THE METABOLIC RESPONSE TO FASTING IN CHILDREN HOMOZYGOUS FOR THE c.1436C→T VARIANT OF THE *CPT1A* GENE

by

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ABBREVIATIONS :

CPT1A – Carnitine Palmitoyltransferase 1A (italic indicates reference to the gene)

CPT1A - Carnitine Palmitoyltransferase 1A (not italic indicates reference to the protein)

ACC - acetyl Co-A carboxylase

MCAD – Medium chain acyl-CoA dehydrogenase deficiency

LCHAD- long chain 3 hydroxy acyl-CoA dehydrogenase deficiency

VLCAD – very long-chain acyl-CoA dehydrogenase deficiency

YSI - Yellow Springs Instrument glucose oxidase analyzer

THL - Tetrahydrolipostatin

FFA – Free Fatty Acids

KB – ketone bodies

3-OH-B- 3 hydroxybutyrate

AC/AC-acetoacetate

FAOD - fatty acid oxidation disorders

UNITS:

mg/dL- milligrams per deciliter

mmol/L – millimole per liter

umol/L – micromole per liter

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ABSTRACT

THE METABOLIC RESPONSE TO FASTING IN CHILDREN HOMOZYGOUS FOR THE c.1436C→T VARIANT OF THE *CPT1A* GENE

Background: Carnitine Palmitoyltransferase 1A (CPT1A) catalyzes the rate limiting step for hepatic fatty acid oxidation and is critical for normal ketogenesis during fasting. Through advances in newborn screening by tandem mass spectrometry(MS/MS), a sequence variant in the *CPT1A* gene that decreases the enzyme's activity in the liver has been identified in the Native Alaskan population as well as other Arctic populations. Over the past four years, over 300 Native Alaska infants have been identified with this specific sequence variant of *CPT1A* by newborn screening. All are homozygous for the same c. 1436C→T variant in *CPT1A*, resulting in approximately an 80% reduction in CPT1A activity. The clinical implications of the c.1436C→T variant of *CPT1A* are unknown. The goal of this study was to determine the metabolic response to fasting in Native Alaskan children homozygous for the c.1436C→T sequence variant in *CPT1A*.

Method: Recruitment of children between 3-5 years of age that had been identified with the specific c.1436C→T variant of *CPT1A* via newborn screening was done in collaboration with the Alaska Department of Health and Social Services. Subjects were admitted to the inpatient research unit in Doernbecher Children's Hospital for evaluations

which included an 18 hour medically supervised fast. Fasting began at approximately 6:00pm, after the participants were given an ad lib diner at 5:00pm. Glucose, ketone bodies (KB), free fatty acid (FFA) and the ratio of FFA to KB (FFA/KB) were measured over the course of the fast, and levels were compared to published fasting data from age-matched controls.

Results: Two of the subjects became hypoglycemic during the fast (blood glucose less than 55 mg/dL), and developed symptoms of hypoglycemia including lethargy. Hypoglycemic symptoms were quickly resolved when the subjects were administered an intravenous solution of 25% Dextrose (D_{25}) along with initiation of oral carbohydrate consumption. Among the other 3 subjects, there was no difference in mean glucose or free fatty acid concentrations between the control children and the children with the c.1436C→T variant of *CPT1A*. The maximum ketone (KB) production in children with the c.1436C→T variant of *CPT1A* was approximately 10% of that in control children (0.233 mmol/L vs. 2.4mmol/L) .The average FFA/KB ratio was significantly elevated in children with the c.1436C→T variant of *CPT1A* (4.9 at 12 hrs; 7.6 at 18 hrs), compared to controls (<2 at 12 and 18 hours), suggesting an inability of these children to utilize fats for energy during the fast.

Discussion: Children with the c.1436C→T variant of *CPT1A* had reduced ketone production and an elevated FFA/KB ratio in response to fasting, indicating that homozygosity for the c.1436C→T sequence variant impairs the normal ketone production in the liver in response to fasting. Two of the participants developed

hypoketotic hypoglycemia before the 18 hour completion of the fast. These two subjects had final FFA/KB ratios of 4.1 and 16. The ratio in the participants who did not develop hypoketotic hypoglycemia was 6.3, 8.7, and 7.07. One of the two participants who developed hypoketotic hypoglycemia was the youngest participant in the study, 3.5 years old compared to and average age 4.6, this participant also had the lowest FFA/KB ratio. The cause of hypoketotic hypoglycemia can not be attributed to altered fatty acid mobilization, due to a normal rise in free fatty acid levels in children with the specific c.1436C→T variant of *CPT1A.*

Conclusion: Children with c.1436C→T variant of *CPT1A* have an altered response to fasting, presenting with hypoketosis as well as an increased risk for developing hypoketotic hypoglycemia. Thus, sequence variant c.1436C→T in the *CPT1A* gene impacts fasting tolerance, can present with clinical symptoms of hypoglycemia under conditions of environmental stress, and therefore should not be considered a benign variant.

Introduction

Through advances in newborn screening by tandem mass spectrometry (MS/MS) a sequence variant (c.1436C→T) in the carnitine palmitoyltransferase 1A (*CPT1A*) gene has been identified in the Native Alaska population. The c.1436C→T variant results in the substitution of a leucine for proline at amino acid position 479 (P479L) in the CPT1A protein, which is associated with an approximately 80% reduction in catalytic activity when assayed in patient fibroblasts (2). This sequence variant is also prevalent in the Inuit populations of Canada and Greenland (6, 2). A recent study by Greenberg et al. suggests that children homozygous for this variant may be at risk of hypoglycemia with prolonged fasting or during times of stress, such as during infections (2). The clinical impact of this sequence variant on the metabolic response to fasting is the focus of this study.

Over the past four years, over 300 Alaska Native infants have been identified with the c.1436C→T variant of *CPT1A* via MS/MS(1). Prior to this testing only 30 published cases of CPT1A deficiency were known worldwide (1). In contrast to complete loss of function (0% catalytic activity) as seen in classic CPT1A deficiency, Native Alaskan infants identified with the specific c.1436C→T variant of *CPT1A* have approximately a 20% residual CPT1A activity (1).

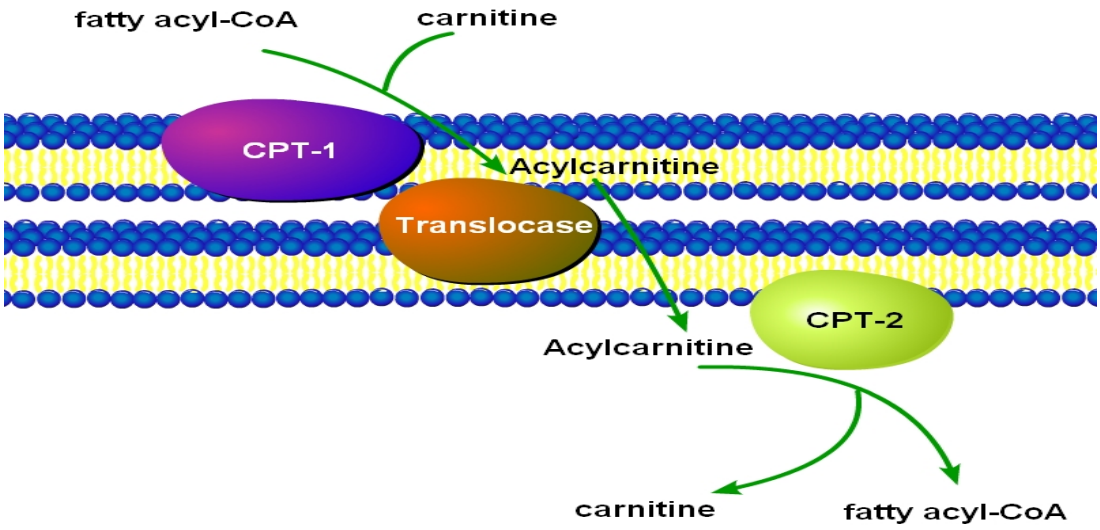
The clinical implications of the c.1436C→T variant of *CPT1A* are currently unknown. There is no research data on the fasting tolerance of subjects with this variant to date. However, children with CPT1A deficiency due to mutations that result in near or complete loss of enzyme activity show an increased risk for hypoketotic hypoglycemia, liver dysfunction and sudden unexplained death (3). Currently there is insufficient evidence to determine if treatment is necessary or the best approach to treatment for children with the c.1436C→T variant of *CPT1A* which is prevalent in the Alaska Native population. Current treatment and management of children with the c.1436C→T sequence variant is based on data from patients with severe forms of CPT1A deficiency and other fatty acid oxidation disorders (FAOD). This may not be appropriate for infants and children who are homozygous for the c.1436C→T variant of the *CPT1A* (1). The goal of this study was to collect physiologic data on affected children that can be used to aid in the development of evidence-based treatment guidelines.

Background and Significance

Fatty Acid Beta-Oxidation

Fatty acid oxidation occurs in several different tissues in the body, including the muscle, adipose and liver. There are three isoforms of CPT1 expressed from 3 different genes which include liver-type (CPT1A), muscle-type (CPT1B), and brain-type (CPT1C) (4). Only CPT1A deficiency has been recorded in humans (4).

Fatty acid metabolism begins with the fatty acid crossing into the mitochondrial matrix in the liver to undergo beta-oxidation. The first step of bringing long chain fatty acids into mitochondria requires CPT1 (3). CPT1 catalyzes the synthesis of long chain fatty acylcarnitine esters from fatty acyl-CoAs and free carnitine, and serves as the rate limiting step for fatty acid oxidation (4). The carnitine/acyl-carnitine transferase (CACT) then moves the acylcarnitine into the mitochondrial matrix (5). Once inside, CPT2 catalyzes the reverse of the CPT1 reaction, producing fatty acyl-CoAs that subsequently undergo beta-oxidation (4). Fatty acid beta oxidation produces acetyl-CoA for oxidative phosphorylation or ketone body production in the liver (4).



