
**A Workshop Presentation given by Doctor David.A.Weinstein M.D., M.M.Sc.
(University of Florida) for the AGSD (UK) Conference October 2007**

Doctor Weinstein began his report with a brief explanation of the restrictions on the pathway of glycogen due to the absence or malfunction of the enzyme glucose-6-phosphatase and the areas of the body where damage could occur such as the liver, kidneys, and brain. He then considered the following:

1. Treatment strategies for type I and including new research on cornstarch
2. Overview of complications and treatments
3. Potential and difficulties with gene therapy and stem cell therapy
4. New collaborations and research

1. Treatment Strategies for Type I

The goal of treatment for GSD Type I is to provide a continuous supply of glucose to maintain normal plasma glucose concentrations so that glycogen breakdown does not occur:

Plasma glucose \geq 4.2 mmol/L
Blood lactate \leq 2.2 mmol/L

Therapy for GSD Type I

- To achieve these goals, either cornstarch therapy or continuous feeds can be used.
- Treatments are individualized based upon schedule, diet, and results of annual metabolic studies
- No children and almost no adults can make it through overnight fast with cornstarch and maintain good metabolic control

Treatment of GSD Type I in 2007

The timing and doses of cornstarch are individualized, but usually doses are given every 3-5 hours during the day and every 4-5 hours at night

At the University of Florida GSD clinic, patients have traditionally been hospitalized once a year for individualized protocols through the end of puberty during which cornstarch doses are titrated based upon hourly glucose and frequent lactate determinations

Monitoring in GSD Type I

- Home glucose and lactate monitoring performed
- Lactate monitoring particularly useful in conditions where hyperlactatemia can occur in the setting of normoglycemia e.g.
 - Illness
 - Pregnancy
 - Puberty
 - Medications
 - Patients with milder GSD type I where partial enzyme activity is present

Doctor Weinstein recommended the lactate meter “Lactate Pro” found at www.fact-canada.org. The glucose meter Dr. Weinstein recommends is the “Freestyle Flash”.

Discussion Period in Workshop

Doctors in the USA begin to switch babies over to a cornstarch regime from continuous feeding as young as six months if they can tolerate 5 grams of cornstarch without having diarrhoea.

The general view in the USA is that cornstarch is more effective and user-friendly than continuous feeding and the earlier cornstarch is used the better but the European doctors prefer a more gradual transition from continuous feeding to cornstarch. In Dr Weinstein's clinic, each patient is monitored in hospital to determine exact dosages of cornstarch, and Dr Weinstein recommends that the dosage of cornstarch consumed should be weighed precisely using a gram scale. Only brands like Argo or Kingsford in the US should be used since all cornstarch brands are not equivalent. He recommends that cornstarch is given on average 6 times daily, but dosing is individualized. On average, patients receive 3-4gms/kg of cornstarch throughout the day, but significant variability exists. Ideally Doctor Weinstein would like to fine tune the cornstarch intake so that the doses are different for day and night and different levels are taken depending on activities and amount of food consumed. He says that the 'Freestyle Flash' meters are good for measuring glucose levels at home, but warns that other meters brands report glucose values that are inaccurate because some of the other meters are distorted by lactates.

In the Netherlands, continuous feeding is preferred to cornstarch for young children as it has achieved better results especially growth and producing less complications. In Dr. Weinstein's experience, growth is better with cornstarch, and the complication rate is lower.

Cornstarch Trials

The greatest daily life improvement for Type I patients would be the ability to sleep through the night without waking to take cornstarch. A product and dose of a that product needs to be found so that patients maintain ideal lab values on only 1 dose of starch a night.

To this end a study has taken place using a modified cornstarch.

- 5 products were identified over 2 years.
- 2 products were tested unsuccessfully in previous studies.
- New product identified in England, and initial studies were performed by Dr. Lee's group in 20 patients with GSD type I and type III
- Studies transferred to Florida to allow testing at night where glucose levels were monitored down to a level of 3.3mmol/L
- Patients were 13 years and older

2 night study where subjects were given the traditional Argo starch 1 night and the new experimental starch on the other night.

