

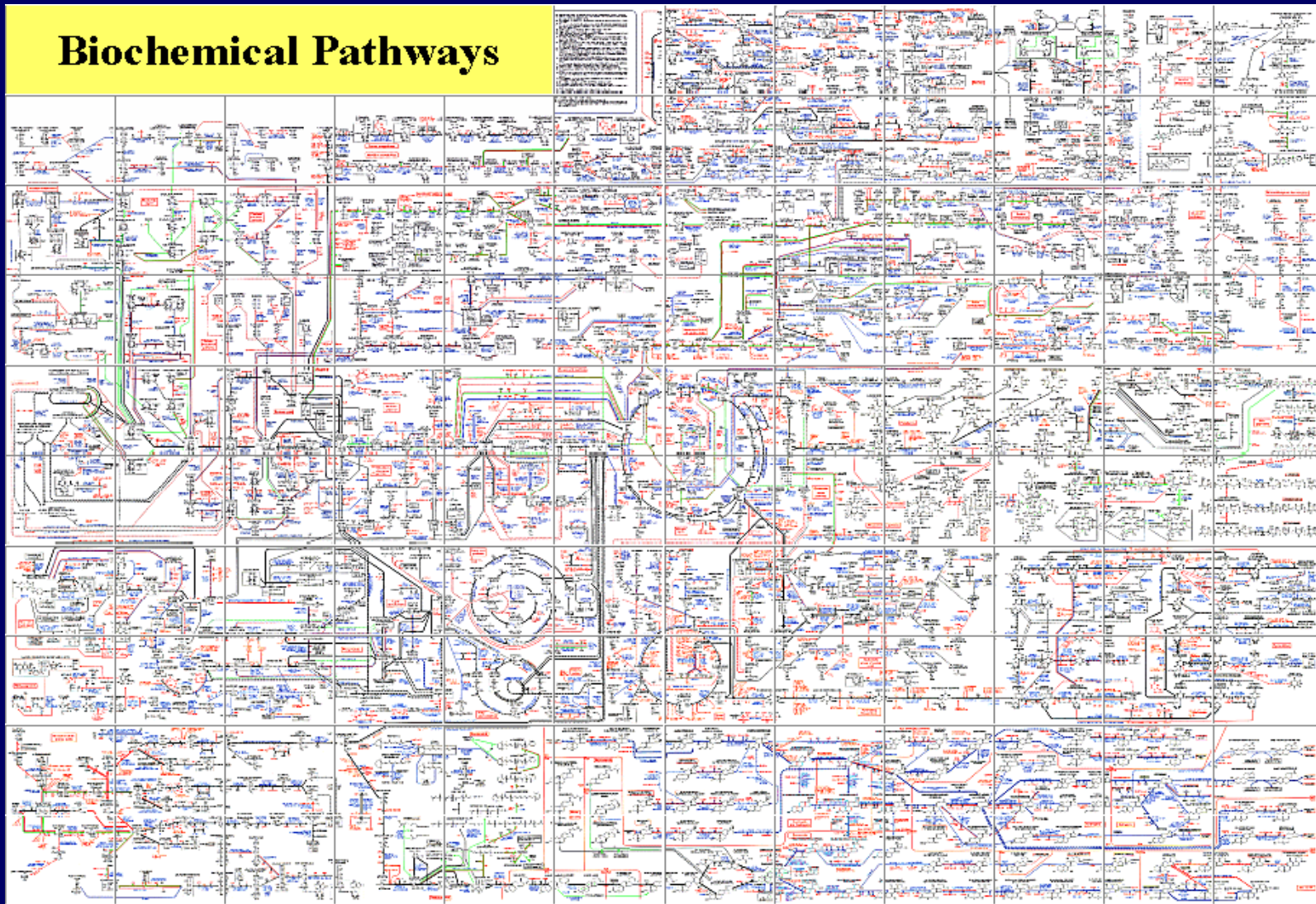
Micronutrient Undernutrition in Americans

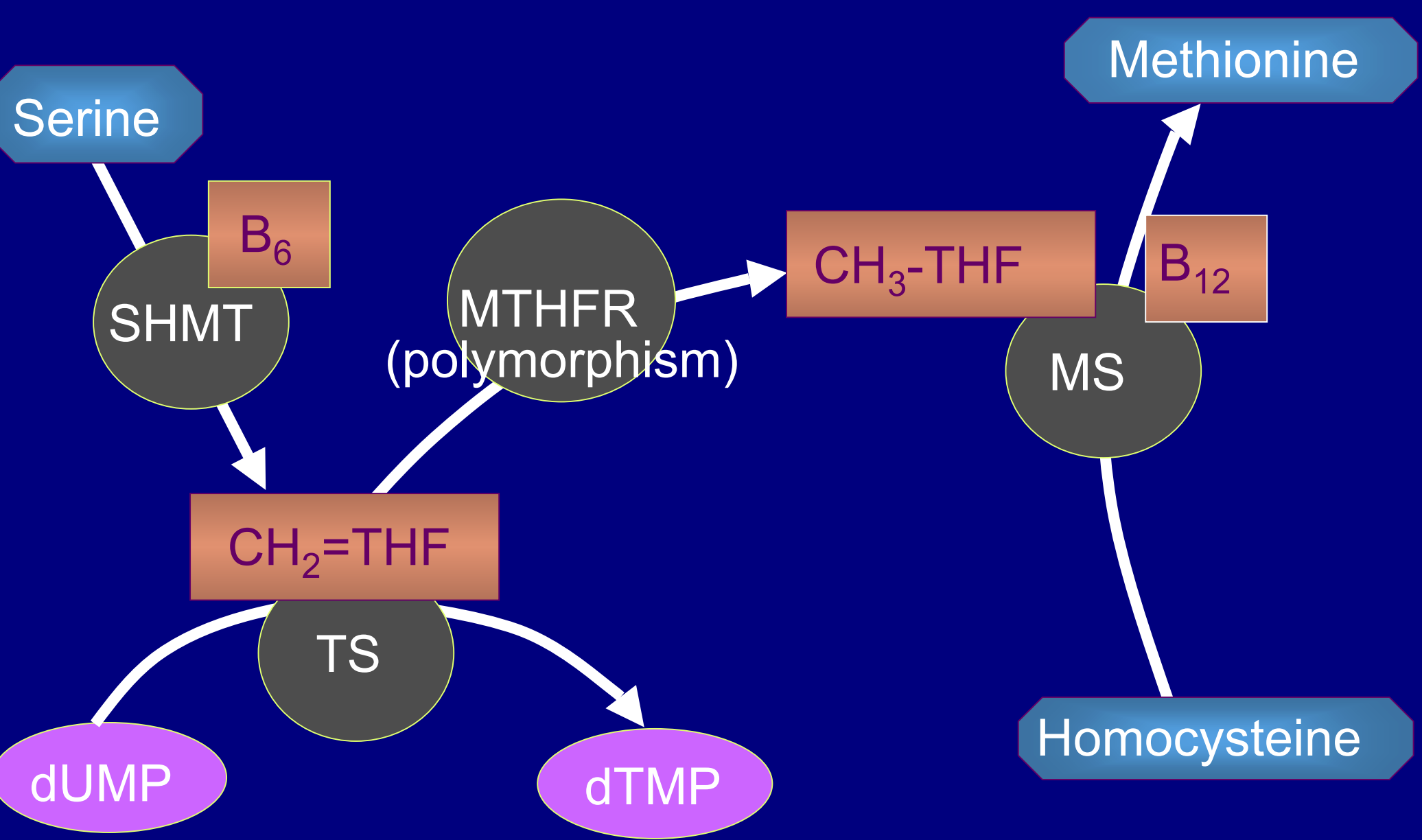
Nutrient	Population Group	RDA	% ingesting < RDA	% ingesting <50% RDA
Minerals				
Iron	Women 20-30 years	18 mg	75%	25%
	Women 50+ years	8 mg	25%	5-10%
Zinc	Men; Women 50+ years	11; 8 mg	50%	10%
Vitamins				
B6	Men; Women	1.7; 1.5 mg	50%	10%
Folate**	Men; Women	400 mcg	75%	25%; 50%
B12	Men; Women	2.4 mcg	10-20; 25-50 %	5; ~10-25%
C	Men; Women	90; 75 mg	50%	25%

•Wakimoto and Block (2001) *J Gerontol A Biol Sci Med Sci.* Oct; 56 Spec No 2(2):65-80.

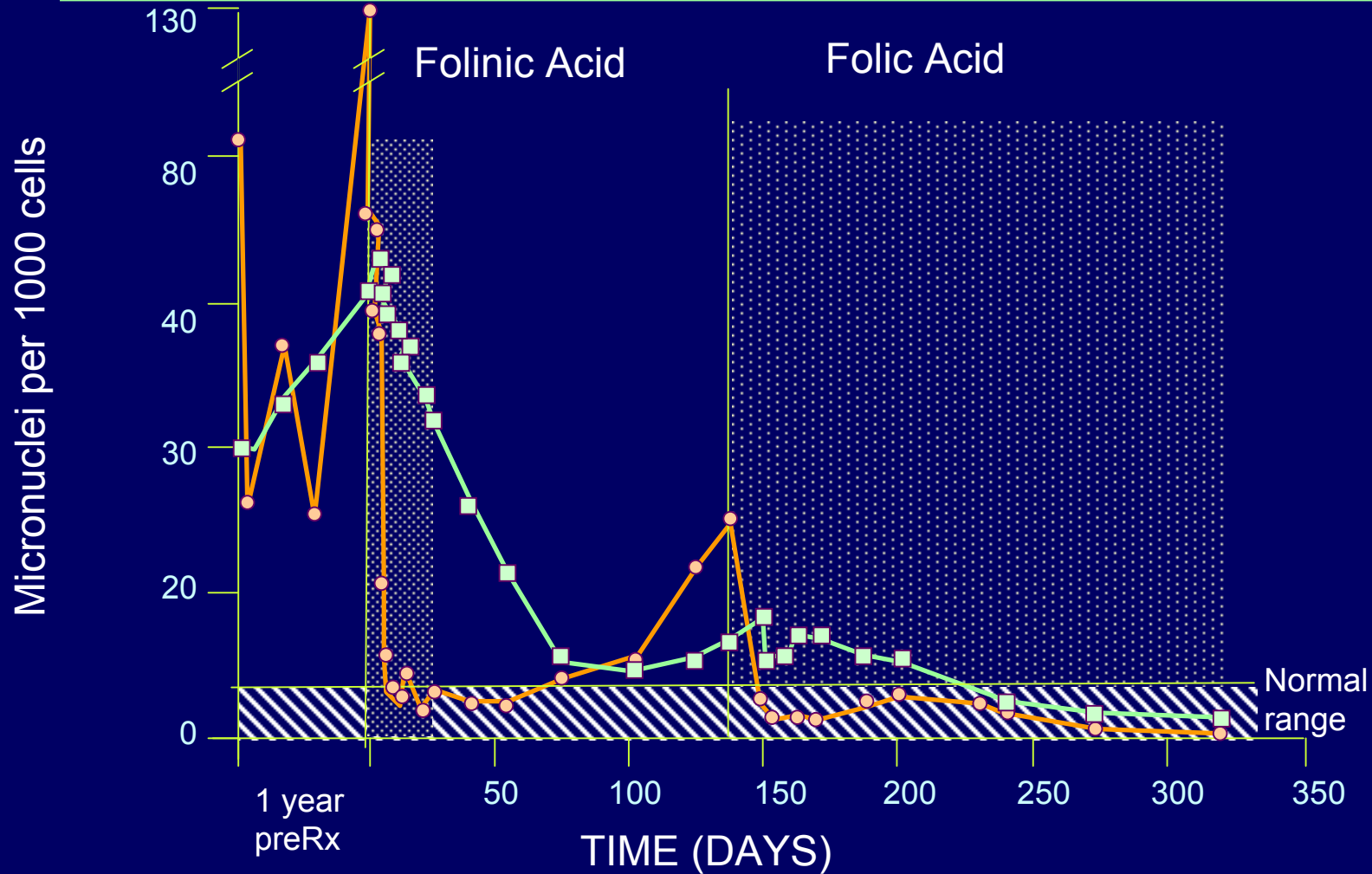
** Before U.S. Food Fortification

Biochemical Pathways

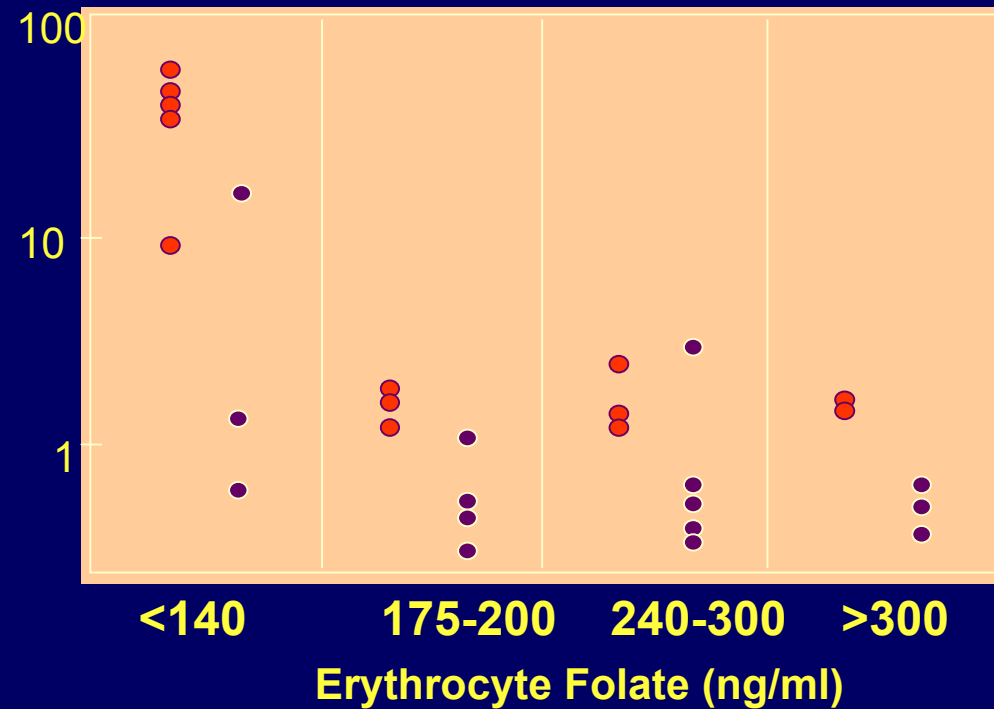




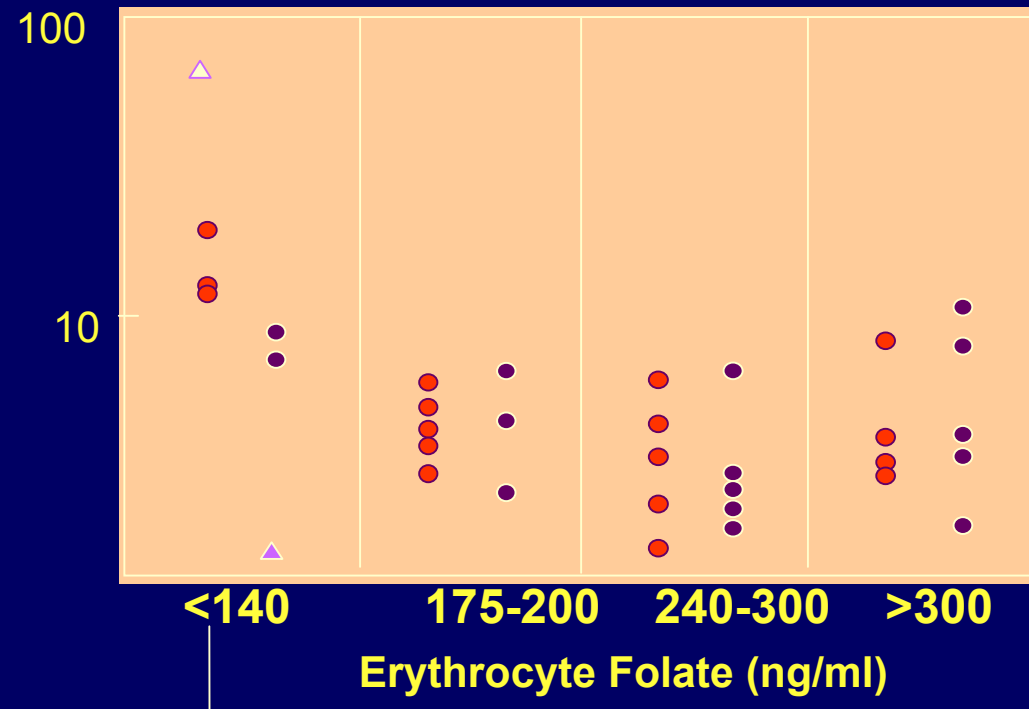
Micronuclei in: RNA positive reticulocytes RNA negative erythrocytes



