

# 分析粒線體功能與 代謝研究的最佳工具

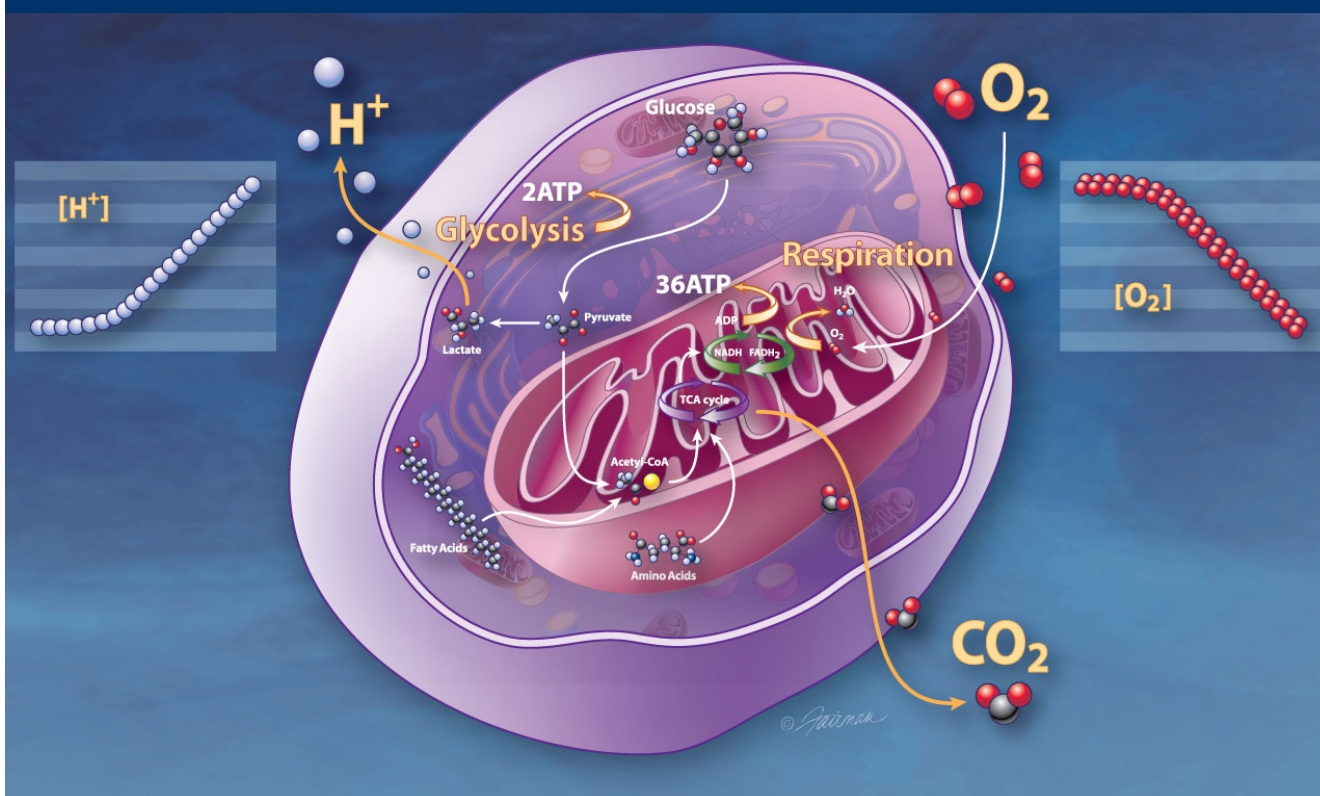
海馬生物能量測定儀  
Seahorse XF analyzer

Product specialist  
李文瑋 David Li

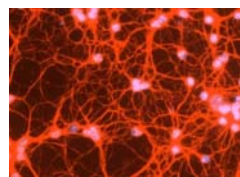
 **尚博生物科技公司**  
Cell-Bio Biotechnology Co., Ltd.

Office: 02-27855860 Mobile: 0919830158  
david.cellbio@gmail.com

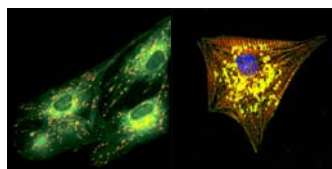
## Mitochondria



## Decade of the mitochondria



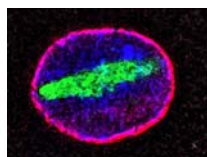
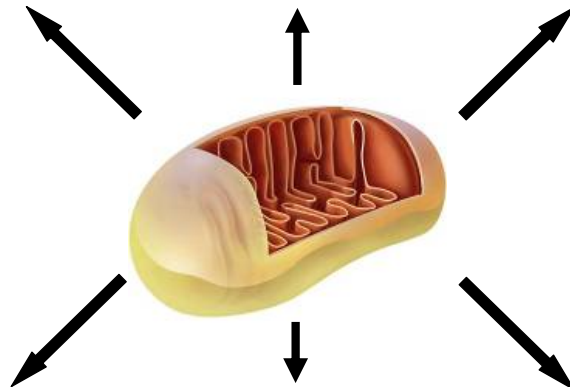
Neurodegenerative Disease



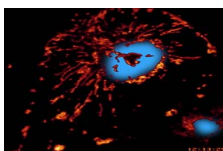
Obesity/Diabetes



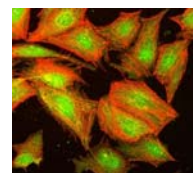
Cancer



Aging



Hepatobiology &  
*In vitro* toxicity

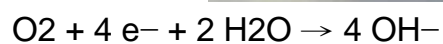


Cardiovascular

## Legacies from Leland Clark

- End-point assay
- Single parameter
- Full user attention
- Low through-put
- High sample amount

