

Mitochondrial Cytopathies: A Primer

Mitochondrial Cytopathies 2000

Reprinted with permission of

Bruce H. Cohen, MD
Cleveland Clinic Foundation
Staff, Department of Neurology
June 2000

Objectives

1. Review of the basic biochemistry of oxidative metabolism
2. Describe mitochondrial molecular genetics and biochemistry of mitochondrial disorders
3. Acquire information needed to recognize the patient at risk for a mitochondrial cytopathy
4. Assess which patient needs referral for further evaluation
5. Organize a care plan
6. Package includes: Primer (Biology, Genetics and Overview of Evaluation, Details of Evaluation Guidelines, Primer for Management)

Introduction:

- Heterogeneous group of disorders
- Primary defect is a deficit of energy output
- Genetic defects are due to alteration of mitochondrial enzyme function, due to either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations
- diagnosis is clinical and usually should be confirmed with laboratory testing

Pediatric Case Reports:

1. 1982 DF was born at term and had no problems until she was 4 months old when it was found she was anemic during an evaluation for fever. She had multiple courses of antibiotics for UTIs and otitis media. She developed a picture of overwhelming sepsis and her lactic acid was elevated at 12 mM. She subsequently went on to have a chronic sideroblastic anemia, pancreatic failure, cardiac dysfunction, hearing loss, ptosis, myopathy and retinal degeneration. She died at the age of 15 from cardiac failure. Her mtDNA showed a typical deletion seen in Kearns Sayre syndrome.
2. 1984 CP was born at term weighing 1900 gm after an uncomplicated pregnancy, labor and delivery. He had mild dysmorphic features, along with the fact he was SGA, with rocker bottom feet, small upturned nose and epicanthal folds. He was noted to be hypotonic at birth and at 19 hours of life had his first seizure. His initial evaluation showed a metabolic acidosis with a lactic acid of 6 mM, with a lactate to pyruvate ratio of 10:1. Seizures continued and he was transferred to our hospital. Over the next several days his acidosis worsened, and fluids of D10+ ½ Normal bicarbonate were used to attempt to correct his acidosis. A urine organic acid showed enormous lactate excretion. A tentative diagnosis of pyruvate dehydrogenase deficiency was made, and the fluids were changed to a D2.5 solution with amino acids and intralipids. Within hours, the acidosis reversed and the child was extubated 2 days later. He was treated with high dose B1 and lipoic acid (stimulators of PDH), as well as with polycitra. He was given a regular infant formula with MCT oil and the lactic acid remained in the 4-6 mM range. He died in his sleep at 8 months of life. The E1- α subunit of PDH was absent. This is an X-linked disease.

1 Bruce H. Cohen, MD

3. 1984 A 7 day old boy was transferred to our hospital for jaundice. He was born at term without problems. He began regular infant feeds and ate well initially. He became jaundiced at 3 days of life and was placed under bililights. He became increasingly lethargic and developed diarrhea. He was placed in an incubator for hypothermia, despite bundling him in blankets. He continued to bottle feed, despite his increasing lethargy. His bilirubin was as high as 15 with about 50% conjugated. On arrival the transport team checked his urine for reducing substances (positive) and glucose (negative). A tentative diagnosis of galactosemia was made and subsequently confirmed. He was treated with IV glucose and prophylactic antibiotics for a few days and recovered. He was started on a soy-based formula and did well until 3 weeks of life when he developed e. coli. sepsis. At this time (1999) he is alive and aside from learning problems, doing well on a lactose free diet.
4. 1984 A 5 week old girl was seen in the emergency room for dehydration. She was born at term weighing 3100 grams and had been a poor feeder since discharge. She had not gained any weight by her two-week pediatric visit and at the time of the ER visit had weighed 2800 grams. She was thought to be 5-10% dehydrated. A sepsis work up was performed in the ER and antibiotics started. Her initial bloods tests showed glucose of 46, Na 148, CO2 15, Cl 113, K 4, BUN 1, WBC 2.0, Hb 8.8, Plts 49K. Before a bed was available a ammonia was drawn (2200 mg/dl) and organic acids (huge propionic acid peak).
5. 1987 SC was born weighing 3250 gm at term following a normal pregnancy, labor and delivery. A rapid respiratory rate was noted on the first day of life and initial labs showed an anion gap of 22. A sepsis work-up was initiated and antibiotics begun. The initial lactic acid was 20 mM (normal < 2.0 mM). The child was ventilated and bicarbonate was begun. The child was transferred to our hospital where the lactate increased to 60 mM over the next few days, with a lactate to pyruvate ratio of 40:1, and the child died on day 3 of life. Liver tissue was analyzed and there was no COX (complex IV) activity present.
6. Circa 1990 A 3 week-old infant was admitted to a Cleveland hospital with e. coli. sepsis and meningitis. He responded to antibiotics and was discharged. Over the next several years he was admitted repeatedly for vomiting, dehydration and presumed sepsis. During a legal review, a positive State infant screening test was found in the doctor's files, positive for galactosemia.
7. 1998 A infant girl was born at CCF following a normal pregnancy, labor and delivery. She was very hypotonic at birth and was transferred to Metro for respiratory support. She underwent a muscle biopsy procedure, which showed giant abnormal mitochondria. After discharge, she returned to CCF for ongoing neurologic care. The subsequent muscle biopsy determined the etiology to be a complex III ETC deficiency.
8. 1999 A 12 week old was admitted to CCF with intractable seizures. She had been healthy at birth but developed seizures in the first month of life. Her initial laboratory work-up showed a peak of 3-OH isobutyric acid.

