

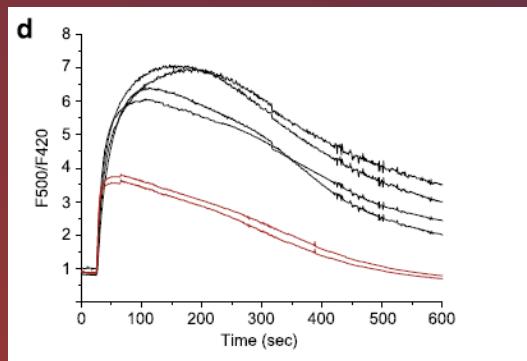
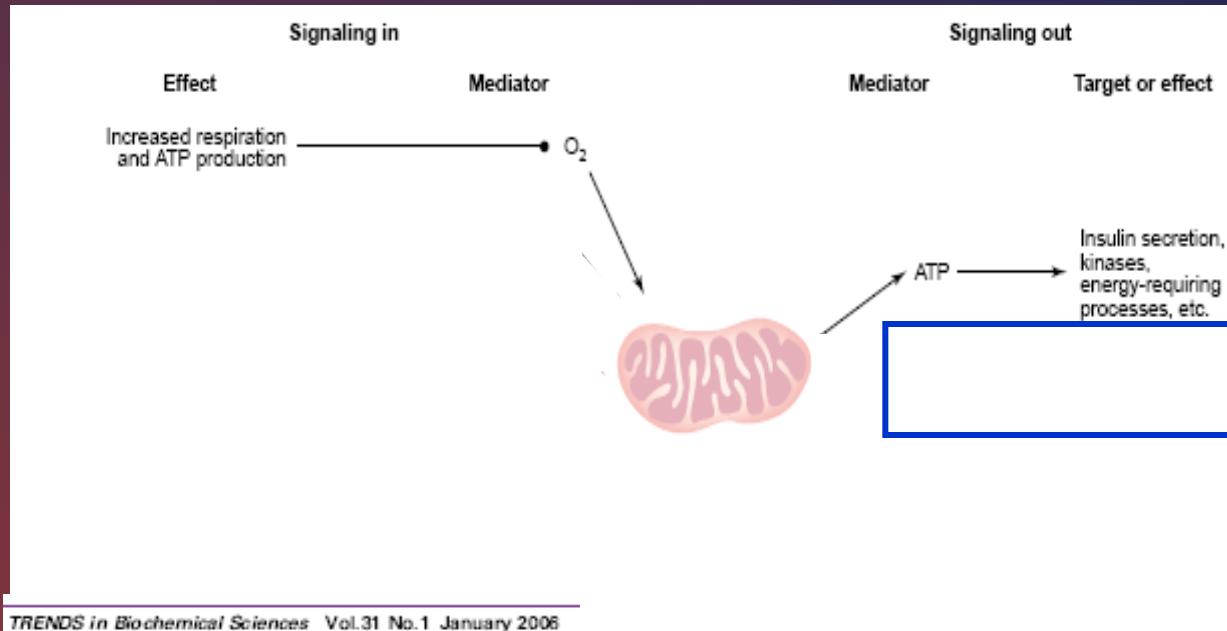
Nutrient Overload and Divergence in Adaptive Redox Responses between Heart and Skeletal Muscle

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Roles of Mitochondria in the Cell

No longer viewed as simply the 'engine of the cell'



$\uparrow 100 \mu M H_2O_2$

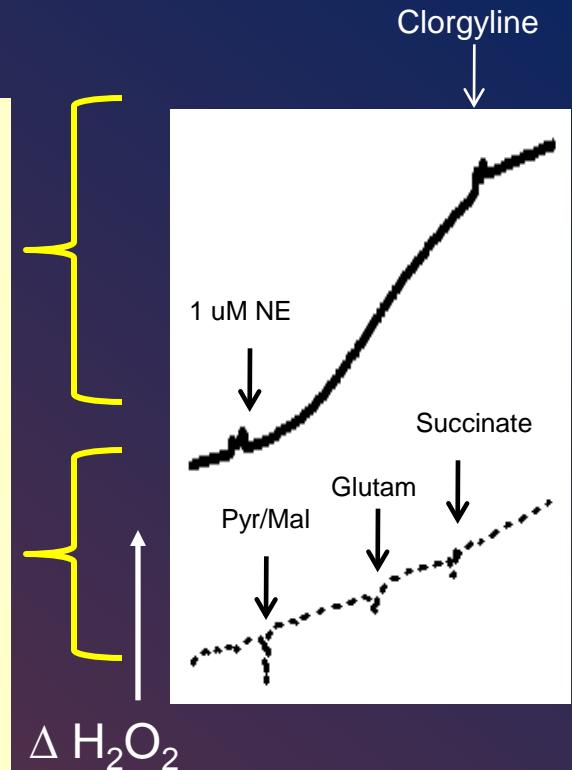
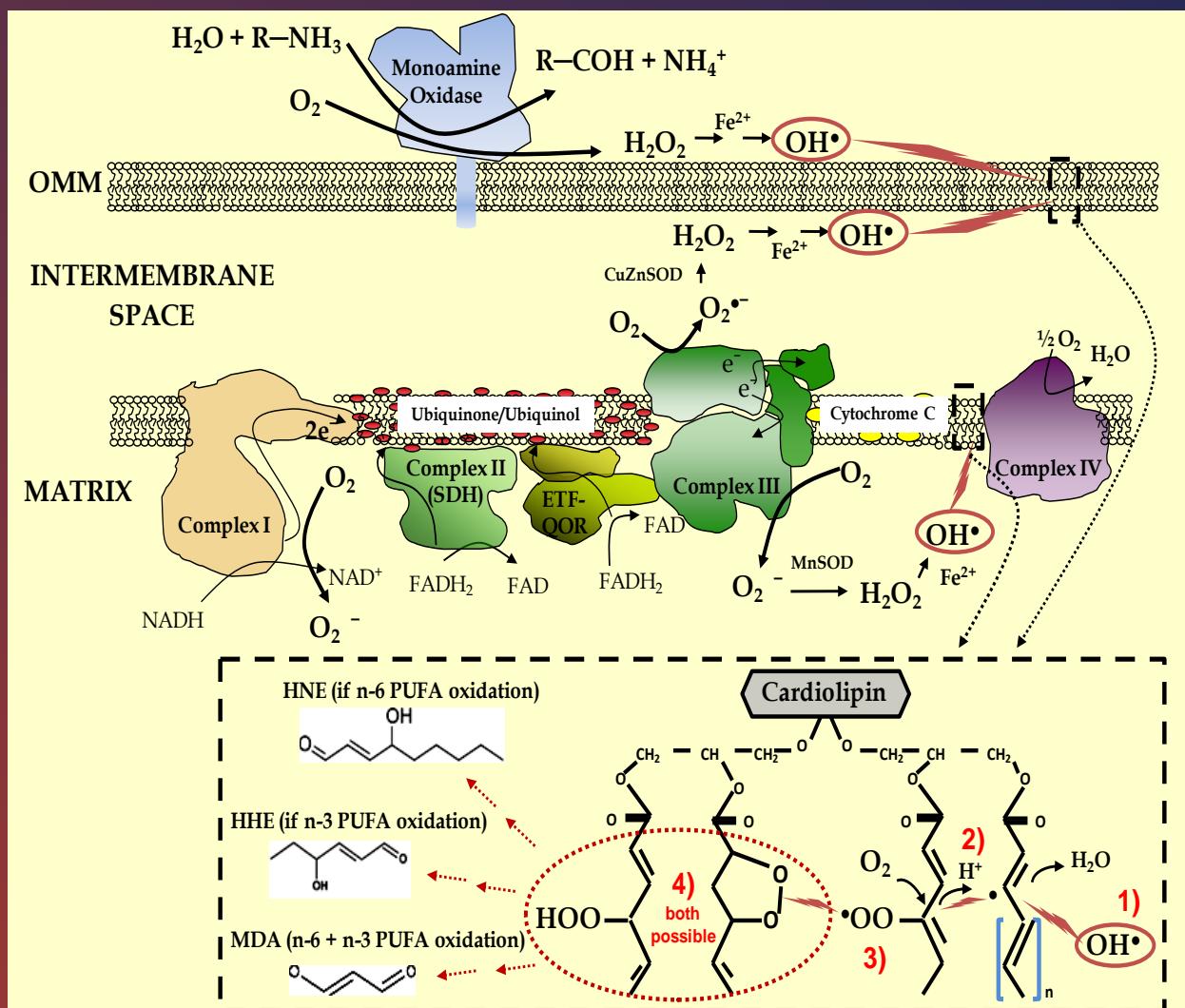
Physiological levels of ROS can only be transmitted via REDOX pathways

