Drug-Induced Mitochondrial Dysfunction: An Emerging Model for Idiosyncratic Drug Toxicity

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Drug-Induced Mitochondrial Toxicity: A New Model of Idiosyncratic Adverse Drug Responses

Overview

- **Mitochondrial failure**
  - Complex organelle can fail in many ways.
  - $\Delta\Psi$ bioaccumulates some drugs
- **Drugs with Mitochondrial Liabilities**
  - OXPHOS Inhibitors & Uncouplers = acute liabilities
  - Inhibitors of Expression/Replication = chronic treatments
- **How Was it NOT Discovered??**
- **New Preclinical Screens**

1. Mitochondrial Respiration in 96-well Plates
2. Metabolic Profiling
3. Identifying Site of Action
6. Cell Models to Facilitate Detection of Mitochondrial Liabilities

- **New Model of Idiosyncratic Toxicity**

Some of the 44 Drugs Withdrawn Since 1960

Drug name	Withdrawn	Remarks
thalidomide	1960s	teratogenicity
lysergic acid diethylamide (LSD)	1960s	abused
diethylstilbestrol	1970s	teratogenicity
phenformin and buformin	1978	lactic acidosis
ticrynafen	1982	hepatitis
zimelidine	1983	Guillain-Barré syndrome
methaqualone	1984	addiction and overdose
triazolam	1991	UK - psychiatric
fenfluramine	1997	hepatotoxicity
dexfenfluramine	1997	hepatotoxicity
terfenadine	1998	arrhythmias
mibefradil	1998	interactions
troglitazone	2000	hepatotoxicity
alosetron	2000	constipation
cisapride	2000s	arrhythmias
cerivastatin	2001	rhabdomyolysis
rapacuronium	2001	bronchospasm
rofecoxib	2004	myocardial infarction
Adderall XR)	2005	Canada - stroke
hydromorphone	2005	overdose with alcohol
pemoline	2005	hepatotoxicity
natalizumab	2005-2006	CNS viral inflammation

Mitochondrial Impairment of Drugs Receiving Black Box Warnings

Hepatotoxicity

Antivirals
Abacavir
Didanosine
Emtricitabine
Entecavir
Emtricitabine
Lamivudine
Nevirapine
Telbivudine
Tenofovir
Tipranavir
Stavudine
Zalcitabine
Zidovudine

Anti-Cancer
Flutamide
Dacarbazine
Gemtuzumab
Methotrexate
Pentostatin
Tamoxifen

Antibiotics
Isoniazid
Ketoconazole (oral)
Streptozocin
Trovafloxacin

CNS
Dantrolene
Divalproex Sodium
Felbamate
Naltrexone
Nefazodone
ValproicAcid and Alper's

Hypertension
Bosentan

Elevated serum liver enzymes (AST, ALT) reflect hepatocyte death.

Lactic acidosis reflects mitochondrial impairment.

Cardiovascular

Anthracyclines
Daunorubicin
Doxorubicin
Epirubicin
Idarubicin

NSAIDs
Celecoxib
Diclofenac
Diflunisal
Etodolac
Fenoprofen
Ibuprofen
Indomethacin
Ketoprofen
Mefenamic acid
Meloxicam
Naproxen
Nabumetone
Oxaprozin
Piroxicam
Salsalate
Sulindac
Thioridazine
Tolmetin

Anaesthetic
Bupivacaine

Anti-Cancer
Arsenic Trioxide
Cetuximab
Denileukin diftitox
Mitoxantrone
Tamoxifen

Beta-Blocker]
Atenolol

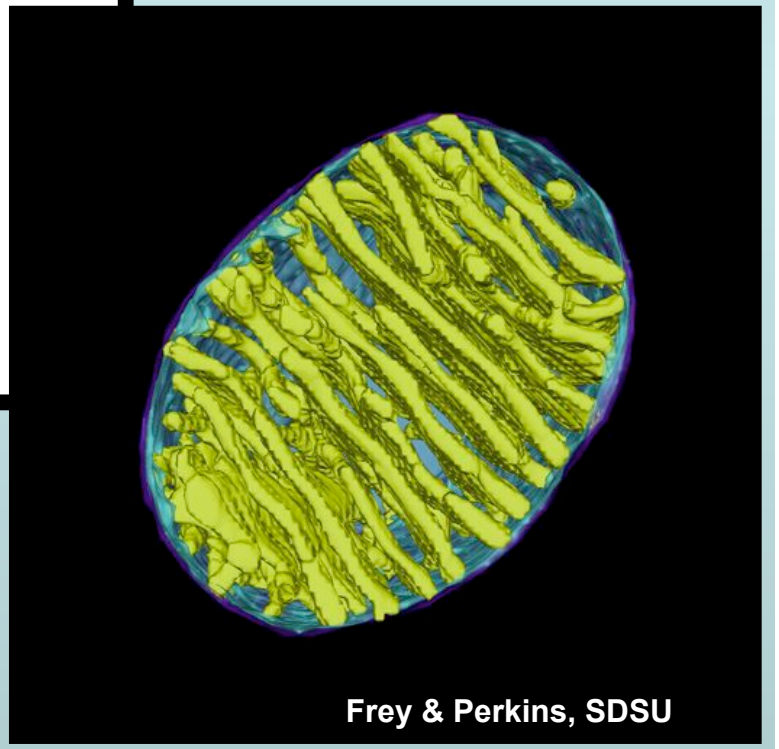
Antiarhythmic
Amiodarone (oral)
Disopyramide
Dofetilide
Ibutilide

CNS
Amphetamines
Atomoxetin
Droperidol
Methamphetamine
Pergolide

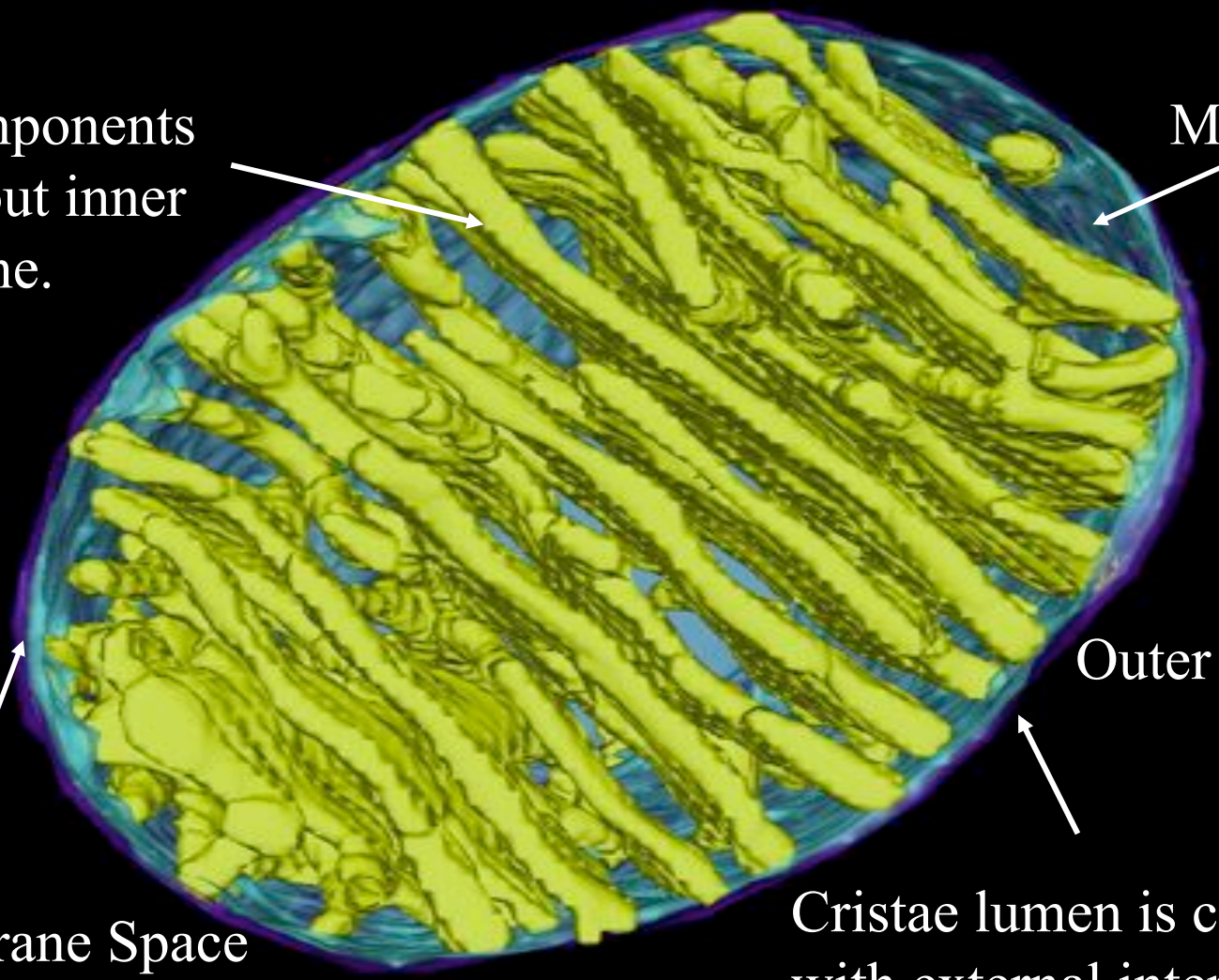
Diabetes
Pioglitazone
Rosiglitazone

Mitochondria: Bioenergetics, Oxidative Pathology and Cellular Viability Converge

- *Cytoplasmic Organelles*
- **Generate > 90% of cellular energy**
- **Generate 90% of radicals**
- **Gatekeepers of cell death (apoptosis & necrosis)**
- Steroid synthesis; b-oxidation...
- Endosymbionts co-evolved from
- ancient bacteria
- Mitochondrial DNA = the only non-nuclear genome in all animals
- Replication independent of cell replication



Mitochondrial Compartmentalization



ETS components throughout inner membrane.

