HYPOVITAMINOSIS D IN GLYCOGEN STORAGE DISEASE: A RETROSPECTIVE CHART REVIEW

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ABSTRACT

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The purpose of this study was to determine the extent of vitamin D deficiency and insufficiency in a cohort of glycogen storage disease patients. In addition, the level of vitamin D supplementation required to increase serum 25-hydroxyvitamin D [25(OH)D] was assessed. Data was obtained via a retrospective chart review of GSD patients followed by the Department of Pediatrics Medical Genetics from the McGovern Medical School in Houston. GSD patients had significantly lower levels of serum 25(OH)D concentrations ($p>0.000$; CI 95%) compared to recommended levels. GSD patients were at increased risk of inadequate vitamin D status based on serum 25(OH)D levels with 48% deficient and 40% insufficient. Finally, findings indicated that vitamin D supplementation above the normal recommended level of 400 - 1000 IU/day was required to raise serum 25(OH)D concentrations to desirable levels.

Word Count: 133

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LIST OF ABBREVIATIONS

1,25(OH)D	One, twenty-five-hydroxyvitamin D; Calcitriol
25(OH)D	25-hydroxyvitaminD; Calcidiol
7-DHC	Seven-dehydrocholesterol
AGL	Glycogen debranching enzyme
CAMP	Cathelicidin antimicrobial peptides
CDC	Centers for Disease Control and Prevention
DBP	Vitamin D-binding protein
ER	Endoplasmic Reticulum
G-1-P	Glucose 1-phosphate
G-6-P	Glucose 6-phosphate
G6PT	Glucose 6-phosphate translocase
GCKR	Glucokinase regulatory protein
GLUT	Glucose transporter isoform
GSD	Glycogen Storage Disease
GYS	Glycogen synthase
IBD	Inflammatory bowel disease
PGM	Phosphoglucomutase isoenzyme
PHK	Glycogen phosphorylase kinase
PP1	Protein phosphatase-1
PTH	Parathyroid hormone
PYG	Glycogen phosphorylase

PYGB	Brain glycogen phosphorylase
PYGL	Liver glycogen phosphorylase
PYGM	Muscle glycogen phosphorylase
UGP	UDP-glucose pyrophosphorylase
VDR	Vitamin D receptor
Vitamin D3	Pre-vitamin D
WHO	World Health Organization

CHAPTER I

INTRODUCTION

GLYCOGEN

Glycogen is a branched-chain polymer of glucose used for energy storage in humans (Figure 1a and 1b).[1] Glycogen contains up to 55,000 glucose residues or moieties as well as small amounts of glucosamine and phosphate. The source and function of glucosamine and phosphate within glycogen is unknown. Glycogen is predominantly stored in the liver and skeletal muscle to maintain blood glucose levels during fasting or between meals, or to provide energy while exercising. Lactate produced during muscle contraction is transported to the liver where it is used to help replenish liver glycogen stores.[1] Glycogen makes up approximately 8% of liver mass and 1% of muscle mass.[2] However, the body's glycogen stores are largely contained within the muscle due to greater muscle mass.[2] Free glucose enters most human cells via facilitated transport utilizing a glucose transporter (GLUT-1, GLUT-2, GLUT-3, and GLUT-4).[1] GLUT1 is responsible for transporting glucose across the blood-brain barrier and caries most of the glucose used by the body in the post-absorptive state. In skeletal muscle, GLUT1 and GLUT4 are predominantly responsible for glucose uptake. GLUT1 is an integral membrane protein that brings glucose into the muscle fiber. GLUT4 normally resides inside the intracellular storage vesicles and is translocated to the plasma membrane after stimulation by muscle contraction or insulin. Thus, glucose uptake by skeletal muscle is increased during endurance training and insulin release by increasing

the level of GLUT4 on the plasma membrane.[1] GLUT2 transports glucose down the concentration gradient into hepatocytes and pancreatic beta-cells.[1]

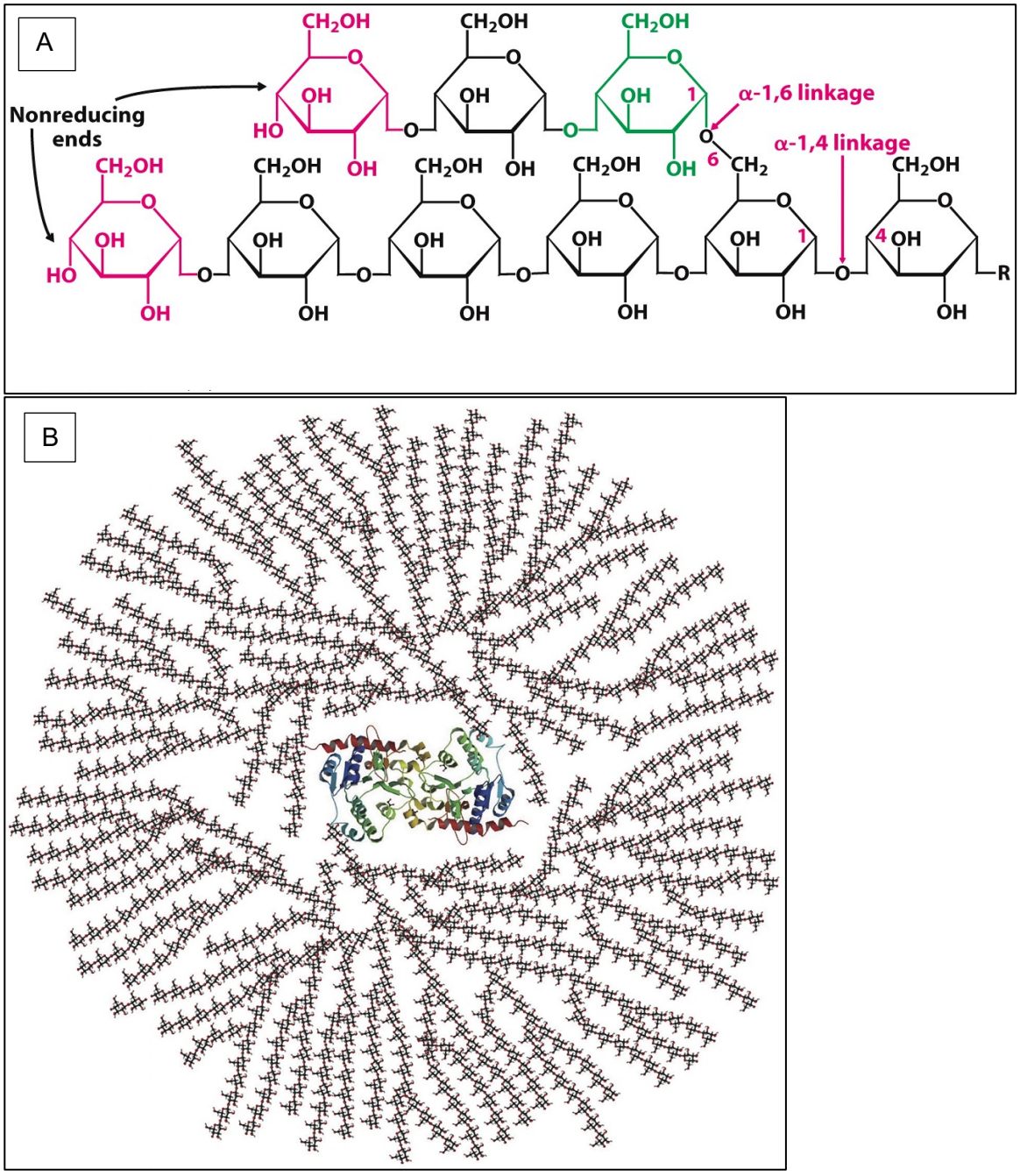

Figure 1. A, Structure of Glycogen Branch Point[3]. B, Full Glycogen Structure[4].

