison of Clinical Complications and Mortality

Patients treated with conventional diets before the TH trial did not routinely receive carnitine supplement. The frequency of each clinical complication was markedly reduced with the TH diet and carnitine compared with conventional diets. ($P < 0.0001$) (Table 2) Acidosis, hypoglycemia, and hyperammonemia did not occur during the TH trial. A recent retrospective chart review study of 20 of these same patients concluded that extension of the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

TH diet for three years reduced days required in hospital for clinical complications. [29] Recent animal studies with TH report significant benefit for serious complications such as correction of ventricular hypertrophy and pressure overload in rats [30], relief of ischemic stroke [31] and reduced seizures [32]. These observations indicate that an unrecognized energy deficiency exists in broad areas of disease and may also benefit from TH and carnitine therapy in humans.

Mortality for LC-FOD patients receiving conventional diet therapy has not improved over the past decade. Previous reports of mortality included many more patients with the neonatal-onset form of CPT II deficiency and CACT patients and did not describe the causes of death. [2–4] The TH trial included only 1 patient with neonatal CPT II and two patients with CACT deficiency. This difference made comparison unequal. For example, mortality in the previously reported studies was 51.2% [2], and 65.1% [3,4] and was reduced to 11.5% in the TH trial. (Table 3)

In order to equate these mortality rates, comparison was also examined between the previous studies and the TH trial only with CPT-I, late-onset CPT II, VLCAD, and LCHAD/TFP deficiencies. The adjusted mortality rates were only slightly reduced from 51.2% to 45% [2] and from 65.1 % to 60 % [3,4] compared to 8% for the 49 patients in the TH trial. The mortality rate with the TH trial was significantly reduced by either analysis. ($P < 0.0001$)

When the cause of death due to surgical/medical malpractice (*VLCAD-3 & TFP-5*), and parental withdrawal of all therapy (*CACT-1 & CACT-2*) were excluded from this analysis, the mortality rate due to metabolic failure despite being treated with TH and carnitine was only 3.8%.

Conclusions

1.) This study supports the existence of an energy deficiency in LC-FOD patients that can be corrected more effectively and safely with anaplerotic diet therapy and carnitine supplement than with conventional diet therapy. Clinical complications were reduced from ~ 60% with conventional diet treatment to 10 % with TH and carnitine and mortality reduced from ~ 65 % to 3.8 %. 2.) Carnitine supplementation was uncomplicated and did not increase long-chain acylcarnitine levels and is important for maintaining mitochondrial homeostasis during illness. 3.) TH interventions with infants were safe and could be implemented following early diagnosis by either expanded newborn screening or pre-natal diagnosis. 4.) Enteric delivery of TH was anaplerotic for all patients even during crisis. 5.) Evidence for an associated methylation defect, unaffected by anaplerosis, was observed in all patients and may reflect reduced creatine-PO_4 availability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We wish to thank Dr. Wolf-Ulrich Brewer and Barbara Pagliocca, formerly of SASOL GmbH, Witten, DE for providing TH oil and consultations for this investigation. Special appreciation is expressed to Mary Wallace for her

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

exemplary assistance in dietary design and patient management and to Drs. Larry Sweetman, Teodoro Bottiglieri, Diane Roe, Jiahuan Ding, and Bing-Zhi Yang. We give special thanks to Dr. Gerry Vockley for accepting 20 of these patients in 2009 who would otherwise have been unable to continue TH therapy. Funding for this study was provided from the Baylor Health Care System Foundation, and generous donations from Mr. William L. Hutchison.

ABBREVIATIONS

CAC	*citric Acid Cycle*
LC-FOD	*Long-Chain Fat Oxidation Disorder(s)*
CoA	*co-enzyme A*
EMS	*electrospray mass spectrometry*

Enzyme Deficiencies

CPT-I	*carnitinepalmitoyl transferase I*
CACT	*carnitine-acylcarnitine translocase*
CPT II	*carnitinepalmitoyl transferase II*
VLCAD	*very-long chain acyl-CoA dehydrogenase*
VLCAD-C	*cardiac phenotype*
TFP	*trifunctional protein*
LCHAD	*long-chain 3-hydroxyl acyl-CoA dehydrogenase*
MCT	*medium-chain triglyceride(s)*
TH	*triheptanoin*

