Original Investigation

Triheptanoin for Glucose Transporter Type I Deficiency (GID) Modulation of Human Ictogenesis, Cerebral Metabolic Rate, and Cognitive Indices by a Food Supplement

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IMPORTANCE Disorders of brain metabolism are multiform in their mechanisms and manifestations, many of which remain insufficiently understood and are thus similarly treated. Glucose transporter type I deficiency (GID) is commonly associated with seizures and with electrographic spike-waves. The GID syndrome has long been attributed to energy (ie, adenosine triphosphate synthetic) failure such as that consequent to tricarboxylic acid (TCA) cycle intermediate depletion. Indeed, glucose and other substrates generate TCAs via anaplerosis. However, TCAs are preserved in murine GID, rendering energy-failure inferences premature and suggesting a different hypothesis, also grounded on our work, that consumption of alternate TCA precursors is stimulated and may be detrimental. Second, common ketogenic diets lead to a therapeutically counterintuitive reduction in blood glucose available to the GID brain and prove ineffective in one-third of patients.

OBJECTIVE To identify the most helpful outcomes for treatment evaluation and to uphold (rather than diminish) blood glucose concentration and stimulate the TCA cycle, including anaplerosis, in GID using the medium-chain, food-grade triglyceride triheptanoin.

DESIGN, SETTING, AND PARTICIPANTS Unsponsored, open-label cases series conducted in an academic setting. Fourteen children and adults with GID who were not receiving a ketogenic diet were selected on a first-come, first-enrolled basis.

INTERVENTION Supplementation of the regular diet with food-grade triheptanoin.

MAIN OUTCOMES AND MEASURES First, we show that, regardless of electroencephalographic spike-waves, most seizures are rarely visible, such that perceptions by patients or others are inadequate for treatment evaluation. Thus, we used quantitative electroencephalographic, neuropsychological, blood analytical, and magnetic resonance imaging cerebral metabolic rate measurements.

RESULTS One participant (7%) did not manifest spike-waves; however, spike-waves promptly decreased by 70% (P = .001) in the other participants after consumption of triheptanoin. In addition, the neuropsychological performance and cerebral metabolic rate increased in most patients. Eleven patients (78%) had no adverse effects after prolonged use of triheptanoin. Three patients (21%) experienced gastrointestinal symptoms, and 1 (7%) discontinued the use of triheptanoin.

CONCLUSIONS AND RELEVANCE Triheptanoin can favorably influence cardinal aspects of neural function in GID. In addition, our outcome measures constitute an important framework for the evaluation of therapies for encephalopathies associated with impaired intermediary metabolism.

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Glucose is the principal cerebral metabolic substrate under normal circumstances. Glucose provides energy (adenosine triphosphate) to neural cells and carbon for neurotransmitter production, one of the numerous biosynthetic reactions that the brain carries out, via glycolysis and the tricarboxylic acid (TCA) cycle. However, glucose metabolism also fulfills other, less-acknowledged cerebral biosynthetic requirements, which are no less important from a clinical standpoint when they remain unmet, such as anaplerosis (ie, the replenishment of intermediates that are normally lost from the TCA pool). Thus, a myriad of cerebral energetic and carbon fluxes, many of which are poorly understood quantitatively, stem from brain glucose availability. This is, in turn, contingent on the activity of the facilitative membrane glucose transporter type I (GLUT1), which permits glucose to cross the blood–brain barrier and subsequently transit into, and probably through, astrocytes.

Human GLUT1 deficiency (G1D) due to mutation of the gene SLC2A1 (OMIM 606777) is associated with partial loss of function (ie, GLUT1 activity is never abolished) and results in decreased brain glucose accumulation. Glucose transporter type I deficiency manifests as an encephalopathy frequently (but not exclusively) characterized by medication-refractory infantile-onset seizures, diminished encephalopathic mass, intellectual disability, and complex (ie, multiform) movement disturbances (spasticity, ataxia, chorea, dystonia, and combinations thereof). This encephalopathy is often accompanied by a reduced cerebrospinal fluid glucose concentration, even though cerebrospinal fluid glucose abundance primarily reflects secretion by the choroid plexus and exceeds the concentration typical of the brain’s interstitial space. Notably, a series of important neural function defects in G1D is independent of blood–brain barrier GLUT1 dysfunction and can be ameliorated by increasing the brain tissue glucose concentration, as has been shown in the G1D mouse. We noted that these findings might bear relevance to human G1D, thus inviting treatment development based on the enhancement of brain glucose influx and/or the supply of an effective substitute substrate.