Structure

Glycogen moieties are linked via alpha-1,4 bonds; branch points are linked to the main chain via alpha-1,6 bonds.[1] The structure of alpha-1,6 bonds cause the chain to kink allowing the new strand to lengthen alongside the main chain. Branches are added at even intervals resulting in a spherical shape. Each glycogen structure contains a glycogenin protein at its core that is responsible for catalyzing the initial reaction.[1]

Metabolic Pathway

Formation. Once free glucose enters a cell it is phosphorylated via the action of a hexokinase isoenzyme, resulting in glucose 6-phosphate (G-6-P).[1] Hexokinases are numbered either I, II, III, or IV. Hexokinase II is expressed in skeletal muscle and hexokinase IV (glucokinase) is expressed in hepatocytes and pancreatic alpha- and beta-cells. Hexokinases I to III have a high affinity for glucose and are inhibited by G-6-P. Glucokinase has a lower affinity for glucose and is not inhibited by G-6-P. Glucokinase activity is regulated by the glucokinase regulatory protein (GCKR), which inhibits glucokinase during the fasting state. In the postprandial state, glucokinase is unrestrained in the cytosol leading to increased G-6-P and promoting glucose metabolism. Glycogen 6-phosphate is isomerized by phosphoglucomutase isoenzymes (PGM1, PGM2, PGM3, PGM4, and PGM5). PGM1, utilizing a divalent metal ion (predominantly Mg2+), catalyzes the reversible isomerization of phosphate between the 1- and 6-positions of the glucose molecule. Glucose 1-phosphate (G-1-P) is reversibly transformed into UDP-glucose via the action of UDP-glucose pyrophosphorylase (UGP). UDP-glucose is the immediate glucose donor for the initiation and elongation of glycogen. Glycogenin catalyzes the transfer of a glucose residue from UDP-glucose to

itself, forming alpha-1,4-glycosidic linkages creating a linear polymer of approximately 10 - 20 glucose moieties.[1] This chain forms the base for glycogen formation via the combined action of glycogen branching enzyme and glycogen synthase. Glycogen synthase catalyzes the elongation of the main glycogen chain by adding glucose residues from UDP-glucose, forming alpha-1,4 linkages and releasing UDP. Glycogen synthase has two isoforms, GYS1 and GYS2. GYS1 is present in the heart and skeletal muscle, while GYS2 is present in the liver.[1] A deficiency of glycogen synthase results in post-prandial hyperglycemia and hyperlactatemia, as well as ketotic hypoglycemia during fasting.[1,5] Glycogen synthase activity is stimulated by G-6-P and inhibited by increasing glycogen stores. Protein phosphatase-1 (PP1) catalyzes the dephosphorylation and subsequent activation of glycogen synthase. Protein phosphatase-1 is activated by insulin which is believed to be part of the mechanism by which this hormone activates liver and skeletal muscle glycogen synthesis. Glycogen branching enzyme catalyzes the transfer of a chain of 6 - 8 glucose residues via the formation of alpha-1,6 linkages making glycogen a branched chain polymer.[1] Glycogen branching enzyme deficiency causes glycogen to form with fewer branch points resembling the structure of amylopectin.[5] Glycogen metabolism is summarized in Figure 2.

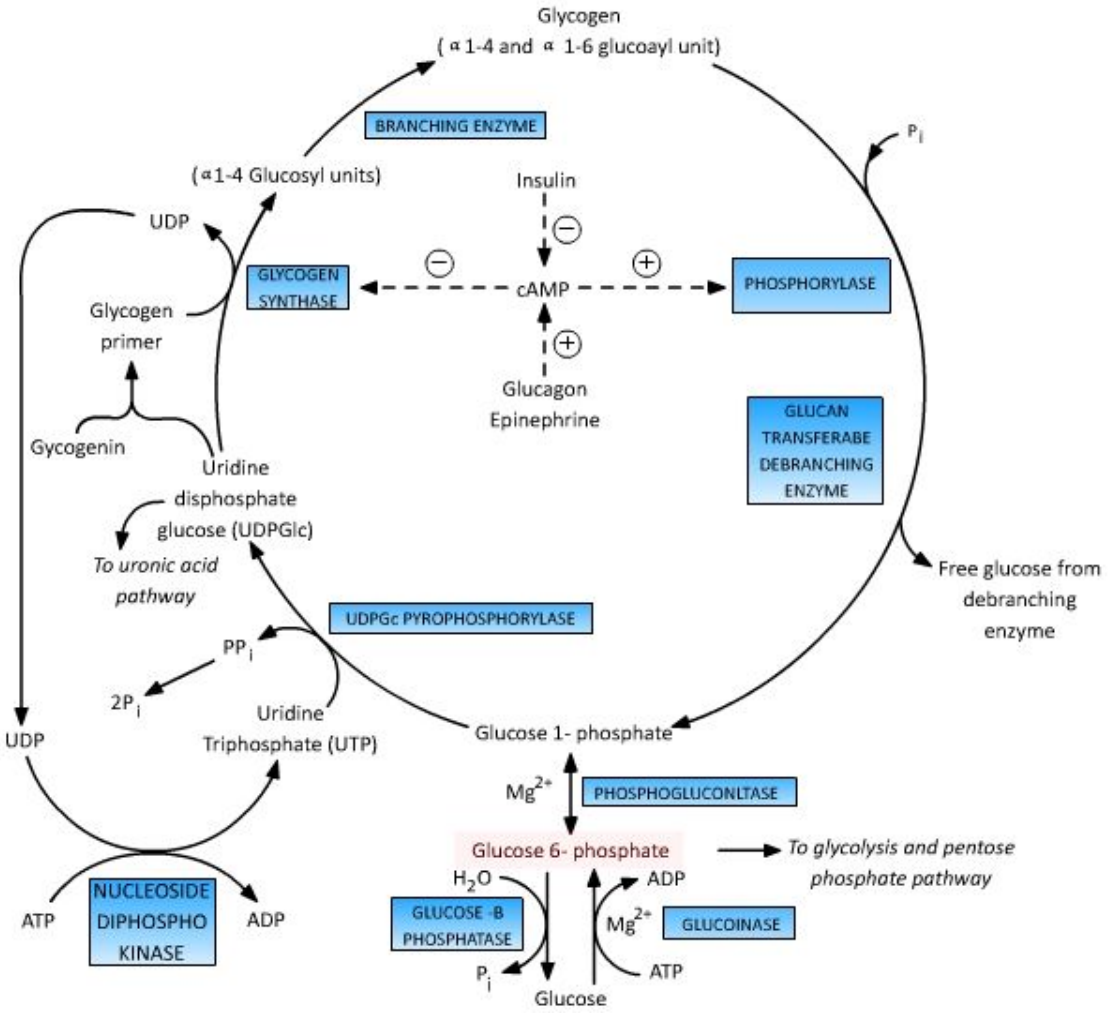

Figure 2. Glycogen Metabolism Pathway[6]

Breakdown. Glycogen can be broken down either inside the lysosomes or in the cytosol.[1] Inside lysosomes glycogen is broken down into glucose by the enzyme acid alpha-1,4-glucosidase, 1,4-alpha-glucan hydrolase, or acid maltase.[1] Any loss of function of these enzymes leads to glycogen accumulation in the lysosomes causing cell injury and loss of normal function.[5] Breakdown in the cytosol occurs via the action of glycogen phosphorylase (PYG) and glycogen debranching enzyme.[1] PYG releases G-1-

P from the linear glycogen chain by untying alpha-1,4 bonds. PYG action is blocked once it is four glucose residues from a branch point. There are three isoenzymes of glycogen phosphorylase; muscle (PYGM), liver (PYGL), and brain (PYGB).[1] Deficiencies of PYGM or PYGL result in an almost complete inability to convert glycogen into glucose in the muscle and liver.[5] PYGL deficiency causes glycogen to build up in the liver resulting in hepatomegaly.[5] The brain isoform is found in the brain, heart and liver.[1] In the muscle PYG is inhibited by G-6-P and UDP-glucose, and activated by AMP and increasing glycogen concentrations. The reversible phosphorylation and dephosphorylation either activates or deactivates PYG, respectively. Phosphorylation of PYG is catalyzed by glycogen phosphorylase kinase (PHK) and dephosphorylation is catalyzed by PP1. In the liver glucagon effectively activates PYG via an increase in cAMP.[1] Once PYG has released G-1-P from the linear chain, four glucose residues remain attached that must be released by glycogen debranching enzyme (AGL).