Adult Case Reports:

1. R.L. 71 year old man with repeated ER visits and hospitalizations due to altered mental status. The evaluation determined a cirrhotic liver and it was assumed his alteration in mental status was due to alcohol ingestion, despite ethanol levels of zero. He was treated with IV fluids and seemed to be in his normal state within hours. During one ER visit, the neurology consult was a resident that just rotated off pediatric neurology and performed a mini-metabolic evaluation in the ER (elevated NH₃, muscle CK and lactic acid and no ketones). Dx: Long-chain acylCoA dehydrogenase deficiency. Comments: LCAD is the first step in the sequential beta-oxidation of long chain fats (dietary or stored). With LCAD deficiency, one cannot rely on fats for energy. R.L. relied on his wife to cook his meals, and when she died, R.L. was on his own for the first time in his life. He began skipping meals, and if the fasts were long enough, glycogen stores were depleted and he was not able to generate energy from fat stores. Hypoglycemic hypoketonuria is an important clue to detecting disorders of fat metabolism. Interviews with the family indicated that R.L. never consumed more than a glass of red wine a day. He has responded to a diet very

2 Bruce H. Cohen, MD

low in fats, with frequent meals (including bedtime snacks) high in complex carbohydrates. Supplements include levo-carnitine & multivitamins.

2. S.K. 57 year old man came from India for evaluation of his cardiac conduction defect. His family history is significant for his mother dying at a young age of a cardiomyopathy, and that his brothers have similar problems to his. He was the president of a successful business in India. As a young man he was diagnosed with calcifying pancreatitis, and had been on replacement digestive enzymes for years. He has had numerous admissions for non-surgical bowel obstruction and has had several exploratory laparotomies, the etiology was never determined. Over the last 15 years he lost 75 pounds, and developed aching in his limbs. In January 1996 he was admitted for a stroke, and a cardiac evaluation determined he was in A-fib. He was placed on Coumadin. He was readmitted for a stroke several months later, and was again found to be in A-fib. His neurologist and cardiologist referred him to CCF Cardiology for placement of a pacemaker. In October 1996, after a 24 hour journey, he visited our cardiology department, and was told that a pacemaker was not indicated. While resting in the waiting room, trying to gather the energy to walk back to the hotel, he collapsed. He spent the next two weeks in a stupor in the NICU, and again, another stroke was detected, along with his A-fib. The patient was found to have an elevated lactate, ammonia and CK (MM). Polarography revealed decreased oxidation of substrates that donate reducing equivalents to complex I and beta oxidation. An evaluation determined that he had a deficiency in carnitine palmitoyl transferase II activity. Treatment was started and included a low fat diet with frequent meals rich in complex carbohydrates, along with levo-carnitine, CoQ10 and other vitamins. He has had no further events since his hospitalization in October 1996. Comments: In order for the mitochondria to burn fats, the free fatty acid must first enter the mitochondrial inner membrane. CPT I catalyzes the conversion of the activated free FA (acyl CoA) + carnitine to the acyl-carnitine. A carnitine translocase exchanges the acyl-carnitine across the inner membrane for a free carnitine molecule. CPT II catalyzes the conversion of the acyl-carnitine to the acyl-CoA and free carnitine. The acyl-CoA can then enter the beta-oxidation spiral. CPT II deficiency usually results in exercise intolerance, muscle cramping and fatigue in young adults, but is also known to cause an early cardiomyopathy.

3. P.L. is a 40 year old woman with "CFS". She has been evaluated by dozens of CCF doctors and seems to have secondary gain issues. After a surgical procedure she did not recover normally from anesthesia, and remained apnic for 30 minutes, and was referred for evaluation. An extensive laboratory evaluation was not helpful. A muscle biopsy was performed and demonstrated mild mitochondrial proliferation and mild abnormalities in electron transport chain activity. It is not clear if she does or does not have a genuine disorder.

Catastrophic Presentations of Metabolic Disease in the Newborn

- nonspecific finding
- lethargy, irritability, hyperactivity
- failure to feed well
- hypothermia or fever
- cyanosis
- seizures
- vomiting
- RTA
- jaundice (early and/or prolonged)
- diarrhea or abdominal distension

Brief Differential Diagnosis

3 Bruce H. Cohen, MD

- organic acidemias: MSUD, propionic, isovaleric, methylmalonic, others
- urea acid cycle defects: carbamyl phosphate synthetase deficiency, OTC, citrullinemia, argininosuccinic aciduria
- carbohydrate disorders: galactosemia, hereditary fructose intolerance
- aminoacidopathies: homocystinuria, tyrosinemia, nonketotic hyperglycinemia
- endocrinopathies: “CAH, congenital diabetes”

Exam

- odor
- neurologic: tone, level of alertness, DTRs
- general: dysmorphic features

Lab Evaluation

- glucose, glucose, glucose
- electrolytes, calculate anion gap
- CBC (look for low counts)
- BUN (low BUN indicates failure of urea acid cycle, either primary or secondary)
- Lactate, pyruvate, and L/P ratio
 - ↑ lactate with L/P 10-20 indicates a disorder of pyruvate metabolism such as PDH deficiency
 - ↑ lactate with L/P of > 20 indicates a disorder of oxidative phosphorylation
- Ammonia
- CK
- Biotinidase level (usually causes problems after 6 months)
- VLCFA (neonatal peroxisomal disorders)
- Amino Acids (blood and urine)
- Organic Acids (quantitative)
- Acyl carnitines (blood and urine)
- Skin biopsy for EM and fibroblast culture
- Muscle biopsy

Treatment

Supportive and varies according to diagnosis

Presentation of Mitochondrial Disease in Adults

As varied as in children, more complicated to diagnosis because adults have acquired other diseases

- Childhood onset mitochondrial diseases
- Muscle: new muscle weakness, cramping
- Brain: migraine, stroke or stroke-like events, dementia, MS-like presentation
- Endocrine: diabetes (~5% of DM in Great Britain may be due to the mtDNA 3243 mutation)
- Cardiac: early cardiomyopathy, cardiac conduction defects (association of LHON with WPW, etc).
- Systemic: CFS-like illness