Uracil/10,000 thymine

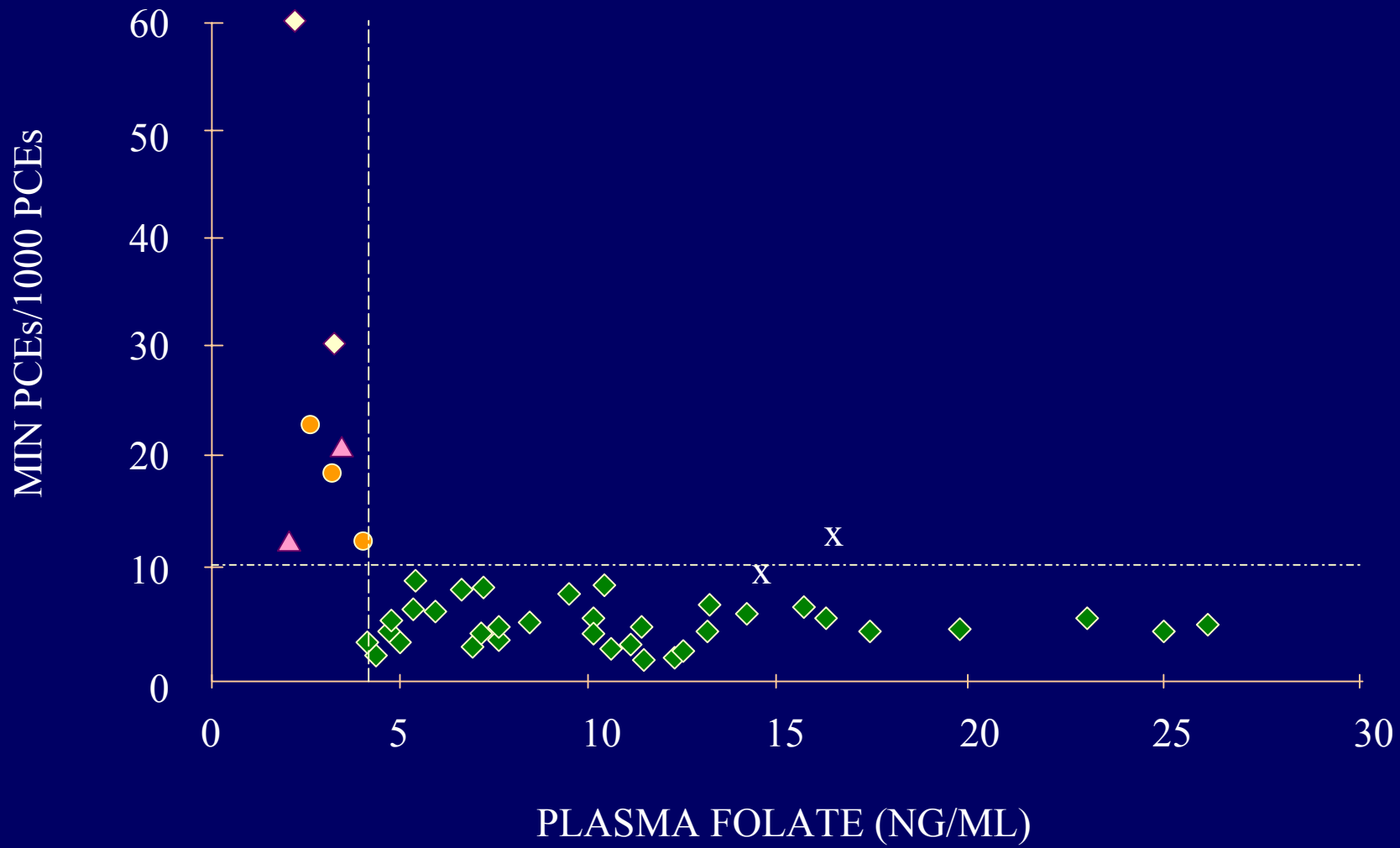


Micronuclei (mn reticulocytes/1000 reticulocytes)

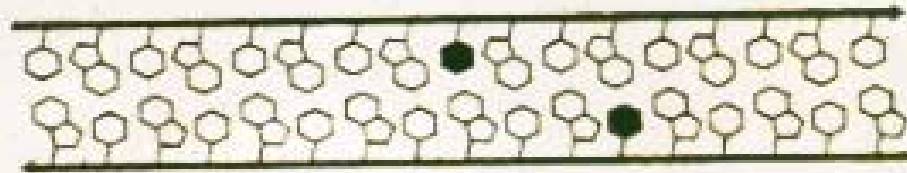


10% US population
50% Low income minority

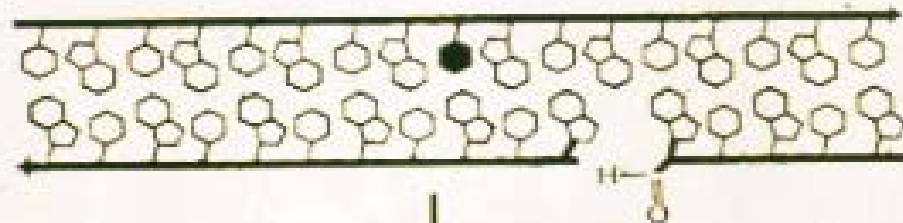
- Presupplementation
- Postsupplementation



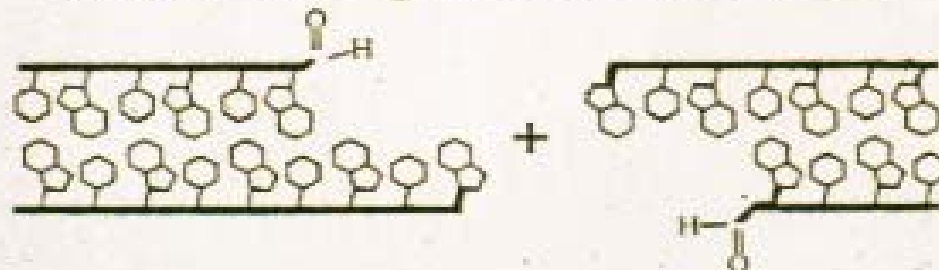
Base excision repair processing of opposed lesions



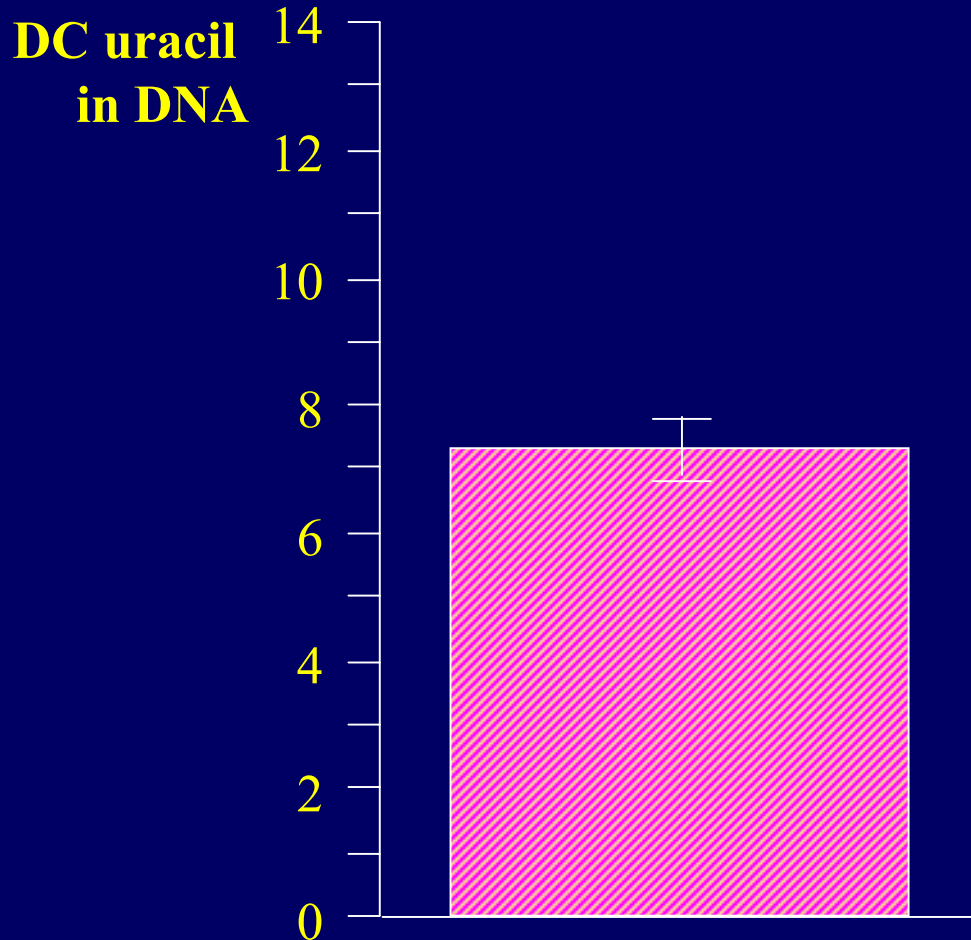
Gap three or more nucleotides away from base lesion



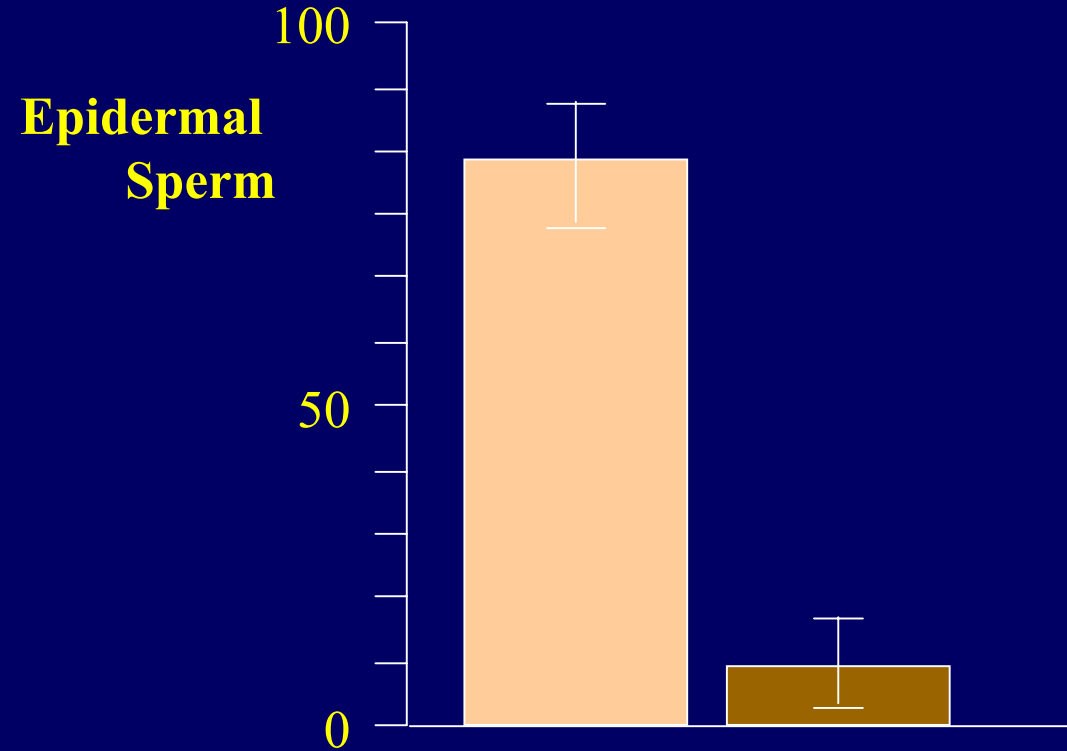
DNA double strand break formed by processing the second lesion



Mean uracil content in sperm DNA from 23 men on diets low in fruits and vegetables