AGL functions in a two-step process by first removing the remaining four glucose residues and transferring them to a different strand. It then hydrolyzes the alpha-1,6 bond of the branch point allowing PYG access to the alpha-1,4 bonds.[1] Any deficiency of AGL leads to an accumulation of abnormal glycogen characterized by very short outer chains.[5] Once released, G-1-P undergoes isomerization to G-6-P by phosphoglucomutases.[1] G-6-P is then transported from the cytosol to the endoplasmic reticulum (ER) by glucose 6-phosphate translocase (G6PT). Once in the ER G-6-P is dephosphorylated by glucose 6-phosphatase isoenzymes to free glucose. G6PT is an integral membrane protein which exchanges G-6-P in the cytosol for inorganic

phosphate in the lumen of the ER.[1] If G-6-P is not dephosphorylated it enters the glycolysis pathway leading to increased lactate levels and potentially lactic acidosis.[5]

GLYCOGEN STORAGE DISEASE

Glycogen Storage Disease (GSD) is a group of disorders caused by inborn errors of metabolism that affect the structure, use, and/or function of stored glycogen.[5] GSD is broken down into 13 subtypes, each one characterized by their own unique pathophysiology, clinical and biochemical features, and treatment and prognosis.[5] Except type 0 all GSD forms are characterized by an accumulation of glycogen in various tissues leading to both muscular and hepatic symptoms. The different causes of altered glycogen metabolism are related to deficiencies of various enzymes depending on the GSD type. General signs are a combination of both muscular and hepatic symptoms, hypoglycemia, increased serum lactate and urate, as well as elevated creatine kinase and muscle weakness.[5] Inappropriate storage of glycogen in the liver leads to hepatomegaly in multiple types of GSD including types I, III, VI, and IX. GSD types I, III, and VI also present with mild to severe hyperlipidemia.[5] Many GSD types are treated by prescribing patients regular frequent feedings of uncooked cornstarch, which provides a slow release of free glucose in order to maintain normal blood glucose levels.[5] GSD types I, III, and IX have an incidence rate of 1 in 100,000.[7-9] The types, signs, symptoms, complications, and methods of treatment are summarized in Table 1.

Table 1. Glycogen Storage Disease: Subtypes, Signs, Symptoms, and Treatment[5]

Type	Enzyme Deficiency	Signs	Symptoms	Complications	Treatment
Ia and Ib	G-6-P Phosphatase	• Hepatomegaly • Hyperlipidemia • Liver and kidney glycogen accumulation • Elevated serum lactate	• Hypoglycemic seizures	• Liver adenomas • Hepatocellular carcinoma • Insulin resistance • Progressive kidney disease	• Frequent meals of uncooked starch • Nasogastric tube feedings • Avoidance of fructose and galactose containing foods
Ib Only	G-6-P Transferase	• Myeloid Dysfunction • Chronic Neutropenia • Neutrophil and monocyte dysfunction • Bacterial infections • Inflammatory Bowel Disease			
III	Glycogen Debranching enzyme	• Hepatomegaly • Hypoglycemia • Hyperlipidemia • Stunted growth • Myopathy		• Ketotic hypoglycemia • Hepatocellular carcinoma • Liver cirrhosis • Abnormal cardiac rhythms	• Frequent carbohydrate-rich feedings • High protein diets

Table 1. Treatment, cont.[5]

Type	Enzyme Deficiency	Signs	Symptoms	Complications	Treatment
V	Skeletal Muscle Phosphorylase	• Myoglobinuria	• Muscle weakness, aches, cramps • Second-Wind phenomenon • Persistent weakness with age	• Rhabdomyolysis • Renal failure	• Excessive weight gain • Gentle exercise
IX	Phosphorylase b Kinase	• Hepatomegaly • Growth retardation • Minor motor developmental delay • Dyslipidemia			• Frequent feedings • Carbohydrate-rich diet
0	Liver Glycogen Synthase	• Ketotic hypoglycemia • Postprandial hypoglycemia • Increased lactate			• Protein-rich meals • Frequent feeds of uncooked starch

GSD Type I

Although there are two separate subtypes of GSD I the same therapy is utilized as both are characterized by an inability to dephosphorylate G-6-P into glucose leading to a build-up of glycogen within the liver and kidneys.[5,10] GSD I is the most common and severe childhood form with most patients usually presenting in early infancy.[5] The liver is unable to generate free glucose in response to hypoglycemia and as G-6-P accumulates in the cytosol it enters glycolysis leading to a build-up of lactate in the cell and possible lactic acidosis.[5,11] There is also increased uric acid due to decreased clearance and increased production.[5,11] GSD Ia is identified by a deficiency of G-6-P phosphatase which dephosphorylates G-6-P once it has been transferred into the ER.[5,10] GSD Ib is characterized by a deficiency of G-6-P transferase which transports G-6-P from the cytosol into the ER.[5,10] Mutations on chromosomes 17 and 11 are responsible for GSD Ia and Ib respectively.[1,11] Aside from the different enzyme deficiencies GSD Ib distinguishing features include myeloid dysfunction, chronic neutropenia, and functional deficiencies of neutrophils and monocytes.[10] Also, GSD Ib patients often develop inflammatory bowel disease (IBD) and recurring bacterial infections.[10] GSD I patients present in early infancy with hepatomegaly, and hypoglycemic seizures accompanied by hyperlipidemia due to increased synthesis of triglycerides.[5,11] Complications are associated with poor metabolic control and include liver adenomas, hepatocellular carcinoma, insulin resistance, and progressive kidney disease.[5] Minarich et al. found that GSD I was associated with low bone mineral density likely related to low vitamin D and associated disease complications.[10] Early diagnosis and management is the best way to mitigate symptoms.[5] Diagnosis is made using clinical presentation, abnormal lactate and

lipid values, and genetic analysis. Treatment for both GSD I types is mainly focused on maintaining glucose homeostasis. This is achieved via multiple methods including regular consumption of uncooked cornstarch, and nasogastric tube feedings. GSD I patients should also avoid foods, such as dairy, that contain fructose and galactose as these cannot be converted to free glucose.[5]

GSD Type III

GSD III is characterized by a deficiency of glycogen debranching enzyme leading to an accumulation of glycogen with very short outer chains in the liver, skeletal muscle, and heart.[1,5] The gene coding for glycogen debranching enzyme has been mapped to chromosome 1.[1] GSD III symptoms are similar to GSD I in that patients present with hepatomegaly, hypoglycemia, hyperlipidemia, and stunted growth.[5] Approximately 75% of GSD III patients present with myopathy and hepatopathy with only 15% presenting with hepatopathy only.[1,5] Inadequate management can lead to ketotic hypoglycemia, hepatocellular carcinoma, liver cirrhosis, and abnormal cardiac rhythms with increasing age.[1,5] Melis et al*,* found reduced bone mineral density in GSD III patients as well as decreased levels of IGF-1, insulin, calcitonin, and osteocalcin.[12] There have also been reports of ovarian polycystosis, diabetes mellitus, and osteopenia/osteoporosis.[12] GSD III may be diagnosed by observing elevated liver transaminases, splenomegaly without enlarged kidneys, or abnormal glycogen and debranching enzyme deficiency.[5] Treatment of GSD III is similar to GSD I in that hypoglycemia is avoided using frequent feedings of carbohydrate-rich foods.[5] However, GSD III patients do not need to avoid fructose, galactose, or dairy, and may benefit from high protein diets.[5]

Type V

GSD V is caused by a deficiency of skeletal muscle phosphorylase (PYGM) leading to a complete inability to convert glycogen to glucose in the muscle.[1,5] The gene for PYGM is encoded on chromosome 11.[5] GSD V patients cannot mobilize muscle glycogen stores during anaerobic activity leading to muscle weakness, aches and pains.[5] In children GSD V presents as muscle pain and weakness along with dark colored urine from myoglobinuria.[5] These symptoms are usually not discovered until patients are older. Many patients experience the second-wind phenomenon where they experience pain after starting an activity but are able to finish the activity after a short rest. GSD V is a progressive disorder of altered muscle metabolism leading to muscle cramps, myoglobinuria, rhabdomyolysis, and renal failure if not adequetly managed.[5] Older adult patients experience persistent weakness and increasing disability. GSD V is diagnosed via muscle histochemistry from a biopsy showing PYGM deficiency or molecular analysis. There is no specific treatment for GSD V although patients often benefit from excessive weight gain, which lowers their metabolic threshold, along with gentle exercise. Outcome is usually mild with only rare cases leading to rhabdomyolysis and possibly acute multi organ failure.[5]

