

Protective effects of palm fruit juice (PFJ) against mitochondrial DNA damage caused by drug toxic effects or disease.

Adam E. Osborne

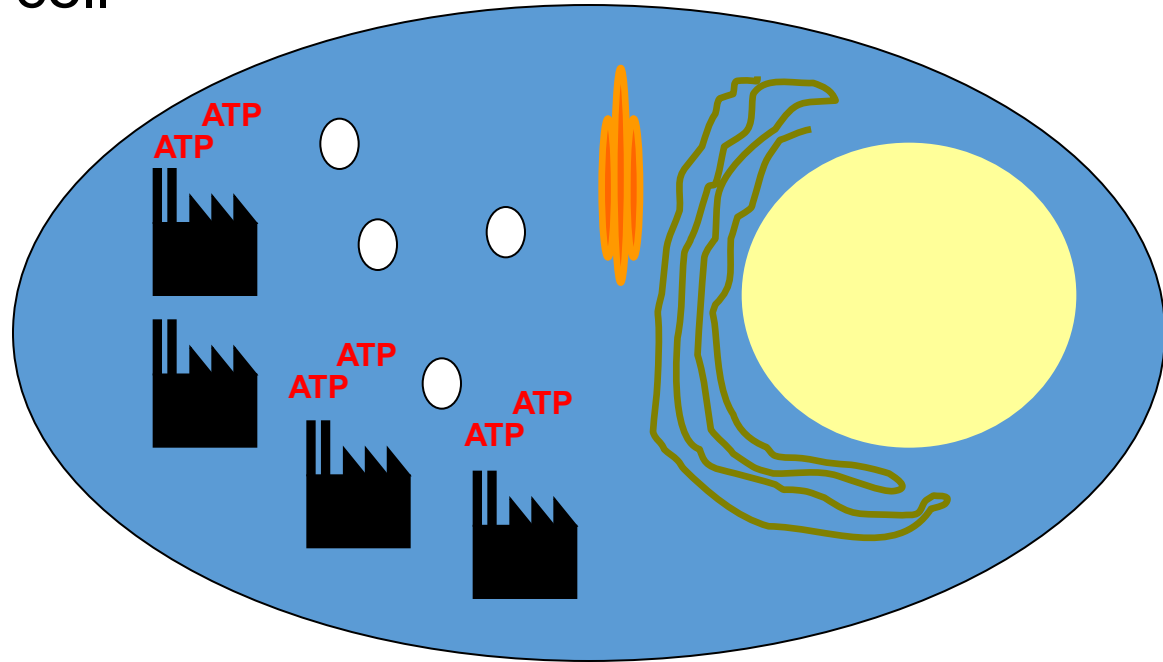
J. Aquiles Sanchez, K.C. Hayes, and Lawrence J. Wangh
Brandeis University

August 4, 2015

Palm International Nutra-Cosmeceutical Conference

Mitochondria

- “Power Houses” of the cell
- Also regulates:
 - Programmed cell death
 - Insulin release
- Multiple mitochondria per cell
- Multiple genomes per mitochondria

