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HKCNDP

The Hong Kong Society of
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ANNUAL SCIENTIFIC MEETING

16 - 19 November 2007

Hong Kong

Energy Crisis of Nervous System

Table of Contents

Course Director

Dr. Ingrid Tein is curently an Associate Professor of Paediatrics at the University of Toronto and Director of the Neurometabolic Clinic, Neuroinvestigational Unit and Neurometabolic Research Laboratory at the Hospital for Sick Children (HSC). She also is a Senior Scientist in the Research Institute at HSC. Dr. Tein holds a cross-appointment in the Department of Laboratory Medicine and Pathobiology at the University of Toronto for the teaching and supervision of graduate students.

Dr. Tein obtained her BSc and MD degrees at the University of Toronto. She then completed her residency in Paediatrics followed by a residency in Paediatric Neurology at HSC. Following this, she completed four years of post-doctoral reseach training in fatty acid oxidation disorders beginning at the Clinique et Unite de Recherche Genetique Medicale at the Hopital Necker-Enfants Malades, Faculte de Medecine, Inserm U75 in Paris for one year under the supervision of Professor Agr. J.M. Saudubray. This was followed by three years in the Department of Neurology at Columbia University, New York under the joint supervision of Dr. Darryl DeVivo and Dr. Salvatore DiMauro.

Dr. Tein currently directs the Neurometabolic Clinic and Neuroinvestigational Unit for the investigation, management and treatment of children with a variety of neurometabolic diseases including mitochondrial disorders, fatty acid oxidation defects, peroxisomal disorders, glycolytic/glycogenolytic defects, organic acidurias, etc. The Neuroinvestigational Unit includes prospective multidisciplinary cross-over cofactor trials in children with mitochondrial disorders and the investigation of metabolic myopathies.

Dr. Tein also directs the Neurometabolic Research Laboratory whose fundamental mandate is the biochemical and molecular characterization of genetic defects of fatty acid oxidation including most importantly the plasmalemmal carnitine transporter defect, short- and long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiencies, short-chain acyl-CoA dehydrogenase deficiency and carnitine palmitoyltransferase deficiency. Another key mandate of the lab is to develop in vitro models to examine the mechanisms of cellular toxicity in fatty acid oxidation defects for the development of novel treatment strategies to reverse the clinical pathology. She runs an active training programme for post-doctoral fellows and graduate students.

Dr. Tein is also an active member of the Executive Board of the International Child Neurology Association since 2002 for the promotion of excellent standards in clinical practice in Child Neurology, global education and research collaborations around the world. She serves as Chair of the Oversight Committee for the development of the Child Neurology Knowledge Environment and is Chair of the Scientific Programme Committee for the XIth International Child Neurology Congress in Cairo, May 2010. In addition she serves on the Research Task Force and the Editorial Board of the International Review of Child Neurology Book Series.

PROGRAMME-AT-A-GLANCE

Date	Time	Session	Topic	Speaker
16 Nov (FRI)	1800 - 2000	*Registration and Light Buffet Dinner*		
	2000 - 2200	Seminar I	Approach to Fatty Acid Oxidation Disorders. Novel Neuromuscular Presentations and Treatments	Dr. Ingrid Tein
17 Nov (SAT)	1230 - 1400	*Registration and Light Buffet Lunch*		
	1400 - 1500	Seminar II	Primary and Secondary Disorders of Carnitine Metabolism	Dr. Ingrid Tein
	1500 - 1530	*Tea Break*		
	1530 - 1600	Local Presentation I	Local Scenario of Neurometabolic Diseases - Experience of a Tertiary Referral Center	Dr. Cheuk-wing Fung
	1600 - 1700	Seminar III	Approach to the Diagnosis and Management of Mitochondrial Disorders Part I	Dr. Ingrid Tein
18 Nov (SUN)	0900 - 0930	*Registration*		
	0930 - 1030	Seminar IV	Approach to the Diagnosis and Management of Mitochondrial Disorders Part II	Dr. Ingrid Tein
	1030 - 1130	Free Paper Session	Oral Presentations of Free Papers	
	1130 - 1200	*Tea Break*		
	1200 - 1300	Local Presentation II	Laboratory Diagnosis of Neurometabolic and Other Common Inherited Metabolic Diseases Genetic Study of CNS Diseases	Professor Nelson Tang Dr. Ivan Lo
	1300 - 1430	*Light Buffet Lunch*		
	1430 - 1600	Case Presentation	Case Discussions by Hospital Neurology / Neurometabolic Teams	
	1600 - 1630	*Tea Break*		
	1630 - 1710	Seminar V	Application of Exercise Physiology Testing to the Diagnosis of Metabolic Myopathies	Dr. Ingrid Tein
19 Nov (MON)	1830 - 1900	*Registration*		
	1900 - 2000	Keynote Lecture	Approach to the Diagnosis and Management of Muscle Cramps, Exercise Intolerance and Recurrent Childhood Myoglobinuria	Dr. Ingrid Tein
	2000 - 2200	*Chinese Banquet*		

Venues:

16 November 2007: Lecture Theatre, 12/F., Block R, Queen Elizabeth Hospital, Jordan

17 - 18 November 2007: Lecture Theatre, G/F., Block M, Queen Elizabeth Hospital, Jordan

19 November 2007: Centenary Ballroom, G/F., Marco Polo Hong Kong Hotel, Tsim Sha Tsui

Approach to Fatty Acid Oxidation Disorders. Novel Neuromuscular Presentations and Treatments

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Defects in fatty acid oxidation (FAO) are an important group of disorders because they are potentially rapidly fatal and a source of major morbidity encompassing a spectrum of clinical disorders including recurrent myoglobinuria, progressive lipid storage myopathy, progressive hypertrophic and/or dilatative cardiomyopathy, cardiac arrhythmias, recurrent hypoglycemic hypoketotic encephalopathy or Reye-like syndrome, seizures, pigmentary retinopathy, neuropathy and mental retardation. Pathologically, they may present with microvesicular steatosis of the liver and muscle and endocardial fibroelastosis of the heart. Acute metabolic decompensations are often triggered by precipitating factors such as infection with vomiting, fever, cold exposure with shivering thermogenesis, fasting, prolonged exercise and emotional stress. As all of the known conditions are inherited as autosomal recessive diseases, there is oftentimes a family history of sudden unexpected death or SIDS in siblings, and thus screening of family members is critically important. The early recognition and prompt institution of therapy as well as the institution of appropriate preventative measures, and in certain cases specific therapy, may be life-saving and may significantly decrease long-term morbidity, particularly with respect to secondary central nervous system sequelae. In addition, heterozygous mothers carrying infants who are homozyous or compound heterozygous for a defect in the trifunctional protein or long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD), carnitine palmitoyltransferase I (CPT I), medium-chain acyl-CoA dehydrogenase (MCAD) or short-chain acyl-CoA dehydrogenase (SCAD) enzymes may develop liver disease such as acute fatty liver of pregnancy or HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome. The first FAO enzyme defect was reported in 1973 by DiMauro, namely carnitine palmitoyltransferase II (CPT II) deficiency which presented with recurrent myoglobinuria. There are now at least 18 recognized enzyme defects in FAO, most of which have been diagnosed in the last 15 years. With each newly described disorder and with significant advances in biomedical technology, there has been a rapid increase in the number of subsequently diagnosed cases. In a recent survey from the Pennsylvania newborn screening program using tandem mass spectrometry and confirmed by molecular analysis for several mutations, the incidence for medium chain acyl-CoA dehydrogenase (MCAD) deficiency was found to be 1 in 8,930 live births (Ziadeh et al 1995).

