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Overview:

The energy that the body needs in order to function is obtained from carbohydrates that we get through our diet. These carbohydrates are monosacharides, disaccharides and polysacharides. The polysaccharides and disaccharides are hydrolyzed to monosaccharide's such glucose (which comprises roughly 80%) fructose and galactose. Most cells convert the fructose and galactose to glucose. The body can use the glucose or store it. If energy is needed, glucose can be oxidized through the many reactions of glycolysis which gives a net production of 2 ATP and 2 NADH from one molecule of glucose. In the presence of oxygen the product of glycolysis pyruvic acid will be decarboxilated to Acetyl Coenzyme A (Acetyl COA) in the mitochondrial matrix forming carbon dioxide and 2 NADH in the process. The acetyl COA subsequently enters the citric acid cycle (the Krebs cycle) where after a series of reactions, 2 molecules of ATP, 4 molecules of carbon dioxide, 6 NADH and 2 FADH2 are produced. The NADH's enter the electron transport chain and over a series of reactions with the proteins in the mitochondrial matrix form 3 ATP per molecule of NADH and 2 ATP per molecule of FADH2. (Murray)

In accordance with homeostasis, the presence of high energy will cause the body to balance energy levels by forming storage molecules of energy for use in times of need. This storage form of glucose is called glycogen; the synthesis is called glycogenesis. This glycogen can later be degraded into is monomer units for the use in oxidative phosphorylation. (Champe)

Glycogen is a branched chain of alpha-D-glucose. Most of the glucose molecules are attached to each other via a carbon to oxygen to carbon bond known as a glycosidic bond. In the linear portions of glycogen the glycosidic bond is known as the 1,4 linkage this is a bond between the carbon labeled 1 and the carbon labeled 4 on the next glucose. Every 8-10 glucose molecules there is a 1, 6 glycosidic bond causing the above mentioned characteristic branching of glycogen. (Murray) [Figure 1]

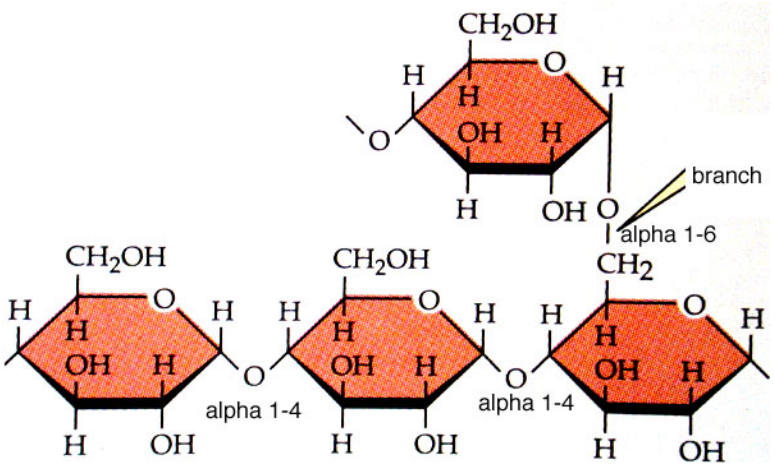

Figure 1 1,4 and 1,6 Glycosidic Bonds in Glycogen (Brooklyn College, 2009)

Glycogen is mainly stored in the skeletal muscle and the liver while it has been found in almost every cell in the body in trace amounts. The liver cell has the most glycogen consisting of up to 6% of its total weight. Alternatively in muscle cells glycogen is never more than 1% its total mass. However, because the total mass of muscle in the body is larger than that of the liver, overall, muscle contains more glycogen than the liver. (Murray)

Muscle contains glycogen for its own use. When in need of energy it will break down the glycogen via glycolysis in order to synthesize ATP for muscle contraction. Muscle will replenish

its reserves of glycogen after exercise for example and will not be affected during short periods of fasting. Yet, the liver stores glycogen in order to maintain blood glucose between meals; higher levels of glycogen are found after meals and lower levels during fasting. (Murray)