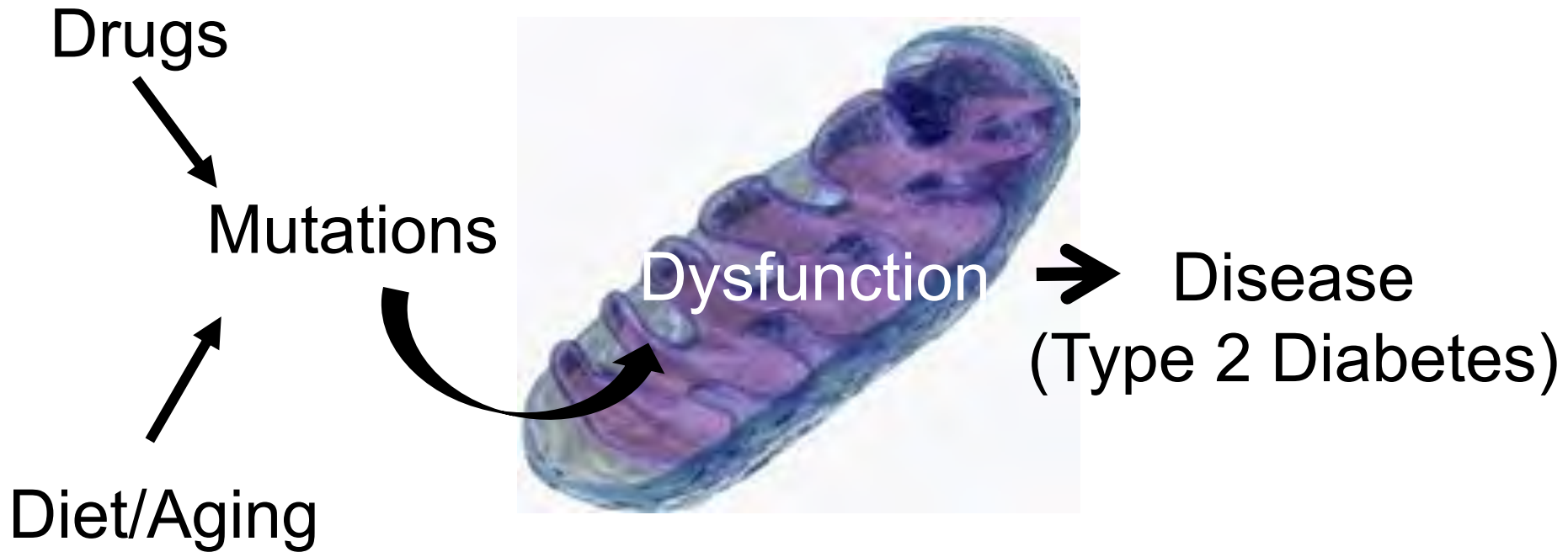


Mitochondrial Dysfunction and Disease

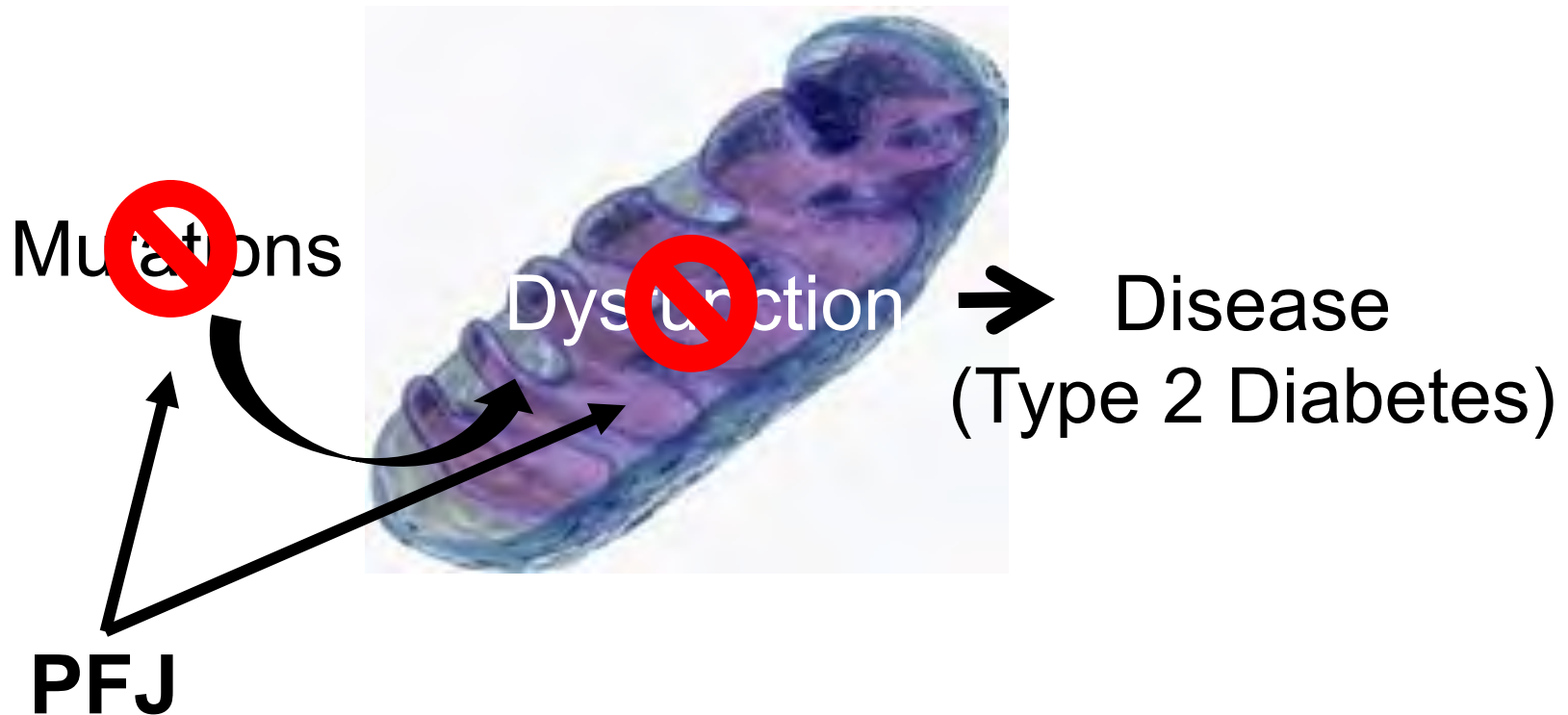


Dysfunction → Disease

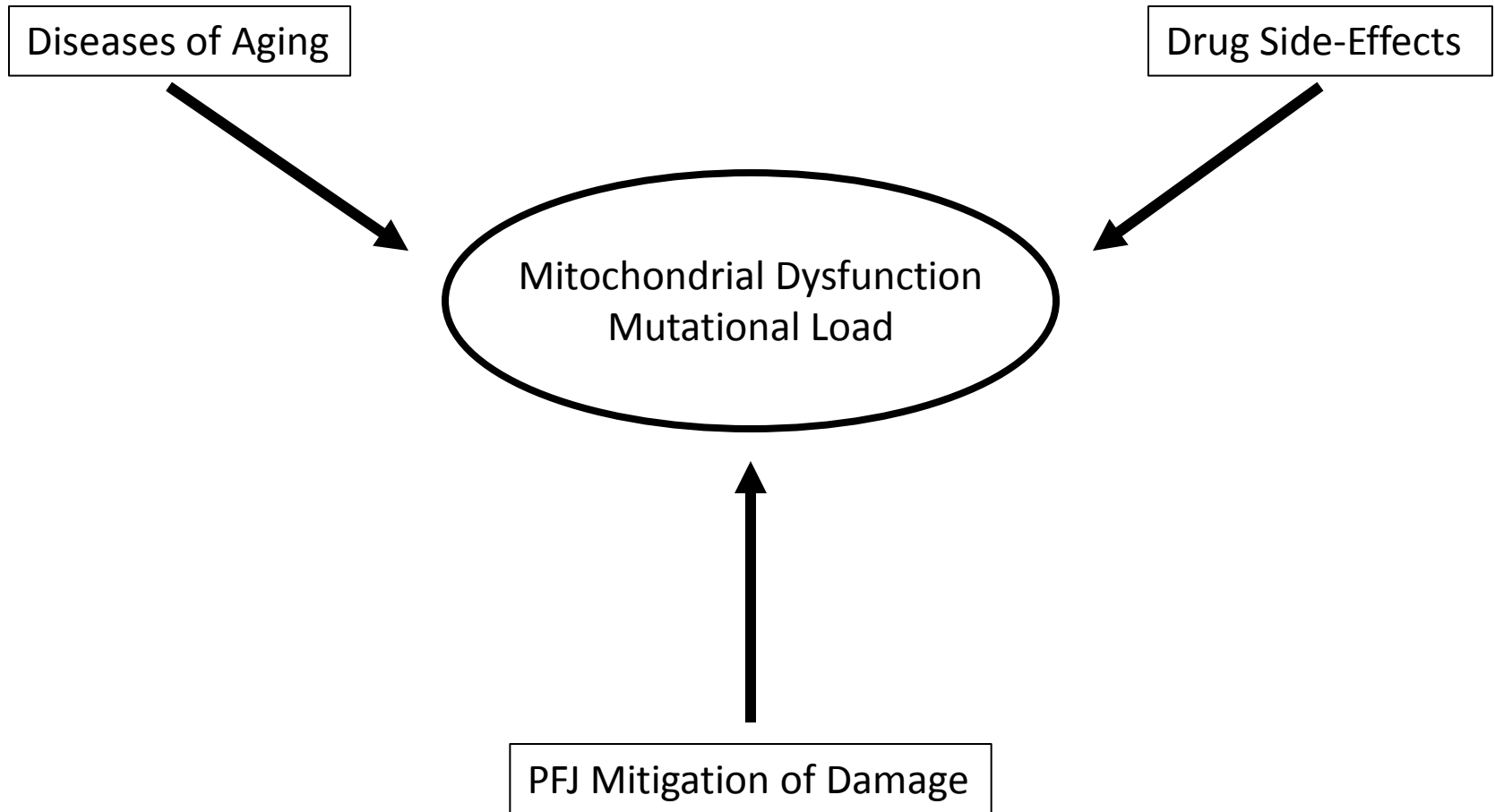
Mitochondrial Dysfunction and Disease



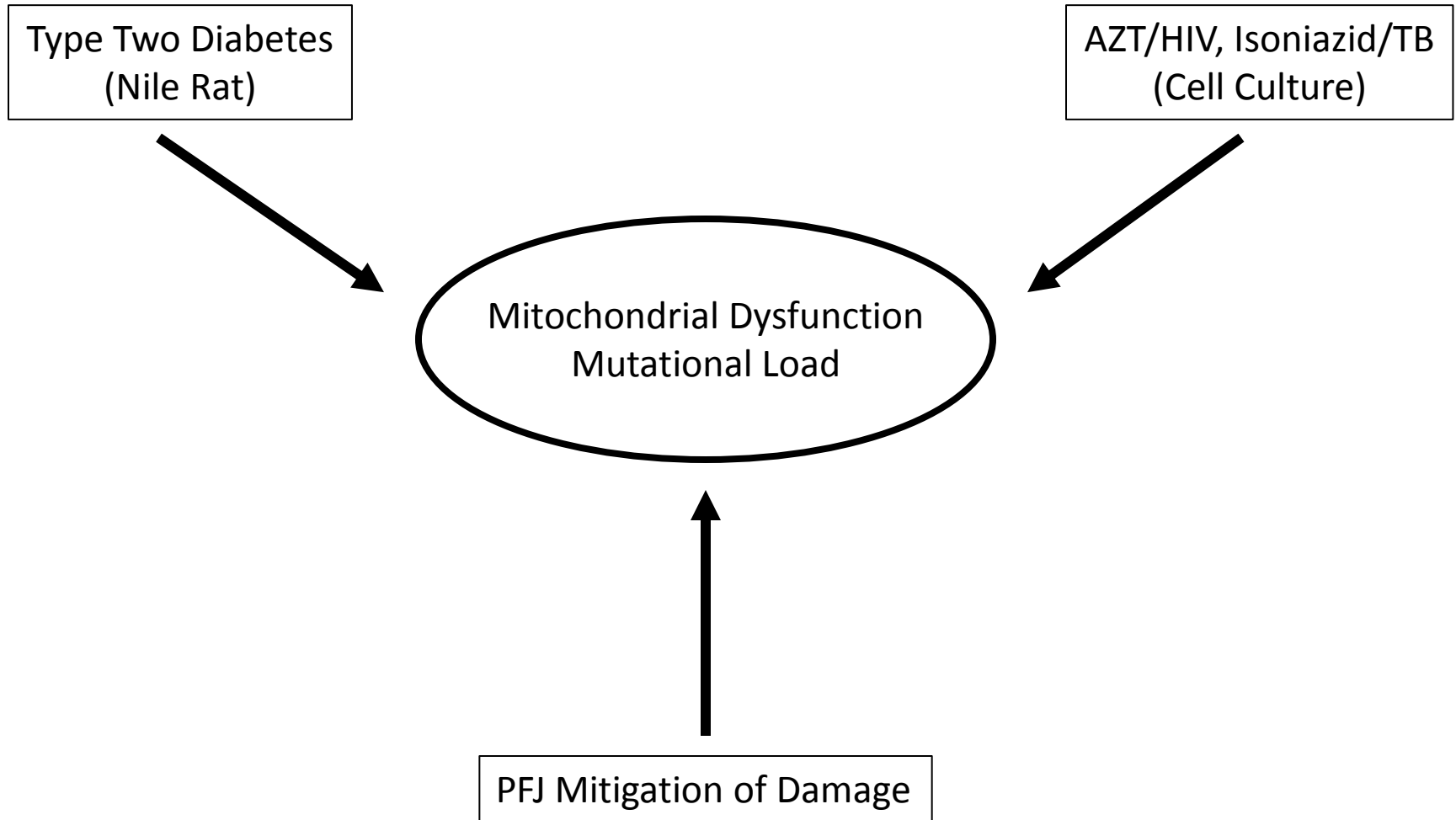
Mitochondrial Dysfunction and Disease



Two Approaches to Mitochondrial Damage and Mitigation



Two Approaches to Mitochondrial Damage and Mitigation



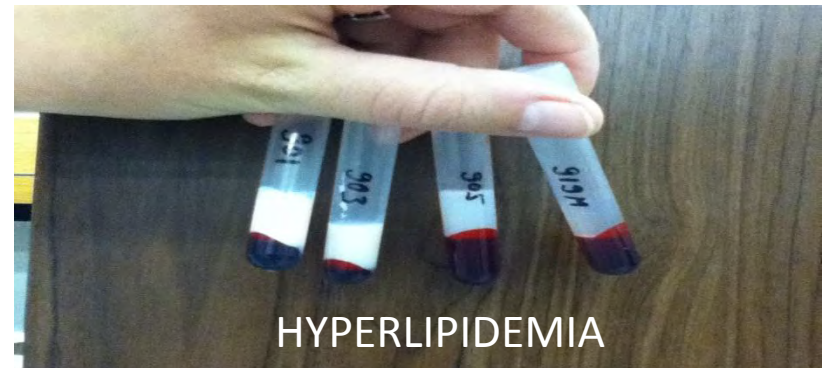
Mitochondrial Mutational Load in Nile Rat Type 2 Diabetes



The Nile grass rat (*Arvicanthis niloticus*)

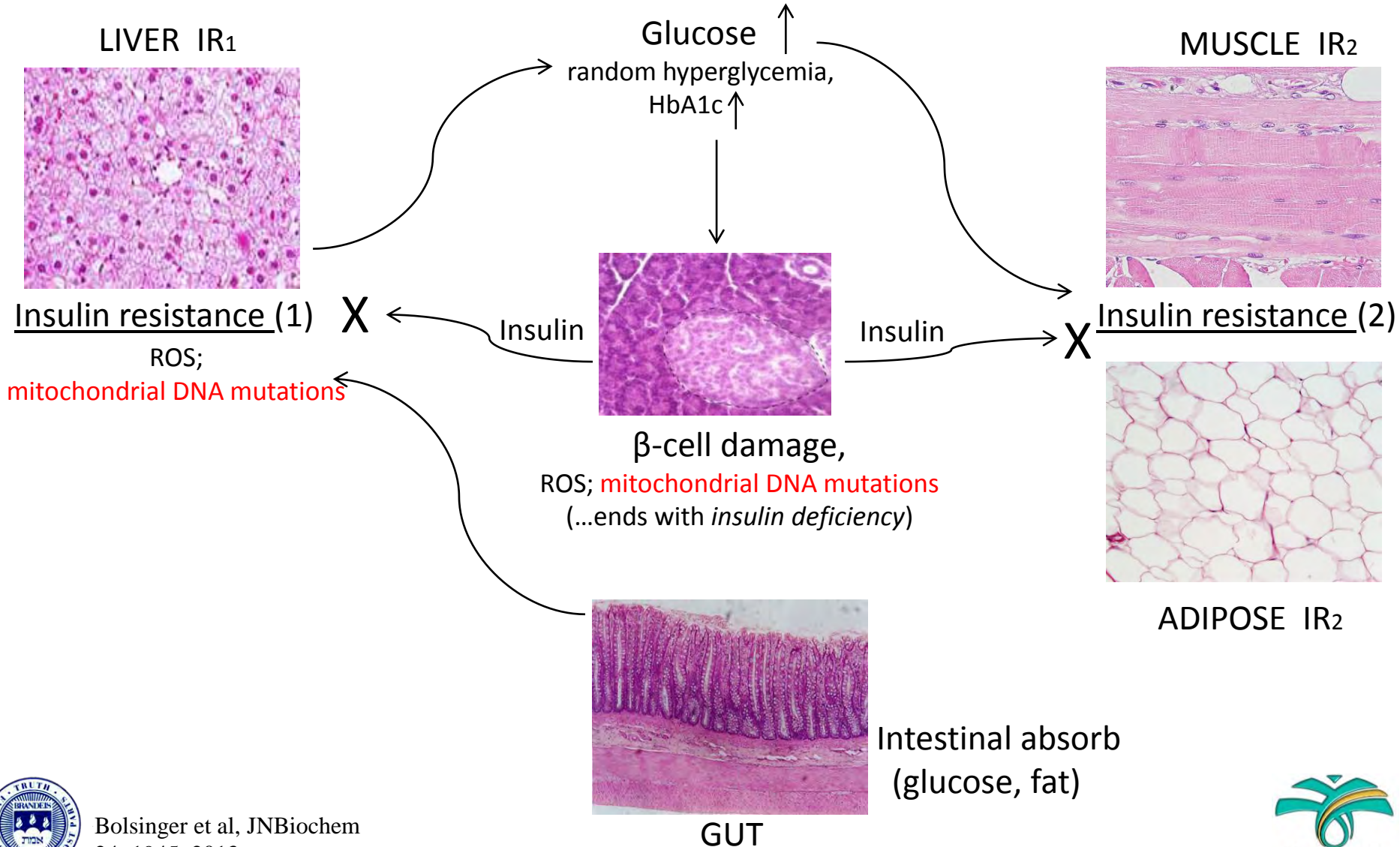


- Natural habitat: sub-Saharan Africa
- Original stock used in research for circadian studies
- But.. extremely susceptible to **T2DM** and **Metabolic Syndrome** when fed lab chow!

