Glycogen Storage Disease Type I: Laboratory Data and Diagnosis

Nabil W. Waked,1 John G. Bitar,2,4 and Charles K. Allam3

A study of 20 cases of glycogen storage disease type I has shown differences from the classical picture. Hyperuricemia was observed in fewer than half of the cases. All patients had increased triglycerides in serum, but fewer than two thirds had increased concentrations of total cholesterol. There was a consistent increase of aminotransferases in serum. Many textbooks discuss hyperuricemia, lactic acidemia, and lipemia in this disease without mentioning aminotransferases, and above-normal values for these enzymes ought to be given consideration, to avoid misdiagnosis. Glycogen storage disease type IB was detected by comparing glucose-6-phosphatase (EC 3.1.3.9) activity in frozen and unfrozen portions of the same liver biopsy. Latent activity, which appeared after freezing, increased the total activity to within the normal range (4.7–9.1 μmol/min per gram of tissue, wet weight) in type IB, but not in type IA.

Additional Keyphrases: liver disease · glucose-6-phosphatase · triglycerides · aminotransferases · type IA and IB diseases differentiated

Glycogen storage disease type I, von Gierke's disease, is characterized by a liver enlarged with deposits of glycogen and fat. The glucose concentration in the blood is low because of a hepatic deficiency of glucose-6-phosphatase (EC 3.1.3.9; G6Pase). On the other hand, blood lipids are increased as a result of fat mobilization for energy requirements. Lactic acidemia and hyperuricemia are also characteristic features (1).

Here we present a study of 20 cases of von Gierke's disease, in which laboratory findings for the glucagon tolerance test, uric acid, aminotransferases, and cholesterol differed from those commonly ascribed to this disorder. We also report that freezing and thawing of liver tissue may be used to distinguish type IA from type IB.

Materials and Methods

Glucagon tolerance tests were performed by administering glucagon (Novo, Copenhagen, Denmark) intravenously, 50 to 100 μg/kg body weight, after a 3- to 6-h fast. Blood was sampled for glucose and lactate determinations immediately before and then at intervals up to 90 min after giving glucagon.

Chemical tests on blood samples included determinations of glucose by the neocuproine method (2) and lactate in whole blood by the method of Marbach and Weil (3). In the last two cases examined, glucose was determined enzymatically by the hexokinase method (4). Total cholesterol and triglycerides were determined by an extraction procedure, with the Technicon AutoAnalyzer (5, 6), or by enzymatic methods (7, 8). Uric acid was determined by the phosphotungstic acid method (9). Aminotransferases of the earliest cases (cases 2 to 4) were assayed by the method of Reitman and Frankel (10); in the later cases, they were determined at 30 °C with kits based on the method of Henry et al. (11) and supplied by Union Carbide, Rye, NY 10580, or by Baker Instruments Corp., Allentown, PA 18130.

Liver-biopsy specimens were obtained with a Menghini needle, with local xylocaine anesthesia after sedation with diazepam. Fresh tissue (10 to 43 mg) was used for the assay of G6Pase. This was performed as described by Harris and Olmo (12) except that the tissue was homogenized in sodium citrate (0.1 mol/L, pH 6.5) instead of in glucose solution. A portion of the tissue was also frozen and thawed before assay, to test for glycogen storage disease type IB. A unit of enzyme activity is defined as 1 μmol of glucose 6-phosphate hydrolyzed per minute per gram wet weight of tissue at 37 °C. The remaining portion of the biopsy was fixed in absolute ethanol for histological examination. The periodic acid–Schiff stain was used for glycogen, alternate sections having been treated with amylase before staining. Sections for routine study were stained with hematoxylin and eosin.

Results

Table 1 presents results of a study of glycogen storage disease type I, which was begun in 1974. Patients are listed in their chronological order of admission to the American University Medical Center, Beirut. All the patients, except a newborn, presented with abdominal distension, which is what drew the attention of the parents and the referring physician.

Histological examination showed excess glycogen and fat in the livers of all patients. Of the 20 cases, 16 had subnormal values for blood sugar during fasting. Glucagon-tolerance tests were carried out as a supportive test before obtaining a biopsy (13). In earlier cases only glucose was determined after administering glucagon. Subsequently, lactate was measured concurrently, because an increase in blood lactate would distinguish glycogen storage disease type I from type VI, which is due to liver deficiency of phosphorylase (EC 2.4.1.1). Results were variable: three cases with near-normal glucose values showed a moderate increase after glucagon; seven other cases showed an unexplained decline in blood glucose; and two cases showed no increase in blood lactate.

Case 8 was of particular interest, because her near-normal value for blood glucose measured during fasting did not increase after administration of glucagon. She was then given a galactose tolerance test (3 g/kg body weight), which resulted in an increase in blood sugar from 3.5 to 6.8 mmol/L and in blood lactate from 1.7 to 9.4 mmol/L. The increase in blood sugar, which peaked at 30 min, may be interpreted as an increase also in blood galactose, because the neocuproine method does not distinguish between reducing sugars. However, the increase in blood lactate was consistent with glycogen storage disease type I (14). Now 10 years old, she still has a protuberant abdomen and is living on a regimen of frequent short meals, but attends school regularly.