Folate Deficiency Study epidermal Sperm Count



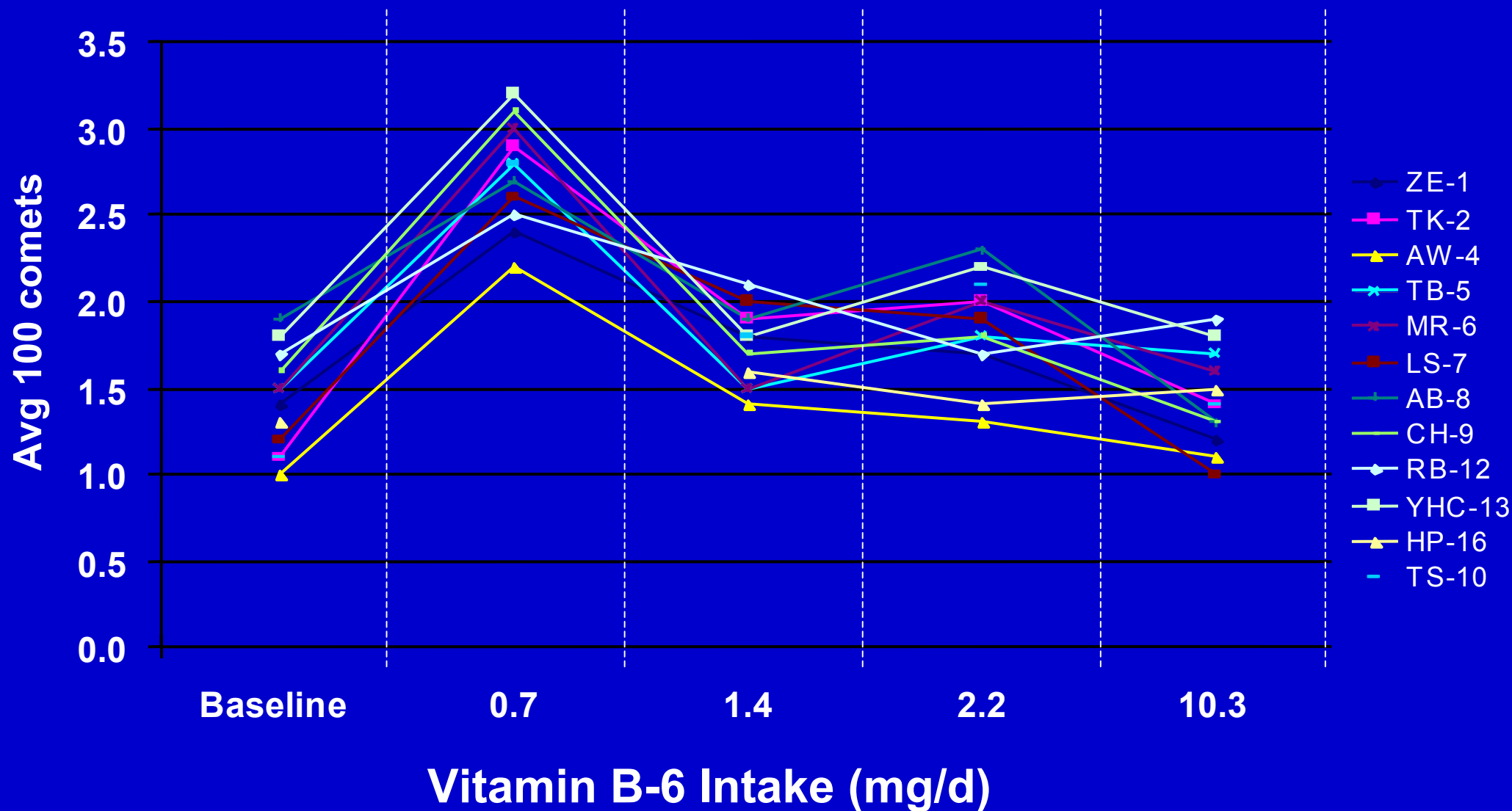
Seminal plasma folates vs. semen quality

Correlation coefficient (r); n=48

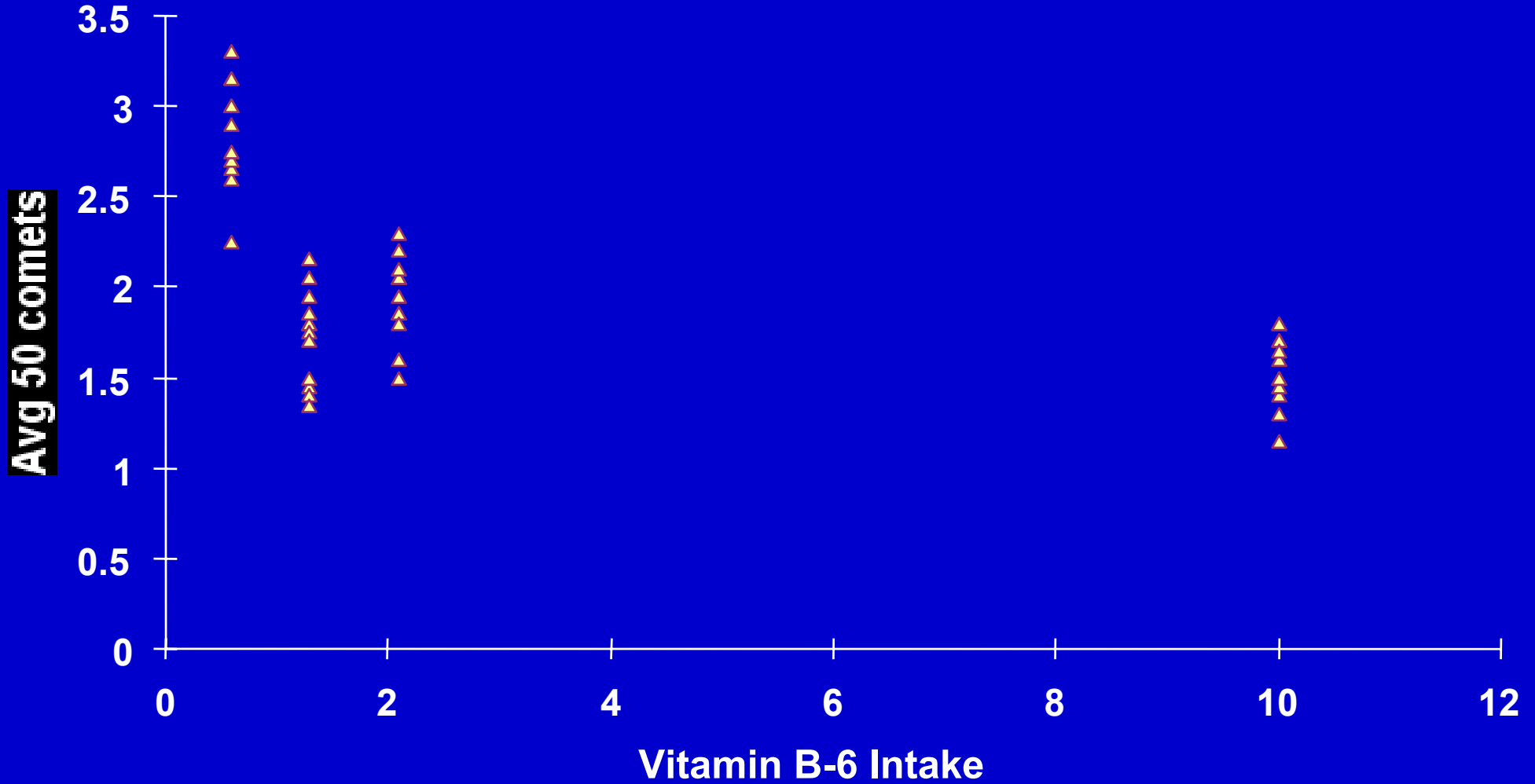
<u>Seminal Plasma</u>	<u>Sperm Density ($10^6/\text{mL}$)</u>	<u>Total Sperm Count (10^5)</u>
Non-methyl THF (methylene-THF, etc.)	* 0.37	* 0.31
5-Methyl THF	0.08	0.07

* $p < 0.05$

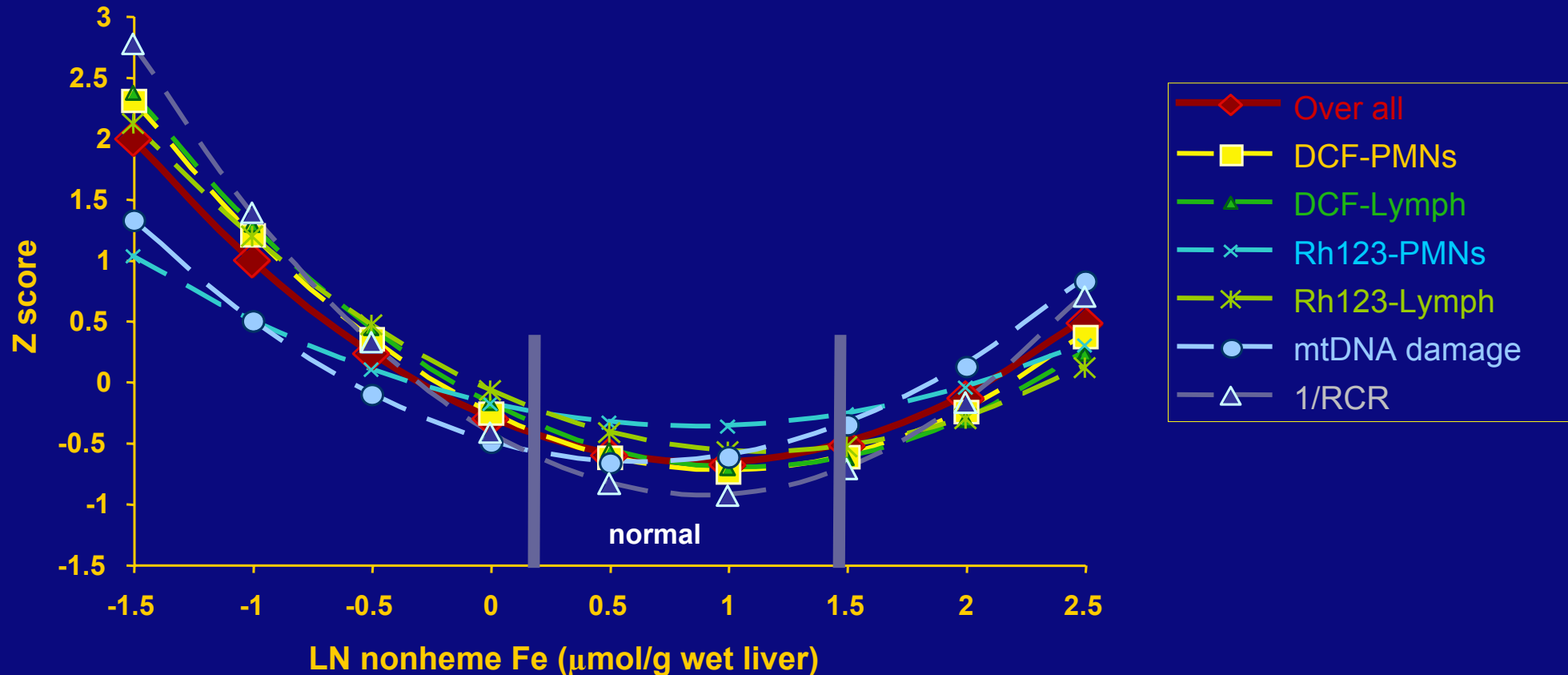
Human Lymphocyte DNA Strand Breaks (Comet Assay) vs. B-6 Intake



Human Lymphocyte DNA Strand Breaks (Comet Assay) vs. B-6 Intake



Analysis of nonlinear regression models: comparison of an overall model and individual models of Z-transformed values vs. ln- nonheme liver iron



. Each of the six dependent variables (that were analyzed by nonlinear regression in former figures) were transformed to Z scores and modeled as a quadratic function of the ln-liver nonheme iron as the independent variable. The equation for the RCR ratio's Z score was obtained from inverted RCR values (1/RCR) so that normal rats had the lower instead of the higher values. For presentation purposes each model line was obtained from 9 values of liver iron. All statistics were performed as in materials and methods.