CPT1A is the first control point for regulation of hepatic beta oxidation. In the fed state, glucose serves as the primary energy source in the liver, and acetyl Co-A carboxylase (ACC) catalyzes the formation of malonyl-CoA. Malonyl-CoA binds to a site on CPT1A and reduces its activity, directing fatty acids towards triglyceride production and storage rather than oxidation (3). When ACC activity decreases, such as in a fasting state, malonyl Co-A levels also decrease, resulting in an activation of CPT1A. This allows fatty acids to enter the mitochondrial matrix to be oxidized, providing acetyl CoA for ATP production and the synthesis of ketone bodies (3).

Malonyl CoA is known to interact and suppress the activity of all three isoforms of CPT1. CPT1A contains two allosteric binding sites for malonyl-CoA, which are distinct from the carnitine binding site (6,2). Mutations in the *CPT1A* gene that result in changes

in the protein structure of the enzyme can either enhance or inhibit the interaction between CPT1A and malonyl-CoA (7). The specific c.1436C→T variant of *CPT1A* results in a structural change effecting the function and interaction of the protein with malonyl-CoA (6). This structural change does not inhibit CPT1A completely (6), but rather affects the affinity of CPT1A for malonyl-CoA, limiting malonyl-CoA's inhibitory effect on CPT1A while preserving some limited function of the protein (2, 6).

Glucose Homeostasis:

Glucose from a meal is used throughout the body to supply cellular energy. During the fed state, and in the presence of adequate oxygen, glucose is oxidized to ATP via two processes, glycolysis and the Krebs cycle to provide ATP. To remain in homeostasis, excessive glucose can enter the liver and muscle and be stored as glycogen or converted into fatty acids and stored as triglycerides. This stored glucose can be used at a later time when the body needs energy. To maintain glucose homeostasis when fasting, glucose is synthesized in the liver via gluconeogenesis. In addition glycogen is broken down via glycogenolysis to provide glucose.

Some tissues, such as skeletal muscle, can use fatty acids for energy which spares glucose for tissues in which it is the preferred energy source. Free fatty acids derived from the hydrolysis of triglycerides are released from adipose tissue and then are transported to the liver and muscle to be used as energy. In the liver, fatty acids are also oxidized to

produce ketones. Some tissues, such as those from the central nervous system, are able to use ketones instead of glucose.

When fasting, a typical metabolic response will provide adequate energy and prevent hypoglycemia from occurring (8). Hypoglycemia is defined based on the age of the child, and blood glucose. A blood glucose of <55 mg/dl in children and <35-45 mg/dl in neonates defines hypoglycemia (11). Hypoglycemia presents with some common signs and symptoms including irritability, tachycardia, tremors, lethargy, apnea, seizures, diaphoresis, anxiety, headache, tachypnea, weakness, confusion, stupor, ataxia, and coma (11). The normal physiologic response to decreased glucose production is increased mitochondrial fatty acid beta-oxidation and the production of ketones (11).

Diagnosis

Classically, patients with 100% loss of CPT1A activity are usually diagnosed within the first few days of an infant's life through tandem mass spectrometry (MS/MS) newborn screening (4). During times of inadequate intake, hypoketotic hypoglycemia has been identified in these patients, with low or absent urine ketones (4). Plasma carnitine levels are normal, or may be elevated (4). Diagnosis is based on the ratio of increased free carnitine, along with low levels of long chain acylcarnitine (C0/ C18+C16) resulting from the deficiency of CPT1A (4).

Several mutations of the *CPT1A* gene have been identified and there appears to be a correlation between the severity of the deficiency of CPT1A activity and the clinical symptoms, which include hypoketotic hypoglycemic, altered mental status and hepatomegaly (4). Elevated liver function tests and elevated free fatty acids have been identified in patients with classic CPT1A deficiency (4).

Recently a high rate of the c.1436C→T variant of *CPT1A* was identified among Native Alaskan infants. The Northwest Regional Newborn Screening Program has identified more than 300 infants with a high C0/C18 + C16 acylcarnitine ratio. Confirmatory tests found all infants were homozygous for the same DNA sequence variant c.1436C→T (3).

Fatty Acid Oxidation Disorders

Fatty acid oxidation disorders are a group of inherited metabolic conditions that lead to a decrease in the ability to use fatty acids for energy (3). Each fatty acid oxidation disorder is associated with a specific enzyme defect in the fatty acid metabolic pathway and affects utilization of dietary and stored fat (3). Most individuals diagnosed with a fatty acid oxidation disorder have elevated levels of free fatty acids and low levels of ketones during times of fasting (8). This has been documented in other forms of CPT1A

deficiency but is currently unknown among individuals with the c.1436C→T variant of *CPT1A*.

When the oxidation of fatty acids is defective free fatty acids are still released from adipose tissue during fasting. When they reach the liver, skeletal muscle and heart, they can accumulate if not utilized for energy (4). The inability of the liver to oxidize fats may result in steatosis and decreased production of ketones. Limited hepatic fatty acid oxidation decreases the energy available during fasting from two key energy sources; fatty acids and ketones, which increases the need for a constant supply of glucose and limits an individual's ability to fast (4).

Fasting guidelines are a mainstay of treatment for fatty acids oxidation disorders. Under normal circumstances children have an accelerated physiological response to fasting due to lower glycogen stores than adults (9). This shortens the time between utilizing glycogen stores and beginning fatty acid oxidation for energy. Fasting duration for normal children as well as children with fatty acid oxidation disorders increases as a child ages due to an increasing capacity to store glycogen (10).

Treatment

Fibroblasts from patients who are homozygous for the c.1436C→T variant have approximately 20% residual function of the enzyme (1). A higher residual enzyme

activity might suggest a milder clinical phenotype (1, 2). Individuals with classic CPT1A deficiency present with hepatomegaly and episodes of hypoglycemia (10). Individuals who are homozygous for c.1436C→T variant may or may not present with any of the symptoms of classic CPT1A deficiency (2). Therefore it is unknown if treatment is needed or if this is a benign variant (2).

Treatment for CPT1A deficiency includes avoiding fasting, and in some cases, treating with cornstarch at night when a diagnosed individual has a decreased intake such as during an illness. Utilizing dietary medium chain triglycerides and a carnitine supplement has been successful in other fatty acid oxidation disorders, although currently these treatments have not been investigated among subjects with CPT1A deficiency (1). Patients with CPT1A deficiency often have elevated free carnitine, so supplemental carnitine is not needed or advisable (10). Intervention during illness is crucial and can be life saving (13). Illness increases energy requirements, while decreasing appetite along with a potential energy loss from vomiting and/or diarrhea (1, 13).

Patients with CPT1A deficiency who develop mild hypoglycemia and who are capable of eating or drinking are treated with orange juice or some other carbohydrate containing beverages. Plasma glucose <45 mg/dl with or without symptoms requires treatment and intravenous glucose maybe required if oral intake is not possible.

Goal

During prolonged fasting, patients with fatty acid oxidation disorders have the potential for developing complications. Successful fasting guidelines have been followed with good outcomes for patients with VLCAD and MCAD deficiency. The general fasting guidelines for healthy children with MCAD and VLCAD are as follows; infants 0-4 months should fast for no longer than 4 hours, for each month thereafter an hour is added to the maximum fasting time (12). As a child ages, a normal eating schedule of meals and snacks should be followed, and any change in oral intake needs to be closely monitored (12).

It is currently unknown if prolonged fasting in children who are homozygous for the c.1436C→T variant of *CPT1A* increases their risk for metabolic decompensation or hypoglycemia. If fasting does place children at risk, a working tool such as the guidelines above may be beneficial for families with children who are homozygous for the c.1436C→T variant of *CPT1A*. Providing an understanding of the time limitations to fasting may help reduce the risk of hypoglycemia. The goal of this study will be to determine if fasting is abnormal for children who are homozygous for the c.1436C→T variant of the *CPT1A* gene and if fasting guidelines for treatment are necessary in this population (1).

Hypothesis and Aims

Hypothesis 1: Children who are homozygous for c.1436C→T sequent variant in the *CPT1A* gene will have an abnormal fasting response.

Aim 1: To characterize the metabolic response to fasting of Alaska Native children with the specific c.1436C→T sequent variant of *CPT1A* via a medically monitored fast.

Aim 2: To compare the fasting response of Alaska Native children with c.1436C→T variant of *CPT1A* to the fasting response of normal healthy children.

Methods

Overall study design: The metabolic response to a medically supervised fast will be assessed in children who are homozygous for c.1436C→T variant in the *CPT1A* gene. Results will be compared to published data on the fasting response in control children.

Patient Recruitment: In collaboration with the Alaska Department of Health and Social Services, children between the ages of 3-5 years old that were identified with the specific c.1436C→T variant of *CPT1A* by newborn screening from the Norton Sound region of Western Alaska were contacted to participate in the study (6).

Inclusion Criteria for children to participate in the study are as follows:

Identified with CPT-1 deficiency

homozygous for the c.1436C→T sequence variant of CPT1A

greater than 6 kg

otherwise healthy

Exclusion Criteria for children to participate in the study are as follows:

liver dysfunction

diabetes

renal disease

metal plate in body

The families, who were invited to participate received a letter from the Alaska State government about the study and were given the option to receive more information from Oregon Health and Science University (Appendix B). If a family agreed to participate in the study, consent was obtained from the parents/guardians and contact information for future travel arrangements were made (1). The family flew to Portland, Oregon for 4 days to participate in the supervised fasting study. The medically supervised fast occurred during days 2 and 3 of their stay and families left to return home on day 4 as long as the child was medically stable. This pilot study included five families with a child $\leq$5 years of age.

Metabolic Studies: Subjects were admitted to the Clinical & Translational Research Center (CTRC) inpatient unit in Doernbecher Children's Hospital for a medically supervised fast. This procedure carries some risk, but is a standard procedure used in the evaluation of children with unexplained hypoglycemia (1). After consuming a self-selected dinner meal, an IV of ½ normal saline was started after which subjects were allowed only water or other non-caloric beverages to drink during the eighteen hour fast. Blood glucose was measured by Yellow Springs Instrument glucose oxidase analyzer (YSI) at hour 6 and then every hour until completion of the eighteen hour fast, or until termination of the fast. If the blood glucose was < 40 mg/dl at any time during the study, then a blood sample was collected, and the fast terminated by giving $D_{25}W$ IV and feeding orally.