In this regard, the ketogenic diet, which is used for the treatment of G1D solely on the basis of biochemical assumptions and empirical observations and has not been the subject of a controlled clinical trial in G1D, ameliorates some movement disorders and epilepsy in two-thirds of the patients who receive it (J.M.P., unpublished data, 2012). Unfortunately, neurologic deficits, particularly those centered on other aspects of movement coordination (eg, ataxia and dystarthis) and cognition, tend to persist while the diet is used, with a significant portion of patients experiencing recurrent or incompletely treated abnormalities. The remaining one-third of the patients who are poorly responsive to the diet face an even more problematic clinical course. The diet, given with the therapeutic intent of stimulating brain metabolism in G1D, may act as an anticonvulsant through several potential mechanisms, including the production of acetyl-coenzyme A that can stimulate the neural TCA cycle, but the diet’s mode of action has not been investigated in G1D. In addition to insufficiently alleviating all incapacitating features of G1D, ketogenic diets are not universally tolerable, and few adults receive them, whereas other interventions, such as the modified Atkins diet, have not been subject to rigorous investigation in G1D. Thus, most therapeutic efforts have been limited to early diagnosis and initiation of a ketogenic diet during childhood.

In contrast with this form of therapy, studies have shown that nutrients with additional potential to refill TCA cycle intermediates via anaplerosis, such as triheptanoin (an edible triglyceride of the 7-carbon fatty acid heptanoate that can yield molecules of heptanoate and other anaplerotic metabolites in the liver), offer therapeutic benefit in diseases in which metabolic precursor depletion or overuse is suspected or identified. Dietary triheptanoin gives rise (after hepatic metabolism) to plasma heptanoate and 5-carbon ketone bodies (β-heptapentanoate and β-hydroxyheptanoate), all of which can penetrate and be readily metabolized by the brain (eFigure in the Supplement), as has been shown in rodents including G1D mice. Neoglucogenesis can also be observed after heptanoate infusion, and this process may act synergistically with the other heptanoate metabolites in brain glucose-deficient states, such as G1D. Heptanoate metabolism exerts anticonvulsant effects in an unrelated animal model of experimental pilocarpine-induced epilepsy and refills depleted brain TCA cycle intermediates. Therefore, the rationale for the present work combines potential biochemical benefits, experience with other neurometabolic disorders that we have treated with triheptanoin, and our ex vivo G1D rodent model evidence of the metabolic effects of triheptanoin on the brain, all in the context of limited therapeutic alternatives.

Methods

This study was conducted with institutional review board approval of The University of Texas Southwestern Medical Center and was referenced under the US Food and Drug Administration Investigational New Drug 59303 (sponsor-investigator C.R.R.). Written informed consent was obtained from each participant older than 18 years, and informed consent was obtained from one parent or guardian of all younger participants. Assent was obtained from children between 10 and 18 years. Travel and lodging costs up to a total of $500 per family were reimbursed for each visit.

Participants

A summary of patient age, SLC2A1 G1D causative mutation, and other factors is given in eTable 1 in the Supplement. Study participants were recruited (1) from the Rare Brain Disorders Program at The University of Texas Southwestern Medical Center and Children’s Medical Center Dallas, including existing patients and those responding to our official website announcement, and (2) via public announcement by the GLUT1 Deficiency Syndrome Foundation. Patients enrolled included males and females, ages 2 years to 28 years, and English or Spanish speakers. Participants were consecutively enrolled from all eligible patients who contacted us. There was no consideration given to geographic location (including the United States and Canada), disease severity, or selection factors other than those listed here. Exclusion criteria included the current use of a ke-
togenic diet. Participants with metal implants incompatible with exposure to a research magnetic field or unable to tolerate magnetic resonance imaging (MRI) because of anxiety were excluded from the MRI procedures. Participants who were not previously genetically verified for G1D received genotyping via the National Institutes of Health Office of Rare Diseases Research Collaboration, Education, and Test Translation (CETT) program for G1D at the University of Texas Southwestern Medical Center and Children’s Medical Center Dallas. Because approximately 15% of patients with G1D do not harbor identifiable pathogenic mutations in SLC2A1, one patient, who exhibited clinical symptoms, a decreased cerebrospinal fluid glucose concentration, and a characteristic brain fludeoxyglucose F18 positron emission tomography scan indicative of G1D, was enrolled despite normal clinically available DNA sequencing of SLC2A1 and standard chromosomal microarray analysis. No medications were changed immediately before or during the study for any participant.

Dietary Supplement
Triheptanoin is a naturally occurring fat that is readily synthesized from castor bean oil for use in the human food industry as an additive to dairy products or as an emollient in cosmetics. In the United States, food-grade triheptanoin first received orphan designation for the treatment of fatty acid oxidation disorders after work performed under our Investigational New Drug 59303 status (sponsor-investigator, C.R.R.), and, more recently (2012), it achieved an equivalent legal status in the European Union for the treatment of very-long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (designation EU/3/12/1081).

Participants received open-label adjunctive supplementation with food-grade triheptanoin, a medium-chain triglyceride of heptanoic acid that is colorless and odorless. Triheptanoin was manufactured in oil form (Sasol Germany GmbH) and permitted, per the manufacturer (product information documentation, version 4.02; revision January 16, 2008), for applications in the food industry as a food additive (butter marker-fat) and commercialized as Spezialöl 107 (European Inventory of Existing Commercial Substances Chemical Abstracts Service numbers: 620-67-7/210-647-2; minimum 95% triheptanoate and 99% 7-carbon fatty acid as determined by gas chromatography by the manufacturer). The triheptanoin oil used in this study was purchased from the Sasol North American distributor.

