Oxidative Damage and Antioxidant Defenses in Mitochondria

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- **Burke Medical Research Institute**
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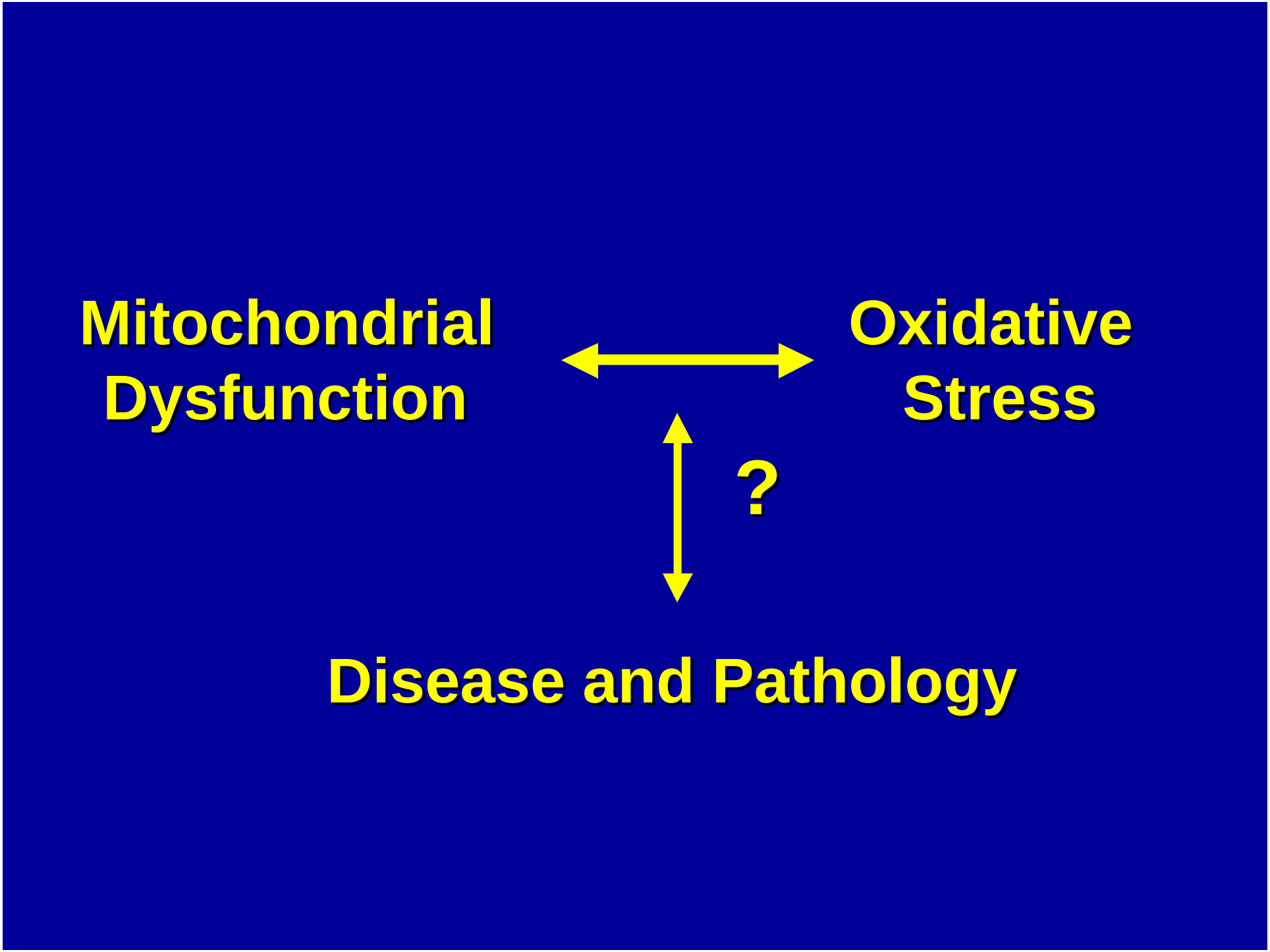
Acknowledgements

Many thanks to the researchers whose findings will be summarized


Mitochondrial Dysfunction
Oxidative Stress
?
Disease and Pathology

Diseases/Pathologies

"Acute"

e.g., Ischemia-Reperfusion

Keys: energy and inducing cell death

"Chronic"

e.g., Alzheimer's, Diabetes, Parkinson's

Key: First system to fail (?)

Mitochondria as a Target

Oxidative Stress → Mitochondrial Dysfunction

Different Target Levels -- And Concerns

Whole Mitochondria

Mitochondrial Systems

Macromolecules

“Whole Mitochondria”

“Incomplete Experiments”

Mitochondrial Lysis

Permeability transition and/or cytochrome *c* release

Mitochondrial Permeability Transition (PT or mPT)

Cyclosporin A sensitive, Ca^{2+}-mediated induction of a specific proteinaceous pore in the inner mitochondrial membrane

Induction involves

- Ca^{2+}-cycling dependent and independent events
- Oxidative stress

Consequences

- Free diffusion of solutes <1500 Daltons
- Prevents oxidative phosphorylation
- ATP synthase converted to an ATPase
- Efflux of matrix Ca^{2+} stores

May contribute to ischemia-reperfusion injury

May propagate apoptotic cascades

Oxidants and PT

***tert*-BuOOH, phenylarsine oxide, diamide, Menadione, $ONOO^-$,**

4-hydroxyalkenals, DOPEGAL (3,4-dihydroxyphenylglycolaldehyde)

Oxidants and the PT

Oxidants appear to act at two sites to stimulate induction of the PT by shifting the gating potential to progressively more negative values of $\Delta\Psi$.