-S – S-
-SO₂[·],
-SO₃[·]

-Lipid-OOH
- Aldehydes

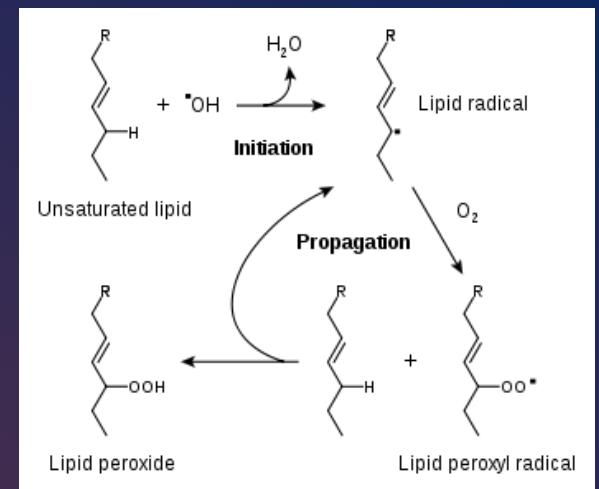
-Stable
electrophiles
(i.e. proteins)

Mitochondrial ROS and Lipid Peroxides (LOOH)



Mitochondria prepared
from Human Heart biopsy

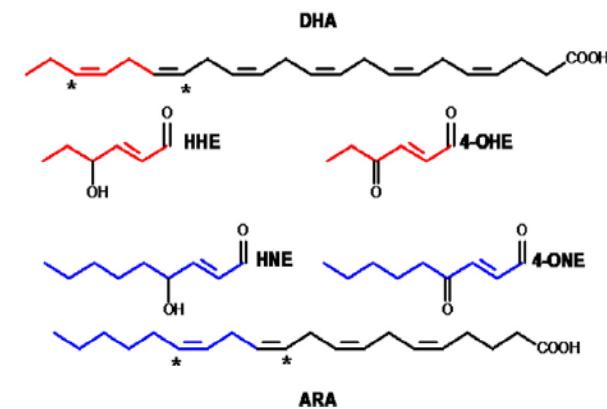
Lipid peroxidation and reactive aldehyde (HA E) formation



Reactive Aldehydes



Protein,
DNA
Adduct
formation



n-3 PUFA-derived

n-6 PUFA-derived

..... In
Skeletal Muscle

Consequences of Nutrient Overload

(i.e. High Fat, High Sucrose Diet)



Research article

Mitochondrial H₂O₂ emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans

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..... In Heart

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High Dietary Fat Selectively Increases Catalase Expression within Cardiac Mitochondria*

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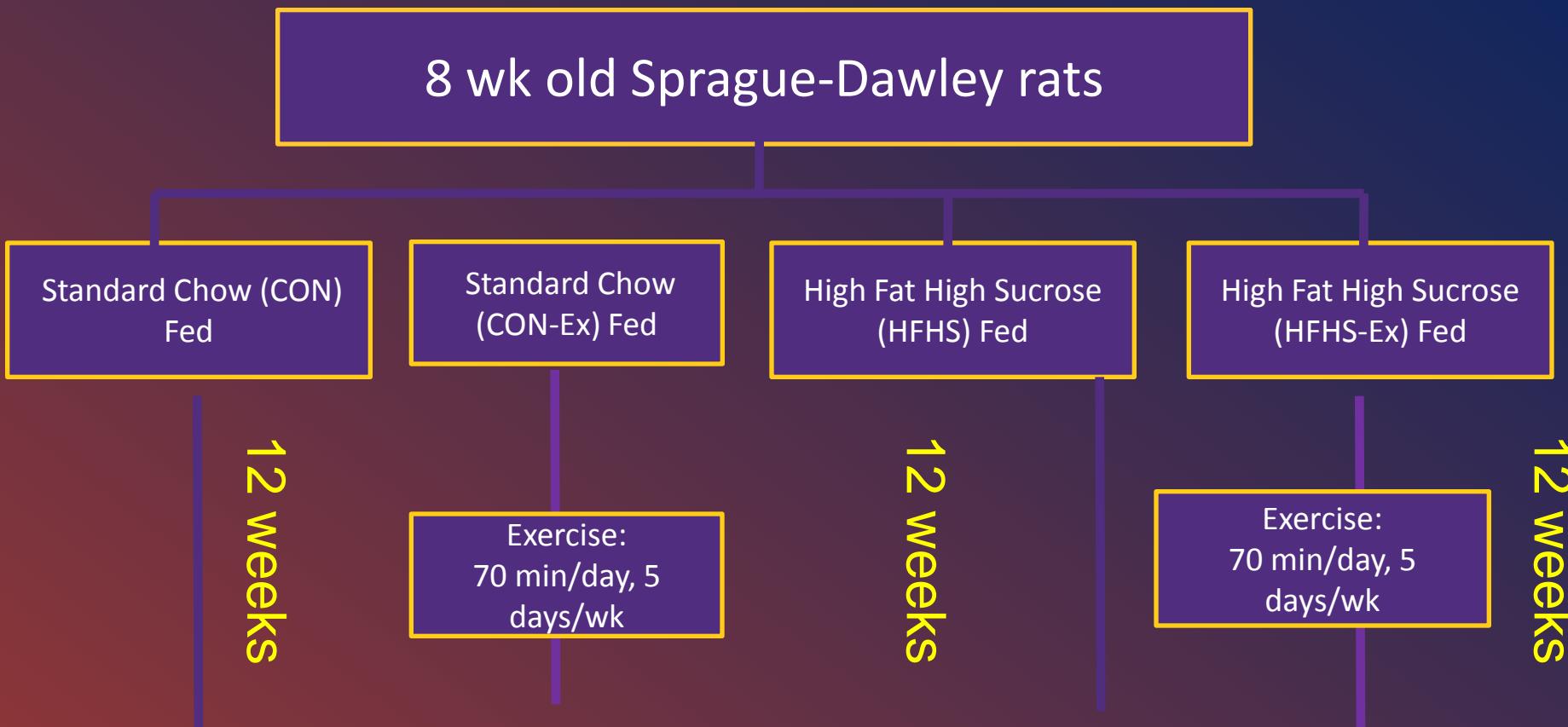
Question 1 What are the mechanisms underlying this disparity between Skeletal Muscle and Heart with nutrient overload??