Procedures
The experimental procedures are illustrated in Figure 1. Each patient underwent a pretreatment medical history, complete physical and neurologic examinations, general analytical laboratory evaluation (following overnight fasting), electroencephalogram (EEG), and neuropsychological evaluation. Participants who consented to imaging also underwent a 30-minute MRI examination of the total rate of oxygen consumption by the brain (ie, the cerebral metabolic rate [CMRO2]).

All participants underwent EEG recording. The EEG was acquired by placing an MRI-compatible electrode cap containing 32 active electrodes except where noted below. An additional electrode was placed directly on the skin of the chest. All preparation equipment coming into contact with the skin was discarded after a single use, and electrodes and caps were disinfected after use following standardized University of Texas Southwestern Medical Center guidelines.

Baseline (resting) EEG recordings after overnight (>8 hours) fasting (except for water and medications) were obtained for 30 to 45 minutes. Baseline neuropsychological testing, lasting approximately 30 minutes, occurred before baseline EEG recordings were performed. Parents and patients (when sufficiently mature) were asked to note all visible seizures, and their observations were contrasted with EEG recordings. After a sufficiently informative baseline EEG (ie, defined as a tracing containing a steady rate of spike-wave discharges) had been established and recorded (for a minimum of 15 minutes), participants consumed a single dose of triheptanoin oil over 1 to 3 minutes in amounts ranging from 15 to 60 mL according to body weight (0.75-1.0 g/kg). Electroencephalographic monitoring was continued for at least an additional 90 minutes after participants consumed the oil. Neuropsychological testing was repeated approximately 90 to 120 minutes after oil ingestion. Blood samples were collected from each participant before and at the conclusion of the EEG. A seizure rate, defined as the total duration of EEG-recorded spike-wave seizures divided by the total duration of the EEG recording, was calculated for the baseline period (before triheptanoin oil consumption) and posttriheptanoin period (from 5 minutes after triheptanoin oil consumption to the conclusion of the EEG recording).

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**Figure 1. Flow Diagram of Study Visits and Procedures**

<table>
<thead>
<tr>
<th>Visit 1: Baseline (fasting)</th>
<th>Visit 2: 3-mo follow-up</th>
<th>Visit 3: 6-mo follow-up/study exit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose and other safety blood testing</td>
<td>Fasting glucose and other safety blood testing</td>
<td>Fasting glucose and other safety blood testing</td>
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<tr>
<td>Neuropsychological testing</td>
<td>Neuropsychological testing</td>
<td>Neuropsychological testing</td>
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<tr>
<td>EEG</td>
<td>(optional)</td>
<td>(optional)</td>
</tr>
<tr>
<td>MRI (optional)</td>
<td>MRI (optional)</td>
<td>Discontinue triheptanoin therapy</td>
</tr>
<tr>
<td>Administration of triheptanoin</td>
<td>Wait 60-90 min</td>
<td>Physical and neurologic examination</td>
</tr>
<tr>
<td>Random glucose measurement</td>
<td>EEG</td>
<td>Physical and neurologic examination</td>
</tr>
<tr>
<td>Neuropsychological testing</td>
<td>MRI (optional)</td>
<td>Fasting glucose and other safety blood testing</td>
</tr>
<tr>
<td>MRI (optional)</td>
<td>Neuropsychological testing</td>
<td>Neuropsychological testing</td>
</tr>
<tr>
<td>Start daily triheptanoin therapy</td>
<td>Exit study</td>
<td></td>
</tr>
</tbody>
</table>

Each patient participated in 3 visits. A limited number of patients underwent electroencephalogram (EEG) and magnetic resonance imaging (MRI) at the 3-month follow-up visit.
Participants who consented to imaging underwent MRI before and after triheptanoin administration. The second MRI took place 60 to 90 minutes following triheptanoin consumption. This time interval was selected to capture the peak metabolism of triheptanoin in blood as separately determined by us in 11 participants (C.R.R., unpublished data, 2006). Magnetic resonance imaging was performed before and after triheptanoin consumption and immediately after each session of neuropsychological testing, followed by a 5-minute rest period. The EEG was recorded continuously during the MRI scan.

Magnetic resonance imaging included a routine axial T1-weighted sequence to establish that brain configuration was normal in all participants and, more importantly, allowed for CMRO2 quantification,42-47 with a test-retest CMRO2 reliability of less than 5% error. In brief, using phase-contrast MRI and T2-relaxation underspin tagging MRI measurements, the whole-brain arteriovenous oxygenation gradient was determined and used to calculate brain oxygen extraction with the aid of full-brain volumetric measurements excluding cerebrospinal fluid. Scan acquisition time was 5 minutes per MRI. No sedation or stimulation was administered.

Neuropsychological testing consisted of the Peabody Picture Vocabulary Test, Fourth Edition,48 (PPVT-4) and the Expressive Vocabulary Test, Second Edition (EVT-2).49 These 2 tests were chosen for their ease of administration, relatively short administration time, and availability of parallel forms, allowing for quick readministration without the concern of practice effects.50 Administration of the tests took approximately 30 to 45 minutes per testing session.