1953 - Clark Electrode

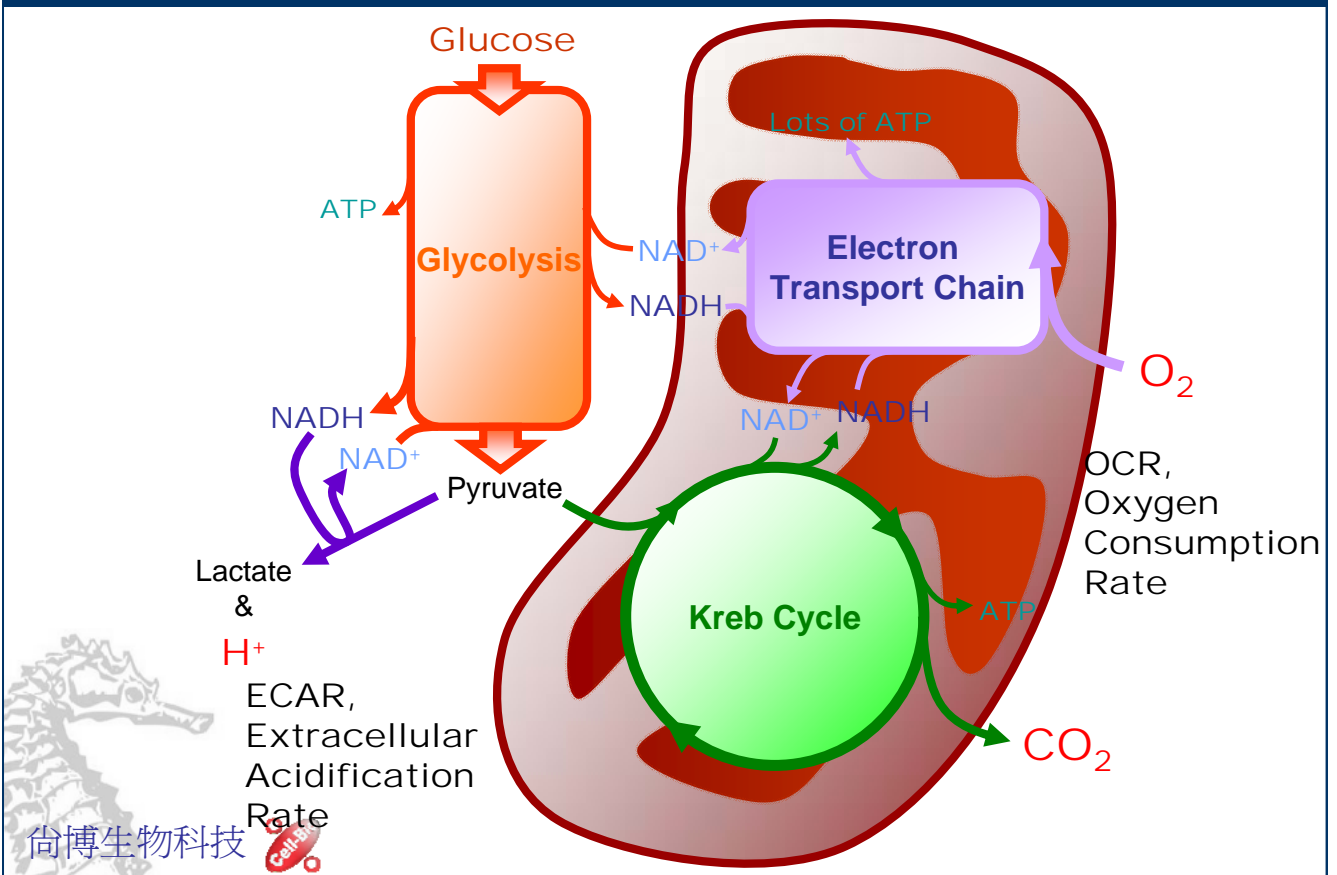


# Cellular Bioenergetics for the 21<sup>st</sup> Century

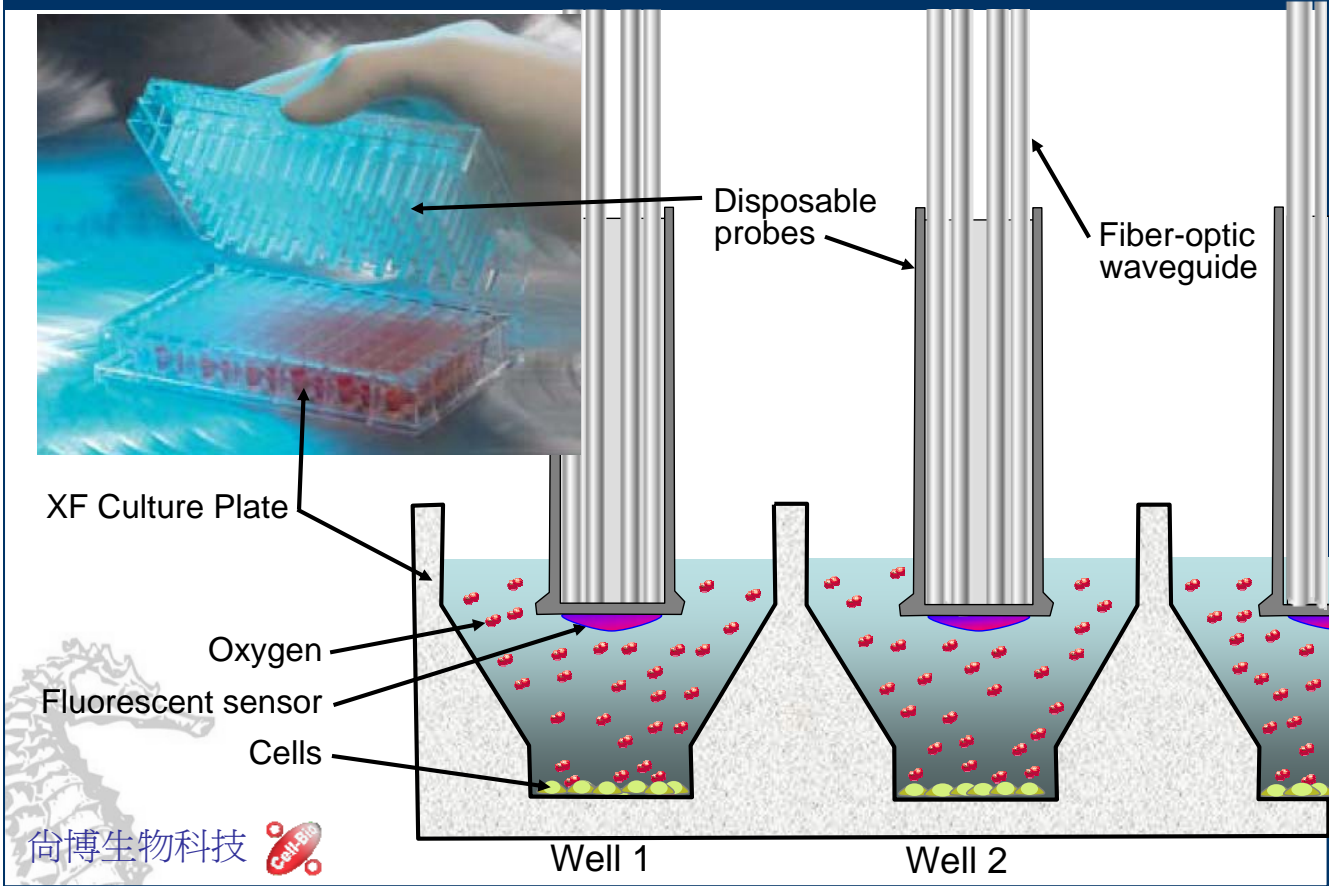
## Extracellular Flux (XF) Analyzers



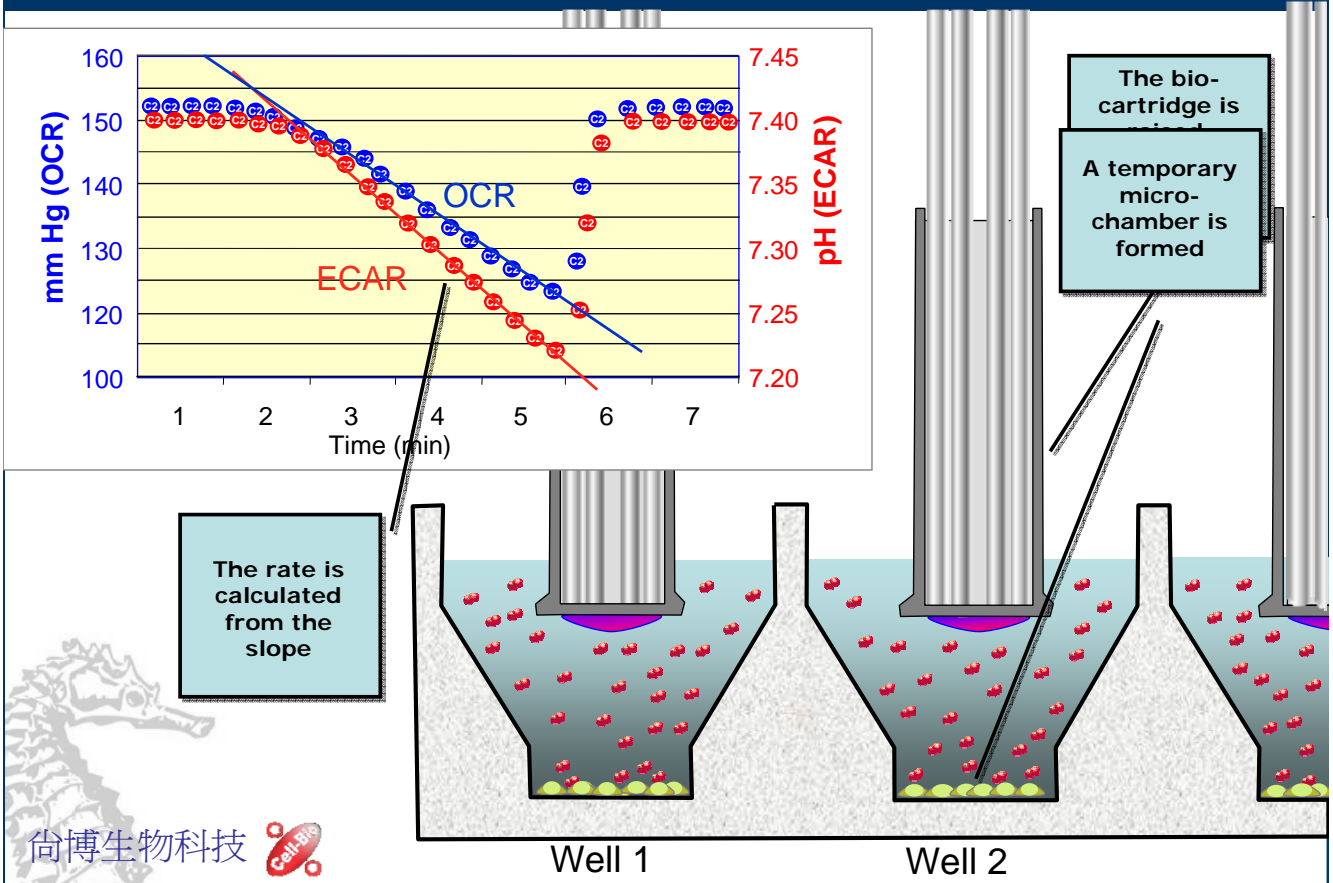
## Major Bioenergetics Pathway



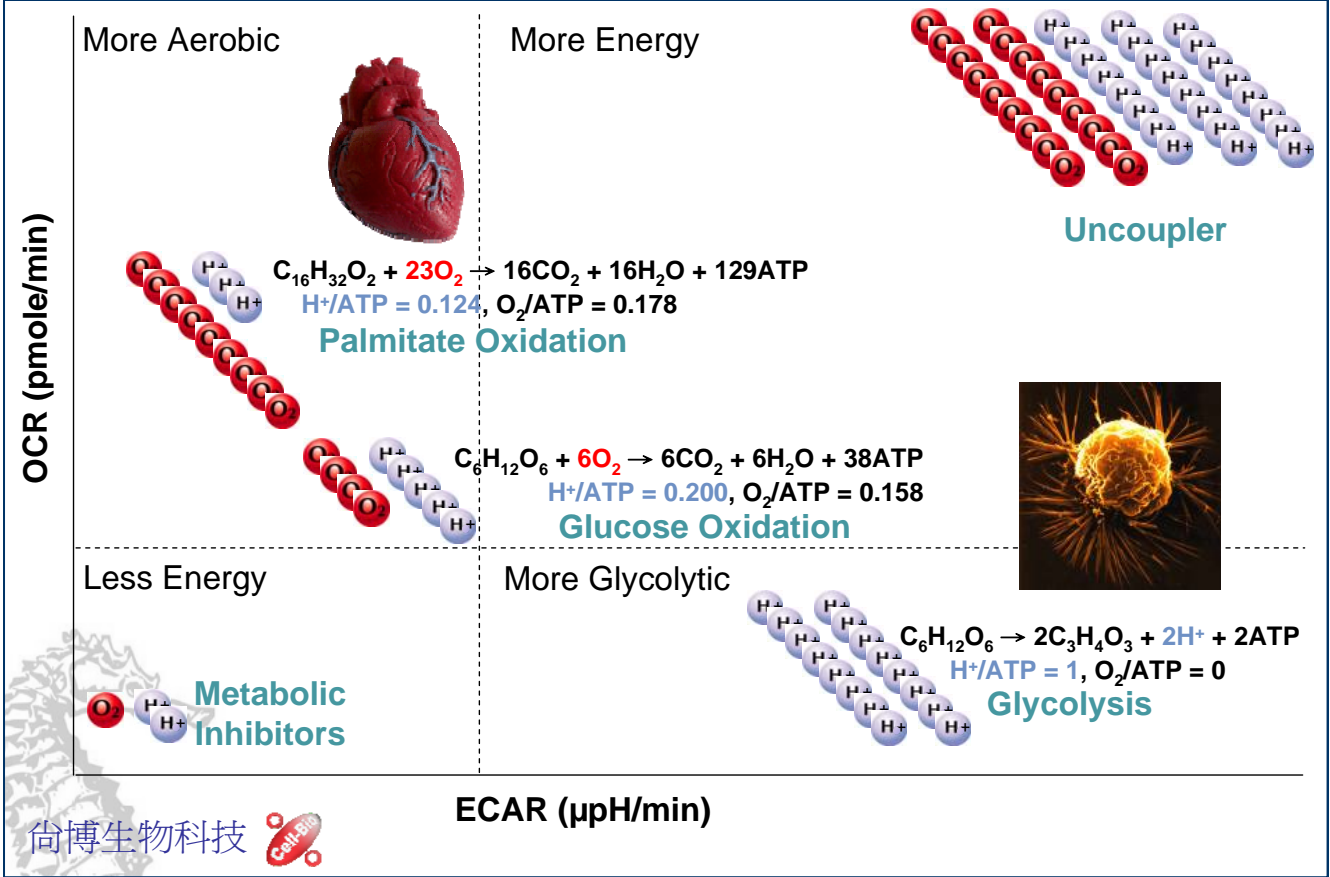
# Measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)



