Recurrent rhabdomyolysis due to decreased glycolysis occurred during strenuous exercise by patients with lactate dehydrogenase-A subunit (LDH-A; muscle) deficiency. We report the glycolytic features of 4 patients from 2 families in whom the severity of the disease differed. There was no difference in the gene abnormality. The enzyme activity of LDH in the muscle was less than 5% that of the control value. Glycolysis in the muscle showed that the respective sums of the pyruvate and lactate levels in the patients with mild and severe symptoms were reduced to approximately 65% and 35% that of the control value. Comparable amounts of glycerol 3-phosphate were produced. Glycerol 3-phosphate dehydrogenase activity in the muscles of patients with mild symptoms was three times the control value. These findings suggest that the disease severity in our patients may be related to the degree of NADH reoxidation by glycerol 3-phosphate dehydrogenase substituting for LDH.

CHARACTERIZATION OF THE GLYCOLYSIS IN LACTATE DEHYDROGENASE-A DEFICIENCY

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Lactate dehydrogenase (LDH), the enzyme in the final step of the glycolytic pathway, catalyzes the interconversion of pyruvate and lactate, with nicotinamide adenine dinucleotide (NAD) as the coenzyme. Five isozymes of tetrameric LDH, present in different proportions in the various somatic tissues, are produced by a combination of the LDH-A (muscle) and LDH-B (heart) subunits. The LDH-A gene product predominates in skeletal muscles. Decreased glycolysis created by a deficiency in the LDH-A subunit causes muscle cramping and myoglobinuria during extended periods of exercise. We have reported the gene abnormality of a 20-nucleotide deletion in exon 6 in 4 patients from 2 families in whom the severity of the disease varies. To determine the metabolic basis for the clinical differences, we investigated carbohydrate metabolism in these patients’ muscles and compared the results with those for 4 normal subjects.

CASE REPORTS

Patient 1. A 34-year-old man complained of severe muscle pain and myoglobinuria after judo practice at age 16. These symptoms, induced by heavy exercise that included judo, swimming, and a short track run, disappeared within a few days without treatment. No consanguinity was found through four generations of his family. Neurologic examination showed neither muscle atrophy nor weakness, and the deep tendon reflexes were normal. There was no apparent sensory disturbance. The respective serum concentrations of creatine kinase (CK) and LDH were 26,290 U/L (normal < 120) and 466 U/L (normal 200–370). Isozyme analysis of his serum LDH showed only one band, B4. Serum electrolytes and thyroid function were within the normal ranges. Electromyography showed a normal neuromuscular unit. In the semischemic forearm work capacity test, muscle pain and stiffness appeared about 30 s after the test started, and the patient could not continue with the testing. Serum pyruvate was elevated, but lactate was below the control values. Twelve hours after the ischemic test, the serum CK level had risen to 15,650 U/L, but the LDH had increased to
only 381 U/L. No glycogen storage was found in a muscle biopsy specimen.

**Patient 2.** A 30-year-old man, the brother of patient 1, also experienced recurrent episodes of muscle pain and rhabdomyolysis during his teenage years. No abnormality was found on physical examination. Isoenzyme analysis of his serum LDH showed a defect in the A subunit. The same symptoms as those of patient 1 were induced by the semi-ischemic forearm work test. His serum CK level rose to 26,290 U/L, but the LDH concentration was only 431 U/L.

**Patient 3.** A 27-year-old woman complained occasionally of being easily fatigued. Her parents' marriage was consanguineous. No myoglobinuria had been found previously. Her serum CK level had increased gradually during a pregnancy and, because her uterus was too stiff during the early stage of delivery, a cesarean section was performed. When she presented, neither muscle wasting nor weakness was detectable, and all the deep tendon reflexes were normal. Laboratory tests showed respective serum CK and LDH concentrations of 84 and 225 U/L. Her serum LDH isozyme pattern showed only one band, LDH-B. An electromyogram showed no abnormality. There was no increase in the venous lactate concentration, but a marked increase in venous pyruvate occurred under anaerobic conditions. After the work loading test, her serum CK level rose to 1574 U/L and her LDH to 234 U/L.