3-Month Triheptanoin Consumption
To investigate its long-term effect on outcome measures, triheptanoin oil was administered for 3 months at approximately 1 g/kg of body weight per day, divided into 4 doses per day, given 30 to 60 minutes before a routine meal. This dose represents approximately 30% of the total dietary fat. Participants followed additional dietary modifications with the goal of maintaining a constant total daily fat intake and body weight. A reduction in simple carbohydrate consumption was instituted with the intent of lowering the glycemic index so as to minimize insulin release, interference with ketogenesis, or excessive weight gain. Daily caloric and protein intake remained unmodified.

Long-term Follow-up
Participants were evaluated at 3 months and 6 months for follow-up testing and posttreatment advice as necessary. Triheptanoin was discontinued at the 3-month visit. Follow-up visits included complete physical and neurologic examinations, general analytical laboratory analyses, and neuropsycholog-

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Figure 2. Electroencephalogram (EEG)-Captured Spike-Wave Seizures in Patients With Glucose Transporter Type 1 Deficiency

A, An example segment of an EEG recording containing 3 spike-wave seizure periods. Electrode positioning followed standard abbreviated nomenclature. Vertical bars indicate 1 second. B, Illustration of the identification of seizure duration. C, The seizure-nonseizure binarized time course of a representative participant. Spike-wave periods are represented as bar plots against an EEG time course in the setting of a nonseizure baseline state. The pink bar indicates triheptanoin (oil) administration.
Triheptanoin in Glucose Transporter Type 1 Deficiency

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Longer-term (Indefinite) Consumption of Triheptanoin

All participants who reported clinical benefit and requested to continue triheptanoin beyond the 3-month visit were removed from the initial research protocol. To continue triheptanoin for an indefinite period, patients voluntarily submitted a signed letter requesting continuation and then were enrolled in a second institutional review board–approved protocol, agreeing to provide semiannual blood tests and general physical examination reports. Additional contact was maintained with each of these participants via telephone or secure, Health Insurance Portability and Accountability Act–compliant e-mail communications as needed.

Statistical Analysis

The primary outcome of our study was a reduction in electrographic seizures as measured by seizure rate (total time of the seizures divided by the total EEG-recorded time) and increased neuropsychological performance as measured by the PPVT-4 and EVT-2. Secondary outcomes included CMRO2. Descriptive statistics are provided for demographic and clinical measures. Wilcoxon matched-pairs signed rank tests were performed on EEG and CMRO2 data before and after triheptanoin consumption at baseline and at baseline and 3-month measures of EEG, neuropsychological measures, and blood analyses (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and β-hydroxybutyrate levels). SPSS, version 20 (SPSS Inc), was used to analyze these data using a 2-sided P value (unless otherwise noted).

Results

Patient Characteristics

Fourteen patients with a G1D diagnosis were enrolled (eTable 1 in the Supplement). The median age at enrollment was 12.5 years (range, 2-27 years), and 6 of 14 patients (43%) were female. Most patients identified themselves as white (12 [86%]) and non-Hispanic (13 [93%]); 1 patient (7%) was African American and 1 patient (7%) was white and Asian. The median age at the onset of symptoms based on 13 (93%) patients was 8.5 months (range, 1 month to 30 months), and the median age at diagnosis based on 11 (79%) patients was 7.3 years (range, 2-27 years). Patients were enrolled from the United States and Canada.

Of the 14 patients enrolled in the trial, 5 participated in imaging. These patients were older than 15 years. The 9 patients who elected not to participate in imaging did so for reasons including the inability to remain immobile owing to movement disorder or anxiety (5 [36%]), immaturity (2 [14%]), metal implants preventing exposure to research MRI (1 [7%]), and personal choice (1 [7%]).

Adverse Events

No patient experienced serious or unexpected adverse events. One patient (7%) discontinued triheptanoin therapy after 3 weeks owing to gastric discomfort. One other patient (7%) experienced significant (10%) weight gain at 2 months that did not lead to discontinuation. This patient did not follow the recommended dietary and nutritional advice by decreasing extra sources of fat and simple sugars. Two patients (14%) experienced diarrhea and/or digestive discomfort within days of treatment initiation, but these symptoms resolved by reducing the dose of triheptanoin by one-half and gradually increasing the amount to the target levels over several days.

Correlation Between EEG and Absences

All 14 patients were observed by their primary caretaker while undergoing EEG monitoring. Patients considered to be mature and their caretakers were asked to report all absence events that they noticed. Patients and caretakers had no access to the EEG tracing and were visually supervised in a separate room through a window by the EEG recording operator. The EEG operator (P.L. and D.M.) and an expert neurologist (J.M.P.) noted the correlation between reported absence or any other potential seizure events and the EEG tracing. All of the participants who manifested spike-waves, including the caretakers, underreported EEG spike-wave seizures. For 7 patients, more than 50% of spike-wave seizures lasting longer than 3 seconds (ie, EEG absence seizures that are typically documented in clinical neurophysiological reports) were not noticeable by the observer as absences or other abnormal behavior. For 4 participants, no absence or other abnormal manifestation was reported despite the occurrence of abundant spike-wave seizures lasting longer than 3 seconds.