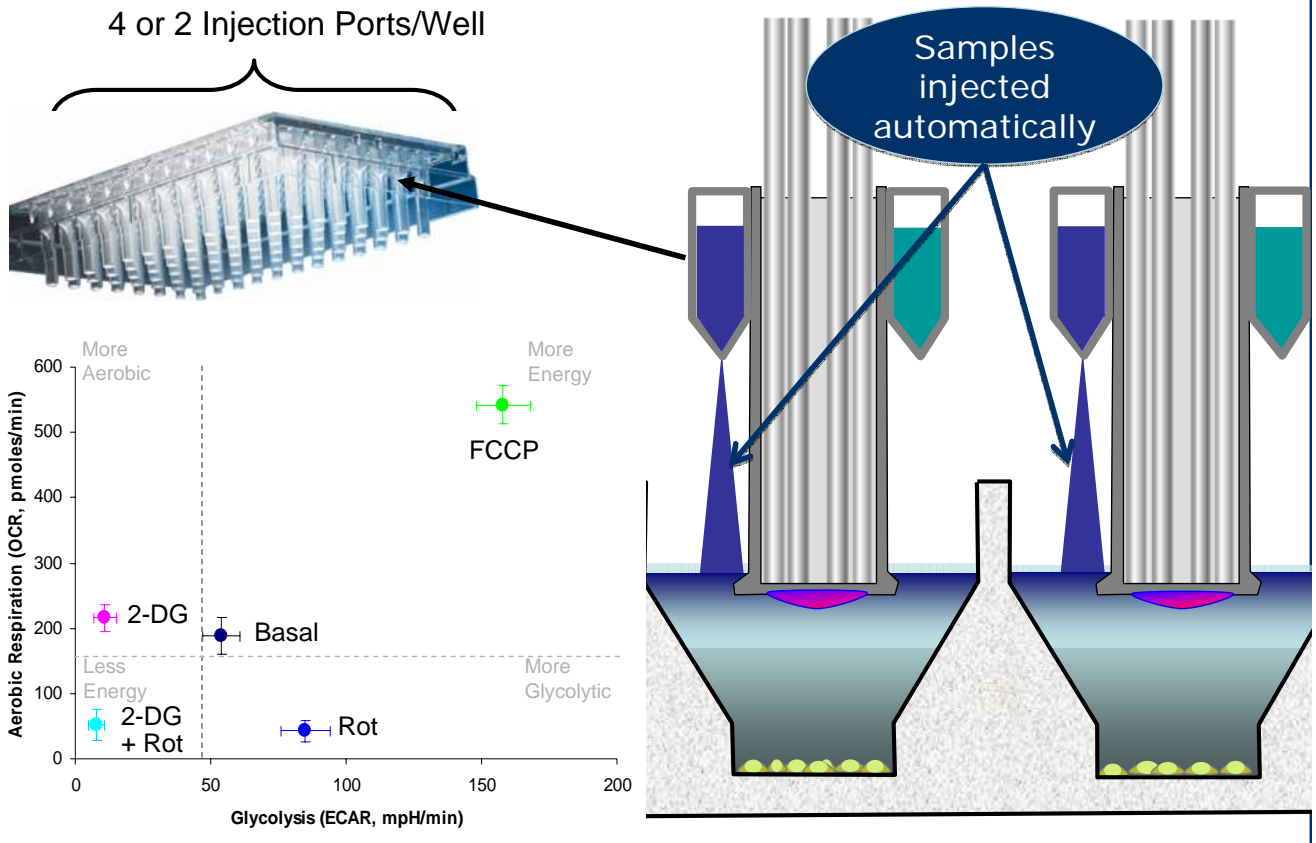
# Measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)



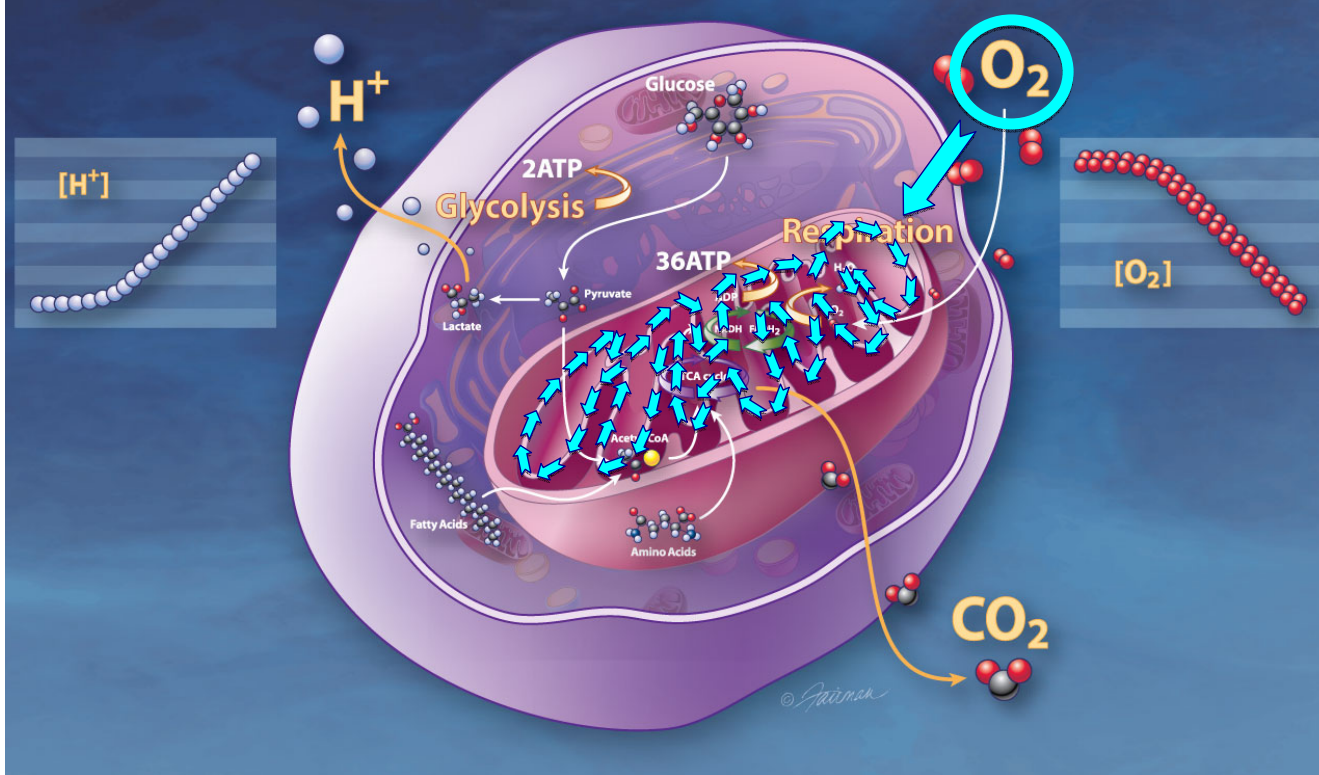
# Quantifying Bioenergetics and Substrate Utilization



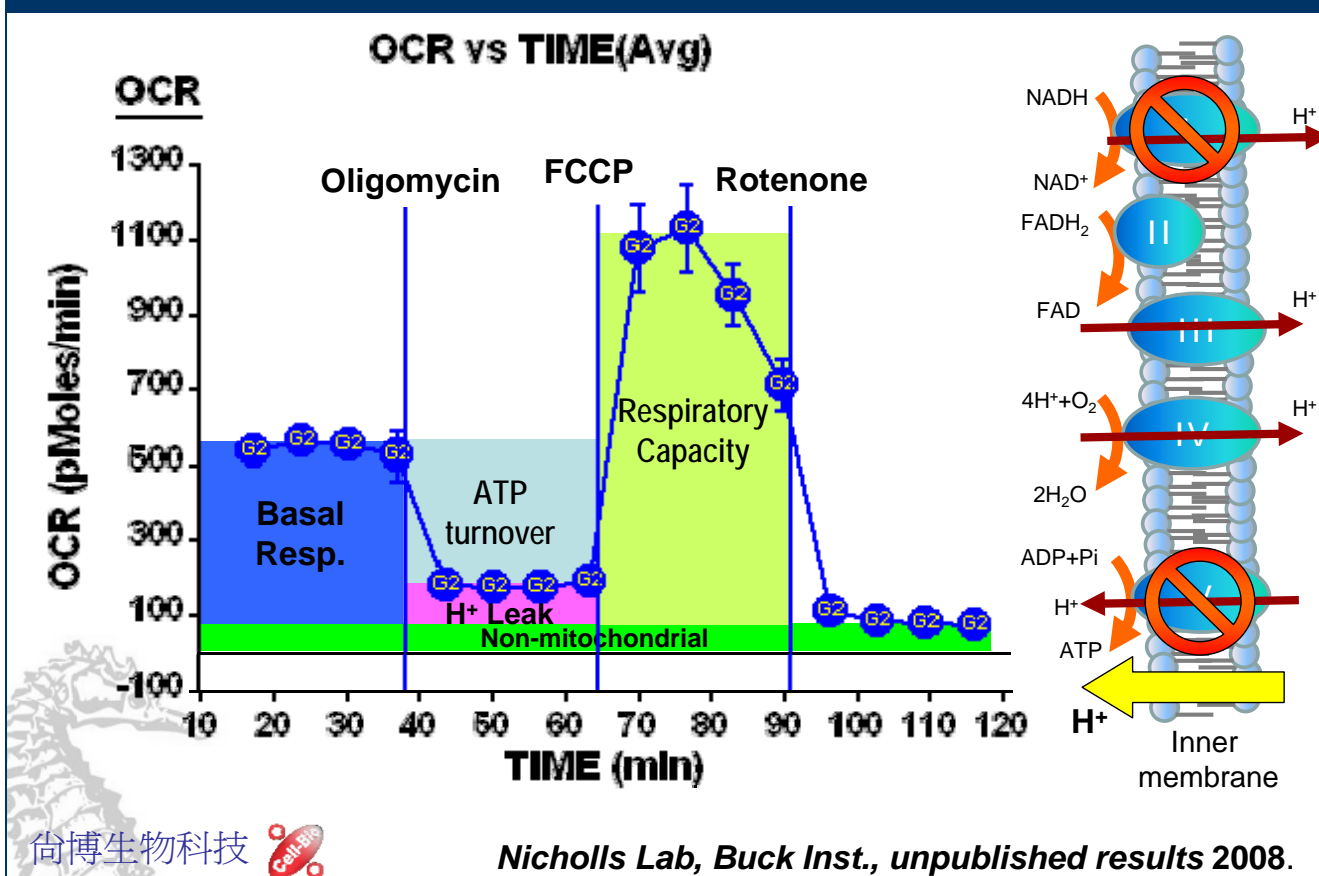
# Multiple Automatic Injection Ports Allows More Conditions to be Assessed Per Experiment



