Increased Serum Lactate Dehydrogenase Isoenzyme 1 and "Flipped" LD-1/LD-2 Ratio in Myopathy Associated with Partial Carnitine Palmitoyltransferase Deficiency

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We describe a case of a limb-girdle myopathy presenting with myoglobinuria. A partial deficiency of muscle carnitine palmitoyltransferase (EC 2.3.1.21) may also have been present. All "muscle-type" serum enzymes were markedly increased (to between 30- and 400-fold their respective upper reference limits) and creatine kinase (EC 2.7.3.2) isoenzyme 2 (CK-MB) was increased 130-fold but was still <2% of the total creatine kinase activity. The isoenzyme pattern of lactate dehydrogenase (EC 1.1.1.27) in serum was "anodic," with isoenzyme 1 > isoenzyme 2—an unusual pattern for myopathies. The possible physiological basis for such a finding is discussed.

Additional Keyphrases: myoglobinuria · creatine kinase

Myopathies increase activities of enzymes in serum, notably creatine kinase (CK; EC 2.7.3.2), lactate dehydrogenase (LD; EC 1.1.1.27), aspartate aminotransferase (EC 2.6.1.1), and aldolase (EC 4.1.2.13). The LD isoenzyme pattern tends to be "cathodal"; i.e., LD-4 and LD-5 predominate (1). However, a few cases of unusual LD isoenzyme patterns have been reported. For example, patterns with increased anodal fractions (LD-2 and LD-3) have been detected in patients with polymyositis and other chronic muscle diseases (2–4). More recently, an atypical LD isoenzyme pattern, in which LD-1 and LD-2 were increased, has been reported to be associated with rhabdomyolysis (5) probably owing to poisoning with an intermediate-acting barbiturate.

In these cited cases, LD-1 activity did not exceed LD-2. This appears to be a characteristic finding in all the unusual patterns reported thus far with myopathies. In general, increased LD-1 in serum and LD-1/LD-2 ratios >1.00 are associated only with myocardial or renal infarction, in vitro or in vivo hemolysis, or germ-cell tumors (6, 7). However, we report a case with an unusual increase of LD-1 with a "flipped" LD-1/LD-2 ratio in the serum of a patient with low activity of muscle carnitine palmitoyltransferase (EC 2.3.1.21), which may have indicated a partial deficiency of this enzyme (8).

Materials and Methods

Total LD (9) and CK (10) were determined, at 37 °C, with a "Reaction Rate" analyzer (Model 2086; LKB-Produkter AB, Bromma, Sweden); aspartate aminotransferase (9) and alanine aminotransferase (EC 2.6.1.2) (9) at 37 °C with a Multistat centrifugal analyzer (Instrumentation Laboratory Inc., Lexington, MA 02173); gamma-glutamyltransferase (EC 2.3.2.2) (9) at 37 °C with a TR Enzyme Analyzer (Beckman Instruments Inc., Fullerton, CA 92634); and aldolase at 37 °C with a Calbiochem Kit (Hoechst Canada Inc., Behring Diagnostics, Montreal, Quebec, Canada) and a Beckman DU 8 spectrophotometer.

Isoenzymes of LD (6) and CK were separated by electrophoresis on thin-layer agarose (LD isoenzyme assay: Corning Universal Electrophoresis Film, Corning Medical and Scientific, Palo Alto, CA 94306; CK isoenzyme assay: Beckman Reagent System, Beckman Instruments, Inc., Brea, CA 92621) and quantified by scanning with a Clinician fluorescence densitometer (Helena Laboratories, Beaumont, TX 77704).

Upper reference limits for serum established by this laboratory were: aspartate and alanine aminotransferase and gamma-glutamyltransferase, 30 U/L; total LD, 378 U/L; total CK, 174 (males) and 140 (females) U/L; LD-1, 26%; LD-1/LD-2 ratio, 0.75; CK-2 (CK-MB), 10 (males) and 8 (females) U/L; and aldolase, 6.0 U/L.

Case History

A 17-year-old young woman with a history of mental retardation since birth (and bouts of idiopathic seizures satisfactorily controlled with phenytoin) was admitted to University Hospital for investigation of progressive weakness and a "brown" urine, which had started some two weeks earlier after a virus-like illness with cough, mild fever, and decreased appetite, and which appeared to have been exacerbated by heavy exertion in a snow storm. Results of physical examination at the time of admission were unremarkable except for the marked weakness in limb-girdle muscles. She had neither skin lesions nor muscle tenderness, but she complained of marked weakness in all proximal limb-girdle muscles, particularly of the legs. Laboratory findings on admission included a normal value for hemoglobin, marked myoglobinemia and myoglobinuria (confirmed by a specific radioimmunoassay), increased serum enzymes (see below), negligible concentration of phenytoin in serum, sinus tachycardia, and an electrocardiogram showing QT prolongation.

She was transferred to the Cardiac Care Unit because of these abnormalities. Subsequent electrocardiograms and ventricular wall-motion studies suggested normal cardiac function and she was transferred to the general-medical ward. Myoglobinuria disappeared on hospital day nine. Results of an ischemic lactate stress test done during her hospital stay were normal.

During her stay in hospital she steadily regained muscle power, and her activities of serum enzymes decreased to more nearly normal values (see below); she was discharged 21 days after admission.

On admission, total CK, aspartate aminotransferase, LD, alanine aminotransferase, and aldolase activities were 395-, 72-, 46-, 32-, and 31-fold their respective upper reference limits (Figure 1), thus indicating an abnormality of skeletal muscle. CK-2 activity was also increased 130-fold, but was initially less than 2% of the total CK activity (Figure 1a).