There are five enzymes that catalyze the reactions of glycogenesis. The first enzyme glucokinase which is found in liver cells and beta cells of the pancreas phosphorylates glucose. Additionally, hexokinase, a similar enzyme phosphorylates glucose in most of the other cells of the body. This phosphorylation creates Glucose 6-PO4 which is the substrate for the enzyme phosphoglucomutase which transfers the phosphate group to the 1st carbon. Next, uridine triphosphate (UTP) a high energy molecule reacts with glucose 1 PO4 and the enzyme UDP-pyrophosporylase forming UDP-glucose. Being that glucose cannot be added to other single molecules of glucose UDP glucose attaches to glycogenin a molecule with a tyrosine side chain to form the primer for additional glucoses to be attached. After adding two molecules of glucose to this base stand, the enzyme glycogen synthase continuously acts with UDP-glucose to form a long linear chain of glucose attached via alpha-1,4-glycosidic bonds. The ends of the chain to which this enzyme attaches to are called nonreducing ends. Branches are formed in the chain in order to increase solubility and to increase nonreducing ends. By increasing non reducing ends enzymes can easily remove and attach glucose molecules thereby increasing the rate of synthesis as well as degradation of the chain. (Champe)

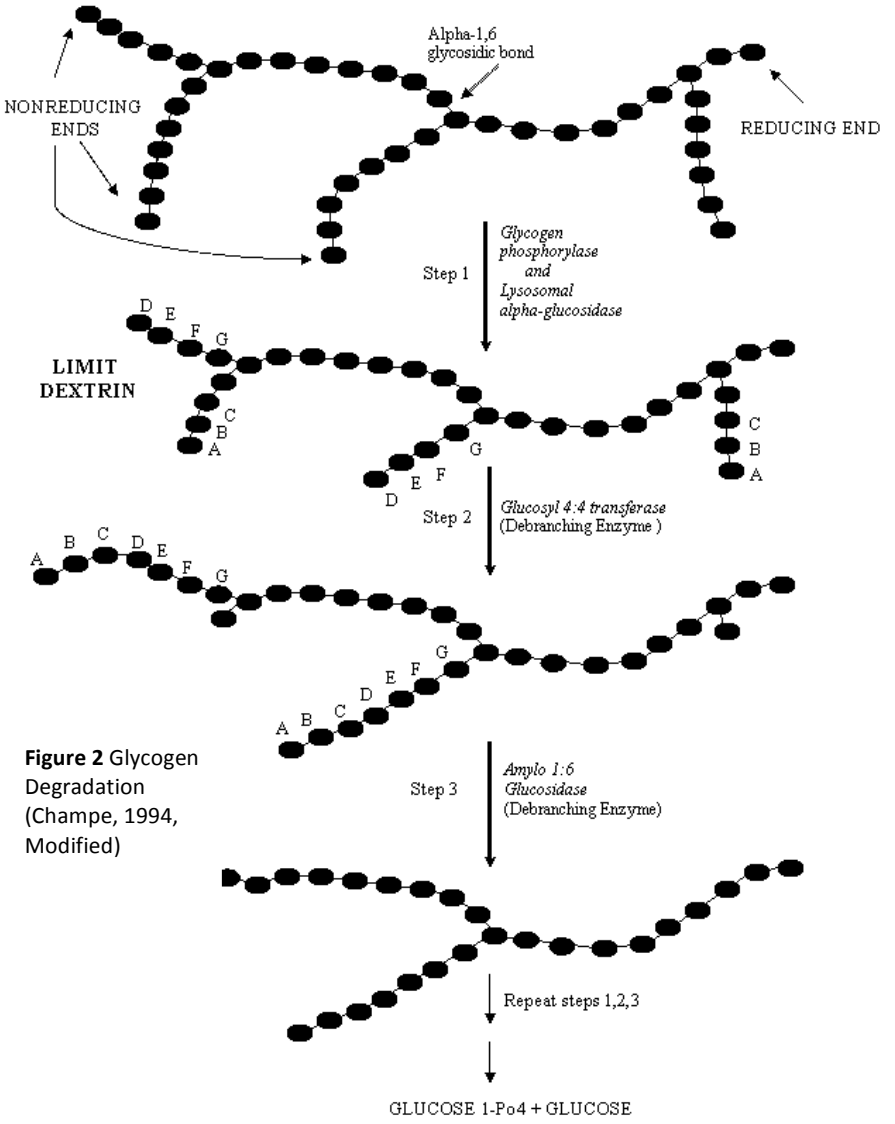

Figure 2 Glycogen Degradation (Champe, 1994, Modified)

The branches are formed by the enzyme glucosyl 4:6 transferase. The enzyme breaks a 1,4 bond in the linear portion of the chain usually forming 5-8 glycosyl chains and attaches this small chain to another spot on the chain via a 1,6 linkage. This results in two more nonreducing ends.

