2015 Glut1 Deficiency Foundation
Conference Summary Report

gratitude and credit to Kris Engelstad and all the speakers for helping prepare the presentation summaries

Glucose Transporter Type 1 Deficiency Syndrome is regularly referenced using a variety of terms, and these individual summaries were no exception. In the interest of clarity and uniformity, we have used the term **Glut1 Deficiency** throughout the summary.

Glucose Transporter Type 1 Deficiency Syndrome is also known and referenced as: Glut1 Deficiency, G1D, Glut1 DS, Glut-1 DS, Glut1, Glut-1, Glut 1, GLUT1, and De Vivo Disease

**2015 Glut1 Deficiency Foundation
Conference Summary**

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Glut1 Deficiency 2015 Update

I. Glucose is the essential fuel for the brain. Glucose transporters *(proteins)* transport glucose into the brain. Patients with Glut1 Deficiency have low CSF glucose. Symptoms of Glut1 Deficiency include: seizures, movement disorders, cognitive disturbances.

Glucose is transferred to the brain and it is converted into energy. However, body fat can be made into ketones which enter the energy cycle and produce energy. In this situation, we are just running the brain with a different fuel *(ketones instead of glucose).* Dr. Eric Kossoff initiated a website that helps patients to find a ketogenic diet center in various places in the world.

II. What has happened in the field of Glut1 Deficiency in the past two years?

Glut1 transporter:

An article in "Nature" (2014) describes the crystalline structure of the human glucose transporter Glut1. The crystal structure is subdivided into three domains: the C-domain and N-domain serve like pincers, the ICH domain forms a latch that operates the other two domains for glucose transport. SLC2A1 mutations were modelled and explained by this mechanism.

Symptoms and diagnosis

CSF glucose levels in Glut1 Deficiency and controls were reported by Leen (2013) . Normal control CSF glucose values were in 70's and CSF glucose in Glut1 Deficiency patients is lower than 70 mg/dl.

Glut1 Deficiency Type 2 has been proposed (OMIM) but is not considered a separate entity by many scientists in the field. It describes Glut1 Deficiency variants with completely different clinical symptoms to classical Glut1 but also caused by mutations in the SLC2A1 gene including: paroxysmal exercise induced dyskinesia, +/-epilepsy, +/- hemolytic anemia (caused by stomatin deficiency cryohydrocytosis).

III. Diets and Treatments

1) The ketogenic diet is the first choice for treatment options. The classical ketogenic diet is a 4:1 or 3:1 *(fats:proteins and carbohydrates).*

2) The Modified Atkins Diet is also used in Glut1 Deficiency, but currently there is not enough data available.

3) The low glycemic index diet has not been tried in Glut1 Deficiency.

4) Regular diet

Be careful as not all diets are appropriate for Glut Deficiency in general and for special age groups in particular!

The Modified Atkins Diet (MAD) is best for adults who do not want a straight ketogenic diet. However, in Glut1 Deficiency we don't know if the MAD diet supplies enough energy. Not a lot of patients have tried it and been reported as having been successful on the diet.

Selenium deficiency can be a problem in ketogenic diets as it can lead to cardiac issues such as prolonged QT.

The high fat content of ketogenic diets may increase the risk of atherosclerosis. Impaired carotid distensibility and thicker carotid intima may be an early marker Coppola (2014) and Kapetanakis (2014). However this was only seen for the 1st year of the diet, then the distension disappeared. More data is necessary and we should monitor carotids by ultrasound to monitor potential atherosclerosis. My unpublished data indicates that long term use of the ketogenic diet doesn't seem to promote the development of elevated lipids and cholesterol as long as the fats are being burned off by energy use. If hyperlipidemia is present use formula instead of solid KD in infant, lower the ketogenic ratio, supplement with carnitine, increase PUFAs and add MCT oil or pancreatic enzymes.

Growth is a concern as it can decelerate, but not that dramatically.

We should also focus on the positive side effects of the ketogenic diet: anticonvulsant medication, improved cognition, altertness and behavior improves and thus life quality. Additionally, seizures are improved with ketogenic diet. Motor development improves somewhat less. Language abnormalities respond least to the ketogenic diet.

Acetazolamide has also been used in Glut1 Deficiency in response to paroxysmal exertional dystonia. There are several case reports with conflicting results and responses, so currently it is difficult to assess this treatment option.

IV. The Changing Face of Glut1 Deficiency

Initially, we thought that seizure treatment is most important. In time, other questions arose, such as how to treat paroxysmal movement disorders. Epilepsy and developmental delay dominate during infancy and early childhood and respond best to a ketogenic diet. Paroxysmal movement disorders emerge during late childhood and puberty and we have

not quite understood why this is the case. Paroxysmal non-epileptic events often are triggered by exercise.

Early ketogenic use may be beneficial and puberty can be an issue as children don't want to use the diet. Adults with Glut1 Deficiency present with: 1/3 seizures, 1/3 spasticity, and 1/3 encephalopathy.

Triheptanoin, also called C7, is an artificial ketone ester made of 3 ketones linked to central ridge. It is a liquid at room temp and can work just as ketones do, entering the TCA cycle through Acetyl Co-A. Normal fats have even carbon numbers. Triheptanoin has an odd number of carbons (n=7) and boosts the energy cycle termed anaplerosis.