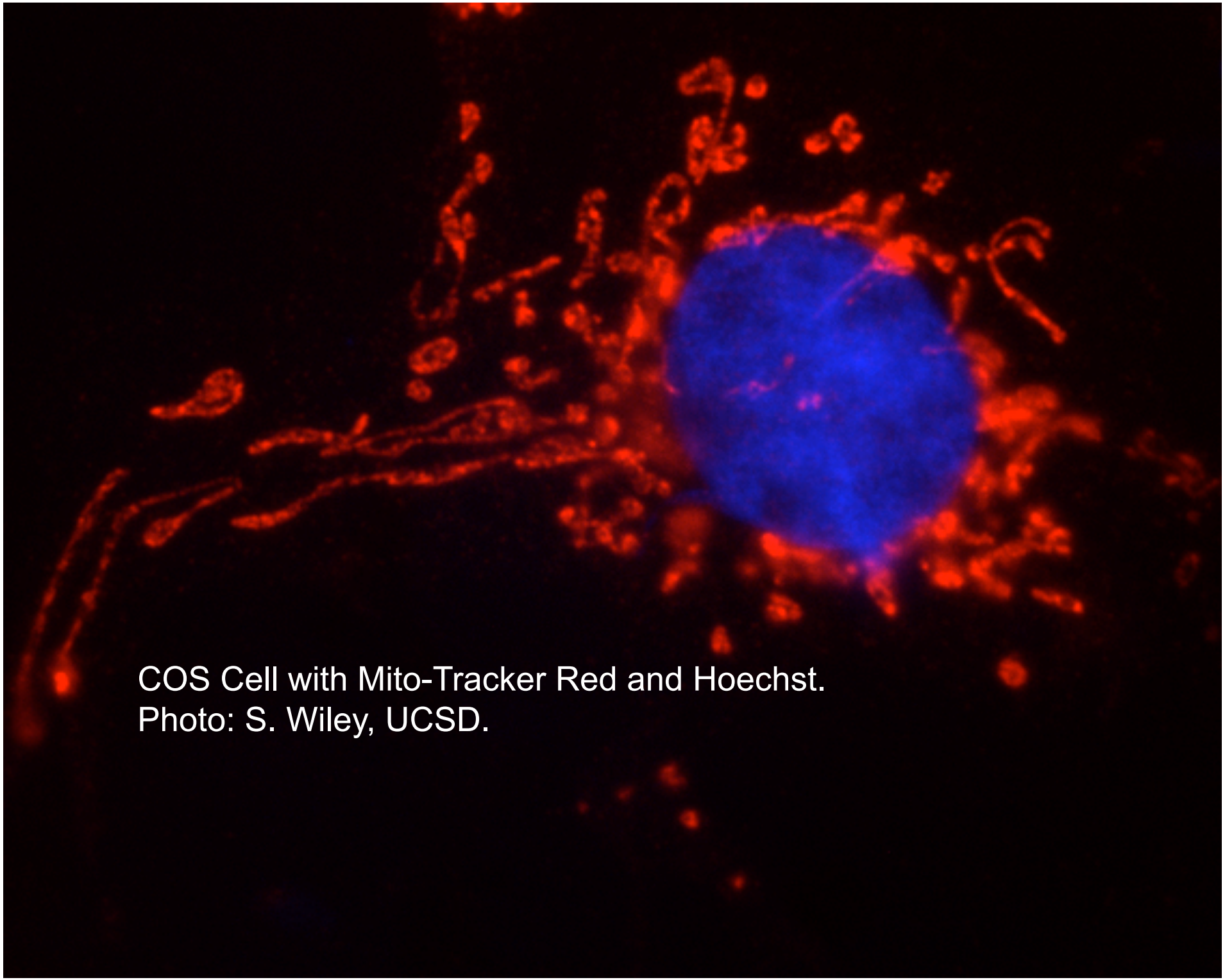
Matrix

Outer membrane

Intermembrane Space

Cristae lumen is contiguous with external intermembrane space via cristae junctions.

see Perkins and Frey, *Micron*, 31:97, 2000.
University of California at San Diego Super Computer Center
San Diego State University



COS Cell with Mito-Tracker Red and Hoechst.
Photo: S. Wiley, UCSD.

ATP Turnover and Human Metabolism

1) Resting metabolism:
   Female = 6127 kJ/24 hr
   Male = 7983 kJ/24 hr
   (DeLorenzo et al, Eur. J. Clin Nutr, 55:208, 2001)

2) ATP hydrolysis under *physiological* conditions = 42-50 kJ/ mol *(Campbell Biology, Third Edition. Benjamin Cummings, 1993:97-101.)*

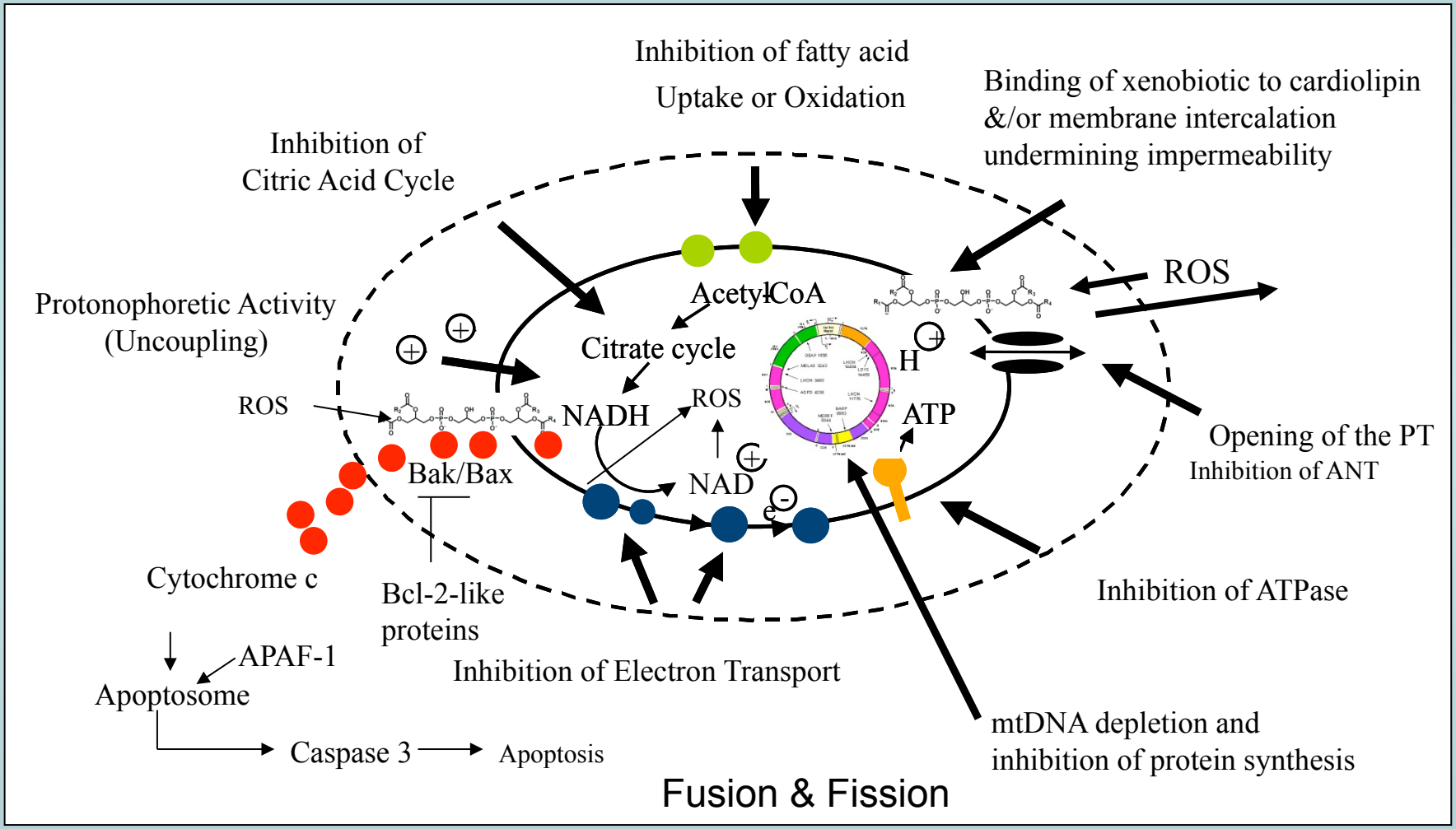
3) Females turn over 133 mol ATP/da; Males 173 mol ATP/da

4) ATP = 507g/mol

Therefore:
Females turn over 67,431 g/da = 148 lbs of ATP per day
Males turn over 87,711 g/da = 193 lbs of ATP per day

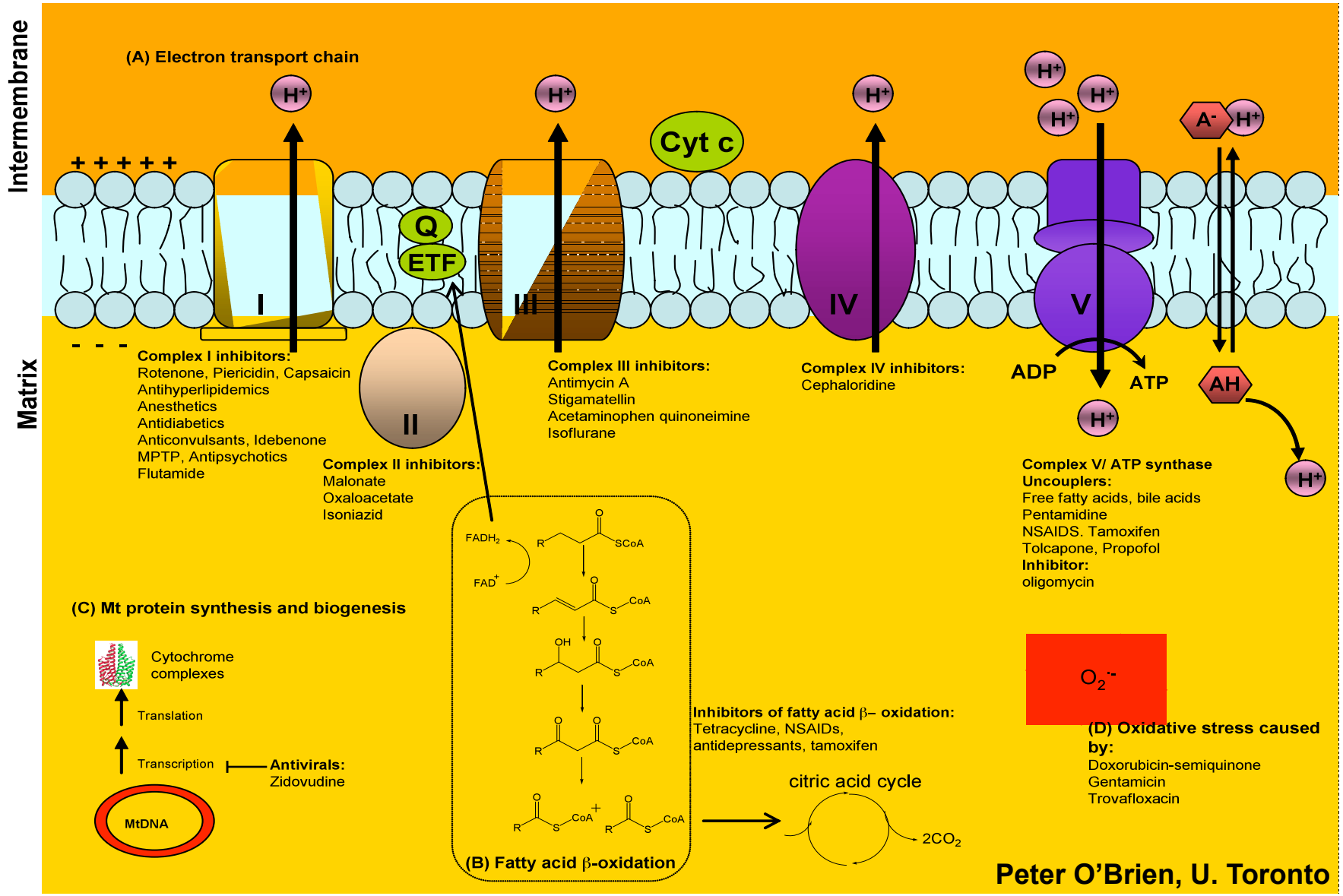
Aerobic Physiological Scope = 10-20X
(Hoppeler & Weibel, Encyclopedia of Life Sciences, John Wiley & Sons, 2001)

Mitochondria: Complex Organelle Can Fail in Many Ways



Dykens & Will, Drug Discovery Today, 12:777-785, 2007.

