Impaired fasting tolerance among Alaska native children with a common carnitine palmitoyltransferase 1A sequence variant

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ARTICLE INFO

Article history:
Received 7 May 2011
Received in revised form 21 June 2011
Accepted 22 June 2011
Available online xxx

Keywords:
Fatty acid oxidation
Carnitine palmitoyltransferase 1A
Ketones
Hypoketotic
Hypoglycemia
Fasting

A B S T R A C T

A high prevalence of the sequence variant c.1436C→T in the CPT1A gene has been identified among Alaska Native newborns but the clinical implications of this variant are unknown. We conducted medically supervised fasts in 5 children homozygous for the c.1436C→T variant. Plasma free fatty acids increased normally in these children but their long-chain acylcarnitine and ketone production was significantly blunted. The fast was terminated early in two subjects due to symptoms of hypoglycemia. Homozygosity for the c.1436C→T sequence variant of CPT1A impairs fasting ketogenesis, and can cause hypoketotic hypoglycemia in young children.

Trial registration www.clinicaltrials.gov NCT00653666 “Metabolic Consequences of CPT1A Deficiency”

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1. Introduction

Carnitine palmitoyltransferase 1A (CPT1A) catalyzes the synthesis of long chain acylcarnitines and is required for mitochondrial fatty acid oxidation in the liver. CPT1A deficiency is an inherited disorder of hepatic fatty acid oxidation that affects the tolerance to fasting and presents clinically with hypoketotic hypoglycemia. A unique polymorphism in the CPT1A gene (c.1436C→T) is associated with decreased enzyme activity and altered regulation [1], and has been identified among the Canadian and Greenland Inuit, and some Alaska Native populations [2–4]. This sequence variant results in a proline to leucine substitution at amino acid 479 (p.P479L) of the CPT1A protein. The prevalence of this genetic variant is extremely high in some regions, with reports of up to 80% of individuals being homozygous in some Alaska Native populations [2,3]. The observation that the c.1436C→T variant is the most prevalent or “normal” allele within certain populations suggests positive selection for some unknown beneficial effect. The potential benefit of the c.1436C→T variant has not been elucidated, but Greenberg and colleagues [3] have proposed that because the P479L protein has a high residual enzyme activity (20% of normal) and is relatively insensitive to malonyl-CoA inhibition, it may allow some post-prandial hepatic ketogenesis to occur in the context of the traditional high fat, high protein diet consumed by indigenous Arctic people.

There are also potential negative consequences associated with the c.1436C→T variant. CPT1A is required for fasting ketogenesis, and severe deficiency is associated with hypoketotic hypoglycemia, liver dysfunction and sudden infant death [5]. Infant mortality rates are significantly higher in regions where the variant is most prevalent [6,7], and our preliminary evidence indicates that Alaska Native infants who are homozygous for the variant have an increased risk of infant death [8]. Our hypothesis is that the increased infant mortality rate associated with the c.1436C→T variant is due to its effect on fasting ketogenesis.

During the initial phase of fasting the breakdown of liver glycogen supplies systemic glucose needs. Glycogen depletion leads to a gradual decrease in serum glucose and insulin, with activation of hormone sensitive lipase in adipose tissue and release of free fatty acids into the blood. The liver oxidizes fatty acids to generate ketones, which serve as an alternate energy source, sparing glucose for glucose-requiring tissues. ATP derived from fatty acid oxidation also energizes de novo...
glucose synthesis from amino acids and other substrates in the liver. CPT1A is required for mitochondrial fatty acid oxidation and is therefore essential in the metabolic response to fasting.

In this study we evaluated the fasting response of five children homozygous for the c.1436C→T variant to determine the physiologic significance of the decreased CPT1A activity associated with this polymorphism.

2. Methods

2.1. Subject recruitment

The Alaska Newborn Screening Program sent a letter to all nine eligible families with children age three and older that had been identified as homozygous for the c.1436C→T variant via newborn screening and lived in the Norton Sound area of Alaska inviting them to participate. Six interested families contacted the principal investigator (MBG), who provided details of the study and handled travel logistics for families. Five families decided to participate and they flew from their home in Alaska to Portland, OR. Informed consent to participate in the study was obtained from the parents of all subjects. The Institutional Review Boards of Oregon Health & Science University (OHSU) and the Alaska Area Indian Health Service (OHSU IRB3556, Alaska Area IRB 2007-9-30, NCT00653666) approved this study. Tribal approval was obtained from the Research Ethics Review Board of the Norton Sound Health Corporation (Nome, Alaska) and the Southcentral Foundation (Anchorage, Alaska).

