



**Module 3**  
Chapter 3:  
Function Testing





## Function Testing as a **Diagnostic Target**

Since different forms of mitochondrial dysfunction may affect the brain, heart, peripheral nervous and endocrine systems, eyes, ears, guts, and kidneys, amongst other organs, mitochondriopathies have been proposed as an attractive diagnostic target to be investigated in any patient with an unexplained progressive multisystem disorder.

Generalized approaches, such as physical exercise, which demonstrate neuroprotective, cardioprotective, and anti-cancer effects, are clearly associated with mitochondrial function support.

Function of the mitochondria can be changed by lifestyle and therapeutic interventions. Functional testing provides a means to stratify and monitor progress

Phytochemicals, naturally occurring compounds used due to their neuroprotective, cardioprotective, and anti-carcinogenic effects, have been demonstrated to modulate mitochondrial function, structure, and related mechanisms.

Recent progress in the area of mitochondriopathies revealed that patient stratification and risk assessment are supportive of effective treatments, considering the level of mitochondrial impairment and individual predisposition to associated pathologies.



Common Method Used to Measure

# Mitochondrial Function



## Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)

The measurement of OCR and ECAR provides a systems-level view of cellular metabolic functions. OCR is an indicator of mitochondrial respiration, while ECAR reflects glycolysis. These measurements are typically performed using the Seahorse XF Analyzer, which allows real-time monitoring of cellular bioenergetics in live cells (Nature).



## High-Resolution Respirometry

High-resolution respirometry involves measuring the oxygen consumption of isolated mitochondria or permeabilized cells using a Clark-type oxygen electrode. This method provides detailed information on the activity of the electron transport chain (ETC) complexes and overall mitochondrial respiration (Nature).



## Fluorescent Probes for Membrane Potential

Fluorescent probes, such as tetramethylrhodamine methyl ester (TMRM) and JC-1, are used to measure the mitochondrial membrane potential. These probes accumulate in mitochondria in a potential-dependent manner, allowing for the assessment of mitochondrial health and function (PubMed).



Modern analytical instrumentation has the ability to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in live cells making it a more broadly available method and process.

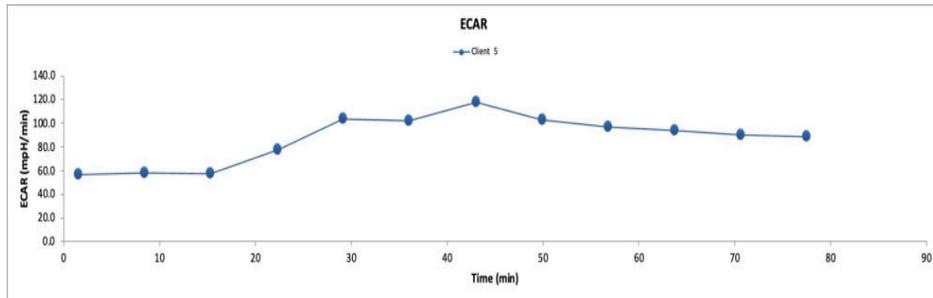
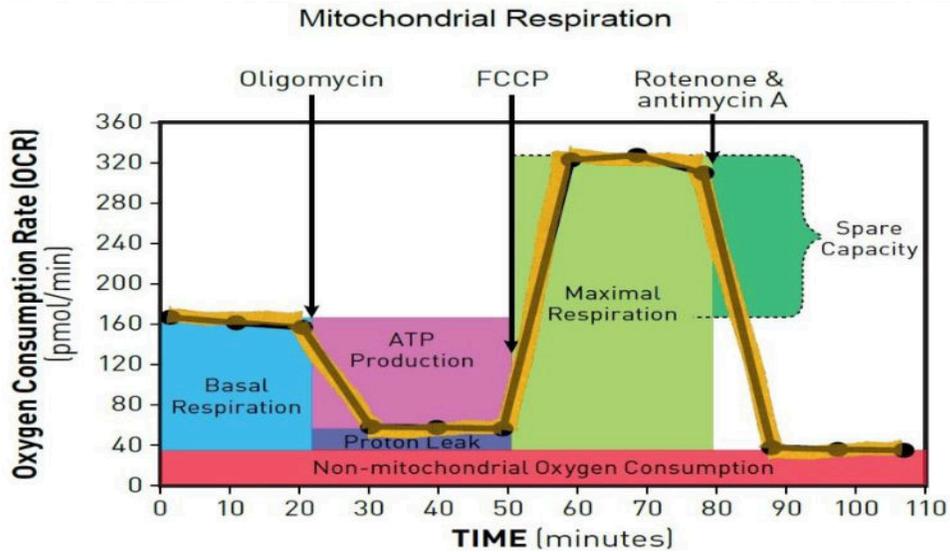
OCR and ECAR rates are key indicators of mitochondrial respiration and glycolysis.