Each hour, a registered nurse (RN) drew approximately half a milliliter sample and provided this sample to be analyzed on site. The whole blood was centrifuged for eight minutes and then the serum was removed and stored in a clean microfuge tube. Each glucose sample was analyzed immediately after the sample was drawn using YSI and then recorded on a flow sheet to keep an accurate account of the hourly serum glucose in each subject.

Blood samples of 5mls were drawn at baseline, at 6 hours, 12 hours and 18 hours of the fast. One ml was analyzed for a basic metabolic panel, by the Oregon Health and Science University lab and results were retrieved through EPIC. One ml was used to analyze plasma ketones. The final 2mls were used to analyze the acylcarnitine profile, and serum free fatty acids. 2 mls were collected into a red top tube and were centrifuged and the plasma was collected and stored at -80 degrees Celsius until analyzed for ketones. 2 mls were collected into a purple top EDTA tube. 1 ml whole EDTA blood was moved to a tube containing added Tetrahydrolipostatin (THL). Both tubes were centrifuged. Plasma with THL was stored at -80 degrees Celsius until analyzed for free fatty acids. EDTA plasma was stored at -80 degrees Celsius until analyzed for acylcarnitines.

Ketones were analyzed by a GC/MS in the laboratory of Dr. James Shoemaker, St Louis University. Acylcarnitines were analyzed at the Biochemical Genetics Laboratory, Mayo Clinic, Rochester, MN using tandem mass spectrometry (MS/MS). Free Fatty Acids were

analyzed using a colorimetric assay kit from WACO. Results were compared to published normal fasting data.

Outcome Variables:

Key outcome variables were the time for blood glucose to drop to <40mg/dl, development of symptoms of hypoglycemia (termination of fast) or 18 hour blood glucose concentration, and free fatty acid (FFA) and ketone body (KB) levels, and the FFA/KB ratio.

Statistics:

This pilot study will use primarily descriptive statistics to characterize the fasting response among children who are homozygous for c.1436C→T variant of CPT1A gene. It is the first study of its kind looking at this specific variant of *CPT1A* and will be used to calculate variation and sample size for further studies.

Results:

For this study five children who had been identified via newborn screening as homozygous for the c.1436C→T variant of the *CPT1A* gene were fasted and blood samples were drawn during the fast at hours, 0, 6, 12, and 18 post-prandial. Each of the five subjects were fasted individually when in their normal state of health. The results were than compared to known control values from children of a similar age for each analyte.

Table 1: **Participant Demographics**

	Age	Weight (kg)	Height (cm)	BMI	Gender
Subject 1	4yrs 8 months	20.3	109	17.09	Male
Subject 2	3yrs 6 months	16.6	92	19.6	Male
Subject 3	4yrs 2 months	23.9	111.5	19.2	Male
Subject 4	4yrs 8 months	16.4	98	17.1	Male
Subject 5	4yrs 9 months	21.9	110.5	17.94	Female

BMI = body mass index calculated a kg/(m^2)

Glucose:

Three of the five subjects with the c.1436C→ variant of *CPT1A* were able to fast for the full 18 hours while maintaining normal glucose levels and without developing symptoms

of hypoglycemia (Fig 2). One subject, subject 5, became hypoglycemic (glucose = 51 mg/dl at 17.5 hours post prandial) and lethargic, and required IV glucose infusion. The fast was terminated at 16 hours for subject 2 due to lethargy and hypoglycemia (glucose = 24mg/dl at 15.5 hours post prandial), requiring IV glucose administration.

The individual change and the mean glucose values at the end of the fast were similar between subjects with c.1436C→ variant of *CPT1A* and published control data except subject 2, who fell to 24mg/dL. There was a fairly wide variation among both subjects with c.1436C→T variant of *CPT1A*, and the published normal controls.

Figure 2 Plasma Glucose during an 18 hour supervised fast

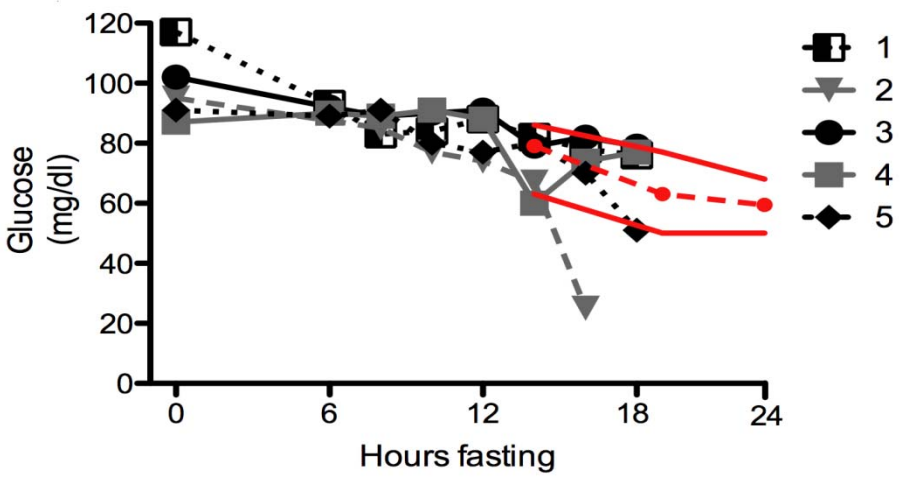


Figure 2 Legend: Change in plasma glucose in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 normal children age

1-7 years. Control data is presented as the mean (•----•----•) and the 10th and 90th percentiles (-) at 15, 20 and 24 hours of fasting (14).

Free Fatty Acids

The majority of FFA arises from lipolysis of triglycerides from adipocytes. During fasting, FFA's are expected to increase to provide an endogenous source of energy when there is no exogenous caloric intake (15). Under normal fasting conditions, FFA rise to approximately 3.32 mmol/L in a 24hr fast of control children (15).

The pattern and rate of increase in free fatty acids with fasting was similar between subjects with the c.1436C→T variant of *CPT1A* and controls. This suggests that children with the specific c.1436C→T variant of *CPT1A* are mobilizing fatty acids at a similar rate as control children.

Figure 3: Plasma Free Fatty Acids during an 18 hour supervised fast

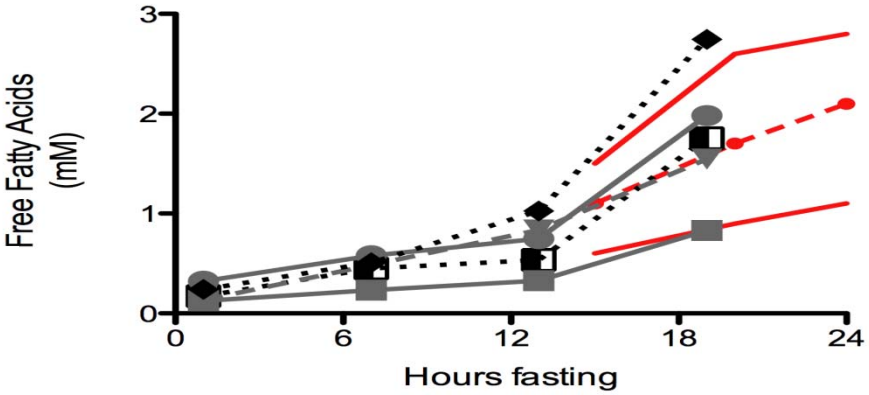


Figure 3 Legend: Change in plasma free fatty acids in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 normal children age 1-7 years. Control data is presented as the mean (•-----•-----•) and the 10^{th} and 90^{th} percentiles (-) at 15, 20 and 24 hours of fasting (14).

Ketones

Ketones were analyzed to assess the subjects' ability to utilize free fatty acids for ketone production, which provides an alternate source of energy when exogenous glucose is not available. In the literature, a ketone concentration of less than 1.5mmol/L at 20 hours post prandial has been used to diagnose hypoketotic states in children 7 years and younger (14).

Children with the c.1436C→T variant of *CPT1A* had plasma ketone concentrations less than 20% of normal children at similar time points. After 12 hours of fasting, subjects with the c.1436C→T variant of *CPT1A* had plasma ketone concentrations 5 to 10 times lower than concentrations reported in published normal controls.

In control children, when fasting glucose was less than 54mg/dL, ketones were always over 1.8mmol/L. In contrast, children with FAO disorders always had ketone concentrations less than 0.8mmol/L. Both of the subjects in our study who's glucose fell below 54mg/dL had ketone levels less than 0.8 mmol/L (0.37 and 0.17mmol/L).

Figure 4 Plasma Ketones during an 18 hour supervised fast

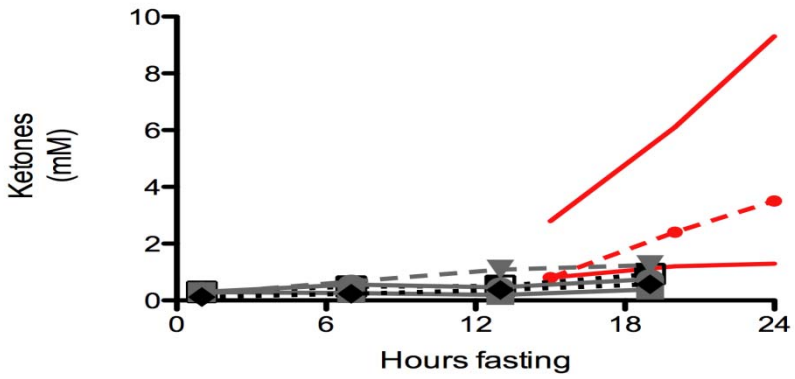


Figure 4 Legend: Change in plasma ketone concentrations in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 control children age 1-7 years. Control data is presented as the mean (•-----•-----•) and the 10^{th} and 90^{th} percentiles (-) at 15, 20 and 24 hours of fasting (14).

Ratios

A hypoketotic state in fasting has been defined as a ratio of FFA/ketone bodies (KB) greater than 2. Bonnefont et al reported that children with a FAOD had a ratio >2.5 while control children had a ratio <0.6 at the end of a fast (14). Children in our study had an

average FFA/KB ratio of 4.9 at 12hrs and 7.6 at 18 hrs. This represents a much greater FFA/KB ratio than control children.