There are at least four clinical and laboratory features that should lead the clinician to suspect a genetic defect in FAO: (1) acute metabolic decompensation in association with fasting, (2) chronic involvement of tissues highly dependent on efficient FAO (e.g., heart, muscle, liver), (3) recurrent episodes of hypoketotic hypoglycemia, and (4) alterations in the quantity of total carnitine or of the percentage of esterified carnitine in plasma and tissue. Children with FAO defects are most prone to decompensation, within the context of depleted glycogen and glucose reserves, during conditions that place stress on the FAO pathway for fuel generation. These conditions include prolonged exercise (particularly after 1 hour of mild to moderate aerobic exercise), fasting, infection with vomiting, and cold-induced shivering thermogenesis. In cold exposure, ketogenesis is stimulated in normal individuals and shivering, which is an involuntary form of muscle activity, is also dependent on long-chain FAO. After an overnight fast, children are most likely to be found comatose in the early morning hours. During infection, there may be an added problem with vomiting and decreased oral intake. Children may also present with a Reye-like syndrome. Infants and younger children are at greater risk during fasting because of their decreased abilities for fasting adaptation.

Tissues such as skeletal muscle, heart, and liver have high energy demands and are therefore highly dependent on efficient FAO. When there is a defect in hepatic ketogenesis, glucose becomes the only available fuel and thus becomes rate-limiting under conditions of FAO stress when glycogen and glucose stores have been depleted. As a result, free fatty acids, which are liberated during fasting and cannot be metabolized because of the block, may be stored in the cytosol as triglycerides. This produces a progressive lipid storage myopathy with weakness, a hypertrophic and/or dilatative cardiomyopathy, and a fatty liver. Increased content of short- or medium-chain fatty acids and in particular their dicarboxylic metabolites, from compensatory omega oxidation, may cause a variety of secondary biochemical abnormalities, including an impairment of gluconeogenesis, ß-oxidation, and the citric acid cycle, leading to a further decrease in

cellular ATP production. In addition, in the long-chain FAO disorders, which may have recurrent episodes of acute muscle breakdown or myoglobinuria (e.g., CPT II, VLCAD, trifunctional enzyme deficiencies), the accumulation of long-chain fatty acids and long-chain acylcarnitines may have detergent-like actions on muscle membranes. Excessive amounts of palmitoyl-CoA and palmitoylcarnitine have detergent properties on isolated canine myocytic sarcolemmal membranes and potentiate free radical-induced lipid membrane peroxidative injury in ischemia. Long-chain acylcarnitines also activate calcium channels in cardiac and smooth muscle myocytes. They may thus potentiate the increase in cytosolic calcium associated with arrhythmogenesis, as seen in ischemic myocardium.

The pattern of hypoketotic hypoglycemia reflects the accelerated rate of glucose utilization that occurs when fatty acids cannot be used as fuels and ketone bodies are not generated to spare glucose/glycogen stores. An increase in the ratio of serum free fatty acids to ketones from the normal ratio of 1:1 to greater than 2:1 therefore would suggest a block in beta oxidation.

In most cases of intramitochondrial beta oxidation defects, the total carnitine concentration is decreased (<50% or normal), and the acylcarnitine fraction is increased (>50% esterified; normal is 10% to 25% in the fed state and 30% to 50% in the fasted state). In the intramitochondrial ß-oxidation disorders, the excessive acyl-CoAs that accumulate proximal to the metabolic block may be converted into acylcarnitines by chain length-specific carnitine acyltransferases. These acylcarnitines when filtered through the kidneys compete with free carnitine at the renal tubular reabsorptive site. Because the longer chain-length acylcarnitines have an increasingly higher affinity for the carnitine transporter than does free carnitine, the free carnitine will be excreted, leading to a decrease in free carnitine in the serum. In the case of the plasmalemmal carnitine transporter defect, the total carnitine is markedly reduced (e.g., <5% of normal) and the esterified fraction is normal because the transporter defect, which is also expressed in kidney, leads to a decreased renal threshold for carnitine reabsorption. In contrast, in CPT I deficiency, the total plasma carnitine concentration may be normal or increased with a decreased esterified fraction because the esterification of palmitate to carnitine is defective.

During acute catabolic crises, other biochemical derangements may be noted. A modest hyperammonemia accompanied by threefold to fivefold elevations of liver transaminases may be documented during the Reye-like syndrome presentation. In acute myoglobinuria there are marked increases in the sarcoplasmic enzymes, including serum CK, which may rise higher than 100,000 IU/L (normal < 250 IU/L). The serum concentrations of amino acids (especially taurine), creatinine, potassium, phosphate, and urate may also be increased. The hyperphosphatemia may lead to secondary hypocalcemia. These changes may have deleterious renal and cardiac effects, thereby further exaggerating the damage. During acute myoglobinuria, one most be careful to monitor for renal failure, respiratory failure and cardiac arrhythmias. Lactic acidosis may also be noted during the acute catabolic presentations. This may reflect either poor tissue perfusion or inhibition of critical enzymes, such as pyruvate carboxylase, by accumulated metabolites. Urine organic acid screening may demonstrate unusual or excessive amounts of organic acids, which may be diagnostic of a specific ß- oxidation block.

In terms of the approach to investigation, if the child arrives in the emergency room with an acute decompensation, it is important to obtain blood and urine samples, prior to the institution of intravenous glucose. It is most important to obtain a serum glucose, free fatty acid to ketone body ratio, lactate, serum carnitine (total and free) and serum acylcarnitines. Urine should be obtained for determination of urine ketones, organic acids, acylglycines and acylcarnitines. The chain length and species-type of the serum acylcarnitines, urinary organic acids and urinary acylglycines may help to identify the specific site of FAO block and the chain-length specificity of the defect. Loading tests with L-carnitine and the measurement of specific metabolites may also be helpful. Depending on the suspected site of defect, a direct enzymatic assay may then be performed for the specific enzyme in cultured skin fibroblasts or in biopsied muscle or liver. Following this, molecular mutation analysis can be done to identify homozygotes and carriers.

General treatment approaches include the strict avoidance of precipitating factors such as prolonged fasting, prolonged aerobic exercise (>30 minutes), and cold exposure leading to shivering thermogenesis. Prolonged fasting would be 6 to 10 hours for the infant younger than 1 year of age or 12 hours for the

child between 1 and 4 years of age. In the event of progressive lethargy or obtundation or an inability to take oral feedings because of vomiting, the child should be taken immediately to the emergency room for intravenous glucose therapy. Intravenous glucose should be provided at rates sufficient to prevent fatty acid mobilization (8 to 10 mg/kg/min glucose infusion). This regimen should be continued until the catabolic cascade has been reversed and the child is able to take oral feedings again. It is wise to avoid prolonged exercise (> 30 minutes) because during this time there is increased fat mobilization. A high-carbohydrate load before exercise is advisable with a rest period and repeat carbohydrate load at 15 minutes. Avoidance of cold exposure is essential.