Question 2: Increased FFA metabolism and oxidative stress occur with exercise (Ex) as well as HFHS diet, yet Ex is beneficial to 'cardiometabolic' health. Why is this?

A Hypothesis... differences in redox adaptations between Heart and SkM , particularly in mitochondria, explain these disparate responses

Hormetic effects of Exercise and Dietary Fats (especially PUFAs)
Known to be potent inducers of antioxidant enzymes and mitochondrial biogenesis

Experimental Model and Study Design:



Determine effects on Cardiac and Skeletal Muscle Mitochondria, focus is on Redox Adaptations

Diet Composition:

	<u>Ctl Diet</u> TD110367	<u>HFHS Diet</u> TD110365
Protein (% kcal)	15	14.8
Carbohydrate (% kcal)	72.4	40.7
Sucrose (g/kg)	150	340
Corn Starch (g/kg)	445	62
Fat (% kcal)	12.7	44.6
Anhydrous Milkfat	26	120
Soybean Oil	18	30
Safflower Oil	6	80
Total kcal/g	3.67	4.68
Fatty Acid Composition		
SFA (% total fatty acids)	40	39
MUFA (% total fatty acids)	26	24
PUFA (% total fatty acids)	33	37
C18:2 linoleic (% by wt.)	15.01	8.2
C18:3 linolenic (% by wt.)	1.57	0.3
n/6 to n/3 ratio	9.6	27

Metabolic Endpoints

	<u>Ctl Sed</u>	<u>Ctl + Ex</u>	<u>HFHS Sed</u>	<u>HFHS + Ex</u>
Terminal Body Wt. (g)	458 ± 8.0	424 ± 12.5	499 ± 11.1 **	456 ± 17.2
Heart Wt. (g)	1.14 ± 0.08	1.03 ± 0.13	1.12 ± 0.1	1.15 ± 0.11
Fat Mass (g)	52.0 ± 5.97	34.8 ± 7.48	76.9 ± 4.93 §	44.3 ± 8.48
Lean Mass (g)	341 ± 6.1	328 ± 7.8	356 ± 8.3	349 ± 10.3
Body Fat %	13.1 ± 1.30	9.3 ± 1.82	17.7 ± 0.98 §	10.9 ± 1.52
Glucose (mg/dl)	107 ± 3.5	99 ± 1.6	121 ± 3.5 §	109 ± 3.7
Insulin (pM)	125 ± 43.5	142 ± 28.1	174 ± 24.3	116 ± 23.4
HOMA-IR	4.94 ± 1.84	4.87 ± 0.95	7.34 ± 1.04	4.51 ± 1.09
Cholesterol (mg/dl)	39.0 ± 4.53	34.0 ± 2.59	30.2 ± 1.91	27.1 ± 2.14 *
Triglycerides (mg/dl)	58.0 ± 9.8	54.8 ± 12.2	45.3 ± 10.0	27.3 ± 6.1
Citrate Synthase-Heart	108.8 ± 11.62	119.6 ± 3.01	130 ± 3.29	120 ± 3.59
Citrate Synthase-Sk M	66.81 ± 2.28	76.41 ± 3.80	59.8 ± 3.55 **	69.86 ± 2.71

Mitochondrial experiments using permeabilized myofibers



Bundle of muscle fibers ~10 mg wet wt.

Trimmed to 2-mm wide x 5-mm long (12 mg wet wt)