Immediate Response to Therapy

EEG Recordings

The EEG recordings were analyzed as described in previous work51-53 with the exception that only the clinically relevant aspects of the EEG (ie, those that are part of a standard EEG report) were analyzed. After the standard consent procedure, triheptanoin was urgently initiated in the inpatient setting in 2 of the 14 patients (14%) because of unmanageable epilepsy (absence seizures more frequent than hourly). Thus, baseline research EEG recordings were not performed in these patients as described above and were not included in the quantitative EEG analysis. However, these patients were receiving EEG monitoring just as triheptanoin was initiated, and its administration was followed by the termination of absences and a decrease in EEG spike-waves within 2 hours after triheptanoin ingestion. Of the 12 remaining patients, 1 patient (8%) exhibited no EEG-documented or observable absence seizures at baseline for the duration of the EEG recording and was excluded from the EEG data analysis. The overall self-reported or parent-reported absence seizure rate was below 20% of all electrographic spike-wave seizures regardless of EEG seizure frequency or duration.

Electrographic spike-wave seizures were precisely captured by EEG recording (Figure 3). There were no other types of EEG-documented seizures in any of the patients. The seizure rate was calculated both before and after triheptanoin administration for each participant by dividing the total duration of recorded spike-wave seizures by the total duration of the EEG recording (eTable 2 in the Supplement and Figure 2).
As shown in Figure 2B, the duration of each spike-wave segment was delimited by the onset of the first spike and the return to the baseline rhythm immediately after the last wave. Therates of seizures before and after triheptanoin oil administration at baseline decreased in all patients ($P = .001$) (Figure 3).

Neuropsychological Indices

Eight patients (57%) completed baseline neuropsychological testing. The first 3 participants did not undergo neuropsychological testing owing to logistic limitations, and 2 participants (14%) were urgently enrolled as inpatients as described above. Therefore, the PPVT-4 and EVT-2 tests could not be administered to them. In addition, 1 patient completed baseline neuropsychological testing but did not return for testing at 3 months and was therefore excluded from the analyses. In all, the PPVT-4 and EVT-2 could not be administered to 6 patients.

Vocabulary performance improved acutely and over long-term follow-up with triheptanoin supplementation. The neuropsychological scores of all 8 participants before and after triheptanoin use are represented. Standardized Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4), and Expressive Vocabulary Test, Second Edition (EVT-2), ratings were obtained in the fasting state (baseline) at time 0 minutes (PPVT-4 1A) and 60 minutes (PPVT-4 1B) following triheptanoin ingestion and then after 3 months of daily triheptanoin supplementation (PPVT-4 2). PPVT-4 and EVT-2 scores were below normal for the age ranges and increased at subsequent time points in rigorously statistically significant fashion. The interquartile ranges (IQRs) for the PPVT-4 scaled scores at each of the 3 time points were 59-75, 62-86, and 61-84, respectively. PPVT-4 scores improved significantly over time (Friedman test, $P = .03$). The IQRs for the EVT-2 scaled scores at each of the 3 time points were 60-80, 57-84, and 65-89, respectively. EVT-2 scores improved significantly over time (Friedman test, $P = .02$).

At the baseline visit, patients underwent testing while fasting and then again approximately 1 hour after ingesting triheptanoin. During this visit on the PPVT-4 evaluation, the receptive vocabulary improved in 7 of 8 participants (88%) and worsened in 1 participant (12%) ($P = .04$). On the EVT-2 evaluation, 5 of 8 participants (62%) improved and 2 participants (25%) worsened ($P = .12$). Overall, 8 of 8 participants showed improved scores on some aspect of neuropsychological testing between the fasting baseline evaluation and testing an hour after administration of triheptanoin, but these changes were not significant (Figure 4).

Blood Glucose

Blood glucose levels were measured 90 to 120 minutes after triheptanoin consumption at the baseline visit and compared with fasting levels collected earlier that morning. A Wilcoxon
matched-pairs signed rank analysis yielded a significant decrease (fasting glucose median, 87 mg/dL; posttriheptanoin glucose median, 81 mg/dL; \( P = .02 \)) to convert glucose to millimoles per liter, multiply by 0.0555).

**Cerebral Metabolic Rate**

Five patients completed imaging at baseline. The median percentage change in CMRO2 from baseline following the first administration of triheptanoin was 5.9%, but this change was not significant (\( P = .31 \)). Three participants (60%) experienced an increase in the cerebral metabolic rate, and 2 (40%) experienced small changes (Figure 4).

