7.1 INTRODUCTION

Carbohydrates play both a structural role in the cell, as elements of nucleic acids and glycoproteins, and a metabolic role, as a major energy source. There are several key clues to the diagnosis of an inherited disorder of carbohydrate metabolism, but in most cases, the differential diagnosis is quite broad. Glucose is the primary carbohydrate energy source, and hypoglycemia is a common presenting symptom among the various disorders. When not being used by the body, glucose is stored in the muscles and liver as glycogen, and thus hepatomegaly or hypotonia and muscle weakness may also be seen. As with other biochemical pathways, the biomarker of choice to measure for either diagnosis or follow up for disorders of carbohydrate metabolism depends on which pathway has the blockade. In most cases, this involves determining the enzymatic activity of the dysfunctional enzyme or measuring the concentration of metabolites that accumulate due to the obstruction. This chapter describes the clinical picture, biomarkers for diagnosis, and treatment for disorders of carbohydrate metabolism including galactosemia, glycogen storage diseases (GSDs), and disorders of fructose metabolism.

7.2 GALACTOSEMIA

Galactosemia is a family of autosomal recessive disorders in which the metabolism of the sugar galactose is disrupted. There are three forms of galactosemia, depending on which enzyme in the Leloir pathway of galactose metabolism is nonfunctional (Fig. 7.1). Classic galactosemia is the most common of the three disorders, and it is caused by mutation in the gene for the galactose-1-phosphate uridylyltransferase (GALT) enzyme.\(^1\)–\(^4\) Defects in this enzyme result in the accumulation of galactitol and galactose-1-phosphate metabolites.\(^1\) Galactokinase (GALK) deficiency is a much rarer form of galactosemia, and it is caused by mutation in the gene that encodes the enzyme GALK, causing an accumulation of galactose in the blood and tissues.\(^2\) The
The third type of galactosemia is termed epimerase deficiency galactosemia and is caused by mutation in the gene for the enzyme UDP-galactose-4'-epimerase (GALE). These patients also accumulate galactose and galactose-1-phosphate in erythrocytes as well as high levels of UDP-galactose.

### 7.2.1 CLINICAL PRESENTATION

Patients with classic galactosemia are asymptomatic at birth, but develop life-threatening complications after exposure to milk. Symptoms include feeding difficulties, hypoglycemia, renal tubular dysfunction, vomiting, diarrhea, hepatomegaly, *Escherichia coli* sepsis, and cataracts. Long-term complications can include speech and cognitive disabilities, decreased bone mass and hypergonadotrophic hypogonadism in the majority of females. In contrast to patients with classic galactosemia, patients with GALK deficiency do not have the same issue with consuming milk-based products. They do have high levels of galactose and galactitol in their blood and tissues and can develop cataracts, and rarely, central nervous system abnormalities including mental retardation and pseudotumor cerebri, but these conditions can resolve after eliminating galactose from their diet early in life. Patients experience no long-term complications as long as the dietary restriction is followed. Epimerase deficiency results in a lack of phenotype in most patients, as the enzyme deficiency is usually restricted to red and white blood cells, but there is a very rare manifestation of the disease that has a symptom profile similar to that of classic galactosemia.
7.2.2 TREATMENT

Treatment for all forms of galactosemia is immediate dietary restriction of galactose-containing foods. Infants can be given soy milk or formula containing other carbohydrate sources, or amino acid–based elemental formulas.\(^1,6\) When the patients reach childhood and beyond, galactose restriction is still recommended, but it is increasingly difficult to remove all galactose from the diet, as trace amounts are found in fruits, vegetables, bread, and legumes.\(^1\) A study that compared treatment variation and outcomes in different countries around the world found that in spite of widely disparate manners of monitoring patients, timing of treatment initiation, and levels of dietary restriction, negative outcomes still occurred in the majority of cases.\(^3\) It has been hypothesized that increased concentrations of galactose-1-phosphate is the cause of the pathogenesis in classic galactosemia, and that small inhibitors of GALK could decrease the buildup of this metabolite. Identification of inhibitors of GALK is in the early stages of scientific discovery, and use of these molecules is far from being implemented clinically.\(^7,8\)