Acylcarnitines

C2AC	*acetyl-*
C3AC	*propionyl-*
C7AC	*heptanoyl-*
C8AC	*octanoyl-*
C16AC	*palmitoyl-*
C18:1AC	*oleoyl-*
C16-OHAC	*3-hydroxy-palmitoyl-*

Free Fatty Acids

C7	*heptanoate*
C8	*octanoate*

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Urinary Metabolites

OAA	*oxaloacetate*
BHP	*3-hydroxypentanoate*
BKP	*3-ketopentanoate*
BHB	*3-hydroxybutyrate*
AcAc	*acetoacetate*
Cr	*creatinine*
GGT	*gammaglutaryltransferase*
SAM	*s-adenosyl-methionine*
SAH	*s-adenosyl-homocysteine*

REFERENCES

1. Spiekerkoetter U, Lindner M, Santer R, Grotzke M, Baumgartner MR, Boehles H, Das A, Haase C, Henermann JB, Karall D, de Klerk H, Knerr I, Koch HG, Plecko B, Roschinger W, Schwab KO, Scheible D, Wijburg FA, Zschocke J, Mayatepek E, Wendel U. Management and outcome in 75 individuals with long chain fatty acid oxidation defects: results from a workshop. J Inherit Metab Dis. 2009; 32:488–497. [PubMed: 19399638]
2. Saudubray JM, Martin D, de Lonlay P, Touati G, Poggi-Travert F, Bonnet D, Jouvet P, Boutron M, Slama A, Vianey-Saban C, Bonnefont JP, Rabier D, Kamoun P, Brivet M. Recognition and management of fatty acid oxidation defects: a series of 107 patients. J Inherit Metab Dis. 1999; 22:488–502. [PubMed: 10407781]
3. Baruteau JP, Sachs P, Broué P, Brivet M, Abdoul H, Vianey-Saban C, Ogier de Baulny H. Clinical and biological features at diagnosis in mitochondrial fatty acid beta-oxidation defects: a French pediatric study of 187 patients. J Inherit Metab Dis. 2013; 36:795–803. [PubMed: 23053472]
4. Baruteau JP, Sachs P, Broué P, Brivet M, Abdoul H, Vianey-Saban C, Ogier de Baulny H. Clinical and biological features at diagnosis in mitochondrial fatty acid beta-oxidation defects: a French pediatric study of 187 patients. Complementary Data (Letter to the Editors). J Inherit Metab Dis. 2014; 37:137–139. [PubMed: 23807318]
5. Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Effective dietary treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. J Clin Invest. 2002; 110:259–269. [PubMed: 12122118]
6. Roe CR, Yang BZ, Brunengraber H, Roe DS, Wallace M, Garritson BK. Carnitine palmitoyltransferase II deficiency: successful anaplerotic diet therapy. Neurology. 2008; 71:260–264. [PubMed: 18645163]
7. Roe CR, Bottiglieri T, Wallace M, Arning E, Martin A. Adult polyglucosan body disease (*APBD*): Clinical Benefits with anaplerotic diet therapy and identification of secondary methylation deficits. Mol Gen Metab. 2010; 101:246–252.
8. Pascual JM, Peiying L, Mao D, Kelly DI, Hernandez A, Sheng M, Good LB, Ma Q, Marin-Valencia I, Zhang X, Park JY, Hynan LS, Stavinoha P, Roe CR, Lu H. Triheptanoin for glucose transporter type 1 deficiency (G1D): modulation of human ictogenesis, cerebral metabolic rate, and cognitive indices by a food supplement. JAMA Neurol. 2014; 71:1255–1265. [PubMed: 25110966]
9. Leclerc J, Des Rosiers C, Montgomery JA, Bruunet J, Ste-Marie L, Reider MW, Fernandez CA, Powers L, David F, Brunengraber H. Metabolism of R-beta-hydroxypentanoate and of beta-ketopentanoate in conscious dogs. Am J Physiol. 1995; 268:E446–E452. [PubMed: 7900792]
10. Deng S, Zhang GF, Kasumov T, Roe CR, Brunengraber H. Interrelations between C4 ketogenesis, C5 ketogenesis, and anaplerosis in the perfused rat liver. J Biol Chem. 2009; 284:27799–27807. [PubMed: 19666922]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