Mitochondrial Drug Interactions



Many Drugs Have Mitochondrial Liabilities

- Many, but not all, drugs with organ toxicity have a mitochondrial liability.
  - Screen of > 550 drugs reveals 34% have mitochondrial liabilities.
- Depending on potency, if a drug has a mitochondrial liability, it will have deleterious consequences.
  - Bioaccumulation alters PK.
  - >10,000-fold concentration of some cations in matrix over plasma.
- Severity of such adverse effects is idiosyncratic.
  - Function of organ history and genetics (incl. mtDNA).
    - Preclinical assessments are done in young, perfectly healthy animals.
  - Threshold effects and physiological scope.

Evidence of Drug-Induced Mitochondrial Dysfunction is Rapidly Accumulating

Selected drugs associated with idiosyncratic DILI that exhibit a clear mitochondrial hazard

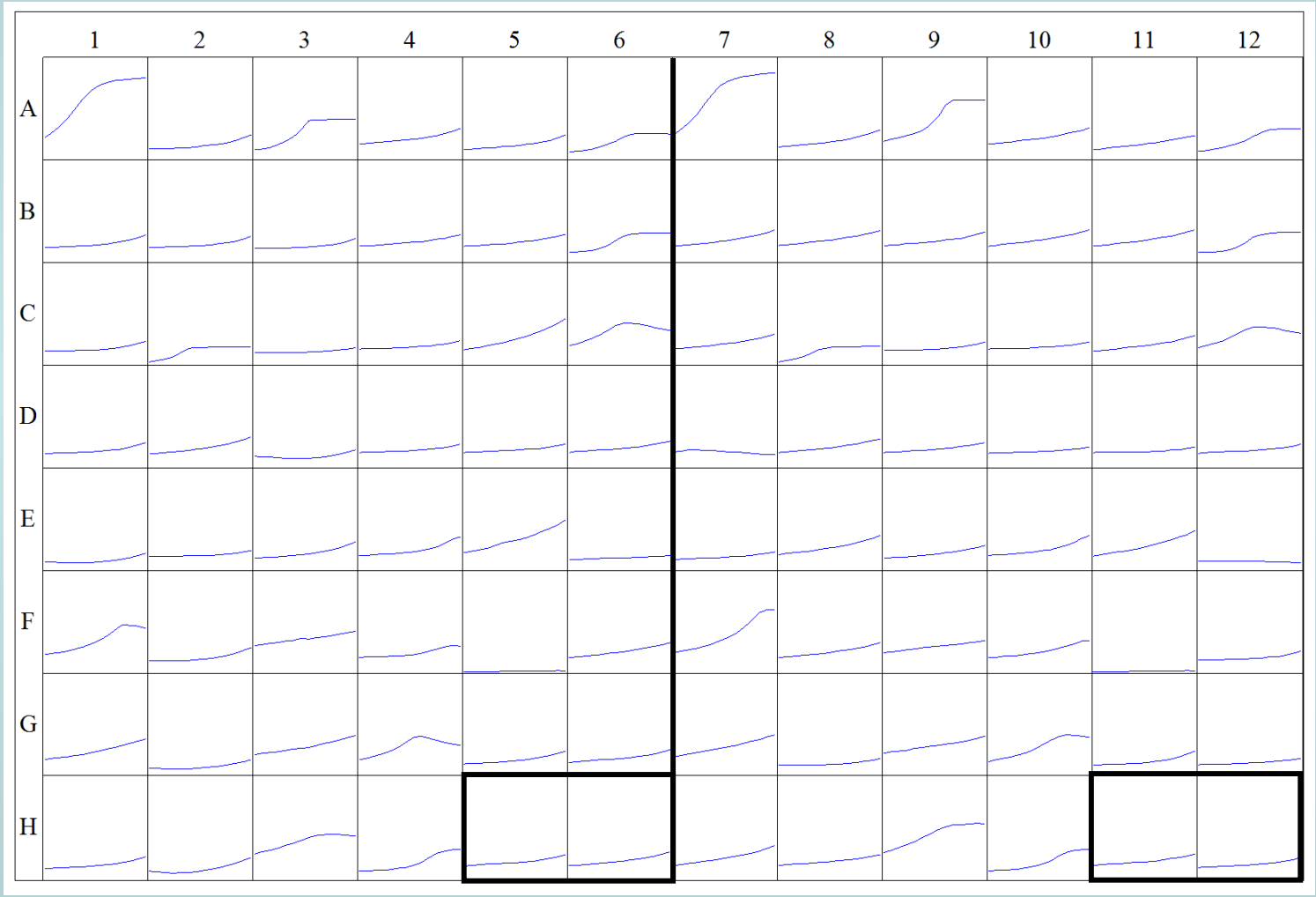
Drug	Mitochondrial liability in hepatocytes
Troglitazone	Bedoucha et al. (2001); Haskins et al. (2001); Tirmenstein et al. (2002); Narayanan et al. (2003); Shishido et al. (2003); Bova et al. (2005); Masubuchi et al. (2006); Ong et al. (in press)
Diclofenac	Petrescu and Tarba (1997); Bort et al. (1998); Masubuchi et al. (1998); Masubuchi et al. (1999); Masubuchi et al. (2000); Masubuchi et al. (2003); Gomez-Lechon et al. (2003a); Gomez-Lechon et al. (2003b); Lim et al. (2006)
Nimesulide	Mingatto et al. (2000); Caparroz-Assef et al. (2001); Mingatto et al. (2002); Tay et al. (2005); Ong et al. (2006)
Mefenamic acid	McDougall et al. (1983); Masubuchi et al. (2000)
Tolcapone	Haasio et al. (2002a,b,c)
Valproic acid	Bjorge and Baillie (1991); Keller et al. (1992); Ponchaut et al. (1992); Tang et al. (1995); Trost and Lemasters (1996); Sobaniec-Lotowska (1997); Tong et al. (2005)
Leflunomide	Spodnik et al. (2002)
Amiodarone	Fromenty et al. (1990); Berson et al. (1998); Spaniol et al. (2001); Kaufmann et al. (2005)
Trovafloxacin	Liguori et al. (2005)
Simvastatin	Velho et al. (2006)
Perhexiline	Deschamps et al. (1994); Berson et al. (1998)
Isoniazid	Schwab and Tuschl (2003); Chowdhury et al. (2006)
Dantrolene	Darios et al. (2003); Munns et al. (2005)
Sulindac	Leite et al. (2006)
Fialuridine	McKenzie et al. (1995); Horn et al. (1997); Lewis et al. (1997)
Lamivudine	Note et al. (2003)
Stavudine	Gaou et al. (2001); Gerschenson et al. (2001); Pace et al. (2003); Velsor et al. (2004)

Boelsterli & Lim. Mitochondrial abnormalities--a link to idiosyncratic drug hepatotoxicity? *Toxicol Appl Pharmacol* 220:92-107, 2007 .

Screens to Detect Mitochondrial Toxicity

State 2

Screen #1 Mitochondrial respiration in 96 well format.

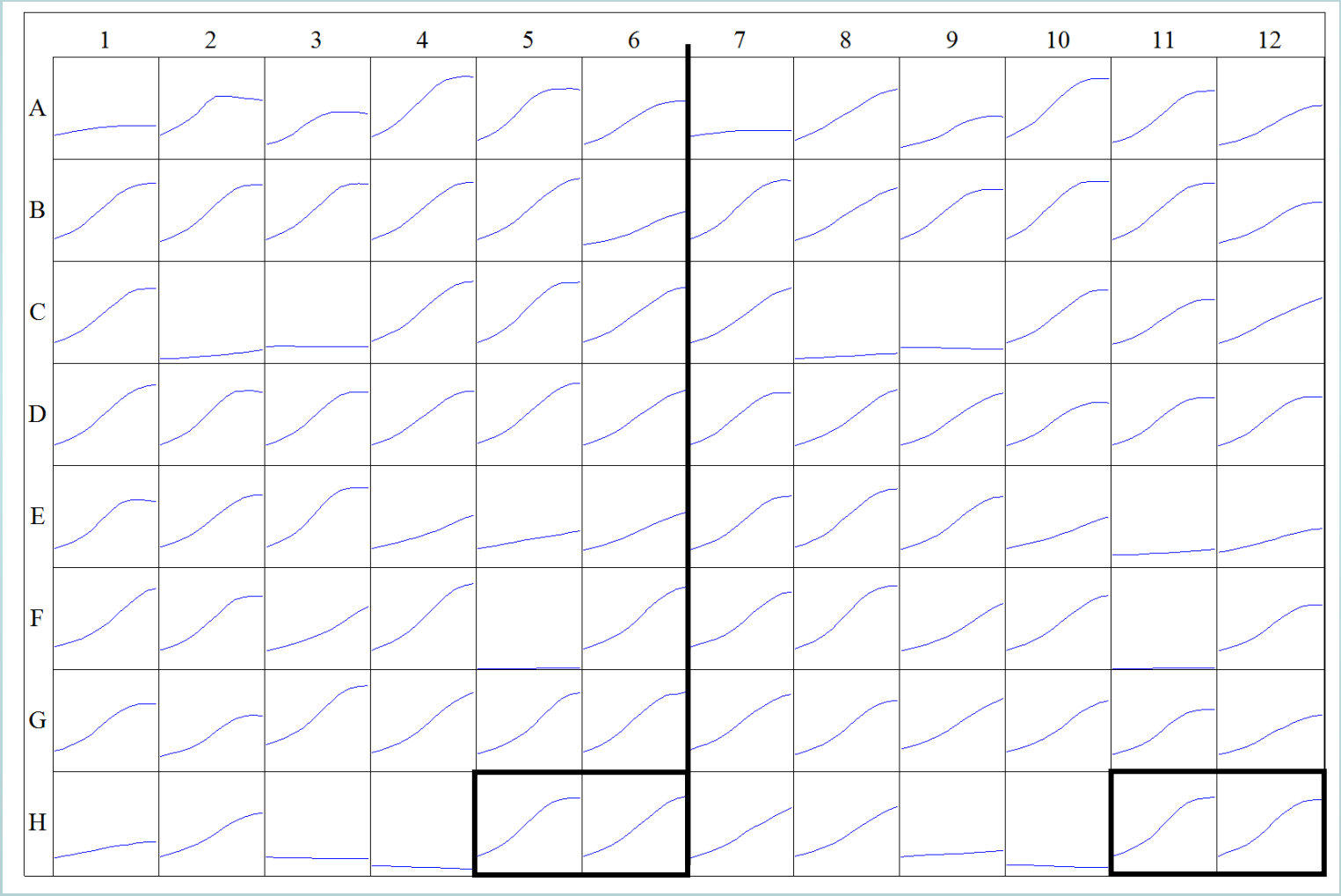


LUXCEL BIOSCIENCES

Dykens et al., Expert Rev. Mol. Diagnostics, 7: 161 (2007)
Will et al., Nature Protocols 1: 2563 (2007).