Brief Differential Diagnosis:

4 Bruce H. Cohen, MD

primary endocrine disease
 vitamin deficiency: B12
 homocystinuria and associated disorders
 primary muscle disease: polymyositis, dystrophin associated glycoprotein muscular dystrophies
 “chronic fatigue syndrome”
 autoimmune disorders
 glycogen storage disorders
 depression and related psychosomatic disorders
 other neurodegenerative disorders (MS, ALS, HD, combined systems degeneration)

History of Mitochondrial Diseases:

- 1962 Luft describes first case of a euthyroid woman with extreme hypermetabolism and gigantic mitochondria in muscle
- 1962 Milton Shy describes mitochondrial proliferation in myopathic patients
- 1962 W. King Engel applies histochemical techniques to muscle, uses modified Gomori trichrome stain
- 1975 L.P. Rowland lumper/splitter debate regarding KSS/progressive ophthalmoplegia
- 1975 Koenigsberger describe a case of MELAS
- 1981 MtDNA genome mapped
- 1982 Rowland and Fukuhara present independent papers regarding KSS and MERRF
- 1984 MERRF, MELAS, KSS paper in Ann Neurol by Pavlakas, Phillips, DiMauro, DeVivo and Rowland
- 1985 Carnitine palmitoyltransferase (CPT) deficiency described by DiMauro
- 1991 Biochemical and molecular analysis becomes commercially available
- 1995 Review articles appear in major medical journals

When To Suspect Mitochondrial Dysfunction:

There is no one identifying feature of mitochondrial disease. Patients can have combinations of problems whose onset may occur from before birth to late adult life. Mitochondrial diseases should be considered in the differential diagnosis when there are these unexplained features, especially when these occur in combination.

- Encephalopathy
 - Seizures
 - Developmental Delay or Regression (including early and late-onset dementia)
 - Myoclonus
 - Movement Disorders (dystonia, dyskinesias, chorea, etc.)
 - Complicated Migraine
 - Stroke
- Neuropathy
- Cardiac Conduction Defects or Cardiomyopathy
- Hearing Deficits
- Short Stature
- Disorders of Extraocular muscles including ptosis, acquired strabismus and ophthalmoplegia
- Diabetes
- Renal tubular disease
- Visual Loss (retinitis)

5 Bruce H. Cohen, MD

- Lactic acidosis, which may be mild

Ultrastructure & Function

- mitochondria are intracellular double-membrane organelles
- role is to generate ATP (the universal currency or fuel)
- Defects include:
 1. mitochondrial transport
 2. substrate utilization
 3. citric acid cycle
 4. oxidative-phosphorylation coupling
 5. respiratory chain defects
- From a molecular “point of view” the defects of these genes include:
 1. defects in transcription, translation or post-translational processing of mitochondrial proteins coded for by nuclear genes (Complex II, PDH, or CPT-II deficiency)
 2. defects in mtDNA genes (including protein, rRNA or tRNA)
 3. defects of nuclear-encoded factors that modulate mtDNA genes
 4. Defects in non-protein parts of the mitochondria (CoQ10 deficiency, prosthetic groups, Menkes)

Mitochondrial DNA

- Circular gene
 - 16,569 base pairs (exactly)...these are numbered 1 to 16,569
 - Heavy and Light strand, each with its own origin of replication
 - All coding sequences are contiguous (no introns)
 - Each mitochondria contains 2 to 10 copies of the mtDNA
 - Each cell can have hundreds of mitochondria
 - mtDNA mutates 6 - 17 times faster than nuclear DNA
 - The only non-coding region is a 1 kB region which contains the origin of replication of the H strand and the promoters for the L and H strand transcription
 - The mitochondrial genetic code differs from the universal code....therefore the mitochondrial protein synthesis relies on nuclear encoded transcriptional and translations factors with tRNA and rRNA derived from mitochondrial genes
- *mit* genes: 13 protein-encoding genes
 7 subunits for NADH dehydrogenase (complex I) [25 total subunits]
 3 subunits of cytochrome oxidase (COX) (complex IV) [13 subunits]
 2 subunits of ATP synthetase (complex V) [12 subunits]
 apocytochrome b (complex III) [9-10 subunits]
 - *syn* genes: protein-synthesis genes
 2 rRNA (12 and 16s)
 22 tRNAs

Genetics of mtDNA

During fertilization the sperm “donates” its nuclear DNA. The sperm contributes little to no mitochondria and therefore no mtDNA. *Therefore, our mitochondrial are our mothers.*