GSD Type IX

GSD IX is characterized by a deficiency of phosphorylase b kinase.[5] Phosphorylase b kinase is composed of four subunits which are encoded by different genes on different chromosomes and are differentially expressed in different tissues. GSD IX can be either X-linked or autosomal recessive with the X-linked type accounting for 75% of patients. X-linked GSD IX patients experience phosphorylase kinase

deficiency in the liver, erythrocytes, leukocytes, and fibroblasts.[5] Autosomal recessive GSD IX patients can have either liver, liver and muscle or muscle only forms. This type typically has more severe clinical symptoms including progressive muscle disease. GSD IX patients typically present at 1-5 years of age with a swollen abdomen from hepatomegaly, growth retardation, minor motor development delay, dyslipidemia, and a slight elevation of liver transaminases. These issues tend to disappear with age until patients are largely asymptomatic except for a clinical phosphorylase kinase deficiency. Very rarely do some patients experience mild hypoglycemia. Diagnosis of GSD IX is challenging as the enzyme deficiency is not always detectable, and a liver, muscle, or heart biopsy is often necessary to determine type. Treatment includes a high-carbohydrate diet, and frequent feedings to prevent hypoglycemia with most patients not needing specific treatment beyond that.[5]

GSD Type 0

GSD 0 is characterized by a deficiency of liver glycogen synthase with a muscle variant possible.[1,5] The gene for hepatic glycogen synthase is coded for on chromosome 12, and the muscle version on chromosome 19.[1] Patients present in early infancy with early morning fatigue, and occasionally convulsions related to ketotic hypoglycemia.[5] Although patients may develop a short stature they do not present with hepatomegaly or hyperlipidemia. Postprandial hyperglycemia and increased lactate are common.[5] Some patients present with muscle cramping suggesting a muscle variant of GSD 0.[1] Treatment consists of high protein meals and frequent uncooked cornstarch feeds.[5]

VITAMIN D

Vitamin D is a fat-soluble secosteroid hormone which has multiple, simultaneous beneficial effects such as cellular proliferation, differentiation, and immunomodulation.[13] The five vitamin D metabolites are 7-dehydrocholesterol, cholecalciferol (previtamin D3), ergocalciferol (previtamin D2), calcidiol [25(OH)D], and calcitriol [1,25(OH)D] (Figure 3).[14] Seven-dehydrocholesterol (7-DHC) is otherwise known as provitamin D3 and is used as a lipid in cell membranes.[14] The current recommended intake levels for vitamin D are 10 μg/d (400 IU/d) for children 0-1 year old and at least 15 μg/d (600 IU/d) for children 1 year and older.[15] Whether from exogenous sources or endogenous formation vitamin D is either stored in adipocytes or processed and activated via the kidneys and liver.[5] Risk factors for suboptimal vitamin D levels include obesity, fat malabsorption, and nephrotic syndrome.[16]

Vitamin D status is categorized based on serum 25-hydroxyvitamin D [25(OH)D] concentrations: sufficient (>30 ng/mL), insufficient (21 - 29 ng/mL), and deficient (≤20 ng/mL).[17] The most accepted function of vitamin D is its relationship to bone health via increasing calcium and phosphorus absorption in the intestines.[16] If vitamin D intake is insufficient calcium is not readily absorbed in the intestine leading to increased bone resorption in order to maintain blood calcium levels. Without an increase in vitamin D and calcium intake, bone breakdown continues, potentially leading to osteoporosis.[16]

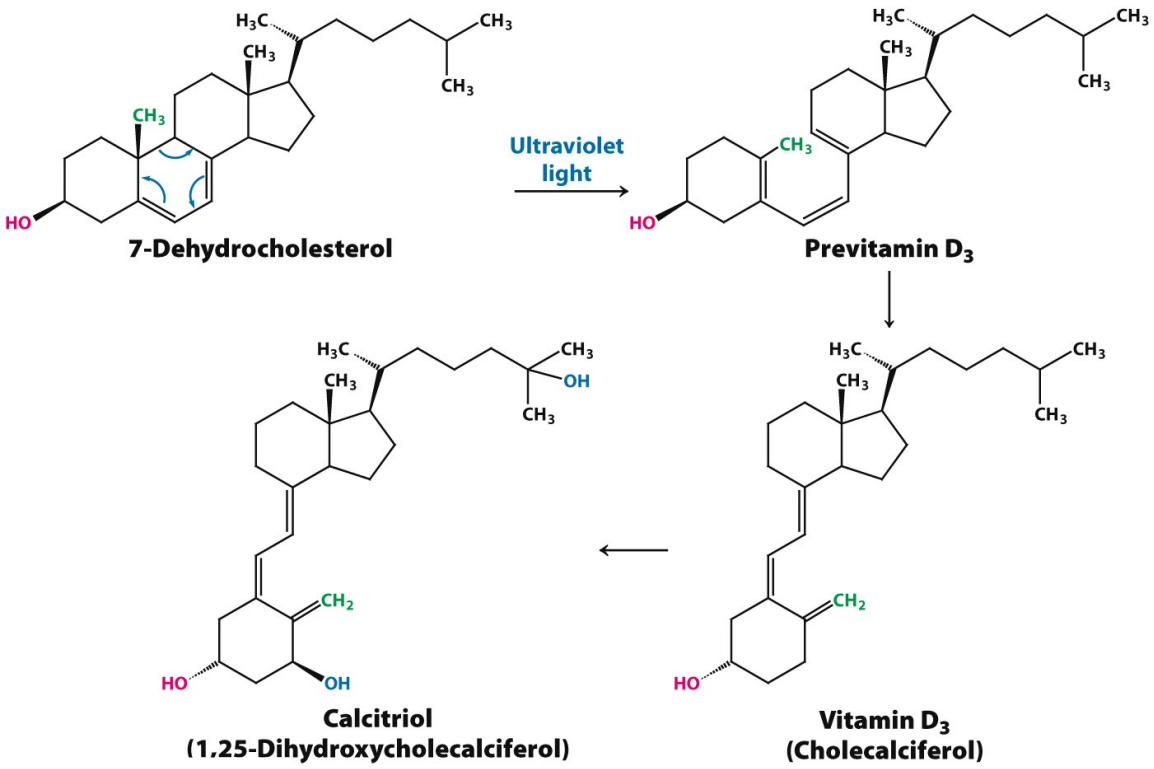

Figure 3. Vitamin D Types and Structures[18]

Sources of Vitamin D

Endogenous formation. The majority of vitamin D is obtained via *de novo* synthesis through the conversion of 7-dehydrocholesterol to pre-vitamin D (vitamin D3) after the skin is exposed to sunlight.[13] Once formed vitamin D3 is either stored in adipocytes or is transferred to the liver for hydroxylation.[16] It is transferred to the liver via vitamin D-binding protein (DBP).[13] DBP is a protein synthesized by the liver which carries 80% of the body's 25(OH)D.[13] Hydroxylation occurs in the liver to produce 25(OH)D.[16] The formation of 25(OH)D is unregulated and has a half-life of 2-3 weeks, whereas 1,25(OH)D has a half-life of 4-6 hours; therefore circulating 25(OH)D is thought to best reflect vitamin D status in the body.[16] After hepatic hydroxylation 25(OH)D is carried to the kidneys where it is further hydroxylated to 1,25(OH)D, or calcitriol, which is the biologically active form of vitamin D.[13] Renal hydroxylation is accomplished by renal 1-alpha hydroxylase which is regulated by parathyroid hormone (PTH).[16]

In the presence of an infection certain immune cells, notably macrophages, acquire the ability to convert 25(OH)D into calcitriol.[19] It has also been recently discovered that almost all tissues are capable of converting cholecalciferol to 25(OH)D.[16]

Exogenous sources. Per the United States Department of Agriculture nutrient database the best dietary sources of vitamin D include cod liver oil, fatty fish, fortified dairy and non-dairy drinks and foods, fortified cereals, UV-treated mushrooms, and eggs.[20] Once ingested vitamin D leaves the lumen of the intestine and enters the cytosol via passive diffusion.[21-23] In the cytosol vitamin D is packaged into chylomicrons which enter the lymph in order to be transported to the liver.[21-23] In the liver, vitamin D is

hydroxylated into 25(OH)D in the same manner as endogenously formed vitamin D.[16] Vitamin D metabolism is summarized in Figure 4.