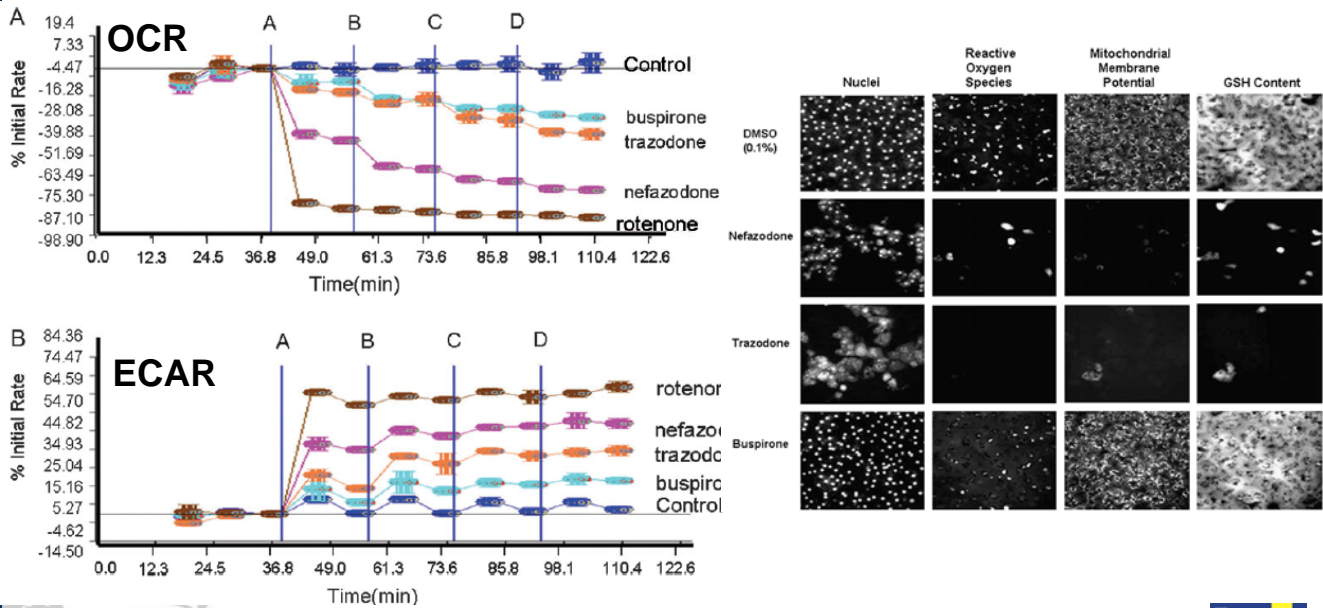
# Mitochondrial Function



## Increased Physiological Content in a Single Well



# In Vitro Assessment of Mitochondrial Dysfunction and Cytotoxicity as an indicator of potential toxicity



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*Toxicol Sci* 2008; 103 (2):335-45

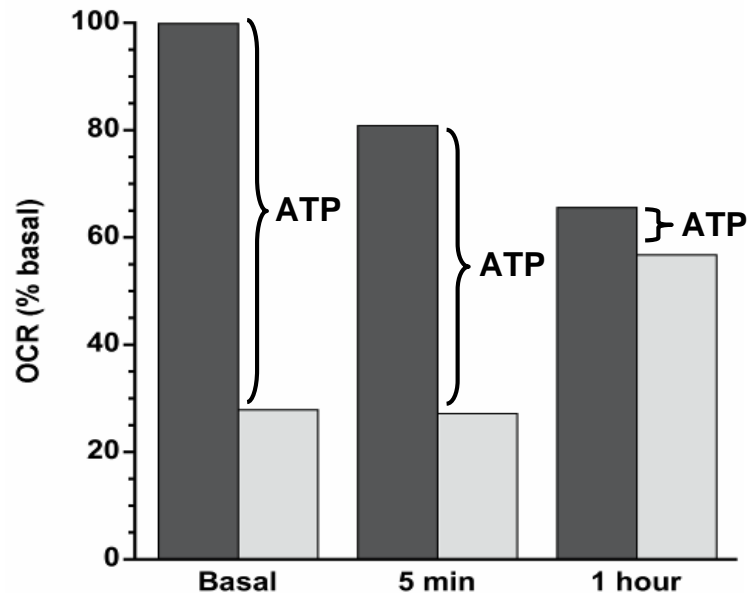


# Identifying compounds that cause oxidative stress



*Drug Discov Today* 2008; 13 (5-6):268-74

- Primary human hepatocytes + 50  $\mu$ M dantrolene
- Primary human hepatocytes + 50  $\mu$ M dantrolene + 10  $\mu$ g/ml Oligomycin



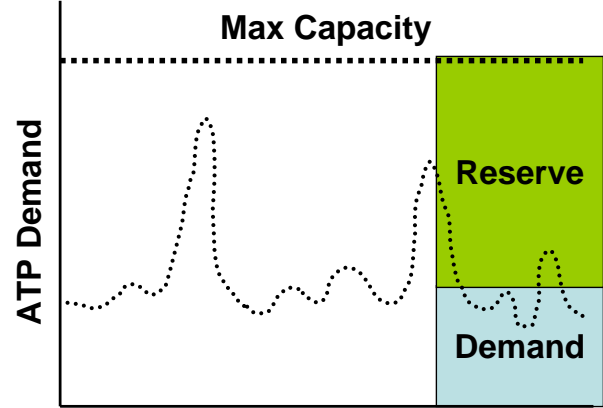
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# All cells carry an energy reserve that determines how they will handle an overload or chronic stress



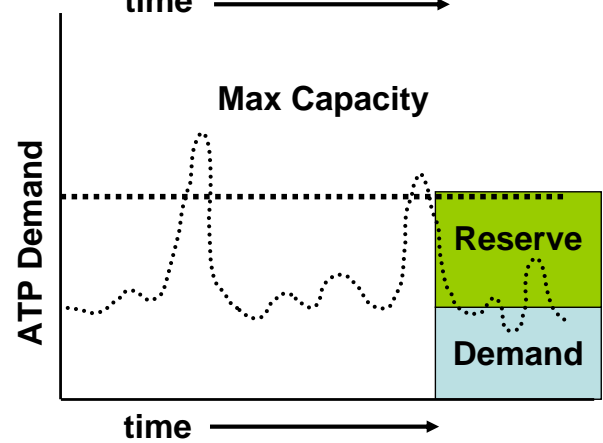
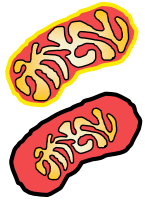
*J Neurochem* 2009; 109:1179-91.

Functional,  
Healthy



*Biochem J* 2009; in revision.

Disease,  
Aging

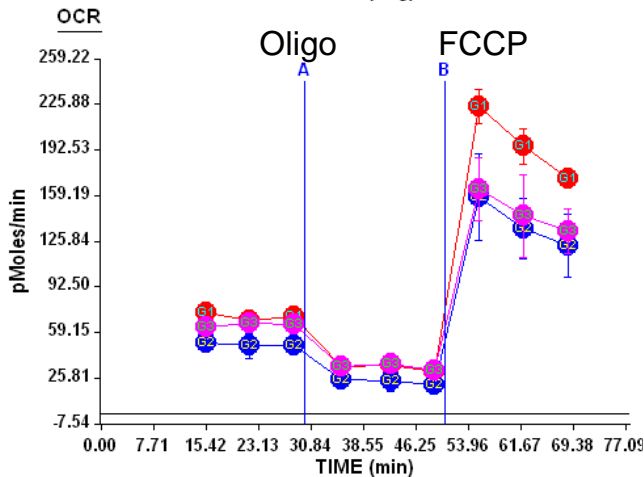


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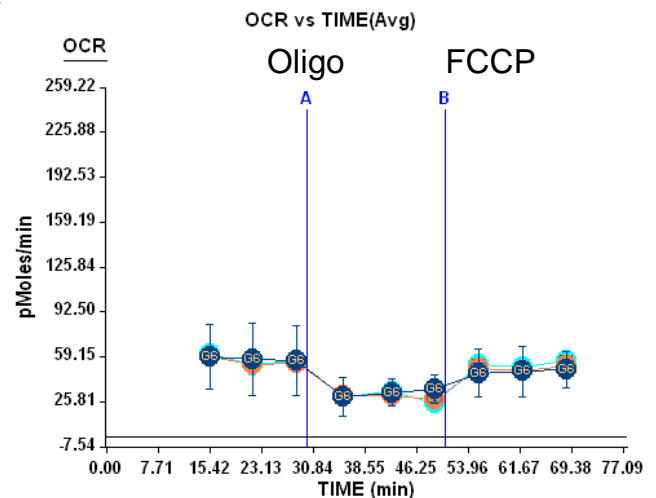


## SOD<sup>-/-</sup> shows dramatic decrease in respiratory capacity

3 independent +/+ mice preps  
OCR vs TIME(Avg)



3 independent -/- mice preps  
OCR vs TIME(Avg)



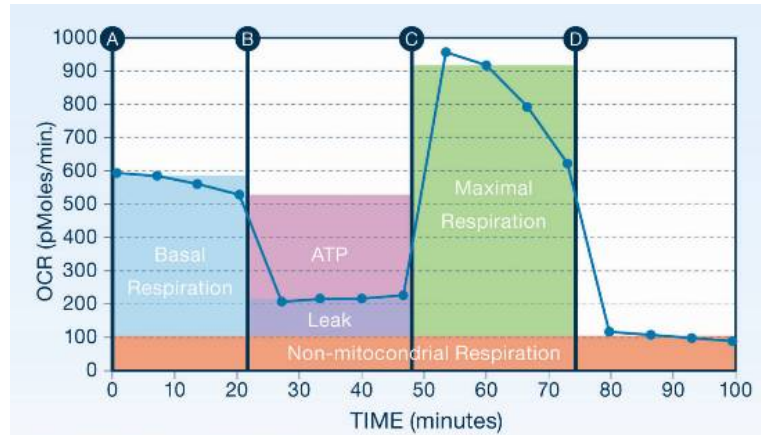
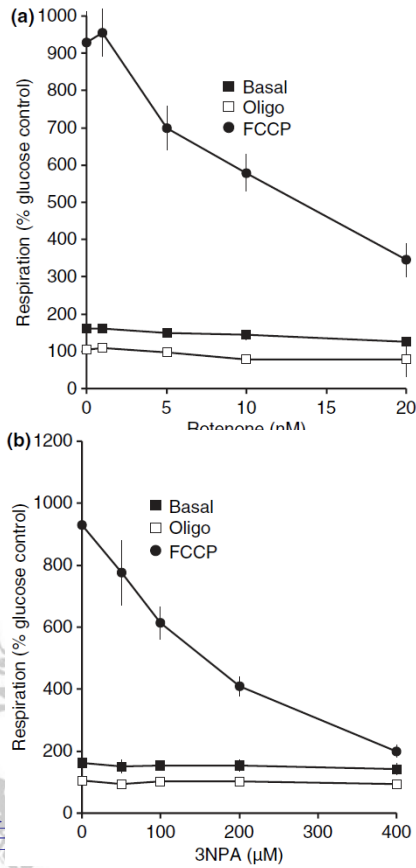
尚博生物科技



Nicholls Lab, Buck Inst, unpublished results 2009.



