



# Glut1 Deficiency Foundation Professional Conference

Poster Session Abstracts  
July 8, 2015

## **#1**

### **Triheptanoin Improves Performance on Cognitive Indices in Glucose Transporter Type I Deficiency (G1D)**

#### **Objectives:**

This study measured neuropsychological performance in a group of children with Glucose Transporter Type I Deficiency (G1D) before and after receiving food-grade triheptanoin as part of a larger study conducted to understand the influence of triheptanoin on 2014).

#### **Methods:**

This study was part of an unsponsored, open-label case series conducted at the University of Texas Southwestern Medical Center (UTSW). Out of 14 children and adults enrolled in the study, 8 completed neuropsychological assessments. After initial medical screening, overnight ( $\geq 8$  hours) fasting. Testing took place again 60 to 90 minutes following triheptanoin consumption. This time interval was selected to capture the peak metabolism of triheptanoin in blood. To investigate its long-term effect on outcome measures, triheptanoin of the Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4) and the Expressive Vocabulary Test, Second Edition (EVT-2). These were chosen for their ease of administration, relatively short administration time, and availability of parallel forms, allowing for quick re-administration without practice effects.

#### **Results:**

Eight participants completed baseline neuropsychological testing. During the baseline visit on the PPVT-4, 7 of 8 participants (88%) showed improvement in receptive language shortly following administration of Triheptanoin ( $P = .04$ ). On the EVT-2, 5 of 8 participants scores on some aspect of testing between the fasting baseline evaluation and testing an hour after administration of triheptanoin, but these changes were not statistically significant in this small sample. When baseline fasting performance was compared with performance the first triheptanoin dose) were maintained on the PPVT-4 (i.e., all 7 participants [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 participants (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).

#### **Conclusion:**

Overall, all participants with G1D who received triheptanoin tended to experience improvement on measures of expressive and receptive language. The reasons for these improvements may include dimensions not measured during this study such as improved participants experienced decreased spikewave activity, and several participants exhibited a rapid increase in the cerebral metabolic rate. These findings suggest a mechanism for improved neuropsychological performance, suggesting that triheptanoin is effective encephalopathy and is associated with clinically meaningful functional improvement. The results establish that triheptanoin has sufficient potential to favorably and significantly affect outcome measures directly relevant to G1D and encourage further trials.

## **#2**

### **Adaptive Behavior and Emotional Functioning in Glucose Transporter Type I Deficiency (G1D) Syndrome**

#### **Objectives:**

This study characterized the adaptive, emotional, and behavioral functioning of a series of children with Glucose Transporter Type I Deficiency (G1D). Currently, there is almost no literature on functional behavioral strengths and weaknesses in this population, address adaptive behavioral and emotional needs in this population, a better understanding of areas of strength and weakness is necessary.

#### **Methods:**

This study was part of an unsponsored, open-label case series conducted at the University of Texas Southwestern Medical Center (UTSW) to understand the influence of triheptanoin on oxygen cerebral metabolic rate (CMRO<sub>2</sub>), seizure activity, and neuropsychological. Participants were recruited from the Rare Brain Disorders Program at UTSW. Out of 14 children and adults enrolled in the larger study, 8 completed complete baseline assessments of adaptive, emotional, and behavioral functioning. Patients enrolled who ages 9 years to 27 years. After initial medical screening, parents completed two questionnaires assessing baseline functioning: 1) The Adaptive Behavior Assessment System-Second Edition (ABAS-II) is a parent/caregiver rating scale that assesses adaptive the Child Behavior Checklist (CBCL) or the Adult Behavior Checklist (ABCL) a parent/caregiver rating scale that assess emotional symptoms such as anxiety and depression as well as behavioral difficulties such as with attention aggressive behavior. Parent individuals in a similar age group, and standardized scores are derived for composite and individual scale ratings.

#### **Results:**

Descriptive statistics were used to summarize parent reported ratings of adaptive functioning, as well as emotional and behavioral functioning. Mean ratings of overall adaptive functioning (i.e., General Adaptive Composite) indicated significant difficulty with behavior domains indicated significant weaknesses relative to the normative sample across most adaptive domains. Analysis of mean ratings of emotional and behavioral functioning did not reveal clinically significant elevations in syndrome scales; however, syndrome scales.