- 100s of mitochondria per cell
- 2 - 10 copies of mtDNA per mitochondria

6 Bruce H. Cohen, MD

- 1000s of mtDNAs contribute to the mitochondrial genotype of each cell. Remember that one (or two) copies of nDNA contribute to the genotype of a cell, and except for those diseases with mosaicism, all tissues in nDNA disorders are genotypically identical.
- Heteroplasmy: During mitosis the mitochondria are randomly distributed to daughter cells. Therefore if the original cell has a mixture of different mtDNAs (an example is the situation where a single mitochondria has one mutant mtDNA and 9 wild mtDNAs in the fertilized ovum), the distribution of the mutant mtDNA will vary widely from cell to cell and organ to organ. i.e.: The newly formed mtDNA will be distributed randomly into the newly formed mitochondria of these daughter cells. What ends up happening is that variable ratios of normal to mutant mtDNA are found in each cell. Because of heteroplasmy, the number of mitochondrial genotypes is enormous. The degree of heteroplasmy can be quantitated if a mtDNA mutation can be detected.
- Homoplasmy: The mtDNA is all one type within a tissue....this is the normal state.
- Threshold expression: All tissues require ATP to survive. Some tissues require a greater flux of ATP production and utilization, and therefore require the integrity of the ox-phos enzyme system. Cellular dysfunction will occur if not enough ATP can be generated. The tissues most affected are those where there is little post-birth mitotic activity (which would cause a selection bias towards cells with healthy mitochondria), i.e.: brain, type I skeletal muscle, cardiac muscle, nerve, liver, proximal tubule of the kidney. Most tissues do not require the ox-phos engine to always be functioning at 100%. In fact, most tissues probably can get by on much less than 100% activity. Therefore, whether or not a tissue is affected depends on the metabolic needs of the tissue and the ability of the mitochondria to meet that need. The phenotypic expression only becomes evident when a proportion of the corresponding mtDNA reaches a critical level.
- Phenotype Variability: The segregated mtDNA leads to graded biochemical defects. Not every affected family member has the same exact phenotype. The genetic defect may not have reached a threshold in the mother, for example.
- mtDNA and Aging: OXPHOS activity declines with age in muscle, liver and brain. In heart muscle, the cytochrome c oxidase activity decreases with age, seemingly due to an accumulation of mtDNA mutations and base substitutions. The extent of damage is tissue specific; for example in the brain, the damage seems most severe in the basal ganglia, followed by the cortex. The cerebellum does not accumulate mtDNA mutations as a function of age. OXPHOS defects are reported in PD, Huntingtons, AD, dystonia. It is unknown if OXPHOS activity diminution is the cause of "aging" (the makers of vitamins want us to think so anyway.)

To complicate matters:

- -environmental factors play a role (EtOH and tobacco accelerate the optic nerve damage in Lebers Hereditary Optic Neuropathy)
- -point mutations may be pathologic or non-pathologic
- -immunologic factors may play a role...for example there is an association between MS and LHON in A¹¹⁷⁷⁸ - positive females....optic nerve damage in LHON may be immunologically mediated and mtDNA may play a role in MS
- -a specific phenotype may have many different genotypes associated with it (LHON has 17 different known mutations, some forms of LHON are more severe than others and in one form, 28% recover vision)

Biochemistry of the Respiratory Chain and Oxidative Phosphorylation (OXPHOS)

7 Bruce H. Cohen, MD

- Complex I: Transfers e^- from NADH to coenzyme Q.
- Complex II: Transfers e^- from FADH or FMNH to coenzyme Q. Succinate dehydrogenase (Krebs cycle) is part of the complex. This is the only part of the chain that is not coded in part by mtDNA, and succinate dehydrogenase deficiency is the only identified nDNA disorder causing an OXPHOS disorder.
- Complex III (coenzyme Q-cytochrome c reductase): Transfers e^- from reduced CoQ to cytochrome c. The apoprotein of cytochrome b is a mtDNA coded polypeptide.
- Complex IV (cytochrome c oxidase or COX): reduces molecular oxygen to water, using the e^- donated from cytochrome c.
- Complex V (ATPase): Converts ADP and P_i to ATP.
- Coenzyme Q_{10} and cytochrome c act as shuttles between complex I and III and II and III. Coenzyme Q_{10} also is a potent antioxidant.

Evaluation of a patient with suspected mitochondrial disease:

- History
- Physical Exam
- Lactate, Pyruvate (blood and CSF)
- Amino Acids (serum, urine and CSF)
- Organic Acids (urine, CSF)
- Carnitine & Acyl Carnitine
- Audiogram
- ECG
- Eye exam
- Blood for mtDNA (if you know what you are looking for....search and detect missions blindly have less of a chance in finding the mutation)
- Blood for nuclear DNA (limited availability, only a few defects have been identified)
- Muscle for mtDNA (same as above)
- Muscle of OXPHOX analysis (spectrophotometry or polarography)
- Muscle of immunologic staining of mtCOX subunits and nCOX subunits
- Fibroblast Culture for OXPHOS analysis

Pearls

1. Mitochondrial cytopathies are not one disease.
2. Keep in mind that there are mitochondrial diseases that are due to inherited mutations (germline mutations) and those due to acquired mutations (somatic mutations). Furthermore it is reasonable to think that there are those that are primary (something inherently wrong with mitochondrial function) and those that are secondary (the mitochondria is injured as a bystander to another process).
3. Not all patients with mitochondrial cytopathies have systemic lactic acidosis. As a general rule, aside from certain mtDNA defects such as MELAS, MERRF, and KSS, lactic acid levels often decrease to normal as the child gets older.
4. A single normal blood or urine lab test does not rule out mitochondrial disease. This is true for organic acids, lactic acid, carnitine analysis and amino acid analysis.
5. Brain dysmorphology (agenesis of the corpus callosum, migrational defects) does not rule out mitochondrial disease.