Screens to Detect Mitochondrial Toxicity

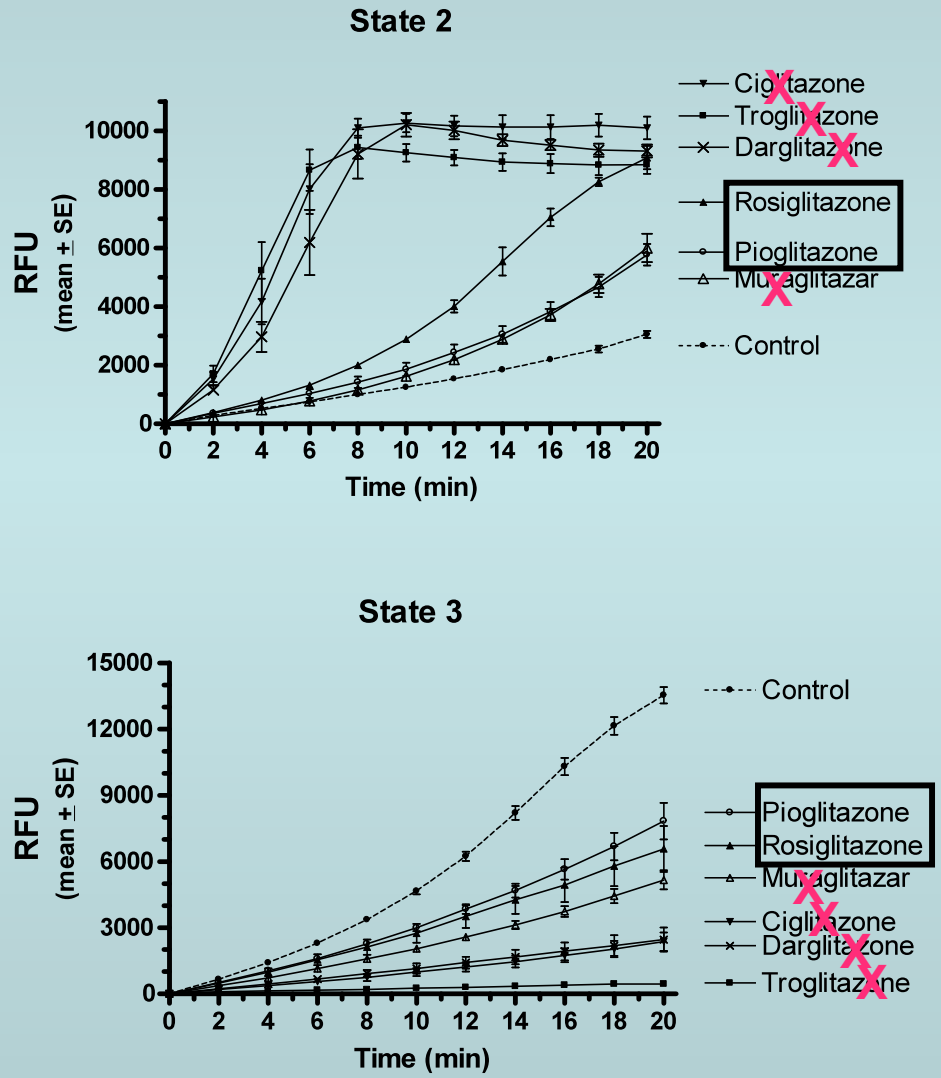
State 3 Screen #1 Mitochondrial respiration in 96 well format.



LUXCEL BIOSCIENCES

Dykens et al., Expert Rev. Mol. Diagnostics, 7: 161 (2007)
Will et al., Nature Protocols 1: 2563 (2007).

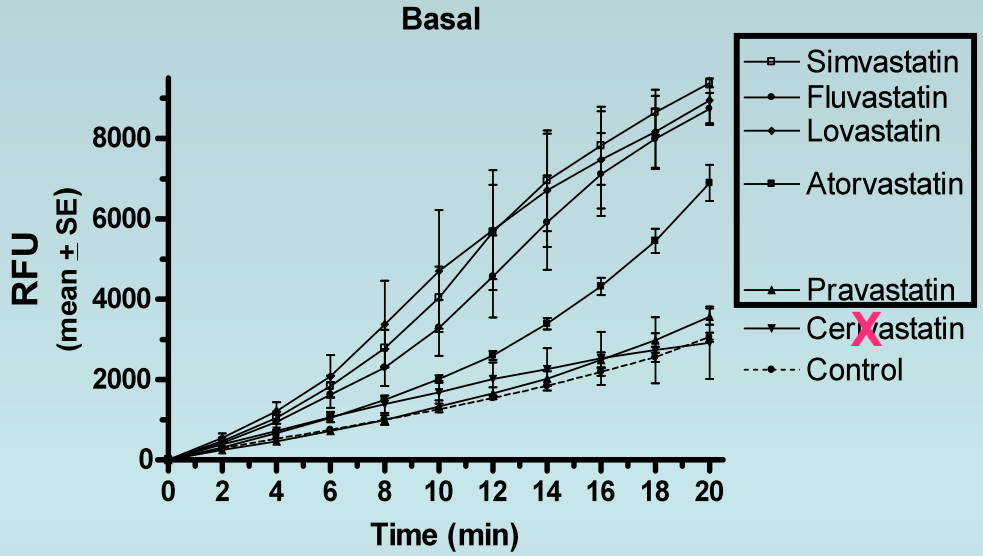
Mitochondrial Effects of Thiozolidinediones Vary

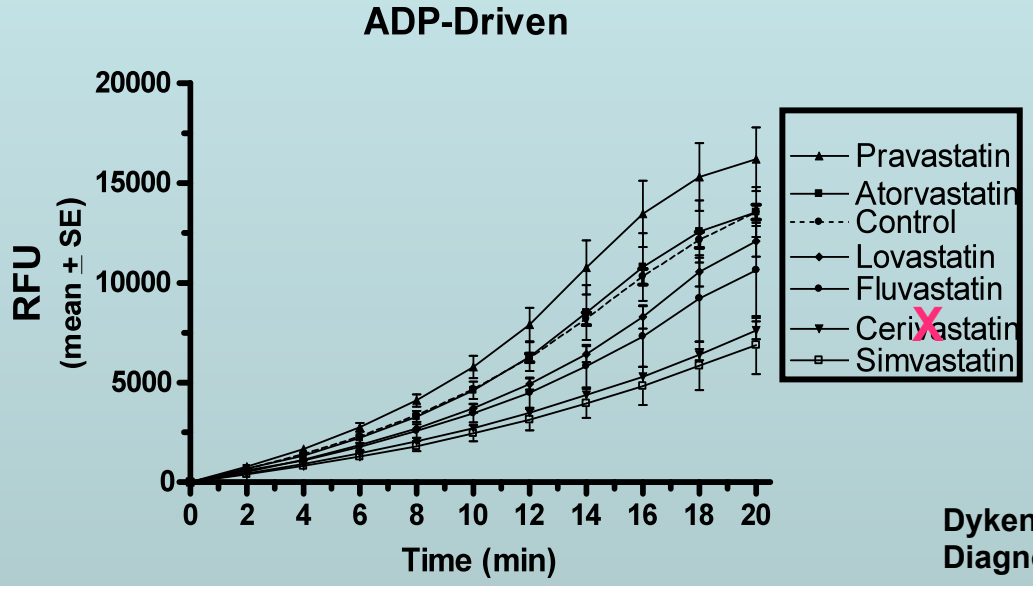


Drugs present at 25nmol/mg mitochondrial protein. N=4, except for controls N=48.

Dykens et al., Expert Rev. Mol. Diagnostics, 7: 161 (2007)

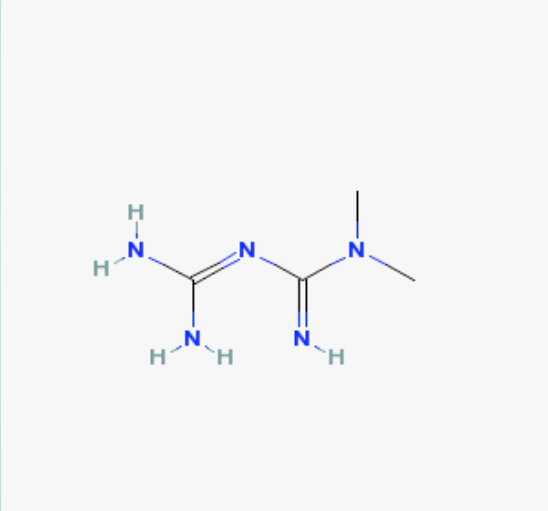
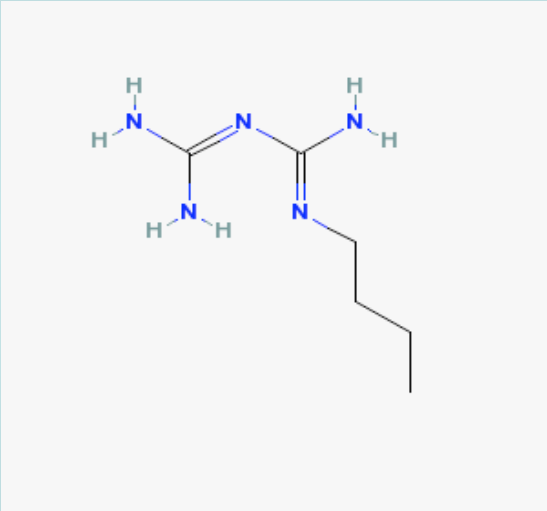
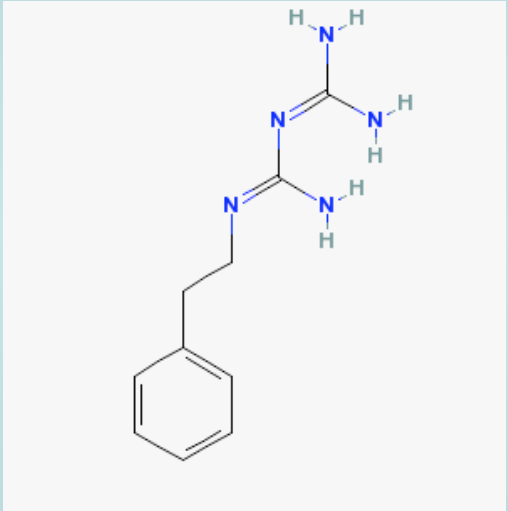
Some Statins Impair Mitochondrial Function