# Modeling stochastic neuron degeneration: The bioenergetic 'hundred year flood'

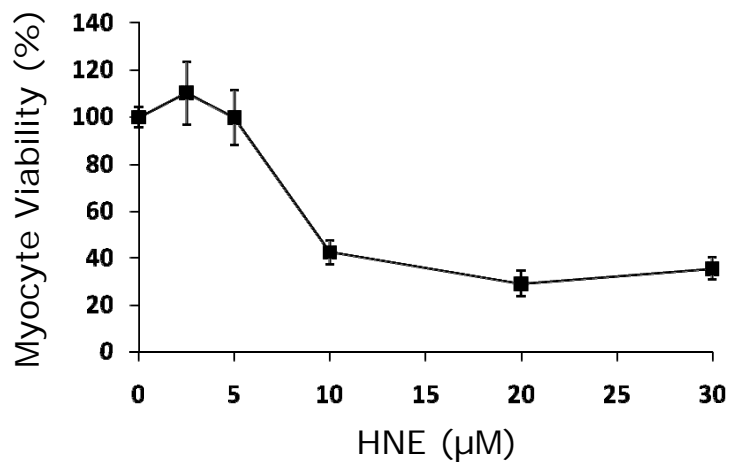
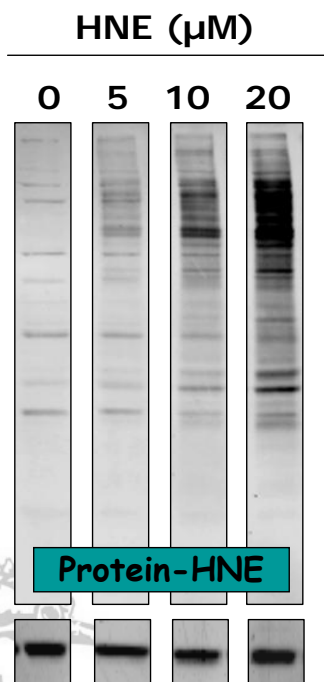


粒線體的四大功能指標：  
基礎代謝率、ATP轉換率、氫離子滲漏以及極限呼吸率。



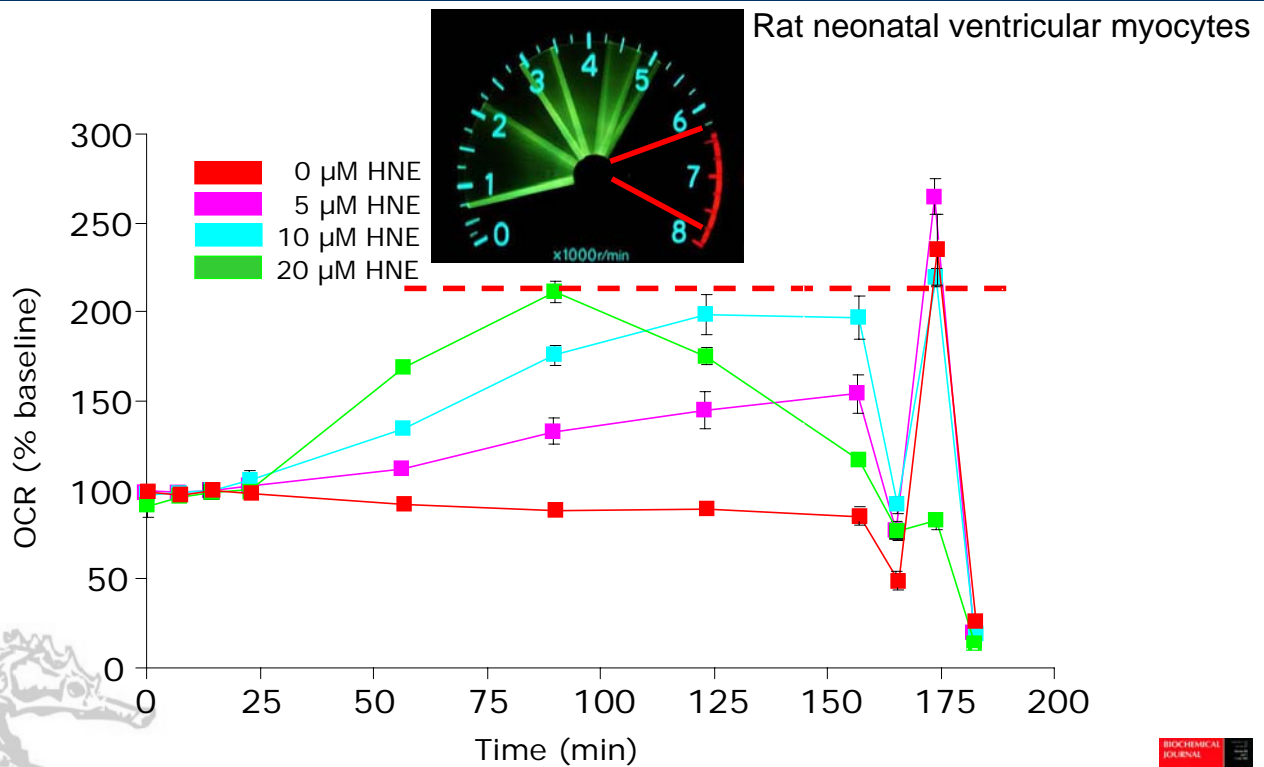
*J Neurochem* 2009; 109:1179-91.

# Protein-HNE modifications are associated with loss of mitochondrial function and cell death



Brad Hill et al., *Biochem J* 2009

## Spare respiratory capacity is an indicator of cell stress



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Brad Hill et al., Biochem J 2009; accepted.



## Mitochondria functional index

### Basal respiration

- Drug toxicity and dose study

### Oxo-pho efficiency

- Identify toxicity mechanism

### Photon leak

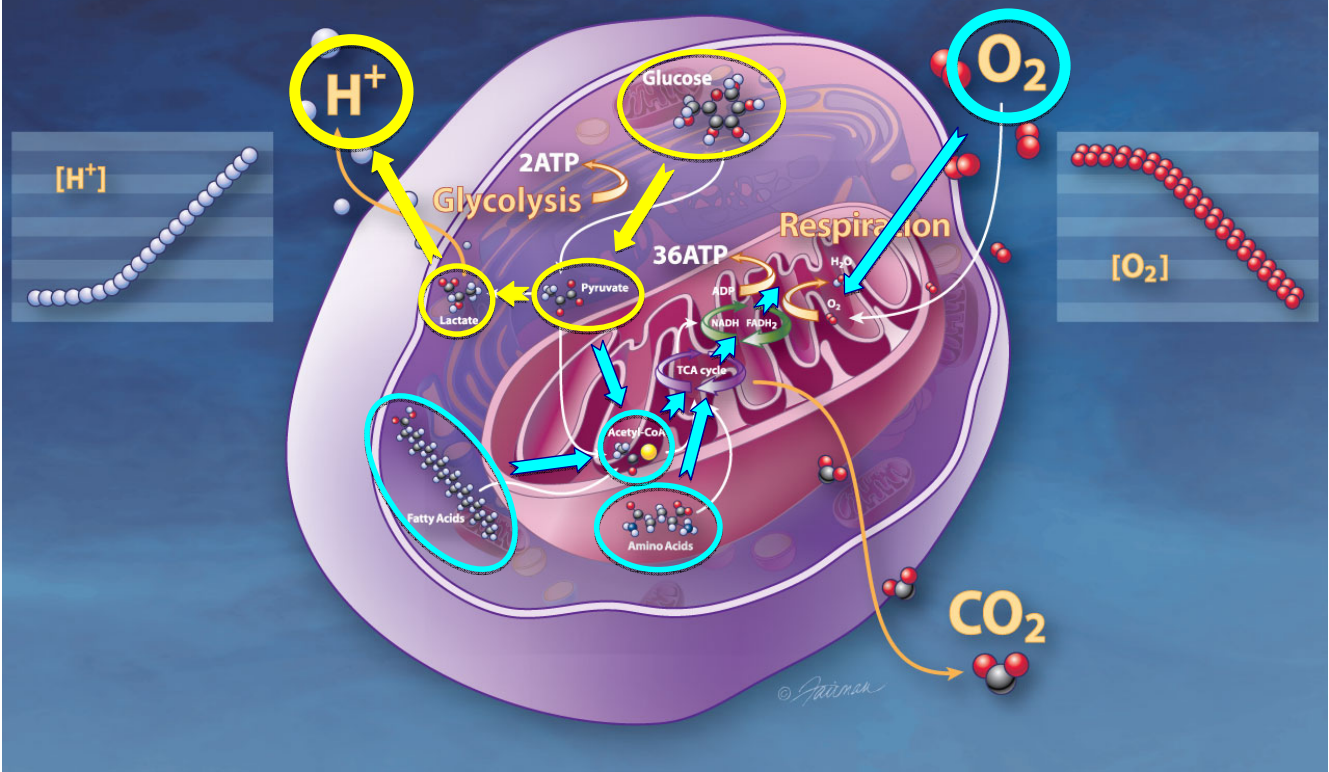
- Structure integrity
- Aging, mutation, oxidative stress and drug

### Respiratory capacity- cellular stress

- Stress capacity

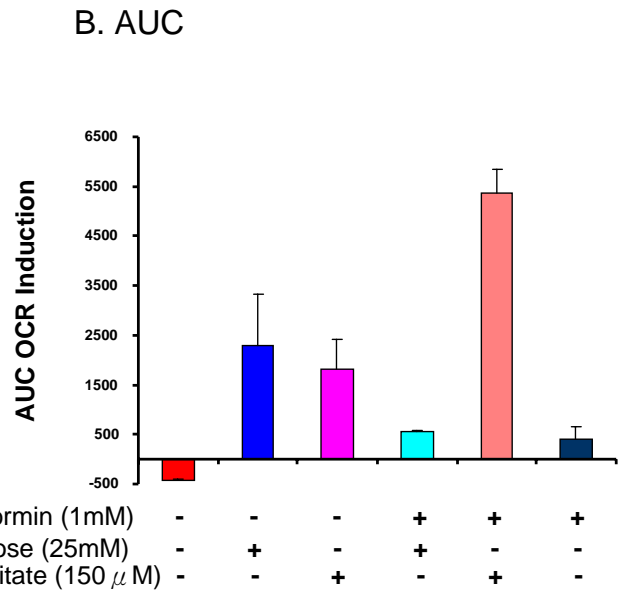
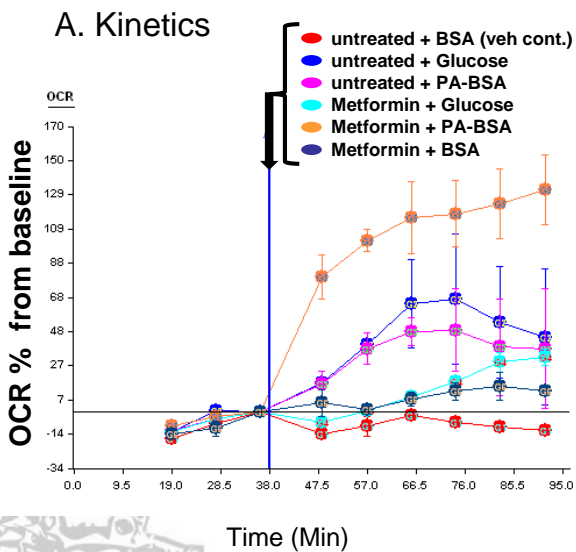
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# Substrate Utilization

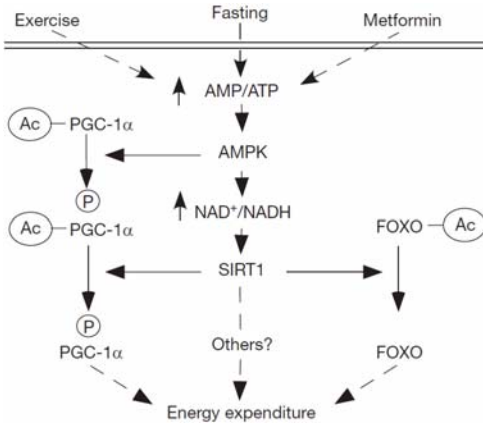


## Metformin Enhances FAO and Lowers Glucose Oxidation in C2C12 Myotubes

Wu Poster; GRC Molecular and Cellular Bioenergetics, 2007.