These measurements provide a systems-level view of cellular metabolic functions, which are precursors to downstream physiologic processes.



# Analytical Process and Calculations

Function is analyzed under basal and stressed conditions



Parameter Value	Equation
Non-mitochondrial Oxygen Consumption	Minimum rate measurement after Rotenone/antimycin A injection
Basal Respiration	(Last rate measurement before first injection) – (Non-Mitochondrial Respiration Rate)
Maximal Respiration	(Maximum rate measurement after FCCP injection) – (Non-Mitochondrial Respiration)
H <sup>+</sup> (Proton) Leak	(Minimum rate measurement after Oligomycin injection) – (Non-Mitochondrial Respiration)
ATP Production	(Last rate measurement before Oligomycin injection) – (Minimum rate measurement after Oligomycin injection)
Spare Respiratory Capacity	(Maximal Respiration) – (Basal Respiration)
Spare Respiratory Capacity as a %	(Maximal Respiration) / (Basal Respiration) × 100
Acute Response	(Last rate measurement before oligomycin Injection) – (Last rate measurement before acute injection)
Coupling Efficiency	ATP Production Rate) / (Basal Respiration Rate) × 100



# Functional Panel Test

## Functional and Dynamic Measures



Non-mitochondrial respiration



Basal respiration



Proton Leak



ATP-linked respiration



Spare capacity



Glycolysis



Oxidative Phosphorylation



Metabolic Potential



Reactive Oxygen Species (ROS)

Modern analytical instrumentation can measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in live cells.

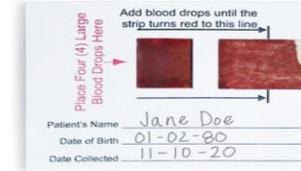
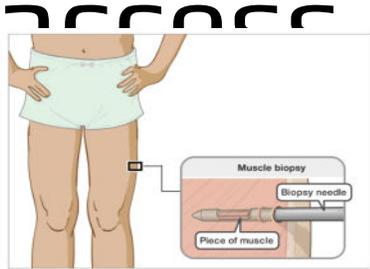
OCR and ECAR rates are key indicators of mitochondrial respiration and glycolysis.

These measurements provide a systems-level view of cellular metabolic functions, which are precursors to downstream physiologic processes.

Paired with other information, the ability to assess function provides a tool to identify with more specificity **what is actually happening** at a cellular level.



# Mitochondrial testing migration from research only to broad



## Gold Standard: Muscle Biopsy

- Gold standard cell based assessment
- Direct measure of function (Multiple bioenergetic measures)
- Link to disease status

## Blood Draw Functional Assay and Standard PBMC

### Advantages

- Validated against biopsy
- Less invasive blood draw
- Equal to biopsy plus ability to capture formed element

## Blood card (mescreen)

- Validated against blood draw
- Non-invasive professional or self collection
- Stable specimen low time burden assay
- Ability for multi-period testing annually
- Variety of endpoint measures correlate to biopsy
- Low cost assay allows for access and scalability

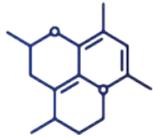
### Disadvantages

- |   |  |  |
|---|--|--|
| <ul style="list-style-type: none"> <li>• Used in research</li> <li>• Painful, invasive, infection potential</li> <li>• Poor adherence and compliance</li> <li>• Expensive time-consuming assay requiring frozen sample shipment</li> <li>• Doesn't provide recommendations</li> </ul> | <ul style="list-style-type: none"> <li>• Used in research</li> <li>• Requires collection site to draw</li> <li>• Adherences issues due to scheduling</li> <li>• High-cost time-consuming assay</li> <li>• Doesn't provide recommendations</li> </ul> | <ul style="list-style-type: none"> <li>• Portion of population doesn't like finger prick collection</li> <li>• Doesn't provide recommendations</li> <li>• Lack of awareness of new functional markers</li> </ul> |
|---|--|--|



# Limitations of Current Methods of Assessing Mitochondrial Function

## Current Methods



**Biomarker & Enzyme  
& Chemistry Testing**

**What MAY be Happening**



**Genetic Testing**

**What MIGHT Happen**



**Function Testing**

**What IS Happening  
(mescreen)**

Blood and urine-based biomarker testing limitations include:

- High inter-person variability
- Measures not directly correlated to specific dysfunction ( i.e. high lactate indicates there may be a dysfunction, or you may have exercised which elevated the level)

Genetic testing limitations include:

Only indicate likelihood of having or potentiating a condition

Previous limitations include:

- Limited access through academic and scientific research and studies
- invasive sample collection in clinical applications
- High cost to perform



# Summary

Genetic testing is effective to establish primary mitochondrial disease.