7.2.3 BIOMARKERS FOR DIFFERENTIAL DIAGNOSIS

Testing for galactosemia is included in the newborn screening panels in all states in the United States and is also included in the newborn screening panels in many countries around the world. The analytes tested vary from state to state, however. Most laboratories measure the activity of the GALT enzyme in dried blood spots, and while this will identify newborns with classic galactosemia, it could miss patients with a milder form of classic galactosemia referred to as the Duarte variant and patients with defects in the GALK and GALE enzymes.\(^9,10\) In addition to testing for GALT activity, some laboratories also measure the concentration of total galactose (galactose plus galactose-1-phosphate), which can identify the other forms of galactosemia.\(^10\) Owing to increased false-positive rate, many newborn screening laboratories have discontinued testing for total galactose. Presence of reducing substances in the urine can also be a clue toward the diagnosis of galactosemia, but this must be confirmed with a more specific test measuring enzyme activity or molecular testing, as this can be a nonspecific finding.\(^4\)

7.2.4 BIOMARKERS FOLLOWED FOR TREATMENT EFFICACY

Dietary compliance in patients with galactosemia is monitored by measuring either galactose-1-phosphate in red blood cells or galactitol levels in the urine.\(^11\) However, levels do not correlate with either the clinical condition or potential complications that can occur in patients, and the levels never decrease to that found in unaffected subjects even when compliance with the diet is very good.\(^11\) Because of the limitations of galactose-1-phosphate and urinary galactitol as biomarkers for treatment
efficacy, many groups have searched for biomarkers with less intraindividual variation and better correlation with patient outcomes. A study led by Coss looked at the differences in N-glycosylation patterns of immunoglobulin G (IgG) molecules to see if they could be used as a more informative clinical marker. When compared to red blood cell galactose-1-phosphate and urinary galactitol levels, IgG N-glycan profiles showed alterations when patients ingested galactose and were more informative than the traditional markers. Ovarian function is monitored in female patients as they reach puberty by measurement of follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol.

7.2.5 CONFOUNDING CONDITIONS
Disorders of galactose metabolism can be mistaken for several other conditions. Presenting symptoms can mimic liver disease or liver failure due to jaundice, hepatomegaly, and elevated liver enzymes. Secondary galactosemia can result from liver dysfunction that is found with congenital infectious hepatitis, hepatic arteriovenous malformations, patent ductus venosus, or tyrosinemia. Untreated patients can present with *E. coli* sepsis, and in the newborn population, galactosemia as a contributing factor to the infection should be included in the differential diagnosis.

7.3 INBORN ERRORS IN FRUCTOSE METABOLISM
Fructose is found in high concentrations in fruits and in an increasing number of processed foods in the form of high fructose corn syrup (HFCS). There are three recognized inherited disorders of fructose metabolism, which vary quite a bit in severity. Essential fructosuria results from a deficiency of fructokinase and leads to benign elevations of fructose in the blood and urine. As this disorder is asymptomatic and usually diagnosed incidentally, it will not be discussed further. Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by a mutation in the gene encoding the enzyme aldolase B. This gives rise to an accumulation of fructose-1-phosphate that inhibits glycogenolysis and gluconeogenesis. Fructose-1,6-bisphosphate (FBP) deficiency is also an autosomal recessive disorder due to mutation in the gene that encodes the FBP enzyme that causes impaired formation of glucose from all precursors.

7.3.1 CLINICAL PRESENTATION
Patients with HFI are usually healthy in the neonatal period and only manifest symptoms once they are weaned and exposed to fructose, sucrose, or sorbitol in fruits and vegetables. Presenting symptoms include bloating, nausea and vomiting, hypoglycemia, restlessness, lethargy, and often with progression to coma. If ingestion of fructose is continued, patients exhibit chronic conditions such as failure to thrive, liver disease, and kidney dysfunction. In contrast to infants with HFI, infants with
FBP can present as neonates with hypoglycemia and severe lactic acidosis due to reduced glycogen stores.\textsuperscript{13} As the patient ages and the tolerance to fasting improves, the symptoms decrease in both frequency and severity. These symptoms can include irritability, hepatomegaly, coma, and somnolence.\textsuperscript{13} In both conditions, exposure to intravenous solutions containing fructose can be fatal, so it is prudent to determine the sugar used in the solution prior to administration.

