FIG. 1. Schematic representation of mitochondrial metabolism. Respiratory chain complexes or components encoded exclusively by the nuclear genome are light orange. Complexes containing some subunits encoded by the nuclear genome and others encoded by mitochondrial DNA are dark orange. CPT, carnitine palmitoyltransferase; PDHC, pyruvate dehydrogenase complex; CoA, coenzyme A; TCA, tricarboxylic acid; CoQ, coenzyme Q; Cyt c, cytochrome c. (Modified from Ref. 12, with permission of McGraw-Hill, New York.)

provokes cramps that are relieved by rest. PFK deficiency can also cause compensated hemolytic anemia, jaundice, and gouty arthritis, which may be helpful in making the diagnosis. Other clinical presentations include hemolytic anemia without myopathy, fixed weakness, and severe and often fatal infantile myopathy sometimes associated with encephalopathy. Laboratory studies generally reveal elevated serum CK, bilirubin, uric acid levels, and reticulocytosis. EMG shows myopathy with irritative features. Inheritance is AR, with men predominantly affected. In the United States, most patients have been of Ashkenazi Jewish origin. Muscle biopsy reveals accumulation of normal-appearing subsarcolemmal and intermyofibrillar glycogen and “pockets” of an abnormal polysaccharide that stains intensely with PAS but is resistant to diastase digestion. Ultrastructurally, this abnormal glycogen has a granular and filamentous appearance, similar to that in branching enzyme deficiency.

PFK (ATP:d-fructose 1-phosphotransferase) is a tetrameric enzyme. The three subunit forms are M (muscle), L (liver), and P (platelet). Muscle exclusively contains the M isof orm, whereas erythrocytes contain both the L and M subunits. PFK deficiency causes a block distal to glucose and fructose metabolism that is not improved by administration of those substances. PFK-deficient patients depend on free fatty acids and ketones for ATP generation; therefore, exercise intolerance is worsened by high-carbohydrate meals that lower the blood levels of free fatty acid and ketones (25). The negative effect of glucose has been aptly described as an “out-of-wind” phenomenon (25). At least 12 distinct mutations in the muscle phosphorylase subunit have been identified (6). Treatment is problematic as in myophosphorylase deficiency because glucose and fructose are not usable substrates.

Type VIII: Phosphorylase β Kinase Deficiency

Phosphorylase β kinase (PKβ) deficiency is associated with four distinct phenotypes based on the mode of inheritance and tissue involvement: liver disease, typically a benign condition of infancy or childhood with hepatomegaly, growth retardation, delayed motor development, fasting hypoglycemia, and usually inherited as X-linked trait; liver and muscle disease with a static myopathy inherited as an AR trait; myopathy alone, inherited in an AR or X-linked recessive pattern; and fatal infantile cardiomyopathy. In patients with myopathy, serum CK is variably increased (9,26). EMG reveals myogenic abnormalities. Muscle biopsy reveals subsarcolemmal glycogen predominantly in type IIb fibers. Ultrastructurally, the glycogen is free in the cytoplasm and appears normal.

PKβ is composed of the subunits α, β, γ, and δ and acts on two enzymes, glycogen synthetase and phosphorylase. Specifically, it converts phosphorylase from the less active β form to the more active α form while converting glycogen synthetase from the more active dephosphorylated form to a less active phosphorylated form. The genes encoding the PKβ subunits have been cloned, and the molecular defects are under investigation. One 48-year-old man with distal limb weakness and low muscle enzyme had a point mutation in the muscle-specific α-subunit gene that converted a codon for glutamic acid to a stop codon (27). No specific therapy has
been effective in this disorder; however, a high-protein diet may be helpful for reasons similar to myophosphorylase deficiency (22).

Type IX: Phosphoglycerate Kinase Deficiency

Phosphoglycerate kinase deficiency can be clinically asymptomatic or present with myopathy in association with hemolytic anemia, mental retardation, and seizures (28). Isolated myopathy with intolerance to vigorous exercise, cramps, and myoglobinuria, was reported in three patients (6). Laboratory findings include a variably increased resting serum CK level and a normal EMG. Muscle biopsy generally shows nonspecific morphologic changes and a normal glycogen content. Muscle phosphoglycerate kinase activity levels are decreased in patients. Phosphoglycerate kinase deficiency is an X-linked recessive disorder. Two different point mutations in this single polypeptide enzyme have been identified in patients with myopathy: a missense mutation and a splice junction mutation (6).