There was a pilot trial of triheptanoin for Glut Deficiency by Dr. Juan Pascual investigating safety, brain MRI, and effect on language. The compound was beneficial in all aspects.

There is a current study with Ultragenyx using Triheptanoin. Patients have 6 weeks of baseline (no study medication), 8 weeks of study (placebo or C7), then open label study medication. If patients are doing well on the ketogenic diet, they should not stop to participate in this research. Caveats of the study are:
- Patients benefitting from a ketogenic diet are not eligible for the study.
- We don't know about the long term side effects of triheptanoin.
- C7 as a food supplement will be considerably cheaper than C7 as a prescription drug

V. Glut1 Deficiency Databank

Dr. Pascual has started a Glut1 Deficiency patient registry.   You can participate at G1Dregistry.org. The registry helps us to answer a lot of questions about Glut1 Deficiency. It is self-entered data.

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Epilepsies of Glut1 Deficiency

I. Epilepsy Related to Glut1 Deficiency

The spectrum of epilepsies associated with Glut1 Deficiency is much broader than previously thought.
- 5% of epilepsy with myoclonic-atonic seizures (previously called myoclonic-astatic epilepsy) is due to Glut1 Deficiency.
- 10% of early onset absence epilepsy is Glut1 Deficiency.
- 1% of genetic generalized epilepsies (previously called idiopathic generalized epilepsies) is Glut1 Deficiency.

There are likely to be many undiagnosed patients in the world. We focus on the *SLC2A1* mutations not just on the CSF glucose.

II. Epilepsy General Information

There are many types of epileptic seizures which have different EEG signatures. Normally brain cells generate "brain waves" which are captured on an EEG. When doing an EEG, we do not expect to capture a seizure but we are looking for the epileptiform discharges that occur between seizures. The type of epileptiform discharge assists in diagnosing a patient's epilepsy syndrome. The diagnosis of epilepsy depends on having seizures; the EEG adds supporting evidence but one has to have seizures to have epilepsy.

Two major groups of seizure types:

Generalized seizures
Involve both sides of the brain at the same time
Seizure types include: absence, myoclonic, generalized tonic clonic seizures

Focal (partial) epilepsies
Begin in one region of brain
Seizure patterns reflect area of brain malfunction
May be associated with focal scar or malformations
May spread to both sides of brain which is now called "evolving to bilateral convulsive seizure" (previously called secondarily generalized which was a confusing term)

Epilepsy syndromes are identified by: type of seizure, anatomy, when they occur, outcome, age of onset. Diagnosis is like a puzzle; an electro-clinical puzzle. Onset age is important as is initial seizures types, other seizures types, development examination, EEG features and MRI features, and genetic testing.

II. Seizures Syndromes that can be Associated with Glut1 Deficiency

Childhood absence epilepsy
> Onset 4-10 years
> 3 hz in 1 second is classical childhood epilepsy
> Seizure types- absence seizures. Many go on to develop generalized seizures
> Sodium valproate is the drug of choice, ethosuximide, lamotrigine
> We evaluated 32 kids with early onset absence epilepsy only <4 years and 4 (10%) had Glut1 Deficiency.

> <u>All children with early onset absence epilepsy should have SLC2A1 genetic analysis!!</u>

Juvenile absence epilepsy
> Onset 11-20 years
> Seizure types: absence, GTCS
> May not lose complete awareness in absence
> There is a polyspike component

Epileptic encephalopathies
> Ongoing epileptic activity much of the time. You may not see anything in clinical features.

Seizures contribute to cognitive and behavioral impairments

Spectrum of Glut1 Deficiency
> Encephalopathy epilepsy: 2.5 hz spike activity, focal slowing, focal discharges, age related changes
> Focal slowing/discharges with onset at <2 yrs,
> Generalized abnormalities at 5 years

Myoclonic astatic epilepsy
> Onset is early to mid-childhood
> Jerk then drop attack
> Responds to ketogenic diet. This responds best to KD of all epilepsies
> We evaluated 84 cases with these types of seizures. All were sequenced for mutations in SLC2A1 (with reflex to MLPA).
> 5% had SLC2A1 mutations

Idiopathic generalized epilepsy
     Early onset absence epilepsy
     Single case with SLC2A1 mutation

Paroxysmal Exertional Dystonia
     Often people don't mention that they have PED's (paroxysmal exertional dystonia).
     Sometimes people don't realize that it is involved with Glut1 Deficiency. Families
     with PED can have several seizure types . Some people only have PED with a
     certain activity (eg. walking in sand for 1 hour). You can have focal and generalized
     epilepsy in one individual or a family

Generalized Epilepsy
     We used to think that patients with genetic generalized epilepsies (25% of all
     epilepsies) were due to polygenic causes (*more than one gene*). In 504 cases
     evaluated; 9/504 patients and 1/470 controls had a mutation in the SLC2A1 gene.

II. Summary:

1% of common generalized epilepsy is due to Glut1 Deficiency.
Movement disorders can be seen in Glut1 Deficiency.