Figure 5

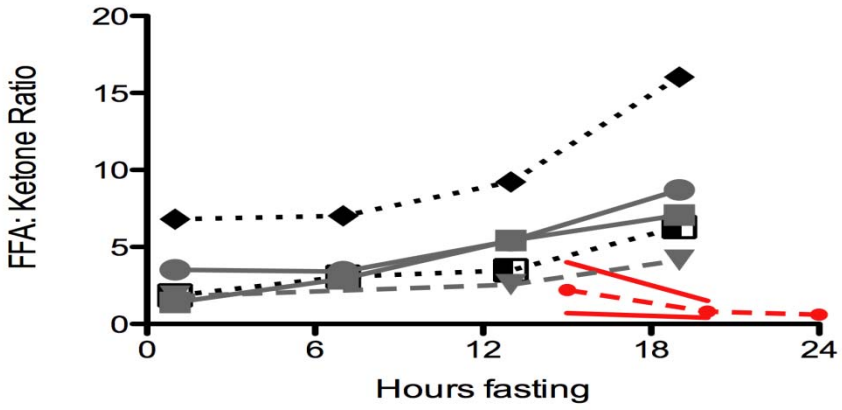


Figure 5 Legend: Change in FFA/KB ratio in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 control children age 1-7 years. Control data is presented as the mean (•----•----•) and the 10th and 90th percentiles (-) at 15, 20 and 24 hours of fasting (14).

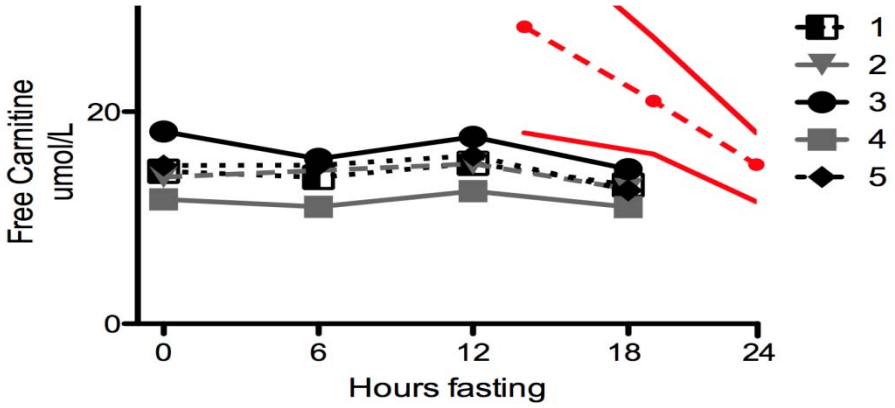
Carnitine

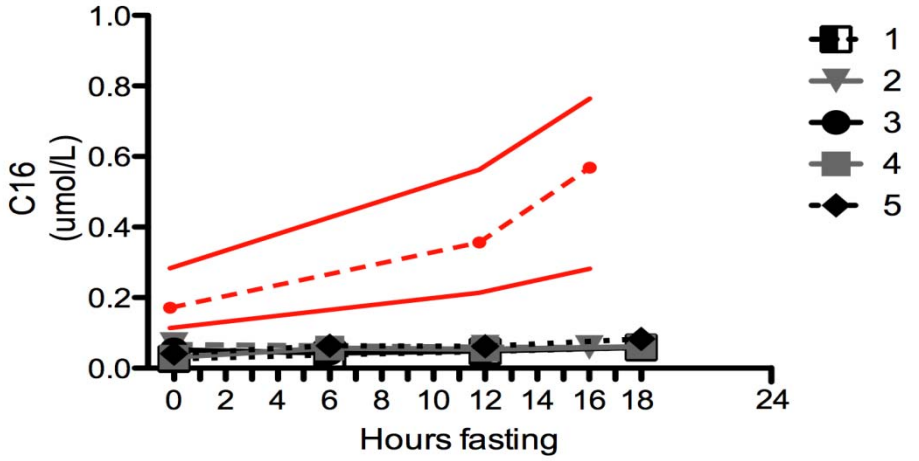
Carnitine is essential in the transport of long-chain-acyl-CoA esters into the mitochondria. It has been reported that plasma free carnitine will decrease with fasting due to an increase in acylcarnitine production (16). As fasting progresses in control

children long chain acylcarnitine (C12-C18) production increases, but the greatest increase is mainly acetyl carnitine (C2) which provides energy during fasting (14, 16).

Classical CPT1A deficiency differs from other fatty acid oxidation disorders in that at diagnosis there is a high free carnitine and low acylcarnitine observed in affected subjects. To date, no report has examined if the high free/ low acylcarnitine ratio observed in patients who are homozygous for the c.1436C-T variant of *CPT1A* changes with feeding and fasting. Here we report the changes with an 18hr fast. Note the method for measuring free carnitine, namely acylcarnitines were measured by MS/MS and free carnitine calculated by the difference. The subjects with the c.1436C→T variant of *CPT1A* had slightly lower free carnitine concentrations using this analytical method than published normal controls but all subjects had levels within the normal range. The free carnitine concentration was stable and did not change during the fast. In contrast, published values in normal controls declined with increased fasting duration.

Figure 6 Plasma carnitine levels during an 18 hour supervised fast





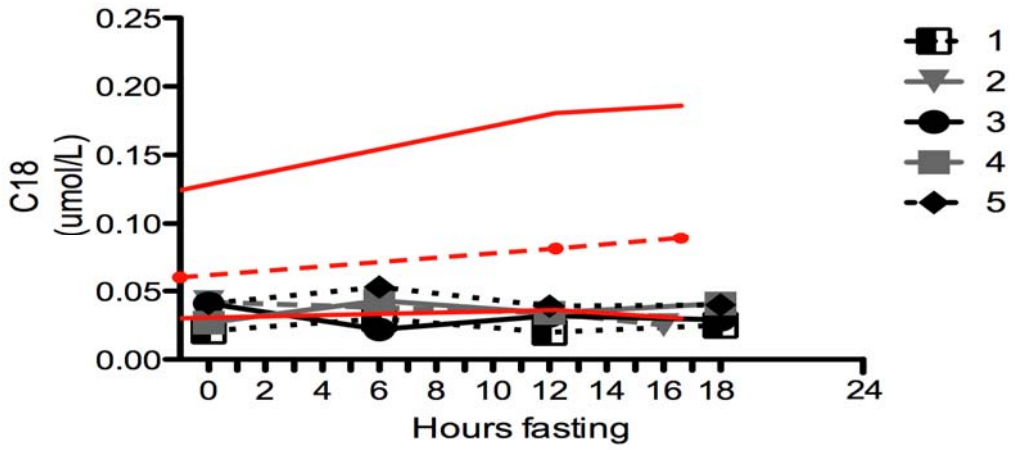


Figure 6 Legend: Change in free carnitine (C0), and acyl carnitines C16 and C18 in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 control children age 1-7 years. Control data is presented as the mean (•----•----•) and the 10th and 90th percentiles (-) at 15, 20 and 24 hours of fasting (14).

Free Carnitine/C16+C18 ratio

There was a large difference in the ratio of free carnitine to acyl carnitine C0/C16+C18 when comparing children with the c.1436C→T variant of *CPT1A* to control children. This difference may be attributed to the large difference in C16 between the two groups. Subjects with the c.1436C→T variant of *CPT1A* had much lower C16 acylcarnitine concentrations that did not change significantly over the course of the fast. Published data in controls indicates total C16 carnitine was higher and increased with prolonged fasting

(16). The large difference in C16 was the largest difference observed with any of the acylcarnitine concentrations, indicating that the c.1436C→T variant of *CPT1A* impaired the synthesis of C16:0 carnitine more than the other acylcarnitine species.

Figure 7: Plasma C0/C16+C18 ratio during an 18 hour supervised fast

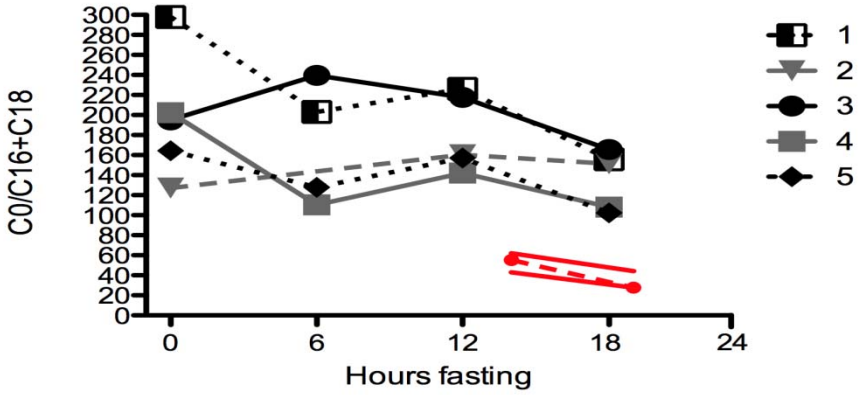


Figure 7 Legend: The change in free carnitine/C16+C18 ratio in 5 subjects who are homozygous for the c.1436C→T variant of *CPT1A* are compared to published data in 27 control children age 1-7 years. Control data is presented as the mean (•----•----•) and the 10^{th} and 90^{th} percentiles (-) at 15, 20 and 24 hours of fasting (14).

Discussion

While there was a range of tolerance to fasting between the five subjects, hypoglycemia was observed in two of the five subjects before completion of the 18 hour fast. The development of hypoglycemia is not an expected result when fasting children ages 3-5 years old. The presence of hypoglycemia in some subjects who are homozygous for the c.1436C→T variant of *CPT1A* suggests this variant can present with symptoms typically observed with a fatty acid oxidation disorder, at least under conditions where people are eating a western diet.