**Patient 4.** A 33-year-old woman, the sister of patient 3, also underwent a cesarean section because of contraction disturbances in the uterine muscle. She showed no muscle weakness or wasting when she presented. The respective serum concentrations of her CK and LDH after the ischemic test were 965 and 218 U/L. The serum LDH isozyme pattern was similar to that of patient 3. Glycolysis retardation also resembled that seen in patient 3.

**MATERIALS AND METHODS**

After receiving the patients' informed consent, we obtained quadriceps femoris muscle tissue by open biopsy. As the control, a specimen of similar tissue was obtained surgically after obtaining the informed consent of 4 subjects with no neuromuscular disorder.

**Enzyme concentrations.** For the glycolytic enzyme assay, the muscle specimen was homogenized with 10 volumes (V/W) of 66.7 mmol/L sodium phosphate buffer (pH 7.4) in an all-glass Potter-Elvehjem homogenizer, after which the homogenate was centrifuged at 14,000 x g for 15 min. Hexokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase (GAPD), and glycerol 3-phosphate dehydrogenase (α-GPD) were assayed according to Bergmeyer, Gawehn, and Grass with slight modifications. The activities of LDH and CK were determined according to the respective methods of Wróblewski and LaDue and Szasz and associates.

**Glycolysis.** For the glycolysis estimation, approximately 100 mg of muscle was minced finely with scissors in the cold then thoroughly homogenized in 9 volumes (V/W) of ice cold 50 mmol/L Hepes buffer (pH 7.4) containing 0.12 mol/L KCl, 5 mmol/L MgSO₄, and 1 mmol/L EDTA in a relatively loose-fitting, all-glass, Potter-Elvehjem homogenizer. The volume of the homogenate was adjusted with the homogenizing medium to 1.0 mL of homogenate per 100 mg of tissue. The reaction mixture (2.0 mL, pH 7.4) contained 0.1–0.5 mL homogenate, 62.5 mmol/L Hepes, 112 mmol/L KCl, 6.25 mmol/L MgSO₄, 0.25 mmol/L EDTA, 1 mmol/L KH₂PO₄, 1 mmol/L ATP, 0.5 mmol/L

### Table 1. Enzyme activities in normal and LDH-deficient muscle extracts.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Patients 1</th>
<th>Patients 2</th>
<th>Patients 3</th>
<th>Patients 4</th>
<th>Control (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>0.24</td>
<td>0.25</td>
<td>0.58</td>
<td>0.63</td>
<td>0.52–0.61</td>
</tr>
<tr>
<td>Aldolase</td>
<td>84</td>
<td>82</td>
<td>94</td>
<td>97</td>
<td>82–94</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>1824</td>
<td>1950</td>
<td>1988</td>
<td>2074</td>
<td>1432–1732</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase (GAPD)</td>
<td>138</td>
<td>140</td>
<td>148</td>
<td>162</td>
<td>98–129</td>
</tr>
<tr>
<td>Glycerol 3-phosphate dehydrogenase (α-GPD)</td>
<td>16.5</td>
<td>17.3</td>
<td>43.5</td>
<td>47.3</td>
<td>13.2–17.1</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>11.7</td>
<td>9.8</td>
<td>11.8</td>
<td>9.8</td>
<td>231–269</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>3012</td>
<td>2963</td>
<td>2581</td>
<td>2465</td>
<td>2314–2860</td>
</tr>
</tbody>
</table>

Values given in μmol/min/g tissue.
NAD, 40 mmol/L nicotinamide, and 1 mmol/L L-cysteine, together with 10 mmol/L glucose, 10 mmol/L glucose 6-phosphate (G6P), 10 mmol/L fructose 1,6-diphosphate (FDP), or 7.2 mg rabbit muscle glycogen as the substrate. The mixture was incubated at 37°C for 5 min before the homogenate was added. The reaction was carried out for 15 min at 37°C, after which 20% HClO₄ was added to the mixture. The concentrations of the substrate and glycolytic intermediates in the neutralized HClO₄ extracts were determined with NAD, NADP, or NADH.¹

**RESULTS**

**Enzyme Activities (Table 1).** LDH activities showed a marked decrease in the patients, being less than 5% of the control value. No remarkable changes were found in the activities of other en-
zymes tested, except for hexokinase and α-GPD. Hexokinase activity for both patients 1 and 2 was approximately 45% of the control value, and the α-GPD activity for both patients 3 and 4 was three times the control value.