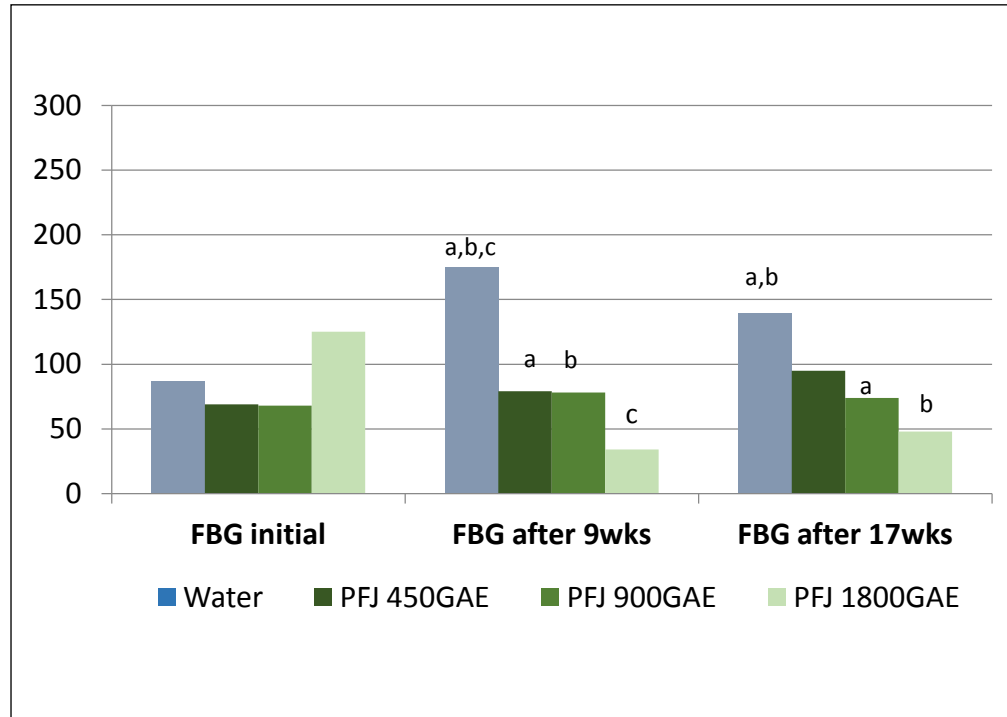


Hypothesis: Pathogenesis of type 2 diabetes

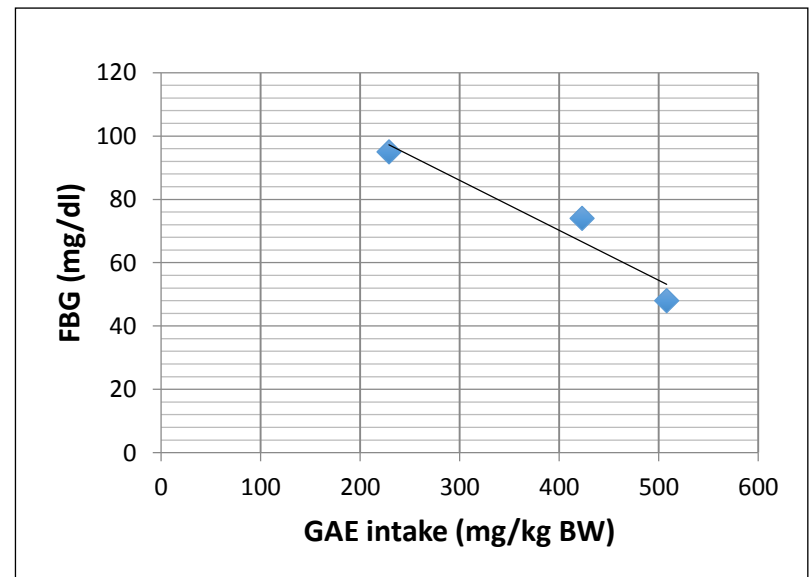
(it all starts with *insulin resistance* caused by diet)



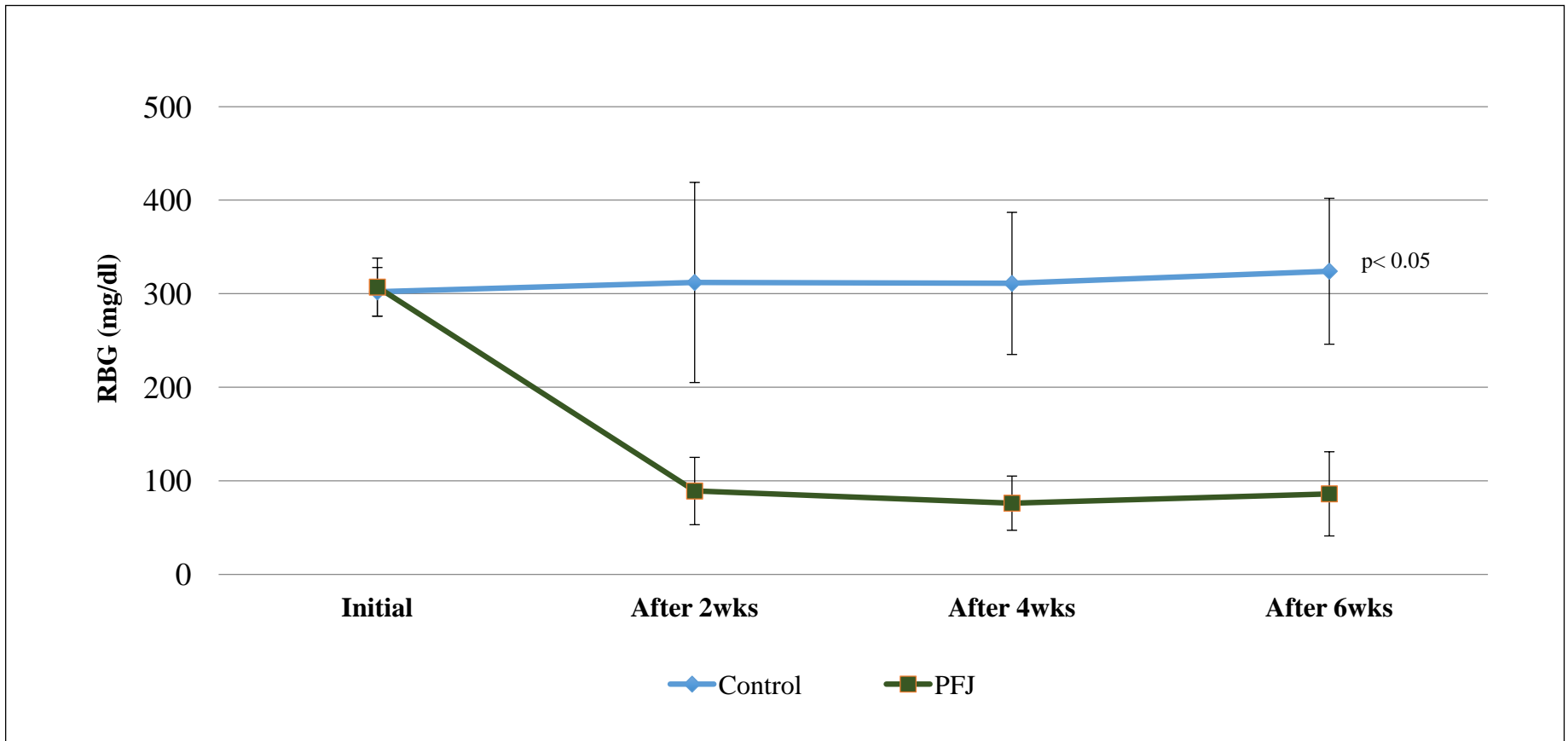
Fasting blood glucose in Nile rats after 9wks and 17wks of graded amts PFJ (0, 450, 900, 1800 GAE of OPP) (NR St. 11)



Correlation between fasting blood glucose and GAE intake of OPP, $r = -0.96$

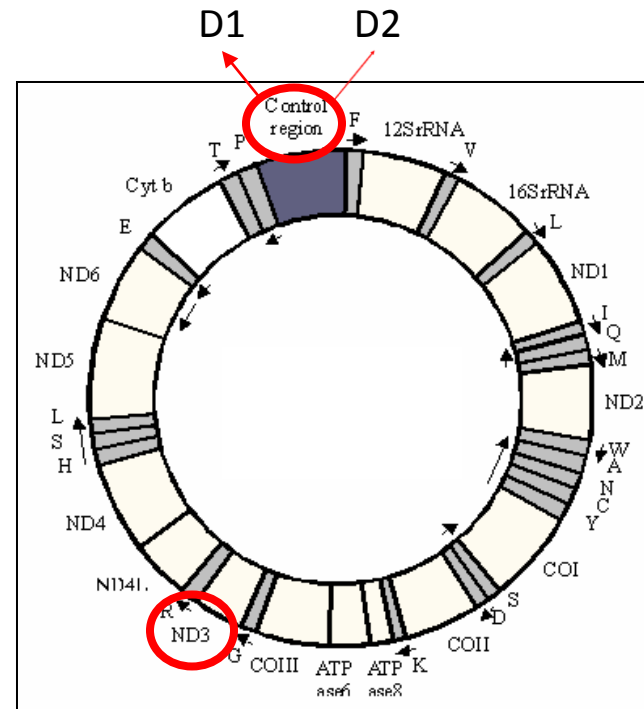


PFJ (GAE intake 319mg/ kg BW) mixed into a **modCHO** diet decreased random blood glucose in diabetic Nile (NR St. 40)



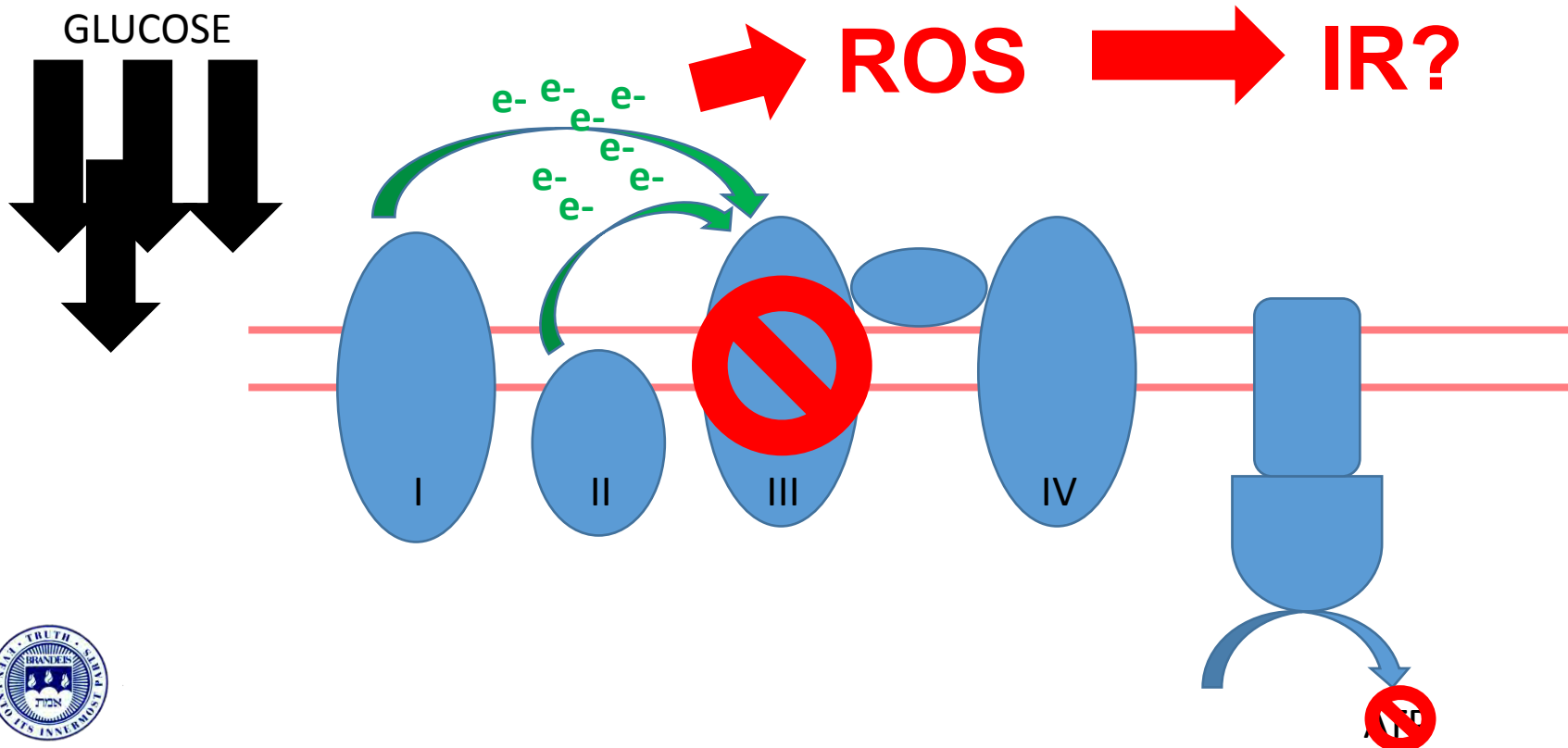
The Nile Rat Assay

- Have sequenced entire mitochondrial genome
- Chose D-Loop and ND3 for assay
- Constructed an assay of three separate monoplexes