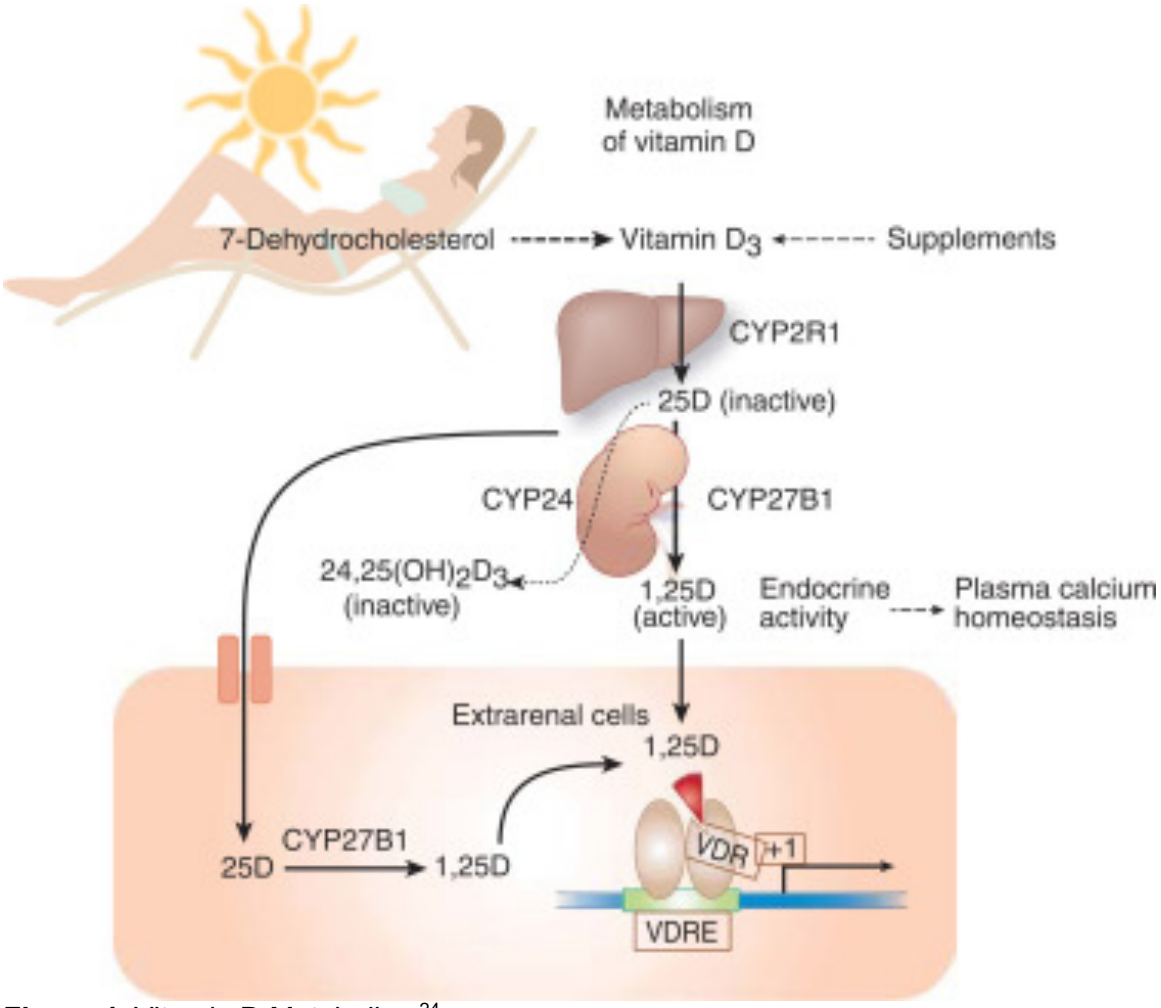

Figure 4. Vitamin D Metabolism[24]

Physiological Functions

Bone Health. Vitamin D has a principle function of maintaining calcium homeostasis by increasing calcium absorption in the intestines.[14] Acting as a hormone, vitamin D stimulates transport of calcium and phosphorus from the lumen of the

intestines into the cytosol.[25] Optimal serum levels of calcium and phosphorus lead to bone health homeostasis via its interaction with PTH.[16,26] PTH acts to increase serum levels of calcium and phosphorus by increasing the activity of osteoclasts.[16] This leads to increased bone demineralization as bones serve as the main pool of calcium and phosphorous in the body.[16] Vitamin D acts as a strong inhibitor of the transcription of the PTH gene leading to PTH suppression when vitamin D levels are optimal.[26] When vitamin D levels are suboptimal PTH is increased.[16,26] When serum vitamin D is closer to optimal, PTH is suppressed regardless of calcium intake and when vitamin D is suboptimal PTH is elevated regardless of calcium intake.[27] If serum vitamin D is not corrected bone turnover may eventually lead to osteoporosis, as well as bone and muscle pain.[16,28]

Immune System. The immune modulating effects of vitamin D are associated with cathelicidins, one of the most important classes of antimicrobial peptides in the body.[16,19] Cathelicidins are necessary for intracellular lysosomal degradation of microbes, and are found in monocytes and epithelial cells of the respiratory tract, skin and gut.[16] Calcitriol (1,25(OH)D) acts as a primary inducer of certain genes that code for antimicrobial peptides in epithelial cells, macrophages, monocytes, and neutrophils.[19] Production of cathelicidin antimicrobial peptides (CAMP) is increased in these cells and is one of the first elements of the innate immune response.[19] Cathelicidins act to disrupt the membrane activity of target cells as they are attracted to the capsular polysaccharides of bacterial membranes. CAMPs attract neutrophils, monocytes, and T cells to sites of infection, and have signal transduction properties including stimulation of

chemokines and cytokines. Thus, optimal vitamin D levels are protective against a variety of bacterial and viral pathogens, as well as cancers of the gut.[19]

General Health. Sufficient vitamin D levels are associated with decreased risk of hypertension, stroke, and several types of cancer as well as improved vascular health and glucose metabolism.[16] Deficiency is associated with diabetes, hypertension, hyperlipidemia, peripheral vascular disease, coronary artery disease, myocardial infarction, heart failure, and stroke.[29] It has been reported that vitamin D also has a number of effects on the human brain.[30] Calcitriol has been shown to have nuclear receptors in neurons and glial cells, and increase the biosynthesis of neurotrophic factors and at least one enzyme involved in neurotransmitter synthesis. Synthesis of nitric oxide is decreased and glutathione levels are increased by 1,25(OH)D.[30] Nitric oxide and glutathione are both involved in brain detoxification pathways. There is also evidence that the hormone may have a role in modulating neurodegenerative and neuroimmune diseases, and management of brain tumors by inducing glioma cell death.[30]

Vitamin D as Gene Inducer. Vitamin D acts as ligand for a nuclear receptor called the vitamin D receptor (VDR) which is expressed in most tissues throughout the body.[16] VDR is activated by 1,25(OH)D at which point it interacts with the vitamin D responsive element of human DNA. The responsive element is part of the promoter region of approximately 200 - 300 genes. This results in transcriptional activity which leads to the generation of specific response proteins being either up- or downregulated.[16]

GSD AND VITAMIN D

To date, there has only been one study examining the relationship between glycogen storage disease and 25(OH)D concentrations.[31] Banugaria and colleagues found that 16 of 26 GSD patients had suboptimal levels of vitamin D, even though 24 of those patients said that they were taking a vitamin D and/or vitamin D plus calcium supplement that met or exceeded the Adequate Intake of 200 IU/day. This lack of research suggested the need for further study regarding the relationship between GSD and hypovitaminosis D.[31]

PURPOSE OF THE STUDY

This study was undertaken to determine the prevalence of vitamin D deficiency and the level of vitamin D supplementation required to reach a sufficient serum 25(OH)D concentration in a cohort of GSD patients followed by the Department of Pediatrics Medical Genetics from the UT Health Science Center McGovern Medical School in Houston.