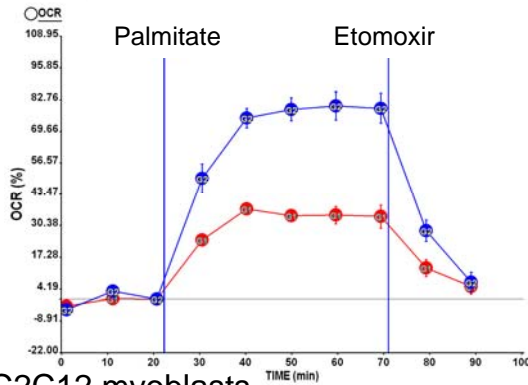
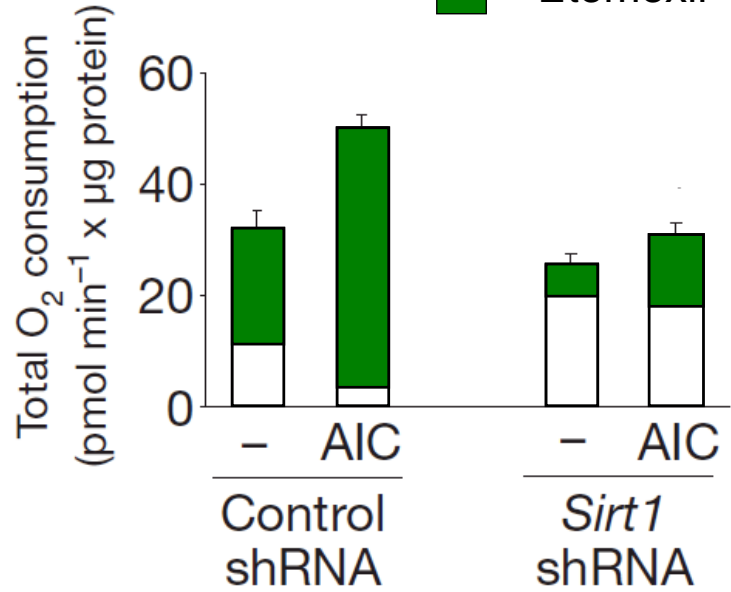


# XF Assays can detect catabolism of exogenous and/or endogenous substrates



C2C12 myoblasts

■ = Etomoxir



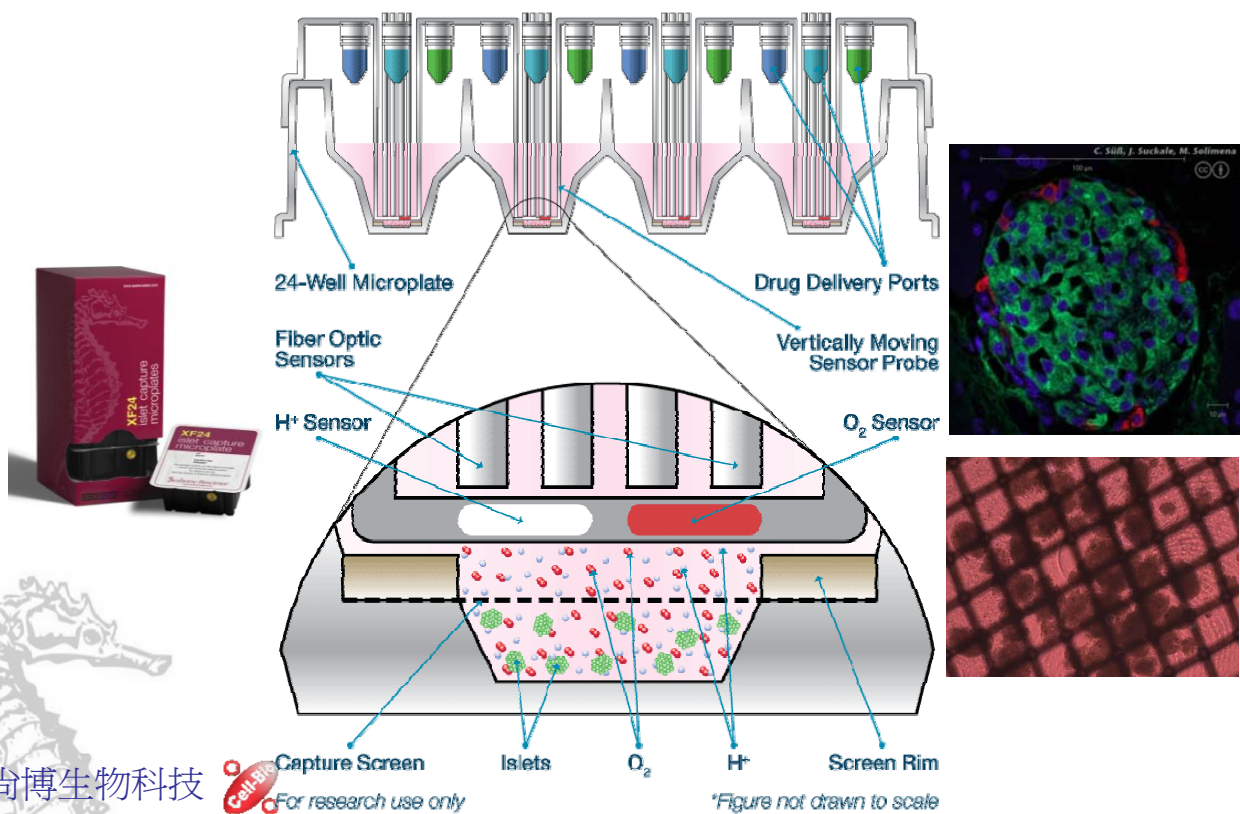
C2C12 myoblasts

Johan Auwerx *Nature* 2009; 458:1056-60



# Islet Capture Microplate

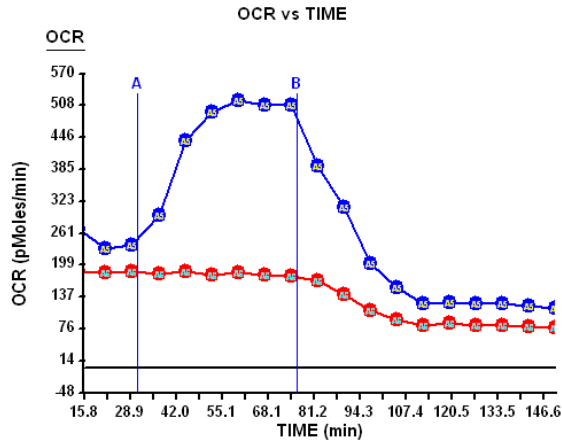
## Cutaway of a Probe & Islet Capture Well



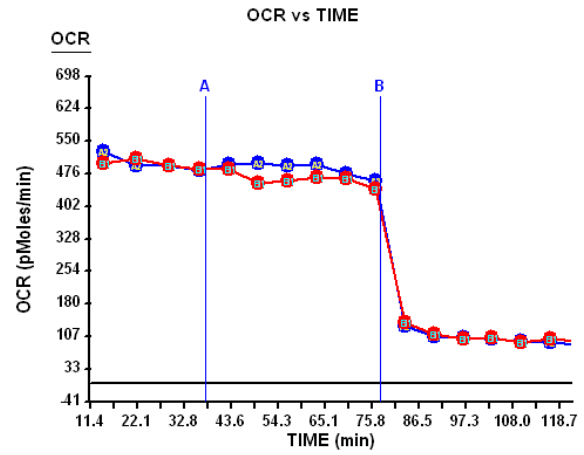
# Oxygen consumption of Mice islets

*"Increase in oxygen consumption with glucose in islets and clonal beta cells; but not in C2C12 myoblasts"*

Islets



C2C12 myoblasts



- Blank injection A; oligomycin at B
- 20mM glucose injection A; oligomycin at B

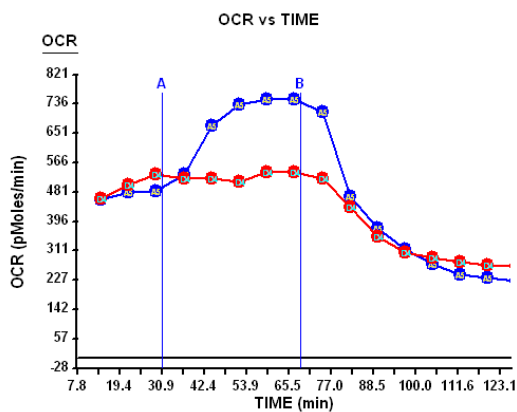


Shirihai Lab, BUMC, unpublished results 2009.