The new reducing ends can then be elongated by glycogen synthase and glucosyl 4:6 transferase usually removing 5-8 terminal molecules and adding them to another point in the chain by 1, 6 linkage and so on forming branched glycogen.(Champe) The remaining branched chain of glycogen after the action of glycogen phosphorylase is called the limit dextrin. Next, the enzyme glucosyl (4:4) transferase

removes 3 of the four remaining glucose units before the branch point and adds them to the remaining linear chain via a 1,4 linkage. Then the enzyme amylo-alpha-(1,6)-glucosidase cleaves the one remaining glucose molecule attached via 1,6 linkage before the branch point to which the cycle starts again with the glycogen phosphorylase removing linear portions of the chain and so on until the glycogen molecule is completely degraded. (Champe)

In response to low blood glucose glycogenolysis is initiated. First the enzyme glycogen phosphorylase cleaves the alpha-1,4 glycosidic bonds of the linear portions of the chain of glycogen. This enzyme stops when there are four glucose units left before a branching point. The enzyme Alpha-1, 4-glucosidase (lysosomal enzyme) is another enzyme that cleaves the alpha-1, 4-glycosyidc bonds until there are four glucose units left in that chain before the branch point. (Champe)

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The products of glycogenolysis are Glucose-1-phosphate and glucose. Glucose-1-phosphate is converted to glucose -6-phosphate by phosphoglucomutase. In the liver Glucose-6 po4 is converted to glucose by glucose-6-phophotase and then released into the blood. In skeletal muscle Glucose-6-po4 is converted to glucose which is used for energy in the form of ATP from glycolysis.(Murray)

Regulation of Glycogen synthesis and degradation in the body occurs through allosteric regulation. This is charachterized by the inhibition of enzyme pathways by changing the shape of a molecule so that it cannot work or activation of a protein to make it work.

Glycogenesis will be initiated when an increased concentration of glucose 6-PO4 is in the cytosol of the cell. The enzyme Glycogen synthase is allosterically activated via positive feedback. This causes the enzyme to synthesize glycogen via pathway mentioned above.

With increased concentration of glucose, glucose 6-PO4, and ATP the enzyme glycogen phosphorylase is inhibited via negative feedback which stops the degradation of glycogen. (Champe)

Glycogen degradation

In order to break down glycogen in a particular cell the glycogen synthesis pathway must be inhibited. This inhibition is carried out by cAMP pathway/ Adenylate cyclase pathway.

In response to hormone such as glucagon attaching to it the membrane receptor couples with the G-protein in the membrane and activates adenylate cyclase enzyme in the membrane. When activated the enzyme catalyzes the reaction of ATP to form 3,5, Adenosine monophosphate (cAMP). CAMP then activates a protein kinase which then phosphorylates a

particular protein substrate in the particular pathway that is occurring. This phosphorylated protein can then act on other substrates. (Champe)

Another process that uses the cAMP pathway is the inhibition of glycogen synthesis. In response to glucagon, epinephrine, or low levels of energy glycogen synthesis will to be stopped. Glucagon or epinephrine activates adenylate cyclase and eventually the cAMP activates a kinase that inactivates glycogen synthase by phosphorylating it. This inactivated enzyme is now referred to as Glycogen synthase b. In the phosphorylated form the glycogen synthase b cannot synthesize more glycogen. (Champe)

Activation of Glycogen degradation is also carried out by cAMP pathway by the binding of glucagon or epinephrine. Activated cAMP dependent kinase phosphorylates and activates phosphorylase kinase. Phosphorylase kinase then phosphorylates the enzyme *glycogen phosphorylase b* to *glycogen phosphorylase a* which starts hydrolyzing glycogen. (Champe)