**Response at 3-Month Follow-up**

**Neuropsychological Testing**

When baseline fasting PPVT-4 and EVT-2 scores were compared with scores at the 3-month follow-up, immediate gains (ie, those detected after the first triheptanoin dose) were maintained on the PPVT-4 (ie, all 7 patients [88%] who exhibited immediate improvement continued to manifest similar improvement at the 3-month follow-up). Immediate gains were also maintained on the EVT-2, with 2 additional patients (25%) demonstrating improvement, for a total of 7 of 8 patients (88%) showing improvement at the 3-month follow-up. Significant improvement in the PPVT-4 and EVT-2 scores were observed from baseline to the 3-month follow-up (\( P = .04 \) and \( P = .02 \), respectively) (Figure 4).

**Laboratory Tests**

Blood glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and β-hydroxybutyrate levels were compared between baseline and the 3-month follow-up using Wilcoxon signed rank tests. All values were within the laboratory reference ranges. There were no significant differences (\( P > .05 \); not shown).

**Cerebral Metabolic Rate**

Two patients (14%) participated in imaging at the 5- to 6-month follow-up evaluation under a separate institutional review board-approved protocol. The first of these participants (designated GD007), who had manifested no EEG-documented seizures and no change in the CMRO2 after triheptanoin ingestion, demonstrated a 17% increase in the CMRO2 from fasting baseline levels. The second patient (designated GD009) manifested no significant change in the CMRO2 relative to a previous significantly increased CMRO2 after triheptanoin initiation (Figure 5).

**Continuation of the Medical Food Supplementation Regimen**

Of the 14 participants, 12 (86%) completed 3 months of triheptanoin consumption and 11 (78%) returned for the 3-month follow-up. One participant (7%) discontinued triheptanoin earlier than stipulated owing to intolerable gastric discomfort, and 1 (7%) discontinued triheptanoin because of parent-reported lack of efficacy. Of the 12 participants who completed 3 months of therapy, 1 individual (7%) declined to return for follow-up owing to financial hardship.

Of the 11 participants (79%) who returned for the 3-month follow-up, only 1 individual (9%) elected to discontinue triheptanoin therapy at that time owing to weight gain. Ten participants (91%) signed informed consent to continue treatment with triheptanoin as described above. Of those 10 participants, 1 patient (7%) discontinued triheptanoin after 5 months of use because of lack of efficacy, although parental reporting indicated nonadherence. All other participants continued to receive triheptanoin without adverse effects at the time of the last assessment for a total of 185 patient-months.

Longer-term blood glucose determinations as described in the Methods section were performed at least semiannually for all patients who continued to receive triheptanoin. No abnormality and no significant change in glycemia were noted on these tests, suggesting that triheptanoin, administered with a concurrent reduction in simple carbohydrates, does not exert its effects via a change in blood glucose levels unless any extra production of glucose is balanced with an equally increased rate of consumption.

**Discussion**

This study was motivated by 2 reasons. First, the pharmacologic treatment of G1D-associated seizures is generally ineffective. A potential role for several specific antiepileptic drugs was suggested by the spike-wave EEG pattern often identified in G1D seizures, which is typical of some absence epilepsies.\(^{54,55}\) Yet this approach has proven to be ineffective.\(^{16}\) In practice, antiepileptic drug use and discontinuation in G1D remains subordinate to trial-and-error interventions.\(^{16,38,56-58}\)

Second, we noted that, in normal conditions, an important brain glucose-derived anaplerotic process is catalyzed by pyruvate carboxylase inside the astrocyte.\(^{59,60}\) which depends on glycolysis (ie, on pyruvate generation) to refuel TCA...
cycle precursors and maintain byproduct output including glutamate, glutamine, and γ-aminobutyric acid. Neurons may carry out only a modest degree of anaplerosis via the malic enzyme. In contrast, common (ie, natural) dietary fats are oxidized into only even-carbon number ketone bodies (β-hydroxybutyrate and acetoacetate), which leads to acetyl-coenzyme A generation, but cannot sustain anaplerosis because the even 4-carbon-number ketone bodies are fully converted into water and carbon dioxide in the TCA cycle. Thus, any ketogenic diet in use today, especially when it results in relative hypoglycemia, represents a rudimentary— if not a restrictive—treatment for G1D when considered from this perspective. Even–carbon number ketones, generated from common dietary fat or a ketogenic diet, ameliorate seizures but are not anaplerotic, as underscored by the fact that the 2 key metabolic roles of glucose are (1) energy production by oxidation of acetyl-coenzyme A and (2) anaplerosis by providing pyruvate for carboxylation. Ketogenic diets can fulfill the first role, but the diet’s fat cannot meet the second role. In contrast, studies have shown that mouse brain nuclear magnetic resonance and mass spectrometry indicate that heptanoate is anaplerotic because it can satisfy both roles. Further observations made in transgenic antisense G1D mice, which appeared normal at birth but later developed frequent spontaneous seizures, decreased brain weight, spasticity, and ataxia (thus, closely mimicking the most commonly recognized human G1D syndrome), also converge on the potential use or overuse of alternative metabolic sources by the brain in G1D. In the state of depleted brain glucose accumulation and spontaneous systemic ketosis typical of G1D mice, whole-brain TCA cycle intermediates and neurotransmitter contents are preserved, undermining the hypothesis of global brain energy failure while suggesting that TCA refilling proceeds via enhancement of alternative metabolic fluxes, such as fatty acids. This was illustrated for heptanoate as noted above.