If children with the c.1436C→T variant of the *CPT1A* are allowed to go for prolonged periods of fasting, then the risk of hypoketotic hypoglycemia may increase, particularly in some children. This was observed in our study. When children with the c.1436C→T variant of *CPT1A* were fasted, there was variation in tolerance to fasting. Three of the five subjects were able to tolerate an 18 hour fast without any complications. Two children, however, become hypoglycemic at 17 hours and 15.5 hours post-prandial. These findings suggest that some children with c.1436C→T variant of *CPT1A* have an increased risk for developing severe hypoglycemia with fasting, and that there is variation even within this specific variant.

The hypoglycemia we observed in two subjects occurred when a significant environmental stress was present, such as prolonged fasting. The risk of developing hypoglycemia increases across pediatric populations under conditions of environmental

stress due to increased energy demand and possibly decreased intake. As with other fatty acid oxidation disorders, episodes of hypoglycemia are observed in conjunction with illness or stress and not during periods of normal health. The fasting tolerance in children with the c.1436C→T variant of *CPT1A* may be further limited during times of stress such as fever, illness, or surgery due to an increased metabolic demand as well as poor oral intake of energy.

Ketone production is an important energy substrate during fasting for children, specifically under the age of 7. Children ages 1-7 have a higher ketone production, lower free fatty acid/ketone ratio and lower glucose levels than older children at similar post-prandial time points (14). Children over the age of 7 have tighter control over glucose homeostasis due to larger glycogen stores. Thus, they rely less on fatty acids for energy(14). Therefore the length of time fasting before glycogen stores are depleted is shorter for children under the age of 7 compared to older children. (14).

All five participants in this study with the specific c.1436C→T variant of *CPT1A* presented with low plasma ketone concentrations, through out the fast as well as at baseline. Baseline ketone concentrations are of particular interest in this population. The participants with the specific c.1436C→T variant of *CPT1A* had detectable ketone levels 1 hour post-prandial. At this time ketone levels are not detectable in control children. The slight elevation in ketones at baseline supports the finding that this specific variant is not fully inhibited by malonyl CoA, such that those with the specific c.1436C→T variant of

CPT1A may have limited but constant fatty acid oxidation, even in the fed state. Further studies evaluating fatty acid oxidation and ketone production in the fed versus fasted state are needed to confirm this initial observation.

The most significant finding of this study is the extreme hypoketosis we observed among all 5 young children who are homozygous for the c. 1436C-T variant of *CPT1A*. The depressed ketone production indicates that there is a severe limitation in hepatic conversion of free fatty acids to ketones in children with c.1436C→T variant of *CPT1A*, resulting in an 80% decrease in ketone concentration when compared to control children. In contrast, the free fatty acids were similar between control children and children with c.1436C→T variant of *CPT1A*. The normal circulating levels of free fatty acids in these children indicates that mobilization of adipose triglyceride stores is not a problem. However, the very low levels of ketones despite elevated free fatty acids indicates that decreased hepatic fatty acid oxidation in turn limits ketone synthesis in children with this specific variant. The theoretical consequences of low ketone concentrations during fasting is further dependence and increased utilization of glucose for energy production increasing the risk of fasting induced hypoglycemia.

Hypoglycemia may be a relatively insensitive marker of a person's metabolic state during prolonged fasting. Therefore using the free fatty acids/ketone ratio is a better indicator of altered metabolic response to fasting when hypoglycemia is not the end result from a supervised fast (14). When the free fatty acid/ketone ratio is greater than 2.6 this indicates altered fasting metabolism (14), and all subjects with the specific c.1436C→T variant of *CPT1A* met this criteria. Similar to reports in other fatty acid oxidation disorders, our data suggests that children with c.1436C→ variant of *CPT1A* can have hypoketosis without developing clinical symptoms of hypoglycemia. Overall the data supports our hypothesis that children with c.1436C→T variant of *CPT1A* have an altered metabolic response to fasting which increases their risk for developing hypoketotic hypoglycemia with prolonged fasting, illness or other environmental stresses.

Hypoketotic hypoglycemia is one of the most common symptoms observed among patients with a variety of fatty acid oxidation disorders. Approximately 25 supervised fasting studies conducted among subjects with MCAD deficiency, the most common of all the fatty acid oxidation disorders, have been published. Derks et al recently published a meta analysis of these studies in order to establish some evidence based guidelines for children with MCAD deficiency (18). Comparing the fasting response of children with MCAD deficiency to the results reported in our study may be helpful in understanding the severity of this particular *CPT1A* variant.

Glucose concentrations in children with c.1436C→T variant of *CPT1A* and children with MCAD deficiency, was not notably different. The glucose differences ranged between 27mg/dL to -10mg/dL depending on time of fast (18). A more formal statistical analysis between children with c.1436C→T variant of *CPT1A* and children with MCAD deficiency could not be completed since the MCAD deficiency studies include only one child at each time point.

Subjects with MCAD deficiency demonstrate hypoketosis with fasting but the severity of depressed ketone production among our participants with c.1436C→T variant of *CPT1A* is greater than that reported in MCAD deficiency (14, 18). Our subjects in this study had approximately 5 times lower ketone production at the end of the 18 hour fast than what has been reported in medically supervised fasts of subjects with MCAD deficiency. Children with the c.1436C→T variant of *CPT1A* have a maximum average ketone concentration of 0.233mMol/L after 18 hours of fasting compared to a ketone concentration of 1.4mMol/L at 24 hours of fasting among subjects with MCAD deficiency (14). Taken together, our results suggest that children who are homozygous for the c.1436C→T variant of *CPT1A* have a more pronounced hypoketosis with fasting than published studies in children with MCAD deficiency.

Acute hypoketotic hypoglycemia is observed in patients with fatty acid oxidation disorders primarily during illness. To prevent these symptoms, parents are instructed to follow a sick day regimen in which fluids and energy are increased. Parents with children

diagnosed with MCAD deficiency are also provided emergency letters and the families are counseled about when to seek medical care if their child becomes very ill. This educational approach decreases the adverse outcomes and death in the MCAD deficient population (17). Perhaps a similar educational approach among families with a child diagnosed with the specific c.1436C→T variant of *CPT1A* could decrease symptoms and lower infant death in this population. Through implementation of an education DVD families are currently provided with some initial education on the specific c.1436C→T variant of *CPT1A*. Further follow up of the effects of this education program are needed to determine its effect on infant and child health.

Overall treatment for fatty acid oxidation disorders is based on fasting avoidance. One of the goals of this study was to begin to determine if treatment was necessary for children with this variant and what treatment might seem appropriate. Establishing treatment recommendations based on fasting studies in 5 children is obviously premature but opens the discussion of how such recommendations have been made in other FAO disorders. Other fatty acid oxidation disorders, such as MCAD deficiency, base fasting guidelines on studies conducted on a small number of subjects. The most comprehensive review of fasting data, as noted above, was published by Derk et al (18). The authors conducted a meta analysis compiling 25 fasting studies conducted in subjects with MCAD deficiency to provided fasting recommendations for this population (18). In clinical practice, these guidelines, published for the treatment of MCAD deficiency, have been applied to children with other fatty acid oxidation disorders, such as VLCAD or LCHAD deficiency.

Based on our preliminary results, children who are homozygous with the specific c.1436C→T variant of *CPT1A* should avoid prolonged fasting. The MCAD deficiency fasting guidelines seem to be an appropriate framework to help define fasting avoidance. The recommendation to avoid fasting is not a meaningful term to most parents and can have a variety of interpretations, depending on the parent's definition of fasting. Providing a quantitative guide to fasting creates a context for parents about what the term "avoid fasting" means for their child. When providing a fasting guideline to parents, it should be emphasized that the guideline is just that, a guideline and not an absolute. Every child will have a different tolerance to fasting, as seen in this study. However, providing some fasting guideline for parents gives a tangible place to start.

Conclusion:

Children with the c.1436C→T variant of the *CPT1A* present with an altered response to fasting compared to control children. The abnormal response to fasting indicates that this variant of *CPT1A* may in fact benefit from avoidance of fasting and precautions during times of limited oral intake. Children ages 3-5 years old with c.1436C→T variant of *CPT1A* have a range of fasting tolerance, as observed in our study. Family education and appropriate feeding guidelines with precautions for altered oral intake may be one component to the intervention that is needed for children with c.1436C→T variant of *CPT1A*.

This report is the first description of the c.1436C→T variant of *CPT1A* having a notable impact on the metabolic response to fasting. Therefore this variant should not be considered a benign variant. The data suggests that children who are homozygous for the c.1436C→T variant of *CPT1A* are at increased risk for severe hypoketotic hypoglycemia with prolonged fasting, illness or other environmental stress. Larger epidemiological data needs to be gathered to determine whether the c.1436C→T variant of *CPT1A* is associated with adverse events in Alaska Native children.

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APPENDIX A

TABLES

Glucose (mg/dL)					
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**
0hr	117	95	102	87	91
6hr	93	35 *(saline draw)	92	90	89
7hr	82	83	96	96	70
8hr	83	85	89	89	91
9hr	87	83	75	89	84
10hr	84	77	90	91	80
11hr	79	77	87	89	83
12hr	88	74	91	88	77
13hr	80	65	84	82	85
14hr	82	67	79	60	80
15hr	55 *(hemolyzed, small blood volume)	54	85	74	78
15.5 hr		43			
16hr	78	25 (fast terminated)	82	74	70
17hr	68	Fast terminated	82	79	60
18hr	76	Fast terminated	79	77	51 (17.5 hr fast terminated)

Table 1
*** denotes data not used in calculating averages**

Glucose (mg/dL) Average		
0hr	98.4	SD 11.78
6hr	79.8*	SD 25.093
7hr	85.4	SD 10.94
8hr	87.4	SD 3.28
9hr	83.6	SD 5.36
10hr	84.4	SD 6.10
11hr	83	SD 5.09
12hr	83.6	SD 7.56
13hr	79.2	SD 8.16
14hr	73.6	SD 9.60
15hr	69.2	SD 13.98

16hr	65.8*	SD 23.24
17hr	72.25*	SD 10.14
18hr	70.75*	SD 13.22

Table 2

*** Denotes 4 subjects used to calculate average**

Free Fatty Acids (mmol/L)					
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**
0hr	0.170925	0.13205	0.32035	0.123568	0.238425
6hr	0.44785	*(saline draw)	0.5753	0.2322	0.505325
12hr	0.534775	0.83395	0.747175	0.3252	1.023825
18hr	1.7514	1.5426 * (drawn at 15.5hr)	1.97755	0.827875	2.74075