“Oxidants” act at P-Site and S-Site

P-site

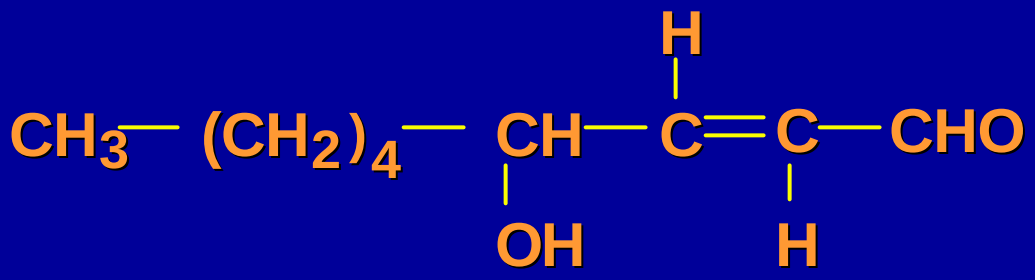
In equilibrium with mitochondrial pyridine nucleotide pool. Oxidants acting at P-site can induce PT without affecting GSH. Reduced pyridine nucleotides delay PT induction, complex I substrates are protective,

S-Site

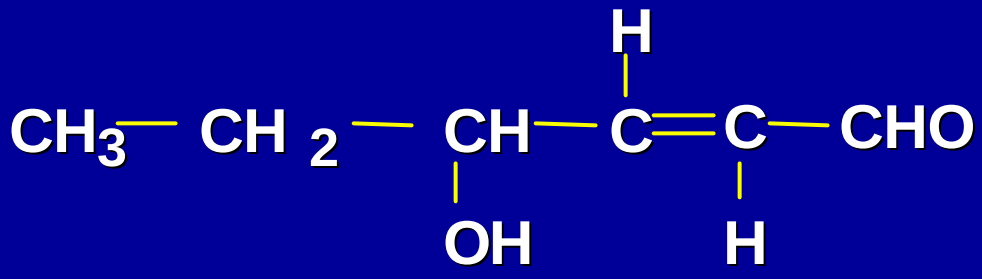
Thiol oxidation associated with PT induction, GSH, DTT are protective

Antioxidants are protective in some models. GSH-GSSG redox couples can link P- and S-sites

4-HHE is ~8 logs more potent as an inducer than 4-HNE



4-Hydroxynonenal (4-HNE)



4-Hydroxyhexenal (4-HHE)

Mitochondrial “Systems”

TCA cycle, electron transport, ion transporters, substrate transporters, protein import systems, mtDNA repair, mtDNA transcription, mRNA translation, mtDNA replication, mtRNA turnover

mtDNA Transcription

Mitochondrial genome essential for OXPHOS

mtDNA not protected by chromatin/histones

mtDNA is attached to inner membrane

- Close to ROS generation sites
- Close to reactive lipid peroxidation byproducts

Peroxyl radicals inhibit mtDNA transcription at concentrations that do not peroxidize lipids, but ADP/Fe/NADPH peroxidizes lipids at concentrations that do not affect transcription.

Targets are differentially sensitive to different ROS species

Specific Molecules

Lipids

Proteins

Nucleic Acids

Lipid Peroxidation -- Why

Mitochondria are rich in PUFAs and transition metals

Strongly reducing environment

Lots of single electrons

Lots of oxygen based chelators (e.g., ADP, citrate)

These may not prevent redox cycling

Phospholipase A_2 may propagate damage reactions

Lipid Peroxidation -- Consequences?

Lysis, PT induction (Generally require high levels)

ROS production

(e.g., HHE, HNE, malondialdehyde)

Induction of generalized and specific protein damage

Alters membrane functional properties

(e.g., fluidity, ion fluxes)

Destruction of critical components

(e.g., cardiolipin, ubiquinone [not ubiquinol])

Lipid Peroxidation -- Modulators

Diet

- Dietary restricted animals more resistant
- Lipid composition of diet affects mitochondria

Age

- Older animals may be more susceptible

Species

- Rat more susceptible than pigeons

Disease/Pathology

- *e.g.*, diabetes, ischemia-reperfusion

Cardiolipin

Only anionic lipid in mitochondria

18% of total mitochondrial phospholipid

90% of its fatty acids are unsaturated

Extremely sensitive to peroxidative damage

Involved in multiple enzyme activities

Complex I, complex IV, several transporters (monocarboxylate, dicarboxylate, oxoglutarate, phosphate, carnitine, ADP translocase)

Damage induces structural abnormalities

Protein Oxidation

Mitochondrial membranes are ~60-75% protein

Mitochondrial proteins rich in thiols, Fe-S clusters

Oxidation reduces system function and efficiency

Energetically costly

Can alter production of reactive species

Specific Proteins

TCA Cycle

PDH, aconitase, isocitrate dehydrogenase, KGDHC, SDH

Fatty Acid Metabolism

3-hydroxybutyrate dehydrogenase

Electron Transport Chain

complex I, II, IV

Phosphorylation System

F_1F_0ATPase, ANT

Miscellaneous

rhodanese, CK, MnSOD

mtDNA Damage

- No protective chromatin/histones
- Hot-spots exist
  - not yet been extensively studied
- mtDNA is attached to inner membrane
  - Close to ROS generation sites
  - Close to reactive lipid peroxidation byproducts
- Repair exists but may be limited
  - Speed (repairs can become limiting)
  - Extent (e.g., no pyrimidine dimer repair)
- 8-OH dG major oxidative lesion
  - increase with age, metabolic rate
  - inversely correlated with MLSP

Steady state damage

1/100,000 bp (1/5 genomes) in rat liver sensitive to formamidopyridine-DNA glycosylase (*i.e.*, 8-OH dG)

Note other studies put number 10x higher

Abasic sites, <1/25 genomes

Damage from some insults may take 2 weeks to resolve

Most oxidatively damaged DNA in broken pieces, not intact circles

Lesions increased mtDNA vs nuDNA
~4- to 50-fold

8-hydroxyguanine

5-hydroxycytosine

5-hydroxyhydantoin

5-hydroxymethyhydantoin

5-hydroxymethyuracil

(Zastawny et al, 1998)