Clinical Outcomes

As indicated by the blood analytical values, there was no significant change in glucose levels or lipid levels, which indicates that the addition of triheptanoin to the diet is safe and metabolically neutral as assessed by these analyses.

Because we identified a prohibitively small detection rate of absences by mere observation, EEG recordings served as the most appropriate quantifiable outcome. The reduction in EEG spike-wave seizures and neuropsychological results of receptive and expressive vocabulary were favorably affected by triheptanoin. Other nonclinical data and nonsystematic evidence also support the clinical improvement noticed by many patients. The voluntary continuation rate (11 of 14 patients at 3 months and 10 of 14 patients at 6 months) is indicative of improvement. Several parents spontaneously reported that other caregivers (school teachers, speech therapists, and physical therapists) who were unaware of the patients’ study participation noted marked improvement in both motor and cognitive skills. This is consistent with improvement on the vocabulary tests, which measure not only language ability but also general intellectual function, because patients are required to maintain attention and concentration for the duration of the task. Perhaps the most striking finding was in the very young participants (aged 2 and 4 years) who were significantly delayed in all aspects of intellectual and motor function; they achieved rapid improvements in developmental milestones, meeting them at age-appropriate intervals.

Cerebral Metabolic Rate

The CMRO2 is diminished in G1D (Figure 5). In contrast with the cerebral metabolic rate characteristic of healthy individuals who were the same age as participants in the present study, patients with G1D demonstrate decreased but uniquely different CMRO2 values. This phenomenon may reflect the broad phenotypic diversity of our case series, of which the CMRO2 is but one aspect. Because G1D is a lifelong genetic disorder that affects the brain in early infancy, the maximum achievable CMRO2 level is unknown should patients experience full restoration of glucose influx after the disorder becomes symptomatic for an extended developmental period of their lives or even if the age-normal CMRO2 level can be exceeded as the consequence of a favorable therapeutic effect. It is not plausible, nor was it attempted, to safely advance general correlations between CMRO2 and EEG spike-wave frequency or neuropsychological indices; this endeavor was outside the scope and inferential power of the present study. Therefore, only individual observations deserve remarks. Of 5 participants who underwent CMRO2 determination, 3 (patients GD008, GD009, and GD011) demonstrated rapid (ie, consistent with the cerebral metabolism of triheptanoin metabolites), significant increases (Figure 5). The CMRO2 of patients GD013 and GD007 did not increase after acute triheptanoin ingestion. Patient GD013 exhibited spike-wave seizures that were abolished by triheptanoin (Figure 3), and patient GD007 did not exhibit spike-wave seizures before or after triheptanoin use. The robustness of the CMRO2 measurements prompts an explanatory framework for these single-patient observations. The simplest interpretation of all of the results reported above, should they prove to be a general feature of G1D, is that triheptanoin metabolism may lead to increased oxygen consumption only while the brain undergoes a reduction of ictogenesis. In parallel to this contention, we hypothesize that, when ictogenesis is abolished by triheptanoin or absent at baseline, triheptanoin exerts little or no effect on CMRO2. Because the relationship between ictogenesis and whole-brain oxygen consumption is unknown (as determined by the global encephalic CMRO2 reported in the present study) and because G1D-associated spike-wave seizures may be caused by aberrant thalamocortical synchronization rather than global or multifocal epileptogenesis, further work currently in progress will aim to elucidate CMRO2 changes in regional ictogenic G1D tissue. In addition, the significant increase in CMRO2 for patient GD007 after 6 months of treatment is notable. Despite the absence of epilepsy as detected by observation and by EEG, this finding is compatible with triheptanoin-induced long-term changes in the nonepileptic G1D brain.

Potential Therapeutic Mechanisms of Triheptanoin in Human G1D

Glucose supports brain metabolism, and neurometabolic diseases involving dysfunctional glucose metabolism are often
associated with intractable absence seizures. Additional molecules, such as fatty acids from ketogenic diets and their derivative ketone bodies, can partially substitute for glucose except that (1) normally or postprandially increased glycemia interferes with ketosis, necessitating that canonical ketogenic diets are paradoxically, for brain states associated with decreased glucose flux, carbohydrate restricted; (2) mitochondrial fatty acid oxidation can compete with glucose metabolism; and (3) all food-derived fats and ketone bodies contain even-chain carbons that are fully consumed in the TCA cycle and excreted as water and carbon dioxide, thus yielding no net carbon to compensate for the natural loss of metabolites. This compensation is normally provided by anaplerosis, which in the brain stems principally from glucose via carbon-donor reactions that are unavailable in the ketogenic diet. In contrast with ketogenic diets, the metabolism of triheptanoin generates both even-carbon and 5-carbon ketones (β-hydroxypentanoate and β-ketopentanoate) in the liver, and the latter are anaplerotic, potentially affording superior benefit. Studies have shown that G1D is associated with cerebral carbon depletion in mice, and the results of the present trial support prior findings of reduced cerebral oxygen consumption (CMRO2) in man.

