Characterization of the Oxidative Metabolism in Lactate Dehydrogenase A Deficiency

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Recurrent rhabdomyolysis due to decreased glycolysis occurred during strenuous exercise in patients with lactate dehydrogenase A subunit (LDH-A) deficiency. We report the features of oxidative metabolism of four patients from two families in whom the severity of the disease differed. Enzyme activities of muscle LDH were decreased to <5% of control. There was no difference in the gene abnormality. Maximal oxygen uptake in the patients with severe and mild symptoms was approximately 73% and 92% of control. Respiratory exchange ratio was increased to more than 1.0 during maximal exercise. These findings suggest that in these patients, the oxidative functions of glycogenolysis in which pyruvate is required for fuel of maximal oxidative metabolism are preserved, and that disease severity may be related to the degree of muscle oxidative capacity.

Internal Medicine 34: 502-506, 1995

Key words: oxidative phosphorylation, glycogenolysis, maximal oxygen uptake, pyruvate, respiratory exchange ratio

Introduction

Lactate dehydrogenase (LDH), the enzyme of the final step of the glycolytic pathway, catalyzes the interconversion of pyruvate and lactate. The five isozymes of tetrameric LDH are produced by a combination of the LDH-A (muscle) and LDH-B (heart) subunits (1, 2). The LDH-A gene product is present predominantly in skeletal muscles (3). The decreased glycolysis created by a deficiency of the LDH-A subunit causes muscle cramping and myoglobinuria during extended periods of exercise (4, 5). We have reported the gene abnormality of a 20-nucleotide deletion in exon 6 in four patients from two families in whom the severity of the disease varies (6, 7). As exercise testing in muscle disorders provides the means to assess exercise capacity quantitatively (8), here, these four patients were asked to perform incremental exercise testing to explore the physiologic implications of the biochemical defect and clarify the difference in disease severity.

Case Reports

Patient 1

A 34-year-old man complained of severe muscle pain and myoglobinuria after Judo practice at the age of 16. No consanguinity was found through four generations. Neurologic examination showed neither muscle atrophy nor weakness and deep tendon reflexes were normal. The respective serum concentrations of creatine kinase (CK) and LDH at rest were 105 U/l (normal <120) and 3 16 U/l (normal 200–370). Isozyme analysis of serum LDH detected only one band of B4. Electromyography showed a normal neuromuscular unit. In the semi-ischemic forearm work capacity test, muscle pain and stiffness appeared about 30s after the test started, and the patient could not continue with the testing. Blood pyruvate concentrations were elevated, but lactate levels were below control values (Fig. 1). 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 seen in a muscle biopsy specimen (Fig. 2).

Patient 2

A 30-year-old man, the brother of patient 1, also had recurrent episodes of muscle pain and rhabdomyolysis during his teenage years. No abnormality was found on physical examination. Isozyme analysis of serum LDH showed a defect of the A subunit. The same symptoms as apparent in patient 1 were induced by the semi-ischemic forearm work test (see Fig. 1). 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 seen in a muscle biopsy specimen (Fig. 2).
Oxidative Metabolism in LDH-A Deficiency

Figure 1. Semi-ischemic forearm work capacity test. In patients 1, 2, 3, and 4, the lactate/pyruvate ratios after exercise were reduced to approximately 84, 86, 69, and 72% of those before exercise in contrast to more than a 4-fold increase in control subjects. ○ patient 1; x patient 2; △ patient 3; □ patient 4.

Patient 3
A 33-year-old woman complained occasionally of being easily fatigued. Her parents' marriage was consanguineous. No myoglobinuria had previously been found. Because her uterus was too stiff in the early stage of delivery, a cesarean section was performed. Neither muscle wasting nor weakness was detectable. Laboratory tests showed respective serum CK and LDH concentrations of 68 U/l and 218 U/l. Her serum LDH isozyme pattern showed only one band, LDH-B₄. 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 (see Fig. 1). After the work loading test, her serum CK level rose to 965 U/l and her LDH to 218 U/l.

Patient 4
A 27-year-old woman, the sister of patient 3, also underwent a cesarean section because of contraction disturbances in the uterine muscle. She had no muscle weakness or wasting. The respective serum concentrations of her CK and LDH at rest were 84 U/l and 225 U/l. The isozyme pattern of serum LDH was similar to that of patient 3. Glycolysis retardation also resembled that seen in patient 3 (see Fig. 1). Twelve hours after the ischemic test, her serum CK level was elevated to 1,574 U/l; LDH was 234 U/l.

Materials and Methods

Enzyme concentrations
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 four subjects with no neuromuscular disorder. The activity of LDH was determined according to the methods of Wróblewski and LaDue (9). Aldolase, triose phosphate isomerase, and glyceraldehyde 3-phosphate dehydrogenase (GAPD) were assayed according to Bergmeyer et al (10) with slight modifications.

Exercise testing
Metabolic and cardiorespiratory responses to exercise were evaluated during an incremental exercise test with a bicycle ergometer according to Elliot and associates (11). Standard cycle ergometry used a 20- to 30-watt work increase every 2 min. Minute ventilation, fraction of expired oxygen, and fraction of expired carbon dioxide were monitored, and calculation of oxygen consumption (VO₂), carbon dioxide production (VCO₂), and respiratory exchange ratio (RER) was performed every 30 s by an automated system (SensorMedics, Yorba Linda, CA, USA). During exercise, the heart rate was monitored by 12-lead continuous electrocardiographic recording, and blood pressure was measured each minute by sphygmomanometry. Two separate tests were run to volitional fatigue. Serum lactate and pyruvate levels were determined before and 2 min after exercise. Three normal controls closely approximated each LDH-deficient patient as matched for sex, age, height, and weight.

