

## Services Offered

### Cultured Skin Fibroblasts

- 1) Integrated Mitochondrial Function Oxidative Phosphorylation
- 2) Analysis of the Electron Transport Chain (ETC)
- 3) Acylcarnitine analysis during fatty acid oxidation

### CULTURED SKIN FIBROBLASTS OXIDATIVE PHOSPHORYLATION: DISEASES UNCOVERED

- Complex I Defect
- Complex II Defect
- Complex III Defect
- Complex IV Defect
- Complex III or Complex IV Defect
  - \* OXPHOS defect BUT velocity of ETC in the control range
- Substrate Utilization
  - \* Pyruvate oxidation selective defect - normal PDH activity, work-up toward pyruvate transporter
- Defect in fatty acid oxidation exploring FAO pathway
- Phosphorylation system—
  - \* Adenine Nucleotide Translocase
  - \* Phosphate Transporter
  - \* Complex V—ATPase

#### Team Members

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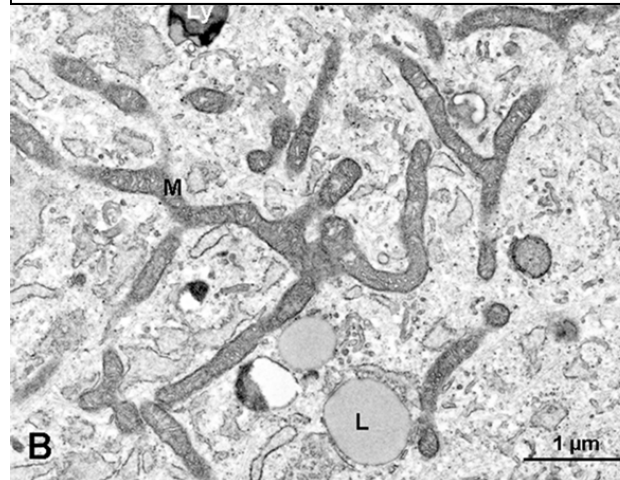
## Center for Inherited Disorder of Energy Metabolism (CIDEM)

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Electron micrograph of a cultured skin fibroblast  
M = mitochondrion; L = lipid.



*Courtesy of Dr. Hisashi Fujioka*

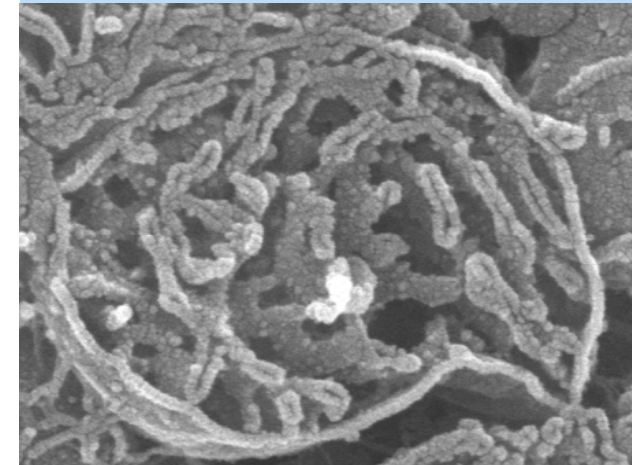
Among the nation's leading academic medical centers, University Hospitals Case Medical Center is the primary affiliate of Case Western Reserve University School of Medicine, a nationally recognized leader in medical research and education.



# CIDEM

Center for Inherited  
Disorders of Energy  
Metabolism  
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## Mitochondrial Diseases Skin Fibroblasts



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## CULTURED SKIN FIBROBLASTS OXIDATIVE PHOSPHORYLATION

### ELECTRON TRANSPORT CHAIN ANALYSIS

- NADH-cytochrome c Reductase (rotenone sensitive I-III)
- Succinate - Cytochrome c Reductase (antimycin A sensitive II-III)
- Decylubiquinol - Cytochrome c Reductase (antimycin A sensitive)
- Cytochrome c Oxidase (cyanide sensitive)
- Citrate Synthetase
- Lactate Dehydrogenase
- Phosphorylation