RNS

Effects of $^{\bullet}NO$ vs $ONOO^{-}$

$^{\bullet}NO$

Reversibly inhibits complex IV

competitive with oxygen

Complex I can become S-nitrosylated if GSH depleted

(also reversible)

May inhibit KGDHC and aconitase

(but may also be $ONOO^-$)

$ONOO^-$

PT inducer

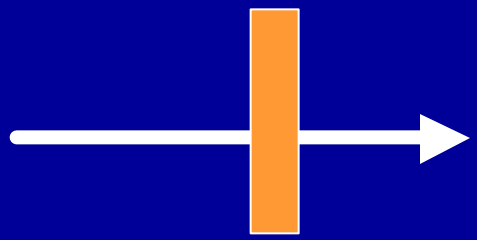
Irreversibly inhibits complex I and II

Can affect ATPase, CK, KGDHC, Aconitase

Reaction with aconitase and other 4Fe-4S proteins can liberate Fe II

Mitochondrial Defenses

Oxidative Stress



Mitochondrial Dysfunction

Defense vs. Repair/Replacement

Repair and Replacement Mechanisms

- **Phospholipase A_2**
- **DNA repair**
- **Gene expression**
- **Import of raw materials**

Redundancy

Respiratory chain may have 2-4 times the levels of required components

Each cell multiple mitochondria, each mitochondria multiple cristae and multiple copies of the genome

“Defense” Concepts

1. The nature of oxygen
2. Primary ROS is $O_2^{\bullet -}$
3. Membranes and thiols
4. Organelle specific defenses
5. Prevention

The Nature of Oxygen

The fundamental reaction of oxidation is the reduction of molecular oxygen

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

Redox potential = -0.18 V

Thus, the oxidation of any and all biological molecules appears to be favored thermodynamically

(Papa and Skulachev 1997)

Nature of Oxygen, Part 2

Thus, the first, and most important direct barrier against oxidative stress is the "use" of molecules that have relatively high activation energies for the process of oxidation

but…

Exceptions abound in the mitochondria, *e.g.*, flavins, quinols, especially semiquinones...

Primary ROS is $O_2^{\bullet -}$

Superoxide is not highly reactive

$$O_2^{\bullet -} \longrightarrow H_2O_2$$

Concern is

$$H_2O_2 \longrightarrow HO^{\bullet}$$

Primary Defense is MnSOD

Matrix enzyme, essentially diffusion limited

Some portion may be associated with inner membrane

Scavenges ~80% of superoxide produced

KO's lethal in days to weeks

(cardiomyopathy, neurodegeneration, more)

hemizygotes may also be sensitized to insult

Transgenics protected against multiple challenges

TNF, paraquat, cigarette smoke, hyperthermia, excitotoxicity, MPTP, stroke, iron overload, etc

Role in cancer (e.g., can suppress malignant phenotype)

May affect cell signaling (e.g., C3H10T1/2 cells)

CuZnSOD may partially localize to intermembrane space

H_2O_2 Removal

Catalase in Heart Mitochondria

GSH-Peroxidase/Reductase

Possibly non-enzymatic scavengers

(e.g., pyruvate)

Protecting Lipids

Tocopherol

$LOO^{\bullet} + \alpha\text{-}TOH \longrightarrow LOOH + \alpha\text{-}TO^{\bullet}$

Ubiquinols (Q_9, Q_{10})

Efficacy enhanced by respiration

May act predominantly by regenerating α-TOH

Semiquinone form propagates reactions!

Ubiquinol

Electron Carrier in Electron Transport Chain

Antioxidant

Biosynthesis takes place in matrix
also Golgi, ER

Found in both inner and outer membrane

Thiols

Overall 100 nmol thiols/mg protein

(90% in proteins)

Protected by three systems

- GSH
- Thioredoxin
- Dihydrolipoic acid and lipoic acid

GSH

Acts both as an antioxidant and to regenerate other antioxidants

Primary Mitochondrial Antioxidant?

- **Severe damage to this system sensitizes to most oxidant attacks**
- **Damage to this system can be a hallmark of irreversible injury**

Constituents:

GSH (~10 mM or 4-8 nmol/mg protein), GSH Reductase, GSH-Peroxidase, $NAD(P)^+$ transhydrogenase, and the GSH transport system, GR, GPx may also be in inter-membrane space

GSH Synthesis and Import

GSH is imported against its concentration gradient

Both low and high affinity transporters exist

At least one component appears to involve dicarboxylate and 2-oxoglutarate carriers

Transport can be impaired by disease (alcoholism)

Transport is sufficiently slow that differences between cytosol and matrix can be observed

Regeneration of GSH from GSSG occurs in matrix

GSH Regeneration

Limiting factor of GSH system in mitochondria may be regeneration

Continued regeneration of GSH requires NADPH, which in turn requires transhydrogenation of $NADP^+$ and NADH

This links $\mathrm{D}Y$, thiol redox status, the redox state of pyridine nucleotides, and mitochondrial antioxidant defenses

Thioredoxin

Small protein involved in maintaining thiols

Mitochondrial forms relatively uncharacterized

Constituents

- thioredoxin, thioredoxin peroxidase and thioredoxin reductase

Potential actions:

- Activates keto-acid dehydrogenases
- Roles in protein folding
- Protects against PT
- Protects radical sensitive proteins

DHLA and LA

Essential cofactor (*e.g.*, KGDHC)

Broadly active antioxidants

Membranes and Thiols

Targets and Defenses

Organelle Specific Defenses

1) Antioxidant Regeneration
2) Proton Gradient
3) α-ketoacids

Antioxidant Regeneration

α-TOH is regenerated by ascorbate, GSH, ubiquinol

DHLA is regenerated by the keto-acid dehydrogenases

DHLA can regenerate ascorbate, GSH, and thioredoxin directly, and α-TOH indirectly *via* GSH