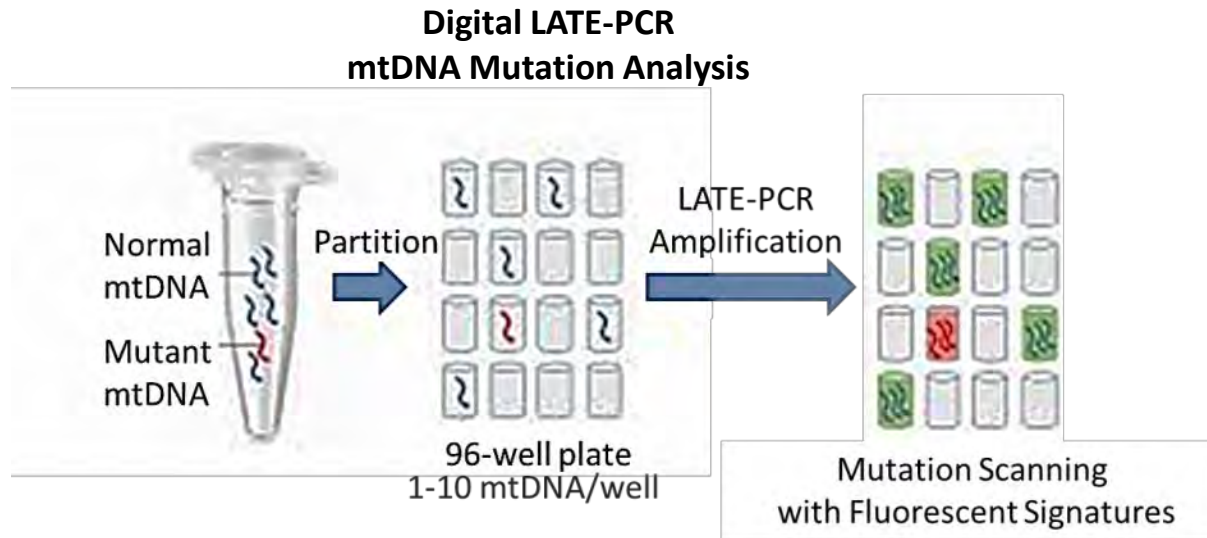


The Nile Rat Assay: Why Liver Mitochondrial DNA?

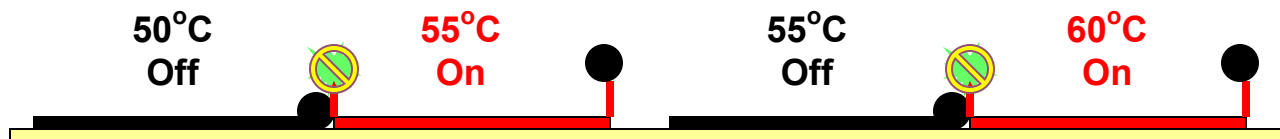
- Integral in glucose homeostasis
- Insulin resistance found early in liver



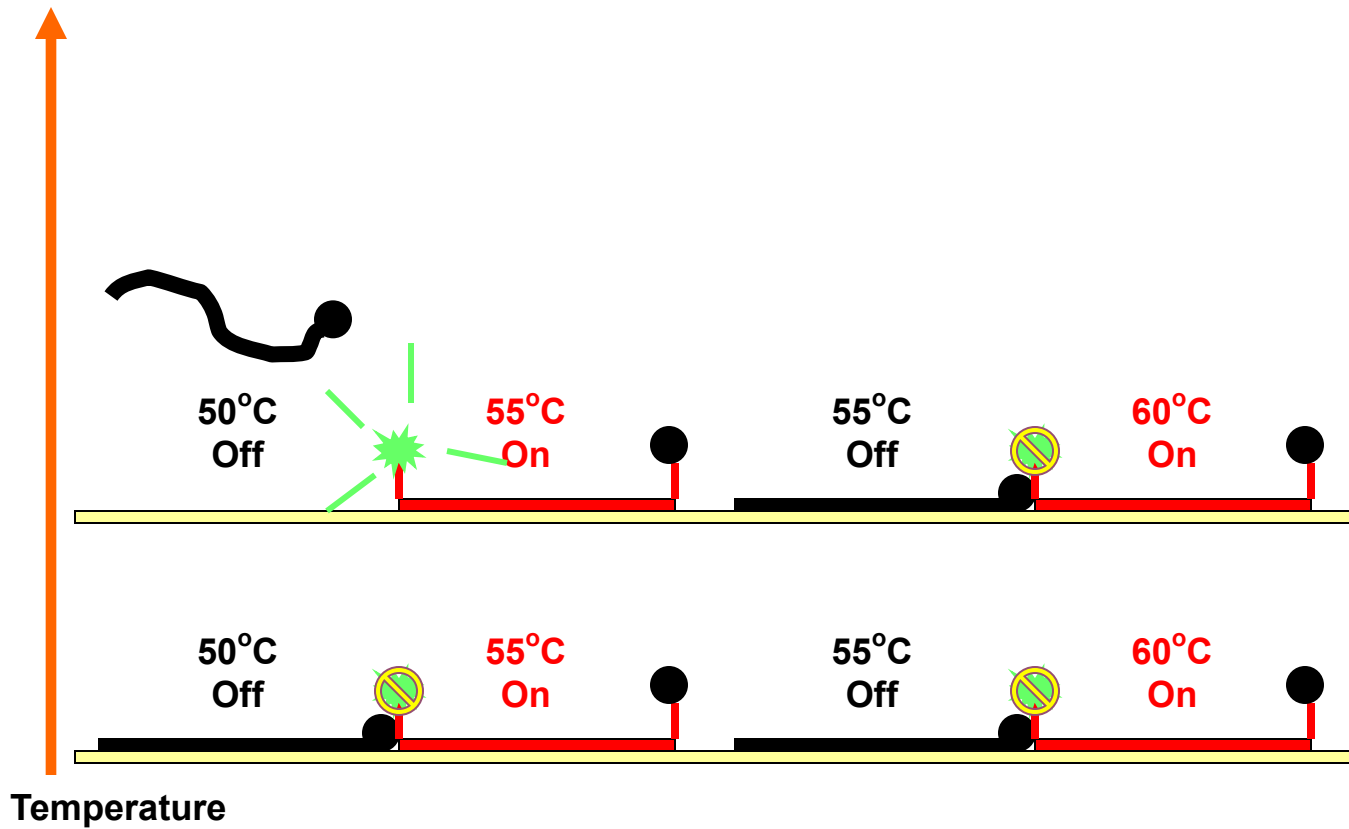
Strategy for Detection of Random Mutations in Mitochondrial DNA