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Movement Disorders of Glut1 Deficiency

I. Introduction to Movement Disorders

Several areas of brain are dedicated to movement. These areas plan, select, balance, and coordinate movement. Problems with voluntary control of movement include: stiffness, awkward, clumsy, unsteady, poorly coordinated. There can also be involuntary movements which include: jerky, fidgety, wriggly, shaking movements, and twisting postures.

Stiffness may be caused by dystonia or spasticity.
Jerkiness may be caused by chorea or myoclonus.
Wriggly movements are usually caused by chorea.
Clumsiness and poor coordination are signs of cerebellar ataxia.

Glut1 Deficiency causes a complex movement disorder. Patients can exhibit several types of movement disorders.

In patients, spasticity is manifested as: stiffness, flexed knees and hips (crouched), over-sensitive reflexes, extension reflex of the big toe with stimulation of the sole of the foot (Babisnki sign).
In patients, dystonia is manifested as involuntary sustained postures.
In patients, chorea is manifested as random appearing brief fidgety movements.
In patients, myoclonus is manifested as rapid jerks.
In patients, ataxia is manifested as slurred speech, inaccuracy when reaching to a target, and a wide-based unsteady gait.

II. Glut1 Deficiency and Movement Disorders

History of Findings
1990's Infantile epilepsy was noted as prominent in Classic Glut1 Deficiency
2000's Glut1 Deficiency can cause both persistent and paroxysmal movement disorders with or without seizures
2010 Movement disorders paper (Pons et al) indicated that of Glut1 Deficiency patients: 86% had dystonia, 75% had chorea, 70% had ataxia, 16% had myoclonus, and 90% had abnormal gait (usually spastic, or spastic-ataxic). Many patients have a combination of movement disorders.

Paroxysmal exercise-induced dyskinesia (PED) are attacks of involuntary movements triggered by exercise. Onset is usually in childhood. Usually, symptoms during an episode appear after 15-20 minutes of exercise (e.g. walking, running, riding a bike), and typically

last for 15 minutes to 1 hour. In 2008 we realized that PED is linked to mutations in the SLC2A1 gene. PED movements can be brief and jerky, or sustained (dystonia). Patients often describe a sensation of their legs feeling shaky or locking up., The most common symptom is leg stiffening when walking.

Glut1 Deficiency can also cause non-motor episodic neurological symptoms, and these can be an important clue to diagnosis. Examples include:
Hemiplegia (weakness on one side of the body)
Migraines
Unsteadiness
Irritable behavior or inconsolable crying
Vomiting
Lethargy
Confusion

III. Diagnostic Clues in Glut1 Deficiency

PED is a strong clue to a diagnosis of Glut1 Deficiency, and assume patients with PED have Glut1 Deficiency until proven otherwise.

In a patient with a complex movement disorder (spasticity, dystonia, ataxia), suspect the diagnosis of Glut1 Deficiency if motor symptoms:
- fluctuate in severity (eg. with exercise, fasting, illness)
- are accompanied by other episodic neurological symptoms.
- are accompanied by learning disability or ADHD

IV. Long Term Outcome and Treatment of Movement Disorders

Movement disorders become more prevalent over time.
Ataxia is present in most patients by early childhood and tends to remain stable over time.
Dystonia becomes more common over time. Severity ranges from mild to severe.
PED starts during childhood: 74% of 27 patients >13 years of age in recent long term follow-up studies had PED. PED may improve in mid-adulthood (40's).

Do movement disorders respond to treatment?
Ketogenic diet is the current standard of care treatment. It is effective in most patients. It is difficult to maintain, especially in adolescence or adulthood. Some symptoms still persist in the long-term on the ketogenic diet.

What other options for treatments are there?
In older patients, the Modified Atkins Diet has been used.

There are some symptomatic treatments.
Spasticity can be treated with botulinum toxin injections.

Dystonia, chorea, and myoclonus are treated with medications.
PED is treated with Acetazolamide which is used in other paroxysmal dyskinesias,
Others medications include: clonazepam, oxacarbazepine (individual patient reports).

V. Conclusion
Persistent and episodic movement disorders are prominent in Glut1 Deficiency. Diagnosis should be suspected if motor symptoms fluctuate, are triggered by exercise, fasting, are accompanied by epilepsy, or learning disability.

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Cognitive Implications of Glut1 Deficiency

 I. Neuropsychology

Cognitive outcomes are final outcomes of brain function, thinking, behaving and interaction with the world. As neuropsychologists, we look at children's strength and weaknesses, parental knowledge, and school knowledge.   We have outcomes research; how well is a child doing?

Domains of assessment include: IQ, memory, language, visual-spatial, attention, cognitive efficiency (speed at response), executive functions (cognitive coordination, think proactively), motor and visual motor, adaptive behavior, emotional functioning, behavioral functioning (eg. depression, aggression), social functioning, academic skills. All of these have subdomains also. It isn't all about the brain; the interaction of person to environment is important.

II.Cognitive Findings in Glut1 Deficiency

We don't know a lot! Initially we presumed that intellectual disability was moderate to severe impairment in cognition as well as self-care. As phenotypes expand, so too have ranges of cognitive functioning in this patient population. Now we know that they range from normal to severe. We now want to know if there are areas of particular strength.