In general, it is advisable to institute a high-carbohydrate, low-fat diet with frequent feedings throughout the day, which would be commensurate with the nutritional needs of the child given his or her age. This goal is best achieved with the aid of a dietitian, aiming toward approximately 70% to 75% of calories from carbohydrate sources, 15% from protein, and approximately 10% to 15% from fat. Monitoring of essential fatty acid levels is important to ensure that the child is receiving adequate essential fatty acids, which may require supplementation. Augmentation of the diet with essential fatty acids (at 1-2% of total energy intake) is often used to reduce the risk of essential fatty acid deficiency. Flaxseed, canola, walnut or safflower oils can be used for this purpose. An older child should have three regular meals per day with three equidistantly placed intermeal snacks, including a bedtime snack. In younger children, oral or nasogastric tube administration of an appropriate formula is indicated. In HMG-CoA lyase deficiency a high-carbohydrate, low-fat, low-protein diet with leucine restriction should be implemented. To delay the onset of fasting overnight in children who manifest symptoms of early morning hypoglycemia, the nightly institution of uncooked corn starch will prolong the postabsorptive state and delay fasting. Cornstarch provides a sustained release source of glucose, thereby preventing hypoglycemia and lipolysis. Cornstarch is usually initiated at 8 months of age when pancreatic enzymes are first able to function at full capacity for appropriate absorption.

Other specific therapies apply to specific FAO defects. For example, the essential indication for carnitine therapy is the high-affinity plasmalemmal carnitine transporter (OCTN2) defect, which is characterized by carnitine-responsive cardiomyopathy, myopathy, recurrent encephalopathy and very low plasma and tissue concentrations of carnitine (generally <5% of normal) in which life-long high-dose oral L-carnitine therapy is life-saving and reverses the pathology in this previously lethal autosomal recessive disease. In the intramitochondrial ß-oxidation defects with secondary carnitine deficiency, the results of carnitine therapy have been highly variable and insufficiently evaluated. Theoretically, carnitine has been given to limit the intracellular concentrations of potentially toxic acyl-CoA intermediates within the cell through transesterification and to thereby liberate CoA, which is a critical intracellular cofactor. However, there has been no objective prospective study to prove that carnitine administration has had a beneficial effect. Furthermore, there is increasing evidence to suggest that carnitine administration may have deleterious effects in the long-chain FAO disorders. In these disorders there is an accumulation of long-chain acyl-CoAs proximal to the metabolic block, which upon esterification become long-chain acylcarnitines. Excessive palmitoylcarnitine may have detergent effects on membranes and arrhythmogenic effects. This field warrants further investigation.

In addition, the daily oral administration of a cod liver oil extract containing high amounts of docosahexaenoic acid (essential polyunsaturated fatty acid) led to marked clinical and electrophysiological recovery of the progressive peripheral sensorimotor axonopathy in one boy with the myoneuropathic form of long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency.

All known FAO disorders are inherited as autosomal-recessive conditions. Therefore, screening for other affected siblings is important because institution of preventive measures is relatively simple, and without treatment there is significant morbidity and mortality. Although screening of family members following identification of an affected individual is of benefit to the family, this approach fails to identify individuals who do not belong to this high-risk group. Tandem mass spectrometry has made screening possible for most FAO defects based upon the profiling of acylcarnitine in newborn blood spots. The inclusion of FAO disorders in screening programs is highly desirable to prevent morbidity and mortaliy, implement preventative measures and treatment strategies and to contain the cost of care of affected patients.

Primary and Secondary Disorders of Carnitine Metabolism

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L-carnitine is a small water soluble molecule that is essential for the metabolism of long-chain fatty acids. It also plays an important role in facilitating branched chain alpha-ketoacid oxidation, shuttling acyl-CoA conjugates from peroxisomes to mitochondria, modulating the intramitochondrial ratio of acyl-CoA to free coenzyme A, and for the esterification of potentially toxic acyl-CoA metabolites which may impair the citric acid cycle, gluconeogenesis, the urea cycle and fatty acid oxidation in acute clinical crises. In non-vegetarians, approximately 75 % of carnitine comes from the diet, principally from red meat and dairy products, and 25 % from endogenous biosynthesis from the two essential amino acids, lysine and methionine, and this process occurs in the liver, kidney and brain. Human milk and most milk-based infant formulas contain adequate amounts of carnitine to sustain early growth and development. Soy bean protein-derived formulas are deficient and serum carnitine concentrations are lower in infants maintained on these feedings unless supplemented with L-carnitine. The plasma carnitine concentration is largely regulated by the renal threshold for this quaternary amine (approximately 40 μmol/L). Approximately 90 % of body carnitine stores are contained within the skeletal muscle and the muscle concentration is approximately 70 times higher than the serum concentration. Tissue carnitine concentrations parallel the capacity of the tissue to metabolize fatty acids.The human tissue concentrations (nmol/g) are estimated as follows; heart (3500-6000) > muscle (2000-4600) > liver (1000-1900) > brain (200-500). Thus carnitine must be transported across a large concentration gradient into the cell by the high-affinity plasma membrane organic cation/carnitine transporter known as OCTN2 which has a binding affinity (Km) for carnitine of 2-6 μM. The serum carnitine concentrations are influenced by age and sex. The values are lower in infants and in females. The total serum carnitine concentrations in healthy individuals range from 30-89 μmol/L with the concentrations in males being ~ 60 μmol/L and in females ~50 μmol/L. The plasma total carnitine concentrations in preterm infants less than 36 weeks postconception are about 30 μmol/L. The total carnitine is the sum of the free and bound fractions. Most clinical investigators consider a free carnitine concentration of < 20 μmol/L after the first week of life or an esterified to free ratio of greater than 0.40 to be abnormal and indicative of relative carnitine deficiency. The etiologies of carnitine deficiency include factors that influence availability and elimination. Carnitine availability is determined by the dietary intake and biosynthesis. Elimination is determined by the formation of acyl conjugates and urinary excretion. Carnitine is not metabolized by mammalian cells.The analysis of serum acylcarnitines by electrospray tandem mass spectrometry has been a very useful tool for the detection of a number of inborn errors of metabolism.

The role of L-carnitine in diverse conditions affecting intermediary metabolism has been defined in recent years. The pathophysiological states associated with carnitine deficiency can be divided into primary and secondary disorders. The primary disorder is due to a genetic defect in the high-affinity plasmalemmal carnitine transporter OCTN2, which results in a profound reduction in serum and tissue carnitine concentrations (often < 5 % of control values) and an inappropriate renal leak of carnitine due to the defective transporter in the kidneys. This is a formerly lethal autosomal recessive disorder of childhood that is characterized by early onset episodic hypoglycemic hypoketotic encephalopathy, progressive lipid storage myopathy with weakness and hypotonia, progressive hypertrophic/dilatative cardiomyopathy, failure to thrive, apathy and anemia which is exquisitely reversible by life-long high dose oral L-carnitine supplementation (100 mg/kg/day) in which L-carnitine therapy is critical and life-saving. The secondary carnitine deficiency disorders are characterized by a less striking decrease in total or free serum carnitine concentrations and often an increase in the esterified to free ratio > 0.4. The secondary forms are more heterogeneous and include underlying genetically-determined metabolic diseases, acquired medical conditions and iatrogenic factors such as drug therapy. Inborn errors of metabolism that result in carnitine deficiency may be due to increased esterification resulting from acyl-CoA accumulation and include intramitochondrial fatty acid oxidation defects and organic acidemias; decreased biosynthesis as seen in homocystinuria and adenosine deaminase deficiency; and increased urinary loss as seen in cystinosis and cytochrome oxidase deficiency. Secondary carnitine deficiency in acquired medical conditions may be due to decreased biosynthesis as in cirrhosis, chronic renal disease and extreme prematurity; dietary deficiency such as chronic TPN (without L-carnitine supplementation), malabsorption (e.g. cystic fibrosis), and unsupplemented soybean protein-derived infant formula; decreased body stores as seen in extreme prematurity, intrauterine growth retardation and infant of a carnitine-deficient mother; and increased urinary loss as seen in Fanconi syndrome and renal tubular acidosis. Iatrogenic factors include increased esterification and impaired hepatic biosynthesis which may be seen with chronic valproic acid administration as well as increased loss which may occur in chronic hemodialysis. Decreased biosynthesis and dietary deficiency are often combined factors. L-carnitine supplementation in the secondary deficiency disorders is generally recommended, with the exception of the long-chain mitochondrial fatty acid oxidation defects where it may have deleterious effects, and will vary according to the degree of the deficiency. The efficacy of L-carnitine supplementation is debated in the secondary carnitine deficiency states, but clinical judgement suggests that hypocarnitinemia should be corrected.