Separate using fine forceps



Permeabilize with Saponin

Permeabilized Fiber Bundles

Mitochondrial Respiration



OROBOROS O₂K
Oxygraph

Mito-H₂O₂ emission



-Ex/ Em 570/ 585

Spectrofluorometer

Mito-Ca²⁺ uptake

Calcium Green 5N

-Ex/ Em 503/ 535

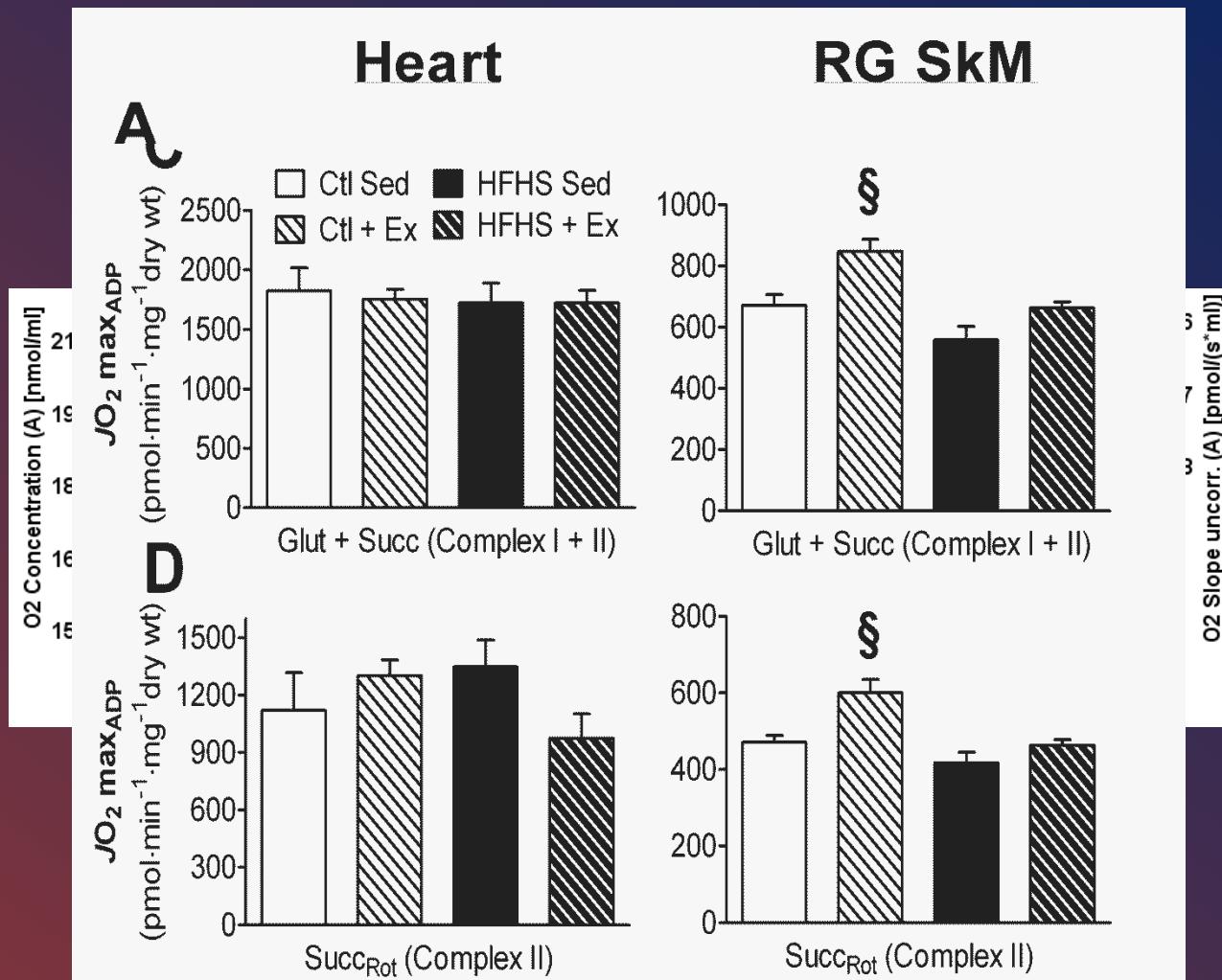
Mitochondrial O₂ capacity in Heart and SkM following HFHS diet and/ or Ex

ATP + Glucose

↓
Hexokinase

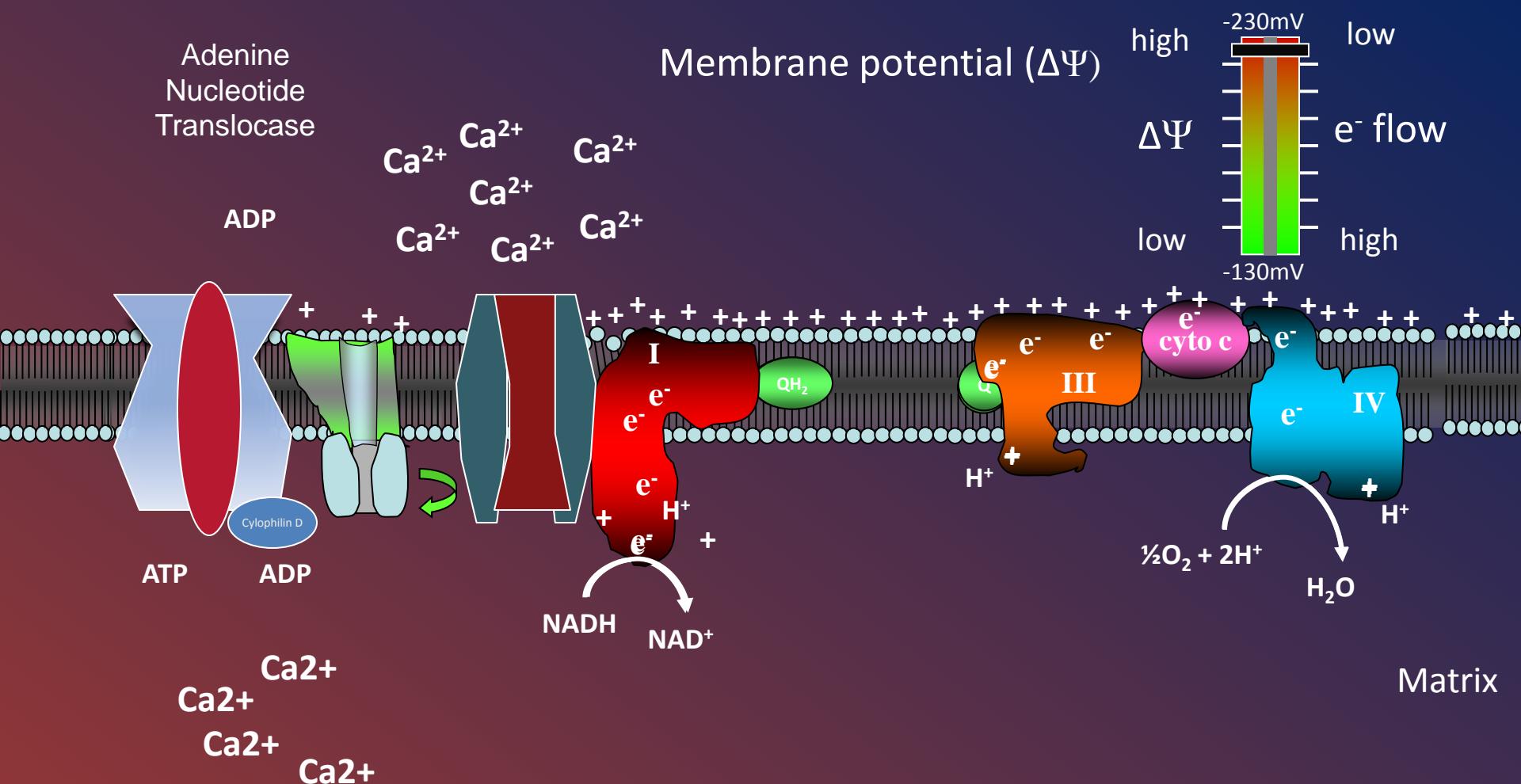
Glucose-6-phosphate

$\Delta G_{ATP} < 0$



Mitochondria maintained in a permanent phosphorylating state – OxPhos constantly maximal