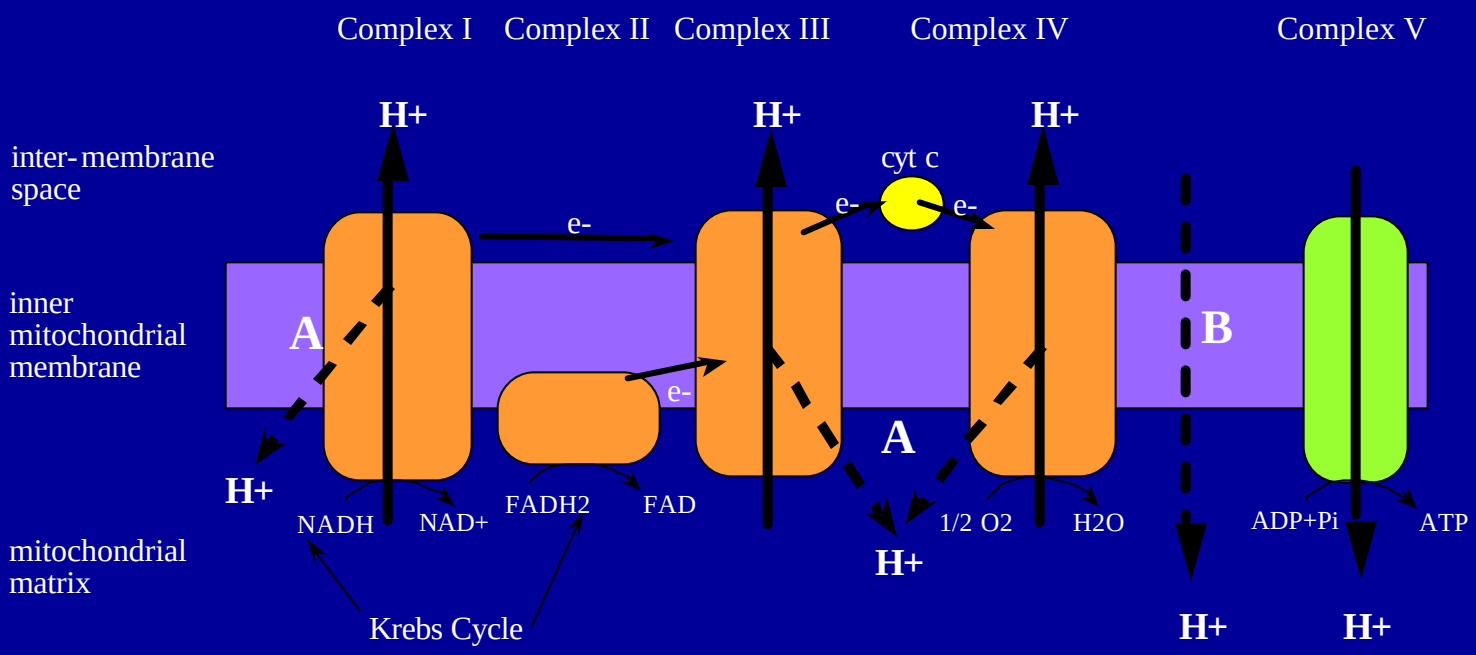
Ubiquinol can regenerate tocopherol

GSH is regenerated *via* transhydrogenation reactions

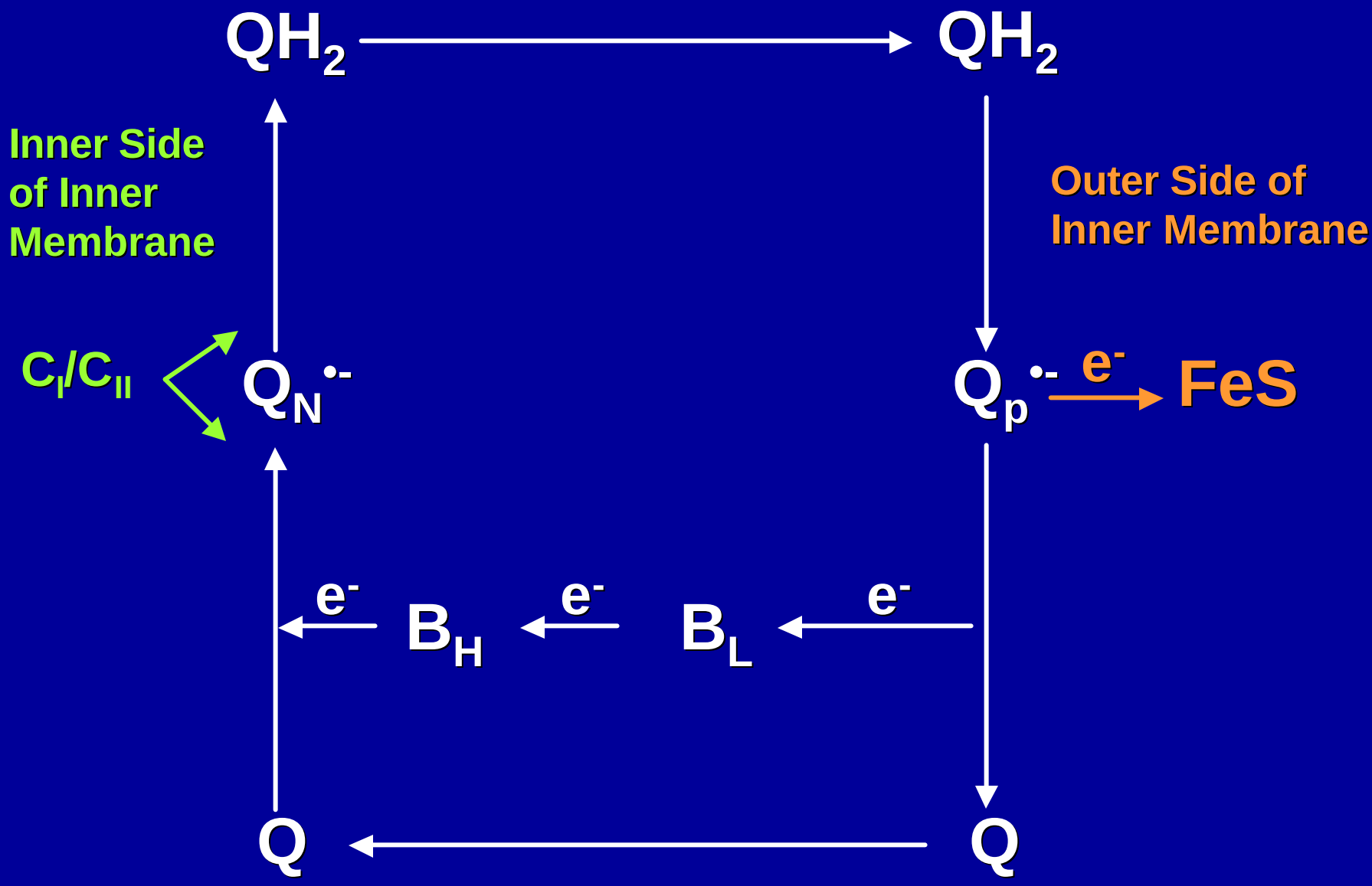
Mitochondria are Reducing Organelles

Antioxidant Regeneration

Mitochondria can subvert reducing equivalents from the production of energy to the enhancement of antioxidant defenses. They therefore have, in theory, a nearly limitless antioxidant capacity as long as respiratory function is maintained.


Complex I
Complex II
Complex III
Complex IV
Complex V
inter-membrane space
inner mitochondrial membrane
mitochondrial matrix
H+
H+
H+
cyt c
e-
e-
e-
e-
A
A
B
H+
H+
H+
H+
NADH
NAD+
FADH2
FAD
1/2 O2
H2O
ADP+Pi
ATP
Krebs Cycle

Q-cycle Regenerates Ubiquinol



Proton Gradient

$4\,(O_2\text{-})_{out} + 4(H^+)_{out} \rightarrow 4\,(HO_2)_{out} \rightarrow 4\,(HO_2)_{in}$