Approach to the Diagnosis and Management of Mitochondrial Disorders Part I & Part II

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Although all of the mitochondrial myopathies that are expressed in muscle are at potential risk for the development of myopathy and exercise intolerance, the specific defects in which acute episodes of myoglobinuria have been documented to date include defects of Complexes I, II, III and IV activities, mtDNA deletions and Coenzyme Q10 deficiency. The following review will summarize and highlight key clinical, morphological, biochemical, genetic and physiological features of this group of disorders.

Clinical Considerations

Clinical heterogeneity is a feature of mitochondrial diseases. There may be variation in the age at onset, course, and distribution of weakness in pure myopathies [DiMauro, 1993]. Additional features may include exercise intolerance and premature fatigue. In the clinical classification there has been controversy between the "lumpers" and the "splitters." Originally, three specific clinical syndromes were described: namely MELAS, MERRF and Kearns-Sayre syndrome, each resulting from three distinct mutations in mtDNA. All three share the features of short stature, dementia, sensorineural hearing loss, lactic acidosis, and ragged red fibres (RRF) [DiMauro, 1993]. Mitochondria and mtDNA are ubiquitous, which explains why every tissue in the body can be affected by mtDNA mutations. The most common presenting clinical features include short stature, sensorineural hearing loss, migraine headaches, ophthalmoparesis, myopathy, axonal neuropathy, hypertrophic cardiomyopathy, diabetes mellitus, and renal tubular acidosis. Additional features may include stroke-like episodes, seizures, myoclonus, retinitis pigmentosa, optic atrophy, ataxia, and gastrointestinal pseudoobstruction. In the case of mtDNA mutations, there may be a diverse spectrum of associated syndromes, even in a single pedigree, because of heteroplasmy and the threshold effect whereby different tissues harboring the same mtDNA mutation may be affected to different degrees or not at all. Furthermore the same mutation can cause different syndromes (e.g. the T8993G mutation can cause either neuropathy, ataxia, retinitis pigmentosa [NARP] or maternally inherited Leigh syndrome [MILS]) and different mutations can cause the same phenotype (e.g. A3243G mutation, single deletion, and multiple deletions of the mtDNA can all cause progressive external opththalmoplegia [PEO]) [Moraes et al., 1993; Vu et al., 2002]. Thus, the diagnosis of mtDNA-related disorders requires a careful synthesis of the clinical history, signs, mode of inheritance, laboratory data, neuroradiological findings, exercise physiology, muscle biopsy, biochemistry and molecular genetics.

The prevalence of mtDNA-related disorders has only been recently estimated and support the conclusion that mitochondrial diseases are among the most common metabolic disorders, at least in northern Europe [Vu et al., 2002]. In northeastern England, mtDNA defects were the cause of disease in 6.57/100,000 adults of working age [Chinnery et al., 2000]. Overall, it was estimated that 12.48/100,000 individuals in the adult and child population either had mtDNA disease or were at risk of developing mtDNA disease. In Western Sweden, the incidence of mitochondrial diseases overall was 1/11,000 pre-school children [Darin et al., 2001]. Certain mtDNA mutations are relatively common, such as the A3243G MELAS mutation in northern Finland which was estimated to occur in 16.3/100,000 in the adult population [Majamaa et al., 1998].

Pathological Considerations

Although the finding of ragged red fibres (RRF) or ultrastructural alterations of mitochondria in muscle biopsy specimens suggests the possibility of a mitochondrial disorder, there are important limitations, as pointed out by DiMauro [1993]:

1) Many primary mitochondrial diseases, such as enzyme defects in metabolic pathways other than the respiratory chain (e.g., the pyruvate dehydrogenase complex [PDHC], CPT, beta-oxidation, and fumarase deficiencies), do not have RRF.
2) Nonmitochondrial disorders, such as muscular dystrophies, polymyositis, and some glycogenoses, may demonstrate RRF or ultrastructural mitochondrial abnormalities in which they represent changes that are likely secondary.

Furthermore, there are defects of the respiratory chain, such as the form of Leigh syndrome secondary to cytochrome c oxidase (COX) deficiency, that tend not to have RRF. Although RRF are most often present in tRNA encoding mtDNA defects, which affect the respiratory chain, protein coding defects, such as neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP), and Leber's hereditary optic neuropathy (LHON), have only subtle mitochondrial changes, without RRF [Uemura et al., 1987]. Furthermore, the appearance of RRF may also depend on the threshold effect (the percentage of mutant mtDNA to wild type mtDNA) and on the stage of the disease. Succinate dehydrogenase (SDH) and COX stains are two other useful histochemical stains. RRF are often COX-negative, although not all COX-negative fibers are RRF [DiMauro, 1993]. COX-negative RRF are seen in patients with progressive external ophthalmoplegia and mtDNA deletions and in MERRF (m yoclonus e pilepsy with r agged-r ed f ibers) syndrome. Conversely, they are not seen in MELAS (m itochondrial e ncephalomyopathy, l actic a cidosis, and s trokelike episodes) syndrome, which usually has COX-positive RRF. On electron microscopy, there may be increased

numbers of mitochondria (pleoconial myopathy), increased size (megaconial myopathy), disoriented or rarefied cristae, or osmiophilic or paracrystalline inclusions [DiMauro, 1993]. Paracrystalline inclusions represent deposits of mitochondrial creatine kinase [Stadhouders et al., 1990]. There may also be lipid or glycogen storage on muscle biopsy, which signifies a defect of terminal oxidation [Jerusalem et al., 1973].

Biochemical Classification

Mitochondrial encephalomyopathies can be classified into five groups [DiMauro, 1993] according to the area of mitochondrial metabolism that is specifically affected: (1) defects of transport, (2) defects of substrate utilization, (3) defects of the Krebs cycle, (4) defects of the respiratory chain, and (5) defects of oxidation/phosphorylation coupling (Figure 80-3) [DiMauro and De Vivo, 1989]. Limitations of this classification scheme relate primarily to the respiratory chain defects that can result from genetic defects of mtDNA, which are usually heteroplasmic, and to deletions of mtDNA or point mutations in tRNA, which affect mtDNA translation as a whole and may lead to multiple respiratory chain defects.

Genetic Classification

Mitochondria are the only subcellular organelles with their own DNA (mtDNA) [Nass and Nass, 1963] that are capable of synthesizing a vital set of proteins. Human mtDNA is a small (16.5-kb), circular, double-stranded molecule that has been completely sequenced [Anderson et al., 1981]. It encodes 13 structural proteins, all of which are subunits of respiratory chain complexes, as well as 2 rRNAs and 22 tRNAs needed for translation. mtDNA has a number of unique features [DiMauro, 1993].