Hours	% remaining in study in subject receiving Argo cornstarch	% remaining in study in subject receiving experimental starch
0-1	100% 12/12	100% 12/12
1-2	100% 12/12	100% 12/12
2-3	100% 12/12	100% 12/12
3-4	100% 12/12	100% 12/12
4-5	100% 12/12	100% 12/12
5-6	75% 9/12	100% 12/12
6-7	58% 7/12	75% 9/12
7-8	42% 5/12	67% 8/12
8-9	25% 3/12	58% 7/12
9-10	17% 2/12	33% 4/12
10+	17% 2/12	25% 3/12

- While the experimental starch was significantly better than Argo cornstarch, no significant difference was found in the time maintained blood glucose above 3.8 mmol/L mg/dL , and the lactate values were not significantly different.
- In addition to offering the potential for extended coverage, the new starch appears to have added safety during the overnight period. Most of the benefit of the new starch was found to be after glucose concentrations fell below 3.8 mmol/L.
- Future studies should be done to determine alternate doses allow for improved control within the therapeutic range (above 3.8mmol/L).

Second Study scheduled to commence in Fall 2007

- Collaboration with GSD teams in Duke, England, and Holland
- Same protocol as the original study, but 3 doses of the new product will be tested
- 54 subjects with type Ia GSD (10 years of age and older) will be enrolled in the studies

Study Team:

Co-Principal Investigators:

- Priya S. Kishnani, M.D. (Duke University)
- David A. Weinstein, M.D., M.M.Sc. (University of Florida)

Additional Investigators:

- Catherine E. Correia
- Anne Boney
- Dwight Koeberl, M.D., Ph.D.
- G. Peter Smit, M.D.
- Philip Lee, M.D.

Gastrostomies

In the USA, most families with Type I opt for a gastrostomy (80 – 90%). The timing for removal of the G-tube is individualized, but it is typical to remove the tube around the age of 12 for boys and 10 for girls.

2. Overview of complications and treatments concerning hepatic adenomas

Despite improvements in therapy, hypoglycemia and long-term complications remain common

Most common complications in adults with GSD type Ia are hepatic Adenomas

Lesions usually develop during puberty with mean age of onset 14.8 ± 4.2 years

- There is no difference between cornstarch and continuous feeding in the prevalence of hepatic adenomas, age of onset, or progression
- Adenoma growth associated with puberty
- Growth of adenomas has been seen in women taking oral contraception and men taking oral steroids
- With the improved survival, an increased risk of hepatocellular carcinoma has been noted
- Series of 8 cases of hepatocellular carcinoma recently reported in patients with GSD type Ia ranging from 19 to 50 years of age.
- No consensus on management of adenomas

Observation

- Cancer
- Bleeding
- Anxiety

Resection

- Surgical Risk
- Experience critical

Ablation

- Technically challenging
- Future imaging extremely difficult

Transplant

- Risk
- Not a cure!

Dr Weinstein said that all of his patients who have taken Citrate supplements haven't had any kidney stones for 8 years. 'Euro CK' is a brand of Potassium Citrate.

Workshop Discussion

- Over 50% of adults with Type I have adenomas (mostly all benign)
- Ultrasound is used to monitor the adenomas and in the USA twice a year MRI scans are also given if lesions are found.
- Progesterone is preferred in oral contraception as estrogen causes an increased level in hormones which can lead to an increase in the growth of an adenoma.
- Of the 8 cases of hepatocellular carcinoma, some of those 8 patients had hepatitis and all had poor control.
- Several patients in USA, Canada, and Holland have had resection. (one 7 years ago)
- Ablation (removing the adenoma) is difficult to follow up and also can scar the liver and so hide further adenomas.

Transplant would be recommended only for management of adenomas that are not able to be resected and are growing in adulthood at an unexpected rate.

5 have been carried out on Type I patients in Dr. Weinstein's practice.

Doctor Weinstein stressed that transplants only take place in extreme cases. Many of the type Ia and Ib patients have had kidney issues following transplant, and most patients with type Ib continue to have neutropenia. Furthermore, people are left with immunosuppression, higher rates of infection, and the risk of organ rejection.

3. Potential and difficulties with gene therapy and stem cell therapy

3 methods are presently being studied aimed at replacing glucose-6-phosphatase activity:

1. Traditional gene therapy using a viral vector to insert the gene
2. Infusion of normal mature liver cells
3. Use of stem cells to regenerate the liver with normal cells

Protocol used in attempted gene therapy in mice

Type Ia mice which manifest the same problems (high cholesterol, triglycerides, uric acid, hypoglycemia) as humans. The mice are not a perfect model of human disease since elevated lactates usually do not occur in the mice.

Gene therapy performed in the laboratory of Dr. Janice Chou from NIH successfully normalized growth and glucose concentrations in the mice.