Fasting protocol: Fasting studies were done on the inpatient unit of the OHSU Pediatric Clinical and Translational Research Center in Doernbecher Children’s Hospital. Subjects were fasted for 18 h following an evening meal, with continuous heart rate and pulse oximetry monitoring. Subjects received maintenance IV fluids with 1/2 normal saline and were allowed to drink calorie-free beverages. Blood samples were obtained after 1, 6, 12 and 18 h of fasting for electrolytes, glucose, insulin, free fatty acids, acylcarnitines and ketones. Beginning at 6 h of fasting, serum glucose was measured hourly on the inpatient unit with a glucose oxidase analyzer (Yellow Springs Instruments, Yellow Springs, OH). The fast was terminated after 18 h and subjects were allowed to eat and drink ad-lib. If the blood glucose dropped below 60 mg/dl monitoring was increased to every 30 min. If the blood glucose dropped below 40 mg/dl or the subject developed clinical signs of hypoglycemia, 2 ml/kg body weight of 25% dextrose was given via IV push and the fast was terminated. Subjects and their families were allowed to return home the following day.

Biochemical analysis: Whole blood was centrifuged within 20 min of collection and aliquots of serum samples were stored at −80 °C. Serum ketones (3-hydroxybutyrate and acetoacetate) were measured by stable isotope dilution gas chromatography–mass spectrometry (GC–MS)[9]. Serum carnitine levels and acylcarnitine profiles were quantified by tandem mass spectrometry (MS/MS) and expressed as μmol/L.[10] Plasma samples were isolated via centrifugation of whole blood collected in EDTA tubes containing a lipase inhibitor (tetrahydrolipos- tatin) and stored at −80 °C. Plasma free fatty acids (FFA) were measured using the NEFA-HR(2) kit (Waco Chemicals, Richmond, VA).

Data analysis: Group data is given as the mean ± the standard deviation of the mean for all subjects (n = 5) unless otherwise stated. Because of the availability of published normal data and the ethical concerns of fasting control children, data from our subjects were compared to published data of children who were free of a fatty acid oxidation (FAO) disorder[11,12].

3. Results

Subject characteristics: Each of the five children were homozygous for the c.1436C→T variant. Height and weight of all subjects were within normal percentiles for age, and the mean body mass index (BMI) was 19.6 kg/m² (92th percentile) (Table 1). All subjects were developmentally normal and had a normal physical exam at the time of the study. Blood chemistries (AST, ALT, bilirubin, albumin, BUN/Cr) were normal in all subjects. To our knowledge, the subjects were unrelated. Parents of all subjects reported that they consumed a regular pediatric diet at home prior to entering the study. Alaska Native families in the Norton Sound region typically follow a diet that includes large amounts fish, land and sea mammals, but is modified from the traditional diet by the inclusion of increased amounts of carbohydrate. During their stay in the pediatric CTRC, subjects were allowed to choose foods from the regular pediatric hospital menu.

3.1. Metabolic response to fasting

Baseline levels of total (54 ± 10 μmol/L) and free (46 ± 8 μmol/L) carnitine were normal in all subjects. In contrast, the ratio of free carnitine (C0) to the sum of the C16 and C18 acylcarnitines (C0/C16 + C18) was significantly elevated (mean 199 ± 68, normal<130 in infants), which is characteristic of CPT1A deficiency, reflecting the decreased ability to generate long chain acylcarnitines[13]. This ratio is used diagnostically to identify infants with CPT1A deficiency by newborn screening, including the five subjects in this study. Over the course of the fast, the expected rise in levels of long chain acylcarnitines and concomitant decrease in free carnitine was reduced (Fig. 1A and B; Table 1)[12,14].

The blunted rise in acylcarnitines was not due to a lack of substrate. Plasma FFA levels rose to a mean of 1.5 ± 0.6 mmol/L after 18 h of fasting, which is similar to levels reported in normal children (Fig. 1C)[11]. In contrast, at the termination of the fast the mean level of serum ketones was decreased (0.21 ± 0.08 mmol/L vs. 2.4 mmol/L), and the FFA:ketone ratio was markedly increased (8.45 ± 4.5, range

Table 1: Demographic data of subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI</th>
<th>Prior symptoms</th>
<th>Plasma acylcarnitine profiles (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post-prandially</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Free carnitine</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>M</td>
<td>109.0</td>
<td>20.3</td>
<td>24.0</td>
<td>None</td>
<td>44.8</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>M</td>
<td>92.0</td>
<td>16.6</td>
<td>19.6</td>
<td>Hypoglycemia and seizures with illness</td>
<td>45.7</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>M</td>
<td>111.5</td>
<td>23.9</td>
<td>19.2</td>
<td>None</td>
<td>55.4</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>M</td>
<td>98.0</td>
<td>16.4</td>
<td>17.1</td>
<td>None</td>
<td>33.6</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>F</td>
<td>110.5</td>
<td>21.9</td>
<td>17.9</td>
<td>Lethargy with illness</td>
<td>51.7</td>
</tr>
</tbody>
</table>

Individual subject data are presented. cm = centimeter; kg = kilograms; BMI = body mass index calculated as weight (kg)/height (m²); prior symptoms = parental reports of symptoms prior to study enrollment; plasma acylcarnitine concentrations of free carnitine, C16:0 and C18:0 acylcarnitine and the C0/C16+C18 ratio one hour after a meal, and C16:0, C18:0, acylcarnitine and the C0/C16+C18 ratio at the time the fast was terminated. The fast was terminated at 18 h of fasting for subjects 1, 3 and 4, at 16 h of fasting for subject 2 and at 17 h of fasting for subject 5.