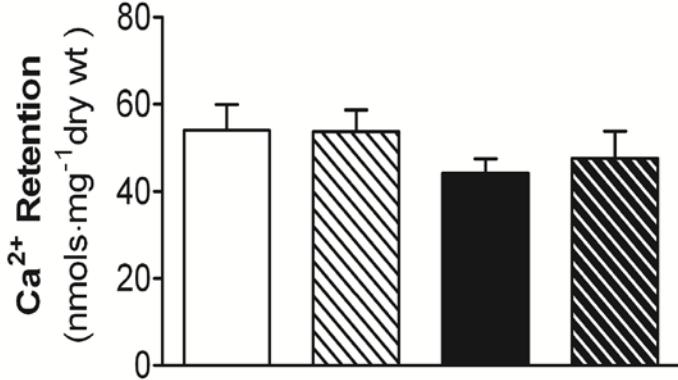
Mitochondrial Ca²⁺ overload and PTP



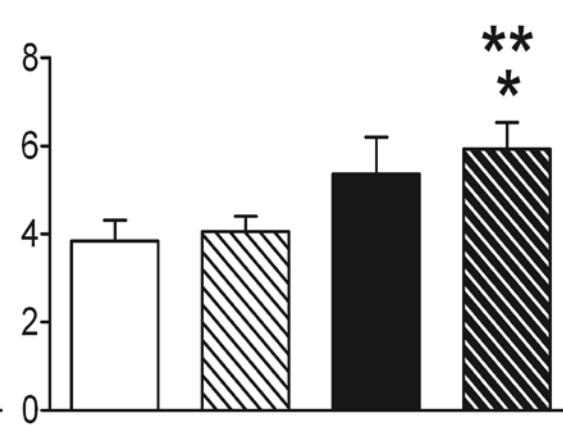
When exposed to elevated levels of cytosolic Ca^{2+} , mitochondria take up the ion due to electrical gradient $\Delta\psi$