8 Bruce H. Cohen, MD

6. Think of mitochondrial cytopathy or other metabolic disease in the setting of atypical white matter disease (atypical multiple sclerosis, work-up negative leukodystrophy)
7. Think of mitochondrial cytopathy when there are a number of organ systems involved.
8. The majority of mitochondrial diseases are probably not due to mutations in the mitochondrial DNA genome.
9. It is not possible to chart the future of a person with a mitochondrial cytopathy. Those with a high degree of pathologic mtDNA heteroplasmy do worse, on average, than those with a lesser degree, but this is only valid for populations of patients and cannot be used to predict what will occur in any one patient. It is not possible to predict the response to vitamins, supplements or diet changes before they are tried. It is not possible to predict the course of siblings or other first degree family members based on what happened with the first family member identified. Remember the literature that is available early in the description of a particular disease (such as exists today with mitochondrial cytopathies) reflects what happens with the sickest patients. Many of those that are not critically ill have escaped detection by doctors, and therefore many of those people that have been diagnosed with a mitochondrial disease in the last 5 years, free of identifiable mtDNA mutations, may have a better overall prognosis than what the literature suggests.
10. Mitochondrial diseases that impact on the developing fetus may cause permanent problems with brain development. During the process of embryogenesis the brain cells 1) undergo rapid cell division, 2) begin migrating to their final destination in the brain, 3) begin connecting to each other and 4) myelinate (the white matter surrounds parts of the nerve cells). The first three processes are finished before a baby is born. The fourth process begins before birth and continues until the 40s. Although the brain continues to develop in some respects after birth, some injuries that occur before birth are not repairable by normal development or by medication or treatments. For example, any metabolic disease that interferes with processes 1,2 or 3 may result in inevitable mental retardation. These injuries have been labeled *mitochondrial embryopathies*. Although treatment may help other aspects of mitochondrial dysfunction, the part of the illness that results in damage to the non-plastic brain functions will not improve. Predicting potential improvement in children under five years of age can be difficult in some circumstances.
11. This is an evolving field. 20 years ago there were fewer identified patients than there are people at this conference. Expect to relearn this year's truth next year.

Laboratory Evaluation for Disorders of Energy Metabolism

Laboratory testing is the usual method physicians go about evaluating patients for disorders of energy metabolism (which include mitochondrial disorders, disorders of oxidative phosphorylation and β -oxidation). Most hospitals do not have a metabolic laboratory and therefore can run only the most basic tests. However, most hospitals will send specimens to any laboratory in the country. Not all laboratory tests are required for all patients, and your physician may decide that some of these tests are not necessary. The lists are authoritative, but are meant to serve as a general guide for evaluation. Not all metabolic disorders primarily affect energy metabolism, but the clinical features may overlap. Testing for these metabolic are listed in a separate table. There is no substitute for good clinical judgement.

The initial laboratory evaluation is generally used as a non-invasive screening for inborn errors of energy metabolism. If the results of this evaluation are suggestive of a specific disorder, a direct test for the disease in question may be able to be performed. If the results of the initial evaluation are normal and there is a strong suspicion of a disorder of a mitochondrial disease, a more intensive evaluation is performed.

The secondary tests are more invasive (and may include a spinal tap) and because some of the tests may require urine specimens collected over time, a bladder catheter may be required in young children. Many of these tests require the specimens to be sent to a special laboratory. Abnormalities found on the secondary tests will guide the physician as to the direction of further testing. However, as with the initial testing, normal results do not eliminate the possibility of a mitochondrial disease, but make it less likely.

The tertiary tests are invasive and/or expensive, and may carry with them some risks, such as metabolic decompensation during a fast. However, if the physician strongly suspects a metabolic illness, these tests may be diagnostic. The muscle biopsy is a tertiary test, but is listed separately because it is the most complicated and invasive of all tests, and in children requires a general anesthesia. Although a muscle biopsy can be performed at any medical center, very few centers have the ability to do all the testing necessary to make a diagnosis. Therefore, the physician must be very conscientious in planning before the biopsy is done.

A lists of tests and centers performing these tests can be found at the following web address:

<http://biochemgen.ucsd.edu/wbgtests/wbgtests.htm>

Initial Laboratory Evaluation

Test	Tissue	Comment
Glucose	B	
Electrolytes	B	
Blood Counts	B	
Lactate	B	Proper technique must be used, tourniquet must be released before blood is sampled
Ammonia	B	
Metabolic Screen	B,U	The metabolic screen varies between hospitals, but may include screening testing for a variety of disorders as well as urine and blood amino acid profile, and screening organic acid testing
Ketones	B,U	Most valuable if collected at the time of an illness

B = blood, U = Urine

Secondary Laboratory Evaluation

Test	Tissue	Comment
Lactate	B,CSF	see above
Pyruvate	B	Proper determination of pyruvate requires the specimen be instantly deprotonized.
L/P Ratio	B	The ratio of lactate to pyruvate can be very helpful in determining which type of disorder may be present
Amino Acids	B,U, CSF	Urine collections may be random or timed; and may be collected after a meal or after a fasting period, depending on the clinical situation. "Generalized aminoaciduria" may indicate the presence of a mitochondrial cytopathy, as well as other medical conditions.
Organic Acids	U, CSF	Samples must be kept refrigerated or frozen. Different techniques, some more sensitive are used by certain laboratories. Urine collections may be random or timed, and may be collected after a fasting period, depending on the clinical situation.
Carnitine Analysis	B,U	Most laboratories determine the free carnitine and total carnitine. Fractionation into specific acyl carnitines may be helpful in some situations. Urine collections may be random or timed, and may be collected after a fasting period, depending on the clinical situation.
Ketones	B,U	Fractionation of ketones into β -hydroxybutyrate and acetoacetate may be helpful. This test is most valuable if collected during an acute illness or after a fast.
Free Fatty Acids	B	
Mitochondrial DNA Point Mutations	B	If a patient fits into a specific, well-described mitochondrial phenotype, testing for specific, known point mutations may be helpful at this stage.
Mitochondrial DNA Southern Blot	B	If a patient fits into a specific, well-described mitochondrial phenotype, Southern blot testing may be helpful at this stage.