GSD

The first known diagnosis of GSD occurred in 1928 by the physicians Snappes and Van Crefald. The case involved was a 7 year old presenting with hepatomegaly or liver tumor and the patient had a very low fasting glucose yet very high acetone and beta-hydroxybuterate in the urine. After various tests the term "insufficient mobilization of glycogen" was used and the disease was defined as a debranching enzyme deficiency. Later, this disease was termed GSD III or Cori's disease. Over the next few years more cases of GSD were found by various physicians and chemists that found increased amounts of glycogen in patients' organs. (Fernandes)

There are more than 14 known glycogen storage diseases. All of these diseases are genetic and characterized by an abnormal type or quantity of glycogen in tissues, usually caused by a missing or defunct enzyme in the pathway. Nearly all of the proteins involved in glycogenolysis and glycogenesis have been the cause of a particular GSD. The defunct or missing enzyme can affect the liver function or muscle function or both. The diseases are diagnosed acc. to types of symptoms that are evident in the patient. (Chen).

Glycogen storage diseases have three general groups hepatic, myopathic and miscellaneous. Clinically, the diseases are numbered but only the first through seventh are traditionally used; the others are described according to enzyme affected (Harrsisons).

Hepatic types are diseases that are grouped acc. to their hepatic hypoglycemic pathophysiology in that those diseases that affect the liver affect sugar levels too because the liver is mainly responsible for carbohydrate metabolism (Chen). These are Von Gierke's disease (Type I) a glucose-6-phosphotase deficiency affects liver, kidney and intestine because of increased glycogen storage which causes severe hypoglycemia, fatty liver and hyperlacticacidemia, Cori's Disease (Type III) which is a debranching enzyme deficiency causing accumulation of branched polysaccharide with symptoms such as hepatomegaly, hypoglycemia and hypotonia and Hers' disease (Type VI) which also has high liver glycogen content and hypoglycemia. Others include Andersen's disease (Type IV) which is caused by a lack of branching enzyme as well as Type IX which is caused by a phosphorylase kinase deficiency. These diseases have characteristic symptoms of expected liver and sugar diseases such as hypoglycemia, hepatomegaly (Chen)

The myopathic type is a group of muscle-energy pathophysiology related diseases. These diseases are McArdle's disease (Type V) a skeletal` muscle glycogen phosphorylase deficiency with symptoms such as myglobinuria, muscle pain, muscle cramps and muscle enzymes in serum after exercise., Tarui's disease (Type VII) which is to type V but also can have symptom of hemolytic anemia. The miscellaneous group includes of Anderson's (Type IV) a branching enzyme deficiency and Pompe's (Type XI). These are different in that their symptoms and causes are not specifically related to any group (Chen). There are other GSD's that are characterized by deficiencies of cAMP dependent protein kinase or adenlyl cyclase.

The overall frequency of glycogen storage disease in populations of European decent is around 1 in 23,000 births with types I,II,III,VI, and IX the most common comprising of approximately 90% of all cases. Inheritance patterns of disease are generally autosomal recessive. However, there are forms that have been found to show x-linked patterns. (Chen)

GSD Type 2 – Pompe's

Glycogen storage disease type ll was discovered by Pompe in 1932 when a 7 year old girl was found with massive accumulation of glycogen in her tissues. The discovery made Pompe the third physician to discover a GSD. In that same year and in the years to come many more cases of cardiomegaly, hypotonia and death before the age of 1 were found with physicians reporting additional symptoms associated. (Hirschorn)

The disease is known as Glycogen storage disease type 2, Pompe's Disease, acid maltase deficiency, acid α-glucosidase deficiency or lysosomal enzyme deficiency. Initially the disease was only referred to as idio cardiomegaly but in 1963 with new biochemical discoveries in cell biology and metabolic pathways it was termed as a glycogen storage disease (Chen). Additionally, it was termed a lysosomal storage disease when Hers eventually defined a lysosomal storage disease as having 1) lysosomal enzyme deficiency 2) deposit build up present in vacuoles (Chen). The discovery by Pompe also led to the startup of research into lysosomal storage diseases with over 40 known today.