Synthesis of Heme

Cytosol



Other intermediates

PBG

2ALA

PPIX

PPGIX

FeII

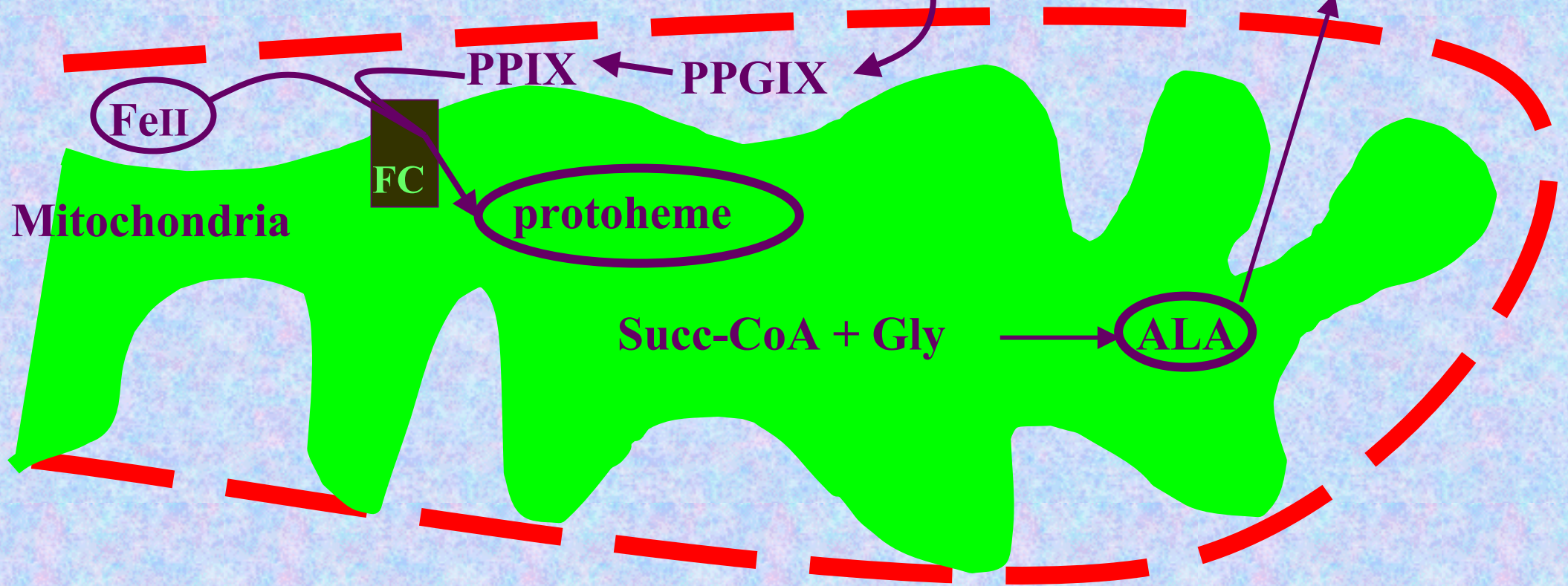
FC

protoheme

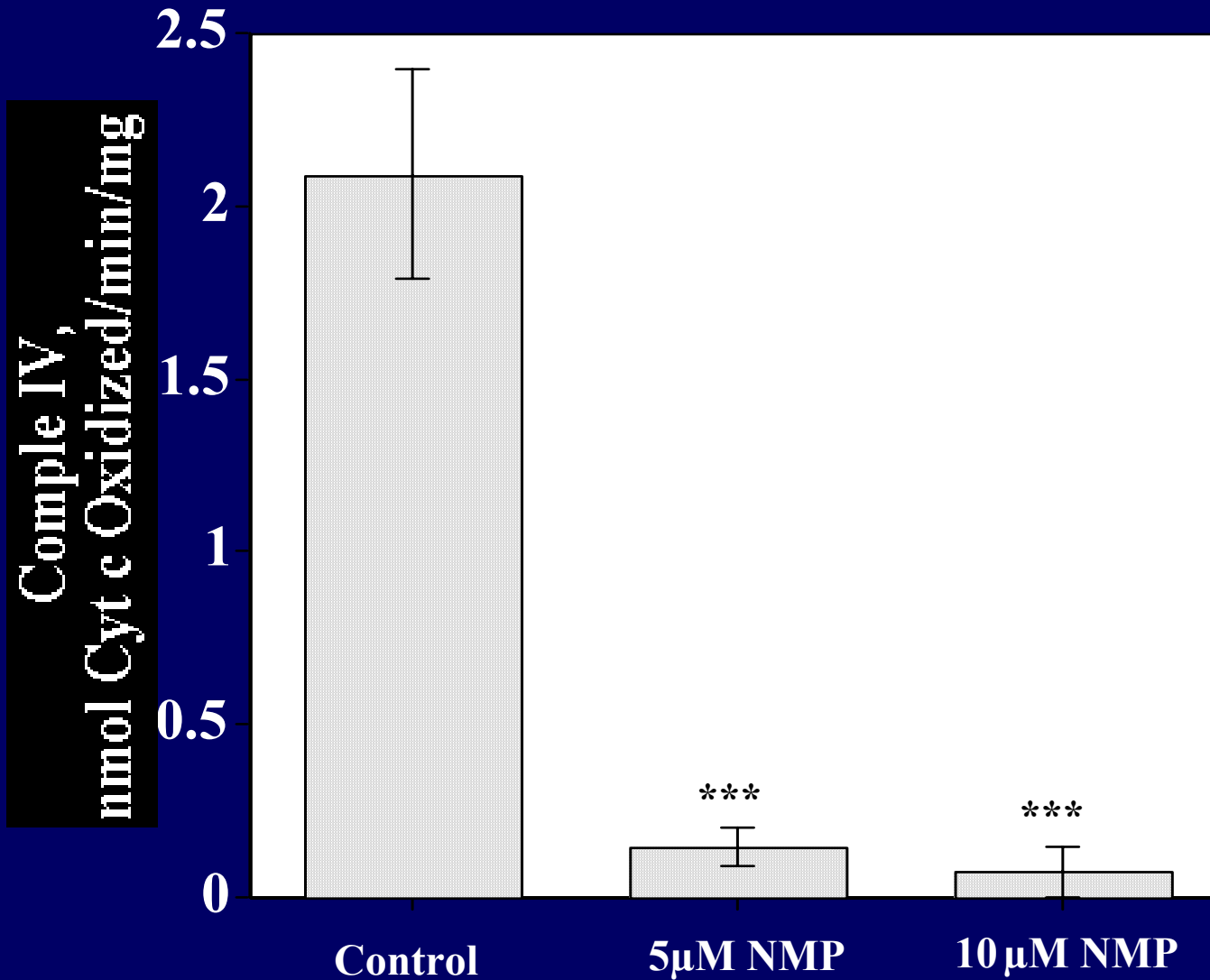
Succ-CoA + Gly

ALA

Mitochondria

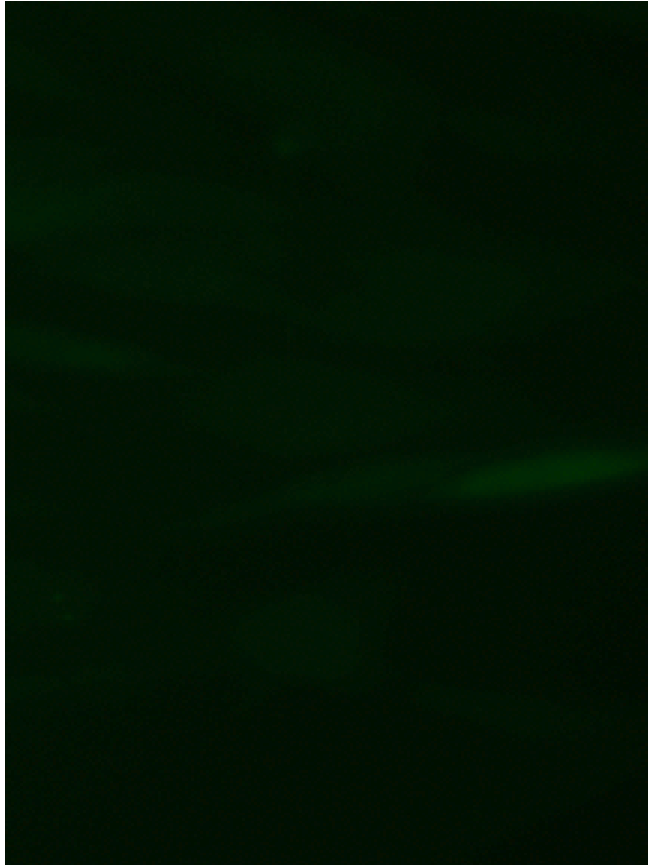


Loss of activity of complex IV in heme-deficient cells

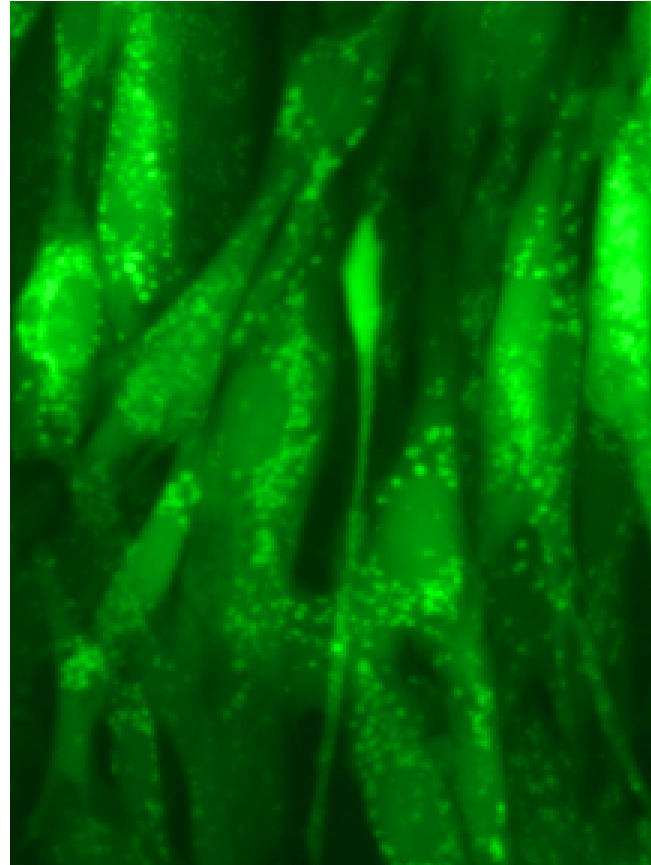


Heme deficiency induces oxidative stress

Control



NMP

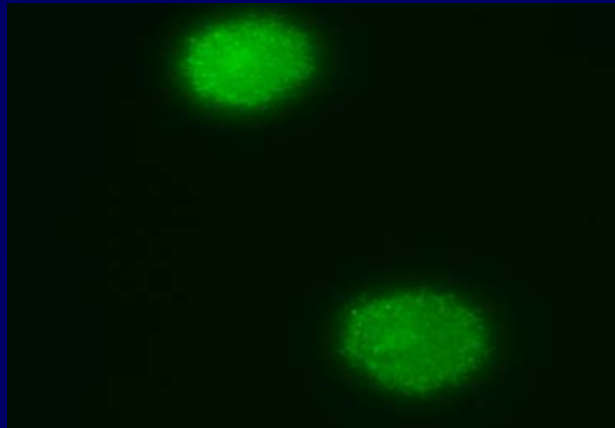


Similarity Between the Consequences of Heme Deficiency and Normal Aging/neurodegeneration

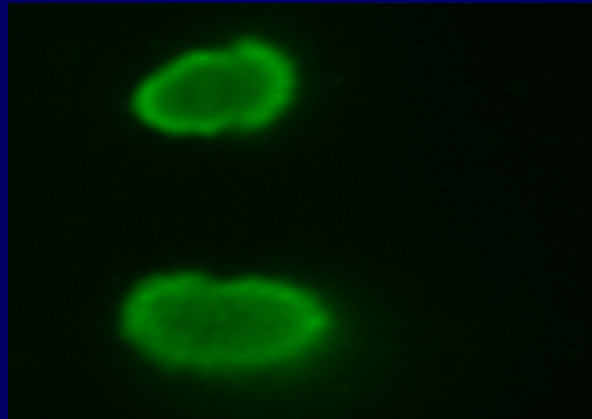
Factor in Study	Heme Deficiency	Aging/Neurodegeneration
Complex IV	Loss of complex IV 9	Loss of complex IV
Iron	Accumulation 11	Accumulation
Oxidative Stress	Increased 9	Increased
APP	Decreased and aggregate appear 11	dimmer or aggregate
NOS	Increased 11	Increased
Cell-cycle and differentiation	Disabled differentiation or proliferation 11	Loss of Axons; neuronal death
Metabolism	Mitochondrial decline 9,10	Hypometabolism
Calcium	Corrupted 9	Corrupted
Ferrochelatase	Increased 9	Increased in senescent cells 9*
Heme synthesis	Decreased 10	Decreased with age**

***Not Determined in vivo. **Not determined in the aging brain**
9) Atamna et al (2001) JBC. 10) Atamna et al (2002) ABB.
11) Atamna et al (2002) PNAS.

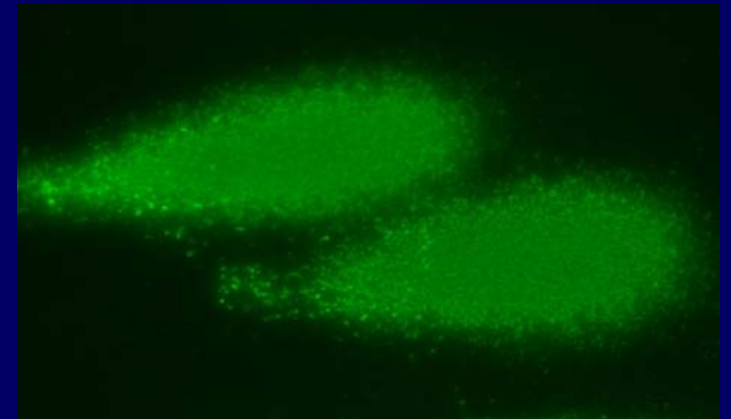
Zinc Deficiency Induces Fapy Glycosylase (Fpg)-sensitive Single Strand Breaks in Human Lung Fibroblasts



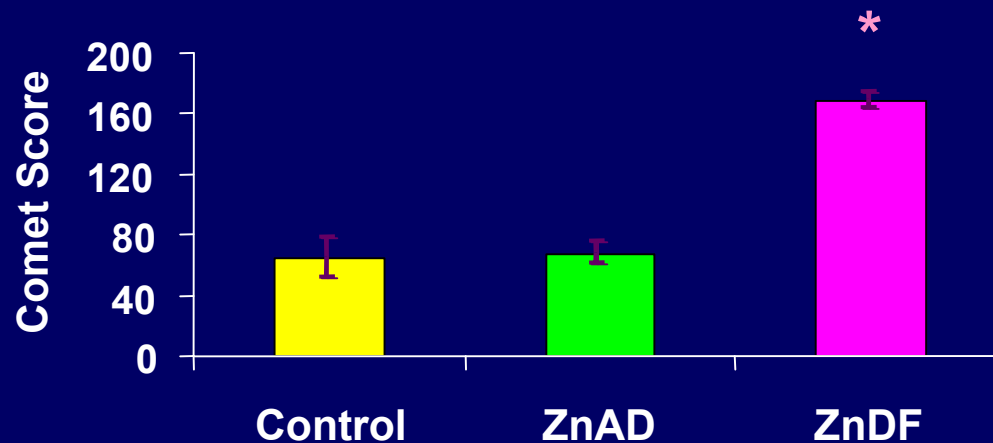
Control (+Fpg)



ZnAD (+Fpg)



ZnDF (+Fpg)



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**High-dose vitamin therapy stimulates
variant enzymes with decreased
coenzyme-binding affinity
(increased K_m): Relevance to genetic
disease and polymorphisms**

Bruce N Ames, Ilan Elson-Schwab, and Eli A Silver,

Am J Clin Nutr 2002; 75:616-658

Summary of Work

Many genetic diseases in humans are ameliorated by the administration of high levels of vitamins. An appreciable percentage of mutations in a gene, perhaps a third, results in the corresponding enzyme having an increased K_m (poorer binding affinity) for a coenzyme or substrate, resulting in a lower rate of reaction. Because the intracellular concentration of coenzyme can often be increased therapeutically, enzymatic activity can be restored at least partially, and the disease phenotype cured or ameliorated. We have documented about 50 human genetic diseases involving dysfunctional enzymes which can be remedied or ameliorated by high levels of the vitamin component of the coenzyme, and a number of other genetic diseases, including some due to polymorphisms, where this approach may be useful.

Mitochondrial Ornithine Amino Transferase & Gyrate Atrophy of the Choroid and Retina

- Defective OAT leads to accumulation of ornithine and sight degradation.
- The K_m of OAT for PLP (B6 cofactor) is increased (7 - 20x) in ~5% of patients.
- B6 therapy lowers ornithine levels.