$4(HO_2)_{in} \rightarrow 4(O_2\text{-})_{in} + 4(H^+)_{in}$

$4(O_2\text{-})_{in} + 4(H^+)_{in} \rightarrow 2(H_2O_2)_{in} + 2(O_2)_{in}$

$2(H_2O_2)_{in} \rightarrow 2(H_2O)_{in} + (O_2)_{in}$

$4\,(O_2\text{-})_{out} + 4(H^+)_{out} \rightarrow 2\,(H_2O)_{in} + 3(O_2)_{in}$

S-S. Liu

a-Ketoacids

Directly scavenge H_2O_2

Pyruvate, a-ketoglutarate

Prevention

vs

Defense

vs

Repair

Prevention

Sequester Reactions

Reduce Oxygen Tension

Reduce Back-pressure

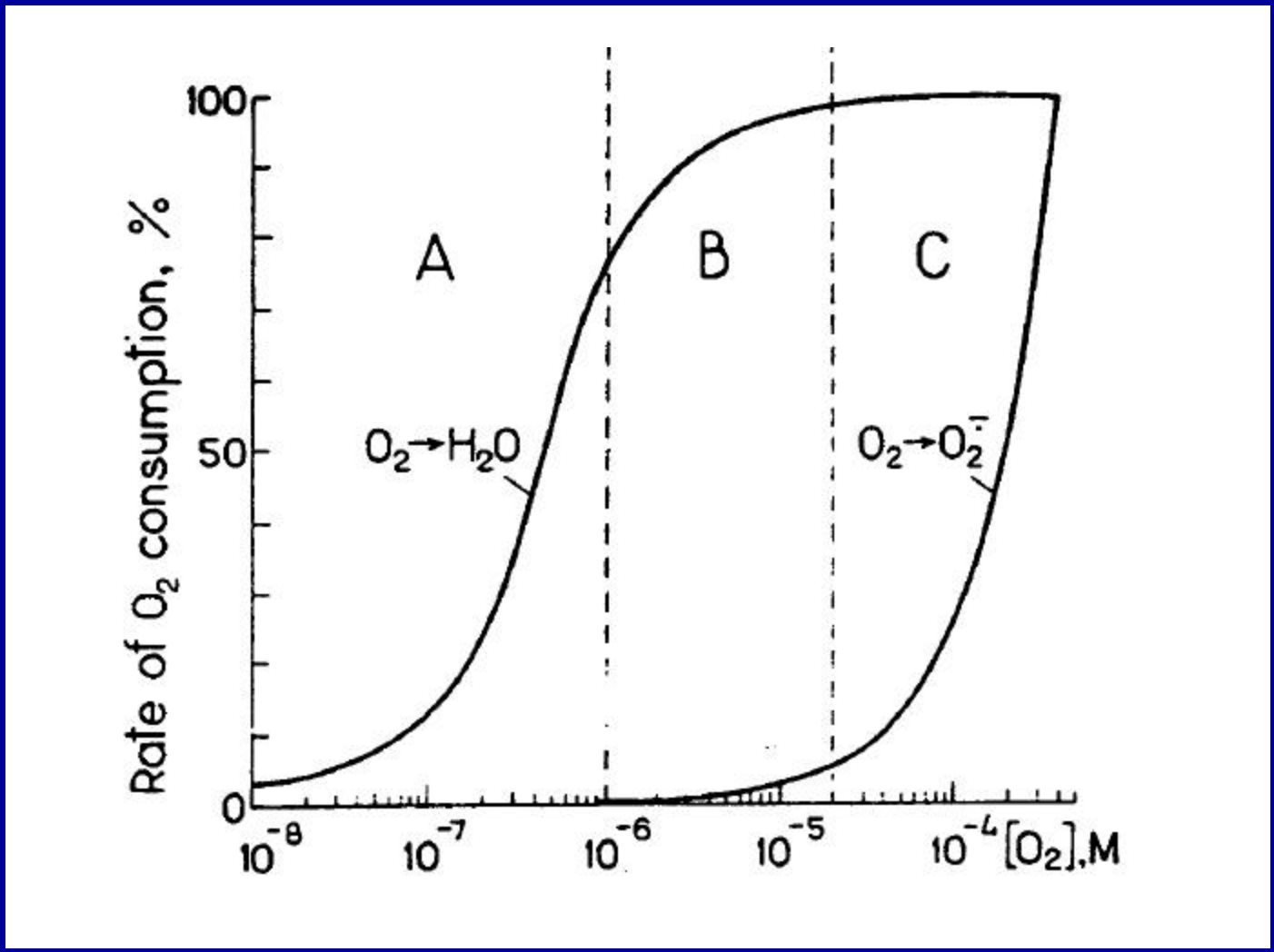
Sequester/Limit Reactions

Mitochondrial ROS production is away from sensitive cellular targets

Fe is sequestered into hemes

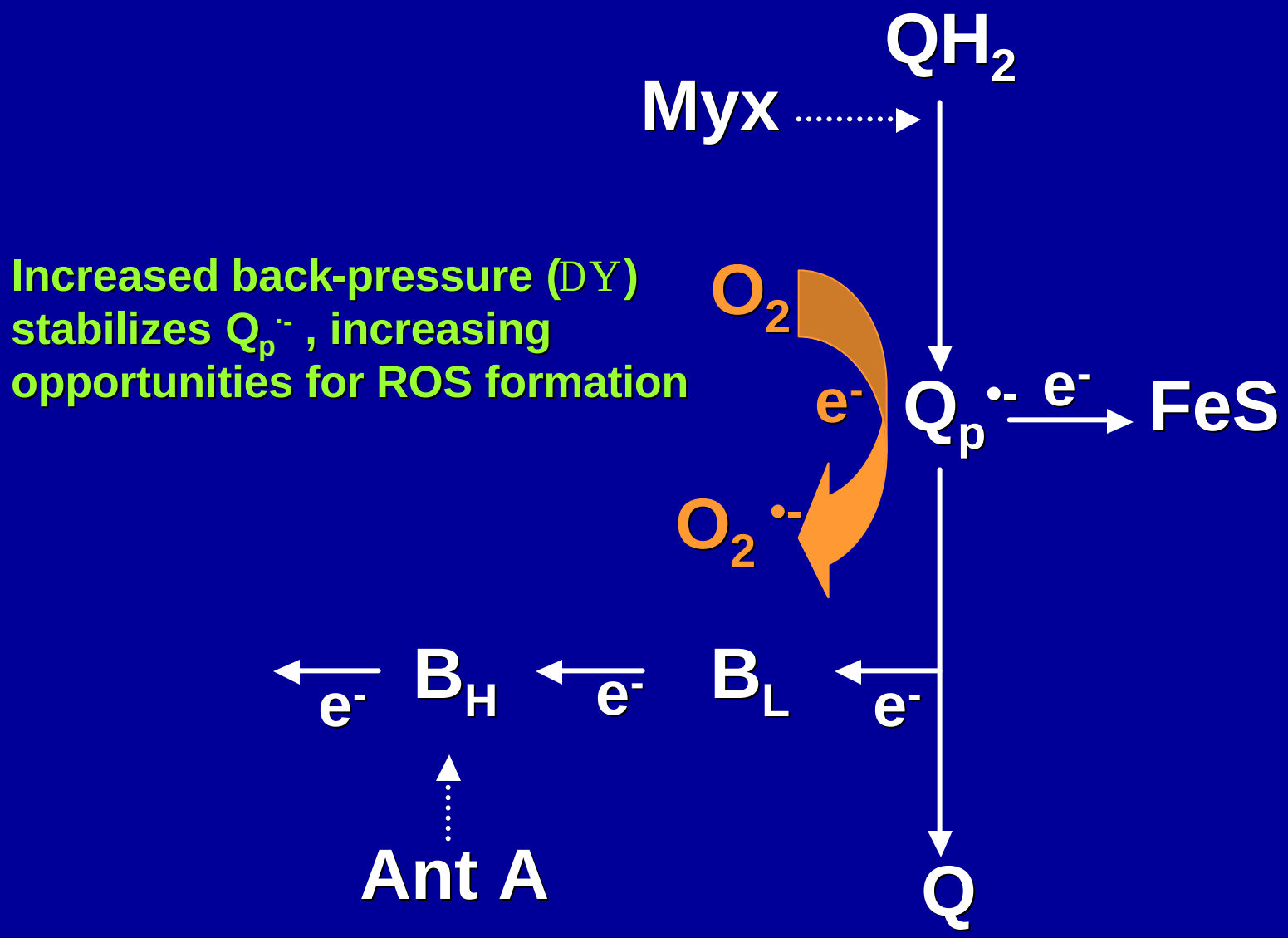
Center P semiquinone is very transient

Reduce Oxygen Tension



Skulachev and Papa

Reduce Back-pressure on ETC



Reduce Back-pressure on ETC

ΔY lower in State 3 (vs State 4)