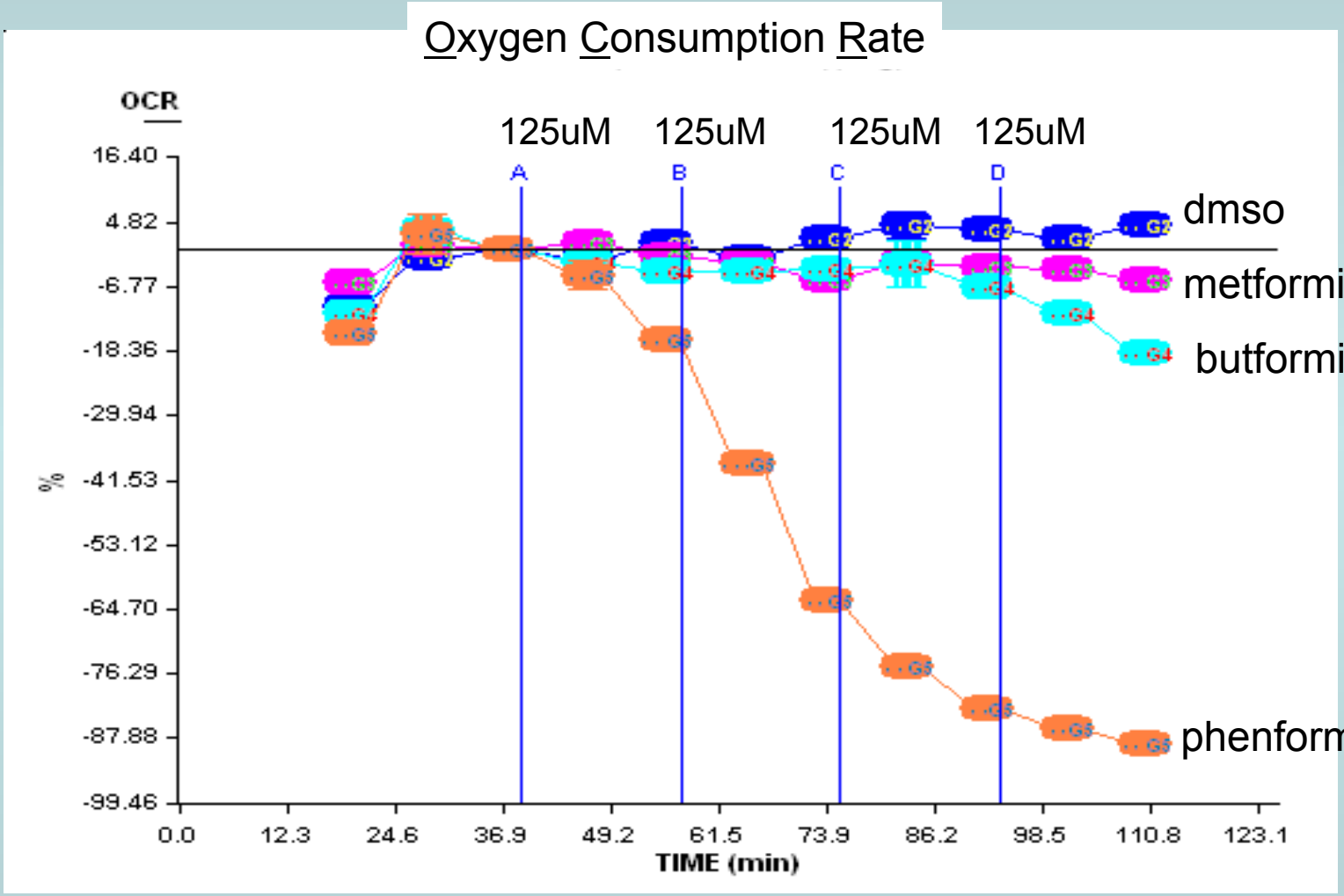
Dykens et al., Expert Rev. Mol. Diagnostics, 7: 161 (2007)

Screen #2: Biguanides Analyzed via Seahorse Technology

Metformin	Buformin	Phenformin
EC50 (μM) for lactic acidosis*		
734 ± 168	**119 ± 18**	**4.97 ± 0.87**
		
Molecular Weight: 129.164 g/mol **Molecular Formula:** C4H11N5 **LogP:** -0.267 **Hydrogen Bond Donor Count:** 3 **Hydrogen Bond Acceptor Count:** 5 **Rotatable Bond Count:** 2 **Tautomer Count:** 3	**Molecular Weight:** 157.217 g/mol **Molecular Formula:** C6H15N5 **LogP:** 0.243 **Hydrogen Bond Donor Count:** 3 **Hydrogen Bond Acceptor Count:** 5 **Rotatable Bond Count:** 4 **Tautomer Count:** 5 formin	**Molecular Weight:** 205.26 g/mol **Molecular Formula:** C10H15N5 **XLogP:** 0.759 **Hydrogen Bond Donor Count:** 3 **Hydrogen Bond Acceptor Count:** 5 **Rotatable Bond Count:** 4 **Tautomer Count:** 5

*** Wang et al., Mol Pharmacol, 63:844, 2003**

Metabolic Profiling to Detect Mitochondrial Toxicity

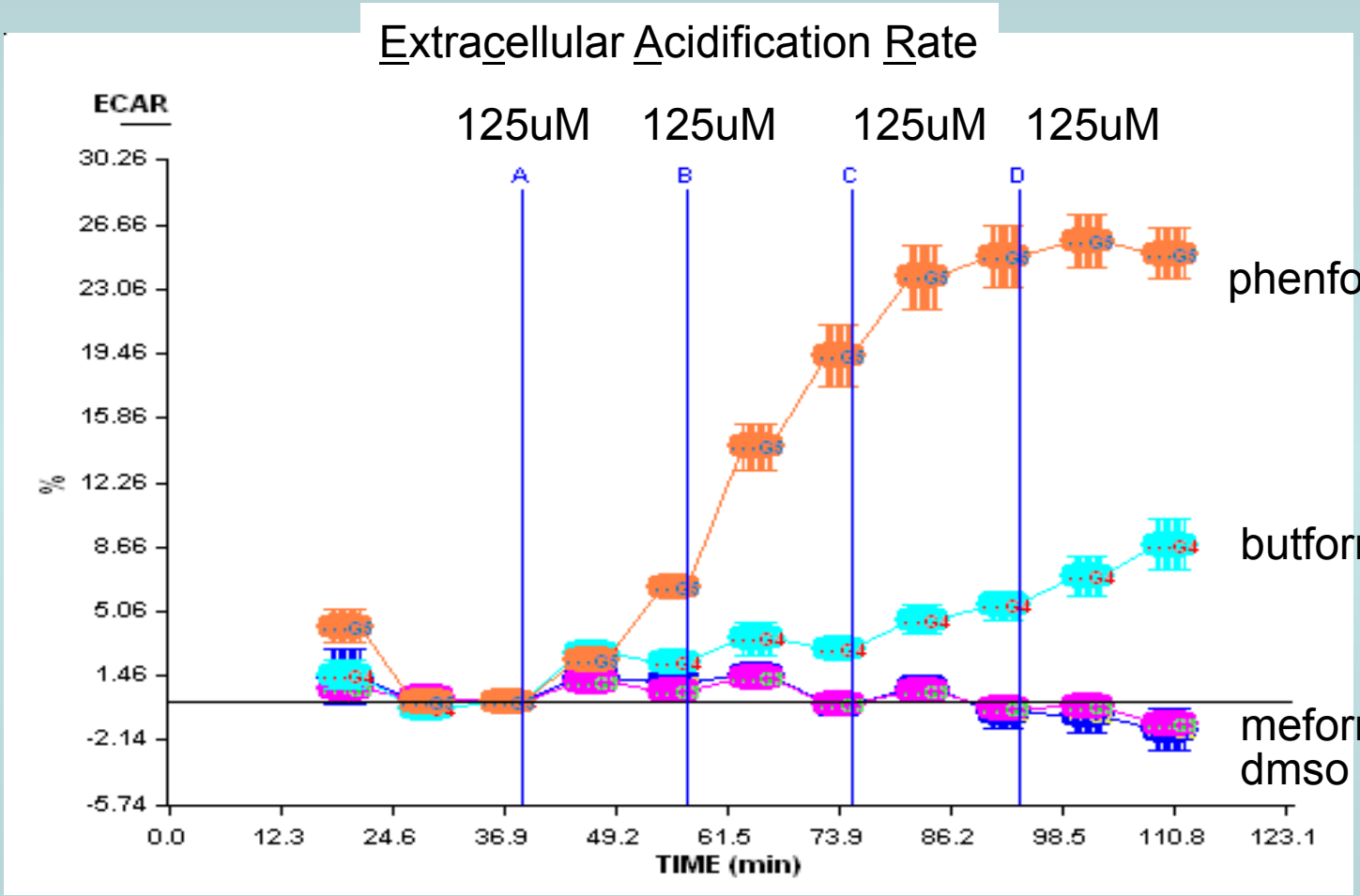


Hep G2 cells

Seahorse Bioscience

Dykens et al, Toxicology Applied Pharmacology, 2008.

Metabolic Profiling to Detect Mitochondrial Toxicity

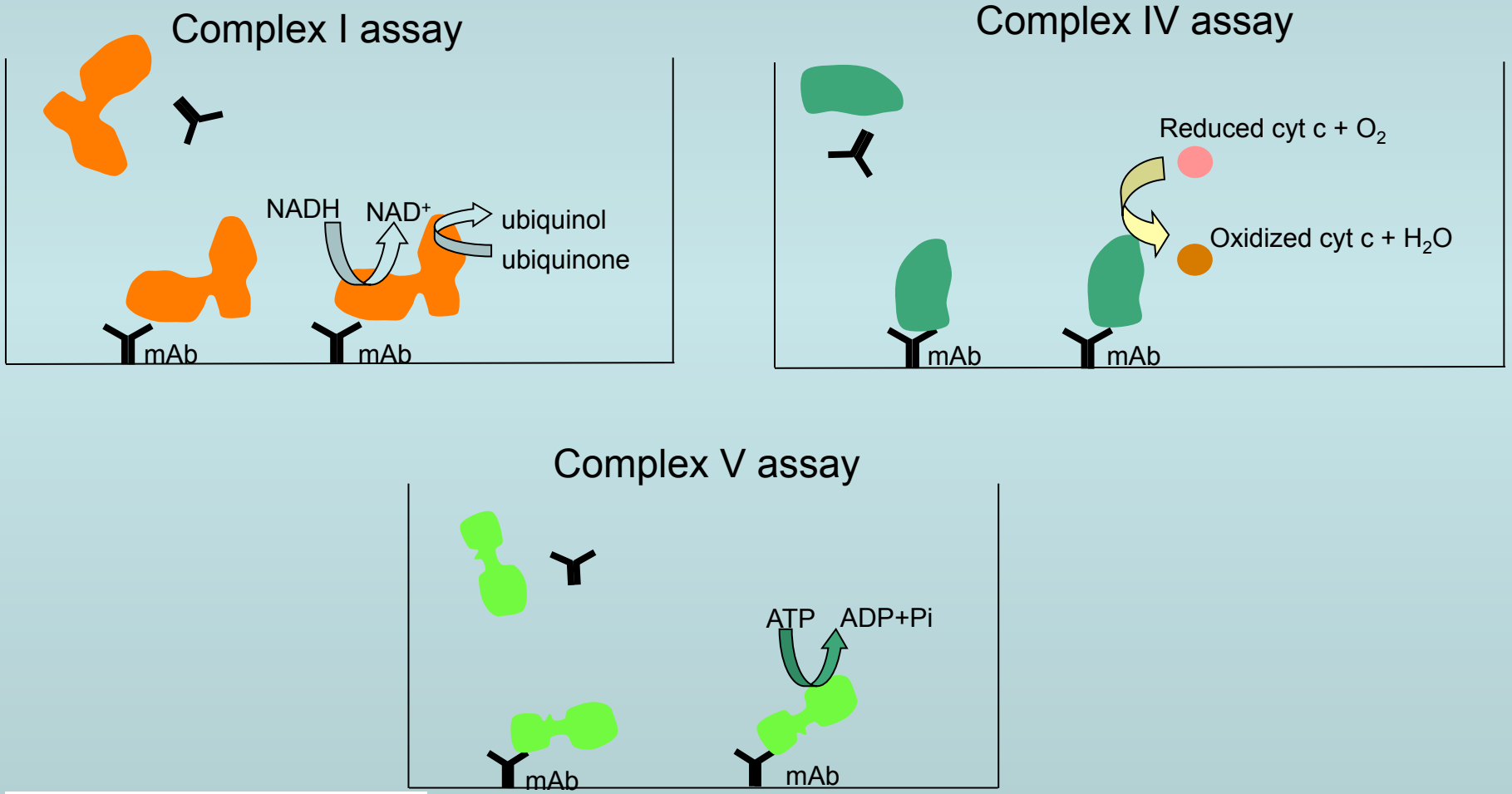


Hep G2 cells

Dykens et al, Toxicology Applied Pharmacology, 2008.