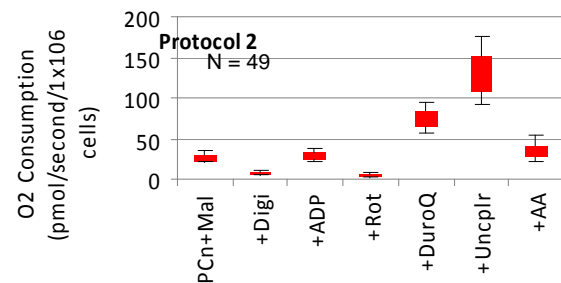
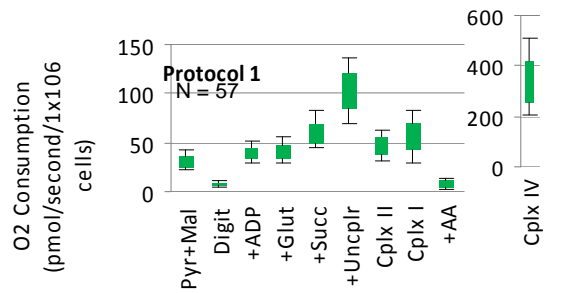
### Protocol 1: Complex I, II and IV respiration

- Oxygen consumption was monitored starting with pyruvate and malate as substrates.
- ADP-stimulated rate was recorded after the cell membrane was permeabilized by digitonin (Dig).
- Glutamate and succinate were sequentially added to obtain the rates with all substrates present.
- An uncoupler, FCCP, was added to provide maximum oxidative rate.
- The addition of rotenone (Complex I inhibitor) allowed us to measure the Complex II dependent rate.
- Antimycin A inhibits Complex III and yielded the non-mitochondrial oxygen consumption.
- Finally, the addition of TMPD + ascorbate reduces cytochrome c and provided assessment of the uncoupled Complex IV respiration, calculated as the azide-sensitive rate.

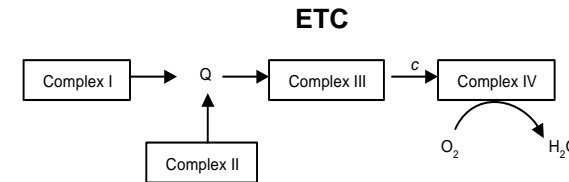
### Protocol 2: Fatty Acid Oxidation and Complex III OXPHOS with DHQ

- Fatty acid oxidation was assayed using palmitoylcarnitine plus malate.
- Cells were permeabilized and stimulated by ADP.
- Following rotenone, Complex III OXPHOS rate was measured using duroquinol
- Maximum CIII capacity with FCCP.
- Last, the Antimycin A-insensitive rate was measured.

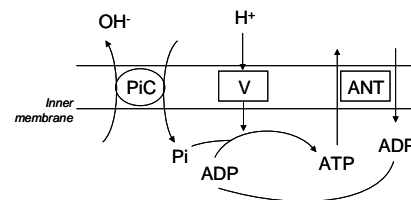
### REFERENCE INTERVALS



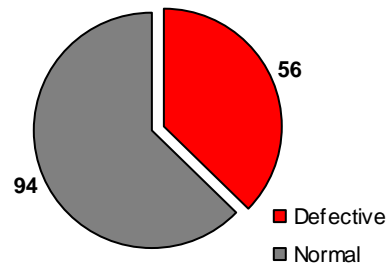
Data shown are mean ± SD (box) and minimum and maximum (whiskers) of different normal human skin fibroblast lines used for reference intervals.



### Phosphorylation System



### Diagnostic Results



### References:

1. Ye F, Hoppel CL. Measuring oxidative phosphorylation in human skin fibroblasts. *Anal Biochem.* 2013 Jun 1;437(1):52-8
2. Wanders RJ, Ruiten JP, Wijburg FA. Studies on mitochondrial oxidative phosphorylation in permeabilized human skin fibroblasts: application to mitochondrial encephalomyopathies. *Biochim Biophys Acta.* 1993 Jun 19;1181(3):219-22.
3. Chretien D, Rustin P, Bourgeron T, Rötig A, Saudubray JM, Munnich A. Reference charts for respiratory chain activities in human tissues. *Clin Chim Acta.* 1994 Jul;228(1):53-70.

### Defects Identified