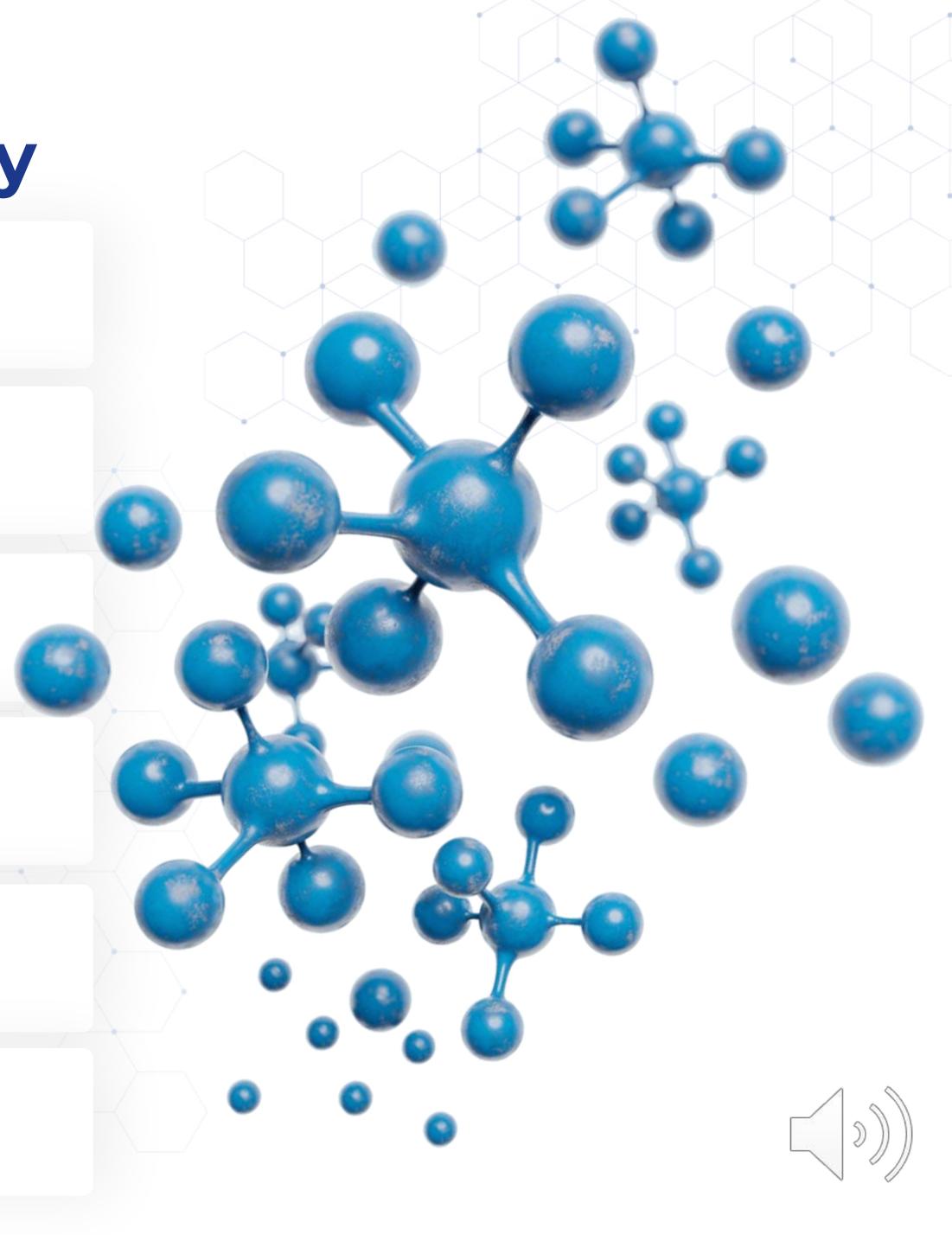
Genetic testing results of having a gene associated with a condition does not mean one will get that disease however facilitates stratifying that individual.

epigenetic testing like bio marker testing has high inter-person variability and is influenced by many factors outside of disease making it difficult to use single markers to diagnose mitochondrial disease (Primary or Secondary).

Biomarkers have high interperson variability and can be influenced factors outside of disease such as diet, exercise, environmental stress.

Functional testing can provide a new data set that provides specificity into degradations in function that can help better understand the cause of one's condition and guide the medical interventions under consideration.

Functional testing until recently has been limited to funded research preventing it from becoming broadly available.



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