The study null hypotheses were:

- GSD patients do not have a higher incidence of vitamin D insufficiency and deficiency based on serum 25(OH)D concentrations than healthy published values from the literature.
- Recommended Dietary Allowances of vitamin D are sufficient to maintain serum 25(OH)D levels of $\geq$ 30 ng/mL in GSD patients.
- Serum 25(OH)D concentrations of a subset of patients receiving 50,000 IU of vitamin D per week for 6 weeks followed by 1,000 IU of vitamin D for 3 months

will be no different than serum 25(OH)D concentrations of non-deficient patients.

- GSD patients do not have abnormal concentrations of serum parathyroid hormone, calcium, and 1,25(OH)D.

CHAPTER II

METHODS

PARTICIPANTS

The study participants were GSD patients followed by the Department of Pediatrics Medical Genetics from the UT Health Science Center McGovern Medical School. The population consisted of patients with GSD types Ia, Ib, III, V, IX, and 0 who were under the care of David F. Rodriguez, MD and Heather Saavedra, RD, LD. The Institutional Review Board of Texas Woman's University approved the study as well as the Committee for the Protection of Human Subjects for The University of Texas Health Science Center Houston.

Chart Review

The study was a retrospective chart review conducted from January 2018 to October 2018. From patient medical records demographic and biomedical information was obtained including sex, date of birth, date of clinic visits, height for adults or length for infants, and body weight. In addition, serum concentrations of 25(OH)D, 1,25(OH)D, PTH, and calcium were obtained as well as the prescribed vitamin D supplementation if any. Although there was insufficient information in the medical and dietary records to analyze total nutrient intake, carbohydrate intake from corn, soy, or Glycosade® starch was estimated. The type of starch consumed depended on the individual tolerance of each patient. Calorie intake from starch was compared to each patient's estimated daily needs based on activity level and age. Data was collected into Excel spreadsheets and patients were assigned identification numbers to maintain patient confidentiality.

Anthropometrics

Weight-for-length, BMI percentile, and BMI were analyzed using World Health Organization (WHO), and Centers for Disease Control and Prevention (CDC) growth charts and formulas.[33-34] Per the WHO, children younger than two years old were analyzed using weight-for-length percentiles with classifications of <2% small, 2 – 98% normal, and >98% large.[33] The CDC weight-for-height percentiles were used for children 2 – 20 years old with classifications of <5% underweight, 5 – 85% normal, 85 – 95% overweight, and >95% obese.[33] Adult Body Mass Index (BMI) ranges were classified as <18.5 underweight, 18.5 – 24.9 normal, 25 – 29.9 overweight, and >30 obese.[34]

Biomedical Indices

Concentrations of serum 25(OHO)D and serum 1,25(OH)D were determined by Mass Spectrometry, and PTH levels were measured using the Electrochemiluminescence Immunoassay Analyzer. Serum calcium was measured by colorimetric assay. Although optimal levels of serum 25(OH)D have been debated, vitamin D deficiency was defined as serum 25(OH)D concentrations less than 20 ng/mL (50 nmol/L) and vitamin D insufficiency as 21 – 29 ng/mL (50 – 80 nmol/L).[17] Mean normal serum 25(OH)D for different age groups are 29.1 for 6 – 10 years, 25.8 for 10 – 19 years, 25.4 for 20 – 39 years, 25.7 for 40 – 59 years, and 26.2 for ≥60 years.[35] The normal reference range for 1,25(OH)D is 15 – 60 pg/mL.[17] PTH is interpreted in the context of calcium and vitamin D serum concentrations and has a range of 18.4 – 80.1 pg/mL.[16,26] Normal serum calcium is defined as 8.5 – 10.5 mg/dL.[36] All baseline data was used for comparing serum 25(OH)D to normal healthy levels regardless of whether or not there was corresponding follow up data.

Vitamin D Supplementation

Vitamin D supplementation prescriptions were based on initial serum 25(OH)D at baseline (Table 2).

Table 2. Vitamin D Supplementation Prescription by Baseline Serum 25(OH)D Levels

Baseline 25(OH)D ng/mL	Vitamin D supplementation level
<22	50,000 IU/week for 6 wks or longer (n = 15)
23 – 29	400 IU/day (n = 2) 800 IU/day (n = 1) 1,000 IU/day (n = 1) 2,000 IU/day (n = 2)
>30	None (n = 4)
Total	25

Patients with an initial baseline serum 25(OH)D <22 ng/mL were prescribed 50,000 IU of vitamin D per week for six weeks followed by 1,000 IU/day for three months. Serum 25(OH)D was remeasured 6 – 12 months after the initial 50,0000 IU prescription. Patients with an initial baseline serum 25(OH)D concentration between 23 – 29 ng/mL were given either 400 IU/day, 800 IU/day, 1,000 IU/day, or 2,000 IU/day. Their serum 25(OH)D was remeasured within 12 – 24 months. Patients with a baseline serum 25(OH)D greater than 30 ng/mL were not prescribed vitamin D supplements.

Statistics

Descriptive statistics were performed in order to describe means and standard deviations for demographic data, serum 25(OH)D and 1,25(OH)D concentrations, serum PTH, and serum calcium. A Wilcoxon Rank Sum test was conducted to examine changes in serum 25(OH)D concentrations before and after vitamin D supplementation.

All data was analyzed in SPSS c23, with a CI of 95% and a *p*-value of <0.05 considered significant.

CHAPTER III

RESULTS

RESULTS

The initial chart review identified a cohort of 27 GSD patients comprised of infants and adults age up to 63 years (Figure 5). Two patients were excluded as there were no others with the same type of GSD. Of the initial remaining 25 patients, only 14 had intervention data available for analysis.

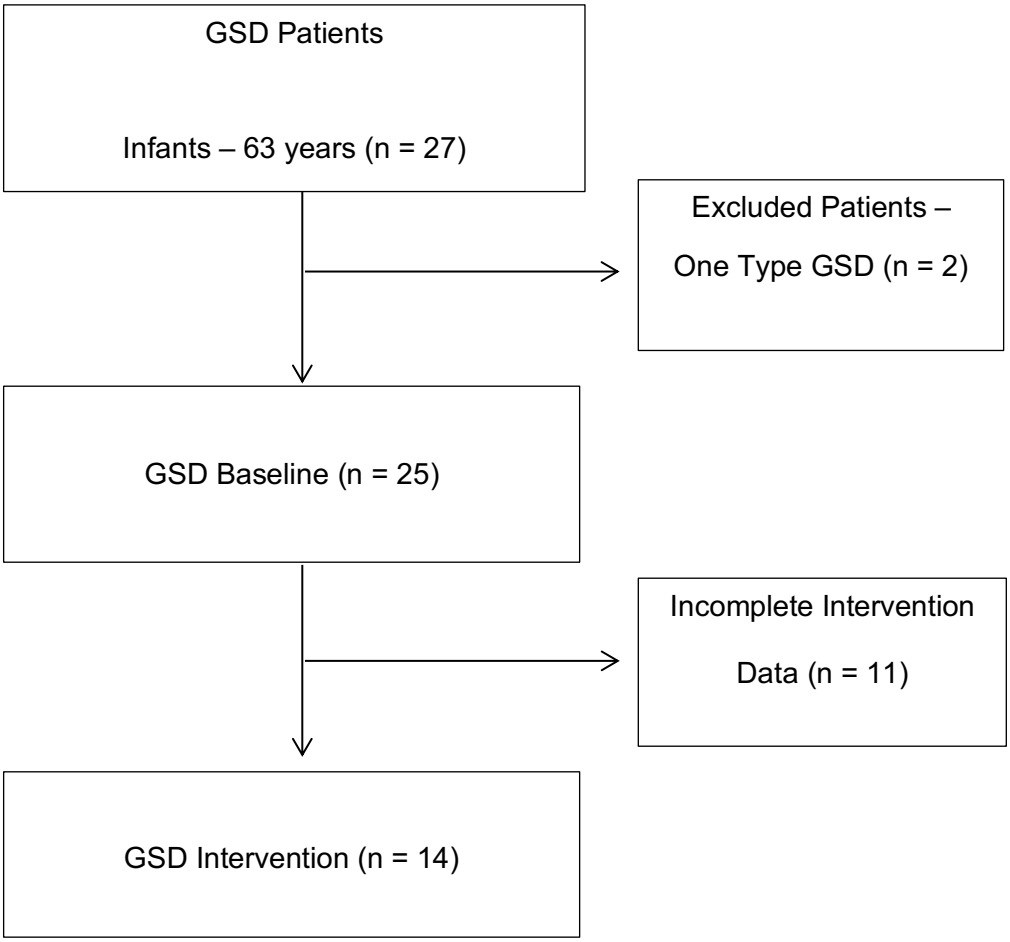

Figure 5. Subject Flow Diagram.