Genetic disorders affect micronutrient *sufficiency* of the following vitamins and nutrients

- 1. Pyridoxine (Vitamin B6)**
- 2. Thiamine (Vitamin B1)**
- 3. Riboflavin (Vitamin B2)**
- 4. Niacin (Vitamin B3)**
- 5. Biotin (Vitamin B7)**
- 6. Cobalamin (Vitamin B12)**
- 7. Folic Acid**
- 8. Vitamin K**
- 9. Vitamin D**
- 10. Vitamin E**
- 11. Tetrahydrobiopterin**
- 12. S-Adenosyl Methionine**
- 13. Pantothenic Acid**
- 14. Lipoic Acid**
- 15. Carnitine**
- 16. Hormones**
- 17. Amino Acids**
- 18. Metals**
- 19. Maxi B Vitamins**

A number of polymorphisms affect coenzyme binding and may be remediable by high-dose vitamins

- **Methylenetetrahydrofolate Reductase C677T and FAD**
- **Glucose-6-Phosphate Dehydrogenase A44G and NADP**
- **NAD(P):Quinone Oxidoreductase 1 (DT-diaphorase) P187S and FAD**
- **Aldehyde Dehydrogenase E487K and NAD**
- **Short-Chain Acyl-CoA Dehydrogenase G209S and FAD**

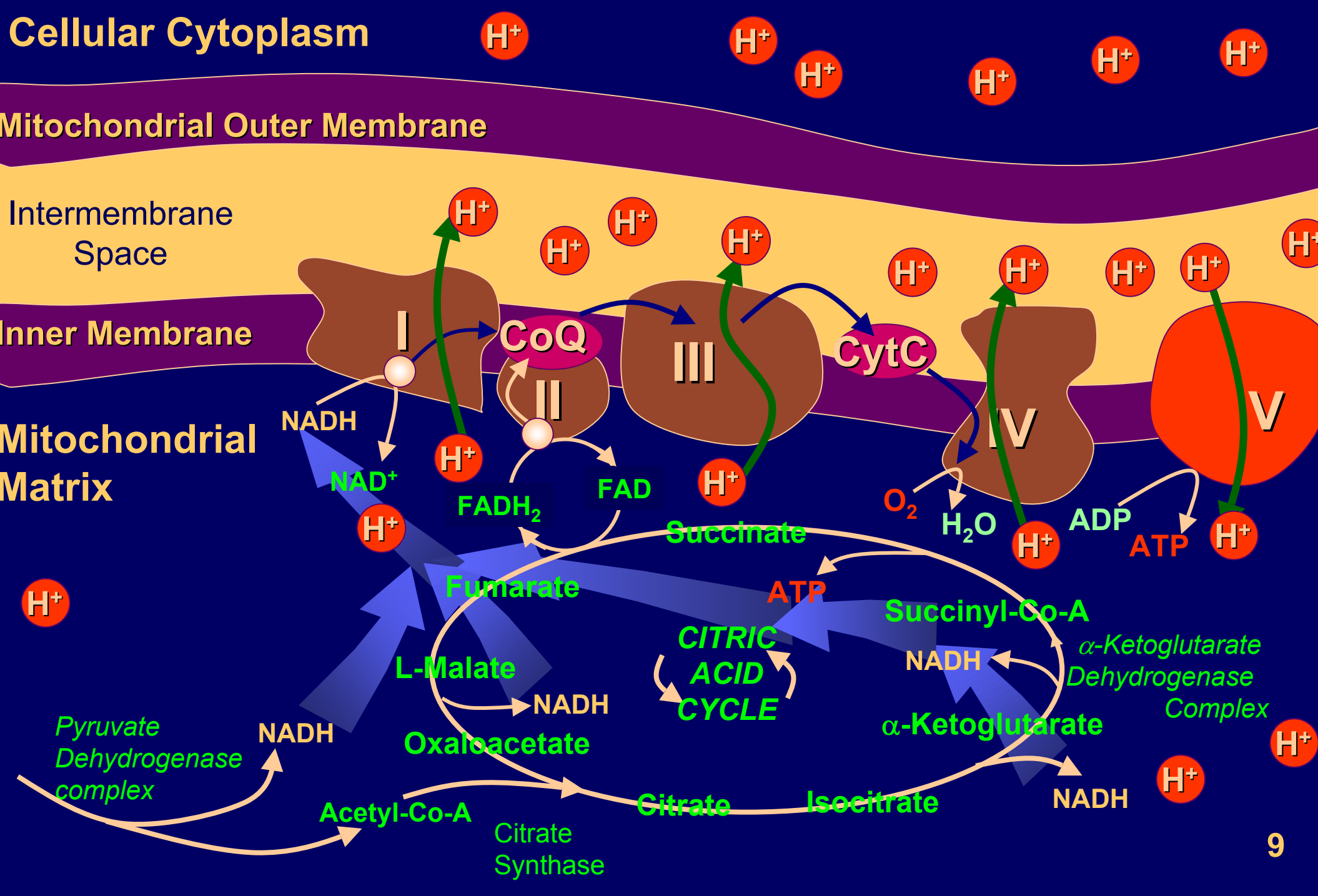
Cellular Cytoplasm

Mitochondrial Outer Membrane

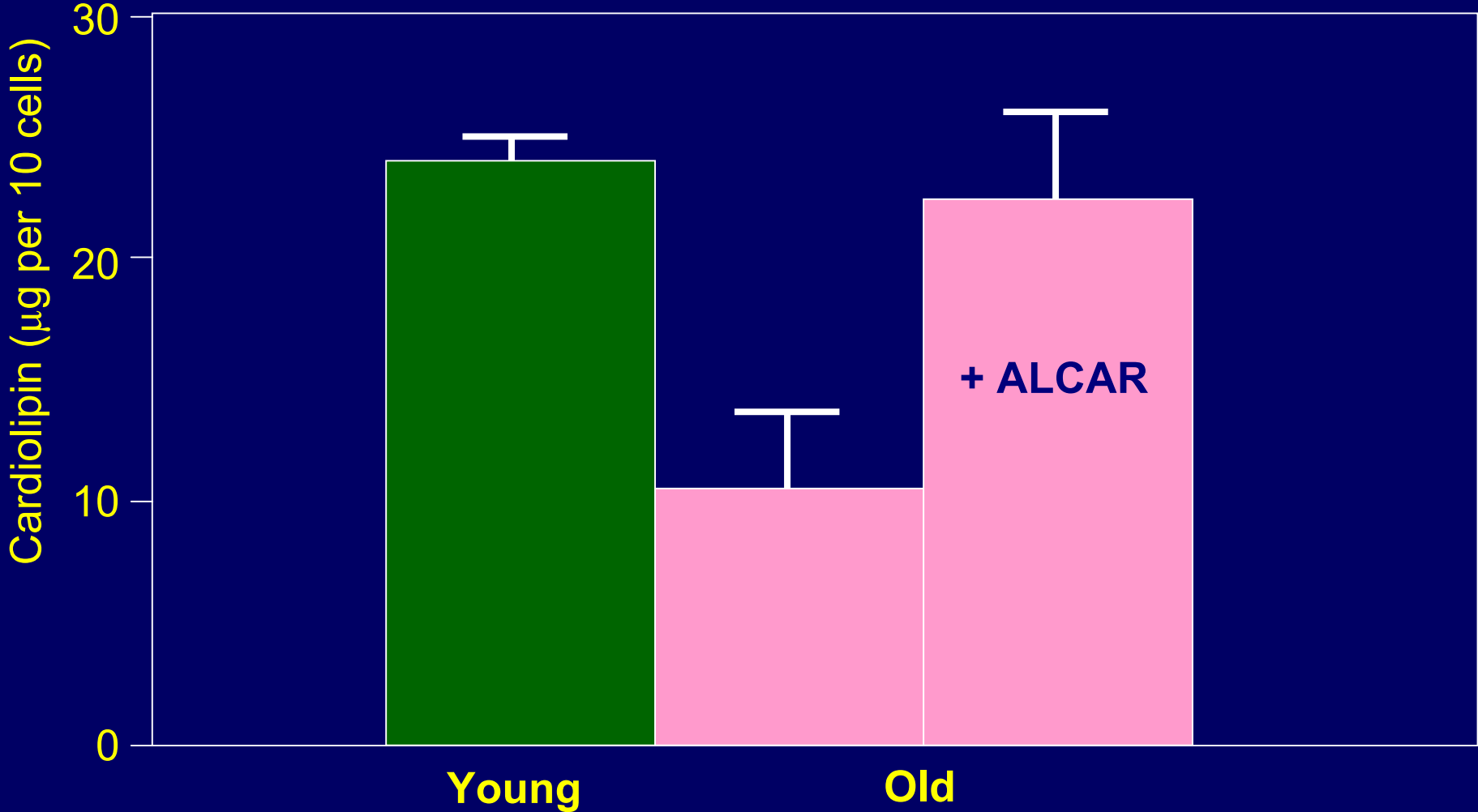
Intermembrane Space

Inner Membrane

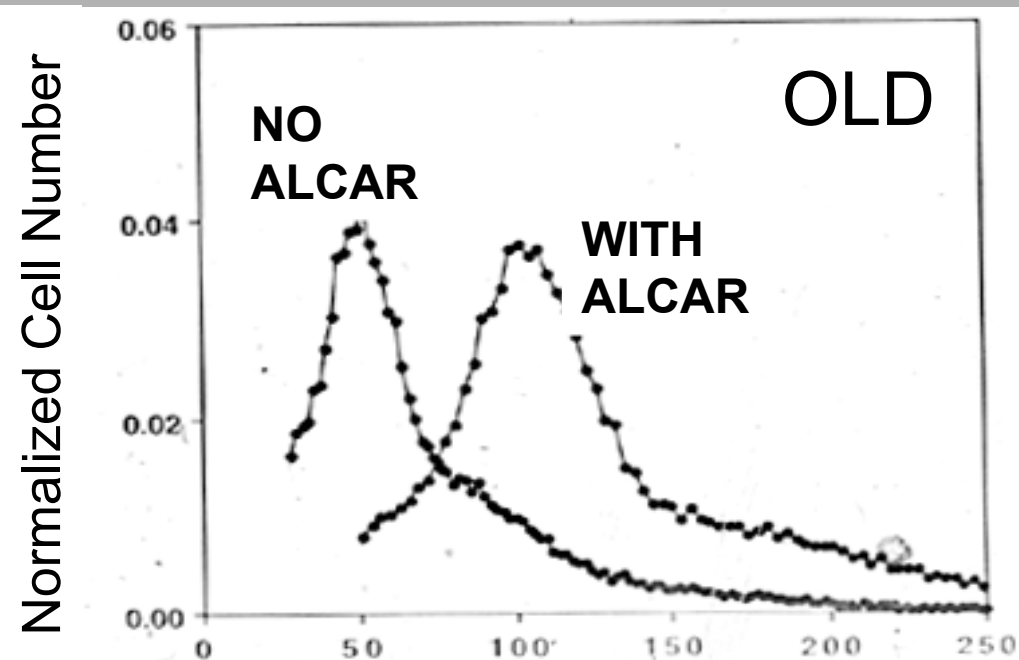
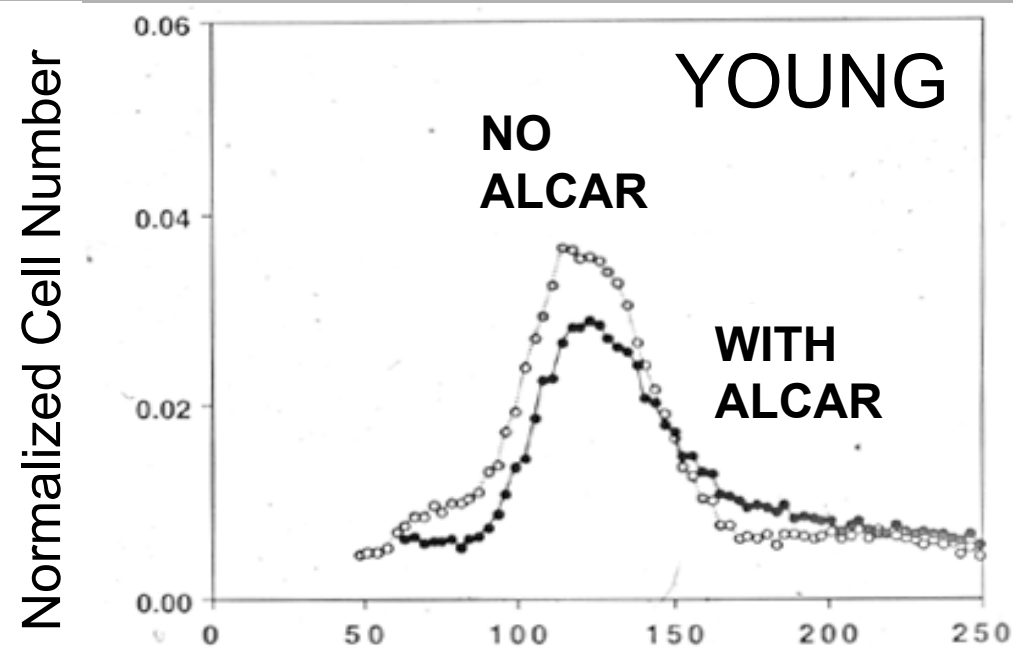
Mitochondrial Matrix



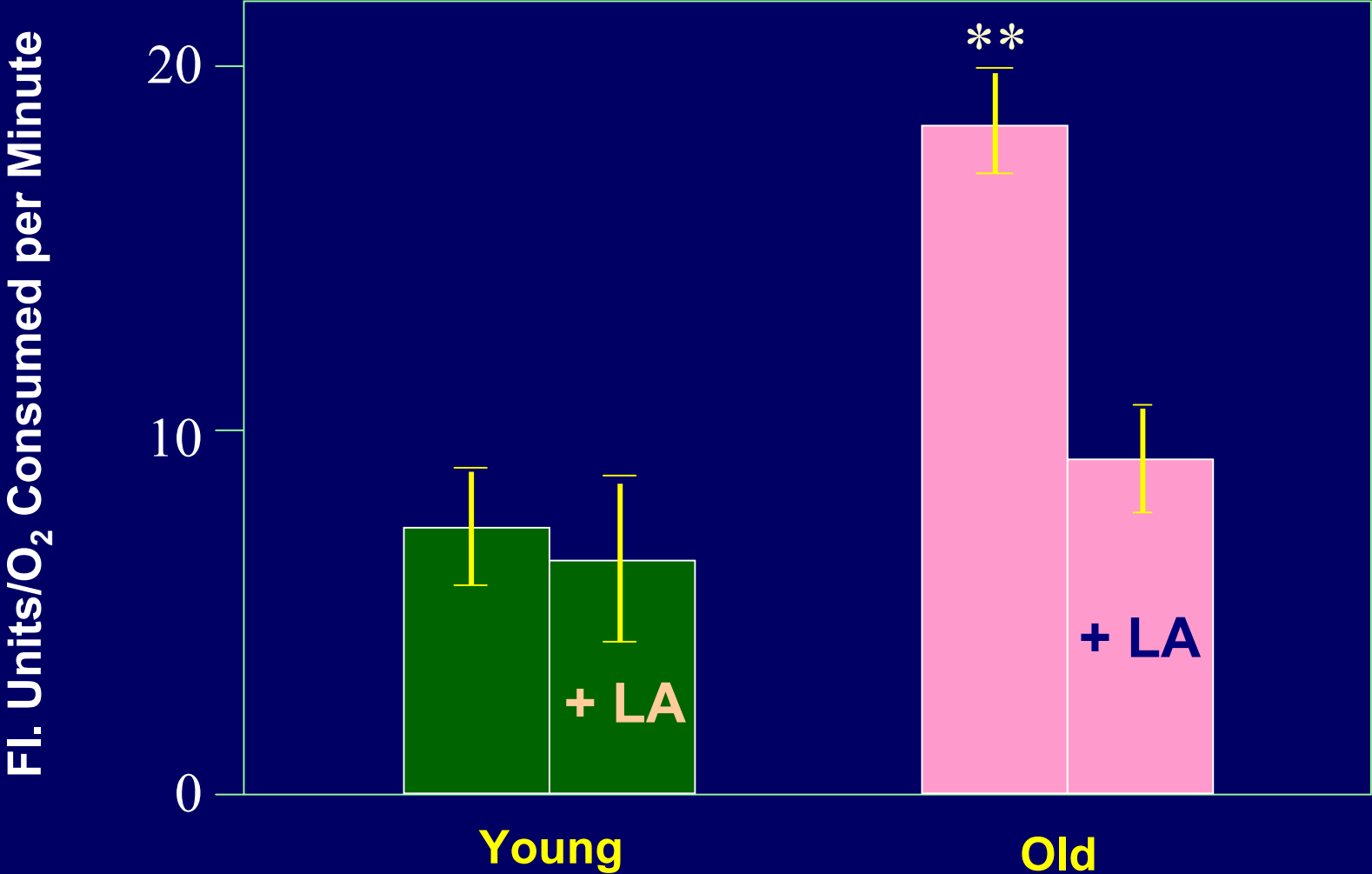
Effect of ALCAR Supplementation on Cardiolipin Levels