#### **Conclusion:**

Results highlight the great difficulty with adaptive behavior skills in individuals with G1D, though clearly more research is need to better understand the nature and extent of deficits in adaptive behavior to provide for more refined treatment and supports for present study is the small sample, and future research should attempt to incorporate larger numbers of individuals with G1D to capture the full range of functional capacities in this population.

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## **#3**

### **A Protein Kinase C Phosphorylation Motif in GLUT1 Affects Glucose Transport and is Mutated in GLUT1 Deficiency Syndrome**

Protein kinase C has been implicated in the phosphorylation of the erythrocyte/brain glucose transporter, GLUT1, without a clear understanding of the site(s) of phosphorylation and the possible effects on glucose transport. Through in vitro kinase assays, mass GLUT1 as a PKC phosphorylation site. Phosphorylation of S226 is required for the rapid increase in glucose uptake and enhanced cell surface localization of GLUT1 induced by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Endogenous GLUT1 VEGF. Several naturally occurring, pathogenic mutations that cause GLUT1 deficiency syndrome disrupt this PKC phosphomotif, impair the phosphorylation of S226 in vitro, and block TPA-mediated increases in glucose uptake. We demonstrate that the phosphorylation modification is important in the physiological regulation of glucose transport.

#### #4

### **Synaptic excitation-inhibition imbalance in glucose transporter I deficiency (G1D) and first treatment of its associated human epilepsy with triheptanoin**

Several findings and principles will be presented: During 3 Hz spike-wave seizures, G1D patients exhibit a specific pattern of regional oxygenation (activation) and decreased oxygenation (deactivation) in the brain. Thalamus and select cortical areas are predominantly activated. Others, overlapping with the default mode network, are deactivated.

- G1D mice are epileptic (3 Hz spike-wave) and display thalamocortical hypersynchronization associated with oscillatory activity in thalamocortical slices, bursts of thalamic action potentials and abnormal cortical synaptic potentials.
- Heptanoate metabolism stimulates the cerebral Krebs cycle and brain anaplerosis.
- The cerebral metabolic rate of G1D patients is decreased.
- Patients receiving C7 can display improvement measurable by several approaches:
- Rapid (minutes) increase in brain metabolic rate
- Substantial long-term (weeks) further increase in brain
- Substantial long-term (weeks) further increase in brain metabolic rate after continued C7 consumption.
- Seizure frequency decreases after C7 ingestion.
- Intellectual performance improves after C7 ingestion.

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#### #5

### **Design of a disease-specific model of the blood-brain barrier using patient induced pluripotent stem cells: the case of Allan-Herndon-Dudley Syndrome**

The blood-brain barrier (BBB) constitutes an important part of the neurovascular unit, providing a physical and chemical barrier to ensure brain homeostasis. Such remarkable barrier is carried by the brain microvascular endothelial cells (BMECs) lining the development of the central nervous system, however their diffusion across the BBB occurs through the recruitment of dedicated TH transporters.

In patients suffering from Allan-Herndon-Dudley Syndrome (AHDS), a rare X-linked mental retardation characterized by high plasma and a low cerebral TH levels. Recent studies linked this disease to mutations in the monocarboxylate transporter MCT8. However, these patients, as rodents display a rodent-specific transporter capable to compensate such impairment.

The aim of this study is to establish and investigate TH uptake at the human blood-brain barrier using an in vitro model based on induced-pluripotent stem cells derived from AHDS patients and using an established differentiation protocol previously published. functional BMECs from both AHDS-iPSCs and from their parental controls. Surprisingly, we noted no differences in terms of barrier phenotype between parental controls and AHDS BMECs. Notably, preliminary results showed a decreased T3 uptake in AHDS-investigating the uptake of T3 and T4 across such model but also demonstrated the ability to transpose our protocol to a patient-specific manner.

We believe that such model can serve as a rationale to develop a patient-derived in vitro model of GLUT-1 deficiency syndrome to provide a patient-specific tool to understand the disease but also to develop novel therapies capable to improve patients outcome.