* B = blood, U = Urine, CSF = Cerebral Spinal Fluid

Tertiary Laboratory Testing

Test	Comment
Repeat Testing	Repeating some of the above listed tests, sometimes under different conditions (such as during an illness), may be helpful.
Provocative Testing	Under monitored conditions, usually in the hospital, repeating some of the above tests after a fast or after a specific meal or intravenous infusion, may be helpful.
Skin Biopsy	A skin (also known as a fibroblast) culture can be established with the skin obtained from a biopsy. This can be sent for testing electron transport chain activity, β -oxidation disorders, as well as for a variety of other specific diseases.
Mitochondrial DNA Point Mutations	If a patient fits into a specific, well-described mitochondrial phenotype, testing for specific, known point mutations may be helpful at this stage.
Mitochondrial DNA Southern Blot	If a patient fits into a specific, well-described mitochondrial phenotype, Southern blot testing may be helpful at this stage.
Coenzyme Q10	Blood Test

Muscle Biopsy

Muscle tissue can be used for tests that can be diagnostic, even when the above tests are normal. Because this is the most invasive test, the risks and costs of the procedure must be weighed against the chance the biopsy will yield positive results and the benefits gained by a diagnosis (treatment decisions, family planning). Before a muscle biopsy is done a plan needs to be arranged for how the muscle is distributed. References labs should be contacted before the biopsy is done so that preparation of the muscle is done correctly. Muscle can be sent for:

- Routine light microscopy including modified Gomori Trichrome stain (checking for ragged red fibers)
- Specific immunohistochemistry (cytochrome oxidase and COX subunits), succinate dehydrogenase, etc.
- Electron microscopy (useful to view the structure of the mitochondria, evaluate for accumulation of excessive mitochondria in the subsarcolemma region and evaluate for mitochondrial proliferation.
- Electric Transport Chain Activity (photometric analysis), preferable performed on fresh muscle but can be done on frozen muscle.
- Oxidative Phosphorylation Activity (oxygen uptake), which can determine the activity of all five complexes, state iii and state iv respiration, respiratory control ratio and estimate efficiency of coupling of electron transport and oxidative phosphorylation. This can be run on fresh muscle only.
- Enzyme activity for β -oxidation disorders including the enzymes of the β -oxidation spiral and carnitine transport.
- Determination of carnitine and acyl-carnitine levels, Co-Enzyme Q₁₀ levels.

Other Metabolic Tests That May Be Indicated in Certain Situations

Test	Tissue	Disease(s)	Comment
Uric Acid, Creatinine	B,U	Lesch-Nyhan	These patients often have lactic acidosis
Copper, Ceruloplasm	B,U	Menkes Kinky Hair Disease, Wilsons Disease, other movement disorders and dementias	
Very Long Chain Fatty Acids	B	Adrenoleukodystrophy and other disorders of peroxisomal metabolism	
Lysozomal Enzymes	B,U	variety of storage diseases and leukodystrophies	

11 Bruce H. Cohen, MD

Testing That May Be Necessary in Patients with Mitochondrial Cytopathies

Brain MRI, MRS

Eye: Retinal exam, electroretinogram

Heart: EKG and echocardiogram

Thyroid Function Tests (blood)

Ears: Audiogram or BAEP

Table 1: Problems Associated with Mitochondrial Cytopathies

Organ System	Possible Problems
Brain	developmental delays, mental retardation, dementia, seizures, neuro-psychiatric disturbances, atypical cerebral palsy, migraines, strokes
Nerves	weakness (which may be intermittent), neuropathic pain, absent reflexes, gastrointestinal problem (ge reflux, constipation, pseudo-obstruction), fainting, absent or excessive sweating resulting in temperature regulation problems
Muscles	weakness, hypotonia, cramping, muscle pain
Kidneys	proximal renal tubular wasting resulting in loss of protein, magnesium, phosphorous, calcium and other electrolytes
Heart	cardiac conduction defects (heart blocks), cardiomyopathy
Liver	hypoglycemia (low blood sugar), liver failure
Eyes	visual loss and blindness
Ears	hearing loss and deafness
Pancreas	diabetes and exocrine pancreatic failure (inability to make digestive enzymes)
Systemic	failure to gain weight, short stature, fatigue, respiratory problems including intermittent air hunger

Invasive Evaluation:

Procedure	Advantages	Disadvantages
Muscle Biopsy	<ul style="list-style-type: none"> -Accurate if positive -Answers available quickly -Two methods of analysis used, oxygen uptake and spectrographic -Can assess both OXPHOS and β-Oxidation (<i>depends on the lab</i>) -Extra muscle can be used for further specific testing such as PDH and PC -Muscle will be processed for electron microscopy and special stains, which are helpful in diagnosis 	<ul style="list-style-type: none"> -Requires an operation and anesthesia in most centers -Expensive -Risk of anesthesia and surgery -One to two inch scar
Skin Biopsy	<ul style="list-style-type: none"> -Results accurate if positive -No anesthesia -Procedure in dermatologist's office -Less expensive -Tests for disorders of PHD and PC (other disorders that cause lactic acidosis) -Scar is less than ¼ inch 	<ul style="list-style-type: none"> -Results can take 1-3 months -If the fibroblast culture fails or becomes infected, the tests cannot be done, which delays the answer for a few more months

Treatment:

- At this time, there is no cure for these disorders.
- Purposes for treatment
 - alleviate symptoms
 - slow down the progression of the disease
- Effectiveness of treatment
 - varies from patient to patient, depending on the exact disorder and the severity of the disorder
 - as a general rule patients with mild disorders tend to respond to treatment better than those with severe disorders.
 - in some circumstances, the treatment can be tailored specifically to the patient, and that treatment is effective, whereas in other circumstance, the treatment is “emperic”, meaning that the treatment makes sense, but that the benefit of treatment is not obvious or proven to be effective
- Benefits of Treatment/Effectiveness of Therapies Vary
 - treatment may be beneficial and noted immediately in some disorders
 - benefit of treatment may take a few months to notice
 - benefit of treatment may never be noticed, but the treatment may be effective in delaying or stopping the progression of the disease
 - some patients may not benefit from therapy
- Key Points to Treatment
 - dietary
 - vitamins and supplements
 - avoidance of stressful factors
 - These recommendations must be tailored by the patient’s physician to meet that patient’s need. Many of these therapies are totally ineffective in some mitochondrial disorders and would be a waste of time, money and effort. In some cases, the treatment could be dangerous.