Proton Leak reduces excess ΔY

Redox slip probably not important

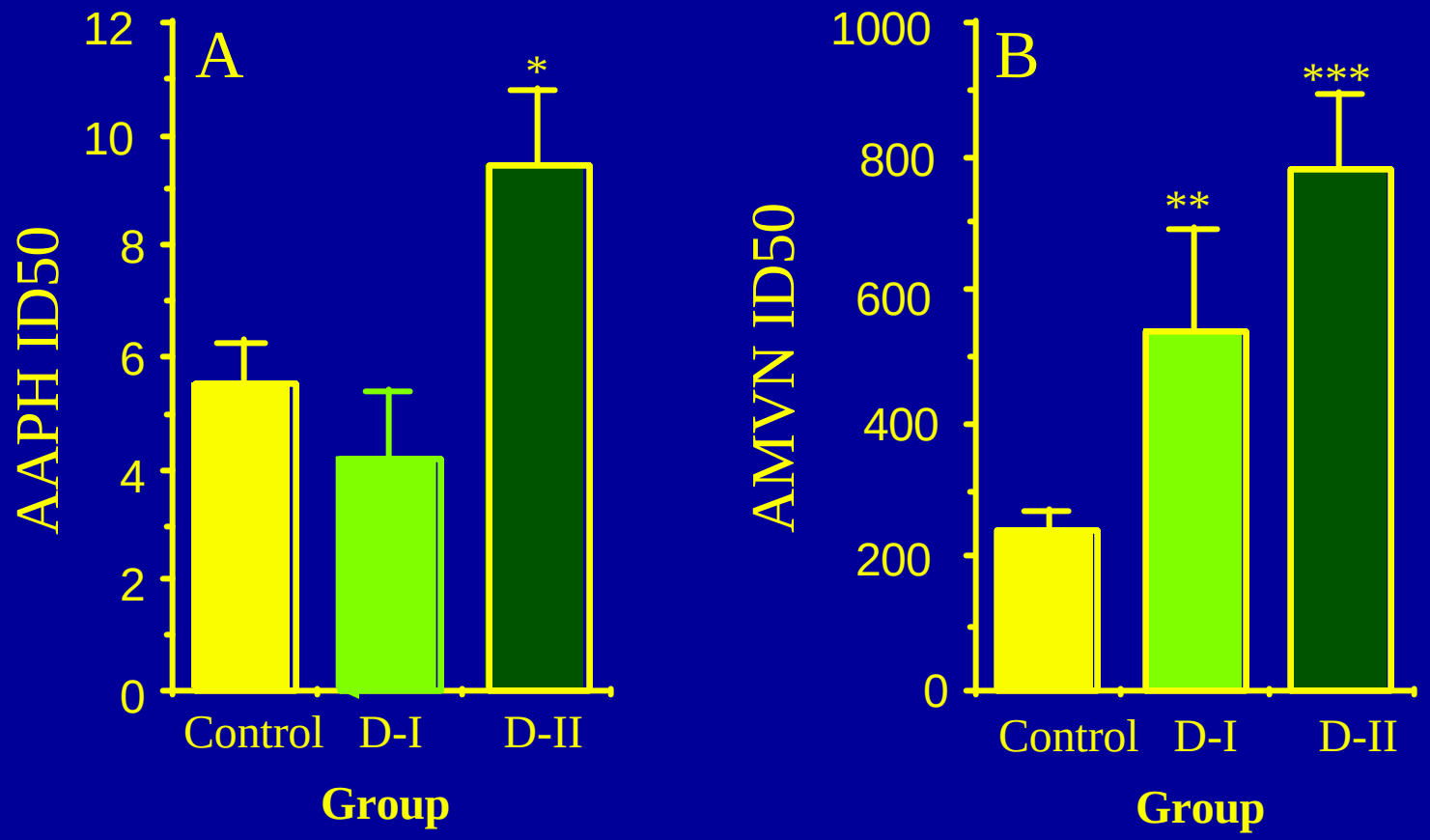
mild uncouplers (*e.g.*, fatty acids)

Aconitase sensitivity?