Demographics and GSD Type

Demographic characteristics and GSD type diagnoses of the patient population are presented in Table 3. The patient population was primarily 19 years old or younger, evenly distributed by sex, and 66% were Hispanic. Children less than two years of age were large (75% (n = 16)) based on growth weight-for-length percentiles, 62.8% (n = 4) of children ages 2 – 19 years were overweight or obese based on BMI percentiles, and 62.8% (n = 5) of the adult patients 20 years and older were overweight or obese based on BMI.

Table 3. Participant Characteristics and Demographics.

	% of Participants
Age at baseline	
0 - 2 yrs	20
2 - 19 yrs	60
20+ yrs	20
Sex	
M	44
F	56
Growth percentile 0 – 2yrs n = 16	
<2	0
2 – 98	25
>98	75
BMI % 2-19 yrs n = 4	
<5	0
5 – 85	37.8
85 - 95	18.9
>95	43.9
BMI 20+ yrs n = 5	
<18.5	0
18.5 – 24.9	20
25 – 29.9	60
>30	20
Ethnicity	
Hispanic	72
Caucasian	28
GSD Type	
I	8
Ia	56
Ib	12
III	16
IX	8

Vitamin D Supplementation

Approximately 57% of GSD patients with supplement intervention follow up data were prescribed 50,000 IU of vitamin D per week for six weeks (n = 8). Substantially fewer patients (21%) with an initial baseline serum 25(OH)D concentration between 22 - 29 ng/mL were given either 400 IU/day (n = 2), or 2,000 IU/day (n = 2). There was no follow up data for the patients prescribed 800 IU/day and 1,000 IU/day. GSD patients with a baseline serum 25(OH)D greater than 30 ng/mL (n = 3) were not prescribed vitamin D supplements.

Biomedical Indices

Participants did not have abnormal serum calcium or PTH concentrations at either baseline or post-intervention. At baseline, 40% (n = 10) of patients were vitamin D insufficient and 48% (n = 12) were vitamin D deficient. Of the patients with follow up data (n = 14), 28.57% (n = 4) were vitamin D insufficient and 7.1% (n = 1) were vitamin D deficient after supplementation. This change represents a statistically significant increase in 25(OH)D from baseline to follow up (*p*-value = 0.001) (Figure 6). Serum 25(OH)D concentrations were compared to published normal healthy values that were based on information from the National Health and Nutrition Examination Survey from 2001 – 2010 (Table 4).[35] Published normal healthy values of serum 25(OH)D concentrations in children 0 – 2 years old was not available.

Table 4. Comparison of GSD Patient Serum 25(OH)D to Normal Healthy Values by Age Group[35]

Age Range	% Deficient <20 ng/mL	% All GSD Deficient	% Insufficient 20 – 30 ng/mL	% All GSD Insuff	% Sufficient >30 ng/mL	% All GSD Suff
0 – 2[a]		25%		50%		25%
2 – 10[b]	10%	40%	49%	40%	41%	20%
10 – 20	25%	50%	47%	50%	28%	0%
20 – 40	31%	80%	41%	20%	29%	0%

a. No reference value available
b. Compared to 6 – 10 year age range

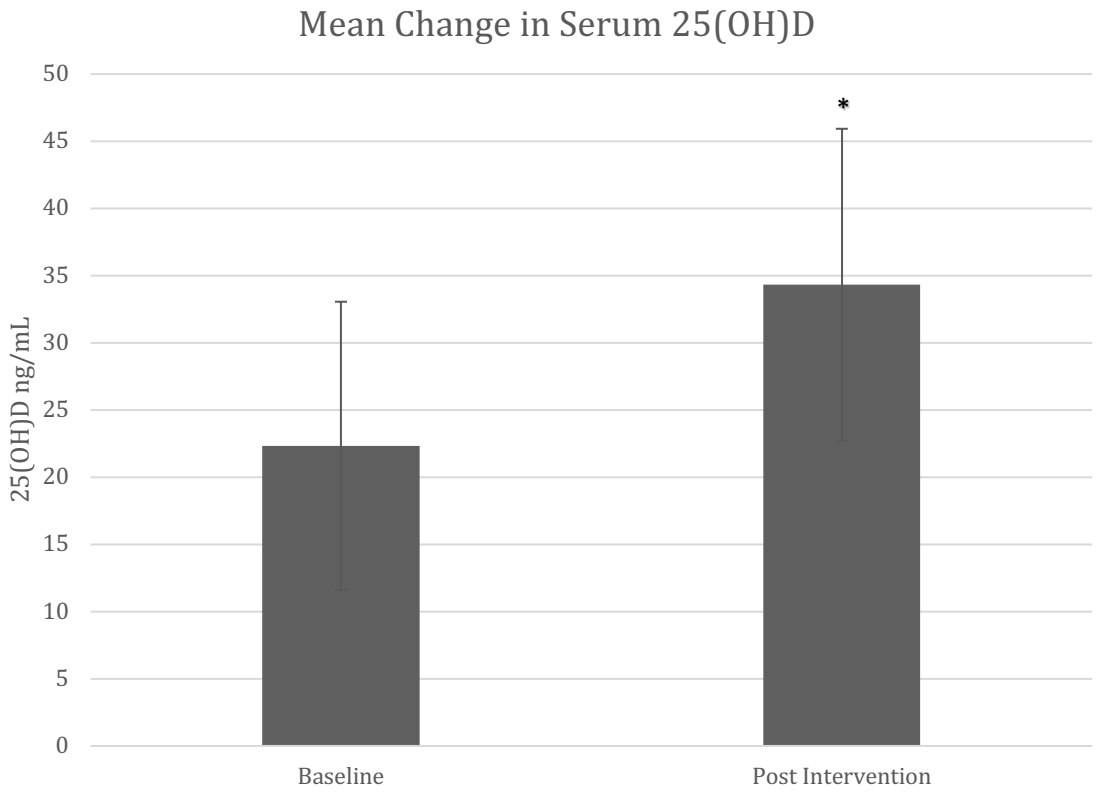

Figure 6. Change in Serum 25(OH)D from Baseline to Post Intervention
*There was a significant difference between baseline and post intervention subjects (*p*-value <0.001).

Serum concentrations of 1,25(OH)D, PTH, and calcium did not change significantly (Table 5).

For most patients a large portion of their daily energy intake was obtained from corn, soy or Glycosade® starch all of which are slow acting sources of free glucose. The type of starch consumed depended on the tolerance of each patient. Depending on type, GSD patients consumed 4.3% - 96% of their daily calorie needs in the form of starch. On average they consumed 51% of their daily calorie needs in the form of starch.

Table 5. Changes in Biochemical Parameters Following Vitamin D Supplementation[a,b]

Parameter	Range	Baseline n = 25	Post Intervention n = 14	*p*-value
25(OH)D		20.52 ± 6.94	34.33 ± 11.60	0.001
	Deficient <19 ng/mL	n = 12	n = 1	
	Insufficient 20-29 ng/mL	n = 10	n = 4	
	Sufficient >30 ng/mL	n = 3	n = 9	
1,25(OH)D	15 - 90 pg/mL	77.28 ± 33.24	82.94 ± 44.79	0.917
PTH, intact	18.4 - 80.1 pg/mL	27.36 ± 20.69	23.92 ± 12.19	0.715
Calcium, serum	8.5 - 9.5 mg/dL	9.76 ± 0.60	9.80 ± 0.55	0.420

a. Values expressed as mean ± SD; *p*-value <0.05.
b. 2. Intervention summary in Table 2.