Please cite this article as: M.B. Gillingham, et al., Impaired fasting tolerance among Alaska native children with a common carnitine palmitoyltransferase 1A sequence variant, Mol. Genet. Metab. (2011), doi:10.1016/j.ymgme.2011.06.017
4.1–16.0, vs. <2.5) in all study subjects when compared to controls at 20 h of fasting (Fig. 1D and E).

Two of the five subjects in our study developed symptoms of hypoglycemia (lethargy and tachycardia) prior to completing the 18-hour fast. Blood glucose dropped to 25 mg/dl after 16 h in subject 2, and to 50 mg/dl at 17 h in subject 5 (Fig. 1F). Symptoms resolved rapidly in both subjects following IV glucose administration and resumption of oral calorie intake. The remaining three subjects remained asymptomatic and maintained a normal serum glucose level (>50 mg/dl) throughout the 18-hour fast.

4. Discussion

The c.1436C→T variant in CPT1A was identified in Alaska Native infants following the implementation of expanded newborn using MS/MS [2]. The subsequent discovery that up to 80% of infants born in some regions are homozygous for this variant has raised significant questions about its health consequences, and whether identification via newborn screening is necessary. The goal of this study was to determine if young children who are homozygous for the c.1436C→T variant in CPT1A identified by newborn screening have an abnormal fasting response. All five of the children in our study had an abnormal metabolic response to fasting, as demonstrated by markedly elevated FFA:ketone ratios. The FFA:ketone ratio is a strong indicator of the capacity to oxidize fatty acids and generate ketones, and the elevated ratios seen in our subjects are characteristic of patients with fatty acid oxidation disorders [11,12,15]. In addition to hypoketosis, patients with disorders of fatty acid oxidation often develop hypoglycemia during fasting, which occurred in two of our study subjects. The impairment of both ketogenesis and glucose homeostasis observed in our subjects indicates that the c.1436C→T variant can have a physiologically relevant impact on CPT1A activity.

It is estimated that more than 700 Alaska Native infants homozygous for the c.1436C→T variant are born each year [2], and yet reports of hypoketotic hypoglycemia are relatively rare and most often associated with fasting related to illness (personal observation—MH and DMK). This suggests that under normal circumstances the impaired fasting ability associated with homozygosity for the variant...
is rarely symptomatic. This may not be true of prolonged fasting associated with viral illness and other stressors. However, we do not know whether all homozgyous children are at risk. Within our small sample only two subjects developed symptoms of hypoglycemia, and there were no obvious differences in baseline lab or anthropomorphic data between them and the other three, suggesting it may not be possible to identify children most at risk. Parents of these two subjects did report prior symptoms in their children at the time of enrollment (Table 1). There are a number of inherited metabolic diseases where only a portion of affected individuals ever present with disease-specific symptoms, and then only when associated with environmental stress. These include other fatty acid oxidation disorders such as medium chain acyl-CoA dehydrogenase deficiency (MCADD) and acute porphyrias.

Newborn screening for MCADD has been shown to be highly sensitive and to decrease infant morbidity and mortality due to this disorder when treatment is initiated in screened populations [16–19]. Treatment for MCADD only requires avoiding prolonged fasting and close monitoring during illness. In our clinical experience, the same approach is also effective for avoiding symptoms of CPT1A deficiency. We predict that only a small fraction of the children identified via newborn screening as homozygous for the c.1436C→T variant will ever have an episode of hypoketotic hypoglycemia. However, in contrast to MCADD, newborn screening by MS/MS identifies only a small fraction (~10%) of the infants homozygous for the c.1436C→T variant due to low sensitivity and specificity [2]. Determination of the need for full asertmation, which would require DNA testing of all infants born in Alaska, will require epidemiologic data to determine the extent and severity of clinical events, including death, associated with the variant allele.

Regardless of future policies regarding the merits of identifying infants with CPT1A deficiency due to the c.1436C→T variant by newborn screening, our results support continuation of the combined educational efforts of the State of Alaska Newborn Screening Program and healthcare providers from the Alaska Native Health System regarding proper care of affected infants and children. These efforts could significantly decrease infant mortality in the Alaska Native population, in which case identification of the c.1436C→T variant by newborn screening would be yet another example of the public health benefits of newborn screening.

Acknowledgments

Supported by the National Institutes of Health grant K01 NIDDK DK071869 (MBG) and the Oregon Clinical and Translational Research Institute (OCTRI) grant UL1 RR024140 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research.

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