Ictogenesis, Spike-Waves, and Absence Epilepsy in G1D
In the present study, we attempted to follow a mechanistic approach by targeting what we believe to be factors that represent primary causal events. This framework is largely based on our work illustrating that decreased glucose flux leads to cerebral metabolic deficit that, in turn, differentially affects excitatory and inhibitory synaptic balance, as well as on the fact that these aberrant phenomena are poised to be relevant to disease pathogenesis based on the plausible direct relationship between metabolism, synaptic function, and epileptogenesis. Furthermore, these phenomena are favorably modifiable in patients with G1D and in mouse brain slices by increasing blood glucose or ketone body availability. We have observed that (1) G1D is associated with diminished cerebral glucose flux (resulting in decreased brain acetyl-coenzyme A); (2) there is no intrinsic neuronal excitability dysfunction or gross structural abnormalities as suggested by the rapid reversibility of abnormalities shown on the human EEG and of key synaptic currents; and (3) brain glucose is used for anaplerosis, and stimulating this process may be of additional benefit when glucose availability is decreased. Given these observations, we reasoned that providing both a source of acetyl-coenzyme A and a source of anaplerotic carbon (via triheptanoin metabolism) results in a treatment strategy based on disease mechanisms and one that antiepileptic drugs or the ketogenic diet do not target. This argumentation represents a reductionist approach such that complementary interventions targeting secondary excitability or other abnormalities may be needed, as is the case with most neurologic diseases. In line with the necessarily reductionist approach outlined above, we selected the EEG as an important outcome. However, there are many nonobservable biological aspects in epilepsy; consequently, the field continues to be driven by the most reliable and accessible indicators, which have been in use for decades (eg, EEG, quality of life, and intellectual assessments). The best treatments include those that target causes, as we believe triheptanoin does. However, observation need not be synonymous with causation: absence seizures, such as those noted in G1D, are temporally well-circumscribed impairments of consciousness associated with visually observable manifestations. During a typical episode of absence, EEG recording reveals repetitive spike-waves that often last more than 3 seconds. However, observable absences, EEG recordings, and sustained attention can be differentially affected by drug treatment, supporting the limited causality clause described above. We anticipate that further ways of assessing epilepsy will emerge and have thus followed a focused approach here.

Implications for Further Trials: Is Triheptanoin a Drug, Prodrug, or a Medical Food?
The International Union of Pure and Applied Chemistry definition of a prodrug is a compound that undergoes biotransformation before exerting pharmacologic effects. Prodrugs are thus canonical drug precursors containing specialized nontoxic protective groups used in a transient manner to alter or eliminate undesirable properties in the parent molecule. In contrast, the term medical food, as defined in section 5(b) of the Orphan Drug Act (21 U.S.C. 360ee [b] [3]), identifies any food that is formulated to be consumed or administered enterally under the supervision of a physician and is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. In this sense, triheptanoin fulfills the requirements of a medical food.

Limitations and New Questions
One patient discontinued triheptanoin because of gastrointestinal intolerance; this unintended effect has been observed with oil consumption and can be ameliorated by varying the administration schedule or by administering triheptanoin in solid form or in an emollient, such as sugar-free and fat-free yogurt or pudding. The age range of the patients enrolled was 2 through 27 years, and this heterogeneity in ages implies heterogeneity in brain metabolism rates. The dose administered (1 g/kg), although appropriate for older adolescents and adults, did not take into account the younger ages, and therefore higher metabolism, of some of the participants. Future trials using biologically relevant age stratification can help to assess the effect of age on outcomes over changing levels of metabolism through a person’s development.

Conclusions
The effect of dietary food-grade triheptanoin on central disease-relevant phenomena is manifest in a broad age range of patients with G1D-associated epilepsy exhibiting varying degrees of disease severity and not receiving a ketogenic diet. To our knowledge, this is the first systematic study of a therapeutically relevant agent in G1D. Based on the glucose dependence of the
abnormalities cited above, we reasoned that, if triheptanoin operates on mechanisms relevant to disease pathogenesis, the therapeutic outcome should be prompt and noticeable (ie, consistent with the intestinal absorption, hepatic metabolism, and brain penetration of triglyceride metabolites) and sustained while the therapy is maintained. All patients with G1D who were receiving triheptanoin experienced decreased spike-wave seizures, and several patients exhibited a rapid increase in the cerebral metabolic rate (ie, those with the highest frequency of spike-waves) and improved neuropsychological performance, suggesting that triheptanoin is effective in ameliorating the brain glucose depletion state associated with G1D encephalopathy. The results establish that triheptanoin is endowed with sufficient potential to favorably and significantly affect outcome measures directly relevant to G1D and encourage further trials. The new trials should include young infants and patients with nonconvulsive forms of G1D and genetically unrelated encephalopathies associated with decreased glucose metabolism or abundance, as noted in several neurodegenerative diseases.

ARTICLE INFORMATION

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