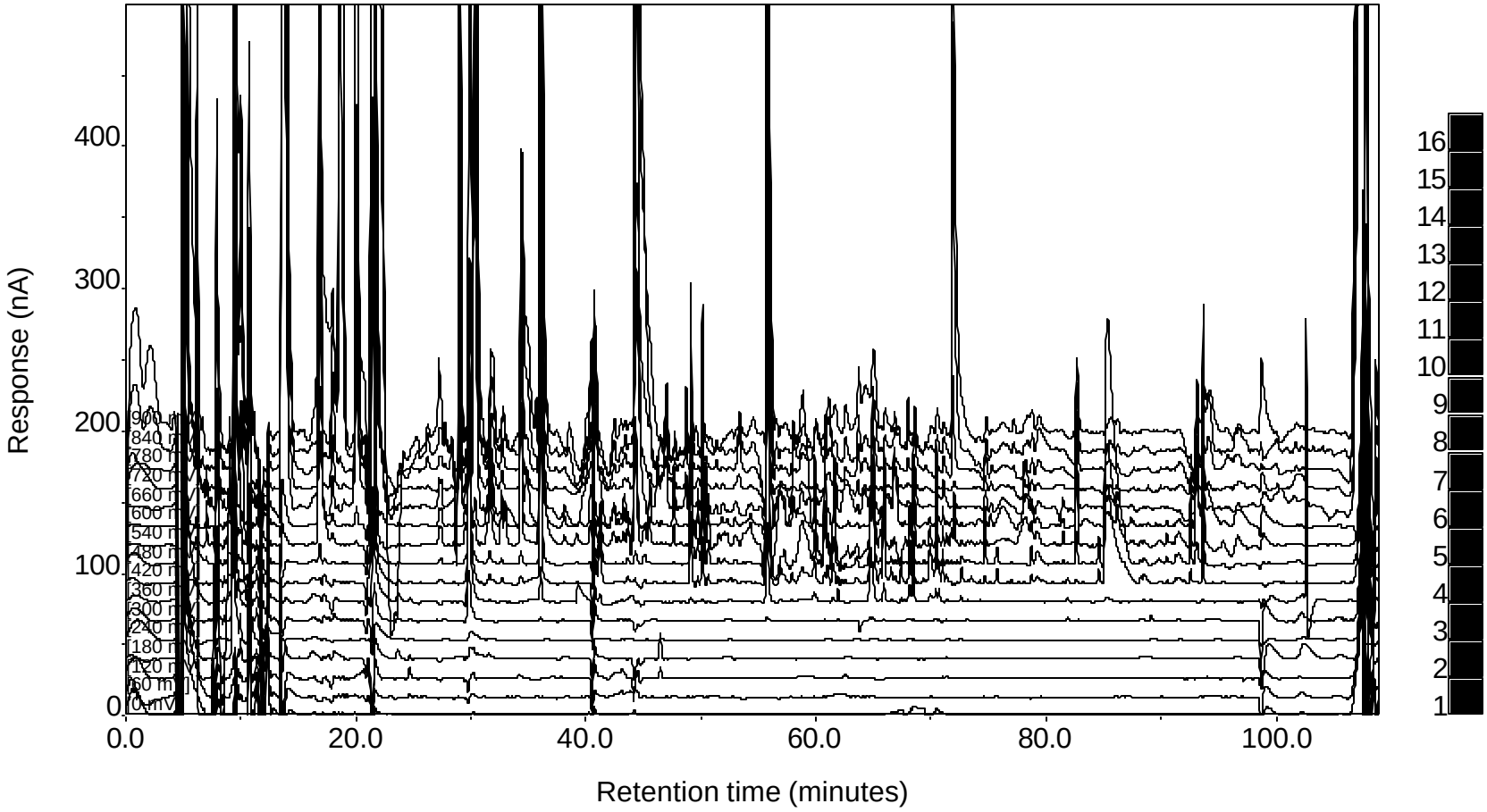
Regulation of Defenses

Uniform or Challenge Specific?

Diabetes Alters Resistance to Oxidant-Mediated Inhibition of mtDNA Transcription

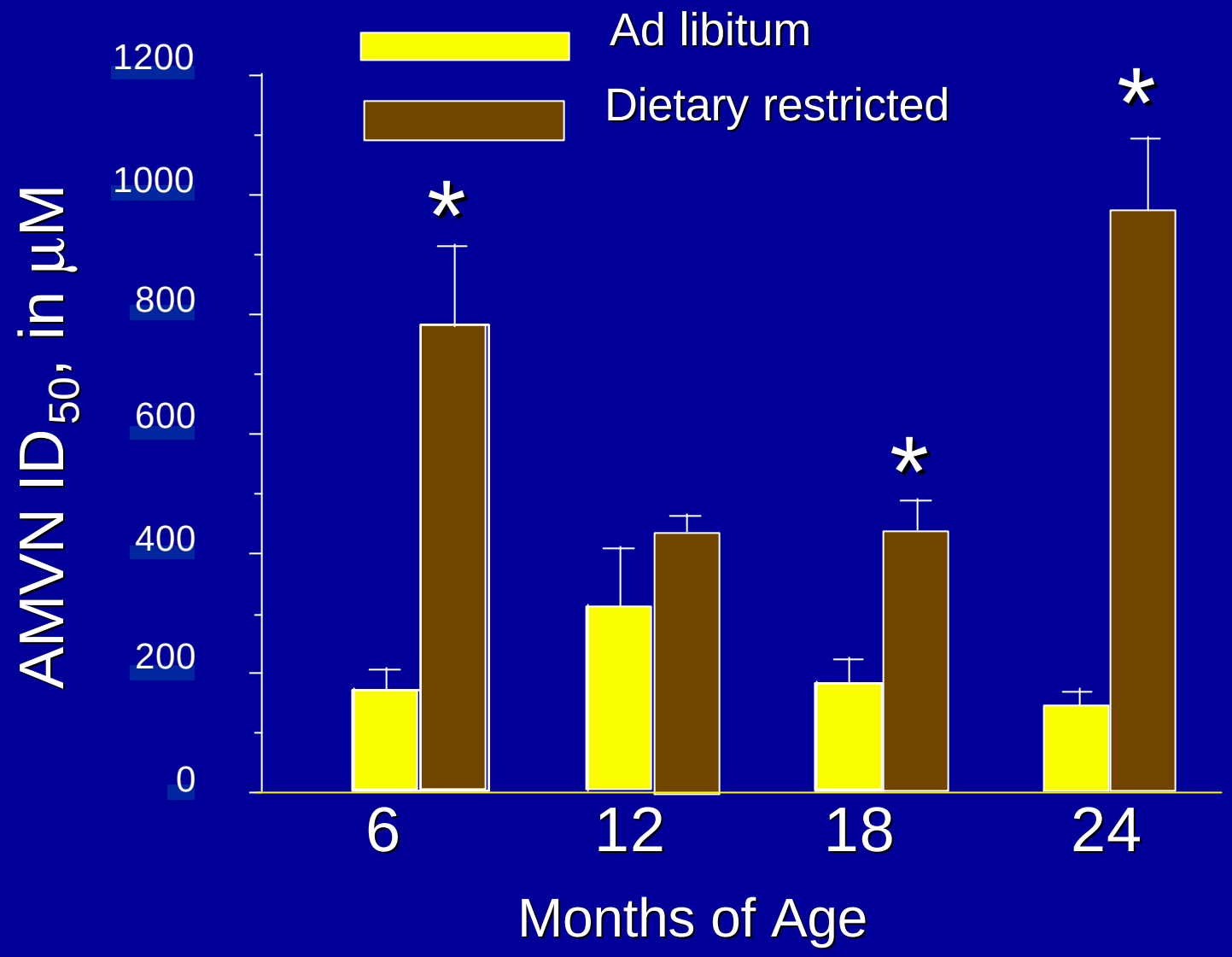


How many Redox Active Compounds?


Response (nA)
400
300
200
100
0
0.0
20.0
40.0
60.0
80.0
100.0
Retention time (minutes)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

Can We Modulate Defense Systems?

Resistance to AMVN-Mediated Inhibition of mtDNA Transcription



Future

Small Molecule Arrays

Proteomics

Gene Arrays

Transgenics/Knock-outs

Small Molecule Therapeutics

Animal Models for Disease