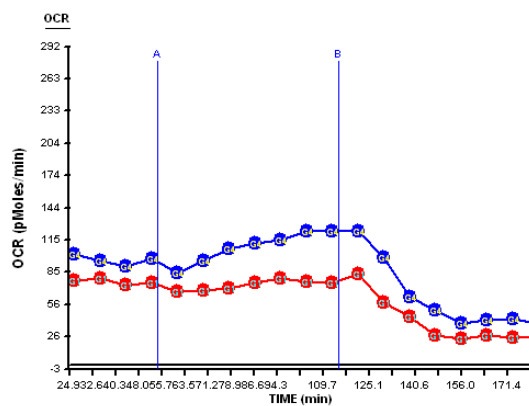
# Oxygen consumption of Human islets

*"Response to glucose blunted in diabetic islets"*

Normal human islets



Type 2 diabetic human islets



- Blank injection A; oligomycin at B
- 20mM glucose injection A; oligomycin at B



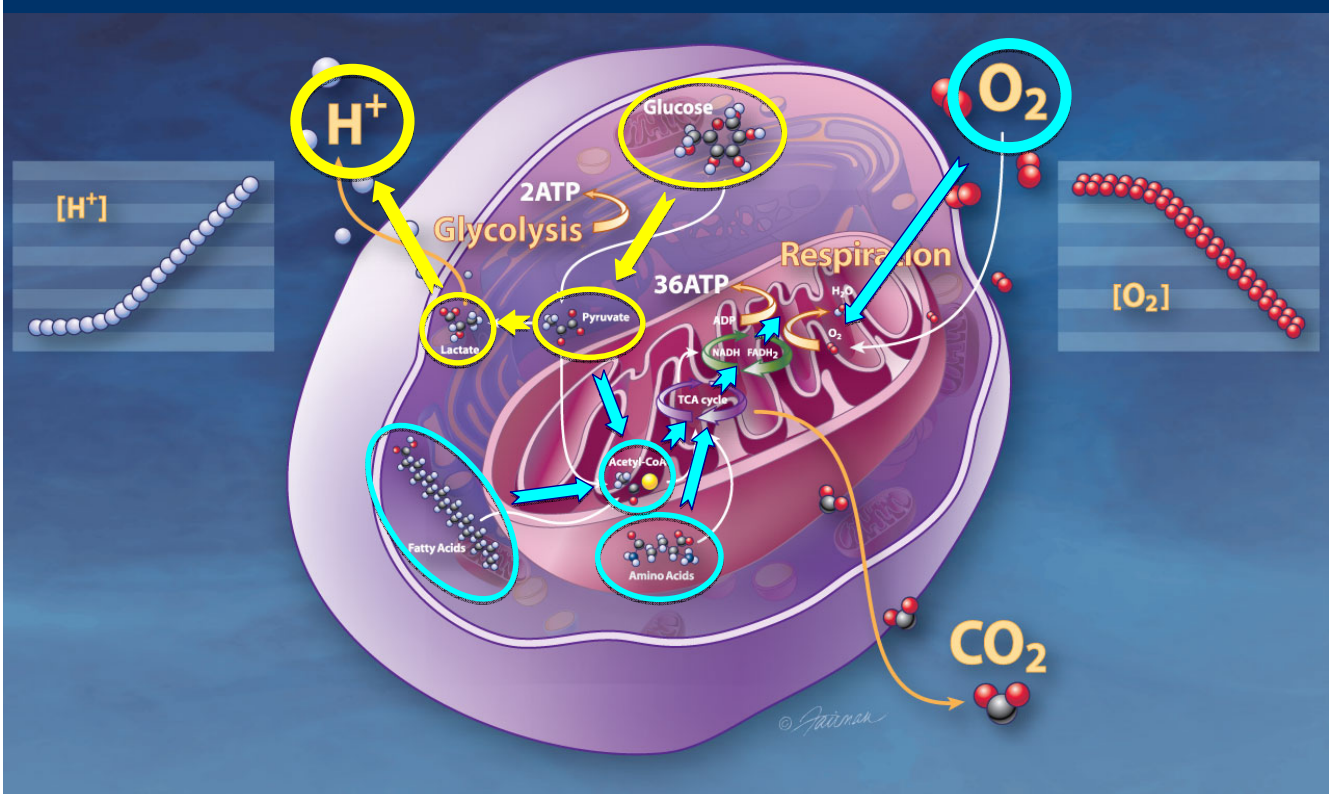
Shirihai Lab, BUMC, unpublished results 2009.

## Substrate utilization

- Metabolic Disease
  - Obesity
  - Diabetes
- Metabolic regulator development



## Energy Expenditure

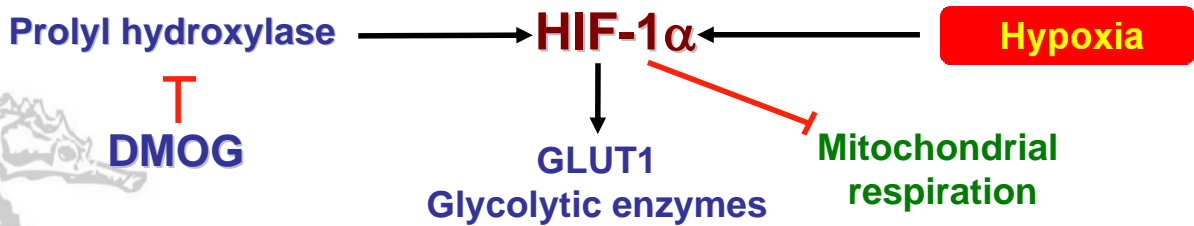


# The Connection Between Cancer and Glucose Metabolism

*“The prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar.”*



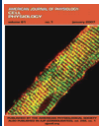
Otto Warburg  
1883 - 1970



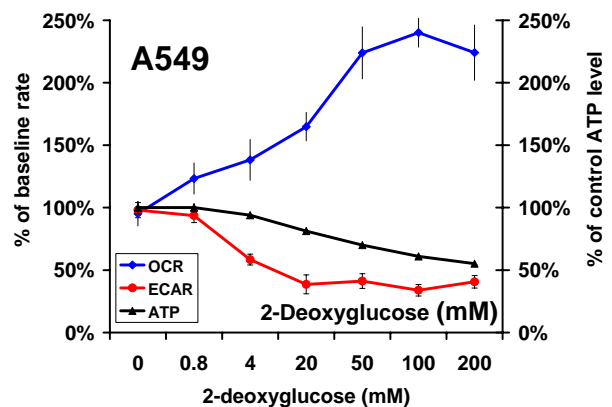
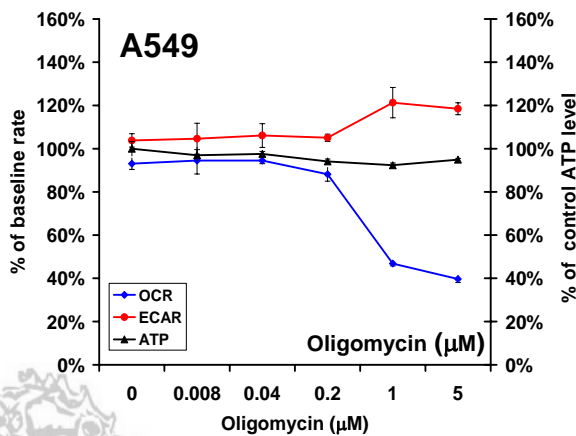
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Modified from: Kelloff GJ et. Al. Clinical Cancer Research 2005 11(8), 2785  
“Progress and Promise of FDG-PET Imaging for Cancer Patient Management and Oncologic Drug Development.”

## High Glycolytic Capacity and Impaired Oxidative Phosphorylation in Tumor cells



*Am J Physiol Cell Physiol* 2007; 292:C125-36.

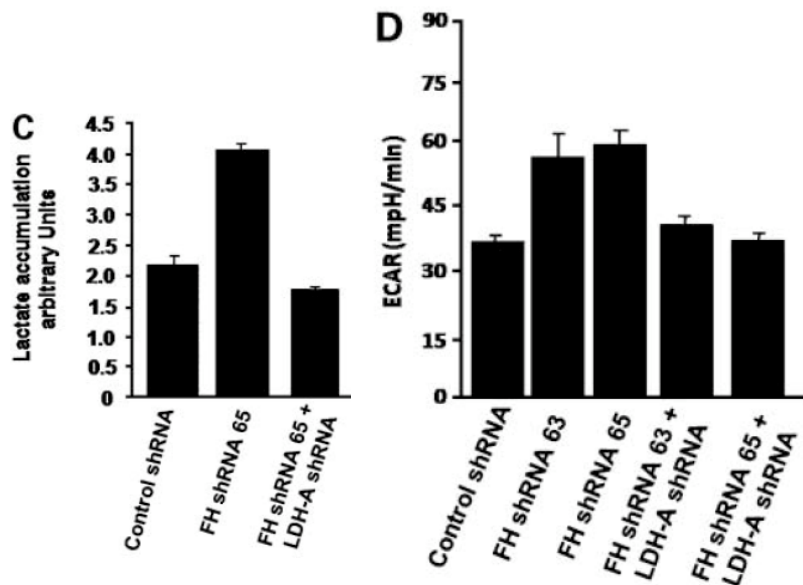


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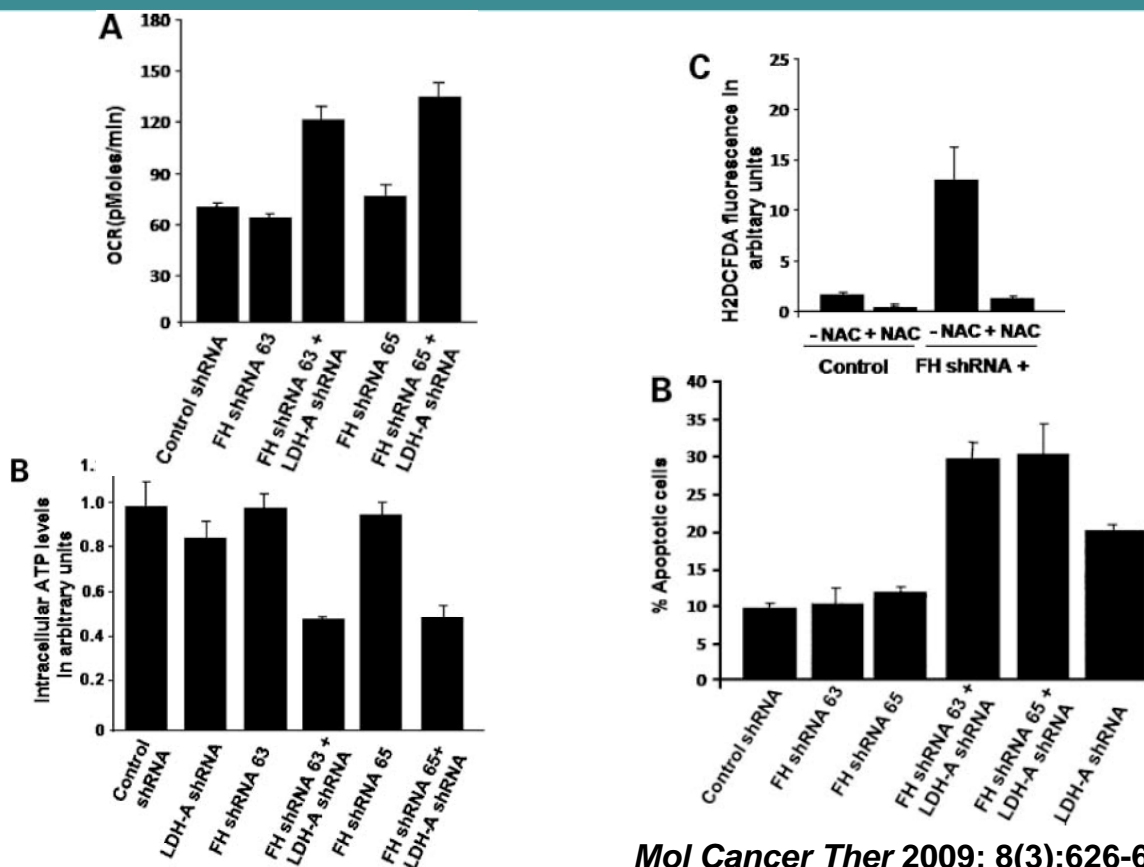
# Hereditary Leiomyomatosis and Renal Cell Cancer Model: Glycolytic Flux in FH/LDH-A deficient cells



*Mol Cancer Ther* 2009; 8(3):626-635.