The lysosome was first discovered by Christian de Duve in 1955 as a membrane bound vacuole in human cells containing hydrolytic enzymes all active at acid pH (de Duve). These enzymes he postulated were able to break down macromolecules one of which is glycogen. (Campell)

Pompe's disease is caused by a genetic deficiency of the enzyme alpha-1,4-glucosidase (lysosomal enzyme) which is found in the lysosome and primary and secondary vacuoles of cells. Normally this enzyme as well as glycogen phosphorylase cleaves the alpha 1,4 glycosidic bonds of the branched chains of glycogen until there are 4 glucose molecules left before the branching point where the enzyme glycosyl 4,4 transferase cleaves the glucose molecules until the branch point. But a deficiency in the lysosomal enzyme will cause a build up in the glycogen branches because of the inability of glycosyl 4,4 transferase to function on the glycogen. This leads to a build up of partially branched glycogen in the cell cytoplasm as well as glycogen accumulation in the primary and secondary vacuoles of the cells. (Chen)

A postulated mechanism of action that causes the symptoms of the disease is that the deficiency of alpha-glucosidase causes a build up in the lysosomes of the cell. After reaching a capacity as well as with mechanical action of muscle the lysosomes rupture spilling their contents into the cytosol. (Griffin) An increase in protein breakdown has been seen in various studies by both radioactive isotope and stable tracers. This breakdown occurs because of the cells' low levels of energy which initiates a cellular response by harvesting energy from the muscle protein and thus the wasting away of muscle and the subsequent symptoms such as muscle pain and hypotonia as well as respiratory problems are seen. (Bodamer)

The clinical representation of Pompe's shows a broad range of phenotypes all of them include myopathy but differ in other symptoms. There are three general groups of GDS 2 infantile onset, juvenile onset and adult onset. The groups differ in their progression characteristics but all are characteristically confirmed by the absence or lack of the alpha-glucosidase. Yet, studies have shown that severities of disease are in correlation with the lack of alpha-glucosidase, with infants and children usually having the worst forms. Additionally, symptoms seem to develop when there is less than 30% enzyme activity and increase in severity with the lessoning of activity. (van der Ploeg)

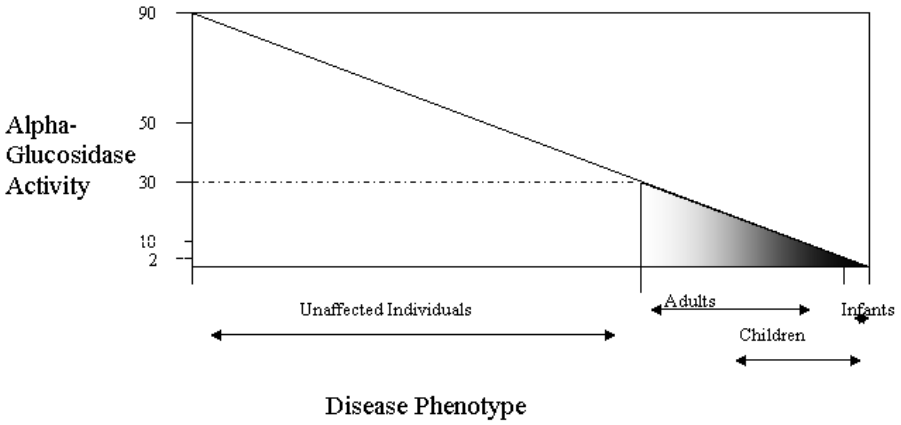

Figure 3 Model Depicting that signs of Pompe's emerge when enzyme activity is below 30% (van der Ploeg, 2008, modified)

Infantile Onset:

Infantile onset, otherwise known as the classic Pompe disease is the most common and the most severe of the three types of GDS2, causing cardio-respiratory death before the age of two years old. Most frequently it is reported in infants in the first months of life. Symptoms are generally severe cardiomegaly and hypotonia as well as macroglossia and rapid progressive weakness. Upon EKG testing as well as during autopsy it was found that both of the ventricles of the heart have increased thickness caused by the excess glycogen. (Chen) [Figure 4]

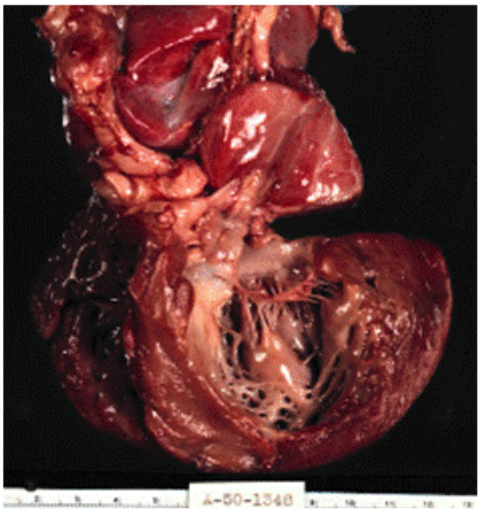

Figure 4 Severe Cardiomegaly in 9 month old child (Johnson, 2009).