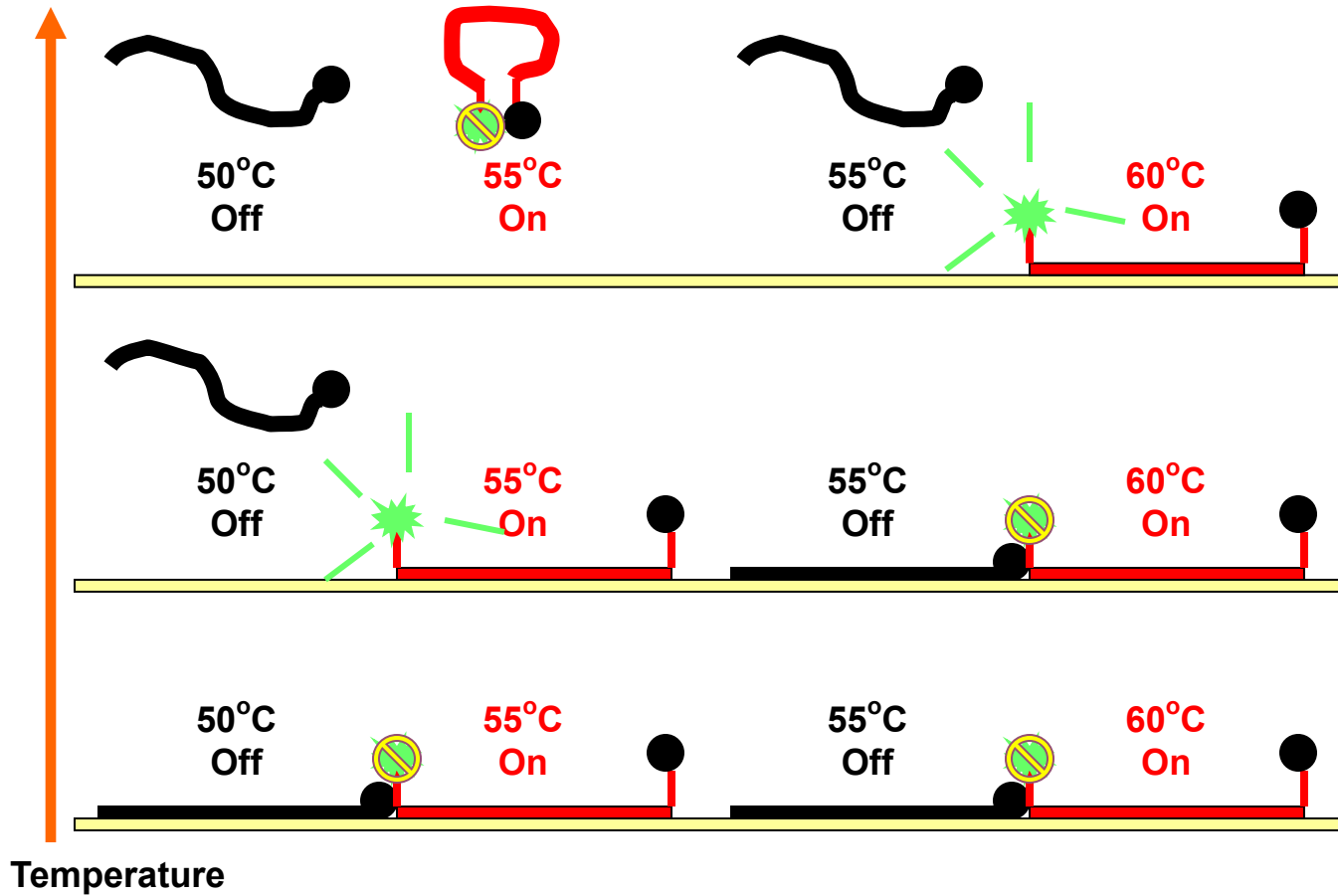
Lights-On/Lights-Off Probes



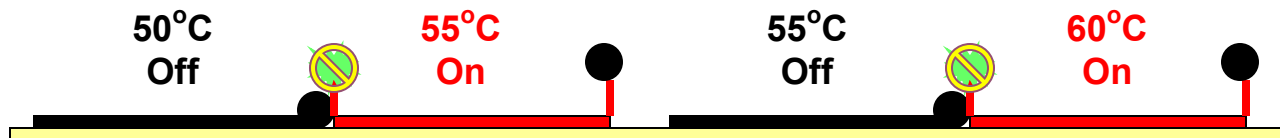
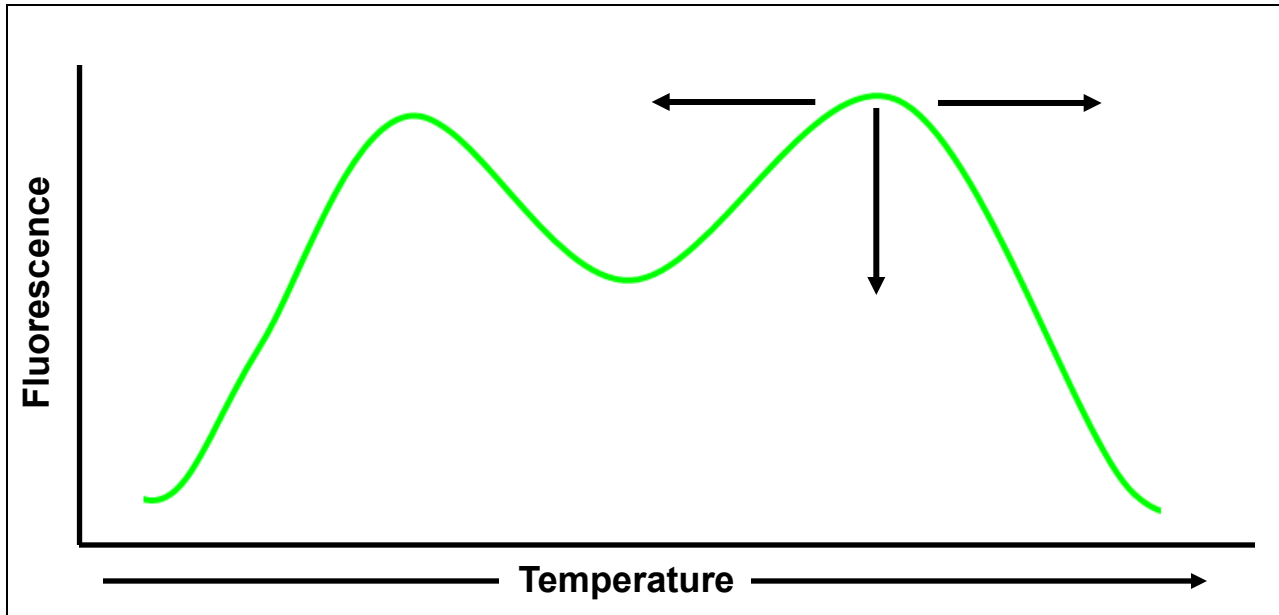
Lights-On/Lights-Off Probes