# LDH-A inhibition results in increased apoptosis via ROS production in FH knockdown cell line

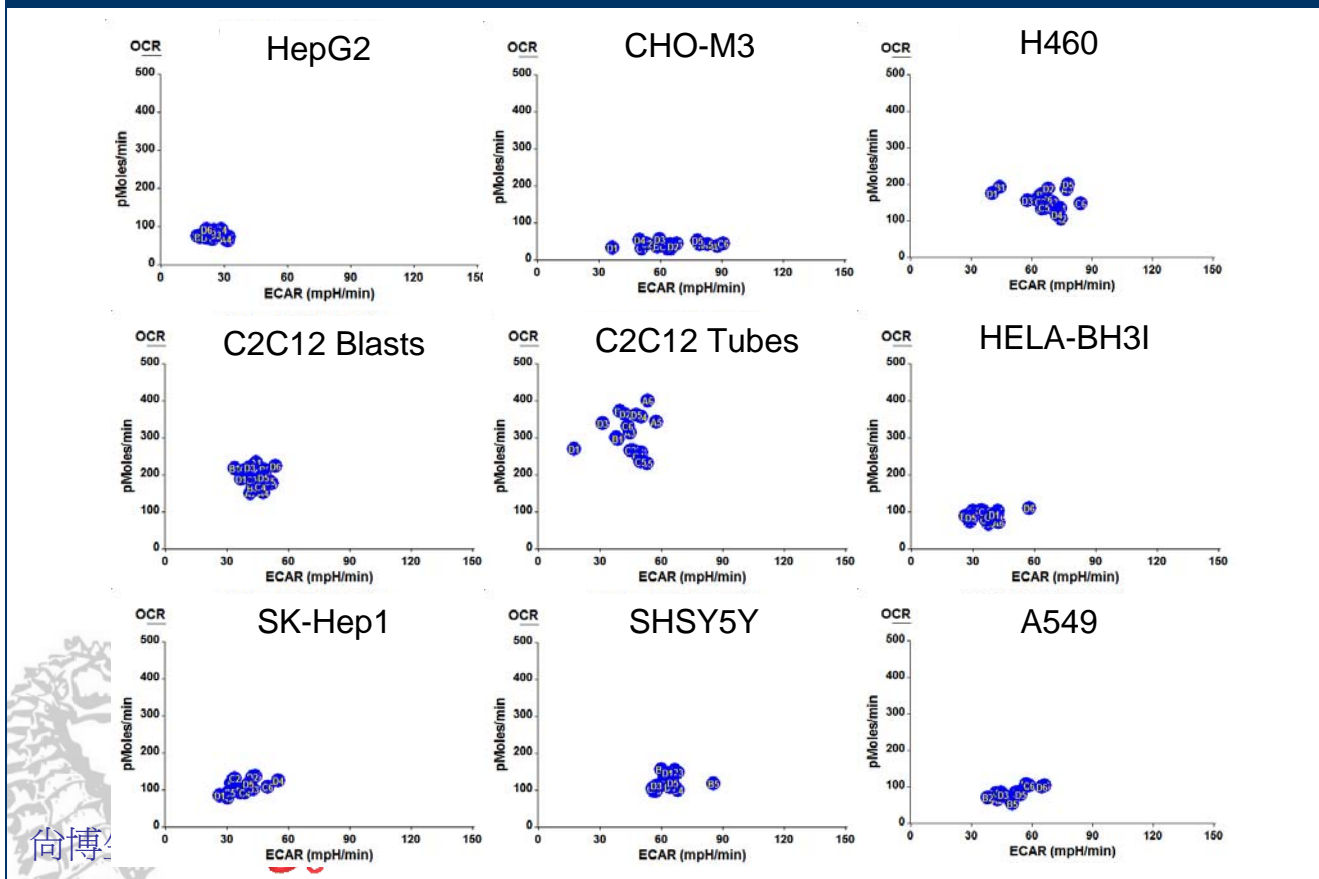


*Mol Cancer Ther* 2009; 8(3):626-635.





# Basal rates of various cell types determined by type of cell, media and environmental conditions



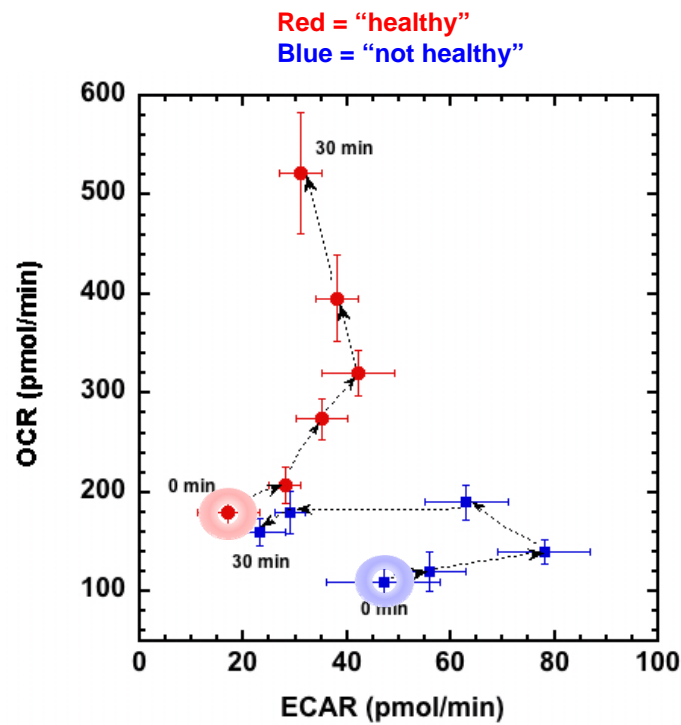
## 細胞健康管理

幫細胞把脈



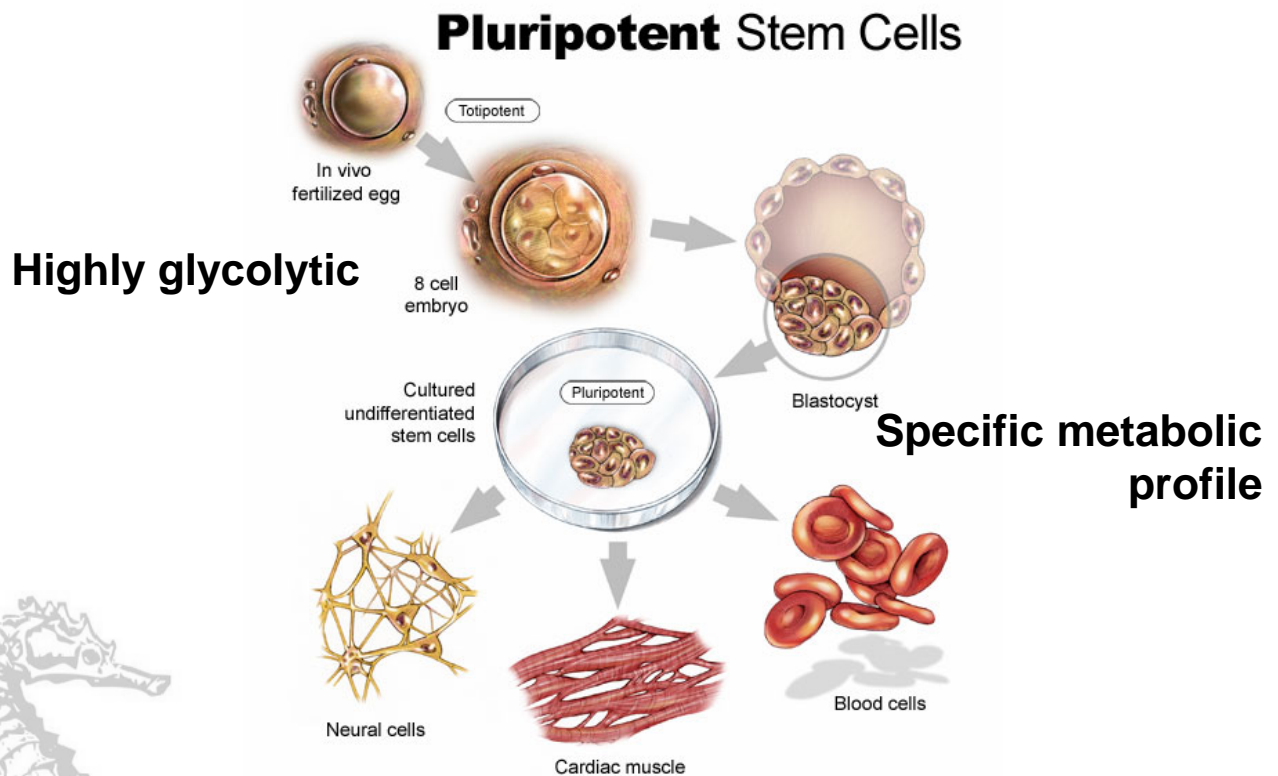
對品質把關

# Cell quality check of primary cardiomyocytes identifies functional cells



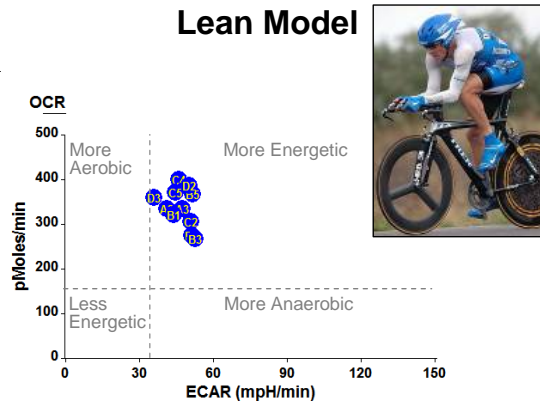
Data courtesy of Craig Beeson, MUSC

# ES cell quality check and differentiation study

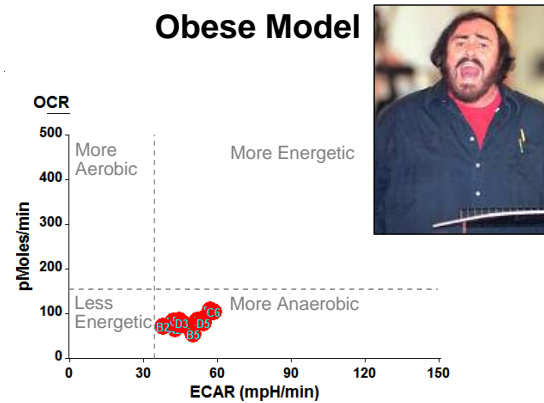


# Determining optimal cell culture conditions to mimic appropriate biology

## Day 7 C2C12 Myocytes Grown Under Different Conditions



- Insulin sensitive
- Good glucose utilization
- Very responsive to exogenous fat



- Insulin insensitive
- Poor glucose utilization
- Low response to exogenous fat

## Summary



Seahorse XF 96 analyzer

非接觸式光學探針設計  
自動化操作與藥物注射系統  
快速檢測

- 粒線體功能
- 代謝系統運作

四不一沒有

- 不用額外試劑
- 不需破壞樣本
- 不用接觸細胞
- 不用人力監控
- 沒有學習門檻

## summary

- Mitochondrial functional index
  - Basal respiration
  - Oxo-pho efficiency
  - Photon leak- structure integrity
  - Respiratory capacity- cellular stress
- Substrate utilization
  - Sugar, fat and amino acid
- Energy expenditure
  - Aerobic or anaerobic pathway
  - 細胞把脈技術



“Measuring mitochondrial function is so easy now”

XF96  
Extracellular Flux Analyzer

Product specialist  
李文瑋 David Li

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