1) It is transmitted by maternal inheritance.
2) Its genetic code differs from that of nuclear DNA (nDNA).
3) It is subject to spontaneous mutations at a higher rate than nDNA.
4) It contains no introns and is therefore tightly packed with information.
5) It has less efficient repair mechanisms than nDNA.
6) It is present in hundreds or thousands of copies per cell.

mtDNA is contributed only by the oocyte in the formation of the zygote [Giles et al., 1980]. Therefore, a mother carrying a mtDNA mutation passes it on to all her children, but only her daughters will transmit it to their progeny. If there is a mutation in some mtDNA in the ovum or zygote, this may be passed on randomly to subsequent generations of cells, some of which will receive primarily or exclusively mutant genomes (mutant homoplasmy); others will receive few or no mutant genomes (normal or wild-type homoplasmy); and still others will receive a mixed population of mutant and wild-type mtDNAs (heteroplasmy).

Maternal inheritance and heteroplasmy have several important implications [DiMauro, 1993].

1) Inheritance of disease is maternal as in X-linked traits, but both sexes are equally affected.
2) Phenotypic expression of a mtDNA mutation will depend on the relative proportions of mutant and wild-type genomes; a minimum critical number of mutant genomes is necessary for expression which is known as the threshold effect. The threshold for disease is lower in tissues that are highly dependent on oxidative metabolism, such as brain, heart, skeletal muscle, retina, renal tubules and endocrine glands. Thus these tissues will be especially vulnerable to the effects of pathogenic mutations in mtDNA.
3) At cell division, the proportion may shift in daughter cells (mitotic segregation), leading to a corresponding phenotypic change. This explains the age-related, and even tissue-related, variability of clinical features frequently observed in mtDNA-related disorders.
4) Subsequent generations will be affected at a higher rate than in autosomal-dominant diseases.

Depending on the specific vulnerability of the tissue to impairments of oxidative metabolism, the critical number of mutant mtDNA needed for the threshold effect may vary. It may also vary according to the vulnerability of the same tissue over time, and this may increase with age [Moraes et al., 1991a; Ozawa, 1995; Wallace et al., 1988b]. Although the mtDNA-encoded peptides are functionally important, they represent only a small proportion of the total mitochondrial protein. Of the approximately 80 proteins that make up the respiratory chain, only 13 are encoded by mtDNA. Complexes I, III, IV, and V contain some subunits encoded by mtDNA; seven for complex 1 (ND1-ND4, ND4L, ND5, and ND6), one for complex III (cytochrome b), three for complex IV (COX 1-COX III), and two for complex V (ATPase 6 and ATPase 8) [DiMauro, 2004]. In contrast, complex II, coenzyme Q and cytochrome c are exclusively encoded by nDNA. Thus the majority of mitochondrial proteins are encoded by nDNA. The nuclear encoded proteins are synthesized in the cytoplasm and then imported into mitochondria. This transport of proteins requires a complex series of postranslational events and translocation machinery. This involves synthesis of larger precursors in the cytosol, amino terminal leader peptides, which function as address signals and recognize specific mitochondrial membrane receptors. This is followed by translocation across the mitochondrial membrane and cleavage of the leader peptides with assembly of mature peptides at their final intramitochondrial location [Schatz, 1991]. Therefore the genetic classification of mitochondrial diseases must take into account defects of nDNA or mtDNA and defects of communication between the two genomes [Vu et al., 2002].

1) Defects of MTDNA

1.1 Defects in Mitochondrial Protein Synthesis
- 1.1.1. mtDNA rearrangements: Single Deletions and Duplications
  - Kearns-Sayre syndrome
  - Sporadic PEO with RRF
- 1.1.2 mtDNA Point Mutations
  - MERRF syndrome
  - MELAS syndrome

1.2 Defects of protein-coding genes
- ATPase 6 mutation
- Leber hereditary optic neuroretinopathy

2) Diseases due to Mutations in nDNA (Mendelian Transmission)

There are numerous disorders due to mutations in nDNA not only because most respiratory chain subunits are nucleus-encoded, but also because the correct structure and functioning of the respiratory chain requires a series of steps, all of which are under the control of nDNA. These steps include the following [DiMauro, 2004];

a) mtDNA integrity and replication requires nDNA-encoded factors and there has been rapid progress in our understanding of the molecular basis of disorders of intergenomic signaling, such as syndromes associated with multiple mtDNA deletions and mtDNA depletion.
b) Hereditary defects in the complex machinery involved in the transport of nDNA-encoded proteins from the cytoplasm into the mitochondria have been documented.
c) nuclear factors are needed for the proper assembly of respiratory chain complexes. Mutations in these ancillary proteins have been associated with numerous disorders, particularly Leigh syndrome.
d) The respiratory chain is embedded in the lipid bilayer of the inner mitochondrial membrane. Alterations of this lipid milieu can lead to disese as seen in Barth syndrome in which there is altered synthesis of cardiolipin.
e) Mitochondria move around the cell, divide by fission and fuse with one another. Disorders of motility can cause disease as seen in autosomal dominant optic neuropathy.

2.1 Mutations in genes encoding enzymes or translocases
- a) Defects of Substrate Transport.
  - OCTN2, CPT I and II deficiency
- b) Defects of Substrate Oxidation.
  - PDHC, Pyruvate carboxylase deficiency
- c) Defects of the Krebs Cycle.
- d) Defects of the Respiratory Chain: Mutations in genes encoding subunits or ancillary proteins of the respiratory chain.
  - Complex I deficiency, Complex II deficiency, Coenzyme Q10 (CoQ10) deficiency
  - Complex III deficiency, Complex IV deficiency, Complex V deficiency
  - Combined defects of the respiratory chain
- e) Defects of oxidation/phosphorylation coupling. e.g. Luft disease

2.2 Defects of mitochondrial protein importation. e.g. *TIMM8a* gene

2.3 Defects of intergenomic dialogue
- a) Multiple Mitochondrial DNA Deletions.
- b) Depletion of Mitochondrial DNA. e.g. TK2 and dGK genes.

2.4 Alterations of the lipid milieu of the inner mitochondrial membrane e.g. Barth's syndrome

2.5 Alterations of mitochondrial motility or fission

Differential Disgnosis of Lactic Acidemia

The ratio of lactate to pyruvate in the blood is a helpful indicator of the underlying problem. It may also help to direct further investigations. A lactate/pyruvate ratio below 25, which is considered to be in the normal range, suggests a defect of either pyruvate dehydrogenase or one of the gluconeogenic enzymes, whereas a consistently elevated lactate/pyruvate ratio, particularly one of 35 or greater, suggests a pyruvate carboxylase (type A) deficiency or a respiratory chain defect [Robinson, et al., 1987, 1989].

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Application of Exercise Physiology Testing to the Diagnosis of Metabolic Myopathies

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The immediate source of energy for contraction and relaxation is provided by the hydrolysis of ATP. Oxidative phosphorylation provides the largest contribution of energy overall, whereas anaerobic glycolysis plays a relatively minor role, primarily limited to conditions of sustained isometric contraction when blood flow and oxygen delivery to exercising muscles are drastically reduced. The dynamic form of exercise, such as walking or running, is primarily dependent on aerobic glycolysis. Therefore the pathophysiology of glycogenoses relates more to the impairment of aerobic than anaerobic glycolysis [Lewis and Haller, 1986; Lewis et al., 1991].