Considering overall intelligence, there is a wide distribution of cognitive ability. A downward shift 2 SD's to left: 100 is normal IQ SD is 15.   Glut1 Deficiency mean is 70 IQ. On the milder end there may be minimal or intermittent impact. On the severe end the cognitive disability is substantial and persistent.

Language is one of the earliest areas and more prominent features. Receptive language is stronger than expressive language. Articulation problems may account for some of the reduced expressive language. This is knowing the answer but the language wasn't recognizable.

In the area of attention, significant fluctuations are reported which may be due to seizure activity. This could limit expression and development of other cognitive functions.

Adaptive behavior is basic self-care and activities of daily living. There is a significant range from normal to severely disabled. Specific areas of weakness that may contribute to this are: motor and movement disorders, attention and alertness problems, language and speech deficits and general cognitive delays.

III. Triheptanoin

Dr. Pascual treated Glut1 Deficiency patients with Triheptanoin (C7)
Receptive vocabulary improved within 1 hour of taking C7 oil. Improvements were still noted after taking C7 for 3 months. Expressive vocabulary also improved with triheptanoin. There was improved ability to do tasks but the patients did not really learn more. In evaluating adaptive behavior, several areas assessed.

Why should you know your child's cognitive profile?
1) Advocating for services (PT, speech, counseling, etc)
2) Prognosis
3) Knowing what to plan for in the future.

IV. Summary

Cognitive difficulties are common and may range from mild to severe.
Speech and language seem more affected.
Attention is more affected.
Many areas of cognition have not been studied.
Emotional and behavioral functioning has not been a topic of study.
We need more refined assessments of neuropsychological functions.

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Exogenous Ketones and Glut1 Deficiency

I. The Use of Exogenous Ketones

We are interested in the possibility of using exogenous ketones for Glut1 Deficiency.

My initial research objective (with the US Navy) was to evaluate cellular metabolism in extreme environmental conditions. One of those conditions was hyperbaric oxygen pressure. Specifically, I looked at cellular and molecular mechanisms of CNS *(central nervous system)* oxygen toxicity resulting in seizures.   The goal was to determine if we can preserve metabolic function under cellular stress, which is associated with impaired glucose metabolism. The research involved utilizing different rodent models of seizures.

The question is whether exogenous ketones can be used as a metabolic therapy. Regarding the armed services, warfighters may benefit from ketones in extreme environments.

II. Oxygen Toxicity

Oxygen (O2) toxicity is created by breathing oxygen at a pressure >2.5 ATA O2. Hyperbaric O2 therapy is similar to navy seal diving *(where concentrated oxygen is breathed through a mask at high pressures)*. There can be CNS oxygen toxicity seizures when diving to certain depths in the ocean and these can be fatal and lead to drowning.

III. How do we study CNS oxygen toxicity?

We know that navy seals must dive within exposure limits or they have seizures. There are various methods that we established to study this phenomenon in the laboratory. We place a rat in a hyperbaric oxygen chamber. At increased O2 levels the rats have seizures. We can look at several things: test various anticonvulsant medications, antioxidants, etc. We can't treat warfighters with seizure medications. We noticed that starvation can delay oxygen

toxicity better than the drugs on the market, so we ask the question- how does starvation change brain energy metabolism?

In a study in 1967 subjects were fasted for 40 days. Ketone bodies were produced naturally as a consequence of burning excess fat. The ketone body Beta-hydroxybutyrate (BHB) exceeded glucose in the blood. Acetoacetate was also significantly elevated. In a fasting condition about 70% of brain energy is from ketone bodies. The subjects were injected with what would typically be a fatal dose of insulin and severe hypoglycemia was created. All subjects survived without brain damage or coma. Interestingly, all were asymptomatic for hypoglycemia! The researchers learned that the physiological state of ketosis is NOT harmful, and more importantly, ketones can preserve brain energy metabolism under conditions of extreme glucose deprivation. Ketone bodies are natural energy substrates from fatty acid oxidation, and ketosis blood levels >0.5mmol/L represents a level of ketosis that contributes to brain energy metabolism. Ketoacidosis (as occurs in type 1 diabetes) is associated with pathologically high ketones (>10mmol/L). Keto-adaptation *(physiological shift toward using fat and ketones for fuel)* occurs with sustained ketosis and results in enhanced brain ketone metabolism over time. A study such as this one would never be approved for use today by an Institutional Review Board, but we can draw very important take home messages from this work.

IV. Exogenous Ketones

Exogenous ketones are natural or synthetic derived substances to artificially produce "instant ketosis". If you can't achieve high levels of ketones through a dietary means then exogenous ketones may be helpful. Note: the definition of exogenous is: introduced from the outside (as a supplement), not created within an organism.

The ketogenic diet is 85-90% fat and 10-15% carbohydrate and protein. Once glucose is depleted, fats are mobilized and ketones are produced which cross the blood brain barrier. This can provide alternative energy source to brain and heart and peripheral tissues. However, because the ketogenic diet is difficult to maintain we are working on ketone esters and ketone salts as an alternative. Consumption of acetoacetate ester produces instant ketosis and rapid and sustained elevation of blood ketones. After 30 minutes we see therapeutic levels. This is sustained up to 8 hours. There can be a small decrease in blood glucose, possible suggesting enhanced glucose uptake. This levels of ketones (beta-hydroxybutyrate) is similar to what we would see in prolonged starvation, but this individual was eating a normal diet.