### 7.3.2 CONFOUNDING CONDITIONS

Disorders of fructose metabolism can be confused with and misdiagnosed as several illnesses including fructose malabsorption, GLUT5 transporter deficiency, food allergy, acute viral gastroenteritis, liver disease, or sepsis. Symptoms that are shared include nausea, vomiting, abdominal pain, and hypoglycemia. Fructose malabsorption and food allergies can present in a similar time frame, after weaning and the introduction of fruit to the diet, but may present earlier, as most commercial formulas contain sucrose. A case report by Wenzel et al.\textsuperscript{15} described a 5-year-old patient who presented with recurrent watery diarrhea, abdominal pain, and food refusal, particularly avoiding sweets and fruit. The patient’s initial symptoms began at the age of 1, after she had been weaned. After ingesting fructose as a part of a fructose breath hydrogen test to confirm or exclude the diagnosis of fructose malabsorption, the patient exhibited a severe reaction including seizures, severe hypoglycemia, and coma, but she recovered quickly after receiving an infusion of glucose. This case exemplifies why a detailed history, including nutritional information, should be obtained before provocative diagnostic testing is undertaken, and why this type of testing is not recommended.

### 7.3.3 BIOMARKERS FOR DIFFERENTIAL DIAGNOSIS

Patients with HFI have elevated levels of fructose-1-phosphate, but there is not a clinical test available for this biomarker. Discovery of fructose in the urine can suggest a disorder of fructose metabolism, but the absence of fructosuria does not rule out these conditions due to variation in the timing of the fructose ingestion. After collecting an extensive dietary and nutritional history of the patient, the least invasive and most common method of diagnosis for both HFI and FBP is DNA analysis. If no mutations are found, then determination of enzymatic activity from liver biopsy can be performed. Provocative testing such as a fructose loading test is not recommended, as mentioned previously.

### 7.3.4 TREATMENT

The treatment for HFI is the elimination of fructose from the diet. Once the patient no longer ingests fructose, sucrose, or sorbitol, clinical symptoms resolve and the prognosis is quite good. As the patient ages, tolerance for fructose increases slightly.\textsuperscript{13} For patients with FBP, restriction of fructose is only recommended in small children. The
most critical feature to control is an avoidance of fasting, especially during a febrile illness, and the ability to fast improves with age.\textsuperscript{13} Treatment for an acute episode is oral or intravenous glucose and long-term treatment includes frequent feeding or use of uncooked cornstarch or other slowly absorbed carbohydrates.\textsuperscript{13}

7.3.5 BIOMARKERS FOLLOWED FOR TREATMENT EFFICACY

There is no current biomarker that is followed in patients with HFI or FBP. Research studies have shown that patients with untreated HFI have defective glycoprotein glycosylation due to the inhibition of enzyme activity by accumulated fructose-1-phosphate.\textsuperscript{16} This defect disappears after fructose restriction and can be detected by analyzing plasma transferrin isoelectric focusing (TfIEF) patterns. Such analysis could be useful for monitoring compliance to dietary treatment as well as diagnosing indolent cases.

7.4 GLYCOGEN STORAGE DISEASES

GSDs comprise a number of disorders that affect the metabolism of glycogen (Table 7.1). Glycogen serves as a reservoir of glucose, and mutations are found in the genes encoding the enzymes that regulate its processing, leading to abnormal concentrations or structures. Glycogenolysis, gluconeogenesis, and the production of lactate and ketone bodies can be affected, depending on the disorder.\textsuperscript{17}

7.4.1 CLINICAL PRESENTATION

The liver and the muscles are the main organs that are affected in GSD, as those organs are the primary sites for storage or utilization of glycogen. Presenting symptoms can

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include hepatomegaly with recurrent hypoglycemia, intermittent myalgia and muscle weakness, and rhabdomyolysis, with slight variations depending on the specific disorder.\textsuperscript{18}

GSD type 0 is due to mutations in the glycogen synthase gene, GYS2, which lead to a decrease in liver glycogen content.\textsuperscript{19} Patients have hypoglycemia and ketosis after short fasts and hyperlipidemia, as excess glucose cannot be converted to glycogen.\textsuperscript{18}

Patients with GSD type I (von Gierke disease) are unable to generate glucose through gluconeogenesis or by the breakdown of glycogen due to a mutation in the G6PC gene, resulting in glucose 6-phosphatase deficiency.\textsuperscript{19} Instead, patients form glucose-6-phosphate, which when utilized in the cell, results in increased lactate, lipids, and uric acid.\textsuperscript{18–21} Patients present in either the newborn period with hypoglycemic seizures or later in infancy, as the time between feedings is increased. By 6 months of age, hepatomegaly and doll-like facies are apparent. Platelet dysfunction and renal tubular acidosis can be added complications.\textsuperscript{18,21} GSD type Ib is caused by mutations in the glucose-6-phosphate translocase gene, SLC37A4, and also includes neutropenia and neutrophil dysfunction, leading to recurrent infections and poor wound healing.\textsuperscript{18,19}