Childhood-Juvenile Onset:

The childhood juvenile onset group is characterized by symptoms occurring after the age of two years old (Chen).

The symptoms usually present are skeletal muscle involved without any major cardiomegaly. The disease progresses more slowly than that of the first type and patients usually die of respiratory failure before thirty years of age. (Chen)

Adult Onset:

Adult onset GSD2 presents as a slowly progressive disease. The disease is usually found in patients in their mid 20's to 60's. In all cases known so far the disease does not cause cardiomegaly but affects other skeletal muscles as well as the diaphragm. Weakness is usually first followed by lower back pain and loss of deep tendon reflexes. Other symptoms include somnolence, orthopnea, exertional dyspnea, and morning headache. (Chen) Death usually occurs from respiratory failure but being that the disease affects the respiratory muscles death may be caused by pulmonary hypertension and cardiac failure.

Documented cases of patients that were athletically active, shows that patients can have completely normal lives before the onset of this disease.

GSD 2b: Danon's disease

First discovered in 1981 in two boys by Morris J. Dannon, this subtype is characterized by patients with GSD2 symptoms such as cardiomyopathy with vacuolar storage of glycogen yet, interestingly, normal alpha-glucosidase activity. Other clinical symptoms are arrhythmias, mental retardation and a Creatine kinase rise to tenfold in some cases. Yet, the hallmark finding is that the vacuoles contained autophagy material with glycogen in skeletal and cardiac muscle. The disease has been shown to be caused by a deficiency of a major lysosomal membrane glycoprotein called lysosomal-associated membrane protein 2 (LAMP-2) with studies mapping the deficiency as originating from the X chromosome and showing x-linked heritability patterns. (Piotrowska-Kownacka)

Genetic causes

The gene that codes for the enzyme alpha-glucosidase is located on chromosome 17 (q21-23) and is designated GAA on the human gene map and has 20 exons, 19 introns and is approximately 20 kb long. There are over 180 mutations that exist with that number increasing. The mutations that are occurring are missense, nonsense, splice site mutations, and small deletions and insertions. However, around half of all the mutations found are due to missense mutations. (Hirschorn)

In 1990 the gene was first cloned and cDNA strands were isolated. It was found that the enzyme created from the cDNA showed the same characteristics as the endogenous enzyme. (Hoefsloot)

Interestingly, carriers of the disease show codominance in that they will have 50% functioning enzyme. Therefore they will not have symptoms of the disease as mentioned above that patients with more than 30% functioning enzyme are not affected. (van der Pleug)

The three clinical manifestations of the disease prompted investigators to conclude that the various phenotypes of the disease are caused by different locations of the various mutations. For instance, in 75% of the patients that had the slow progressive type of Pompe's (which would include child and adult onset) were found to have the particular chromosomal mutation c.-32-13T>G. Yet the other more severe forms were found to have other mutations such as c.1935C>A and c.2560C>T which are found in patients with no enzyme activity at all. (van der Pleug) Additionally, researchers found that the symptoms of the other 25% of the patients with the c.-32-13T>G mutation showed varying amounts of functional enzyme leading to the possible theory that there are modulating factors involved such as diet and exercise. (van der Pleug)

Testing:

Being that in all cases of Pompe's disease there is a deficiency of enzyme prenatal diagnosis is possible in groups that are at risk. Testing of the chorionic villi cells either cultured or uncultured during pregnancy with the artificial substrate 4-methlyumbelliferyl-alpha-D-glucopyranoside (4MUG) will give a probable diagnosis for infantile onset GSD2. This testing is advantageous because it allows for the diagnosis with only 12 weeks into pregnancy and one day to actually diagnose. (Chen) Additionally, a study in Thailand successfully tested an infant with electron microscopy of chorionic villi. Also, amniocenteses as well cordocenteses is possible. (Phupong)

The artificial substrate 4MUG is also used in testing cultured skin fibroblasts. Skin fibroblasts contain large amounts of enzyme so if there is a deficiency it will be noted in accordance with strict standards. This test is advantageous in order to be able to use the same specimen over long periods of time. (van der Plaug)

This same artificial substrate can be used in muscle biopsies. Muscle biopsies can differentiate between the infantile onset and adult onset.