The energy substrates used by muscle for aerobic metabolism depend on the type, intensity, and duration of exercise, as well as on physical conditioning and diet. During intense exercise (close to maximal oxygen uptake, or VO_{2max}, in dynamic exercise or maximal force generation in isometric exercise), energy is derived from anaerobic glycolysis, particularly when there is a burst of activity with rapid acceleration to maximal exercise [DiMauro and Tsujino, 1994]. During low-intensity exercise (below 50% VO_{2max}), the primary source of energy is derived from blood glucose and FFAs. At higher intensities the proportion of energy derived from carbohydrate oxidation increases, and glycogen becomes an important fuel. At 70% to 80% of VO_{2max} the critical energy source is provided by the aerobic metabolism of glycogen, and fatigue occurs when glycogen stores are exhausted [DiMauro and Tsujino, 1994]. Individuals with defective glycolysis/glycogenolysis are most vulnerable during the initial stages of intense exercise, and they must rest soon after beginning exercise because of muscle cramps. However, if they continue to exercise at low intensity, they are able to continue for a longer time. This is known as the *second-wind phenomenon* and has been attributed to a metabolic switch from carbohydrate to fatty acid utilization [Felig and Wahren, 1975] and to increased circulation with increased availability of blood glucose from hepatic glycogenolysis (Haller et al., 1985]. A decrease in ATP levels could first cause muscle contracture. Theoretically a more severe depletion could lead to myoglobinuria [Rowland, 1984], although this has not been proved.

The forearm ischemic exercise test developed by McArdle [1951] is a useful test for the detection of enzymatic defects in the nonlysosomal glycogenolytic and glycolytic pathways. This test can be performed in cooperative children as young as 6 years of age. A catheter is placed in a superficial antecubital vein, and basal lactate and ammonia levels are obtained without stasis. A sphygmomanometer cuff is then placed above the elbow and inflated above arterial pressure. The patient is asked to rhythmically squeeze another rolled-up cuff to well above 120 mm Hg for 1 minute of exercise. This requires constant encouragement from the observer because significant discomfort can occur even in healthy control subjects. The test should be truncated if the patient develops an acute cramp, as myonecrosis and/or a compartment syndrome may occur in an individual with a glycolytic disorder [Meinck et al., 1982; Lindner et al., 2001]. After 1 minute of exercise, the cuff around the arm is deflated and blood samples are sequentially obtained at 1, 3, 5, 7, 10, and 15 minutes. In healthy subjects there is a 4- to 6- fold increase of lactate over baseline with the peak occurring at 1-2 minutes post exercise, which declines to baseline values by 15 minutes. This is paralleled by a similar 5-fold or more increase in ammonia with levels generally peaking at 2-5 minutes after exercise in individuals with normal myoadenylate deaminase activity. In healthy subjects, venous ammonia and lactate are linearly related [Sinkeler et al., 1985]. In individuals with a defect in glycolysis/glycogenolysis, there is an insufficient rise in lactate (less than twofold), with a compensatory and exaggerated increase in ammonia, which also indicates sufficient effort on the part of the individual. This exaggerated rise in ammonia is attributable to high cellular levels of ADP resulting from a combination of blocked glycogenolysis/glycolysis and absent cellular acidosis. An insufficient lactate rise has been demonstrated in PPL, debrancher, PFK, PGK, PGAM, and LDH deficiencies but not in acid maltase or phosphorylase b kinase deficiency. The major limitation of this test is that the rise of venous lactate in individuals who do not have a defect in this pathway is highly dependent on the patient's ability and willingness to exercise. Therefore patients in whom lactate levels are low due to poor effort or to placement of the venous line in other than the median cubital vein, show proportionally blunted ammonia responses.

Given the potential risk of myoglobinuria, alternatives to the traditional ischemic forearm test (IFT) have been described. One of these employs sustained, intense (70% of maximal voluntary contraction, MVC) isometric handgrip exercise [Hogrel et al., 2001]. However, this form of testing is as ischemic as the use of a blood pressure cuff since the intramuscular pressure in isometric contractions of > 50 % MVC completely occludes muscle blood flow. Therefore, any reduction in the incidence of muscle contractures is dependent upon shortening the duration of the test which is also effective in minimizing contractures in the traditional IFT. An alternative non ischemic forearm test (NIFT) has been described and involves 30 maximal handgrip contractions in one minute without a blood pressure cuff [Kazemi-Esfarjani et al., 2002]. The level of increase in lactate and ammonia in control subjects and the diagnostic sensitivity in patients were similar to the ischemic exercise test. However, in contrast to the ischemic tests, the retained oxidative capacity helps to protect individuals from contractures or significant pain [Kazemi-Esfarjani et al., 2002].

Standard exercise physiology tests, such as cycle ergometers or treadmills, can be used to detect alterations of oxidative metabolism _Haller et al., 1989; Lewis and Haller, 1991b]. Maximal oxygen uptake is the most useful indicator of a patient's capacity for oxidative metabolism [DiMauro, 1993]. Typical physiologic responses in patients with defects in oxidative metabolism are as follows [Haller et al., 1989, 1991; Taivassalo et al., 2003]:

1. The increase of cardiac output during exercise is greater than normal relative to the rate of oxidative metabolism. In a study of 40 patients with mitochondrial myopathies using maximal cycle exercise, the increase in cardiac output relative to oxygen uptake (VO_2) (15.0 ± 13.6; range 3.3-73) was found to be exaggerated compared with controls (5.1 ± 0.7).
2. Oxygen extraction per unit of blood remains almost unchanged from rest to maximal exercise. In patients during maximal cycle exercise, the mean peak systemic arteriovenous O_2 difference (a-VO_2) was 7.7 ± 3.5 ml/dl (range 2.7-17.6) compared to controls with values of 15.2 ± 2.1 ml/dl. This leads to a gross mismatch between oxygen transport and utilization.
3. Ventilation is normal at rest but increases excessively relative to oxygen uptake. In patients during maximal cycle exericse, the increase in ventilation relative to VO2 (mean peak $VE/VO_2 = 65 \pm 24$; range 21-104) was exaggerated compared with controls ($VE/VO_2 = 41.2 \pm 7.4$).
4. Venous lactate, which is usually elevated at rest, increases excessively relative to workload and oxygen uptake.
5. Furthermore, during maximal cycle exercise of 40 patients with mitochondrial myopathies, the mean peak work capacity (0.88 ± 0.6 W/kg) and oxygen uptake ($VO_2 = 16 \pm 8$ ml/kg/min) were significantly lower ($P < 0.01$) than in controls (mean work capacity = 2.2 ± 0.7 W/kg; $VO_2 = 32 \pm 7$ ml/kg/min) but the patient range was broad (0.17 - 3.2 W/kg; 6-47 ml/kg/min) [Taivassalo et al., 2003]. It was concluded that the degree of exercise intolerance in mitochondrial myopathies correlated directly with the severity of impaired muscle oxidative phosphorylation as indicated by the peak capacity for muscle oxygen extraction. The exaggerated circulatory and ventilatory responses to exercise were a direct consequence of the level of impaired muscle oxidative phosphorylation and increased exponentially in relation to an increasing severity of oxidative impairment.