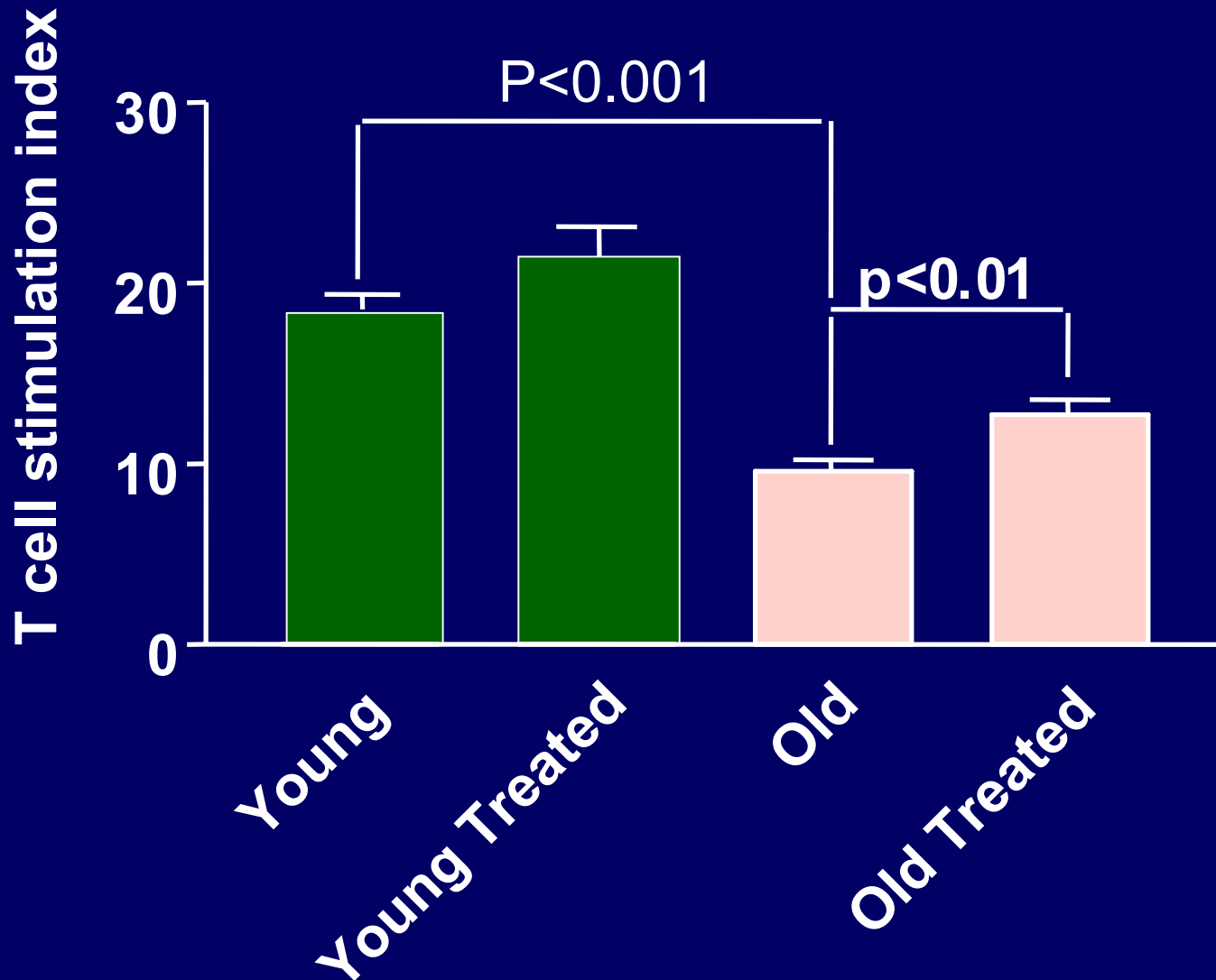
R123 Fluorescence in Young and Old Rat Hepatocytes



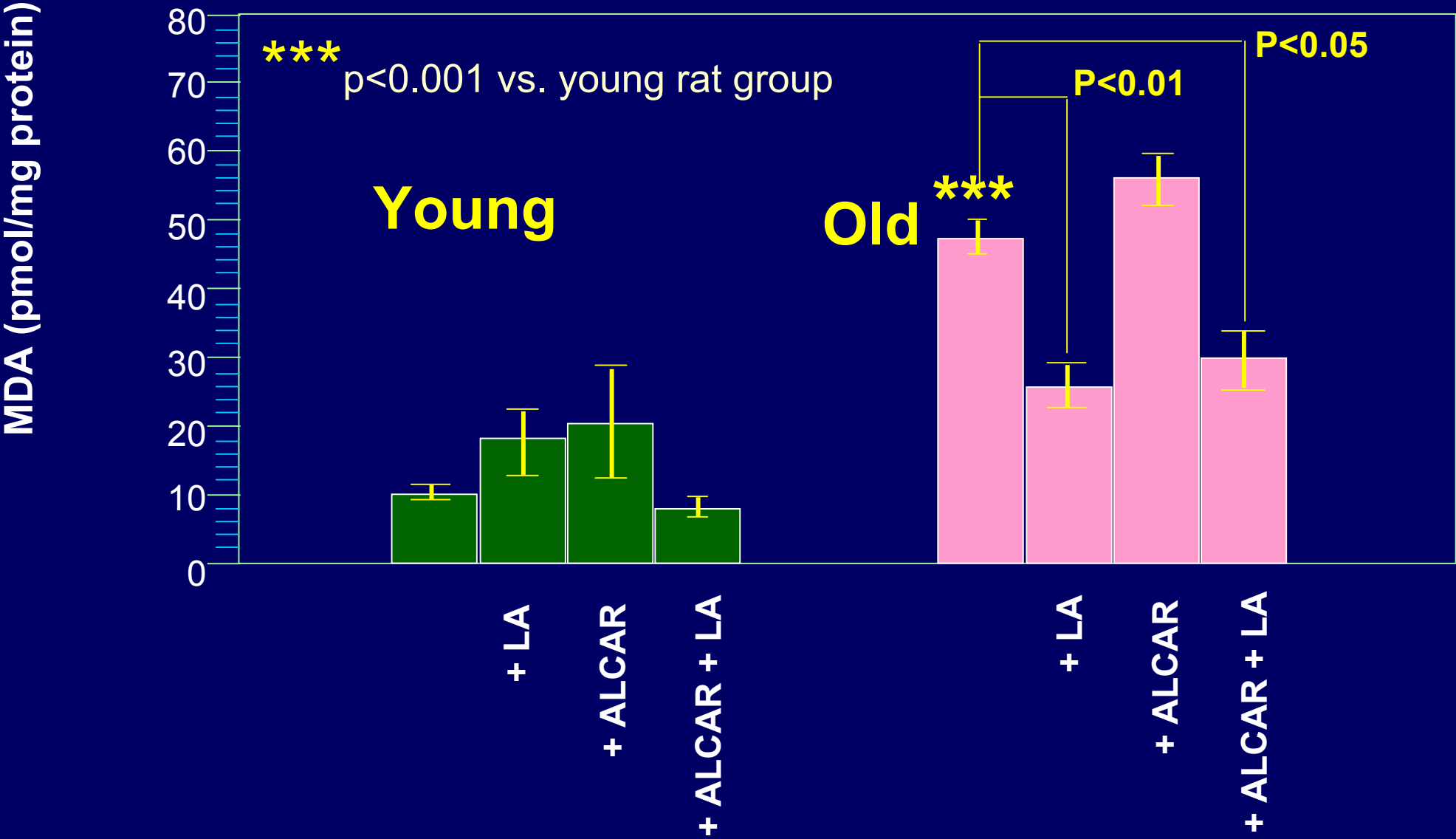
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats



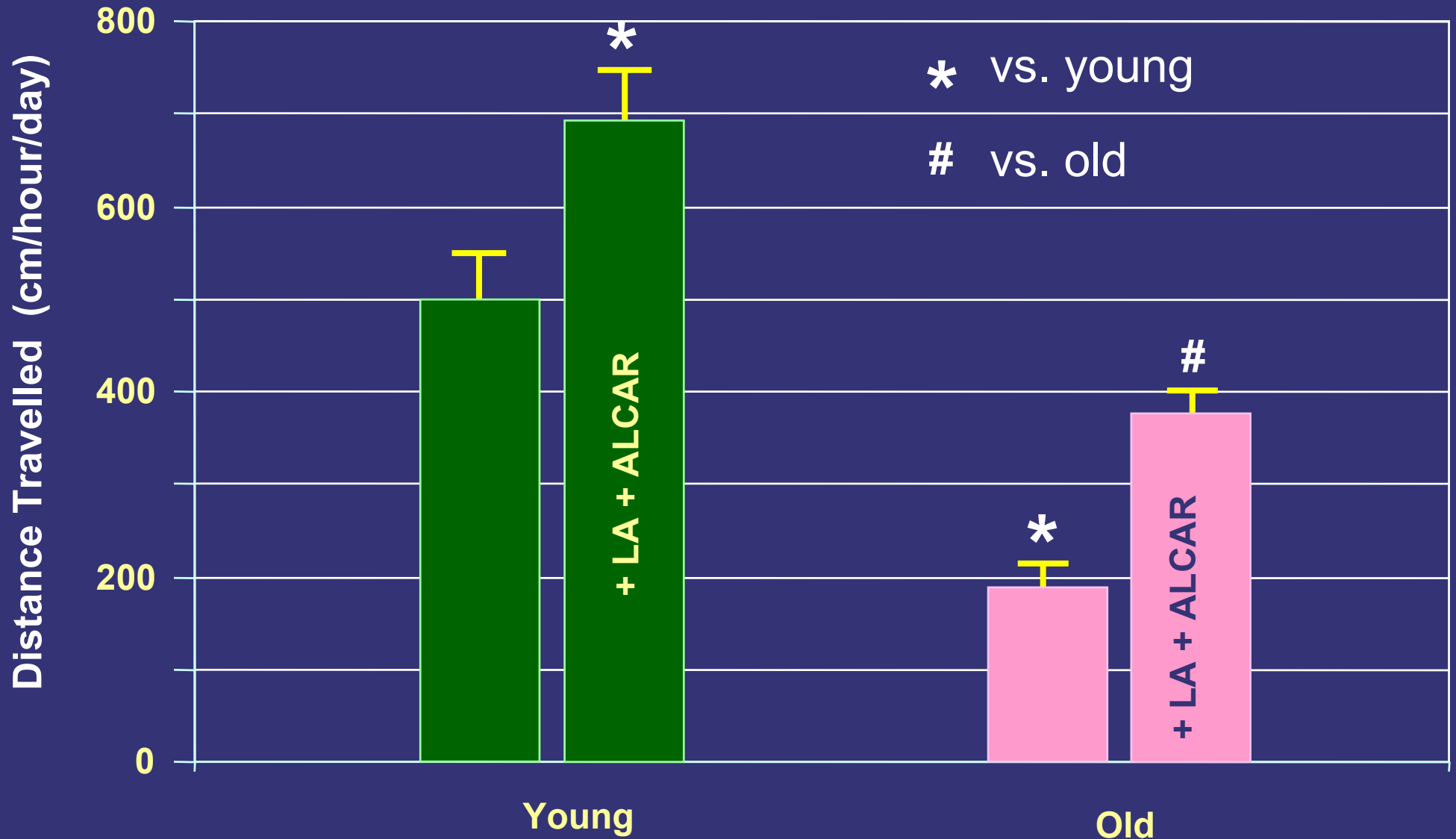
Age-associated decrease in immune function and the effect of ALCAR (0.2%) + LA (0.1%) treatment for 2 months. Values are mean + SEM of 10-11 animals.



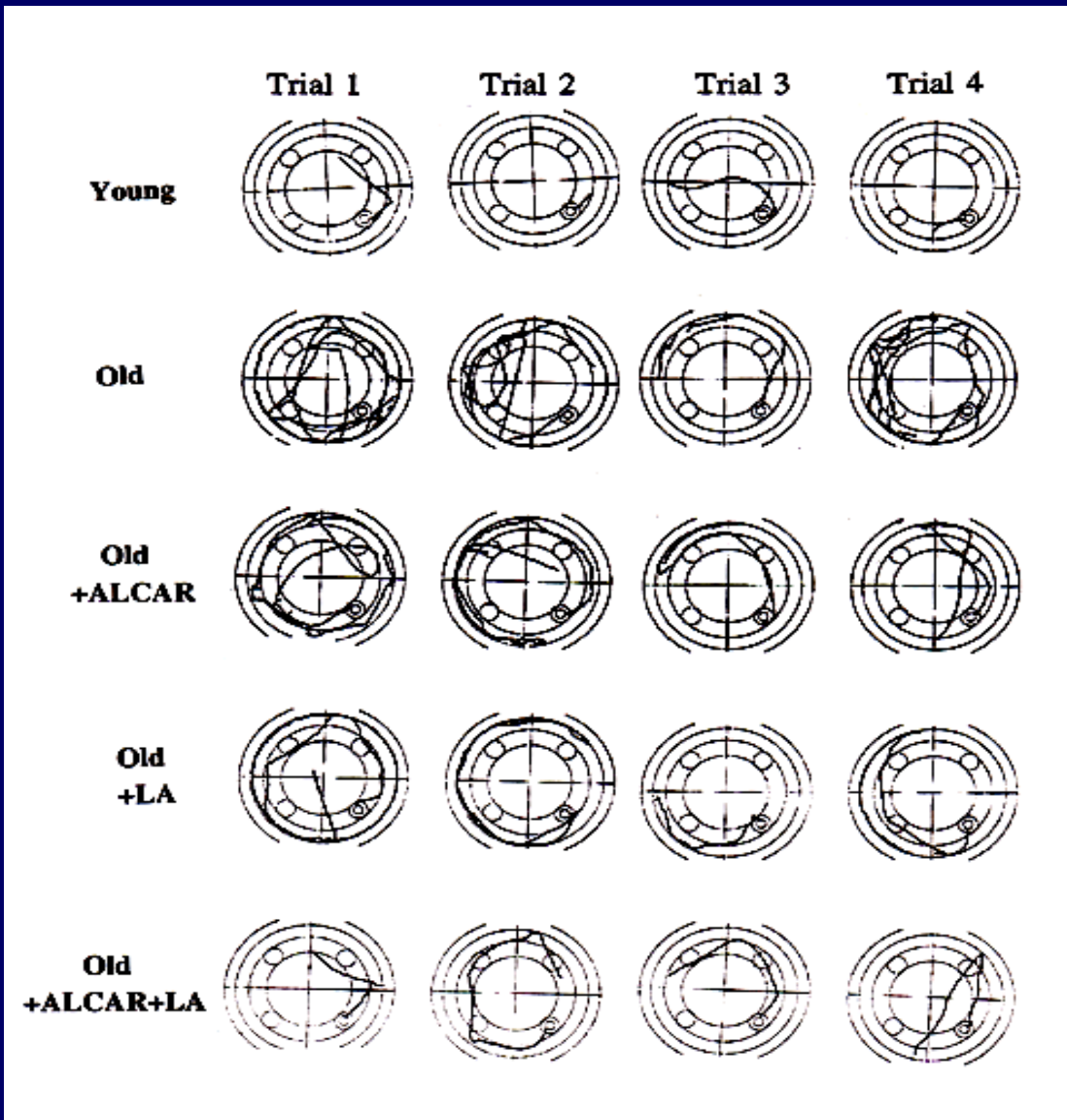
MDA levels in young and old rats with LA, ALCAR, or both



Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)



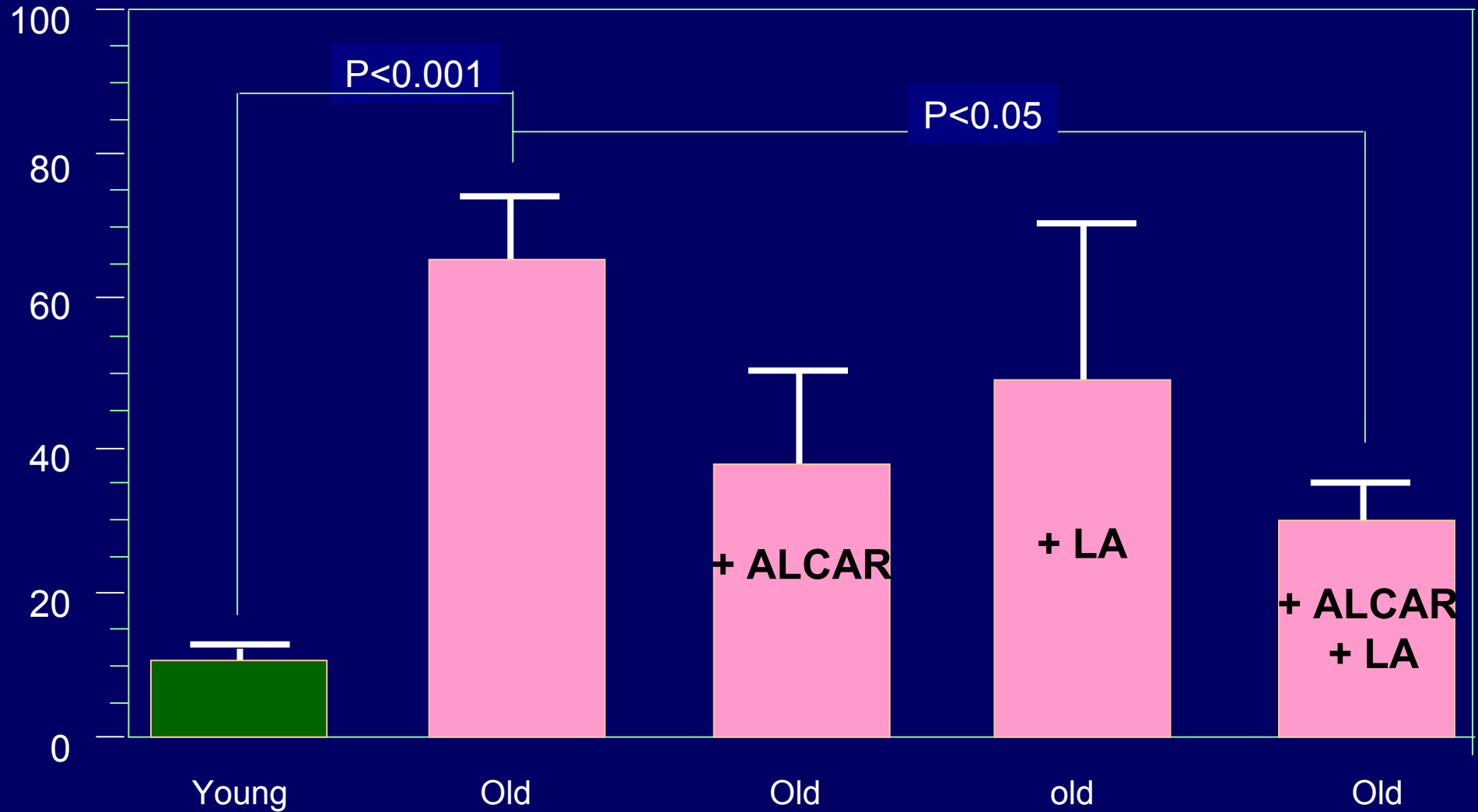
Morris Water Maze for Testing Spatial Memory



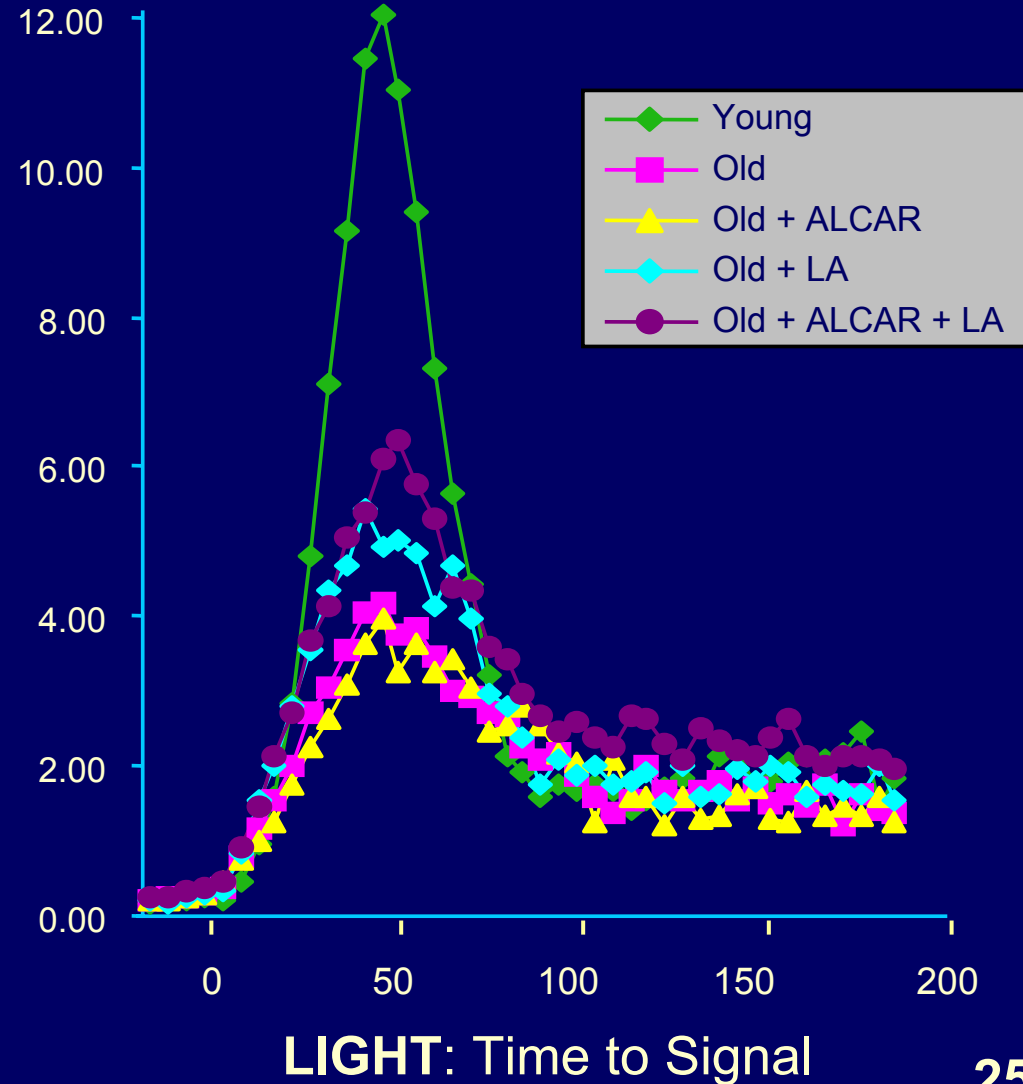
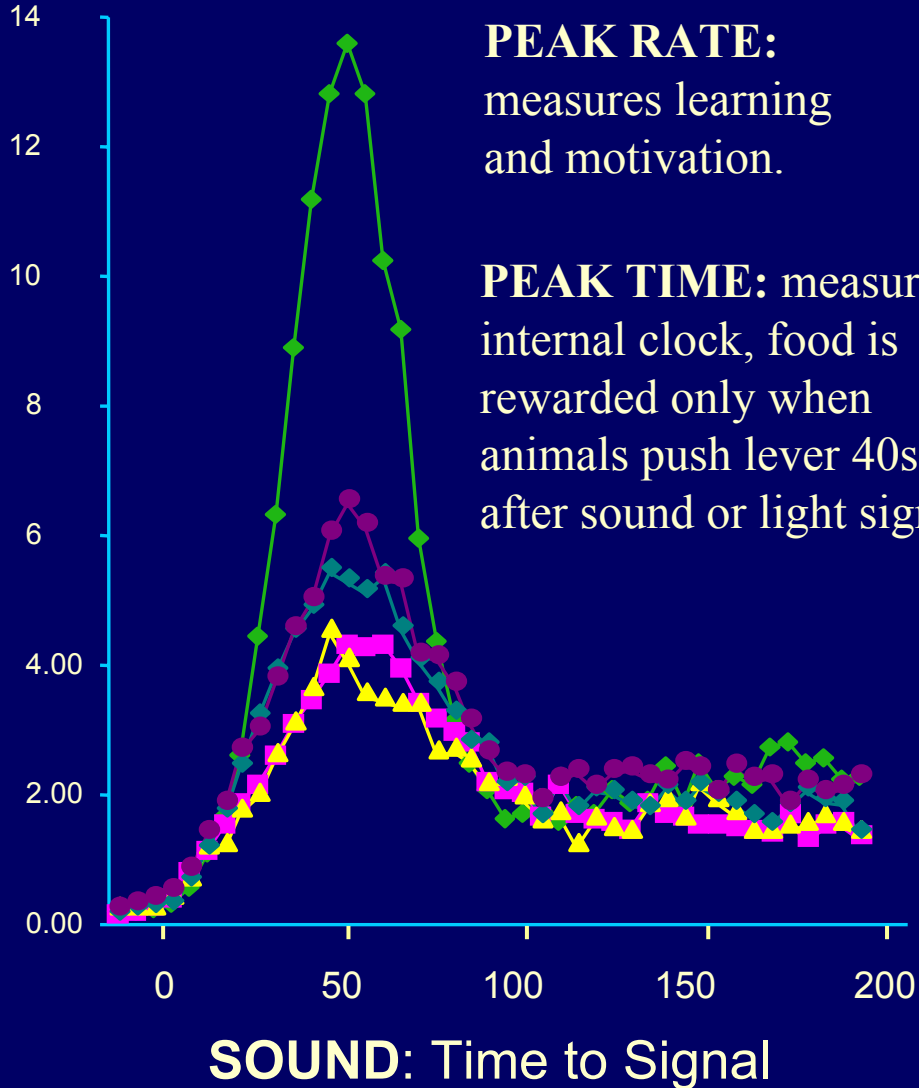
Spatial Memory relies on intact hippocampal function.

Treatments improved poor memory in old rats

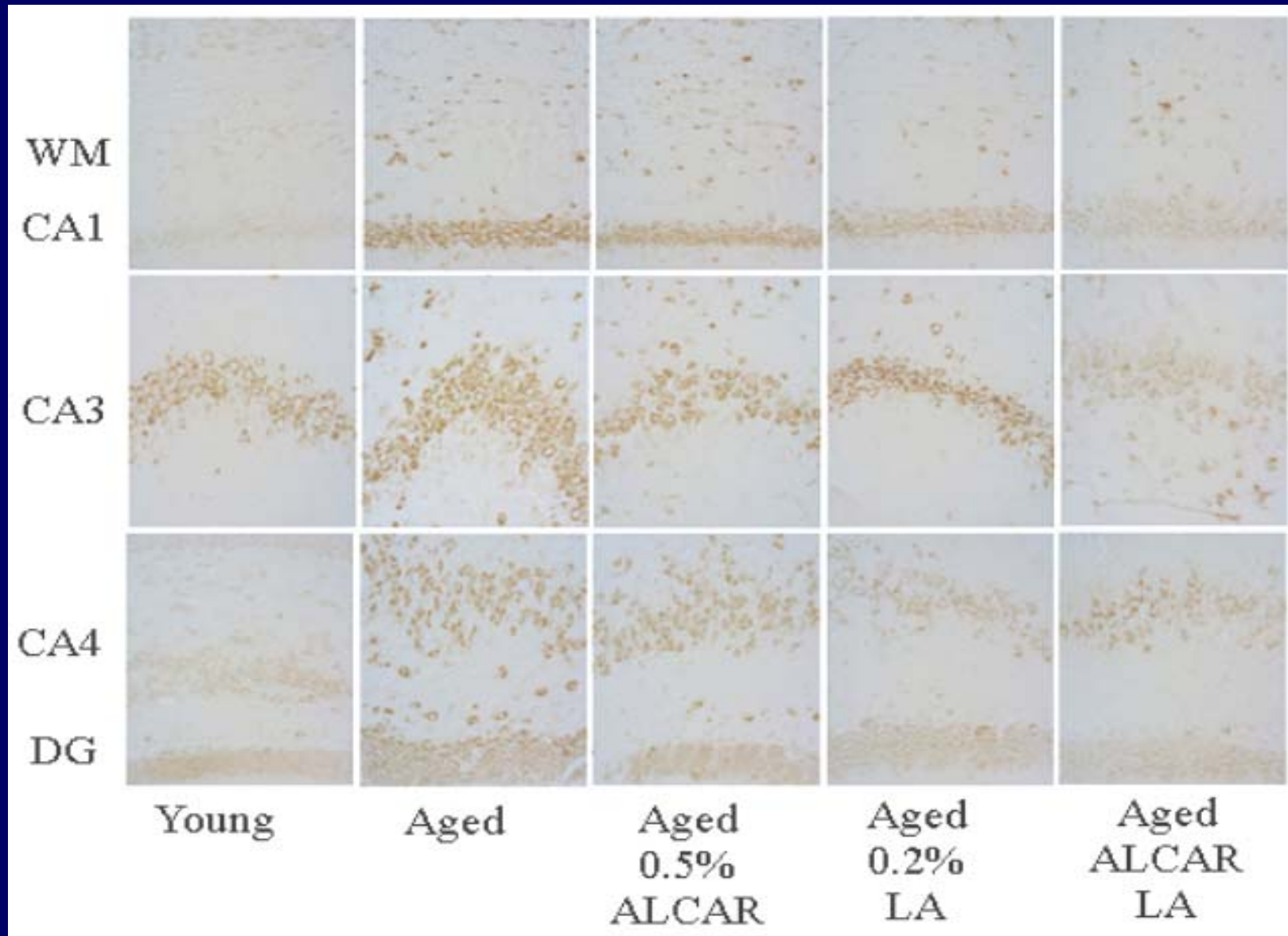
Spatial Memory Tested With Morris Water Maze