There is a new device that allows us to check ketones by breath analysis (Ketonix). The laboratory is testing this machine. We need to consume a relative large amount of this

substance to get to the starvation level beta-hydroxybutyrate levels (i.e. high ketones). The question remains though; will these ketones prevent CNS O2 toxicity?

V. Can Exogenous Ketones Prevent CNS Oxygen Toxicity?

We study this question in rodents/rats. We are able to predict CNS oxygen (O2) toxicity in rats. 5-8 minutes before the onset of CNS O2 toxicity seizures the volume of air (tidal volume) and the respiratory frequency significantly increased and this represents a reliable physiological marker of an impending seizure. We are currently studying the mechanism that correlates the increased respiratory frequency and onset of seizure activity.

We used ketone esters on these rats in order to prevent onset of seizures. It did increase latency to seizure. We found that ketone esters sustained elevation of BHB and delayed seizure onset of about 600%.

In order to test our ketone ester against another model of induced epilepsy, we administered it to a large number of Wistar rats and led them to seizure using the experimental pro-convulsant agent Pentylentetrazole. After an oral administration of our ketone ester, we observed a statistically significant increase of rats' resistance against pentylenetetrazole-induced epilepsy. Moreover, we are currently studying the effects of ketone ester chronically administered (7 and 14 days) in rats, through metabolomics essays and biopsy analyses.

In addition, we defined an algorithmic rule which positively correlates the latency time to seizure and the probability of an impending CNS OT seizure which is going to occur. This represents a new reliable tool to predict the onset of seizure during a hypothetical Navy Seals dive or during a clinical treatment in Hyperbaric Medicine.

The next phase of the research was to use exogenous ketone supplementation as therapeutic for Glut1 Deficiency.

Glut1 Deficiency mice have: ataxia, spontaneous seizures, motor impairments, microcephaly, and normal blood glucose, much like Glut1 Deficiency patients

The groups/therapies tested for G1D could include:
1) Standard diet (SD)
2) Ketogenic diet (KD 75 % fat)
3) Standard diet plus ketone ester (SD+KE)
4) Standard diet plus ketone mineral salt (SD+KS)

Although safe and effective for seizures, ketone esters are very unpalatable and need further work to make them taste better. However, ketone salts are palatable and well tolerated and with ketone salts we see consistent elevated ketone levels. We can use ketogenic fats and ketone salts combined, sustaining the ketone levels longer. With ketone esters (acetoacetate and BHB) we see elevated ketones also in the therapeutic range.

In treated Glut1 Deficiency mice we measured organ weight using SD + KS (standard diet plus ketone salts). The brain weight of KD and SD+KS fed Glut1 Deficiency mice was significantly greater than mice eating SD alone.

We found that SD + KE (standard diet plus ketone esters) improve rotarod function *(motor skills)* greater than SD and KD. Regarding strength and endurance in Glut1 Deficiency mice, KS, KE and KD improved results.

VI. Summary Ketosis as a Metabolic Therapy

Ketones are remarkably neuroprotective signaling metabolites. They may improve oxidative stress, inflammation, longevity, GABA is elevated, glutamate is reduced, anaplerosis is enhanced (energy metabolites), various longevity genes are turned on by ketones, and there is a natural anticonvulsive effect.

We wonder whether these same things happen with exogenous ketones. Medium chain triglyceride oils (MCTs), derived from coconut oil, including caprylic triglyceride (C8) and capric triglyceride (C10) converts in the liver to make ketones. These MCTs also cross the blood brain barrier to provide energy directly in addition to their ketogenic properties.

We learned that: exogenous ketones (BHB and AcAc) have anti-convulsant properties, they protect against hypoglycemia and impaired glucose metabolism. We also found that exogenous ketones may improve brain size and motor function in Glut1 Deficiency mice. The ketogenic diet has already proven to be a remarkably effective therapy for the metabolic management of Glut1 Deficiency. Although exogenous ketones may work well as a stand-alone therapy for Glut1 Deficiency, it is likely that they would further enhance the therapeutic efficacy of the ketogenic diet. Exogenous ketones and ketogenic fats (MCTs) represent a means to circumvent the dietary restrictions needed to achieve a state of nutritional ketosis, thus they may be effective with a standard diet, but more research is needed to further validate these preliminary observations.

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Ketogenic Diets

I. History of the Ketogenic Diet

1921   The ketogenic diet (KD) has been around since 1921.
1993   The Charlie Foundation has increased the popularity of the ketogenic diet.
2008   The first ketogenic conference and the publication of 2 randomized controlled trials
        raise scientific awareness to a new level.
2015   There are a lot of recipe books, cook books, reports on the news, and general books
        on the ketogenic diet.

Diets for epilepsy: advances in the field
1) We realized that we need to know the indications to determine who should go on the diet.
2) There is more research now into ketogenic diets for epilepsy.
3) The KD is going global.
4) We are more aware of Glut1 Deficiency and diets and studies looking at the combination.

Indications for the KD
1993   Myoclonic, absence, atonic (drop), or just try the KD with any type of seizure.
2009   Consensus paper was published on the KD. It describes the best indications of who the ketogenic diet might help. This was a big difference compared to 1993 in knowing who should go on the diet. Glut1 Deficiency is top of the list.