11. Struys EA, Jansen EE, de Meer K, Jakobs C. Determination of S-adenosylmethionine and S-adenosylhomocysteine in plasma and cerebrospinal fluid by stable-isotope dilution tandem mass spectrometry. Clin Chem. 2000; 46:1650–1656. [PubMed: 11017945]
12. Sweetman, L. Organic acid analysis. In: Hommes, FA., editor. Techniques in Diagnostic Human Genetics: A laboratory Manual. New York: Wiley-Liss; 1991. p. 143
13. Vianey-Saban C, Divry P, Roe CR, Nada MA, Brivet M, Zabot MT, Mathieu M. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: Clinical characteristics and diagnostic considerations in 30 patients. Clin Chim Acta. 1998; 269:43–62. [PubMed: 9498103]
14. Roe DS, Yang BZ, Vianey-Saban C, Struys E, Sweetman L, Roe CR. Differentiation of long-chain fatty acid oxidation disorders using alternative precursors and acylcarnitine profiling in fibroblasts. Mol Gen Metab. 2006; 87:40–47.
15. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012; 13:251–262. [PubMed: 22436748]
16. Brunengraber H, Roe CR. Anaplerotic Molecules: Current and Future. J Inherit Metab Dis. 2006; 29:327–331. [PubMed: 16763895]
17. Brunengraber H, Boutry M, Lowenstein JM. Fatty acid and 3-β-hydroxysterol Synthesis in the perfused rat liver. J Biol Chem. 1973:2656–2669. [PubMed: 4697388]
18. Ackrell BA, Kearney EB, Mayr M. Role of oxalacetate in the regulation of mammalian succinate dehydrogenase. J Biol Chem. 1974; 249:2021–2027. [PubMed: 4818821]
19. Muller FL, Liu Y, Abdul-Ghani MA, Lustgarten MS, Bhattacharya A, Jang YC, Van Remmen H. High rates of superoxide production in skeletal-muscle mitochondria respiring on both complex I- and complex II-linked substrates. Biochem J. 2008; 409:491–499. [PubMed: 17916065]
20. Tein I. Disorders of fatty acid oxidation. Handb Clin Neurol. 2013; 113:1675–1688. [PubMed: 23622388]
21. Cox KB, Hamm DA, Millington DS, Matern D, Vockley J, Rinaldo P, Pinkert CA, Rhead WJ, Lindsey JR, Wood PA. Gestational, pathologic and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. Hum Mol Genet. 2001; 10:2069–2077. [PubMed: 11590124]
22. 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. [PubMed: 18317232]
23. Inoue D, Pappano AJ. L-Palmitoylcarnitine and calcium ions act similarly on excitatory ionic currents in avian ventricular muscle. Circ Res. 1983; 52:625–634. [PubMed: 6305529]
24. Mak IT, Kramer JH, Weglicki WB. Potentiation of free radical-induced lipid peroxidative injury to sarcolemmal membranes by lipid amphiphiles. J Biol Chem. 1986; 26:1153–1157. [PubMed: 3003057]
25. Spedding M, Mir AK. Direct activation of Ca^{++} channels by palmitoyl carnitine, a putative endogenous ligand. Br J Pharmacol. 1987; 92:457–468. [PubMed: 2445406]
26. Bakermans AJ, van Weeghel M, Denis S, Nicolay K, Prompers JJ, Houten SM. Carnitine supplementation attenuates myocardial lipid accumulation in long-chain acyl-CoA dehydrogenase knockout mice. J Inherit Metab Dis. 2013; 36:973–981. [PubMed: 23563854]
27. Bakermans AJ, Dodd MS, Nicolay K, Prompers JJ, Tyler DJ, Houten SM. Myocardial energy shortage and unmet anaplerotic needs in the fasted long-chain acyl-CoA dehydrogenase knockout mouse. Cardiovascular Research. 2013; 100:441–449. [PubMed: 24042017]
28. Wyss M, Kaddurah-Daouk R. Creatine and Creatinine Metabolism. Physiological Reviews. 2000; 80:1107–1213. [PubMed: 10893433]
29. Vockley J, Marsden D, McCracken E, DeWard S, Barone A, Hsu K, Kakkis E. Long-term major clinical outcomes in patients with long chain fatty acid oxidation disorders before and after transition to triheptanoin treatment—A retrospective chart review. Mol Genet Metab. 2015; 116:53–60. [PubMed: 26116311]
30. Nguyen TD, Shingu Y, Amorim PA, Schwarzer M, Doenst T. Triheptanoin Alleviates Ventricular Hypertrophy and Improves Myocardial Glucose Oxidation in Rats With Pressure Overload. (J Card Failure ahead of print.). http://dx.doi.org/10.1016/j.cardfail.2015.07.009.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