CHAPTER IV

DISCUSSION

DISCUSSION

Patients with GSD were found to be at increased risk for inadequate vitamin D status. At baseline, 88% (n = 22) of the patients in this study were either vitamin D insufficient or deficient. Therefore, the hypothesis that GSD patients do not have a higher incidence of vitamin D insufficiency and deficiency based on serum 25(OH)D concentrations than healthy published values from the literature is rejected (Table 5). Despite the high dose vitamin D supplementation intervention, 35.7% (n = 5) of the GSD patients remained vitamin D insufficient or deficient at follow up. Therefore, the hypothesis that the Recommended Dietary Allowances of vitamin D are sufficient to maintain serum 25(OH)D levels of $\geq$ 30 ng/mL in GSD patients is rejected. This retrospective chart review found that serum 25(OH)D concentrations increased significantly from baseline following vitamin D supplementation intervention. However, there was insufficient data to compare the subset of patients receiving 50,000 IU/week for six weeks to non-deficient patients. Thus, the hypothesis that serum 25(OH)D concentrations of a subset of patients receiving 50,000 IU of vitamin D per week for 6 weeks followed by 1,000 IU per day of vitamin D for 3 months will be no different than serum 25(OH)D concentrations of non-deficient GSD patients is neither accepted nor rejected.

Finally, study results indicated that serum levels of PTH, calcium and 1,25(OH)D were normal and therefore the hypothesis that GSD patients do not have abnormal concentrations of serum parathyroid hormone, calcium, and 1,25(OH)D is accepted. Serum parathyroid levels were not consistently assessed alongside serum 25(OH)D. It is not expected that serum calcium would be altered due to the action of PTH on the bones. PTH, via increasing bone resorption, ensures that serum calcium levels remain steady in order to provide adequate levels for muscle and heart function. As previously mentioned, vitamin D has strong inhibitory effects on the expression and production of PTH.[26] Normal serum calcium ranges from 8.5 – 10.5 mg/dL, and normal PTH ranges from 18.4 - 80.1 pg/mL. PTH is interpreted in relation to serum calcium. When calcium is normal or high PTH is expected to be in the lower range, and when calcium is low PTH is expected to in the higher range. Clinical hyperparathyroidism is generally defined as a PTH level above 80.1 pg/mL when calcium is within the normal range of 8.5 – 10.5 mg/dL. Therefore, it is expected GSD patients would have normal ranges of both calcium and PTH. PTH is also inhibited by appropriate serum calcium levels which may explain the lack of change in PTH levels at follow up in these GSD patients.[26]

The high prevalence of vitamin D insufficiency and deficiency found in this study confirmed previous investigations. Three studies have reported that GSD patients are at risk of either vitamin D deficiency or osteopenia/osteoporosis.[10,12,31] Vitamin D is an important nutrient with multiple beneficial effects throughout the human body.[16,26,30,19,28] As other studies have shown there are inverse correlations between vitamin D status, liver cirrhosis, and dyslipidemia.[29,37-38] Malham et al. established that vitamin D deficiency as related to liver cirrhosis was associated with the structural and functional

damage, and not the cause of the liver damage.[38] Furthermore, correcting dyslipidemia improves vitamin D status through an unknown mechanism.[37] These studies suggest that vitamin D status in GSD populations should be explored in relation to their altered lipid status and liver glycogen accumulation. Since only one study has specifically examined the link between GSD and vitamin D the mechanism leading to suboptimal 25(OH)D status in this population is unknown.[31] Possible contributing factors include obesity and vitamin D sequestering in adipose tissue, vitamin D dilution due to body volume, hyperlipidemia, and overall liver function in relation to hydroxylation of previtamin D2 and production of vitamin D-binding protein.[37-41]

The high levels of starch consumed by most GSD patients is a potential contributing factor to their elevated BMI. It is also hypothesized that the typical restrictive GSD diet may contribute to nutrient deficiencies.

In order to maintain sufficient 25(OH)D levels this population requires higher than normal levels of vitamin D supplementation. For those patients who are deficient it is recommended that at least 2,000 IU of vitamin D per day be prescribed with close monitoring of vitamin D status. For patients whose vitamin D is not sufficiently elevated it is recommended that they be prescribed 50,000 IU/week for 6 weeks followed by at least 1,000 IU/day.

Strengths and Limitations

Several strengths of the study should be noted. All participants were patients of the UT Health Science Center McGovern Medical School under the care of Dr. Rodriguez and Heather Saavedra MS, RDN. Both serum 25(OH)D and 1,25(OH)D were assessed using Mass Spectrometry which allows for the quantification of both the

circulating and active forms of vitamin D. In addition, a variety of GSD types were represented over a wide range of ages.

Limitations included the small sample size and inconsistent post-intervention time points. Supplementation compliance was also not assessed and a lack of dietary intake data resulted in being unable to estimate vitamin D intake from food. Other limitations include inconsistent vitamin D supplement dosages, lack of follow up data and inconsistent follow up time periods. Finally, vitamin D status across GSD groups could not be performed because of the small sample size.

CHAPTER V

CONCLUSION

CONCLUSION

The current study identified the need for further evaluation of why GSD patients are at increased risk of developing hypovitaminosis D. Findings revealed that vitamin D status was impacted by some underlying mechanism of GSD. Typical dietary intake of vitamin D and/or normal supplementation did not sufficiently raise suboptimal serum 25(OH)D concentrations of GSD patients. Thus, in order to elucidate the underlying mechanisms and the level of appropriate vitamin D intake larger studies are warranted.

The results of the current study show that there is a need for further evaluation into the risk of GSD patients developing hypovitaminosis D and the underlying causes of suboptimal vitamin D status. Future studies should examine the amount of vitamin D supplementation necessary to ensure GSD patients maintain adequate serum 25(OH)D concentrations throughout their life. PTH and liver function should also be closely monitored in these patients in order to determine whether or not they have lower than expected serum concentrations of PTH and any underlying mechanisms.

Given the rarity of GSD it is recommended that future studies be performed using multiple treatment centers to increase the number of patients available for study and strengthen findings. It is further recommended that all relevant laboratory values be consistently assessed including PTH, calcium, and albumin. Finally, liver function tests should be performed including ALT, AST, and circulating levels of vitamin D-binding protein.

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APPENDIX A

IRB Application Approval Letter

Institutional Review Board
Office of Research
6700 Fannin, Houston, TX 77030
713-794-2480
irb-houston@twu.edu
http://www.twu.edu/irb.html

DATE: December 18, 2017

TO: Dr. Carolyn Moore
Nutrition & Food Sciences - Houston

FROM: Institutional Review Board - Houston

Re: Notification of Approval for Modification for Hypovitaminosis D in glycogen storage disease (Protocol #: 19569)

The following modification(s) have been approved by the IRB:

Add Jennifer Melcher #1145088 to the study

APPENDIX B

University of Texas Health Science Center Committee for Review Approval Letter

UTHealth
The University of Texas
Health Science Center at Houston

Committee for the Protection of Human Subjects
6410 Fannin Street, Suite 1100
Houston, Texas 77030

-

NOTICE OF APPROVAL TO IMPLEMENT REQUESTED CHANGES

HSC-MS-17-0535 - Hypovitaminosis D in Glycogen Storage Disease
PI: Dr. Heather Saavedra

Reference Number: 161733

PROVISIONS: Unless otherwise noted, this approval relates to the research to be conducted under the above referenced title and/or to any associated materials considered at this meeting, e.g. study documents, informed consent, etc.

APPROVED: By Expedited Review and Approval

CHANGE APPROVED: Addition of Jennifer Melcher to study team

APPROVAL DATE: 11/27/2017

CHAIRPERSON: Rebecca Lunstroth, JD 

Upon receipt of this letter, and subject to any provisions noted above, you may now implement the changes approved at this meeting.

CHANGES: The principal investigator (PI) must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS. ALL PROTOCOL REVISIONS MUST BE SUBMITTED TO THE SPONSOR OF THE RESEARCH.

INFORMED CONSENT: Informed consent must be obtained by the PI or designee(s), using the format and procedures approved by the CPHS. The PI is responsible to instruct the designee in the methods approved by the CPHS for the consent process. The individual obtaining informed consent must also sign the consent document. **Please note that if revisions to the informed consent form were made and approved, then old blank copies of the ICF MUST be destroyed. Only copies of the appropriately dated, stamped approved informed consent form can be used when obtaining consent.**