## #6

### **Blood beta-hydroxybutyrate measurement and lower ratios in GLUT-1 patients who treated with ketogenic diet**

The aim of this study was to investigate the results of the blood beta-hydroxybutyrate measurement (BHB) in patients with glucose transporter protein 1 deficiency syndrome (GLUT1-DS) who treated with ketogenic diet (KD). Six patients with GLUT1-DS who were referred to Behcet Uz Children's Hospital during the period of June 2012-September 2014 were included. At the 1st month, patients measured BHB (daily, weekly) in capillary blood obtained by finger-prick and the levels of ketone and glucose are recorded. Median BHB level determined  $4.69 \pm 0.6$  mmol/L in patients who were seizure free. The classic KD (ratio 3:1) started at the present time and according the seizure control and BHB levels, 3rd month we decreased the ratio as 2:1 and 1st year 1.5:1 in all patients. Our findings suggest that measurement of the blood BHB levels provide that fine tuning of KD and to decrease the ratios more confident. KD should be continued long period of life who diagnosed with GLUT1-DS and with a less rigorous ratios. In our experience lower ratios are more palatable and sufficient for seizure reduction with follow up by measurement of blood BHB levels.

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## #7

### **Development and characterization of exogenous ketone supplements – novel methods of inducing therapeutic ketosis**

Glucose Type 1 Deficiency Syndrome (GLUT1 DS) is a rare genetic disorder resulting in reduced function of glucose transporter protein type 1 (GLUT1) and impaired brain energy metabolism. Therapeutic ketosis induced with the ketogenic diet (alternate energy source for the starving brain; however, the dietary restrictions of the KD can make it difficult to achieve and maintain therapeutic levels of ketosis long-term. Exogenous ketone supplements are natural or synthetic compounds which dietary intake. We hypothesized that these agents may provide an alternate or adjuvant method of inducing therapeutic ketosis. We have tested the physiological effects of acute (28 day) and chronic (15 week) consumption of five exogenous ketone ketone ester), BMS ( $\beta$ -hydroxybutyrate mineral salt), MCT (medium chain triglyceride oil), and BMS+MCT (1:1 ratio). In the acute study, rats received a daily bolus dose of 5 g/kg or 10 g/kg of one of the five ketone supplements via intragastric gavage sustained therapeutic levels of ketosis ( $>0.5\text{mM} - 5\text{mM } \beta\text{HB}$ ) for 8-12 hours. Overall, ketone supplements had little effect on blood triglycerides, total cholesterol, HDL, and LDL concentration, although BMS-treated rats exhibited a slight elevation HDL compared to controls. Global metabolomics profiling of serum and hippocampal tissue collected from KE and BMS+MCT-treated rats revealed an increase in the concentration of TCA cycle intermediates, medium chain fatty acids, the antioxidants adenosine. Three ketone supplements were tested in the chronic study: KE (LKE: low dose KE,  $\sim 10\text{g/kg/day}$ ; HKE: high dose KE,  $\sim 25\text{g/kg/day}$ ), BMS ( $\sim 25\text{g/kg/day}$ ), and BMS+MCT ( $\sim 25\text{g/kg/day}$ ). Rats consumed a diet ad libitum wherein their respective rodent chow at 5% (LKE) or 20% (HKE, BMS, BMS+MCT) by weight. LKE, HKE, and BMS-fed rats gained less weight than controls and BMS+MCT-fed rats over time, but remained within a healthy weight range for their age. LKE, HKE, and BMS+caloric intake by approximately 25%. Administering ketone supplements in the food induced therapeutic levels of ketosis, elevating blood ketones to approximately  $1\text{mM } \beta\text{HB}$  for the duration of the study. Blood levels of triglycerides, total cholesterol, the end of the chronic study. Serum inflammatory profiling revealed a reduced concentration of several pro-inflammatory cytokines in ketone supplement-fed rats, suggesting that these agents may elicit an anti-inflammatory effect in vivo. There chronic study, suggesting the ketone supplements are safe when consumed for prolonged time periods at a high dose. Exogenous ketone supplements may provide a safe, easy, and effective method of inducing or enhancing therapeutic ketosis.