31. Schwarzkopf TM, KOCH K, Klein J. Reduced severity of ischemic stroke and improvement of mitochondrial function after dietary treatment with the anaplerotic substance triheptanoin. Neuroscience. 2015; 300:201–209. [PubMed: 25982559]

32. Hadera MG, Smeland OB, McDonald TS, Tan KN, Sonnewald U, Borges K. Triheptanoin partially restores levels of tricarboxylic acid cycle intermediates in the mouse pilocarpine model of epilepsy. J Neurochem. 2014; 129:107–119. [PubMed: 24236946]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

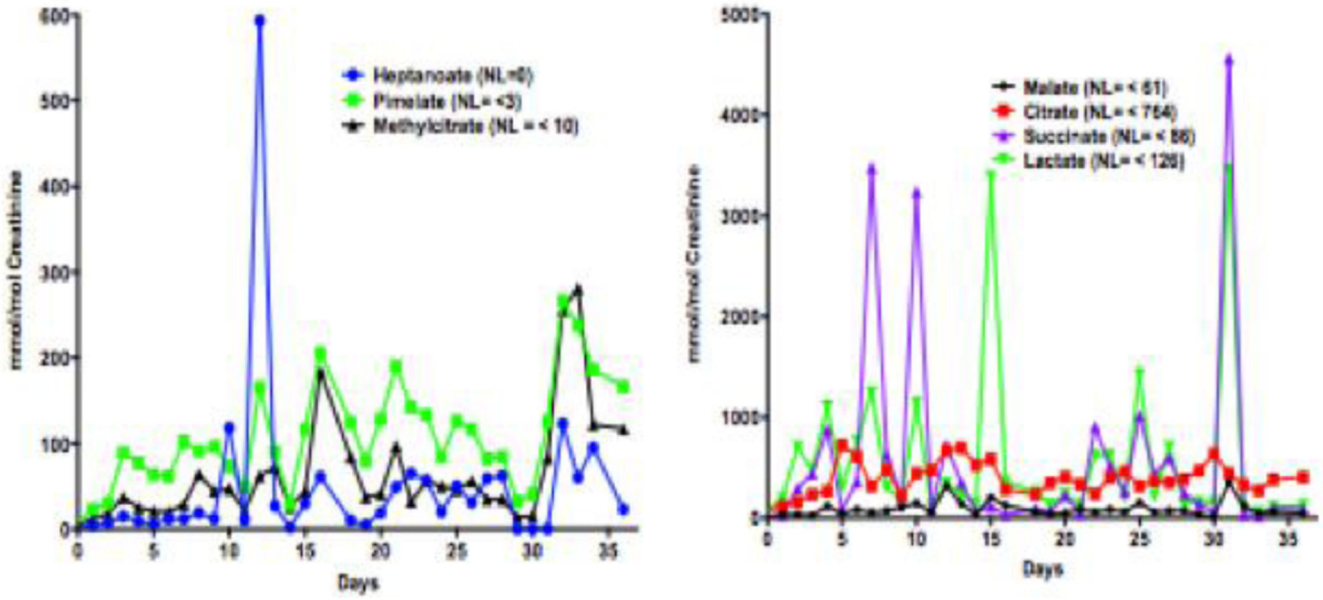

Fig1. TH intervention at Birth in CACT Deficiency

Left Panel: Intermittent increases in heptanoate, pimelate and methylcitrate excretion reflected transient problems with mitochondrial entry of heptanoate and increased excretion of methylcitrate from enhanced propionyl-CoA utilization in the citrate synthase reaction.