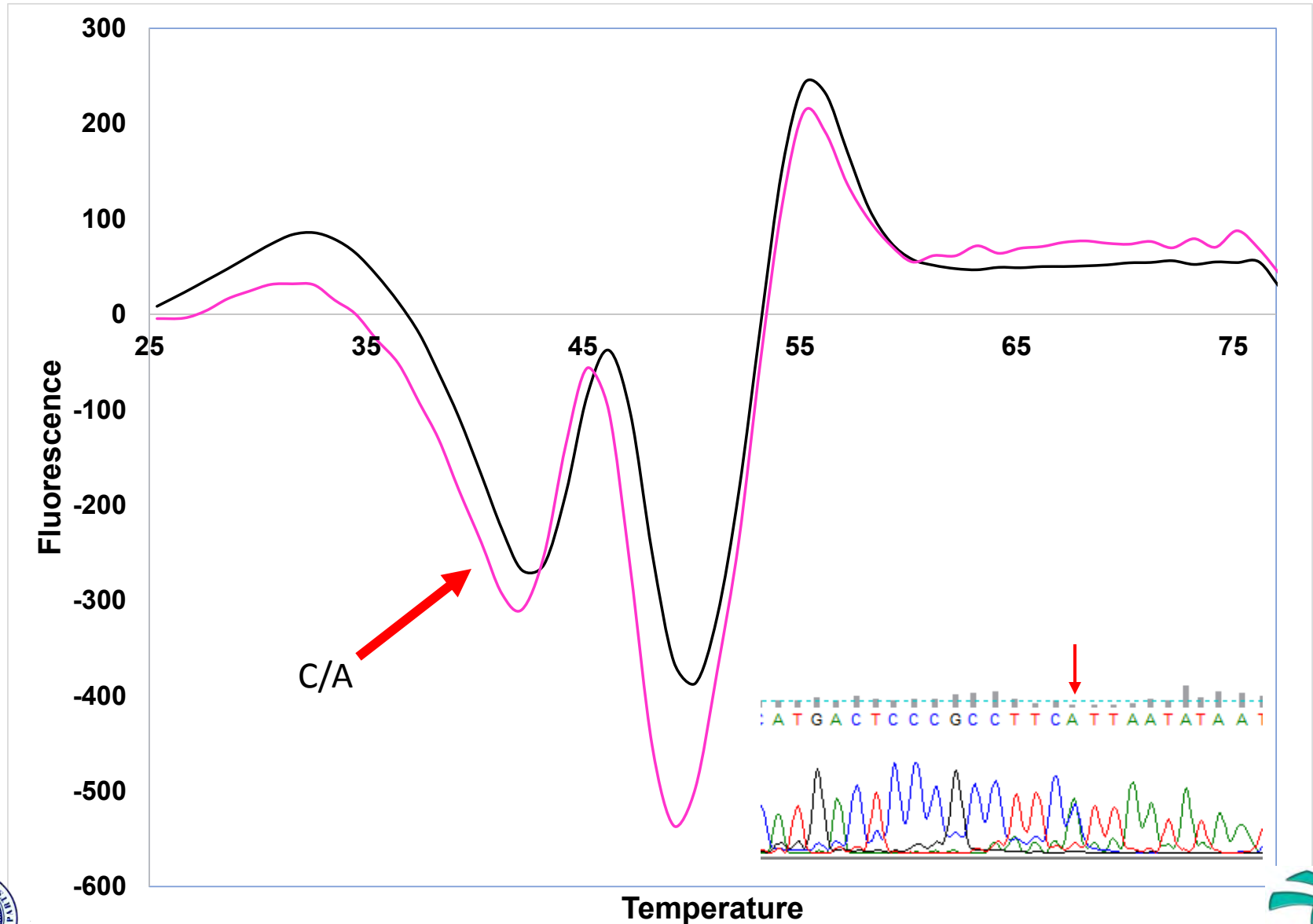
Lights-On/Lights-Off Probes



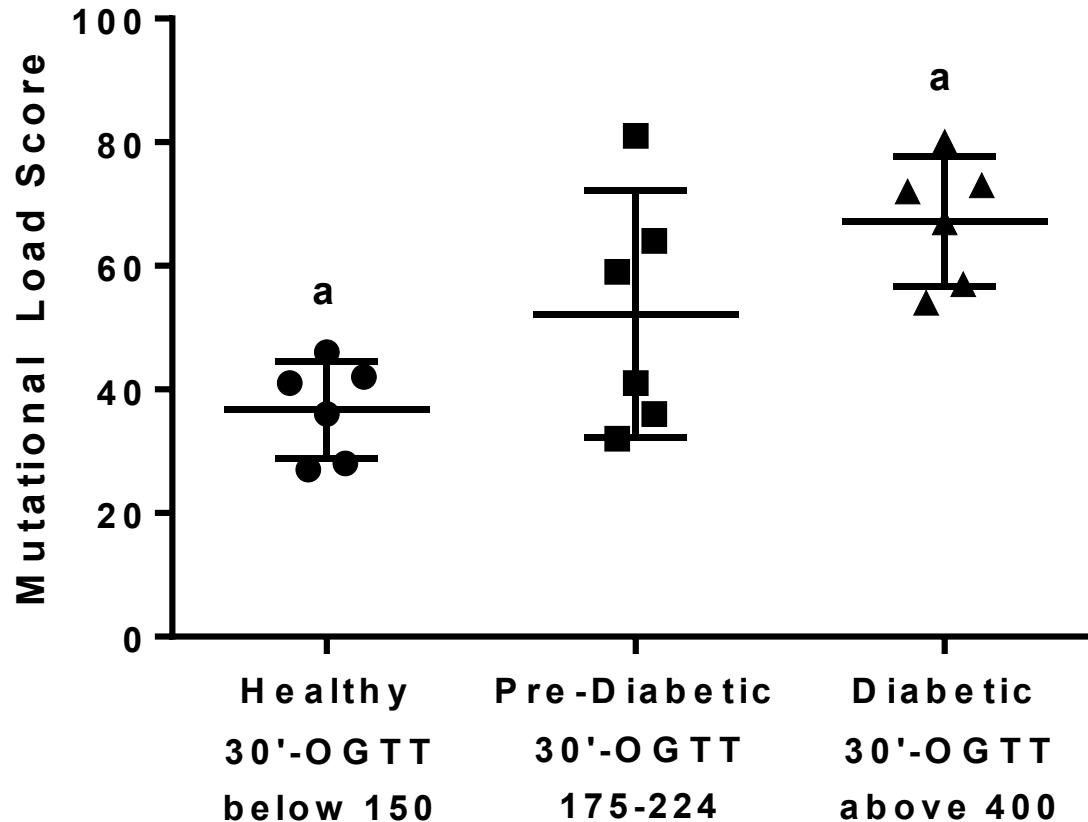
Lights-On/Lights-Off Probes



Example of a Mutational Shift



Mutations Correlate With Diabetes and Onset of Diabetes

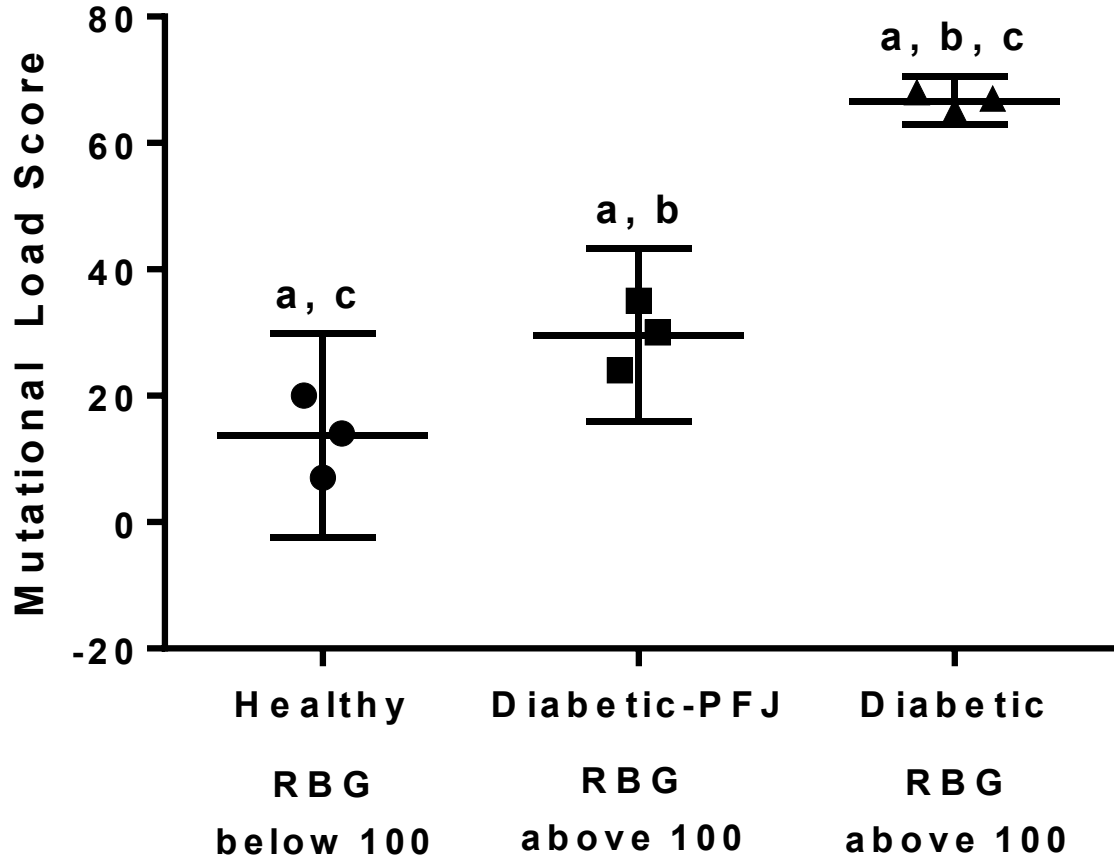


a = p-value of 0.006

Error bars are 95% confidence interval

One-Way ANOVA

PFJ May Also Lower Mutations Diabetic Prone Nile Rats



a = p-value of 0.05

b = p-value of 0.01

c = p-value of 0.01

Error bars are 95% confidence interval

One-Way ANOVA



Mitochondrial Mutational Load in Drug Treatment



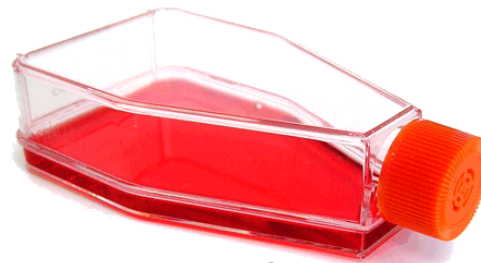
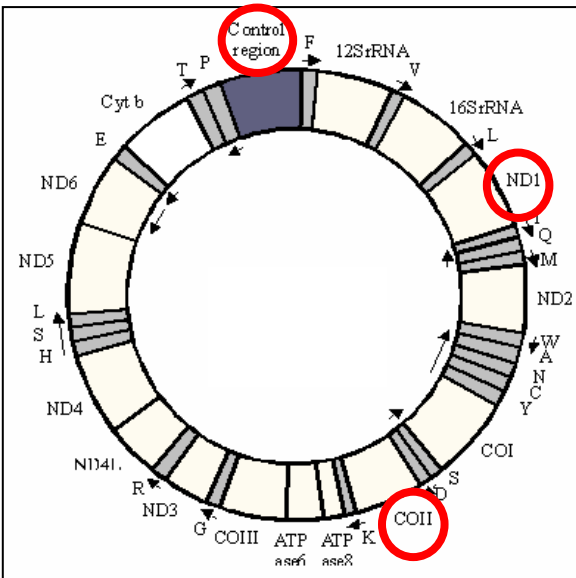
AZT Treatment Increases mtDNA Mutations in HepG2 and CCD-1112Sk Cells

Adam E. Osborne*, John E. Rice, J Aquiles Sanchez and Lawrence J. Wangh
Biology, Brandeis University, Waltham, USA

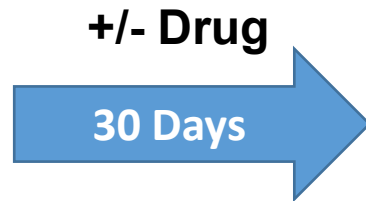
Abstract

Mitochondrial dysfunction is linked to disease, but it remains unclear whether accumulation of random mutations in the mitochondrial genome is the cause of dysfunction. Using digital or near-digital LATE-PCR with Lights-On/Lights-Off probes we have measured the mutational load in mitochondrial genomes. Exposure of HepG2 and CCD-1112Sk cells to AZT for thirty days caused a significant increase in mutations in the three mitochondrial loci examined. These results demonstrate the utility of our method for analysis of mutational load without sequencing and reinforce the fact that mitochondrial DNA damage due to drugs, aging, and disease should be studied in detail.

Development of the Necessary Tools



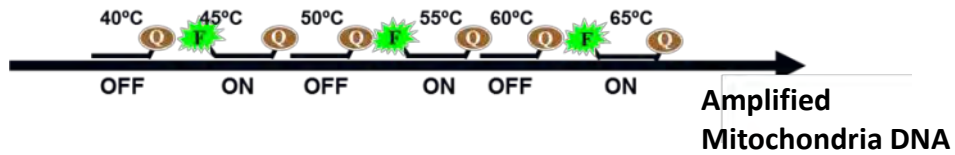
**HepG2
cell culture**



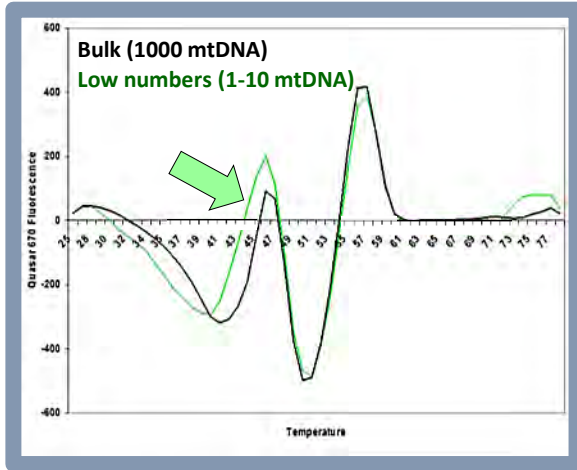
**Mitochondria
Mutational Load
Analysis**

Controls: No Drug*

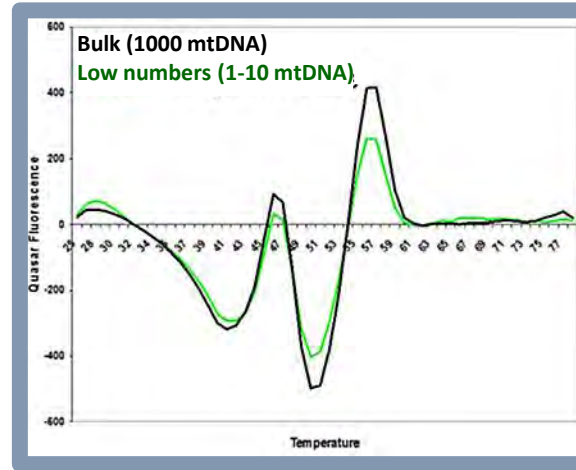
*** Drug replenished every two days**



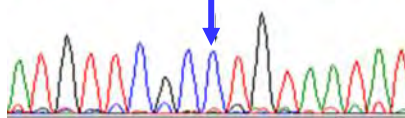
Mutant mtDNA



Normal mtDNA

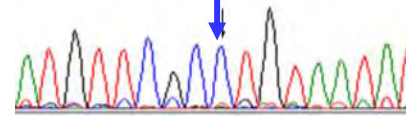


ATGTTTCGCCTGTAATAT



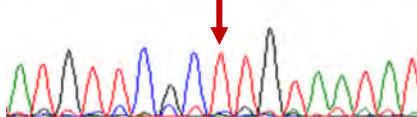
BULK

ATGTTTCGCCTGTAATAT



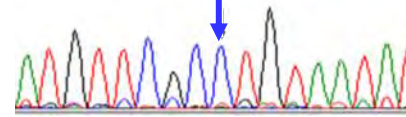
BULK

ATGTTTCGCCTGTAATAT



1-10 MOLECULES

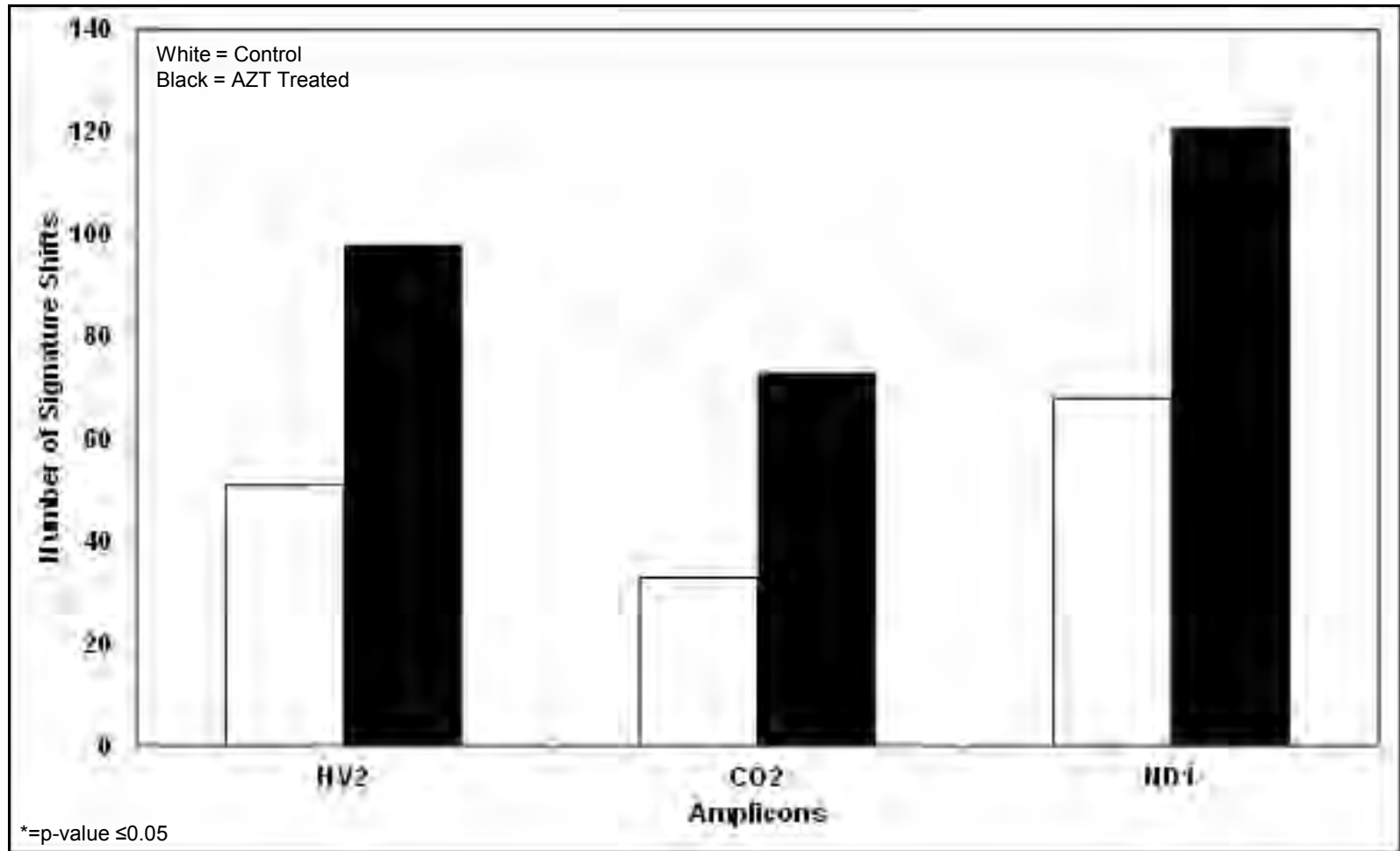
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1-10 MOLECULES