GSD type II (Pompe disease) is caused by mutation in the GAA gene and deficiency of lysosomal acid-\(\alpha\)-glucosidase enzyme (GAA). Pompe disease is the only GSD that is also classified as a lysosomal storage disease.\textsuperscript{22} Patients can present at a variety of different ages, with variable age of onset, severity, and progression of the disease. Despite these differences, all patients show an accumulation of glycogen in skeletal, cardiac, and smooth muscle, which leads to weakness, hypotonia, respiratory distress, and poor linear growth and weight gain.\textsuperscript{22,23} The infantile form tends to be rapidly progressing and lethal, with death due to cardiorespiratory failure by the age of 1 year, while the late-onset form proceeds more slowly and lacks cardiac involvement, with the age of death dependent on the rate of disease progression.\textsuperscript{22,23}

GSD type III (Cori disease) results from a mutation in the AGL gene. This causes a defect in the glycogen debranching enzyme, resulting in a phenotype that includes hypoglycemia with ketosis, hyperlipidemia, hepatosplenomegaly, and myopathy.\textsuperscript{24} This disorder is further stratified by the involvement of skeletal muscle (GSD type IIIa) or nonskeletal muscle (GSD type IIIb).\textsuperscript{18,19} As patients reach adolescence, their hypoglycemia becomes more stable, but myopathy, including cardiomypathy and exercise intolerance, worsens.\textsuperscript{18,19}

GSD type IV (Anderson disease) is caused by a mutation in the GBE1 gene encoding the glycogen branching enzyme and has a variable presentation, depending on where the deficiency is located.\textsuperscript{25} Patients can have severe or mild liver forms, severe or mild neuromuscular forms, or a generalized severe form that is fatal. As expected with numerous forms, the presenting symptoms are quite variable and can include hepatosplenomegaly and hepatic fibrosis, hypotonia, muscular atrophy, myopathy, cardiomypathy, hydrops fetalis, exercise intolerance, and central and peripheral nervous system dysfunction.\textsuperscript{25}
GSD type V (McArdle disease) is due to mutation in the PYGM gene and deficiency of muscle phosphorylase activity, and muscles are not able to utilize muscle glycogen in the initial phase of physical activity. After the blood supply increases and supplies the muscles with energy, patients are able to function normally, in what is referred to as the “second wind” phenomenon. Symptoms don’t present until the second or third decade of life and include exercise-induced muscle pain, fatigue, and, in some cases, rhabdomyolysis.

GSD type VI (Hers disease) is caused by mutation in the PGYL gene and deficiency of the hepatic glycogen phosphorylase and is the rarest of the GSDs. Unlike the other GSDs, it is not associated with hypoglycemia as the presenting symptom, but instead with hepatomegaly, mild liver dysfunction, short stature, and hyperlipidemia. The clinical course for this disorder is quite benign, and most adults are asymptomatic.

GSD type VII (Tarui disease) results from a mutation in the PFKM gene, leading to deficiency of muscle fructokinase. It is clinically very similar to GSD type V, not only with exercise-induced pain, muscle cramps, and fatigue, but also includes nausea and vomiting, hemolytic anemia, and hyperuricemia.

GSD type IX is subdivided into types IXa, IXb, and IXc, depending on which of the genes encoding the subunits of phosphorylase kinase contains the defect, PHKA2, PHKB, or PHKG2, respectively. Most patients have a mild course, with isolated hepatomegaly and fasting ketosis.

### 7.4.2 CONFOUNDING CONDITIONS

Patients who present in the newborn period with hypoglycemia and seizures can be confused for multiple different diagnoses, especially if the secondary symptoms of acidosis and hepatomegaly have yet to manifest. Persistent hypoglycemic hyperinsulinemia of infancy, galactosemia, or fatty oxidation disorders are other diagnoses that should be considered along with one of the GSDs. Hyperglycemia after meals and glucosuria can be confused for diabetes. When Pompe disease is suspected, the differential diagnosis could also include spinal muscular atrophy I, hypothyroidism, myocarditis, mitochondrial/respiratory chain disorders, Danon disease, peroxisomal disorders, muscular dystrophy, myasthenia gravis, and rheumatoid arthritis.