Right Panel: Extreme and often simultaneous elevations of succinate and lactate were observed only in this neonate.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

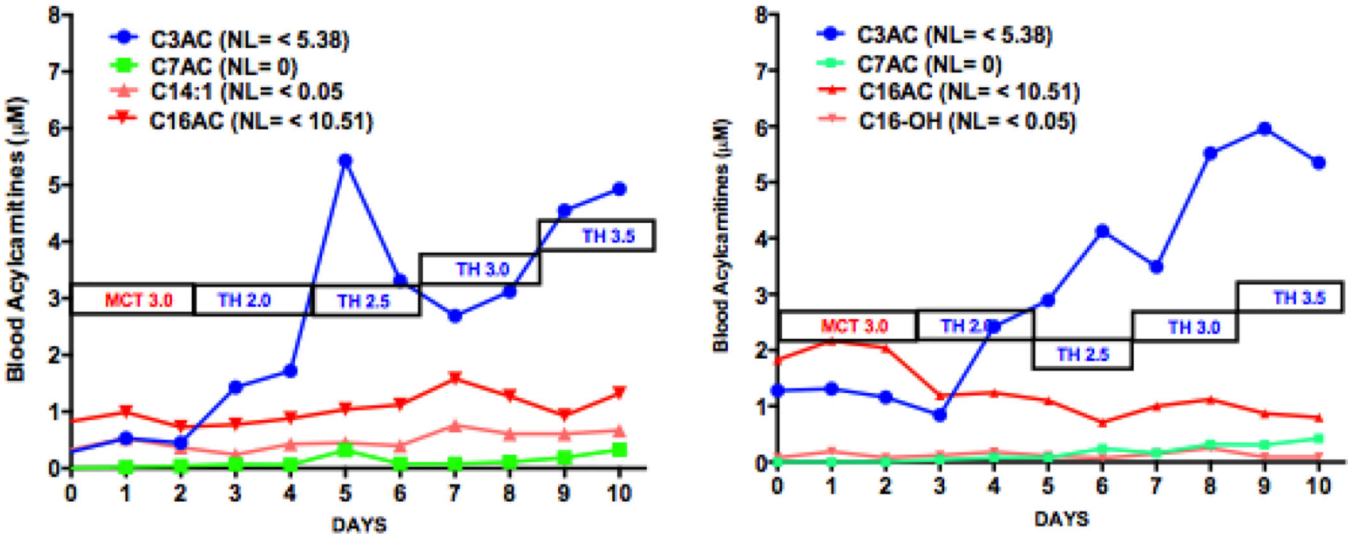

Fig2. Acylcarnitine Analysis of TH Interventions during Infancy

Both infants were initially treated with the MCT diet (*3.0 gm/kg/day*) and carnitine supplement (*100 mg/kg/day*). The TH dose was then increased from 2.0 to 3.5 gm/kg/day with continued carnitine supplement. **Left Panel:** Patient VLCAD-C-9 at 22 days of age. **Right Panel:** Patient LCHAD-5 at 15 days of age.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