All enzyme activities declined after admission—presumably because of bed rest—although both CK-2 and aldolase peaked inexplicably on hospital day six (CK-2 to nearly 5%
of total CK activity) and other muscle enzymes also showed local peaking (Figure 1). Alanine aminotransferase and gamma-glutamyltransferase values remained relatively constant throughout the hospital stay but there was a significant decrease in alanine aminotransferase activities before discharge on day 21 (Figure 1a).

The LD isoenzyme pattern was "anodic" and the LD-1/LD-2 ratio was increased (Figure 2). LD-1 remained increased throughout the hospital stay, whereas LD-2 always remained within the reference range.

A biopsy of the left quadriceps muscle (obtained on hospital day 20) was sent to a reference laboratory for assays of free and total carnitine concentrations (which were normal) and for determination of carnitine palmitoyltransferase activity by the isotope-exchange assay (11). The patient’s carnitine palmitoyltransferase activity was 38.0 nmol/min per gram; the reference interval was 76.1 ± 16.4 (mean ± SD) nmol/min per gram for 68 healthy subjects. In carnitine palmitoyltransferase deficiency, activities are between 5 and 24% of the reference range (11): our patient’s value was interpreted as a low activity but not as low as values obtained from typical cases of carnitine palmitoyltransferase deficiency.

**Discussion**

Anodic serum LD isoenzyme patterns are usually caused by myocardial or renal infarction, hemolysis, or germ-cell tumors—possibilities that were excluded in the present patient. Moreover, the history, physical examination, and laboratory findings all pointed to a skeletal muscle source for the abnormal LD isoenzyme pattern.

Skeletal muscle damage is usually considered a cause of an increase in LD-5 (1). For example, Elliott and Wilkinson (12) described an increase in LD-5 in serum after healthy skeletal muscle had been invaded by the nematode *Trichinella spiralis*, which caused trichinosis, a parasitic myopathy. However, as Rosalki (13) points out, a considerable literature indicates that skeletal muscle damage may also cause mid-zone or anodic LD patterns in addition to the "conventional" cathodic pattern.

Healthy skeletal muscle in adults contains approximately equal proportions of muscle fiber types I ("red" or slow-twitch muscle), II A (intermediate or fast-twitch oxidative type), and II B ("white" or fast-twitch glycolytic muscle). Type II C fibers are present only in fetal muscle (and, under pathological conditions, in adults), although wide variations do occur (14). Type I fibers have a very high oxidative capacity (i.e., very active citrate cycle, fatty acid oxidation, and electron transport chain), whereas type II B fibers have a low oxidative capacity but a high capacity for glycolysis. Type II A fibers have both a high oxidative and glycolytic capacity and are therefore metabolically mid-way between...
the other two fiber types (15, 16). As expected, these metabolic differences are reflected in the distribution of the LD isoenzymes: type II B fibers have a high LD-5 content, whereas type I fibers have a more anodic pattern (although LD-5 is still present in significant quantities); type II A fibers have an LD isoenzyme pattern combining the features of the other two fiber types (17).

The distribution of these fiber types varies both with different muscles in the same individual and with the same muscle in different individuals (18, 19). Superficial muscles tend to have more type II B fibers, whereas deeper-seated tonic postural muscles have more type I fibers (13). There are also age- and sex-related differences in fiber distribution, and indeed immobilization of a subject selectively and significantly reduces the mass of Type I fibers (20). Moreover, the fiber composition of muscles can be altered with training: the trained sprinter has a high proportion of type II B fiber, the long-distance runner a high proportion of type I fibers (15, 16). When these factors are considered with the effects of acquired de-differentiation caused by disease processes (13), it is clear why a wide range of serum LD isoenzyme patterns can be obtained in diseases of skeletal muscle. Obviously in this patient, the disease process involved type I fibers, which is also the fiber selectively affected by immobilization.

Myoglobinuria due to rhabdomyolysis can be classified into five causal groupings of nearly 100 specific diseases: abnormalities of energy production, hypoxia, primary muscle injury, infections, and all other causes (21, 22). From the history and clinical findings in this case, it is likely that abnormalities of energy production and causes due to infection may, together or separately, be the etiological factor(s). This patient complained of distal limb-girdle muscle weakness, particularly of the legs, which suggested a viral myositis. Alternatively, the viral infection may have been the trigger activating a latent defect in muscle metabolism. Loss of appetite and overexertion in the snow storm are likely, and well-recognized, exacerbating factors.

Carnitine palmitoyltransferase deficiency is known to cause recurrent myoglobinuria, which can be precipitated by exercise, fasting, or infection—all conditions in which fatty acid oxidation is increased (17). Carnitine palmitoyltransferase exists in two forms, one form (carnitine palmitoyltransferase I) being bound to the outer face and the other (carnitine palmitoyltransferase II) to the inner face of the inner mitochondrial membrane. These enzymes convert long-chain fatty acids or their coenzyme A derivatives, which cannot enter mitochondria, to acylcarnitine, which is transported across the mitochondrial membrane by a specific carrier. Carnitine palmitoyltransferase II regenerates the acyl-coenzyme A and free carnitine within the mitochondria to acylcarnitine and carnitine, which is transported across the mitochondrial membrane by a specific carrier. Carnitine palmitoyltransferase is an essential component in the major energy-producing pathway of muscle.

In the present patient, carnitine palmitoyltransferase activity was reported as low but not as low as in typical carnitine palmitoyltransferase deficiency, making it doubtful that this case demonstrated true carnitine palmitoyltransferase deficiency. Be that as it may, the combination of all these factors has produced an unusual LD isoenzyme pattern.

References