- Each animal received 2 infusions of AAV1-G6Pase (Adeno Associated Virus 1)
 - 1st infusion of 5×10^{11} particles of in neonatal mice
 - 2nd infusion of 1.5×10^{12} particles at 1 week of age
- G6Pase under the control of the chimeric chicken β -actin promoter and CMV enhancer

Result

All the mice survived without glucose supplementation for the duration of the 57 week study

The growth rates of the AAV1-G6Pase infused GSD-Ia mice are comparable to those of the control littermates

AAV1-G6Pase infusion normalizes plasma glucose, cholesterol, triglyceride, and uric acid profiles

Successful delivery of the G6Pase gene to both the liver and the kidney

AAV1-G6Pase directs hepatic and renal G6Pase expression

The transduced liver tissues show no histological abnormality

The transduced kidney tissues show variable degrees of histological abnormalities particularly 49 to 57 weeks post-infusion

Gene therapy was performed on dogs with GSD type Ia at the University of Florida for the first time on September 11, 2007 so the study is still in progress.

Questions to be answered.

- Will the Adeno Associated Viruses be safe in dogs and humans?
- Can the virus be synthesized for larger animals and humans?
- Will partial enzyme correction lead to normal glucose concentrations but high lactate levels?
- Does glucose-6-phosphatase need to be regulated?
- Is 10% of the liver functioning normally enough to “cure” the disease?

Can bone marrow derived stem cells create enough healthy liver cells?

Several years ago a 19 year old girl with type I needed a bone marrow transplant for an condition unrelated to GSD.

As a result she was able to skip several doses of uncooked cornstarch per day with no hypoglycemia even with significant exertion

In June 2004, the patient was taken off her overnight dose of cornstarch. After 9 hours, her glucose was 89 mg/dL (4.9 mmol/L), and she was able to fast 10.5 hours before she fell to 70 mg/dL (3.9 mmol/L)

Monitoring of “fasting” blood sugars for 4 months revealed no hypoglycemia.

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Evidence to support the formation of healthy liver cells from bone marrow derived stem cells came from a liver biopsy in which the pathology was remarkable for micro and macrosteatosis, but the ‘plant cell appearance’ characteristic of the glycogen storage diseases was not present.

Also the glycogen content: analyzed at Duke University and was found to be 6% (upper limit of the normal range is 6.7%).

The source of the stem cells for the patient was a male. As a result, staining for the X and Y chromosomes could be used to distinguish the patient’s cells from those derived from the donor. Analysis of her liver showed some XY cells that stained with a liver specific probe (Hep Par -1), but not with blood cell markers (CD45).

Summary of evidence

- Clinical evidence of improvement
- Evidence of improved ability to fast
- High normal glycogen content
- 1% of cells found to be XY Hep Par-1/CD45 negative

2. New collaboration and research

- New update on GSD types Ia and Ib
- Update on GSD type III
- Study on hyperlipidemia in GSD type III
- Reports on pregnancy
- New evaluation of treatment strategies

Workshop discussion

Osteoporosis

Osteoporosis is common in all types of glycogen storage disease. In type I GSD, osteoporosis is caused by many factors including vitamin D deficiency, inadequate calcium intake, and chronic acidosis which impairs bone mineralization. With proper treatment, bone mineral density can be normal in GSD type I, but it is important to maximize control and provide proper dietary intake of calcium and vitamin D. Patients in Florida have vitamin D levels screened annually, and bone density testing is performed every five years to screen for osteoporosis.

Diet

Doctor Weinstein recommends a maximum non-utilizable sugar intake of 2.5gms per meal. Fruit sugar not suitable for patients with GSD as fruit sugar cannot be converted to glucose and leads to increased glycogen storage, In addition, when a patient with GSD I consumes fruit sugar (fructose) it is converted to lactate and triglycerides. His patients cut back on table sugar and dairy sugar, and never eat fruit sugar. They completely stay away from Bananas, Apples and Melons.

The diet used in Florida is not universal, and the appropriate diet is still debated.

Patients in Germany have a high intake of pasta and no sugar

UK patients have a less restricted diet

He also recommends no alcohol as it can make the liver more inflamed especially beer which contains maltose.

Cornstarch should be taken ideally half an hour after a meal.

It must be freshly made and can be shaken but milk must not be used as a mixer.

Pregnancy

A recent report on 15 pregnancies in type Ia was just published. One patient followed by Dr. Weinstein with type Ia has had 4 children.

There has been no increase in adenomas or kidney problems with his Type Ia patients who have had babies. Morning sickness has also not been a problem.

There have also been several pregnancies in Type Ib patients, and there have also been no significant difficulties.

Hyperlipidemia

Dr Weinstein commented that he hasn't seen problems using statins to control cholesterol, although he pointed out that patients with Type III must not take them. He recommends using Gemfibrosil to control triglycerides and good metabolic control for lipids.