Aerobic forearm exercise provides an easily performed screening test that sensitively detects impaired O_2 use and accurately assesses the severity of oxidative impairment in patients with mitochondrial myopathy and exercise intolerance. In a study of 13 patients with mitochondrial myopathy and exercise intolerance, the exercise venous PO_2 paradoxically rose from 27.2 ± 4.0 mm Hg to 38.2 ± 13.3 mmHg, whereas the PO_2 fell from 27.2 ± 4.2 mmHg to 24.2 ± 2.7 mm Hg in healthy subjects [Taivassalo et al., 2002]. The range of elevated venous PO_2, during forearm exercise in the patients (32-82 mmHg) correlated closely with the severity of oxidative impairment as assessed during cycle exercise. Impaired oxygen extraction by exercising muscles can also be detected by near-infrared spectroscopy, which measures the degree of deoxygenation of hemoglobin [Bank et al., 1988].

Mitochondrial function in muscle *in vivo* can be quantitatively evaluated using ^{31}P-NMR [Radda et al., 1995]. The ratio of phosphocreatine (PCr) to inorganic phosphate (Pi) can be measured in muscle at rest, during exercise, and during recovery. In patients with mitochondrial dysfunction, PCr/Pi ratios are lower than normal at rest, decrease excessively during exercise, and return to baseline values more slowly than normal [Argov and Bank, 1991].

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Approach to the Diagnosis and Management of Muscle Cramps, Exercise Intolerance and Recurrent Childhood Myoglobinuria

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Defects of energy metabolism may profoundly disrupt the function of muscle and other highly energy-dependent tissues, such as brain, nerve, heart, kidney, liver and bowel. The limits of energy utilization in skeletal muscle are set by the adenosine triphosphatases (ATPases) that couple muscle contraction (myosin ATPase) and ion transport (calcium and sodium, potassium ATPases) to the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi) (Kushmerick 1995). ADP and Pi in turn activate energy producing reactions which regenerate ATP. Without this, ATP stores would be exhausted in seconds. The substrates that are used to replenish ATP are determined by the intrinsic properties of these fuels and by the intensity and duration of exercise that modulates fuel selection (Gollnick, 1985; Astrand and Rodahl 1986). The creatine kinase reaction and anaerobic glycogenolysis are the major anaerobic sources of ADP phosphorylation. Increases in ADP and AMP that occur in heavy exercise are primarily buffered by the coupled adenylate kinase (myokinase), adenylate deaminase (myoadenylate deaminase) reactions. Anaerobic glycogenolysis and phosphocreatine hydrolysis support rates of muscle energy production that are 2- to 4-fold higher than those supported by oxidative metabolism (Sahlin, 1986). Anaerobic energy is crucial for rapid bursts of exercise and to fuel the transition from rest to exercise. The acceleration to high rates of energy production occurs instantly for ATP, in less than a second for phosphocreatine and within seconds for anaerobic glycogenolysis. In contrast, maximal oxidative power requires from 3 minutes (with glycogen as the oxidative substrate) to 30 minutes (for peak fatty acid oxidation). Anaerobic fuels are rapidly depleted and lead to the accumulation of metabolic end products such as protons and Pi which promote fatigue.

If exercise needs to be sustained for more than a few minutes, then oxidative phosphorylation is necessary and provides the most abundant source of ATP synthesis. Glycogen is the major endogenous oxidative fuel of skeletal muscle while blood glucose and free fatty acids (FFA) are the major exogenous fuels. A small percentage of muscle energy needs are supplied by amino acids, predominantly branched chain amino acids, which are oxidized to a limited extent. Oxidative metabolism provides higher yields of ATP per mole of substrate rising from 2 to 36 for glucose and from 3 to 37 per glycosyl unit of glycogen metabolized anaerobically versus oxidatively. Furthermore, the metabolic end products of oxidative metabolism, namely CO_2 and water, are easily removed from working muscle and do not promote fatigue. The most abundant and critical fuel for the support of prolonged, moderate exercise is lipid. Carbohydrate stores in the form of muscle and hepatic glycogen and blood glucose (derived mainly from hepatic glycogenolyis) are limited and can support high intensity exercise for only 1-2 hours. However, carbohydrate, particularly muscle glycogen, is critical for normal oxidative metabolism. Glycogen supports a peak rate of oxidative phosphorylation that is about two-fold greater than fat. Though incompletely understood, this may be based upon a requirement for glycogen-derived pyruvate to support optimal function of the tricarboxylic acid cycle (Sahlin et al., 1990; Sahlin et al., 1995; Gibala et al., 1997). The proportion of carbohydrate relative to lipid oxidation progressively increases as the intensity of the aerobic exercise increases until carbohydrate is the exclusive fuel of maximum oxidative metabolism (Sahlin, 1986; van Loon et al., 2001). Secondly, glycogen is able to accelerate to maximal oxidative power output rapidly compared to other fuels (Sahlin, 1986; Haller and Vissing, 2002). Third, the molar ratio of ATP produced to O_2 consumed is higher for glycogen (6.17) and glucose (5.98) than for fatty acids (5.61) (Rennie and Edwards, 1981). The importance of this point lies in the fact that peak O_2 utilization in healthy humans is limited by O_2 delivery (Saltin, 1988).

The combustion of fuels in oxidative metabolism involves the generation of reducing equivalents in ß-oxidation, glycolysis and the tricarboxylic acid cycle that are oxidized via the respiratory chain where the phosphorylation of ADP is coupled to the reduction of molecular oxygen to water. Normal oxidative metabolism requires a highly integrated physiological support system to regulate the flow of oxygen from the lungs to the respiring muscle mitochondria as well as functional mitochondria that can efficiently extract the available oxygen from blood. Muscle oxygen utilization in oxidative phosphorylation may increase 50 fold or greater from rest to peak exercise. This increase is achieved by increases in the level of oxygen extraction from oxyhemoglobin in red blood cells and in the rate of delivery of oxygenated blood to working muscle by the circulation.

The primary source of energy for resting muscle is derived from fatty acid oxidation (FAO) (Felig and Wahren, 1975). At rest, glucose utilization accounts for 10% to 15% of total oxygen consumption (Wahren, 1977). Both slow and fast twitch fibers have similar levels of glycogen content at rest (Essen, 1978). The choice of the bioenergetic pathway in working muscle depends on the type, intensity, and duration of exercise (Essen, 1977; Gollnick et al., 1974), but also on diet and physical conditioning (DiMauro and Haller, 1999). In the first 5 to 10 minutes of moderate exercise, high-energy phosphates are used to first regenerate adenosine triphosphate (ATP). This is followed by muscle glycogen breakdown, which is indicated by a sharp rise in lactate during the first 10 minutes. Blood lactate levels then drop as muscle triglycerides and blood-borne fuels are used (Felig and Wahren, 1975; Lithell et al., 1979). After 90 minutes, the major fuels are glucose and free fatty acids (FFAs). During 1 to 4 hours of mild to moderate prolonged exercise, muscle uptake of FFAs increases approximately 70%, and after 4 hours, FFAs are used twice as much as carbohydrates.