Dietary Therapy

Many patients, including young children or mentally impaired persons have already “self-adjusted” their diet because they know what foods their body seem to tolerate. The points below are not meant to be suggested therapies for all patients with OXPHOS disorders, and some of the points are dangerous for patients with other disorders (4b could be lethal in pyruvate dehydrogenase deficiency for example). Do not make any of these dietary changes without consulting a physician. A dietitian experienced in metabolic disorders may be helpful.

1. Avoiding fasting is perhaps the most important part of treatment. This means avoid prolonged periods without a meal (even an overnight “fast” from 8 pm to 8 am may be dangerous in some patients). This also means that some patients should not intentionally try to loose weight. In some patients an unintended fast resulting from an illness that causes vomiting or loss of appetite (like the flu) should be hospitalized to ensure continuous nutrition (intravenous glucose for example).
2. Small frequent meals may be better than a typical 3-meal-a-day routine for some patients.
3. A snack before bedtime may be helpful in some patients. This snack should not be mainly “sugar”, like a candy bar, jello or sweetened cereal. It is usually best if the snack consists of a complex carbohydrate. Cornstarch is the best complex carbohydrate, but this is not very tasty. There is a cornstarch bar called ZBar[®] which is not bad. Theoretically, the best snack would be a homemade low-sugar rice pudding thickened with a lot of cornstarch. If you come

up with a tasty recipe, let the UMDF know. Pasta, bread and butter, unsweetened cereal (oatmeal) or a sandwich are acceptable.

- 4a. In patients with complex I deficiency, the addition of extra fat (fats include added oil, butter, & margarine, as well as other “fatty foods”) to the diet should theoretically result in more energy production. This is because the metabolism of protein and carbohydrate produces electrons that must flow through complex I, which is obviously not working properly in complex I deficiency, but fats produce electrons that in addition to flowing through complex I, also produces electrons that can flow through complex II (bypassing complex I). Therefore, if complexes II, III, IV and V are working properly, fats should be slightly more effective in producing energy. A small clinical study yielded mixed results, with some patients improving and others not.
- 4b. In some patients with OXPHOS disorders, reducing fat may be helpful. This includes reducing added oil, butter, & margarine, and cutting down on cheese and fatty meats. This recommendation is not meant to avoid fats altogether. A defect in the OXPHOS can create an “energy backup”, as the respiratory chain cannot handle the flow of electrons coming into it. This backup may result in the formation of excess free fatty acids (fats waiting to be burned), which can poison the enzyme (adenosine nucleotide translocase) that exchanges the low-energy ADP located outside the mitochondria for the high-energy ATP formed at complex V. If you take the approach of limiting fats, extra effort needs to be made to increase the total carbohydrate (in the form of complex carbohydrates) in the diet.
- 4c. In some patients (see #4a and #4b above), adding fat in the form of medium chain triglycerides (MCT), may be helpful. Medium chain triglycerides of 8 to 10 carbons long are easier to metabolize (turn into energy) than the longer chain triglycerides (those with 12-18 carbons) because they do not require carnitine to be transported into the mitochondria. MCT Oil[®] is mainly made of 8 and 10 carbon triglycerides and this type of oil does not occur in nature, but is made from coconut oil. MCT Oil[®] is made by the baby formula company Mead-Johnson. It comes in quart bottles, available by prescription and runs about \$70 a quart. It can be added like oil over pasta and rice. You can cook with it, but this is a light oil and burns easily. The special rules are explained in a recipe book that you can request from the pharmacist. Depending on the situation, a patient may benefit from a few teaspoons to a few tablespoons a day. There are oils sold in health food stores called “MCT Oil” or “medium chain triglyceride oil”. Many of these contain unprocessed coconut oil, which is a 12 carbon triglyceride that requires carnitine for entry into the mitochondria. This would be a waste of money. Unless there is a certified analysis on the label, stay away from these products and stick with the Mead-Johnson brand.
5. Iron generate free radicals under certain conditions, which is especially bad in mitochondrial diseases because the free radicals injure mitochondrial DNA and “poke holes” in the mitochondria, making a bad problem worse. Therefore, iron is theoretically harmful in excess. There is no need to give supplemental iron in vitamins, nor is there a reason to eat foods rich in iron, such as extra red meat, for the purpose of eating foods rich in iron. This does not mean that the person should not eat red meat, especially if they enjoy it. There is no reason to take vitamins with added iron. In addition, vitamin C enhances the absorption of iron from the intestines, and vitamin C should not be given around a meal rich in iron. This is important to remember because some experts feel that vitamin C is a good antioxidant, and also may be helpful in some disorders of OXPHOS.

Vitamins and Cofactors

Vitamins and cofactors are compounds that are required in order for the chemical reactions, which make energy, to run efficiently. By definition, a cofactor can be made by the body, whereas a vitamin cannot, and therefore must be eaten. For most people, a regular diet contains all the vitamins one could possibly need and their bodies can make as much of any specific cofactor that it needs. For those with mitochondrial disorders, added vitamins and cofactors can

be useful. The use of supplemental vitamins and cofactors is controversial in that there are no proven benefits to some of these therapies. For disorders of OXPHOS, coenzyme Q10 is considered as a generally accepted effective therapy, although it may not ultimately be effective for an individual patient. Other treatments are proven therapy is specific disorders, but in other disorders cannot be considered as “proven and effective” but still may be helpful. Some treatments should only be undertaken under the specific guidance of your physician. For specific information about the controversy, as it relates to your or your child’s situation, ask your physician. Most of these vitamins can be purchased from many sources, including the drugstore.