Screen #3: Identifying Site of Action

Activity Assays for Complexes I, IV and V



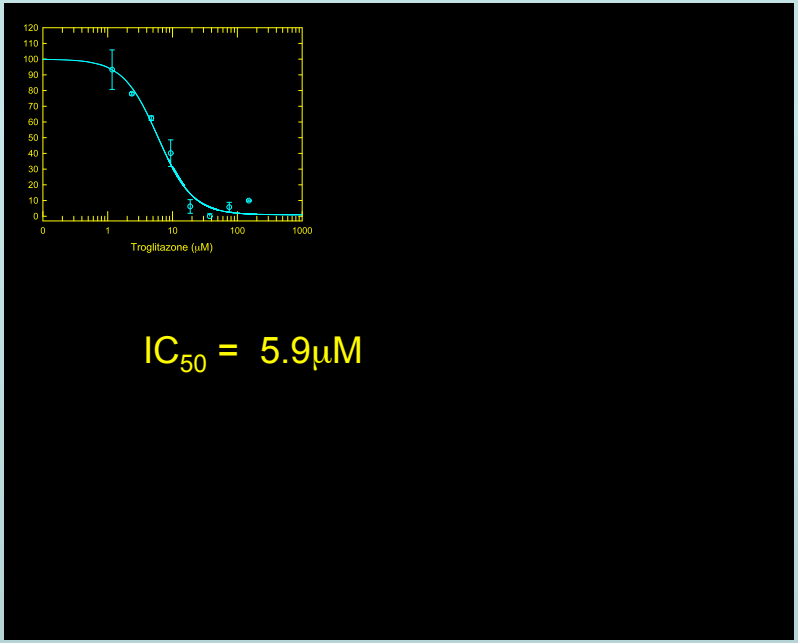
MitoSciences

Nadanaciva et al., Toxicology In vitro (2007).

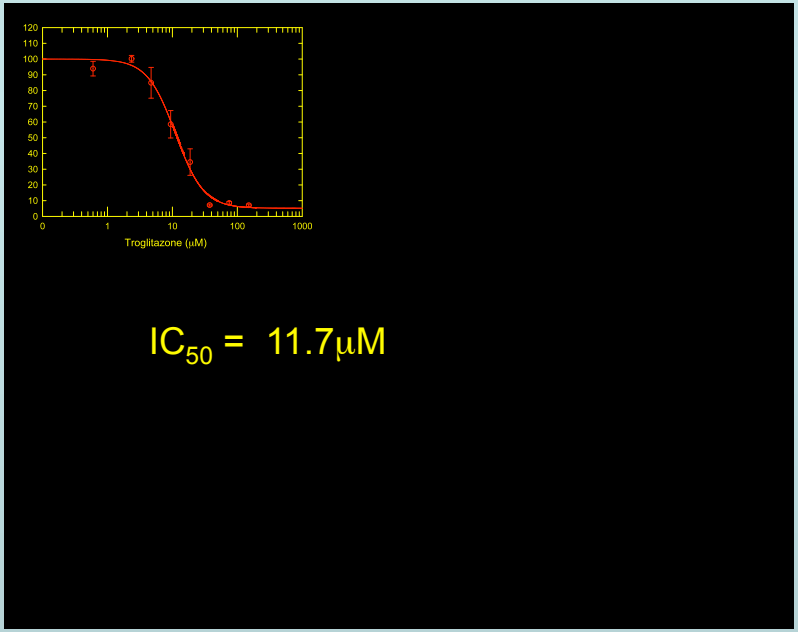
Troglitazone

- **Complex I Activity: Not inhibited at 150 μM.**
- **Complex II/III Activity: Not inhibited at 150 μM**
- **Complex IV Activity: IC_{50} 5.9 μM**
- **Complex V Activity: IC_{50} 11.7 μM**

"Fingerprint", Rank Order Potency



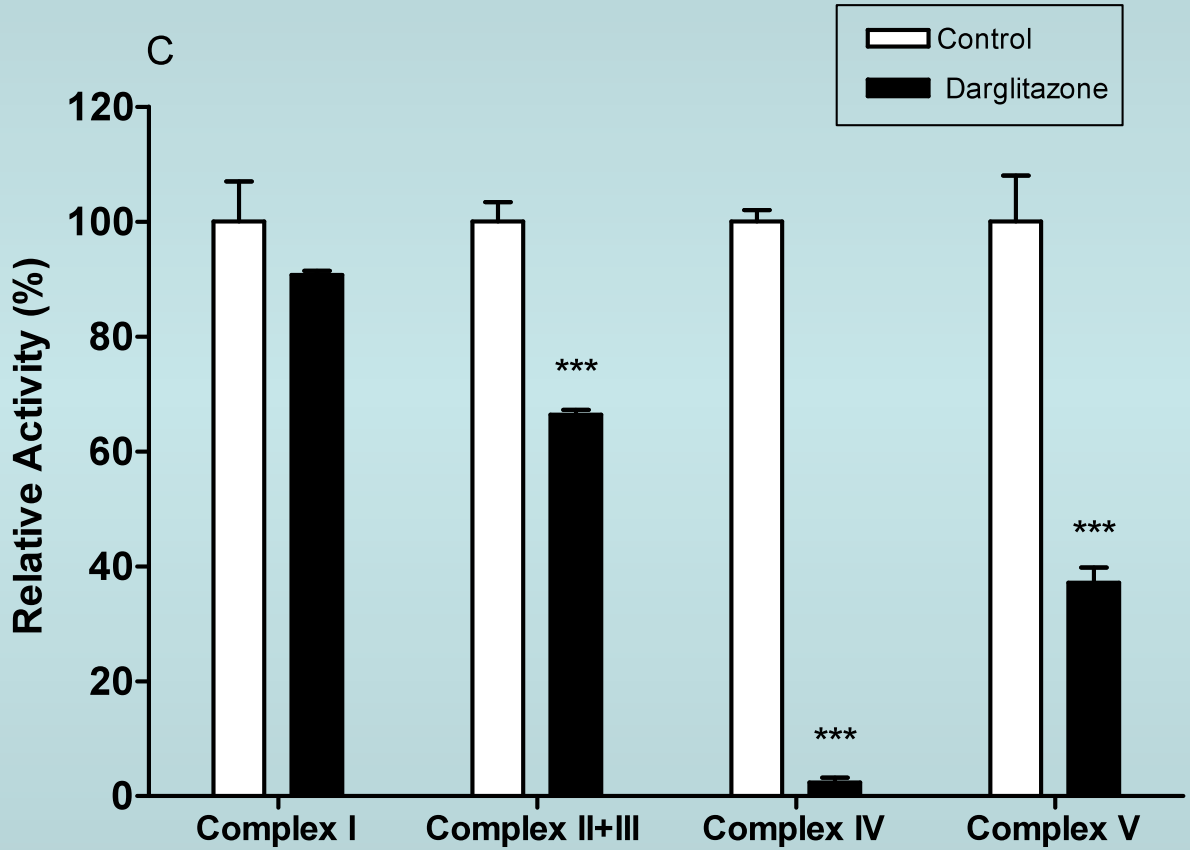
IC_{50} = 5.9μM



IC_{50} = 11.7μM

MitoSciences

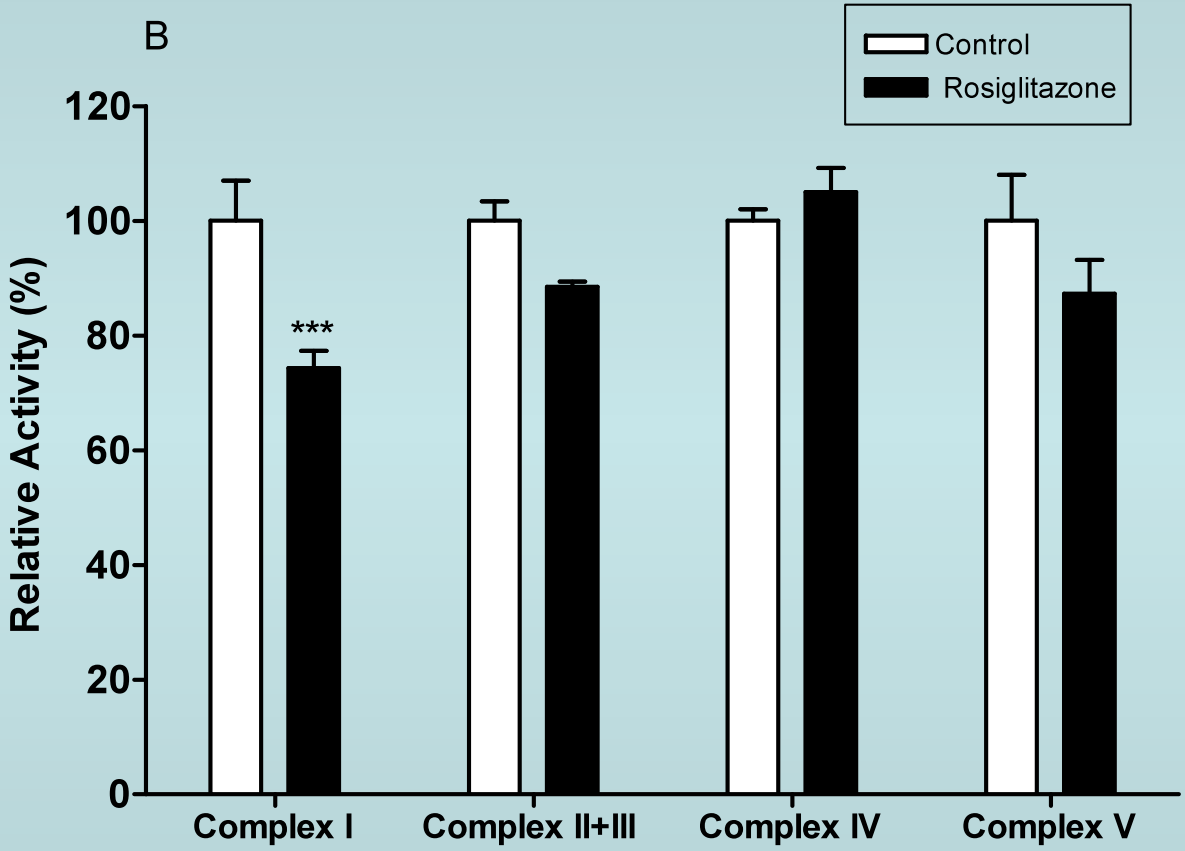
Darglitazone Inhibits Several ETS Complexes



MitoSciences

Nadanaciva et al., Toxicology & Applied Pharmacology, 2007.