Additional testing includes the periodic acid Schiff (PAS) which tests for glycogen. The mechanism occurs through the reaction of periodic acid and glycogen which gives aldehyde products which then react with the Schiff reagent which gives off a purple color. (Kiernan)

Treatments:

The first study on the treatment of Pompe's was done in a Japanese lab. They subsequently showed that they can restore some activity of the acid alpha glucosidase in a mouse model (Kikuchi). Five years later in 2003 the company Genzyme created cloned enzyme recombinant human acid alpha glucosidase (rhGAA) from transgenic rabbit milk or CHO cells and have shown great results. When either of these was given in high doses of 10mg/kg there was a significant diminishing of glycogen in the cells of skeletal and smooth muscle as well as heart and other organs. These results made it possible to apply for FDA testing. (Koeberl)

Studies show that there is a critical threshold of about 30% of the average enzyme activity needed to prevent the symptoms of the disease. Enzyme therapy and replacement are in order to bring the patient up to the threshold but it seems that diet and exercise might lower this threshold. (van der Ploeg)

In 1983 Slonim and coworkers used a high protein diet to cure and raise the muscle function of a child with GSD 2. (Bodamer) This diet relied on the fact that the cell was utilizing energy from the breakdown of protein which is discussed above. Since then however, there is dispute whether such a diet would work, with recent reports showing that only 25% of patients show slight improvement of muscle function. Yet, even if this diet would work it is hard to get patients to eat a 30% protein diet. Furthurmore even if the patients can handle such a diet the patients gain considerable amount of wait which decreases their respiratory function thus removing any positive reasons for the diet.

A recent study was done on the efficacy of rhGAA type called Myzozyme® in the treatment of infantile onset. The study showed that the younger the patients were when the therapy started the better their conditions would be. Infants younger than 6 months showed the best results with all of the patients surviving past 18 months and 83% ventilator free. On the other hand the group of infants that was 6-36 months old when the treatment started did not fair as well. But both groups did show thinning of the cardiac ventricular index, changes in growth and motor development. This study also established that doses 20 mg/kg cause less adverse events than 40 mg/kg of the enzyme. (Koeberl)

There are problems with the treatment of the enzyme just like other synthetic protein therapies. The main problem that impeded the approval of Myzozyme® was the fact that when patients with no GAA activity were treat4ed with Myzozyme® they developed anti-rhGAA antibodies with fatal consequences.

Yet a recent study showed the possibility of elimination of the antibodies with strict immune-modulation therapy and had successful results. Although there are various other reactions to this drug this therapy greatly increases the chance of survival of many of the patients even at later stages. (Mendelsohn) This drug is now being prescribed to Pompe's disease patients of all ages with approval from the European commission and is in the final stages of FDA approval.

There has been promising research regarding the neural deficits in Pompe's patients that was documented just weeks ago. Researchers found that in comparison to a control group mice without the GAA gene have significant glycogen accumulation in the cervical spinal cord and other parts of the CNS. The study also included phrenic nerve monitoring which showed that mice without the GAA gene had deficient neural output. (DeRuisseau) This led to the conclusion that medications should be made to affect the nervous system as well as the musculatory system.

Conclusion

GSD 2 is an inherited autosomal recessive disorder that causes various mutations on the gene that codes for the enzyme acid-alpha glucosidase. The mutation will cause a decrease or stop in enzyme activity leading to a buildup of glycogen in the lysosomes of the cells. Due to the accumulation of the glycogen, severe complications such as cardiomegaly, hepatomegaly, hypotonia and others are seen. However, there is much hope for patients with this disease with the recent approval of the medication Myzozyme® as well as the continuing research into other antidotes that is currently underway.

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