Doctors who evaluate many conditions have figured out whether the ketogenic diet is useful in their patient population. It is useful in many different disorders.

The KD is new in research for epilepsy such as: absence epilepsy, FIRES, Mitochondrial disorder, juvenile myoclonic epilepsy, status epilepticus, etc.

II. Ketogenic Diet New Research for Epilepsy

Do I need to be so strict?
We use no calorie or fluid restrictions, no hospital admission, no fasting required, and no weighing of foods on gram scales for the Modified Atkins Diet (created 2001). The Modified Atkins Diet (MAD) is 2:1 or 1.5 : 1 (this has been around for about 14 years).
A standard diet (not KD or MAD diet) is 50% carbs.

Hopkins has an adult ketogenic diet center which was established in 2010 and is rapidly growing. Many adults are interested today in dietary therapy for epilepsy, including some with Glut1 Deficiency who are 18 years old and looking for a new keto diet center. 12% of patients coming to the center were on diets already.

The potential side effects of a KD include: constipation, weight loss, GI reflux, acidosis, dyslipidemia, renal stones, growth slowing, vitamin D deficiency, kidney stones, bone fractures, and cardiomyopathy (due to selenium deficiency). Most are preventable nowadays with supplements.

Carotid distensibility has also been reported (2014). Carotid stiffness was worse by 12 months and normal by 24 months and also returns to normal when the keto diet stops.

III. Going Global
Many places in the world have the ketogenic diet, however, Central America, South America, Africa, and some parts of Asia do not have the KD.

Two years ago a task force was established to bring the diet to developing countries.

There is a website with recipies, books and articles which are all free!
This was established by the Commission on Medical Therapies of the ILAE.
http://www.ilae.org/Commission/medther/keto-index.cfm

The ILAE task force is working on: training new KD centers, minimum requirements for KD, and raising international awareness.

IV. Glut1 Deficiency and the Ketogenic Diet

Questions that are often asked:

1. Do ketones matter?
2. What happens at puberty
3. Are the diets comparable? Is the MAD equal to the KD?
4. Can we substitute the KD with the MAD in Glut1 Deficiency patients without seizures?
5. Are diet responders really just Glut1 Deficiency patients in disguise?

In puberty we know that seizure activity and ketones may change. It may be a time to watch patients carefully. There is very little information about this currently.

There are a lot of publications for Glut1 Deficiency and the MAD; however patient numbers are small.

Less restrictive diets for reduced symptoms is possible, especially in adolescent years.

The MAD is 1 or 1.5 : 1 ratio which allows for increased protein over the KD. There is no weighing and measuring and protein supply is a less restrictive. The KD is used for infants and young kids; while the MAD can be used in adolescents. We recommend the MAD for patients with Glut1 Deficiency who are teens or adults, do not have epilepsy, and/or have major side effects with the KD.

At Hopkins we have less reliance on ketone levels to determine effect of treatment for patients with epilepsy. There is no scientific evidence that higher ketones are better for Glut1 Deficiency, although many will check blood ketones with a goal of >5 mmol/L. In general, I agree with keeping ketones high for Glut1 Deficiency patients when safe and feasible. We are aware that some patients with high ketones still have seizures, though we don't know why.

The MAD may be practical. If patients on the KD have side effects, intolerability it may be better to switch to MAD versus just stopping the KD. We can always change back.

Should we keep at high level ketones for non-seizure patients? We aren't sure.

Recent studies have looked at large groups of children who respond to the KD with epilepsy and are not diagnosed with a specific problem. They were then screened for the SLC2A1 mutation of Glut1 Deficiency to see if they had Glut1 Deficiency and were not diagnosed. Results showed only 1 patient of about 300 children had it : making this test not necessarily worth doing in all children on the diet who do well.

A big part of the future for diets and Glut1 Deficiency may be the survey I distributed to people in attendance. I asked questions about all these issues to see how Glut1 Deficiency families are REALLY doing the diet out there. Survey results will be reported.

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Gene Therapy Research

I. Rare Genetic Disorders – General Principles

One gene can make several proteins. The total number of proteins in a cell far exceeds the total number of genes (22,000 in human genome). Modifiers are built into our genetic material that effect the expression of disease-causing genes. As a result, all Glut1 Deficiency patients are not the same even if they share the same disease-causing mutation. There are now more than 7,000 rare diseases and 80% are caused by genetic mutations. Children are affected most of the time. 8-9% of the U.S. population is affected by a rare disease (approximately 28 million people). The NIH has gotten involved and created the "Office of Rare Diseases".   Most of the rare diseases have a neurological signature. Commonly, the disease process starts during development. The first decade of life is particularly critical because the brain is growing and maturing. Cell connections are being made and circuits are being established. Much of our genome is dedicated to brain function and development. Glut1 Deficiency, therefore, is a quintessential example of a rare disease that presents with a neurological phenotype.