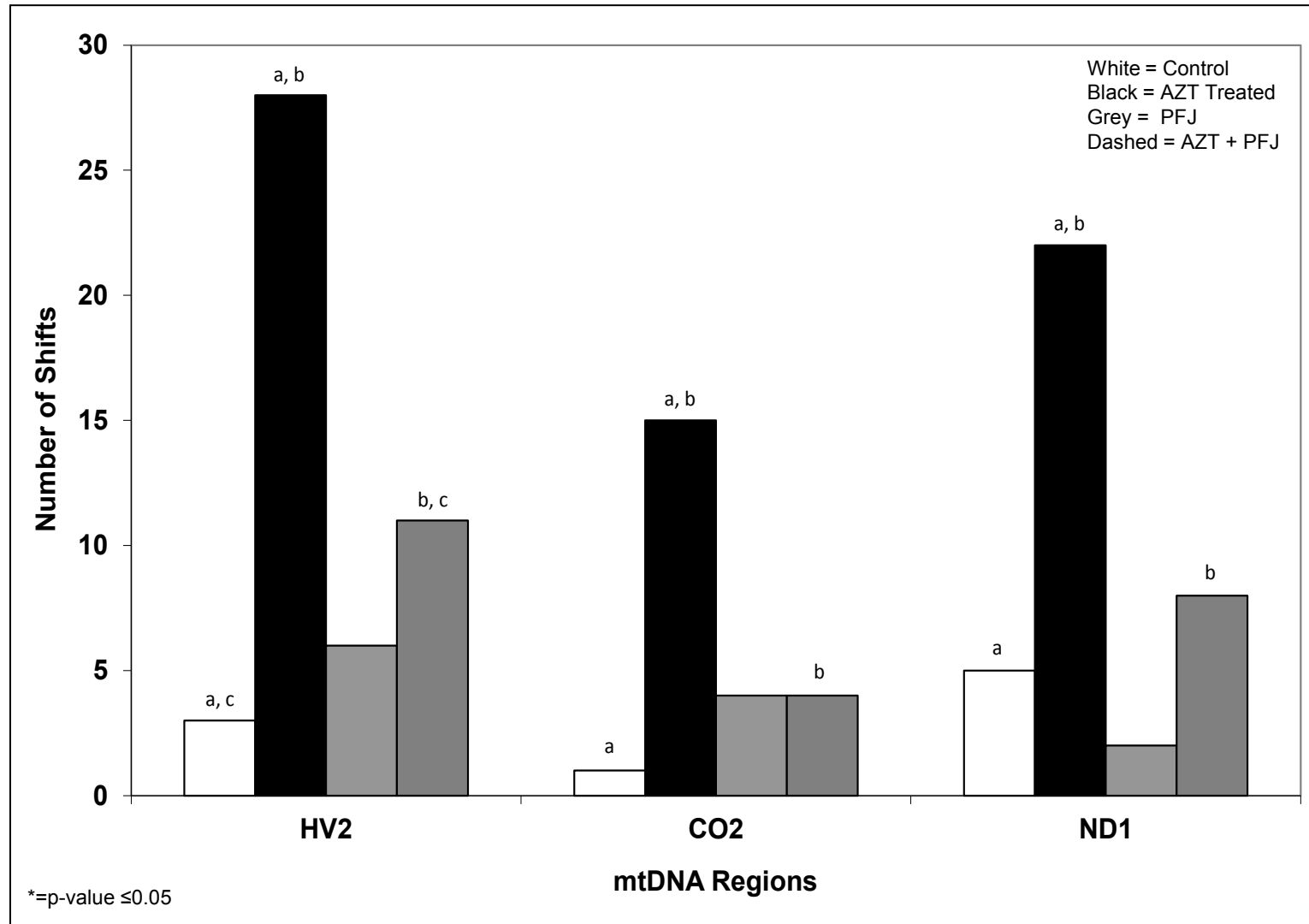
AZT Causes Mitochondrial DNA Mutations



Osborne AE, Rice JE, Sanchez AJ, Wanhg LJ (2013) AZT Treatment Increases mtDNA Mutations in HepG2 and CCD-1112Sk Cells. J AIDS Clin Res 4: 250. doi: 10.4172/2155-6113.1000250



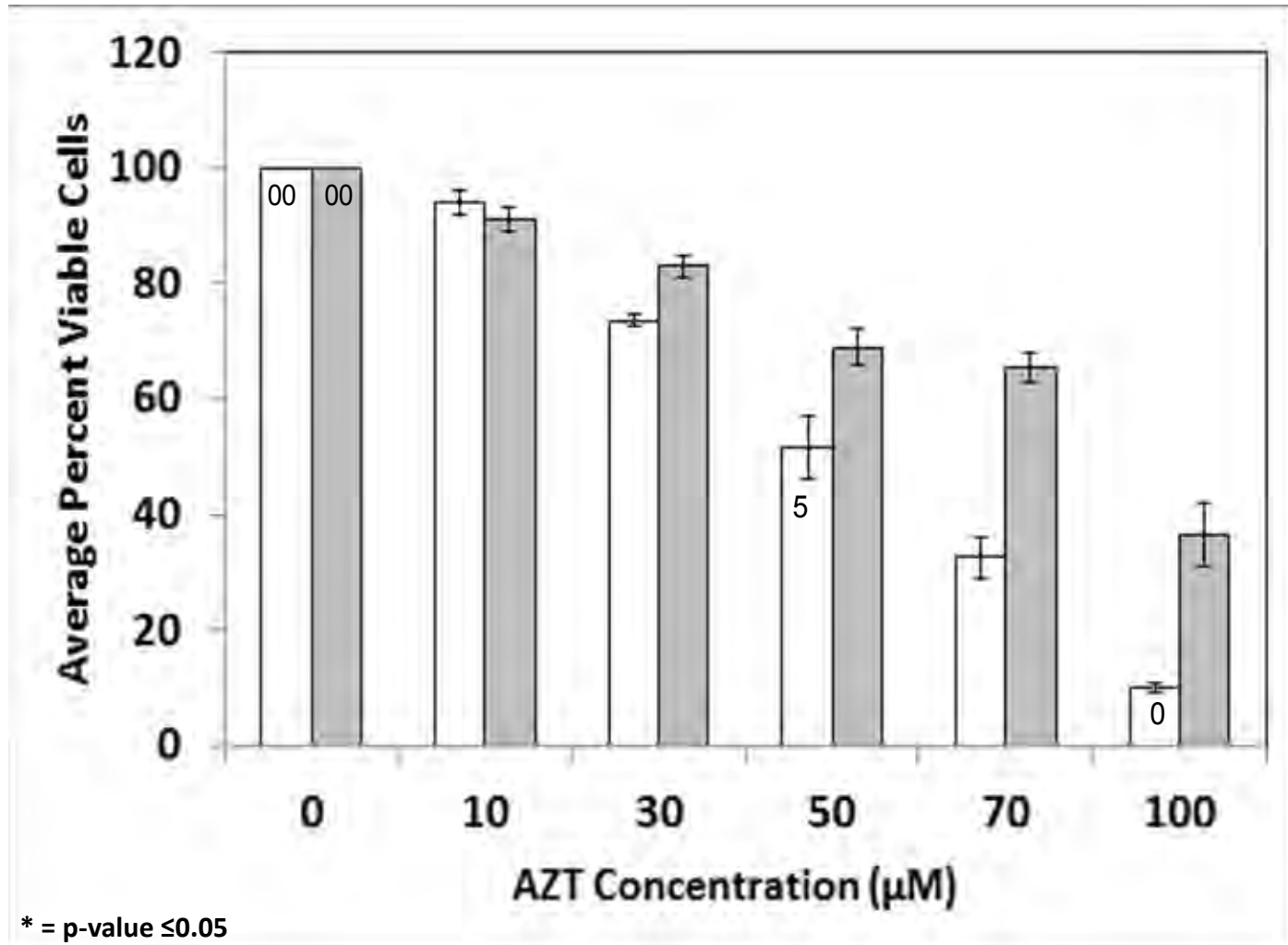
PFJ Reduces mtDNA Mutations



Osborne AE, Sanchez JA, Solomon M, Stopa A, Wangh LJ, et al. (2014) Palm Fruit Juice Mitigates AZT Mitochondrial Genotoxicity and Dose-Dependent Cytotoxicity. J AIDS Clin Res 5:400. doi: 10.4172/2155-6113.1000400