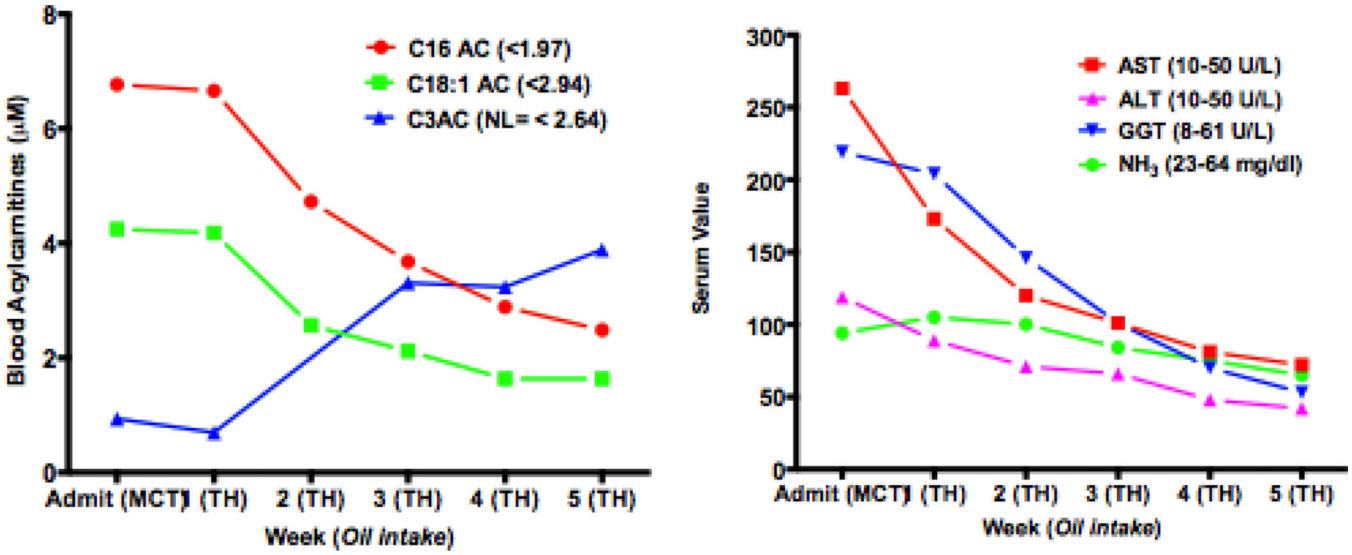

Fig3. Effect of Triheptanoin during Metabolic Crisis in CACT deficiency

Blood levels of acylcarnitines, ammonia and liver enzymes were obtained while initially treated with MCT and subsequently with TH during this 5 week admission. **Left Panel:** The diagnostic acylcarnitines, C16AC and C18:1AC progressively decreased and C3AC increased as the TH dose was increased. **Right Panel:** Serial serum liver enzyme and ammonia levels decreased to near normal levels.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