Table 3

*** denotes data not used in calculating averages**

Free Fatty Acids (mmol/L) Average		
0hr	0.197064	SD 0.825
6hr	0.440169*	SD 0.148
12hr	0.692985	SD 0.2704
18hr	1.768035*	SD 0.694

Table 4

*** denotes 4 subjects used to calculate average**

Ketones (mmol/L)					
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**
0hr	0.092	0.073	0.091	0.088	0.035
6hr	0.146	* (saline draw)	0.169	0.079	0.072
12hr	0.153	0.327	0.138	0.06	0.111
18hr	0.278	0.373* (drawn at 15.5hr)	0.227	0.117	0.171

Table 5

*** denotes data not used in calculating averages**

Ketone (mmol/L) average		
0hr	0.0758	SD 0.0241
6hr	0.116*	SD 0.0483
12hr	0.157	SD 0.101
18hr	0.233*	SD 0.0987

Table 6

*** denotes 4 subjects used to calculate average**

Free Carnitine umol/L					
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**
0hr	14.400	13.846	18.149	11.738	14.948
6hr	13.815	* (saline draw)	15.568	11.039	14.969
12hr	15.148	15.076	17.629	12.510	15.865
18hr	13.095	12.720 * (drawn at 15.5hr)	14.579	11.019	12.583

Table 7

*** denotes data not used in calculating averages**

Free Carnitine umol/L Average		
0hr	14.616	SD 2.32
6hr	13.848*	SD 2.01
12hr	15.246	SD 1.84
18hr	12.799*	SD 1.47

Table 8

*** denotes 4 subjects used to calculate average**

Acylcarnitine Subject 1											
	C2	C12:1	C12	C14:2	C14:1	C14	C16:1	C16	C18:2	C18:1	C18
0hr	5.237	0.024	0.033	0.008	0.011	0.011	0.004	0.026	0.028	0.037	0.021
6hr	7.350	0.028	0.033	0.007	0.014	0.022	0.011	0.038	0.023	0.042	0.030
12hr	9.831	0.010	0.020	0.008	0.020	0.013	0.011	0.047	0.026	0.062	0.020

18hr	15.07	0.058	0.063	0.033	0.061	0.020	0.020	0.059	0.040	0.090	0.025
Acylcarnitine Subject 2											
	C2	C12:1	C12	C14:2	C14:1	C14	C16:1	C16	C18:2	C18:1	C18
0hr	6.583	0.023	0.040	0.008	0.017	0.014	0.007	0.067	0.036	0.070	0.042
6hr	*	*	*	*	*	*	*	*	*	*	*
12hr	14.68	0.026	0.030	0.017	0.031	0.020	0.017	0.060	0.028	0.062	0.034
18hr	18.495 *(15.5 hr)	0.073 *(15.5 hr)	0.065 *(15.5 hr)	0.040 *(15.5 hr)	0.083 *(15.5 hr)	0.022 *(15.5 hr)	0.022 *(15.5 hr)	0.059 *(15.5 hr)	0.043 *(15.5 hr)	0.087 *(15.5 hr)	0.025 *(15.5 hr)
Acylcarnitine Subject 3											
	C2	C12:1	C12	C14:2	C14:1	C14	C16:1	C16	C18:2	C18:1	C18
0hr	6.636	0.027	0.052	0.016	0.026	0.012	0.006	0.052	0.043	0.064	0.041
6hr	8.605	0.031	0.053	0.024	0.040	0.029	0.012	0.043	0.037	0.061	0.022
12hr	10.19	0.047	0.034	0.019	0.034	0.023	0.012	0.049	0.029	0.074	0.032
18hr	14.34	0.125	0.095	0.047	0.091	0.030	0.032	0.059	0.037	0.114	0.029
Acylcarnitine Subject 4											
	C2	C12:1	C12	C14:2	C14:1	C14	C16:1	C16	C18:2	C18:1	C18
0hr	4.949	0.006	0.016	0.007	0.009	0.009	0.007	0.031	0.019	0.033	0.027
6hr	3.703	0.020	0.030	0.012	0.012	0.014	0.010	0.057	0.030	0.067	0.043
12hr	4.189	0.018	0.026	0.008	0.032	0.008	0.010	0.054	0.026	0.059	0.034
18hr	7.657	0.075	0.064	0.025	0.063	0.029	0.016	0.061	0.033	0.104	0.041
Acylcarnitine Subject 5											
	C2	C12:1	C12	C14:2	C14:1	C14	C16:1	C16	C18:2	C18:1	C18
0hr	6.336	0.019	0.036	0.010	0.010	0.012	0.005	0.041	0.047	0.065	0.050
6hr	5.605	0.019	0.033	0.011	0.032	0.024	0.012	0.064	0.040	0.083	0.053

12hr	10.18	0.067	0.050	0.029	0.058	0.035	0.022	0.062	0.055	0.095	0.039
18hr	18.82	0.209	0.112	0.095	0.161	0.073	0.060	0.083	0.062	0.092	0.040

Table 9
*** denotes data not used in calculating averages**

Acylcarnitine umol/L Average						
	C2		C12:1		C12	
0hr	5.948	SD 0.79	0.020	SD 0.0072	0.035	SD 0.013
6hr	6.316*	SD 2.13	0.024*	SD 0.0057	0.037*	SD 0.010
12hr	9.816	SD 3.73	0.034	SD 0.023	0.032	SD 0.011
18hr	14.879*	SD 4.50	0.108*	SD 0.062	0.080*	SD 0.022

Table 10
*** denotes 4 subjects used to calculate average**

Acylcarnitine umol/L Average						
	C14:2		C14:1		C14	
0hr	0.010	SD 0.0037	0.014	SD 0.0068	0.012	SD 0.0019
6hr	0.013*	SD 0.0074	0.024*	SD 0.013	0.022*	SD 0.0062
12hr	0.016	SD 0.0088	0.035	SD 0.014	0.020	SD 0.010
18hr	0.048*	SD 0.027	0.092*	SD 0.04	0.035*	SD 0.021

Table 11
*** denotes 4 subjects used to calculate average**

Acylcarnitine umol/L Average				
	C16:1		C16	

0hr	0.006	SD 0.0012	0.043	SD 0.016
6hr	0.011*	SD 0.00082	0.051*	SD 0.012
12hr	0.014	SD 0.0048	0.054	SD 0.0065
18hr	0.030*	SD 0.017	0.064*	SD 0.010

Table 12

*** denotes 4 subjects used to calculate average**

Acylcarnitine umol/L Average						
	C18:2		C18:1		C18	
0hr	0.035	SD 0.011	0.054	SD 0.017	0.036	SD 0.012
6hr	0.033*	SD 0.0076	0.063*	SD 0.016	0.037*	SD 0.013
12hr	0.033	SD 0.012	0.070	SD 0.015	0.032	SD 0.0068
18hr	0.043*	SD 0.011	0.097*	SD 0.011	0.032*	SD 0.0078

Table 13

*** denotes 4 subjects used to calculate average**

APPENDIX B

Melanie B. Gillingham,
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APPROVED: Aug. 3, 2009

Date: November 24, 2008

Dear Parents,

A new research study for children with CPT-1 deficiency has started. This letter is to invite your child and your family to participate. Participation is voluntary.

Your child has been invited to be in this research study because he or she has been diagnosed with Carnitine Palmitoyltransferase Type 1 Deficiency (CPT-1 deficiency). The purpose of this study is to learn more about how long children with CPT-1 deficiency can wait between meals without developing low blood sugar or symptoms of low blood sugar. The other purpose is to learn more about how much fat is stored in the liver of a child with CPT-1 deficiency.

This study requires 1 trip to Portland, Oregon that will last about 4 days. There are no costs to you to participate in this study. Six children with CPT-1 deficiency between 3 and 5 years of age will be enrolled in this study. If you are interested in participating, or would like to learn more about the study, please return the enclosed response to the researchers or contact them at the numbers below.

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Phone: (503) 494-1682

Email: gillingm@ohsu.edu

Matthew Hirschfeld, MD, PhD

Alaska Native Medical Center

4315 Diplomacy Drive

Anchorage, AK 99508

Phone: (907) 729-1084

eIRB #3556

Detach here:

Date:

To: The Investigators of *Metabolic Consequences of CPT-1 Deficiency in Alaska Native Children*

I am interested in more information about this research study. I may be contacted at the address below.

Name:

Address:

Phone:

APPENDIX C

Oregon Health & Science University Consent Form **IRB#: e3556** **Protocol Approval Date: 07/16/2009**	MED. REC. NO. _____ NAME _____ BIRTHDATE _____ Complete this section only if clinical services are provided.

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form

CHILDREN WITH CPT-1 DEFICIENCY

TITLE: **Metabolic** Consequences of CPT1A deficiency in Alaska Native Children

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

CO-INVESTIGATORS: David Koeller, MD (503) 494-2783

Cary O. Harding, MD (503) 494-2783

Jonathan Purnell, MD (503) 494-1056

William Lambert, PhD (503) 494-9488

SPONSOR: Oregon Clinical and Translational Research Institute (OCTRI)

PURPOSE:

Your child has been invited to be in this research study because he or she has been diagnosed with Carnitine Palmitoyltransferase Type 1 Deficiency (CPT-1 deficiency). The purpose of this study is to learn more about how long children with CPT-1 deficiency can wait between meals without developing low blood sugar or symptoms of low blood sugar. The other purpose is to learn more about how much fat is stored in the liver of a child with CPT-1 deficiency. This study requires 1 trip to Portland, Oregon that will last about 4 days. Six children with CPT-1 deficiency between 3 and 5 years of age will be enrolled in this study. This is a research study and not health care. The participant is free to decline to participate.