Mitochondrial Ca^{2+} overload and PTP

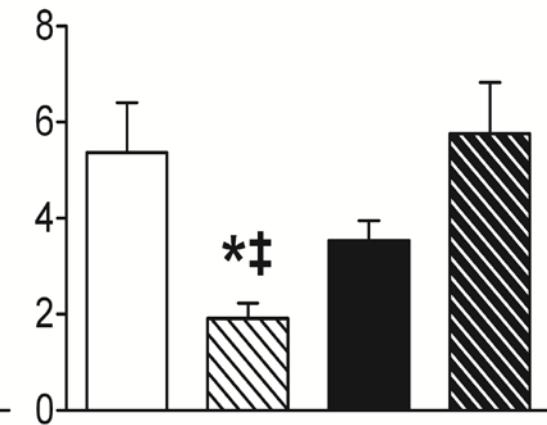
Heart



RG SkM

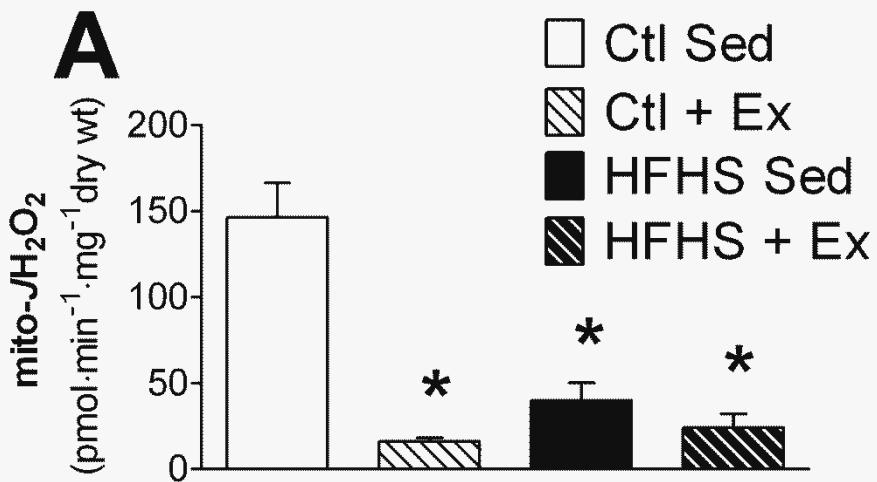


WG SkM

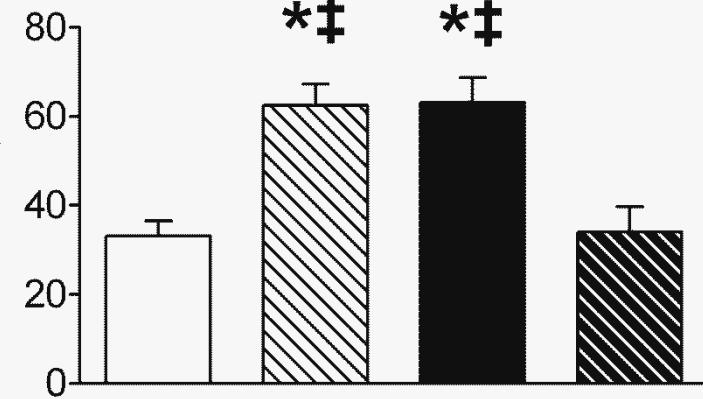


Mitochondrial ROS following HFHS diet and/ or Ex

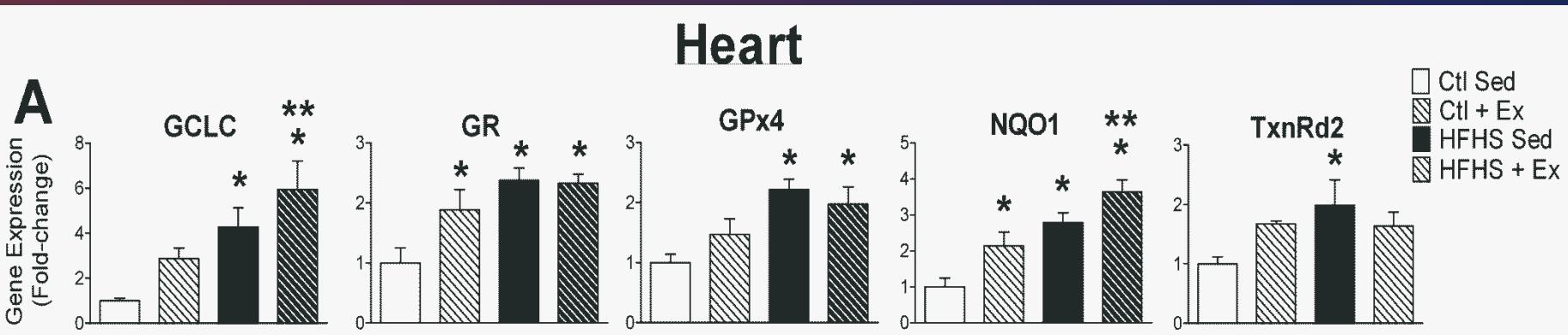
Heart



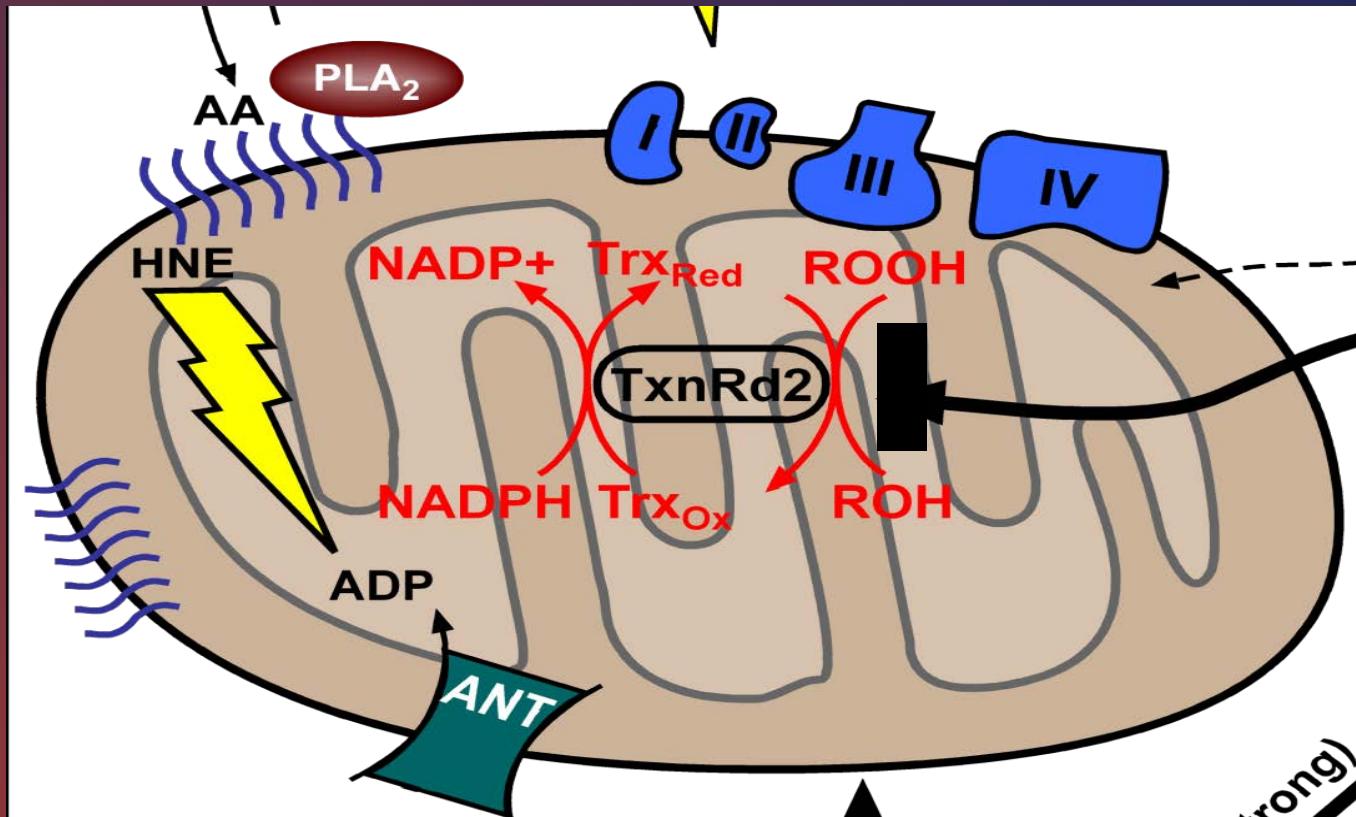
RG SkM



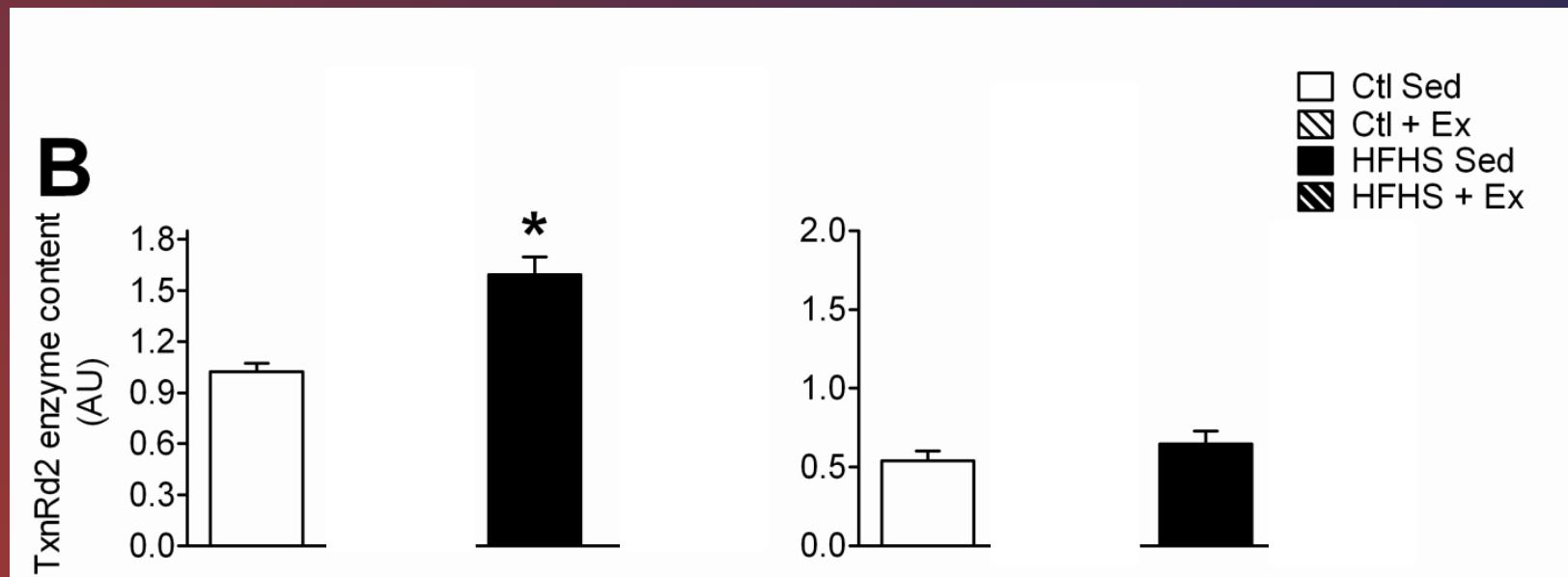
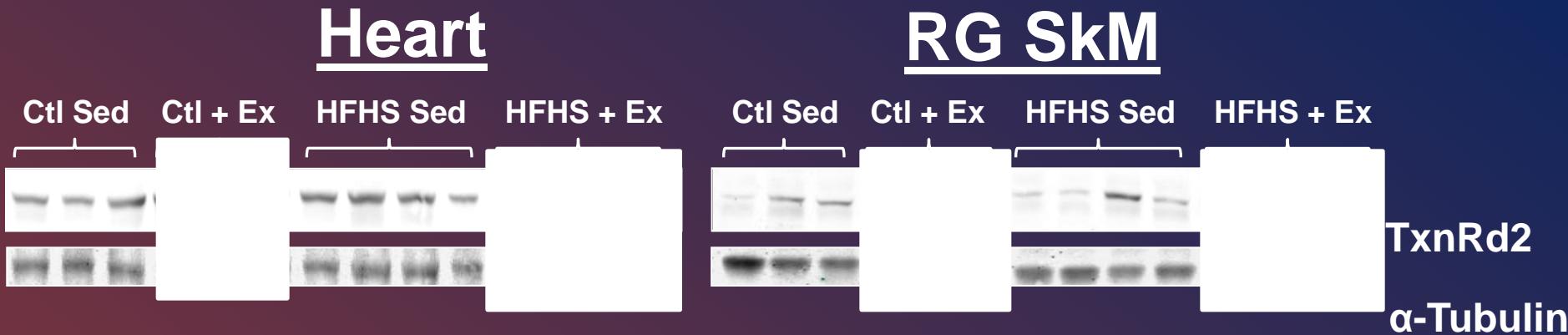
Redox Signaling in Heart and SkM following HFHS diet and/ or Ex