The sources listed below have been found to be fairly priced (often significantly less than the drugstore) and sell very high quality products. These supplemental compounds can serve two functions:

- POSSIBLY ENHANCE ENZYME FUNCTION AND RESULT IN IMPROVED EFFICIENCY OF ENERGY GENERATION
- SERVE AS ANTIOXIDANTS, WHICH MAY SLOW THE PROGRESSION OF THE DISEASE

Table 2: Vitamins and Supplements That May be Helpful

Table 2a: Suggested to most of my patients

Supplement	Dose Range	Patient Dose
CoQ10	5 – 15 mg/kg/day	
levo-carnitine (Carnitor [®])	Variable, starting dose of 30 mg/kg/day, typical max 100 mg/kg/day	
Riboflavin (B2)	50-100+ mg a day	

Table 2b: Second Tier Supplements

Supplement	Dose Range	Patient Dose
Acetyl-L-Carnitine	250 – 1000 mg per day	
Thiamine (B1)	50-100 mg a day	
Riboflavin (B2)	50-100+ mg a day	
Nicotinamide (B3)	50-100 mg a day	
Vitamin E	200-400 IU; 1 - 3 times a day	
Vitamin C	100-500 mg; 1 - 3 times a day	
Lipoic Acid (α-lipoate)	60-200 mg; 3 times a day	
Selenium	25-50 micrograms a day	
β-carotene	10,000 IU; every other day to daily	
Biotin	2.5 – 10 mg a day	
Folic Acid	1 – 10 mg a day	

Table 3: Medication, Minerals, Vitamins, Substrates that May be Helpful (only to be used under a physicians direction)

Supplement	Dose Range	Your Dose
Calcium	Variable	
Magnesium	Variable	
Phosphorous	Variable	
Vitamin K ₃	5 - 30 mg per day (1-800-266-9583)	
Succinate	6 gm per day	
Creatine	5 gm bid after initial load (adults)	
Uridine	To be determined	
Citrates	variable	
Prednisone	variable	

Avoidance of Physiologic “Stress”

Physiologic stress are external factors that may result in worsening the metabolic situation, which may result in temporary, or in sometimes, permanent worsening of the condition. It is impossible to avoid all physiologic stressful conditions, so one should not attempt to do so. However, recognizing what may be stressful for a patients allows one to adjust the lifestyle. Many patients and their parents have already identified these stresses, despite not knowing why the stresses were important, and avoid them.

- Cold Stress is extremely important. Thermal regulation (temperature control) is not always normal in people with mitochondrial diseases and exposure to cold can result in severe heat loss and trigger an energy crisis. When going out into the cold, all exposed body parts should be covered, and exposure to extreme cold be avoided for anything more than a short period. Over bundling can be a problem too (see below).
- Heat Stress can be a problem in some people. This is especially true of those with an inability to sweat normally. Heat exhaustion and heat stroke may occur on hot days. An example of a typical scenario for this situation would be a child would seems to “wilt” in situations like hot classrooms, whereas the other students function normally. Light clothing is important. Patients should avoid direct sunlight on hot days and stay indoors if it is too warm outside. An air conditioned environment may be needed.
- Starvation....see above about fasting
- Lack of sleep may be possibly be harmful.
- Individual distinctive stresses

Avoidance of Toxins

- Alcohol has been known to hasten the progression of some conditions.
- Cigarette smoke, probably due to the carbon monoxide is known to hasten the progression of some conditions. Remember that carbon monoxide kills by inhibiting complex IV of the OXPHOS chain. If there is already a dysfunction of OXPHOS, why make it worse. Cigarette smoke will make it worse.
- MSG (monosodium glutamate) has for years been known to cause migraine headaches in otherwise healthy individuals, and may trigger these events in susceptible people with mitochondrial diseases. MSG is frequently added to Chinese (and other Asian) foods, and is also found in high levels in dried and canned soups. Read the label and avoid MSG.

Mitochondrial Evaluation Worksheet

Name:
DOB

Test	Laboratory	Date	Results
CK			
Lactate			
Pyruvate			
L/P Ratio			
Ammonia			
Free T4, TSH			
Electrolytes			
Glucose			
Ketones; urine			
Ketones; blood			
Amino Acids; blood state:			
Amino Acids; urine state:			
Organic Acids state:			
Carnitine; urine state:			
Carnitine; blood state:			
Acylglycines			
EKG			
Cardiac Echo			
Eye Exam			
ERG			
MRI			
Audiogram			
BAEP			
Molecular Genetics			
Blood			
Southern Blot			
Point Mutations			
Skin EM			
Fibroblast Culture			
Fibroblast Studies			
Muscle Histology			
Muscle EM			
Mito Yield			
Muscle OXPHOS			

Bibliography

Mainstream Journals:

1. Sokol RJ. Expanding spectrum of mitochondrial disorders. *J Peds* 1996;128:597-9.
2. Johns DR. Mitochondrial DNA and disease. *NEJM* 1995;333:638-44.
3. Munnich A et al. Clinical presentation of mitochondrial disorders in childhood. *J Inher Metab Dis* 1996;19:521-527.

Lay Press:

4. Wallace DC. *Scientific American*. August 1997.

Expanding Spectrum into Aging and Common Degenerative Diseases (Alzheimers, Parkinsons, etc.)

5. Beal MF, et al. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? *Trends Neurosci* 1993;16:125-131.
6. Wallace DC. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 1992;256:628-632.

What is next?!

7. Priller J et al. Frataxin gene of Friedreich's Ataxia is targeted to mitochondria. *Ann Neurol* 1997;42:265-269.

Best Overview of Subject:

8. Shoffner JM, Wallace DC. Oxidative phosphorylation diseases and mitochondrial DNA mutations: diagnosis and treatment. *Annu Rev Nutr* 1994;14:535-568.