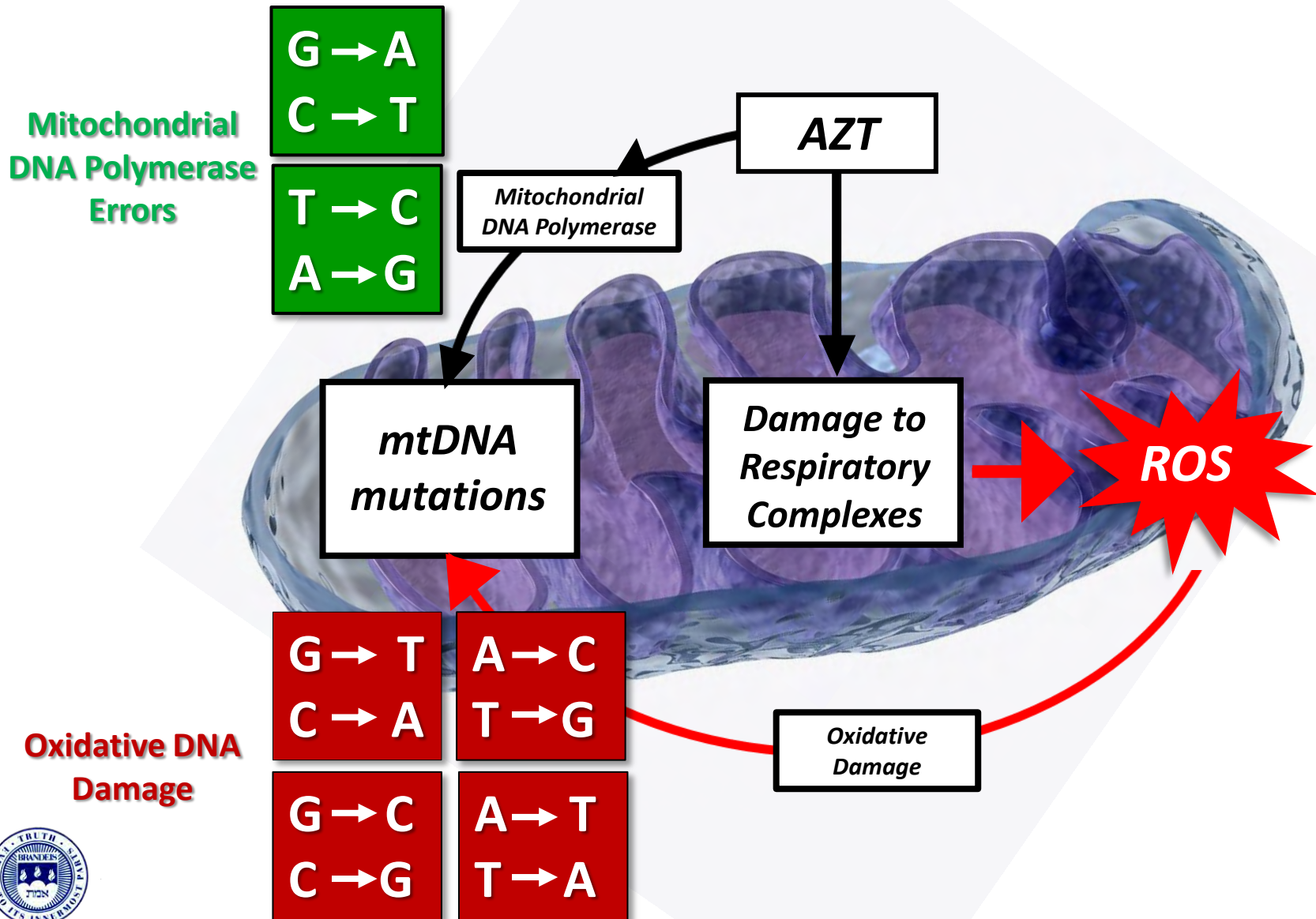
PFJ Reduces AZT's Cytotoxic Effects



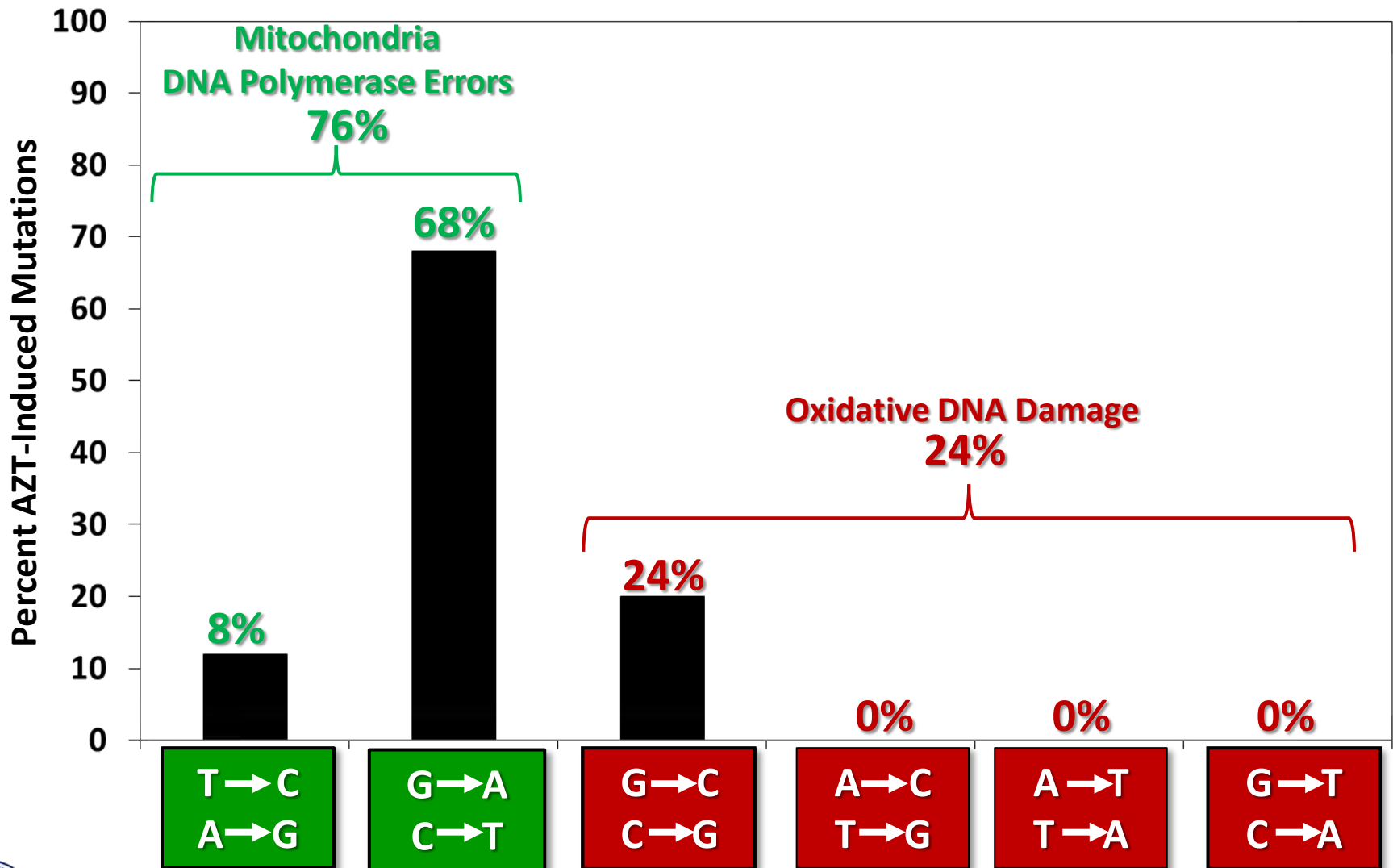
Osborne AE, Sanchez JA, Solomon M, Stopa A, Wangh LJ, et al. (2014) Palm Fruit Juice Mitigates AZT Mitochondrial Genotoxicity and Dose-Dependent Cytotoxicity. *J AIDS Clin Res* 5:400. doi: 10.4172/2155-6113.1000400



Type of Mutations Distinguishes Mechanisms of AZT-Mutagenesis



Oxidative DNA Damage is not the Main Driver of AZT-Induced Mitochondria Mutations

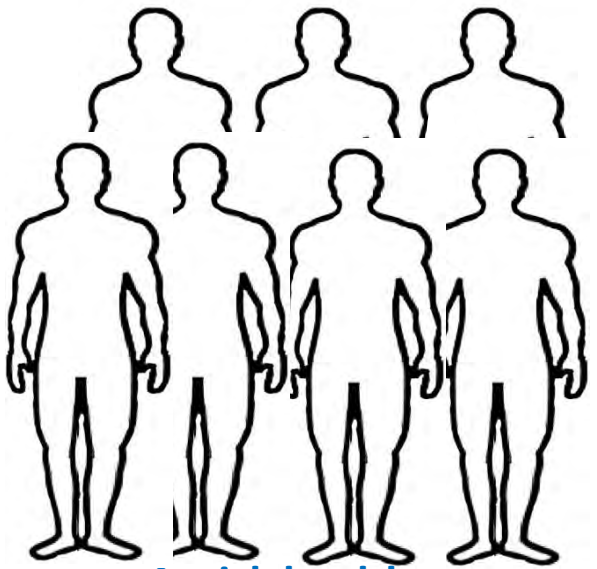
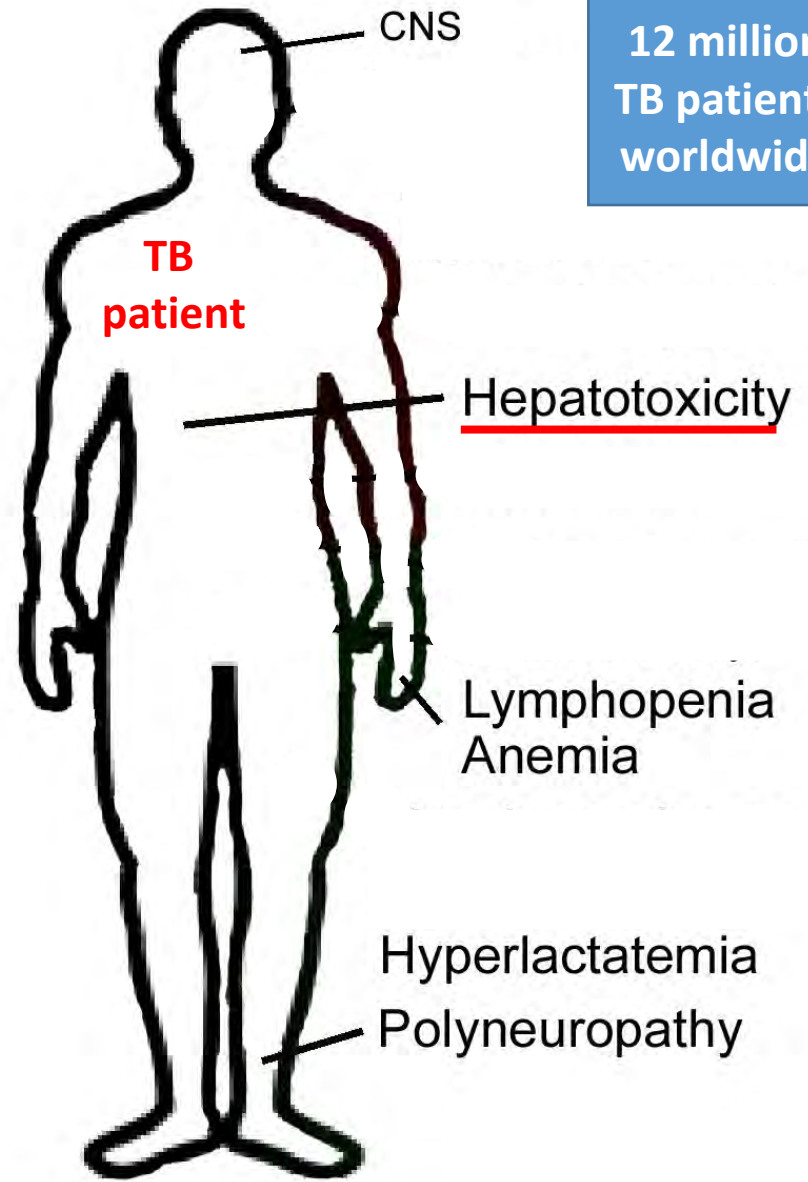


Isoniazid

therapeutic
1-12 months

prophylactic

12 million
TB patients
worldwide

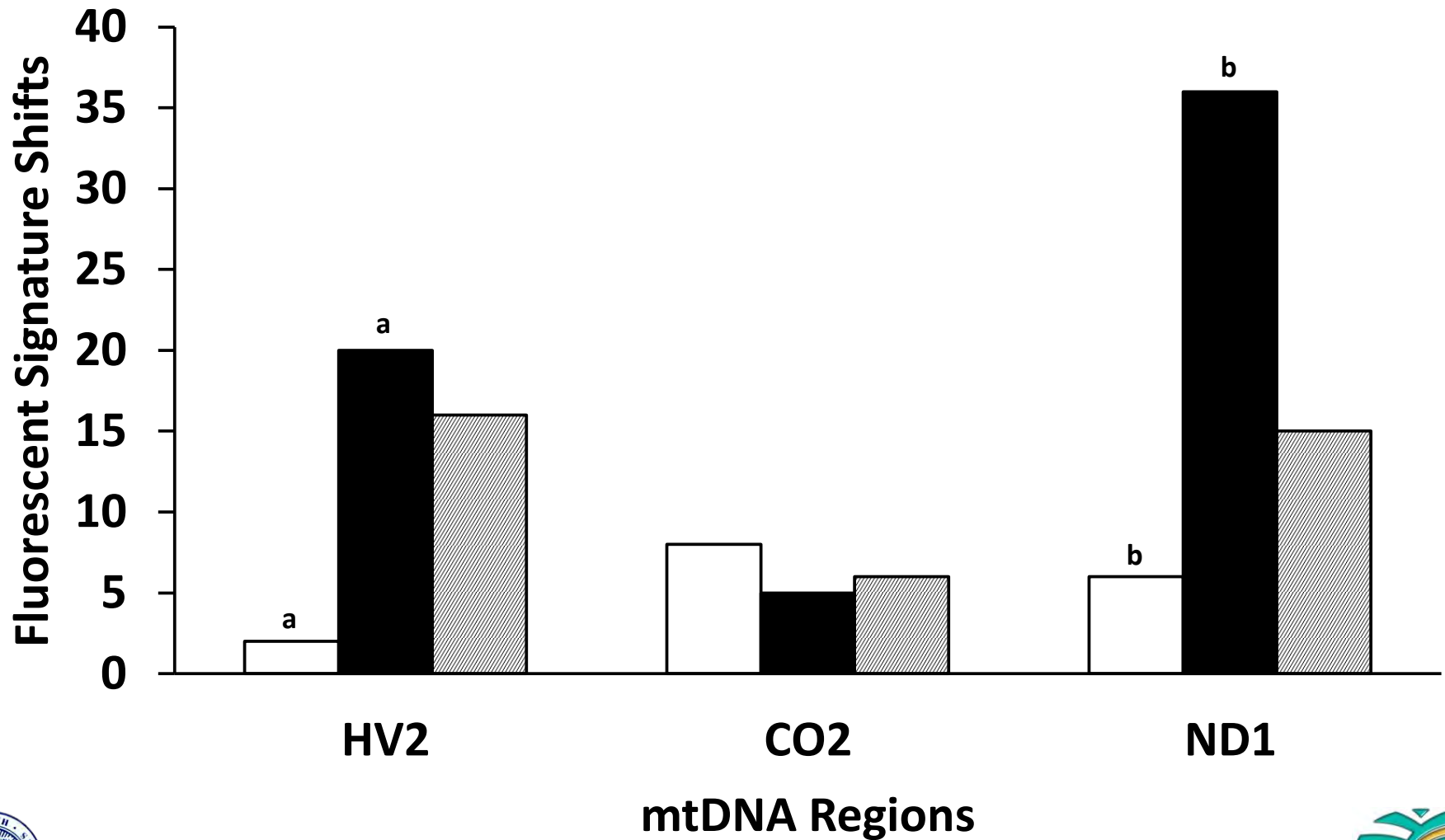


At risk healthy
individuals

Organ manifestations of
isoniazid mitochondrial toxicity



PFJ Reduces but Does not Entirely Mitigate Isoniazid Damage



a, b = p-value ≤ 0.05



Conclusions

- Two model systems that can study the molecular causes of mitochondrial dysfunction
- Mutational load exists
 - Causing problems?
- PFJ may be able to mitigate effects of Diabetes
- PFJ may be able to mitigate adverse drug effects
- Have a research program that not only studies the development of disease/damage as well as its mitigation, but also the molecular mechanisms of disease/damage and PFJ mitigation



Aknowledgements

- Lawrence Wanhg
- K.C. Hayes
- Aquiles Sanchez
- John Rice
- Martha Solomon
- Arielle Stopa
- David Lee
- Avery Dowd
- MPOB