Results

Enzyme activities
A marked decrease in LDH activities occurred in the patients, to less than 5% of the control value [patients 1–4 (µmol/min/g tissue): 11.7, 9.8, 9.8, 11.8; normal: 231–269]. No remarkable changes were found in the activities of other enzymes tested.
Due to leg fatigue, all patients stopped the exercise, but they had no muscle pain or contracture. Maximal heart rates achieved were at least 98% of the expected maximum for all patients. A subject’s approximate maximal heart rate can be estimated on the basis of the following equation: maximal heart rate = 220–(subject age)+10. Maximal oxygen uptake (VO_{2max}) in our patients was more than two-thirds of the normal control value. VO_{2max} in patients 1 and 2 was lower than that in patients 3 and 4. The respiratory exchange ratio (RER), i.e., the ratio of VCO_{2}/VO_{2}, exceeded 1.0 in all patients during maximal aerobic exercise. Blood pyruvate concentrations after exercise in the patients were higher than those in control subjects, although lactate levels in the patients were lower than those in controls. The blood lactate/pyruvate (L/P) ratio in the patients was decreased in contrast to the marked increase in normal subjects.

**Discussion**

The present patients had recurrent episodes of muscle pain and myoglobinuria after brief and intense exercise. These symptoms are well-known hallmarks of the enzyme defects in glycolysis. However, the lack of glycogen storage and the abnormal pyruvate response under anaerobic conditions in these patients differed from patients with glycolytic disorders. Glycogen was a central role in both anaerobic and oxidative muscle metabolism. Its advantage as an oxidative fuel compared to lipid includes a higher power output, more rapid acceleration to maximal power output, and a higher molar ratio of ATP production to O_{2} consumption. Oxidative phosphorylation through glycogenolysis is impaired in patients with muscle glycolytic defects, i.e., muscle phosphorylase deficiency (McArdle’s disease) and muscle phosphofructokinase deficiency (Tarui’s disease) (12, 13). To elucidate the oxidative metabolism in the present patients, we performed large muscle dynamic exercise as exemplified by cycle ergometry (11).

VO_{2max} is an important index of the intensity of muscle oxidative phosphorylation (14). VO_{2max} in patients with McArdle’s disease and Tariui’s disease is low, generally in the range of one-third to one-half of that in sedentary control subjects (11, 12). Carbohydrates are major substrates during intense exercise, and pyruvate formed from carbohydrates through the glycolytic pathway can be oxidized to H_{2}O and CO_{2} in the mitochondria when intracellular oxygen available for metabolism is sufficient. Therefore, individuals with a glycolytic defect that blocks pyruvate production demonstrate reduced VO_{2max}. The present patients achieved significantly greater levels of VO_{2max} despite a metabolic defect. A near normal VO_{2max} in a patient with LDH-A deficiency reported in the United States (15) was similar to that in our patients. Although different levels of cardiorespiratory fitness may have influenced the exercise capability of the patients, VO_{2max} can be estimated from the heart rate corresponding to the VO_{2} at which the patient ceased exercise, by extrapolation to the VO_{2} that would correspond to the subject’s predicted maximal heart rate (13). Heart rates in our patients were similar to those in the sex- and age-matched controls. Exertional pain may preclude continuing exercise sufficiently to obtain the plateau of VO_{2} relative to increasing exercise workloads, which normally defines VO_{2max}. The present patients did not experience muscle pain and contracture. The physiologic data suggested that adequate oxidative capacity for glycogenolysis was preserved in our patients.
patients. The RER provides a measure of the fuel mix supporting oxidative metabolism. The proportion of carbohydrate relative to lipid oxidizes increases with exercise intensity, causing a progressive increase in the RER from approximately 0.7 at rest (reflecting predominant lipid oxidation) to 1.0 (or higher) during maximal exercise, reflecting exclusive oxidation of carbohydrate (16). When ventilation was comparable to that in controls, RER was low (<1.0) in patients with McArdle’s disease and Tarui’s disease during maximal aerobic exercise, indicating substantially reduced carbohydrate oxidation and increased dependence on lipid fuels (11–13). The RER is high (>1.0) in the present patients, indicating exclusive oxidation of carbohydrate. The blood L/P ratio in our patients was decreased after maximal aerobic exercise in contrast to the normal 3-fold increase. These findings suggested that patients with LDH-A deficiency could preserve oxidative function of glycogenolysis that needed pyruvate for fuel. Glycogenolysis in our patients might attenuate the glycogen accumulation in muscle that is typically found in McArdle’s disease and Tarui’s disease.

\[ \text{VO}_{2\max} \text{ in patients 1 and 2 with severe symptoms was 75\% and 71\% of the control value, whereas that in patients 3 and 4 with mild symptoms it was 94\% and 90\%, respectively. In phosphoglycerate mutase deficiency with exertional myoglobinuria, VO}_{2\max} was 76\% of the control mean associated with normal elevation of blood pyruvate in maximal aerobic exercise (17). This finding was similar to patients 1 and 2. The change of L/P ratios in patients 3 and 4 was larger than that in patients 1 and 2, suggesting much more buildup of pyruvate in patients 3 and 4. Some mutations in patients with LDH-A deficiency have been defined: a 2-nucleotide deletion in exon 5, a G-to-A substitution at codon 81, and a G-to-T substitution at codon 328 (18, 19). Clinical phenotypes in these patients might be characterized by molecular genetic heterogeneity (19). No differences in the genetic abnormality, however, were found in the present four patients, suggesting that the differences in disease severity were not due to genomic abnormality in our families (6, 7). The difference between the patients in oxidative capacity, probably due to pyruvate production, might be related to the degree of severity of disease.

### References