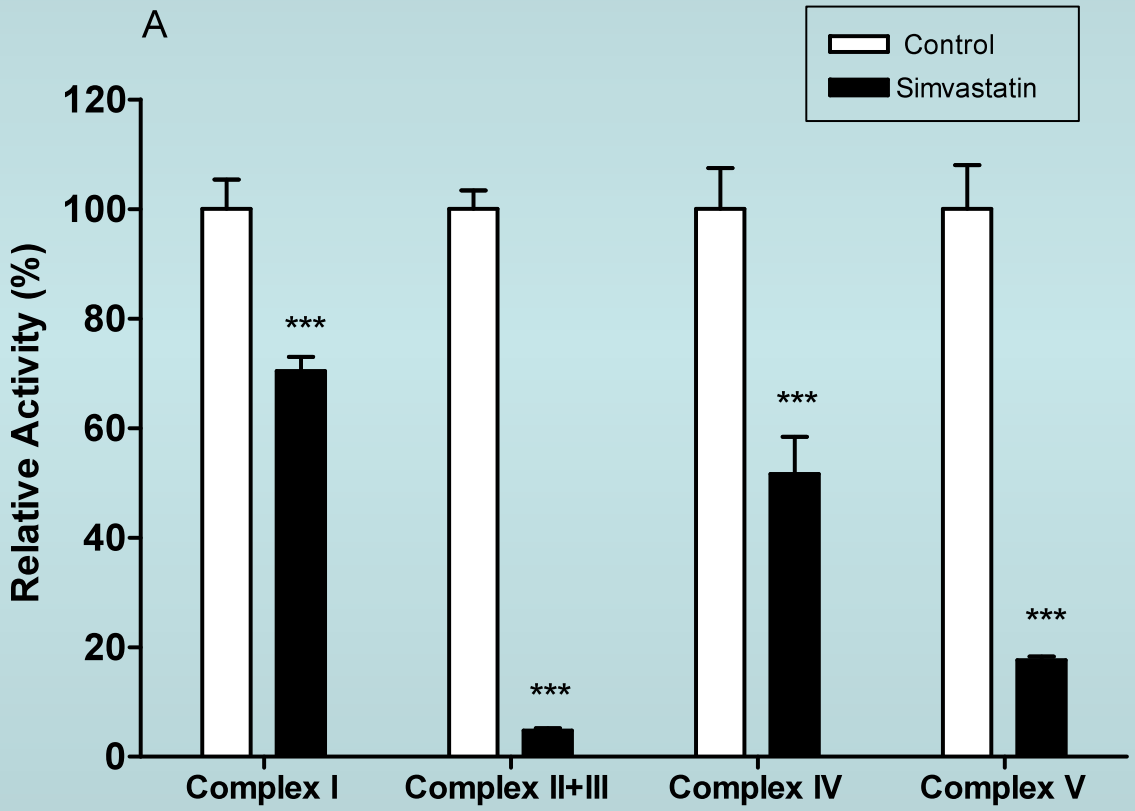
Rosiglitazone Modestly Inhibits CI,



MitoSciences

Nadanaciva et al., Toxicology & Applied Pharmacology, 2007.

Simvastatin also Inhibits Several Complexes



MitoSciences

Nadanaciva et al., Toxicology & Applied Pharmacology, 2007.

Screen #4: Circumventing the Crabtree Effect

Crabtree Effect (1929): inhibition of respiration by elevated glucose.

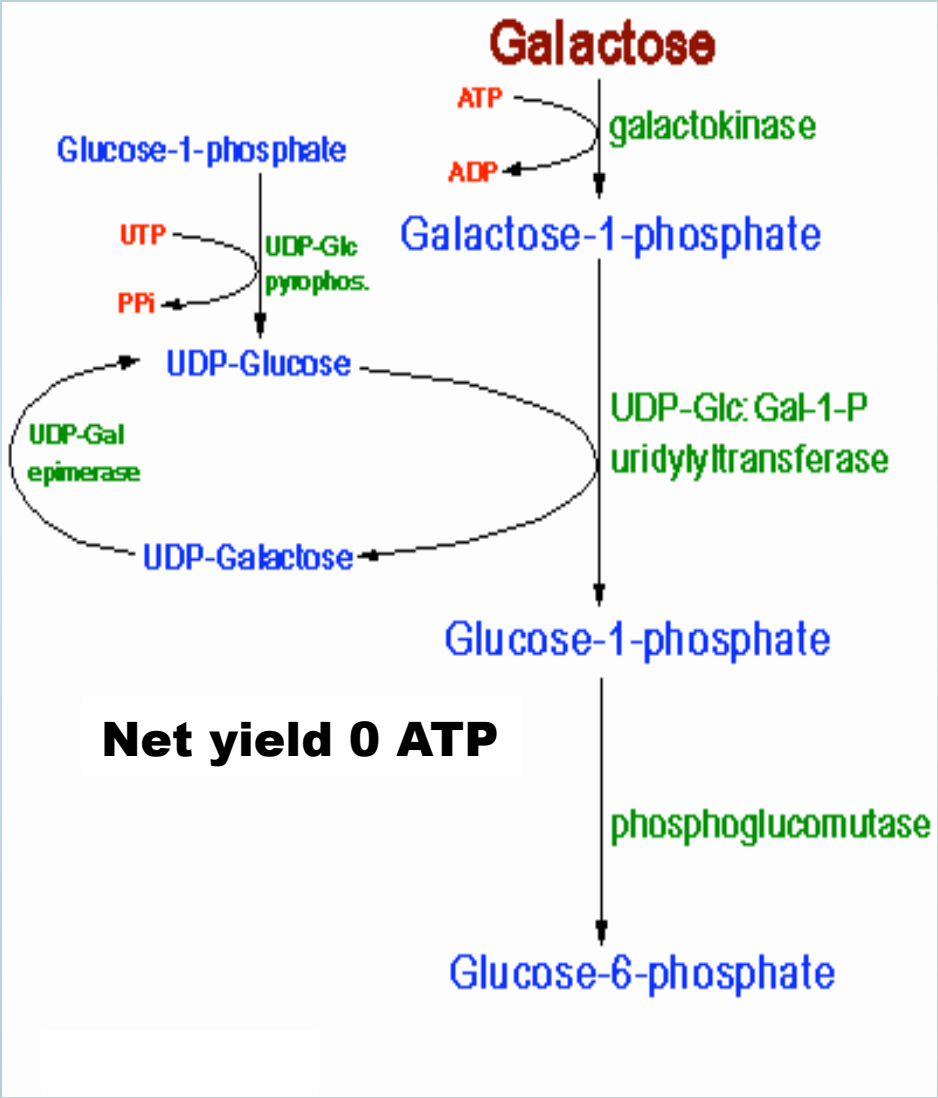
Warburg Effect (1929): aerobic glycolysis yields lactate despite competent mitochondria.

Contemporary cell culture almost uniformly uses 25mM glucose media (5X physiological !)

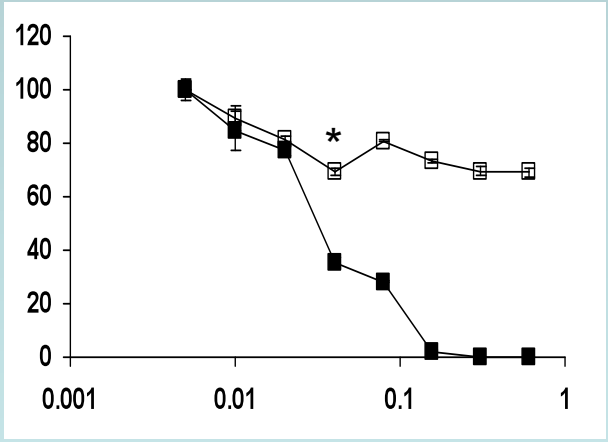
Transformed cells are characterized by low rates of O_2 consumption & resistance to mitotoxicants.

Marroquin et al., Tox. Sci, 97:539, 2007.

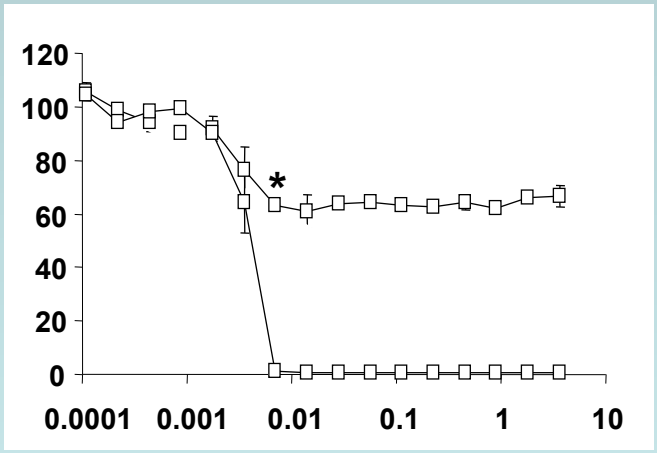
Galactose in Glycolysis yields No ATP



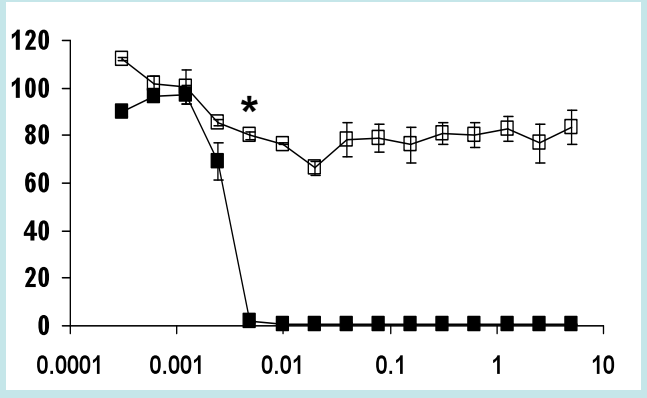
Cells Grown in Galactose Become Susceptible to Mitochondrial Inhibition