## **#8**

### **The effect of ketogenic diet and ketone supplementation on the motor function of GLUT1 deficiency mouse model**

GLUT1 deficiency syndrome is characterized by impaired glucose metabolism in the brain. It has been suggested that elevated blood ketone levels improve symptoms in patients with GLUT1 deficiency syndrome. In order to circumvent the need for strict dietary increase blood ketone level to therapeutic levels. We tested the ketogenic diet and exogenous ketone supplementation in a GLUT1 deficiency syndrome mouse model in order to determine changes in motor function, blood ketone, glucose, lactate levels, and organ weights with either standard rodent chow (SD), ketogenic diet (KD), SD+ ketone ester (SD+KE) or SD+ ketone mineral salt (SD+KS). The body weight was not significantly different between groups during the treatment, except week 2, when the SD+KS group had new diet. The weight of the brain ( $p=0.022$ ;  $0.014$ , respectively) and liver ( $p=0.012$ ;  $0.039$ , respectively) were significantly larger in KD and SD+KS groups at endpoint. The motor function improved in KD and SD+KS groups measured by hanging wire test. The caused faster improvement (from week 1), which improvement disappeared in both groups by week 9 on the hanging wire test. Latency to fall was greater in SD+KE group from week 4 and in SD+KS group during week 6 ( $p=0.021$ ) and 10 ( $p=0.015$ ), measured caused slow and slight elevation, while SD+KS caused significant ( $p=0.038$ ), quick elevation of blood ketone level from week 2, which level remained high until week 10. Glucose level quickly dropped and remained the lowest in SD+KE group, although the difference blood glucose levels which remained low (week 10,  $p=0.05$ ), while SD+KS caused an early decrease (week 2,  $p=0.04$ ) in glucose levels. Lactate levels were not significantly different between groups during the treatment. These preliminary results show that ketogenic ketone levels, reduction in blood glucose levels and improved motor function in GLUT1 deficiency syndrome mice model over 10 weeks.

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## **#9**

### **Elevated blood ketone levels increase the latency of anaesthetic induction in GLUT1 mouse model**

Ketogenic diets have been proven effective in seizure disorders and in several neurological diseases, by supplying alternative energy source to the brain in a form of ketone bodies. Elevated blood ketone levels have been considered to play a role in neuroprotection tested on GLUT1 deficiency syndrome mice model whether elevation of blood ketone levels would result in latency in anaesthetic induction. 3-5 months old GLUT1 deficiency syndrome mice were fed by either standard rodent diet (SD), ketogenic diet (KD) or SD. Induction of mice to isoflurane anaesthesia was video recorded. The time from closure of induction chamber lid until the last movement of mice was measured by a blinded observer. Latency to anaesthesia induction was significantly increased in KD and SD+the same after normalizing the results to body weight. Blood ketone levels were measured immediately after induction. Blood ketone level showed high correlation ( $R=0.99$ ) with latency to induction. Other animal models are to be tested in the future to find out whether latency to anaesthetic induction.

## **#10**

### **Chronic administration of exogenous ketone supplements reduces anxiety in Sprague-Dawley rats**

Nutritional ketosis has been proven effective for seizure disorders and other neurological disorders. GLUT1 deficiency syndrome is characterized by impaired glucose metabolism in the brain, therefore elevated ketone levels can serve as alternative fuel in order nutritional ketosis can promote a reduction in anxiety. The focus of this study was to determine the effects of ketone supplementation on anxiety. We tested three exogenous ketone supplements fed chronically to Sprague-Dawley rats prior to assessment of anxiety with either standard rodent chow (SD) or SD + ketone supplementation. Four treatment groups included low-dose ketone ester (1,3-butanediol-acetoacetate diester, 10g/kg/day, LKE), high dose ketone ester (25g/kg/ day, HKE ), beta-hydroxybutyrate-mineral salt plus maze (Coulbourn Instruments) was used to assess anxiety-related behavior of the rats. Behavioral data collection was conducted manually by a blinded observer and a video-tracking system (SMART V3.0 PLATFORM, Harvard Apparatus). Time spent ( $p=$ were significantly more in BHB-S and BHB-S+MCT groups, while time spent ( $p=0.011$ ;  $0.005$ ) and distance travelled ( $p=0.002$ ) in closed arms were significantly less in these groups, compared to control (SD). Latency to first entrance to closed arms was significantly respectively). Blood ketone levels were elevated in all ketone supplement treatment groups similar to levels reported previously with a ketogenic diet. We conclude that chronic administration of exogenous ketone supplementation reduced an anxiety in Sprague-rats.