**Glycolysis in the Muscle Homogenate (Table 2).**
Glycolysis in the homogenates of the patients' muscles, with glycogen, glucose, G6P, and FDP as the substrates, showed that conversion of pyruvate to lactate was significantly impaired and that similar amounts of glycerol 3-phosphate and glycerol accumulated in the reaction mixture. Although the ratio of the pyruvate and lactate products to the glycerol 3-phosphate and glycerol products was more than 5:1 in the control, it was less than 1:1 in patients 1 and 2 and approximately 1:1 in patients 3 and 4. The sum of the formation of pyruvate and lactate in both patients 1 and 2 was approximately 35% that for the control, whereas in both patients 3 and 4 it was approximately 65%. The velocity at which the glycolytic substrates were used in the muscle homogenates of patients 1 and 2 was decreased as compared with the control muscle specimen. With glucose as the substrate, glycolysis proceeded slowly in patients 1 and 2, probably because of the low hexokinase activity in the muscle tissues. The utilization rate for glycolytic substrates was higher in patients 3 and 4 than in patients 1 and 2.

**DISCUSSION**
During strenuous work, anaerobic glycolysis of glycogen generally provides energy when the intracellular oxygen available for metabolism is insufficient for muscle contraction. Under those conditions, the high concentrations of pyruvate are reduced to lactate by the LDH-A isozyme. Of the enzymes tested, only the activity of LDH in our patients was significantly lower than that of the control muscle. Glycolysis in the homogenates of the patients' muscle tissue with glycogen and glucose as the substrates showed that the FDP and triose phosphates concentrations increased, indicative of a disturbance in glycolysis at the step of glyceraldehyde 3-phosphate dehydrogenase (GAPD). The activity of this enzyme was not, however, reduced. These findings agree with results found for the levels of glycolytic intermediates in muscle specimens obtained just after exercise on a bicycle ergometer. Because the reoxidation of NADH by LDH has been shown to be limited in a patient with LDH-A deficiency, glycolysis is

![Diagram of glycolysis pathway](image-url)

**Normal subject**

**Patient**

**FIGURE 1.** Glycolysis pathway: (1) lactate dehydrogenase (LDH); (2) glyceraldehyde 3-phosphate dehydrogenase (GAPD); (3) glycerol 3-phosphate dehydrogenase (α-GPD); and (4) hexokinase.
The reoxidation of NADH by LDH is limited due to the production of glycerol 3-phosphate and glycerol. Because formation was accounted for mainly by the formation not only of lactate, but of total lactate and pyruvate, was reduced significantly, especially when G6P and FDP were the glycolytic substrates. The sum of the formation of pyruvate and lactate exceeded more slowly than it did in patients with severe symptoms. Glycolysis in the muscle homogenates of patients with severe symptoms proceeded more slowly than it did in patients with mild symptoms.

The decrease in the total pyruvate and lactate formation was accounted for mainly by the formation of glycerol 3-phosphate and glycerol. Because the reoxidation of NADH by LDH is limited due to low LDH activity, a considerable portion of the NADH at the GAPD stage was oxidized to NAD by cytoplasmic α-GPD for the continuous operation of GAPD (Fig. 1). The sum of the formations of glycerol 3-phosphate and glycerol in patients 3 and 4 was higher than it was in patients 1 and 2. The difference may be due to a difference in α-GPD activity. In insect flight muscle, the reoxidation of extramitochondrially formed NADH by LDH is minimal because there is little if any LDH but extremely active α-GPD in the cytoplasm. In contrast, mammalian skeletal muscle contains fairly active cytoplasmic α-GPD. α-GPD activity for both patients 3 and 4 was three times the control value. It is likely that in patients 3 and 4 the low muscle NAD was compensated for and ATP production was maintained to some degree by glycolysis. α-GPD activity may be closely associated with the severity of muscle symptoms. The cytoplasmic α-GPD of skeletal muscle, however, is susceptible to product inhibition by glycerol 3-phosphate; therefore, in our patients the resulting glycolysis would be abortive with markedly reduced efficiency for the immediate supply of ATP.

REFERENCES