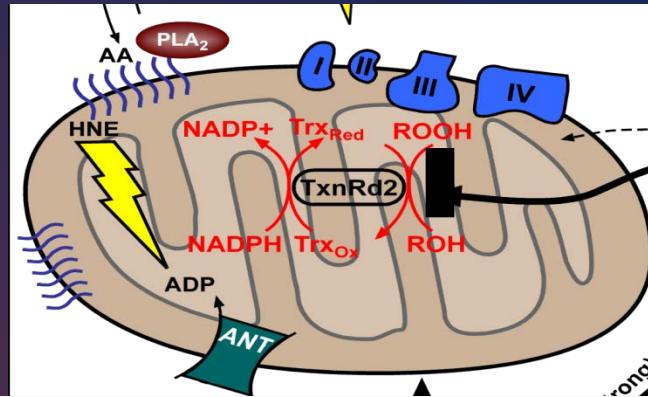
Mitochondrial Redox state and Thioredoxin Reductase-2 (TxnRd2)



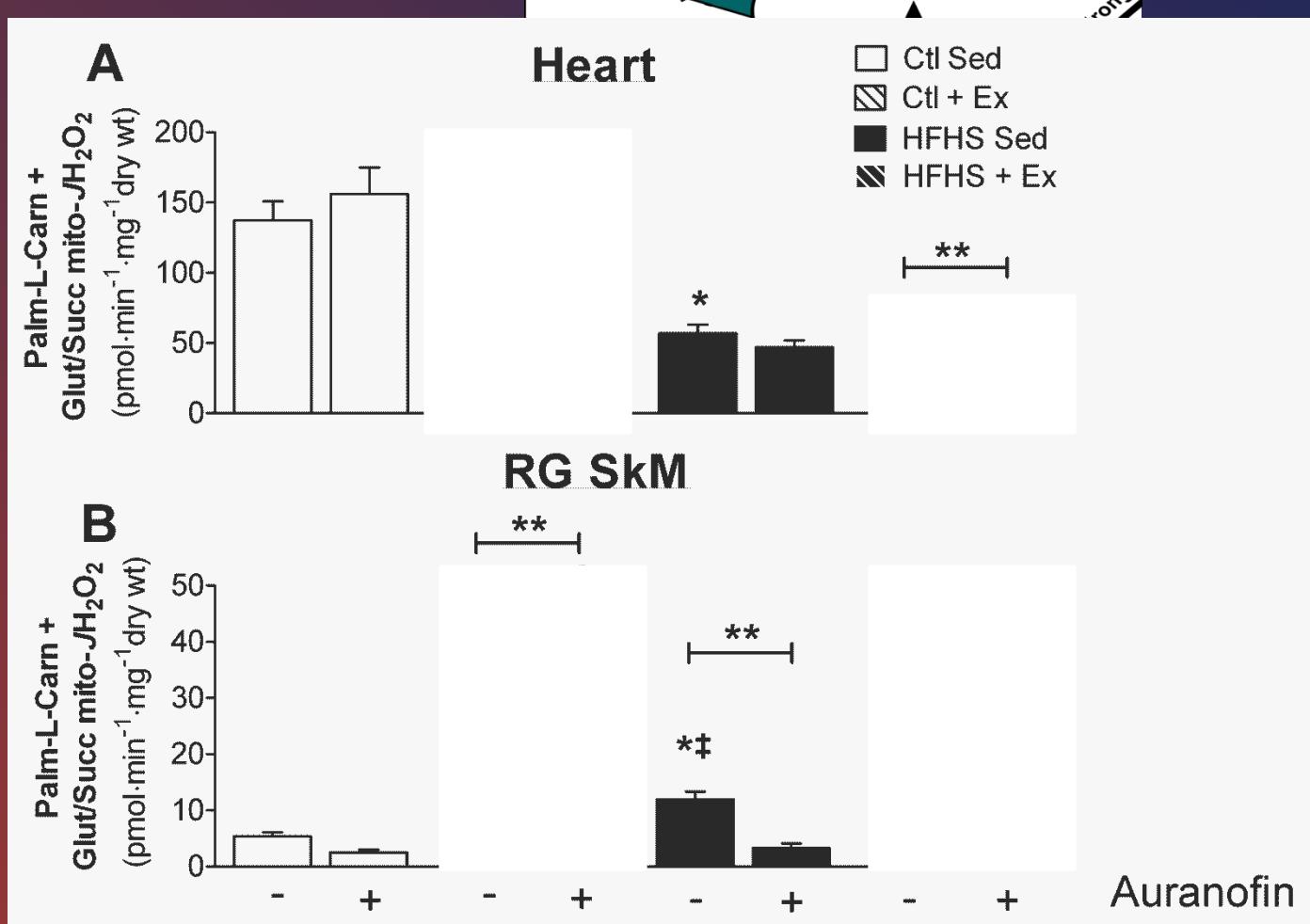
Thioredoxin Reductase-2: A mitochondrial specific antioxidant enzyme