Diagnosis of the disease in each case was confirmed by demonstrating low or reduced G6Pase activity in liver biopsies. Three normal control subjects showed enzyme activity within the range of the disease cases. The G6Pase activity levels of the control subjects were 2.42, 2.51, and 2.60 U/L (mean, 2.50 U/L).

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activities of 4.7, 5.2, and 9.1 μmol/min per gram. Case 6, a newborn, was studied because of a positive family history of glycogen storage disease. However, case 7 was spared a biopsy because of clinical features identical to those of his brother, case 2.

We compared G6Pase in fresh and frozen tissue when we became aware of the work of Narisawa et al. (15). All liver biopsies showed greater G6Pase activity after freezing, but only in case 16 did the activity increase to within the normal reference interval. Thus, it may be classified as glycogen storage disease type IB, in which a defect in the transport of glucose 6-phosphate across microsomal membranes is postulated (16, 17). Earlier cases (3, 6, 8, and 10) with moderate deficiency of the enzyme in unfrozen tissue, ranging between 1.4 and 2.15 μmol/min per gram, may also have belonged to type IB.

Lipid measurements showed in every case that the hyperlipidemia was mainly ascribable to an increase in triglycerides. Cholesterol concentrations were high in only nine cases. Hyperuricemia was also noted in only nine cases (about half), uncorrelated with hypercholesterolemia. Electrophoresis of serum lipoprotein from case 15 showed that this case belonged to Fredrickson's type IV hyperlipoproteinemia (18).

In all the 19 cases for which they were assayed, activities of aminotransferases in serum were above normal, even in cases of moderate G6Pase deficiency. Treatment, which consisted of frequent daytime feedings and nocturnal intragastric glucose infusions, resulted in a decrease in values for serum aminotransferases, an observation that agrees with that of Greene et al. (19).

**Discussion**

The most commonly described laboratory features of glycogen storage disease type I are hypoglycemia, lactic acidemia, hyperuricemia, and lipidemia (1). In our study we found inconsistencies in these features and in the results of glucagon-loading tests. In four cases the concentration of blood glucose after fasting was within the normal range and about half of the patients had normal concentrations of uric acid or cholesterol in their blood. The glucagon-loading test showed a moderate increase in blood sugar in three cases, whereas seven others actually had an unexplained decrease.

The response of lactate and glucose to glucagon also was not consistently as described (1). The most consistent abnormalities were increases in triglycerides and aminotransferases in serum. Although reports of increased serum cholesterol in this disease are many (13, 20–25), the more likely lipid pattern is probably Fredrickson's type IV lipoproteinemia, in which triglycerides are always increased, but not necessarily cholesterol (18).

Hyperuricemia, which is said to be always present in von Gierke's disease (1), was found in about half of our patients. We do not know whether the normal values encountered here reflect regional differences. The causes of hyperuricemia have been the subject of much discussion and controversy (13, 21–23, 25, 26), but increased aminotransferase activity is not even mentioned by major textbooks on pediatrics (27), metabolism (1), and pediatric gastroenterology (28, 29). Increased activities of aminotransferases have already been observed by many workers (19, 20, 23, 24, 30, 31), but as yet there is no explanation for the increase in these enzymes in von Gierke's disease. However, the presence of inflammatory foci in histological preparations from liver biopsies of our patients suggests the existence of cell damage, which itself could result in an increase of serum aminotransferases.

The distinction between glycogen storage disease type IA and type IB was a more recent development in our study. Narisawa et al. (15) demonstrated latency of G6Pase in liver biopsies of type IB subjects by treating homogenates with sodium taurocholate. Corbel et al. (32) disrupted microsom-

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**Table 1. Laboratory Results on Consecutive Patients with von Gierke's Disease**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Glucose, mmol/L</th>
<th>Lactate, mmol/L</th>
<th>G6Pase, μmol/min per gram</th>
<th>Uric acid, mmol/L</th>
<th>AST, U/L</th>
<th>ALT, U/L</th>
<th>Cholesterol, mmol/L</th>
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<tr>
<td>1</td>
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<td>0.54</td>
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<td>4.4</td>
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<td>0.34</td>
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<td>1.8</td>
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<td>0.67</td>
<td>178</td>
<td>232</td>
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**Normal ranges (ages 2m to 2y)*

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<th>Upper</th>
<th>Lower</th>
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<tbody>
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<td>Glucose</td>
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<td>3.3</td>
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<tr>
<td>Lactate</td>
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<tr>
<td>G6Pase</td>
<td>6.2</td>
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*Peak value after glucagon administration. *Reitman–Frankel units (15); normal ranges 8 to 40 for aspartate aminotransferase (AST), 5 to 30 for alanine aminotransferase (ALT). †From ref. 34.
al membranes by homogenizing in water. We adopted the freezing–thawing method and have shown that, in one case, G6Pase activity increased to within the normal range after freezing. In the other cases, enzyme activity remained below normal after freezing, indicating that they were of type IA. Because frozen tissue was frequently used in the past (21, 33), many cases of type IB may have escaped detection and may be more prevalent than currently believed.

We conclude that increased activities of aminotransferases in serum are a more consistent feature of glycogen storage disease type I than is hyperuricemia or hypercholesterolemia. The concentrations of these enzymes should be given due consideration to avoid possible misdiagnosis, and should be included in the general profile of laboratory tests. If sufficient material is available we recommend that latent aminotransferase activity be determined whenever liver G6Pase is assayed.

References