Type X: Phosphoglycerate Mutase Deficiency

Phosphoglycerate mutase (PGAM) deficiency has been identified in only 11 patients; all but 3 were African American (6). Symptoms generally include intolerance to strenuous exercise, cramps, and recurrent myoglobinuria. Muscle biopsies can be normal or show diffuse or patchy increased glycogen accumulation. PGAM activity in muscle tissue ranged from 2% to 6% of normal. The enzyme is a dimer composed of muscle-specific (M), brain-specific (B), or both isoforms. In patients with myopathy, mutations affect the M isof orm. Cardiac muscle expresses both isoforms; hence, the BB isozyme protects the heart from symptoms in PGAM-M deficiency. The disease is transmitted in an AR pattern. Three point mutations have been identified in the PGAM-M gene (29,30). Interestingly, six of seven African American patients were found to be homozygous for a nonsense mutation in codon 79, whereas the seventh patient was a compound heterozygote for that mutation and a missense mutation. By contrast, two Italian patients were homozygous for a third missense mutation.

Type XI: Lactate Dehydrogenase Deficiency

Muscle lactate dehydrogenase (LDH-A) deficiency has been associated with exercise intolerance and myalgia after intense exercise, often followed by myoglobinuria. The first reported patient had serum LDH levels that did not rise proportionally with CK levels during a bout of myoglobinuria (31). Three affected women developed stiffness of the uterine muscle at the onset of delivery necessitating cesarean section, whereas a few patients had a dermatologic disorder characterized by follicular papules and erythematous patches (32). LDH is a tetrameric enzyme comprised of LDH-A and LDH-B subunits. The M4 tetramer predominates in skeletal muscle. In LDH-deficient patients with myopathy, the low residual LDH activity in muscle, about 5% of normal, is due to the small amount of retained LDH-B tetramers. Point mutations, splice junction and microdeletions in LDH-A have been identified in patients (32).

Type XII: Aldolase A Deficiency

In 1996, aldolase A deficiency was identified in a 4-year-old boy with exercise intolerance, mild weakness, developmental delay, hemolysis, and repeated bouts of rhabdomyolysis during febrile illnesses (33). The muscle biopsy did not reveal excess glycogen by histochemistry; however, it did show an aldolase activity level of 3.8% of normal by biochemical analysis and a homozygous point mutation was identified.

Forearm Ischemic Exercise Test

The forearm ischemic exercise test is useful in corroborating the diagnosis of defects in the glycogenolytic or the glycolytic pathways that impair lactate production during ischemic exercise. The protocol, as described by DiMauro and Bresolin (21), is shown in Table 2. An indwelling sterile needle is placed in a superficial antecubital vein and a baseline sample of blood is drawn for serum lactate, ammonia, and CK levels. A sphygmomanometer cuff is placed above the elbow and inflated to about 20 mm Hg above the systolic blood pressure. The patient vigorously squeezes a rolled-up sphygmomanometer cuff.

**TABLE 2. Forearm ischemic exercise test**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Explain the procedure to the patient.</td>
</tr>
<tr>
<td>2.</td>
<td>Insert an indwelling sterile needle into the patient's antecubital vein. We prefer to use 23-gauge butterfly needles. Collect blood for measurements of lactate, creatine kinase, and ammonia.</td>
</tr>
<tr>
<td>3.</td>
<td>Place a sphygmomanometer cuff above the patient's elbow and inflate to about 20 mm Hg above the systolic blood pressure. In children, inflate to, but not above, the mean arterial pressure.</td>
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<tr>
<td>4.</td>
<td>With constant encouragement, have the patient intermittently open and clench his or her fist for 1 min. We prefer to have the patient squeeze a rolled-up sphygmomanometer cuff. The patient should be encouraged to push the mercury column up as high as possible with each squeeze.</td>
</tr>
<tr>
<td>5.</td>
<td>After 1 min of exercise, the blood pressure cuff is deflated and the patient rests.</td>
</tr>
<tr>
<td>6.</td>
<td>If the patient develops a cramp, tell the patient to stop exercising immediately and deflate the sphygmomanometer cuff to avoid excess muscle necrosis.</td>
</tr>
<tr>
<td>7.</td>
<td>Blood samples, drawn at 1, 3, 6, and 10 min after exercise and placed on ice, are sent for lactate, ammonia, and creatine kinase measurements.</td>
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</table>
ter cuff, pushing the mercury column to the top of the scale. After a minute of exercise, which can cause discomfort even in normal individuals and requires constant encouragement by the observer, the blood pressure cuff is deflated. Blood samples are each obtained at 1, 3, 6, and 10 minutes after cessation of exercise. In normal subjects, there is a three- to fivefold increase in blood lactate in the first two samples with a gradual decline to baseline level in later ones that does not generally occur in those with defects of the glycolytic pathway. Serum ammonia should normally rise three- to fivefold after exercise in both normal subjects and in patients with a glycogen metabolism defect. Patients with myophosphorylase deficiency generally show less than 1.5-fold increase in venous lactate levels (21). Many patients also develop muscle cramping during exercise and should immediately stop while the examiner deflates the sphygmomanometer cuff to reduce the risk of muscle necrosis. False-positive results arise because of suboptimal effort that may be suggested by the lack of rise in ammonia after ischemic exercise. If the patient’s lactate rises after exercise but the ammonia fails to rise significantly, then the diagnosis of myoadenylate deaminase deficiency can be considered. The forearm ischemic exercise test can be difficult in children. For that reason, Bruno et al. (34) use a modified forearm semischemic protocol inflating the sphygmomanometer cuff to the mean arterial pressure, which was reliable in patients ranging from ages 7 to 16 years.