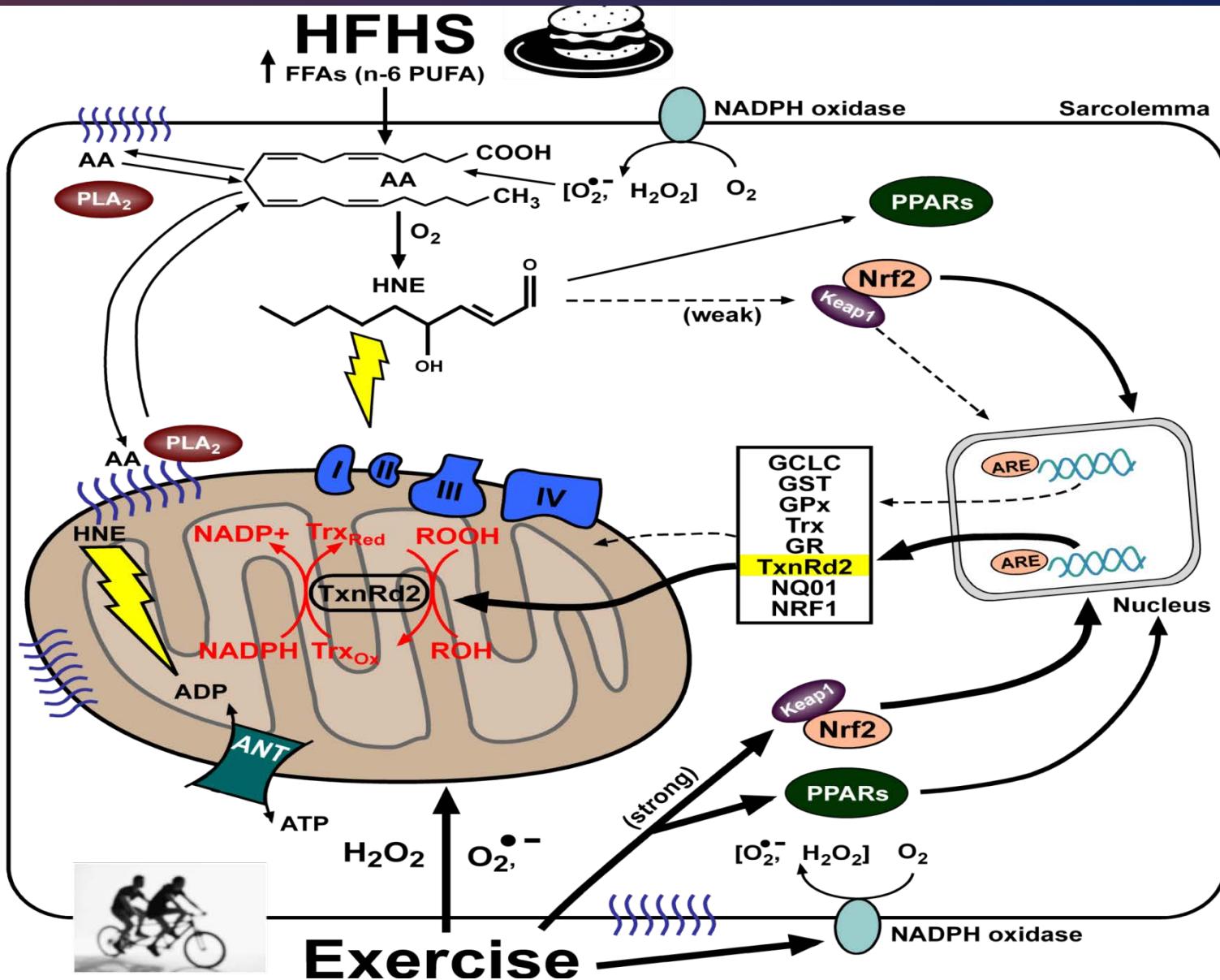
Mitochondrial Redox state and TxnRd2



Auranofin



What might be happening here?



Thank you to....

- Justin La Favor
- Kelsey Fisher-Wellman

My Lab



Kathleen Thayne
- Research Specialist



Timothy Darden
-grad student



Taylor Mattox
-grad student

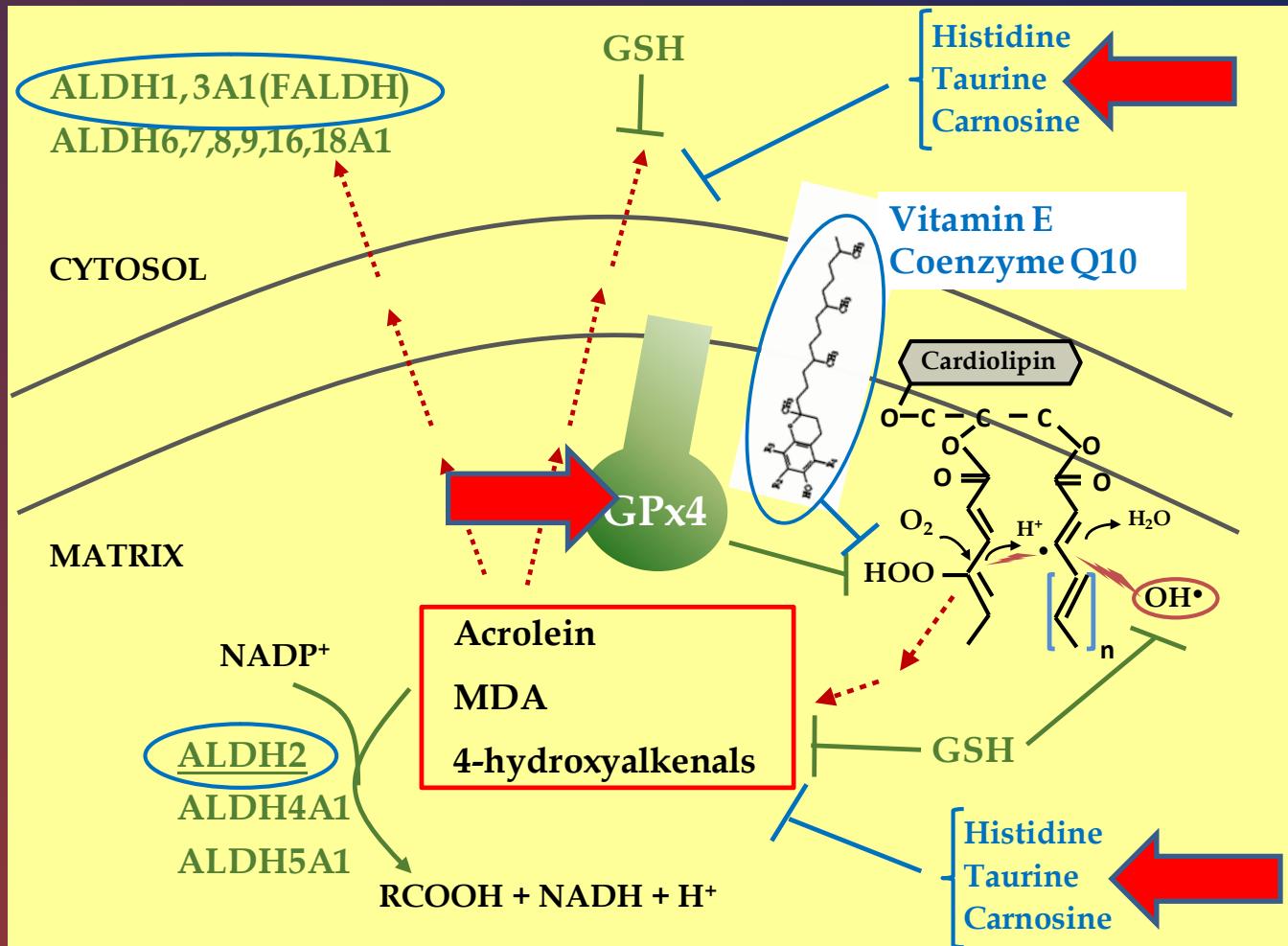


Lalage Katunga
-grad student

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Enzymatic and Non-enzymatic removal of Lipid-OOH and aldehydes in Mitochondria



Anderson, Katunga & Willis (2011) Clin Exp Pharmacol Physiol