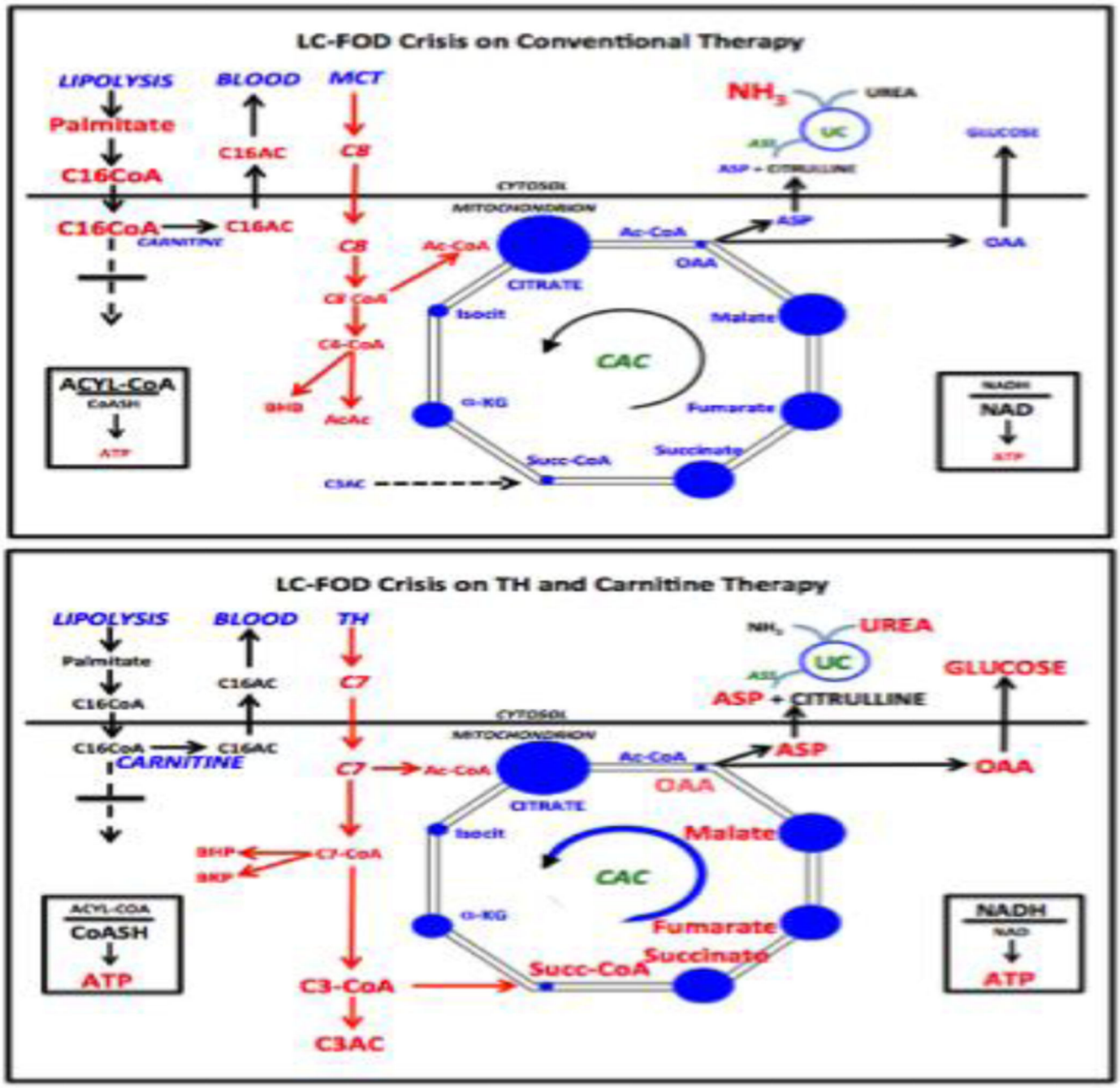

Fig 4. Proposed Biochemical Alterations during Illness in LC-FOD Patients

During illness, lipolysis is activated, blood levels of long-chain acylcarnitines are increased, and acidosis, hypoglycemia, and hyperammonemia can occur due to insufficient anaplerosis and availability of intra-mitochondrial free CoA is reduced (*Upper Panel*). The TH Diet with carnitine supplement would replenish CAC intermediates and stimulate CAC function and ATP production, enhance gluconeogenesis and urea cycle function and maintain intra-mitochondrial homeostasis (*Lower Panel*). (*ASP = aspartate; blue spheres represent the relative sizes of the individual CAC intermediate pools. Turnover of the largest citrate pool*

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

is ~ 5–10 times/minute compared to the highest turnover of 100–200 times/minute of the OAA pool. [16, 17])

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Description of Patients in the Triheptanoin (TH) Diet Protocol

FOD	*Patients*	*Age at Entry (years)*	*Prior Diet*		*TH Intake (months)*
			MCT	**Low Fat**	
CPT-I	2	6, 7	0	2	7
CACT	2	birth, 0.5	1	0	5, 11
CPT II	11	2 – 51	6	5	4 – 43
VLCAD	21	0.2 – 36	16	5	9 – 73
TFP	6	2 – 9	6	0	7 – 29
LCHAD	10	0.1 – 24	9	1	8 – 84
Total:	*52*	*BIRTH to 51 YEARS*	*38*	*13*	*BIRTH to 7 YERAS*

Mol Genet Metab. Author manuscript; available in PMC 2016 December 01.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Clinical Complications with Prior Conventional and TH Diet & Carnitine

Symptom:	***Cardiac***		***Rhabdomyo***		***Weakness***		***Hypoglycemia***		***Hepatomegaly***	
Patients (#)	***Conv**	***TH**	**Conv**	**TH**	**Conv**	**TH**	**Conv**	**TH**	**Conv**	**TH**
CPT-1 (2)	0	0	0	0	2	0	2	0	2	0
CACT (2)	2	0	1	1	2	0	0	0	1	1
CPT-II (11)	1	0	6	1	7	0	4	0	2	0
VLCAD (21)	14	1	21	10	21	3	11	0	13	1
TFP (6)	1	0	6	3	6	2	1	0	1	0
LCHAD (10)	0	0	10	1	10	1	4	0	5	1
TOTAL:	*18*	*1*	*44*	*16*	*48*	*6*	*22*	*0*	*24*	*3*
Frequency on Diets:	*35%*	*2%*	*85%*	*31%*	*92%*	*12%*	*42%*	*0%*	*46%*	*6%*

* Conv = Conventional diet (MCT and/or Low Fat-High Carbohydrate;

* TH= Triheptanoin diet

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3

Mortality in FOD Patients: Conventional vs. Triheptanoin Therapy

Study:	**Saudubray 1999 [2]**		**Baruteau 2013 [3.4]**		***TH Diet Therapy**	
FOD Patients	**Patients**	****Died**	**Patients**	****Died**	**Patients**	*****Died**
CPT-1	4	0	4	1	2	0
CACT	5	5	13	12	2	2
CPT-II (Neonatal)	5	4	15	10	1	0
CPT-II (Late-onset)	5	0	0	0	10	0
VLCAD	8	6	33	20	21	2
LCHAD + TFP	14	6	41	26	16	2
Total:	*41*	*21*	*106*	*69*	*52*	*6*
Overall Mortality:	51.2%		65.1%		11.5%	
Metabolic Death:	Not Indicated		Not Indicated		3.8% (2 patients)	

*No deaths due to TH Supplementation

**Convrntional Therapy [2–4]: No cause of death provided;

***Surgical/Medical malpractice (2); withdrawal of all Therapy (2); Metabolic Failure on TH Diet Therapy (2)