PROCEDURES:

You and your child and other family members will travel from your home to Portland, Oregon (Day 1). A study representative will help you arrange travel and lodging. The following morning, your child will have a magnetic resonance spectroscopy (MRS) study (Day 2). After the study, you and your family are free to relax for the afternoon. That evening, your child will be admitted to the Clinical & Translational Research Center (CTRC) inpatient unit for an 18-hour fasting study (Days 2 & 3). At the end of the fasting study you and your child are free to relax for the evening. The following morning, an investigator will evaluate your child and then you will be free to go to the airport to return home. (Day 4, Table 1)

Table 1: Schedule of Study tests

	Day 1	Day 2	Day 3	Day 4
Travel	X			X
MRS		X		
Fasting Study		X	X	

Physical evaluation	X			X

Each study is described in detail below.

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 child's abdomen, liver, and muscle. During this procedure your child will lie still on a table inside a powerful magnet. There is no radiation (like x-rays) exposure from an MRI machine. If your child has any metal inside of his or her body (plates) they will be excluded from participating in the study. This procedure takes up to 60 minutes. Some children will need to be sedated to lie still in the MRI machine for this test. Your child may not need sedation to complete this test.

Sedation: If your child needs to be sedated, your child will be sedated and closely monitored by the Doernbecher Children's Hospital Pediatric Sedation Team. You will sign a separate consent for the sedation.

Fasting Study: Your child will be admitted to the Clinical & Translational Research Center (CTRC) inpatient unit in the evening before dinner. A slow infusion of salt water (saline) into a vein (IV) will be started in one arm and a second IV will be placed in the other arm for blood sampling. These IVs will be in place until the study is completed (18 hours). Your child will be fed a standardized dinner. One hour after dinner, a blood sample will be drawn. We will draw 10 ml or 2 teaspoons of blood. Your child will be allowed only water or non-caloric beverages such as diet soda or Crystal Light for the next 18 hours. After 6 hours of no food, another blood sample will be drawn from the IV. We will draw another 10 ml or 2 teaspoons of blood. The nurse will check your child's blood sugar level every hour after that until the end of the study. She will draw about 1 ml or ¼ teaspoon from the IV to check your child's blood sugar. If, at any time, your child has a very low blood sugar (<40 mg /dl), the study will be stopped. A sugar solution will be infused with the salt water and your child will be given some food to eat. If his or her blood sugar is not low, the fast will continue. We will draw 10 ml or 2 teaspoons of blood at 12 and 18 hours of no food. At the end of 18 hours, your child will be given a meal to eat and the IVs removed. The study will be concluded.

Blood Samples: About 10 teaspoons of blood will be drawn from your child's arm during the fasting studies. Two teaspoons will be drawn out of the IV at the beginning and after 6, 12 and 18 hours of fasting. One fourth of a teaspoon of blood will be drawn out of the IV each hour beginning 6 hours into the fast. 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 CPT-1 deficiency. You may choose not to have your child's blood sample stored for future use. If you choose not to have your child's blood stored, the samples will be destroyed at the conclusion of the study.

Energy Expenditure Test: During the fasting study, your child's energy expenditure will be measured. "Energy expenditure" is how many calories you use during the day. A clear, colorless, Plexiglass canopy (bubble) will be placed over your child's head and chest while he or she rests on a bed. Samples of the air that your child breathes out will be collected for about 45 minutes. A trained research assistant will perform this test in a private room to make you and your child feel comfortable and relaxed. This test will be performed at night while she/he is asleep.

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

RISKS AND DISCOMFORTS:

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

Travel: Participation requires travel to Portland, Oregon, and approximately 4 days of your time.

MRI/MRS: The magnetic resonance imaging (MRI) machine is a powerful magnet. This magnet may cause any metal in your child's body to move. If you know of any metal in your child's body, you will need to tell the investigator right away. 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 or your child can request they be removed from the scanner and this will be done immediately. The MRI scanner makes a loud beeping sound. Your child may be asked to wear protective earplugs during scanning.

Blood Samples/IV Catheters: Your child will have catheters (tubes) in his or her veins for 18 hours for the fasting study. If your child needs sedation for the MRI/MRS, they will have the IV for an additional 2 hours. The needle used to place the IV may cause bleeding or a bruise. It may

cause some discomfort when the IV is placed. They may get an infection where the tube is placed. This would cause swelling, redness, and pain. These problems are very rare. If your child has these problems, they may need hospital care.

Fasting Study: Children with CPT-1 deficiency can develop low blood sugar and symptoms of low blood sugar with long periods of not eating. The symptoms of low blood sugar include sweating, nausea, weakness and rapid heart rate. During the fasting study your child may experience low blood sugar and/or the symptoms of low blood sugar. If your blood sugar drops too low, you can develop brain damage and/or die. Treatments for very low blood sugar are infusing a sugar solution into a vein, and eating or drinking some food. To protect against a dangerously low blood sugar, the pediatrician will be near your child's room during the whole study. A sugar solution will be placed by your child's bed in case of emergency. The salt solution will be given throughout the study to prevent dehydration and provide a means to give the sugar solution rapidly if needed.

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.

Other Risks: There is a risk of loss of confidentiality. Other people could potentially discover your child has CPT-1 deficiency. There may be emotional harm or distress from a loss of privacy.

BENEFITS:

Results from this study may or may not improve treatment guidelines for children with CPT-1 deficiency. Your child may not personally benefit from being in this research study, and we cannot guarantee that this research study will help your child. However, by serving as a subject, your child may help us learn how to benefit patients in the future.

ALTERNATIVES:

1. You may choose not to be in this study.

2. You may choose to participate. If you choose to participate,

- You may choose to participate and allow your child's blood samples to be stored for future research **or**
- You may choose to participate and request your child's 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.

Blood samples sent to laboratories outside of OHSU will be coded with a unique number. The investigators at OHSU will be the only people with access to the code. No other identifying information will be provided to those laboratories.

Research records may be reviewed and copied by the OHSU Institutional Review Board, the Alaska Native Medical Center Institutional Review Board, the Office for Human Research Protections (OHRP), the Oregon Clinical and Translational Research Institute (OCTRI), and the National Center for Research Resources.

Under Oregon Law, suspected child or elder abuse must be reported to appropriate authorities.

COSTS:

There is no cost to you for the study. The sponsor will cover the travel expenses, lodging and meals for subjects and their families.

LIABILITY:

If you believe your child has been injured or harmed while participating in this research and requires immediate treatment, contact the OHSU paging operator at (503) 494-9000 and ask for Dr. David Koeller or Dr. Cary Harding to be paged.

It is not the policy of the U.S. Department of Health and Human Services to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

In other words, if your child requires medical care as a result of this research project, you will receive medical care but you or your insurance company will be billed for these expenses.

You have not waived your legal rights by signing this form. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

In other words, if you are injured, you may file a lawsuit against OHSU. If you sign this form, you still have all your legal rights.

Oregon Health & Science University is subject to the Oregon Genetic Privacy law (ORS 192.531 through ORS 192.549) and its requirements concerning confidentiality and the legal remedies provided by that law for breach of its requirements. 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.

In other words, the law protects you from having your blood sample be used for other genetic testing without your knowledge. If the researchers do not follow this law, you may file a lawsuit against OHSU.

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.

Your health care provider may be one of the investigators of this research study, and as an investigator is interested in both your clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way involved in this project. You do not have to be in any research study offered by your physician.

You may be removed from the study prior to its conclusion if the study is stopped by the sponsor.

We will give you a copy of this signed form.

SIGNATURES:

Your signature below indicates that you have read this entire form and that you agree for your child to participate in this study. All future research, if applicable, will require review and approval by the Alaska Area Institutional Review Board.

Please initial your choice below.

_____ I give my consent for my child's blood samples to be stored and used for this study only.

_____ I give my consent for my child's blood samples to be used for this study and stored for possible use in future studies of the diagnosis and treatment for CPT-1 deficiency, but I wish to be contacted for permission prior to any future use.

_____ I give my consent for my child's blood samples to be used for this and future studies of the diagnosis and treatment for CPT-1 deficiency including genetic research 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

Aug. 3, 2009

Do not sign this form after the Expiration date of: 07/15/2010

_____
Printed Name of Subject

_____ _____
Signature of Legal Guardian Date

_____

Relationship to Subject

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX D

Oregon Health & Science University Consent Form **IRB#: e3556** **Protocol Approval Date: 07/16/2009**	MED. REC. NO. _____ NAME _____ BIRTHDATE _____ Complete this section only if clinical services are provided.

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form

SIBLINGS OF CHILDREN WITH CPT-1 DEFICIENCY

TITLE: Metabolic Consequences of CPT1A deficiency in Alaska Native Children

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

CO-INVESTIGATORS: David Koeller, MD (503) 494-2783

Cary O. Harding, MD (503) 494-2783

Jonathan Purnell, MD (503) 494-1056

William Lambert, PhD (503) 494-9488

SPONSOR: Oregon Clinical and Translational Research Institute (OCTRI)

PURPOSE:

Your child has been invited to be in this research study because he or she has a sibling that has been diagnosed with Carnitine Palmitoyltransferase Type 1 Deficiency (CPT-1 deficiency). One of the purposes of this study is to learn more about how much fat is stored in the liver of a child with CPT-1 deficiency. To measure this, we are going to look at how much fat is stored in the liver of siblings without CPT-1 deficiency and compare this to the amount stored in children with CPT-1 deficiency. We are asking your child to provide blood to determine if he or she does not have CPT-1 deficiency. The blood sample provided by your child will be analyzed in the laboratory to determine if he or she has a variation in the CPT-1 gene. Genes are the units of DNA--the chemical structure carrying your genetic information--that determine many human characteristics such as the color of your eyes, your height, and whether you are male or female. Your child's blood sample will not be stored or used for other research after the test for CPT-1 deficiency. The blood sample will be destroyed.

This study requires 1 trip to Portland, Oregon that will last about 4 days. Six children with CPT-1 deficiency and 6 siblings will be enrolled in this study. This is a research study and not health care. The participant is free to decline to participate.

PROCEDURES:

A blood sample will be drawn from your child's finger and sent to the laboratory. The sample will be analyzed for the variation in the CPT-1 gene that has already been found in your other child. If the blood test shows that this child also has CPT-1 deficiency, he or she may participate as a subject with CPT-1 deficiency. You will have the option to sign the subject consent form and both children participate or you may choose not to participate at this time.