II. History of Glut1 Deficiency

1991   Glut1 Deficiency phenotype first described in the literature (De Vivo et al).
1998   Pathogenic mutations confirmed in SLC2A1 gene   (Seidner et al).
2001   Expanding phenotypic spectrum with epilepsy presenting in infancy and
         dyskinesias presenting later in childhood and adulthood.
2003   Birth incidence for Glut1 Deficiency is estimated in Queensland, Australia at
         1:90,000 (Coman et al).   If accurate, estimated US prevalence is around 3150 cases.
2006   Glut1 Deficiency mouse model created (Wang et al).
2008   Dystonia 18 is an allelic variant of Glut1 Deficiency.
2011   Dystonia 9 is an allelic variant of Glut1 Deficiency.
2015   Gene therapy with rAAV strategy is feasible (De Vivo, Monani, Gao et al)

III. Etiology of Glut1 Deficiency

Blood vessels carry nutrients to brain, before passing through the blood brain barrier, to support astrocytes and neurons. Most of brain energy is consumed by synaptic activation of cell connections (electro-chemical transmission). Glucose fuels the astrocyte where it is converted through glycolysis to lactate. Then, lactate is transferred to the neuron for oxidation to CO_2 and water. Glycolysis makes 2 ATP, oxidation of lactate makes 34 ATP (*ATP is a unit of energy).* Glut1 Deficiency targets the glial- vascular unit.

Gene therapy primarily targets the endothelial cell in our study design. It is essential to deliver the gene replacement to the most critical cell(s) to maximize benefit and minimize risk.

IV. Mouse Model of Glut1 Deficiency

Our mouse is a heterozygote (1 non-functional SLC2A1 gene and 1 functioning gene). It has reduced Glut1 protein. We use rotarod testing as an outcome measure. Mutant mice don't do very well compared to control mice (wild type). In the heterozygote mouse, the CSF glucose is low (26 mg/dl) compared to control (75 mg/dl).

V. Gene Therapy

What is gene therapy all about? It is the treatment, cure or prevention of disease by modification of ones' genes or the addition of a new gene. We want to be sure that gene therapy is safe and effective (outcome measures are needed).

Strategies to treat a genetic condition with gene therapy:
1) Ex Vivo-fix
2) Take patient's cells into the laboratory, correct the genetic defect ,then transfer the cells back to the patient.
3) In Vivo-fix
4) Gene replacement (would be used for Glut1 Deficiency). Mutations in Glut1 Deficiency are "loss of function" mutations so treating would involve adding genes.
5) Gene silencing - stopping a gene from making a protein.
6) Gene addition - overexpression of a gene.
7) Gene editing - directly repairing the mutated gene in the patient's genome .

Gene delivery vehicles
1) Viral vectors (RNA Virus and DNA virus). This is like the Trojan Horse (it is a virus that likes to go into cells, loaded with the gene).
2) Non-viral vectors.

In 1985 the adenovirus was used as the vector. Unfortunately, a patient with a genetic disease died when exposed to the adenoviral gene construct, bringing gene therapy research to a halt for many years.

Dr. Guangping Gao and others have pioneered the use of the adeno-associated virus, the smallest form of DNA virus, as the vector permitting a resurgence in the field of gene therapy.

The AAV has a lot of great qualities: efficient, stable, minimally immunogenic, and not genotoxic (it does not incorporate into the genome).

The bottleneck in gene therapy is the central nervous system (CNS). How do we deliver the gene to the correct location in the brain?

1) Inject directly into the brain. Used currently in AADC deficiency research with AAV2 as the vector.
2) Inject directly into the cerebral ventricle or spine. Used currently in SMA research with AAV9 as the vector, or with anti-sense oligonucleotides.
3) Inject into the vein. We are proposing this route for Glut1 Deficiency.

In gene delivery, the AAV-gene construct crosses the cell membrane and the nuclear membrane to "set up shop" in the nucleus. The new gene makes RNA in the nucleus and the RNA makes protein in the cytoplasm. We introduce the AAV virus loaded with SLC2A1 gene. It sits in the nucleus and is making protein. The host gene also is making some Glut1 protein.

In the laboratory, cultured human skin fibroblasts have been loaded with the AAV9/ Glut1 gene construct and we have rescued the cellular phenotype. Patient cells can be rescued with Glut1 genes either from mouse or human sources. The control cell lines, overexpressing Glut1 protein, appear to function normally.

The mouse model of gene therapy involves in vivo therapy *(in the live animal)*. The AAV9/ Glut1 construct is injected into mutant and wild type mice. At day 1 of life, the injection is into the cerebral ventricle, at day 3 into the facial vein and at older ages, into the tail vein.

Restoring Glut1 early in life improves motor performance (rotarod and vertical pole tests). Glut1 protein is over expressed in the treated mutant mice, the CSF glucose in treated mice is higher (50-55 mg) compared to the non-treated mice (25 mg/dl). Brain size, also, is improved.

VI. Conclusions

We have shown that we can restore Glut1 protein in vitro in cultured fibroblasts with gene therapy.
We can also restore Glut1 in the model mice early, using gene therapy, and protect them from developing Glut1 Deficiency.

AAV9 gene therapy in humans appears to be feasible and should be performed as early as possible. Rescuing the Glut1 Deficiency phenotype later in life is more difficult.

This research represents a collaborative effort of three laboratories (Monani and De Vivo laboratories at Columbia University, and Gao laboratory at the University of Massachusetts), funded in part by the Will Foundation, the Colleen Giblin Foundation, Milestones for Children, and the Glut1 Deficiency Foundation.