### 7.4.3 BIOMARKERS FOR DIFFERENTIAL DIAGNOSIS

Laboratory testing for GSDs should include glucose, electrolytes, liver function tests, complete blood count, creatine kinase, uric acid, cholesterol, triglycerides, ammonia, and lactate, preferably after the patient has fasted. In most cases, genetic testing has replaced enzyme assays as the diagnostic method of choice since enzyme assays are difficult to perform and must be done by an experienced laboratory. Liver biopsy shows hepatocytes bulging with glycogen and a vacuolated appearance, but this is now rarely performed since genetic testing is readily available and less invasive. To help in the diagnosis of GSDs involving the muscles, the lactate-ischemia test
Glycogen storage diseases can be performed. Compared to healthy controls, the ammonia level is similarly elevated but the lactate response is attenuated after vigorous forearm exercise. For Pompe disease, a chest X-ray and electrocardiogram (ECG) are useful to arrive at the correct diagnosis and will show cardiomegaly and an abnormal, pathognomonic ECG. A diagnosis of Pompe disease can be confirmed by measuring GAA enzymatic activity from cultured fibroblasts, muscle biopsy, or dried blood spot, elevation of glucose tetrasaccharide (GLc₄) in urine, or molecular testing. In 2013, the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children (DACHDNC) voted to add Pompe disease to the recommended uniform screening panel. The recommendation was confirmed by the US Secretary of Health and Human Services in 2015. In 2016, several states have implemented screening for Pompe disease as part of their newborn screening panel, with several other states pursuing implementation. The question remains, however, of how to determine the treatment for the patients diagnosed via newborn screening. Mutation analysis is not predictive of the patient’s phenotype, and patients with infantile-onset cannot be distinguished from those with late-onset or mild disease.

### 7.4.4 TREATMENT

The main treatment for most GSDs is dietary, with the goal to maintain a normal blood glucose concentration via carbohydrates in the diet. Uncooked cornstarch is commonly used, with continuous feeding overnight to prevent fasting and frequent feeding during the day. Continuous glucose monitoring can be utilized to minimize peaks and troughs in glucose levels. Enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) has emerged as a viable treatment for patients with Pompe disease who also have cross-reactive immunologic material (CRIM-positive). An assessment of a cohort of 10 patients with infantile-onset Pompe disease who were identified at birth via newborn screening results and treated with rhGAA for a median time period of 63 months showed the benefits of ERT. The patients showed long-term survival when compared to untreated cases, and all the patients could walk independently and did not require mechanical ventilation. Muscle weakness did appear after 2 years of age, as well as ptosis and speech disorders. CRIM-negative patients, as well as CRIM-positive patients with high-sustained anti-rhGAA IgG antibody titers (HSAT) have been successfully treated with ERT in combination with immunosuppressive regimens including rituximab, methotrexate, intravenous immunoglobulin, and bortezomib.

Treatment of patients with adult-onset Pompe disease appears to be less effective in halting disease progression. A 5-year retrospective study found that pulmonary function was stabilized but muscle endurance was not significantly enhanced as seen in previous studies. A separate study that focused on quality of life in patients with adult-onset Pompe disease reported that ERT had a positive effect on patients’ physical health status and participation in daily life, as well as not only halting the decline of their health status, but improving it in the first 2 years of treatment.
7.4.5 BIOMARKERS FOLLOWED FOR TREATMENT EFFICACY

Patients should be monitored for growth and other markers of glycemic control, including glucose, uric acid, triglycerides, liver function tests, lactate, or ketones, depending on the disorder being managed. Monitoring of the size of the liver by ultrasound is also recommended. Patients with GSD type I should have measured glomerular filtration rates (GFR) performed to screen for developing end-stage renal disease.\textsuperscript{18–20} Patients with Pompe should be monitored for cardiomyopathy, respiratory function, muscle weakness, and neurological sequelae.\textsuperscript{22}

7.5 CONCLUSIONS

Disorders of carbohydrate metabolism include galactosemia, GSDs, and disorders of fructose metabolism. The prognosis of these disorders runs the spectrum from lifethreatening to completely benign. The majority of these disorders can be managed by restricting the diet of the patient and avoiding the specific carbohydrate involved. Emerging treatment for Pompe disease, the only GSD that is also a lysosomal storage disease, includes enzyme replacement therapy. Traditional diagnosis of these disorders is accomplished by assessing enzyme activity, but this is being replaced by noninvasive molecular testing on an increasing basis. The biomarker of choice for monitoring treatment compliance is dependent on the disorder in question, but patients with all disorders require routine laboratory testing for long-term patient management.

REFERENCES