Services Offered

1) Skeletal Muscle - Isolate mitochondria
   Liver – Isolate mitochondria
2) Integrated Mitochondrial Function - 
   Oxidative Phosphorylation
   Skeletal Muscle / Liver Mitochondria
3) Analysis of the Electron Transport Chain (ETC)
   Skeletal Muscle (fresh or frozen), Liver (fresh or frozen), Heart (frozen), and Brain (frozen)

OXIDATIVE PHOSPHORYLATION:
DISEASES UNCOVERED

- Complex I Deficiency
- Complex II Deficiency
- Complex IV Deficiency
- Complex III or Complex IV Deficiency
  - OXPHOS defect BUT velocity of ETC in the control range
- Substrate Utilization
  - Pyruvate oxidation selective defect
    ♦ PDH activity
    ♦ Pyruvate transporter
- TCA cycle defect
- Defect in fatty acid oxidation
  - Long-Chain / Medium-Chain
- Phosphorylation system—
  - Adenine Nucleotide Tranlocase
  - Phosphate Transporter
  - Complex V—ATPase

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Normal Isolated Human Skeletal Muscle Mitochondria

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Among the nation’s leading academic medical centers, University Hospitals Case Medical Center is the primary affiliate of Case Western Reserve University School of Medicine, a nationally recognized leader in medical research and education.

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ELECTRON TRANSPORT CHAIN ANALYSIS

Skeletal Muscle, Liver, Heart, Brain
- Complex I/III linked
- NADH-Cytochrome c Reductase (rotenone-sensitive)
- Complex II/III linked
- Succinate - Cytochrome c Reductase (antimycin A sensitive)
- Complex III
- Decylubiquiol - Cytochrome c Reductase (antimycin A sensitive)
- Cytochrome c Oxidase (cyanide sensitive)
- NADH-Ferricyanide Reductase (first 3 proteins of Complex I)
- Succinate Dehydrogenase
- Citrate Synthetase
- Lactate Dehydrogenase
- Calculate Mitochondrial protein content (mg/g wet weight muscle)

Isolated Skeletal Muscle Mitochondria (and Liver)
- Complex I
  - NADH-Decylubiquione Reductase (rotenone-sensitive)
- Complex II ± duroquinone
- Complex II/III linked
  - NADH-Cytochrome c Reductase (rotenone-sensitive)
- Complex II/III linked
  - Succinate - Cytochrome c Reductase (antimycin A sensitive)
- Complex III
  - Decylubiquiol - Cytochrome c Reductase (antimycin A sensitive)
- Cytochrome c Oxidase (cyanide sensitive)
- NADH-Ferricyanide Reductase (first 3 proteins of Complex I)

ETC Analysis

Homogenate

Subtilisin Pellet EM

A. SSM aggregation. B. Higher magnification of A. Individual organelles are pleomorphic and possess distorted cristae. One mitochondrion has a lipid droplet-like structure inside the organelle. Bars=1µm

Oxidative Phosphorylation
- Glutamate
- Glutamate + Malate
- Pyruvate + Malate
- α-Ketoglutarate + Malonate
- Palmitoylcarmitine + Malate
- Linoleoylacmitine + Malate
- Palmitoyl-CoA + Carnitine + Malate
- Octanoylacmitine + Malate
- Octanoyl-CoA + Carnitine + Malate
- Octanooate + Malate
- cis-4-Decenoylacmitine + Malate
- Acetyl-L-carmitine + Malate
- Succinate (+ rotenone)
- Duroquinol (+ rotenone)
- Glycerol-3-phosphate (+ rotenone)
- TMPD + ascorbate (+ rotenone)
- NADH + Cytochrome C
- Pyruvate + Malonate + Carnitine
- ANT by CarboxyATrK titration

Phospholipids
- Cardiolipin

Blue Native Electrophoresis

Carnitine/ Acylcarnitines

Prepare Mitochondria

EM

Subtilisin ppt

Isolated mitochondria

Three large mitochondria, 5.57µm, 3.7µm, and 3µm, found in a subtilisin pellet.