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## **#11**

### **Mammalian Synthetic Chromosomes for Bioengineering of the Blood Brain Barrier Suitable for GLUT-1 DS Therapeutics**

The mammalian brain depends on plasma glucose as a major energy substrate and nutrient beginning postnatally and extending across the life span. The integral membrane protein Glut-1 is the predominant glucose transporter at the blood-brain barrier (BBB) and is expressed at high levels in brain endothelial cells. Mutations in the human GLUT-1 gene leading to defective glucose transport can result in hypoglycorrachia with associated developmental encephalopathy along with infantile seizures, cognitive and developmental impairment, choreoathetosis and dystonia. Glut-1 deficiency syndrome (GLUT-1 DS) is inherited in an autosomal-dominant pattern and a varied spectrum of heterozygous mutations in the GLUT-1 gene can result in haploinsufficiency.

Bioengineering of stem cells traditionally has involved the introduction of transgenes via viral-based and "naked" DNA delivery methodology. However, several caveats remain as this approach is developed for potential GLUT-1 DS therapy. For example, retroviral-based vectors can lead to variegated gene expression, insertional mutagenesis and oncogenesis. To circumvent many of the limitations associated with plasmid and viral-based gene expression systems, mammalian synthetic chromosomes provide an alternative means to introduce large payloads of genetic information into cells as an autonomously replicating, non-integrating chromosome-based vector system for expression of genes from their native promoters.

Our approach is to populate the brain vasculature with endothelial cells engineered to express the GLUT-1 gene and ameliorate GLUT-1 haploinsufficiency. Our long-term goal is to employ a stem cell-based therapeutic approach to engineer brain endothelial cells to express a genomic copy of the native GLUT-1 locus using a novel, non-viral based mammalian synthetic chromosome (ACE chromosome) expression system amenable to a broad-range of potential gene therapy and stem cell-based applications. Here we present the engineering of the ACE chromosome with genomic copies of GLUT-1 constructs that will be used to complement GLUT-1 deficiency in vitro and in vivo.

Two BAC clones spanning the 33kb genomic GLUT-1 gene and adjacent regulatory sequences were retrofitted to contain the targeting sequence required for integration onto the ACE chromosome. Retrofitted clones were quality controlled by PCR to confirm maintenance of all GLUT-1 exons (10 exons). Confirmed, retrofitted BACs were then integrated onto the ACE using a unidirectional bacteriophage lambda integrase, a component of the ACE bioengineering system. ACE integration is selected by antibiotic resistance and confirmed by PCR of the recombination junctions. Validation of GLUT-1 genomic clone expression was assessed by RT-PCR. This study presents a model by which the BBB may be reengineered for altered functionality. Other applications include the incorporation of transporters that would improve delivery of therapeutics or stem cellbased therapeutic delivery. Thus, the ACE chromosome represents a novel bioengineering platform technology suitable for regenerative medicine applications and potential orphan genetic diseases effecting rare vascular malformations utilizing genomic clones

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## **#12**

### **Two children with glucose transporter type I deficiency syndrome (GLUT1 DS) treated with 2.5:1 ketogenic diet.**

**Purpose:** We describe two children with glucose transporter type I deficiency syndrome (GLUT1 DS) treated with 2.5:1 ketogenic diet. Those children are first cases in our country and were treated with ketogenic diet like all over the world. In many ketogemic ratio. However not for all children those ratios are suitable. We describe two children with GLUT1 DS treated with lower ketogenic ratio.

**Methods:** Two children - 13 and 1/12 years old girl and 6 and 6/12 years old boy - both with with confirmed de novo GLUT1 gene mutation in chromosome 1p34.2 (SLC2A1 gene, OMIM 606777). Children were initially treated with 1.5:1 ketogenic values (and beta-hydroxybutyrate levels), eeg are provided. 11 months observation period. Both children have no epilepsy seizures but were treated with antiepileptic drugs.

**Results:** Very good results in boy - almost all symptoms disappeared. Girl has also majority of symptoms disappeared, but sometimes has had weakness symptoms. Good tolerability of the diet in both children without side effects.

**Conclusion:** Our observation confirm that low ketogenic diet ratio also could be efficacious in some particular children. Lower ketogenic ratio could be associated with a fewer number of adverse events. Gradually increased ketogenic diet is better tolerated. The diet should be "tailored" to the particular patient.