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Current Research Overview
Triheptanoin and Related Research

I. Patient Data Registry

I have established a patient data registry.   Glut1 Deficiency patients enter data about their symptoms so that we can learn more about this disorder. The data is de-identified and secure. 150 patients have already registered. There are 50,000 pieces of unique data in the registry.

II. G1D 2013-2015 The poverty of principles and the hope of research.

What do neuroscientists do? We diagnose, comprehend, and treat. We are good at diagnosis, ok at comprehending, and not so good at treating. You can refer to the book by Rosenberg and Pascual entitled "Molecular and genetic basis of neurological and psychiatric disease".

The research goal is how things change versus what is the absolute amount or level of the disease state.   Flux analysis in neurobiology is the main focus.

There are 3 things in Glut1 Deficiency that are incorrect principles.
1) The disorder is irreversible (severe energy metabolism disorder).
2) Glut1 Deficiency is due to energy failure.
3) Glut1 Deficiency leads to widespread brain dysfunction.

Brain energy metabolism includes glycolysis to citric acid cycle to oxidative phosphorylation for ATP production.

There are many "gatekeepers" that allow the chemistry in the brain to proceed. Flux is a problem with energy loss and neurotransmitters are impacted.

III. Glut1 Deficiency Mouse Model

We have a mouse model of Glut1 Deficiency. We can obtain PET scans on mice. The thalamus is blue on the PET scan which in humans means low glucose metabolism. We inject mice with glucose and follow the EEG to determine what happens. We also inject a magnetic label with C13 so we can see the fate of glucose. We run simultaneous MRI and EEG in mice.

Glucose is transferred from blood vessel, to brain support cells (astrocytes), to neurons.

EEG in the mouse is seen as repetitive electrical discharges which are: rapid at the beginning of this activity, everywhere in brain, and developing at the same time. We want to know the mechanism that all of this can happen at the same time? There must be a way that all of the brain cells "speak" together and seize at the same time. This doesn't make sense!

The mouse EEG is similar to human EEG.

Where in the brain is the pacemaker for the EEG anomaly? We isolate the thalamus to determine where the signal comes from and record this; in the mouse brain tissue slice.

The thalamus is the pacemaker in Glut1 Deficiency. It likely has a low threshold to become excited. Thus it isn't a severe irreversible problem. If you apply glucose to the thalamus tissue you can make it function normal. The thalamus in Glut1 Deficiency has a lot of electrical activity periodic and rhythmic electrical activity.

In humans we run functional MRI and EEG simultaneously. Brain activation is evaluated in functional MRI. We also look at decreased activity. When we also look at EEG data at the same time we can correlate seizures with functional MRI and see where the seizure actually starts from.

Thalamus, cerebellum, cerebral cortex are all active in seizure activity in Glut1 Deficiency. Brain deactivation is also important! In the post seizure setting, there is lower metabolism than at baseline (pre-seizure) time point. The brain has a default mode network which is a localized phenomenon. This is localized within a network not across the whole brain. Epilepsy is circuit dependent.

Regarding energy metabolism in Glut1 Deficiency mice; we cannot find metabolism compounds that are abnormal in Glut1 Deficiency thus there is no energy failure. So what is low when there is low glucose? Other compounds are compensating for low glucose. What did we learn from mice that are ketotic without being on a ketogenic diet? They naturally have high free fatty acids.

Glucose and inhibitory synaptic transmission, there is a problem with the synapse (mechanism for transmission from one neuron to another). Glut1 Deficiency involves a decrease in amount of neuron firing in the brain. The problem is not so much with glucose transport and transport across the supporting cells. There are problems with these transmitters. Glutamate is a weaker signal. GABA is a weaker signal. Thus there is evidence of synaptic failure. Does this inform treatment?

IV. Triheptanoin and Glut1 Deficiency

Maybe we can fix some of the cell to cell functioning by using acetate. We use C7 to restore brain function. This has been tried in mice. Brain metabolism in mice is evaluated with MRI. We inject glucose in the vein and magnetic labeled glucose and can see the chemical plot. We can evaluate many of the chemical reactions after a glucose injection. We can tease out the metabolism of the glucose. C7 is metabolized in the liver which make ketone bodies that go into the brain; at least, that is the theory. We have data on this. When we give heptanoic acid, glucose increases. When we give C7 plus the ketogenic diet, the glucose values can rise and the patients may have seizures.

Human studies with C7

A standard diet is 35% fat. The ketogenic diet is 87% fat. The C7 diet 60% carbs and 35% fat (triheptanoin).

We conducted a clinical research study with Triheptanoin. We ran MRI, neuropsych, blood sampling before and again at the end of treatment with C7.

Results on the 1st patient who had a lot of seizures during initial testing period. When this patient was given just 2 tsp of triheptanoin, the rate was reduced. In fact, everyone who received triheptanoin had reduced seizure rate on EEG post triheptanoin. This is evidence that Glut1 Deficiency is a mild state. This indicates that we can modify brain metabolism.

The synapse is where the problem in Glut1 Deficiency occurs. It is a cell to cell problem in Glut1 Deficiency, not in the whole brain network, rather a selective areas of the brain.