You and your child and other family members will travel from your home to Portland, Oregon (Day 1). A study representative will help you arrange travel and lodging. The following morning, your children will have a magnetic resonance spectroscopy (MRS) study (Day 2).

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 child's abdomen, liver, and muscle. During this procedure your child will lie still on a table inside a powerful magnet. There is no radiation (like x-rays) exposure from an MRI machine. If your child has any metal inside of his or her body (plates) they will not have this procedure. This procedure takes up to 60 minutes. Your child will not require sedation to complete this test.

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

SUBJECT ACCESS TO GENETIC INFORMATION:

The results of these genetic testing for CPT-1 deficiency in your child will be made available to you. The investigator of this study will contact you to tell you the results of this test.

Positive Result: If your child does have CPT-1 deficiency, the investigator will recommend you discuss the results with your health care provider. We will ask your permission to report these results to your primary physician. We will recommend you seek follow-up care at the same genetic clinic your other child attends.

Negative Result: If your child does not have CPT-1 deficiency, the investigator will only report these results to you. Your physician will not be contacted.

Blood sample for CPT-1 deficiency testing: The filter paper card collected to test your child for CPT-1 deficiency will be destroyed after the test is complete. No other genetic tests will be conducted on that sample.

RISKS AND DISCOMFORTS:

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

Travel: Participation requires travel to Portland, Oregon, and approximately 4 days of your time.

MRI/MRS: The magnetic resonance imaging (MRI) machine is a powerful magnet. This magnet may cause any metal in your child's body to move. If you know of any metal in your child's body, you will need to tell the investigator right away. 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 or your child can request they be removed from the scanner and this will be done immediately. The MRI scanner makes a loud beeping sound. Your child may be asked to wear protective earplugs during scanning.

Finger stick Blood Samples: We will draw blood from the tip of a finger. The blood will be dropped onto a card. Your child may feel some pain when his/her finger is poked. There is a small chance the needle will cause a bruise, or an infection.

Other Risks: The risk of genetic testing for CPT-1 is a loss of confidentiality and potential discovery of a genetic disease that you did not previously know about. There may be emotional harm or distress from discovering your child has a genetic disease you did not know about.

BENEFITS:

Your child will not benefit personally from being in this study. However, by serving as a subject, your child may help us learn how to benefit patients in the future.

ALTERNATIVES:

You may choose not to be in this study.

CONFIDENTIALITY:

We will not use your name or your identity for publication or publicity purposes. Research records may be reviewed and copied by the OHSU Institutional Review Board, the Alaska Native Medical Center Institutional Review Board, the Office for Human Research Protections (OHRP), the Oregon Clinical and Translational Research Institute (OCTRI), and the National Center for Research Resources.

Under Oregon Law, suspected child or elder abuse must be reported to appropriate authorities.

COSTS:

There is no cost to you for the study. The sponsor will cover the travel expenses, lodging and meals for subjects and their families.

LIABILITY:

If you believe your child has been injured or harmed while participating in this research and requires immediate treatment, contact the OHSU paging operator at (503) 494-9000 and ask for Dr. David Koeller or Dr. Cary Harding to be paged.

It is not the policy of the U.S. Department of Health and Human Services to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

In other words, if your child requires medical care as a result of this research project, you will receive medical care but you or your insurance company will be billed for these expenses.

You have not waived your legal rights by signing this form. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

In other words, if you are injured, you may file a lawsuit against OHSU. If you sign this form, you still have all your legal rights.

Oregon Health & Science University is subject to the Oregon Genetic Privacy law (ORS 192.531 through ORS 192.549) and its requirements concerning confidentiality and the legal remedies provided by that law for breach of its requirements. 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.

In other words, the law protects you from having your blood sample be used for other genetic testing without your knowledge. If the researchers do not follow this law, you may file a lawsuit against OHSU.

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.

Your health care provider may be one of the investigators of this research study, and as an investigator is interested in both your clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way involved in this project. You do not have to be in any research study offered by your physician.

You may be removed from the study prior to its conclusion if the study is stopped by the sponsor.

We will give you a copy of this signed form.

SIGNATURES:

Your signature below indicates that you have read this entire form and that you agree for your child to participate in this study.

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

OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD
PHONE NUMBER (503) 494-7887
CONSENT/AUTHORIZATION FORM APPROVAL DATE

Aug. 3, 2009

Do not sign this form after the Expiration date of: 07/15/2010

_____
Printed Name of Subject

_____ _____

Signature of Legal Guardian Date

_____

Relationship to Subject

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX E

ADMISSION ORDERS FOR Metabolic Consequences of CPT1A Deficiency in Alaska Native Children (CTRC Protocol # 1029)

Patiennt Name _____ DOB_____ MR#_____

- Admit on _____ to inpatient unit DCH10N
- Attending Physician: Dr. David Koeller Pager #: 10773
- Principal investigator: Melanie Gillingham, PhD cell phone 503-319-2404
- **Medical Coverage**:
- **Please make sure that a copy of the signed consent for the participant is in the front of their chart before proceeding**.

ALLERGIES:

CODE STATUS

- Full Code

NURSING

General

- Vital signs (HR, RR, and temperature) on admit.
- Weigh patient on admit.
- Weigh patient **daily** in a.m. when awake.
- Notify MD or PA-C

Temp> 38.3 degrees C
HR > 125 < 80
RR > 30 < 20
SBP > 120 < 84
DBP > 80 < 40

ACTIVITY

- Up Ad Lib.
- May go off floor with parent(s)

NUTRITION

- Regular diet except where noted.

6.

7. MEDICATIONS

- May apply EMLA/LMX to potential venipuncture/IV/biopsy sites.
- Heparin (10 units/ml) give 1-2 ml IV flush q 8 hours PRN IV patency
- See medication reconciliation form.

8. LAB

- Blood samples per daily orders below.

9.

10. DAILY ORDERS

11. Day 1 Wednesday Oct. 7th:

1. Obtain height, weight and vitals (BP, pulse respiratory rate, and temperature)
2. Patient may select snacks and meals from hospital menu
3. Place peripheral IV for sedated MRS on Thursday, Oct. 8th
4. Regular Diet until 0100 AM.

12.

Day 2 Thursday Oct 8th:

5. Clear liquids from 0100am to 0500am, NPO at 0500 am
6. Begin D10 (10% dextrose) ½ NS at 30 ml/hr at 0500 AM
7. **Wake subject at 6:00 AM**
8. Obtain height, weight and vitals (BP, pulse respiratory rate, and temperature)
9. Send subject to AIRC for sedated MRS with IVF at 0630. Sedation to begin at 0700.
10. Place 2nd peripheral IV and heparin lock while subject is sedated for MRS.
11. After the sedated MRS, discontinue IV fluid infusion and heparin lock the IV when patient is awake and able to eat and drink
12. Patient may select lunch, dinner and snacks from hospital menu
13. The patient and family are free to relax for the afternoon and may order from the hospital menu for snacks and dinner.
14. Feed subject dinner at 1700. The fasting study begins with the end of dinner at 1730.

FASTING STUDY (Day 2 1800 pm- Day 3 1200 pm)

- Begin IV fluids: ½ NS at 30 ml/hr in one peripheral IV one hour after dinner (1800).
- Begin monitoring of HR and pulse-ox throughout fast one hour after dinner (1800).
- One hour after dinner (1800), draw the following labs via the heparin locked IV:
    - 2 ml red top for insulin & ketones
    - 2 ml EDTA (lavender) top for acylcarnitines & free fatty acids
    - give red top and EDTA tubes to study personnel to process

    - 1 ml green top (Li Hep) for complete metabolic panel (CMP)
    - send to OHSU central labs for analysis
- **Subject will be allowed only water or non-caloric beverages for the next 18 hours**
- After six hours (2400) of no food, draw the following labs:
    - 2 ml red top for insulin & ketones
    - 2 ml EDTA (lavender) top for acylcarnitines & free fatty acids
    - give red top and EDTA tubes to study personnel to process
    - 1 ml green top (Li Hep) for complete metabolic panel (CMP)
    - send to OHSU central labs for analysis
- Starting at 6 hours post fasting at 2400, check blood glucose level via YSI every hour until the end of the study.
    - Draw discard 0.5 ml
    - Record glucose result reported by study personnel.
- Observe subject for signs of hypoglycemia such as altered mental status, increased HR, or sweating.
- If at any time the subject has a blood glucose <60mg/dL, the frequency of checking blood glucose levels will increase to every 30 minutes.
- If at any time the subject has a blood glucose < 40mg/dL, the study will be stopped.
- Have at the bedside: 25% Dextrose 2 ml/kg body weight for emergency push if symptomatic hypoglycemia develops
- Indirect Calorimetry: study personnel will measure sleeping energy expenditure when subject is asleep after 6 hour blood draw
- After twelve hours of fasting (0600 am), draw the following labs:
    - 2 ml red top for insulin & ketones
    - 2 ml EDTA (lavender) top for acylcarnitines & free fatty acids
    - give red top and EDTA tubes to study personnel to process
    - 1 ml green top (Li Hep) for complete metabolic panel (CMP)
    - send to OHSU central labs for analysis
- After eighteen hours of fasting (1200 pm), draw the following labs:
    - 2 ml red top for insulin & ketones
    - 2 ml EDTA (lavender) top for acylcarnitines & free fatty acids
    - give red top and EDTA tubes to study personnel to process
    - 1 ml green top (Li Hep) for complete metabolic panel (CMP)
    - send to OHSU central labs for analysis
- Terminate fast.

Day3 Friday Oct. 9th:

1. Fasting study will continue until Hour 18 (1200 pm)

2. Peripheral IVs may be removed after fasting study is completed and the patient is eating normally.
3. Please order lunch from the regular menu for subject to have when fasting study is completed.
4. Subject may order the rest of his/her meals from the regular menu for the duration of his/her stay.
5. As soon as subject is done with the fasting study, he/she and the family are free to relax for the afternoon.
6. Observe subject for signs of return to normal activities with regular food and beverage intake.

Day 4 Saturday Oct 10th:

Subject will be discharged when medically stable.