Symptoms in muscle energy defects are directly related to a mismatch between the rate of ATP utlilization (energy demand) relative to the capacity of the muscle metabolic pathways to regenerate ATP (energy supply). This energy supply/demand mismatch impairs energy-dependent processes that power muscle contraction (weakness, exertional fatigue), mediate muscle relaxation (muscle cramping, tightness) and/or maintain membrane ion gradients necessary for normal membrane excitability (fatigue, weakness) and muscle cell integrity (muscle pain, injury, myoglobinuria). In metabolic myopathies, the specific metabolic mediators of premature fatigue, cramping, pain, and muscle injury are complex and vary among the different metabolic disorders. Disorders of glycogen, lipid, or mitochondrial metabolism may cause two main clinical syndromes in muscle: (1) acute, recurrent, reversible muscle dysfunction with exercise intolerance and acute muscle breakdown or myoglobinuria (with or without cramps) (e.g., phosphorylase [PPL], phosphorylase b kinase, phosphofructokinase [PFK], phosphoglycerate kinase [PGK], phosphoglycerate mutase [PGAM], and lactate dehydrogenase [LDH] among the glycogenoses) and carnitine palmitoyltransferase II (CPT II), very long-chain acyl-CoA dehydrogenase (VLCAD), trifunctional protein (TFP), short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency among the disorders of FAO, and Complex II and Coenzyme Q10 deficiencies among the mitochondrial disorders; and (2) progressive weakness (e.g., acid maltase, debrancher enzyme, brancher, and aldolase enzyme deficiencies among the glycogenoses; LCAD, VLCAD, TFP, glutaric aciduria type II and short-chain acyl-CoA dehydrogenase (SCAD) deficiencies and plasmalemmal carnitine transporter (OCTN2) defect among the FAO defects; and mitochondrial enzyme deficiencies such as Complex I and cytochrome oxidase deficiency and multiple mtDNA deletions and mitochondrial DNA depletion syndrome). Progressive weakness and recurrent myoglobinuria can also occur together in a given disorder (e.g., LCAD, VLCAD, SCHAD, TFP and severe CPT II deficiencies among the FAO defects, as well as multiple mtDNA deletions and Coenzyme Q10 deficiency).

Myoglobinuria is a clinical syndrome, not just a biochemical state _Rowland, 1984). In the alert patient, myalgia and limb weakness are the most common presenting symptoms. Urine color is usually brownish rather than red, and the urine tests positive for both albumin and heme (a concentration of at least 4 _g/ml). There are few or no red blood cells. Myoglobin can be identified by immunochemical methods. The sarcoplasmic enzymes, including serum creatine kinase (CK), are usually elevated to more than 100 times normal. Inconstant features include hyperphosphatemia, hyperuricemia, and hypocalcemia or hypercalcemia. If renal failure occurs, serum potassium and calcium levels may rise. If the patient is comatose or if the presenting disorder is one of acute renal failure, there may be no muscle symptoms or signs. Under these conditions, the diagnosis can be made if (1) there is renal failure and (2) the serum content of sarcoplasmic enzymes is 100 times normal. The potentially life-threatening hazards of an attack of myoglobinuria include renal or respiratory failure and cardiac arrhythmias.

The etiologies of heritable myoglobinuria differ in adults compared with children. In a study of 77 adult patients ages 15 to 65 years, Tonin et al. (1990) identified the enzyme abnormality in 36 patients (47%) as follows: CPT deficiency in 17 patients; glycolytic defects in 15 patients (including PPL in 10, PPL b kinase in 4, and PGK in 1); myoadenylate deaminase in 3; and combined CPT and myoadenylate deaminase in 1. In contrast, in 100 cases of recurrent childhood-onset myoglobinuria a lower percentage of children have been diagnosed biochemically (24%); 16 with CPT deficiency, 1 with SCHAD deficiency, and 7 with various glycolytic defects, including 2 PPL, 1 PGK, 3 PGAM, and 1 LDH deficiency (Tein et al., 1990). These children could be divided into two groups—a type I exertional group in which exertion was the primary precipitating factor (56 cases) and a type II toxic group in which infection and/or fever and leukocytosis were the primary precipitants (37 cases). The type II toxic childhood group was distinguished from the type I exertional childhood- and adult-onset

Table 1: Differentiation Between Disorders of Glycogen vs. Lipid Metabolism. Resulting in Exercise Intolerance and/or Myoglobinuria

	Glycolytic / Glycogenolytic Phosphorylase Deficiency	Fatty Acid Oxidation Carnitine palmitoyltransferase II Deficiency
Symptom onset in exercise	Early (first few minutes)	Late (particulary after 1 hour)
Second wind	+	-
Myalgia	Cramps	Stiffness
Fixed weakness	More common	Less common
Elevated interictal CK	+	-
Abnormal forearm	+	-
ischemic lactate test	+	-
Delayed ketogenesis	-	+
Muscle biopsy	± Glycogen storage	± Lipid storage

groups by its etiologies, which were limited to FAO defects, as well as its slight female predominance, which contrasted with the marked male predominance in the latter two groups. The type II toxic group was further distinguished by the earlier age at onset of myoglobinuria, the presence of a more generalized disease (e.g., ictal bulbar signs, seizures, encephalopathy, developmental delay), and a higher mortality rate. Currently the most common etiology for recurrent myoglobinuria in both adults and in children is CPT II deficiency (Tein et al., 1992).

Table 2: Heritable Causes of Exercise Intolerance and Recurrent Myoglobinuria

I) Biochemical Abnormality Known

1. Glycolysis/ Glycogenolysis
   1) *Phosphorylase (McArdle, 1951)
   2) Phosphofructokinase (Tarui et al., 1965; Layzer et al., 1967)
   3) *Phosphoglycerate kinase (DiMauro et al., 1981a)
   4) *Phosphoglycerate mutase (DiMauro et al., 1981b)
   5) *Lactate dehydrogenase (Kanno et al., 1980)
   6) Phosphorylase "b" kinase (Abarbanel et al., 1986)
   7) Debrancher (Brown, 1986)

2. Fatty Acid Oxidation
   1) *Carnitine palmitoyltransferase II (DiMauro and DiMauro, 1973)
   2) Long-chain acyl-CoA dehydrogenase (Roe, 1987)
   3) Very long-chain acyl-CoA dehydrogenase (Ogilvie et al., 1994)
   4) Medium-chain acyl-CoA dehydrogenase (Ruitenbeek et al., 1995)
   5) *Short-chain L-3-hydroxyacyl-CoA dehydrogenase (Tein et al., 1991)
   6) *Trifunctional protein/ long-chain L-3-hydroxyacyl-CoA dehydrogenase (Dionisi-Vici et al., 1991)

3. Pentose Phosphate Pathway
   1) *G6PDH (Bresolin et al., 1989)

4. Purine Nucleotide Cycle
   1) Myoadenylate deaminase (Hyser et al., 1989)

5. Respiratory Chain
   1) *Complex II and aconitase (Haller et al., 1991)
   2) Coenzyme Q deficiency (Ogasahara et al., 1989)
   3) *Multiple mitochondrial DNA deletions (Ohno et al., 1991)
   4) Complex I deficiency (de Lonlay-Debeney et al. 1999)
   5) Complex III deficiency (cytochrome b) (Andreu et al. 1999)
   6) Complex IV deficiency (Cytochrome oxidase deficiency) (Keightley et al. 1996)

II) Biochemical Abnormality Imcompletely Characterized

1) *Impaired long-chain fatty acid oxidation (Engel et al., 1970)
2) *Impaired function of the sarcoplasmic reticulum (?) in familial malignant hyperthermia (predisposition in central core disease, Duchenne muscular dystrophy, Becker muscular dystrophy, myotonic dystrophy, myotonia congenita, Schwartz-Jampel syndrome, King-Denborough syndrome)
3) *Abnormal composition of the sarcolemma in Duchenne and Becker muscular dystrophy (Bonilla et al., 1989; Hoffman et al., 1989; Medori et al., 1989)

III) Biochemical Abnormality Unknown

1) *Familial recurrent myoglobinuira
2) *Repeated attacks in sporadic cases
3) *Etiologies that have been documented to cause recurrent myoglobinuria beginning in childhood

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