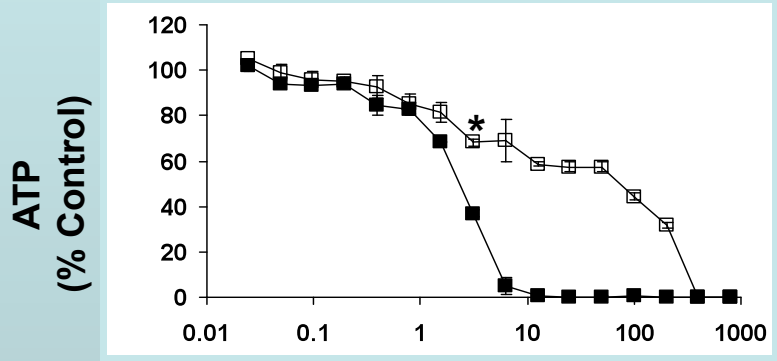
[Rotenone] μM



[Antimycin] μM



[Oligomycin] μM



[FCCP] μM

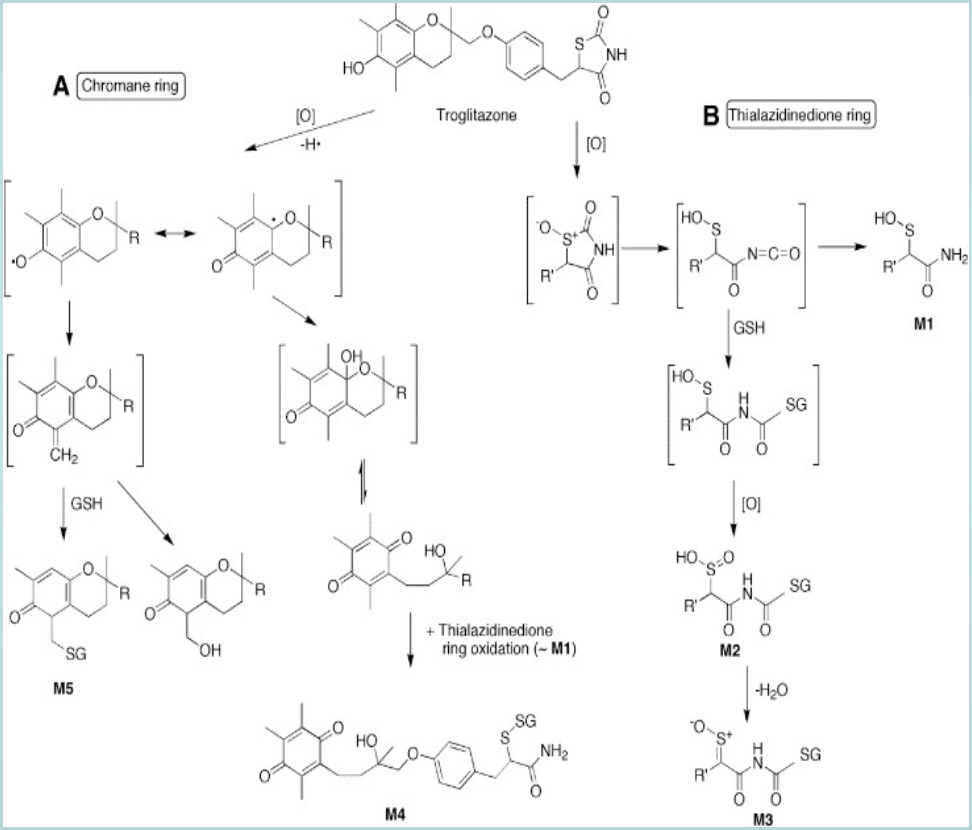
Marroquin et al., Tox. Sci, 97:539, 2007.

Three Hypotheses for Idiosyncratic Drug Response

1. **Haptan**: xenobiotic binds to protein and elicits an immune response. (Landsteiner 1930s)
   - eliminate “non-self” molecules
   - penicillin allergic response

2. **Danger**: immuno-response to cytotoxicity from parent or metabolite. (Matzinger, 1994)

3. **Pharmacological Interaction**: xenobiotic binds to T cell receptor-major histocompatibility complex to yield immune response. (Pichler, 2002)



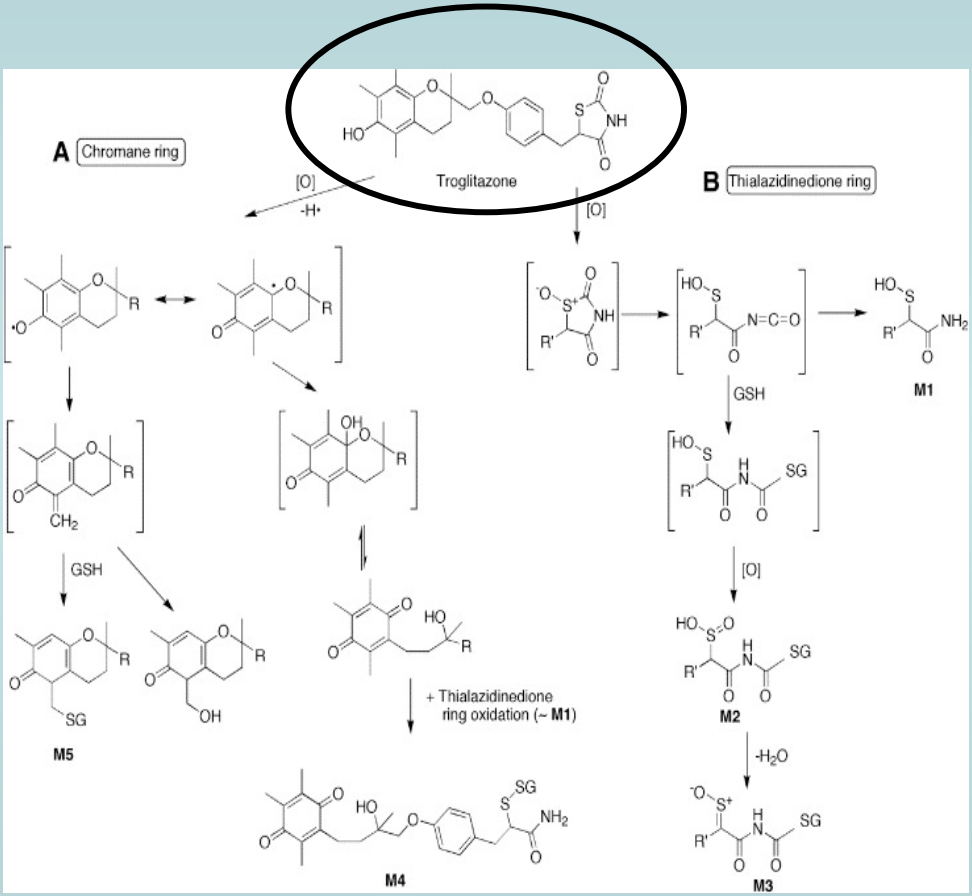
Uetrech, *Drug Metab Rev*, 38:745, 2006.

Masubuchi, *Drug Metab Pharmacokinet*. 21:347, 2006

~~Three~~ Four Hypotheses for Idiosyncratic Drug Response

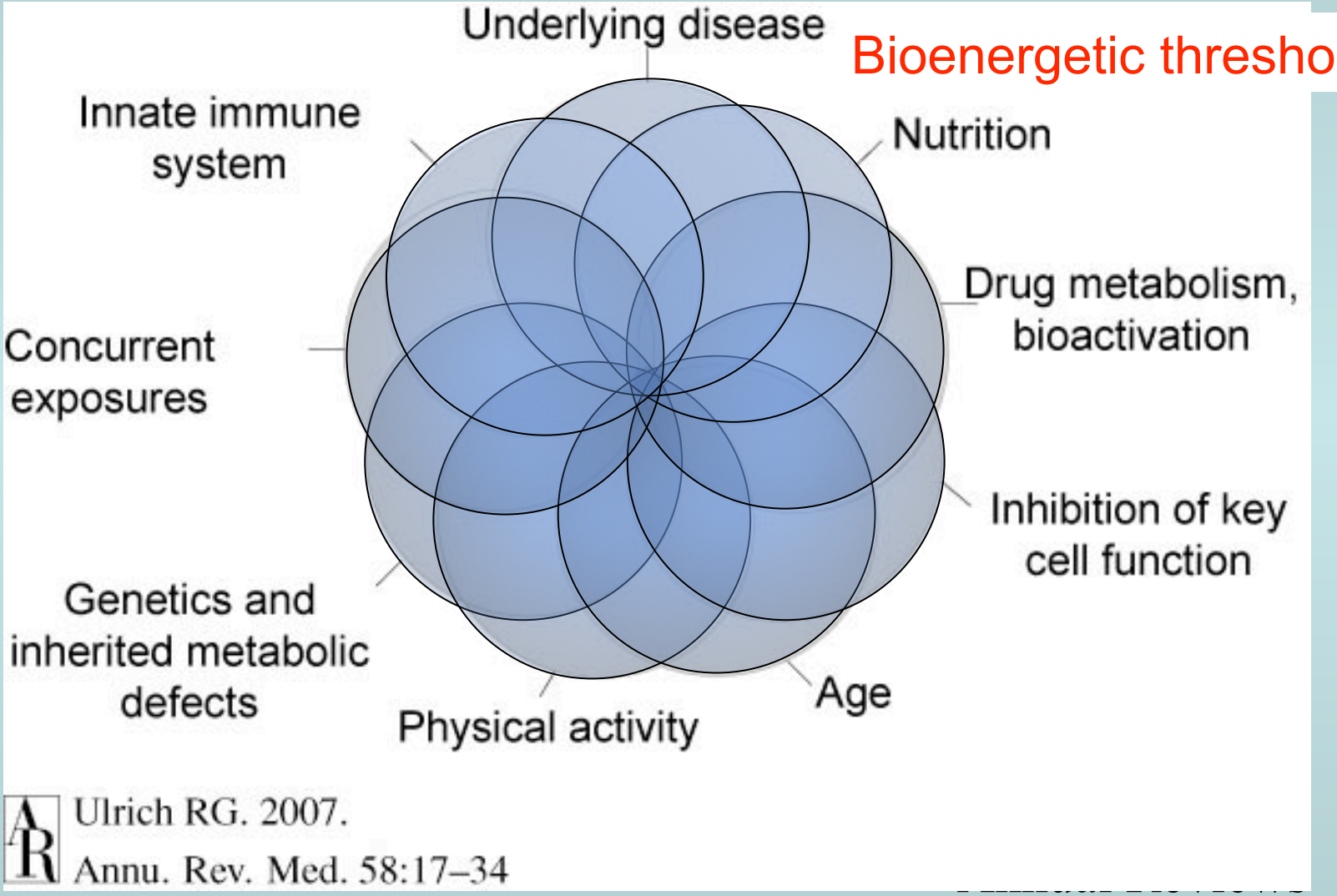
1. **Haptan**
2. **Danger**
3. **Pharmacological Interaction**

4. **Mitochondrial Dysfunction**: "off-target" impairment yields organ tox.
   - Bioenergetic &/or oxidative
   - Parent or metabolite
   - Drug effect a constant
   - Genetics & organ history impart idiosyncratic response.
   - Bio-accumulation exacerbates



Masubuchi, *Drug Metab Pharmacokinet*. 21:347, 2006

Risk Factors Converge to Yield Idiosyncratic Toxicity



Ulrich RG. 2007.
Annu. Rev. Med. 58:17–34

Drug-Induced Mitochondrial Toxicity

- Many, but not all, drugs with organ toxicity have mitochondrial liabilities.
  - **Elevated serum liver enzymes = hepatocyte death**
  - **Lactic acidosis is classic hallmark.**
- Depending on severity, if a drug has a mitochondrial liability, it will have deleterious consequences.
  - **Acute vs. Chronic Exposure**
  - **Bio-accumulation**
  - **Threshold effects**
  - **Combination therapies worse (cervistatin & gemfibrozil)**
  - **Idiosyncratic responses function of genetics and organ history.**

"The first opportunity to prevent hepatotoxicity arises in the early stages of drug development…"

Navarro & Senior, NEJM, 354:731, 2006

Dykens et al., Expert Rev. Mol. Diagnostics, 7: 161 (2007)