DEFECTS OF LIPID METABOLISM

Lipids are the most important and efficient fuel source in the body. Fatty acids are vital during periods of fasting, particularly when liver glycogen stores are depleted a few hours after a meal. They serve three main functions: their partial oxidation in the liver produces ketones that are an important auxiliary fuel for almost all tissues and especially the brain; they provide a major energy source in cardiac and skeletal muscle, particularly during rest and during prolonged exercise; and ATP produced from fatty acid oxidation provides energy for gluconeogenesis and ureagenesis. The normal fatty acid oxidation pathway is outlined in Fig. 2. Adipocytes liberate free fatty acids that are bound to serum albumin or incorporated into triglyceride-rich lipoproteins and transported to other tissues. Short 4-carbon- and medium 8-carbon-chain fatty acids freely cross the outer and inner mitochondrial membranes into the mitochondrial matrix where they are metabolized to CoA esters before beta-oxidation. By contrast, the mitochondrial membranes are impermeable to long-chain fatty acids (LCFA); therefore, at the outer mitochondrial and endoplasmic reticulum membranes, LCFA must first be converted to CoA-thioesters by long-chain acyl-CoA synthetase. To cross the inner mitochondrial membrane, the very-long-chain acyl-CoA (VLCA-CoA) and long-chain acyl-CoA (LCA-CoA) molecules are converted into acylcarmitine with release of free CoA by CPT I located on the inner side of the outer mitochondrial membrane. Carnitine:acylcarmitine translocase transports very long and long acylcarmitine across the inner mitochondrial membrane. Within the mitochondrial matrix, CPT II bound to the inner surface of the inner mitochondrial membrane exchanges CoA for carnitine to reform VLCA-CoA and LCA-CoA and liberate carnitine, which is shuttled back into the cytoplasm by the translocase.

VLCA-CoA, 14 to 24 carbon atoms in length, is processed by VLCA-CoA dehydrogenase bound to the inner mitochondrial membrane that creates 2-trans-enoyl-CoA molecules. The VLCA-CoA molecules are processed by a trifunctional enzyme, which is also bound to the inner mitochondrial membrane and possesses three beta-oxidation enzymatic activities: long-chain enoyl-CoA hydratase, long-chain-1-hydroxyacyl-CoA dehydrogenase, and long-chain thiolase enzymes. The process shortens the acyl-CoA by two carbon molecules.

Acyl-CoAs of 4 to 18 carbons in length are oxidized by the beta-oxidation pathway in the mitochondrial matrix. Each cycle the beta-oxidation system shortens the acyl-CoA by two carbon fragments through four catalytic steps: acyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, 1,3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase. The acetyl-CoA moiety produced at each turn of the beta-oxidation spiral enters the Krebs cycle. There are three different mitochondrial matrix dehydrogenase enzymes, namely, short-chain acyl-CoA dehydrogenase, which acts on fatty acid of 4 to 6 carbon atoms; medium-chain acyl-CoA dehydrogenase (MCAD) for substrates of 4 to 14 carbon atoms, and long-chain acyl-CoA dehydrogenase for substrates of 10 to 18 carbon atoms. Defects of fatty acid oxidation can occur at various points along the pathway from the transport of fatty acid into mitochondria to the beta-oxidation cycle as described below.

Carnitine Deficiency

L-Carnitine or 3-hydroxy-4-N-trimethylammoniobutanoate is a vital molecule for the transport of LCFA into mitochondria. Other physiologic functions of L-carnitine include buffering of acyl-CoA:CoASH ratio, scavenging of potentially toxic acyl groups, and oxidation of branched-chain amino acids (35). About 75% of L-carnitine is derived from dietary sources, whereas the rest is synthesized in the liver and kidney; 95% of the total body carnitine is stored in muscle.

Primary deficiency of L-carnitine is manifested in three phenotypic forms: dilated cardiomyopathy, myopathy, and hypoketotic hypoglycemia with recurrent encephalopathy. Patients frequently show overlapping phenotypes. The age at onset of symptoms ranges from 1 month to 7 years with a mean of 2 years (4). The cardiomyopathy is progressive and rapidly fatal unless treated with L-carnitine supple-