Peak procedure: for measuring temporal memory. Associated with striatum, cerebellum, & hippocampus



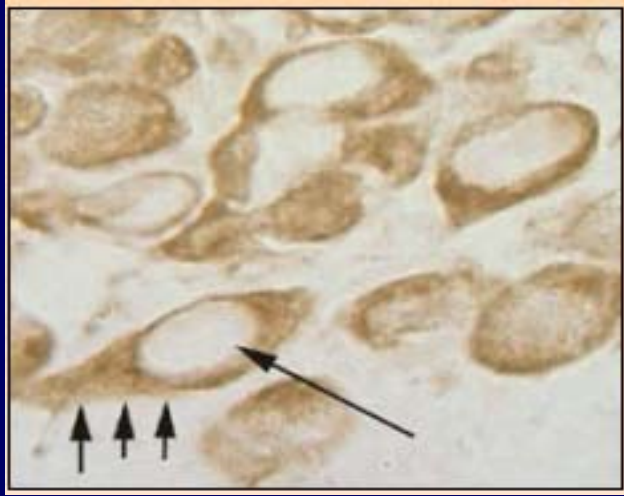
Oxidative Damage to Nucleic Acid in Old Rats by mAb to oxo8G/oxo8dG: Immunohistochemical stain of neurons



Staining of oxidized nucleic acid in neurons (mAb to oxo8dG in DNA/oxo8G in RNA)

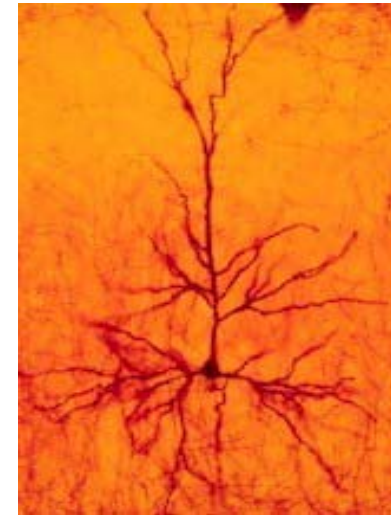
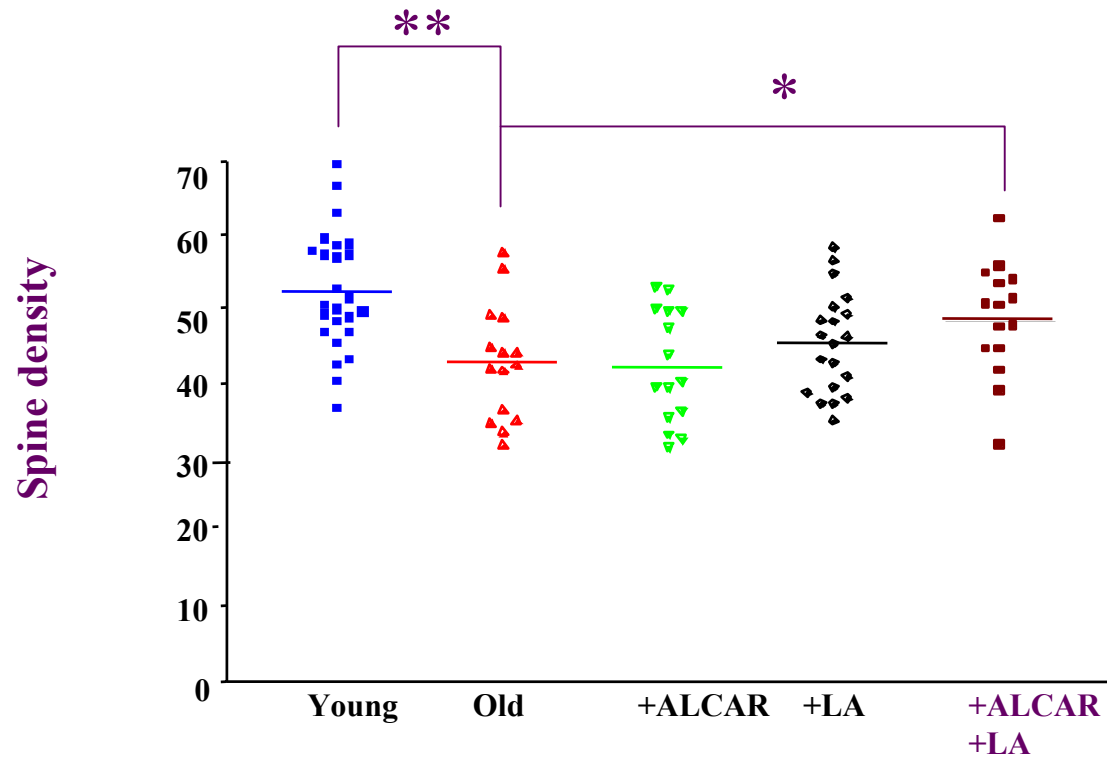
RNA is Oxidized

(92% is removed by RNase)

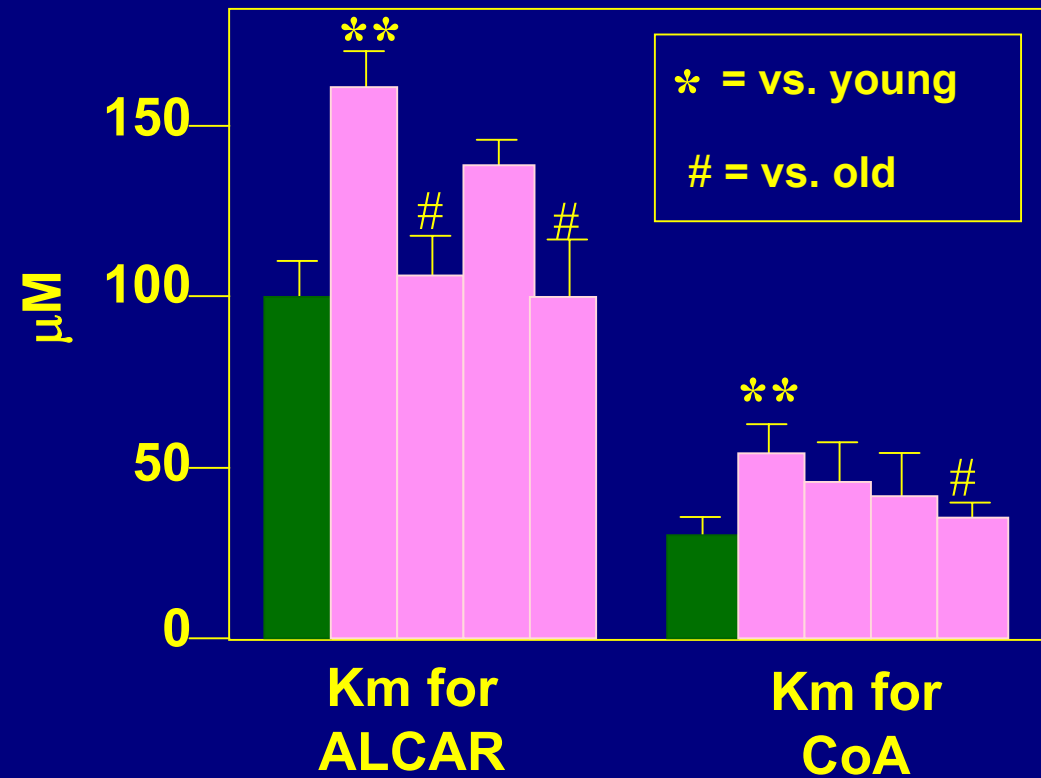
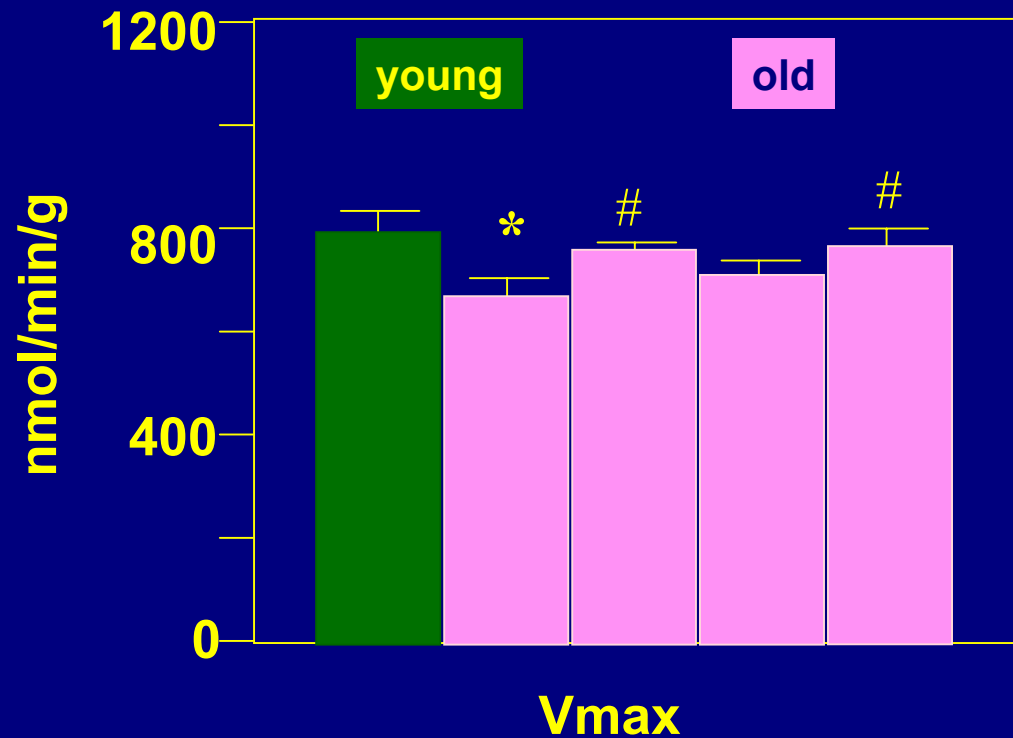
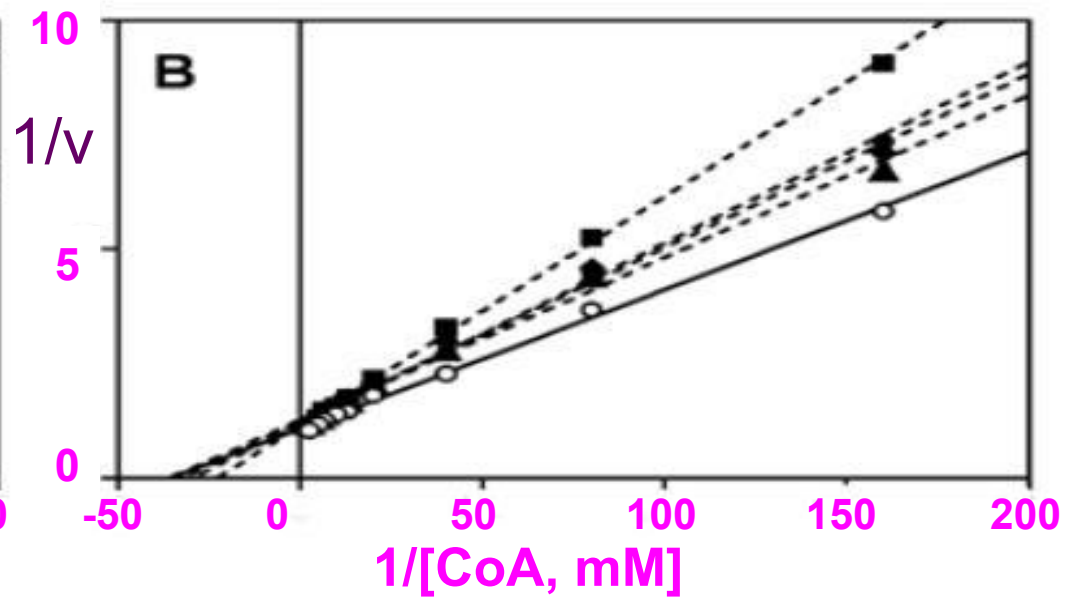
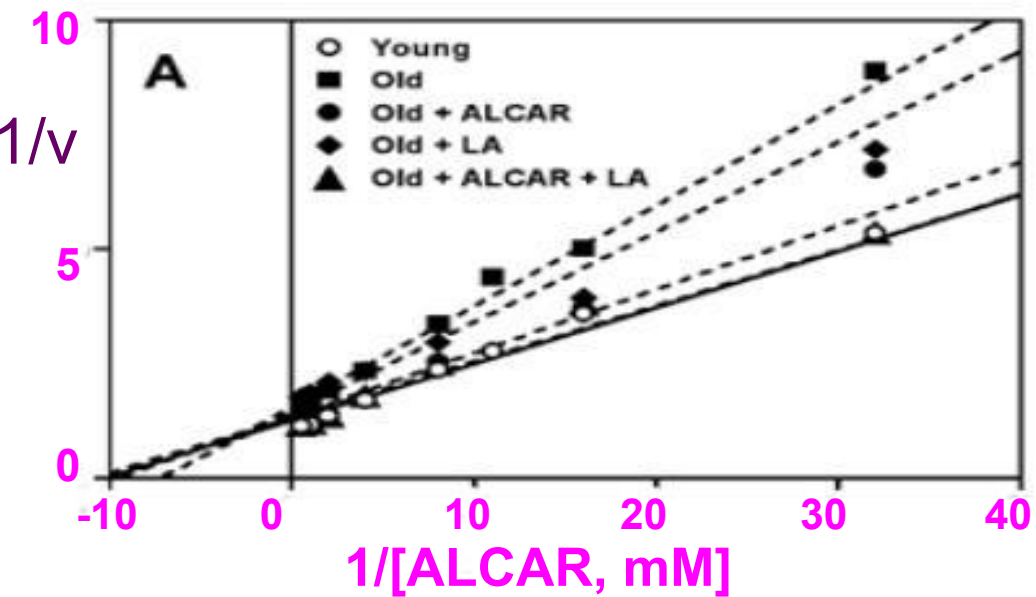


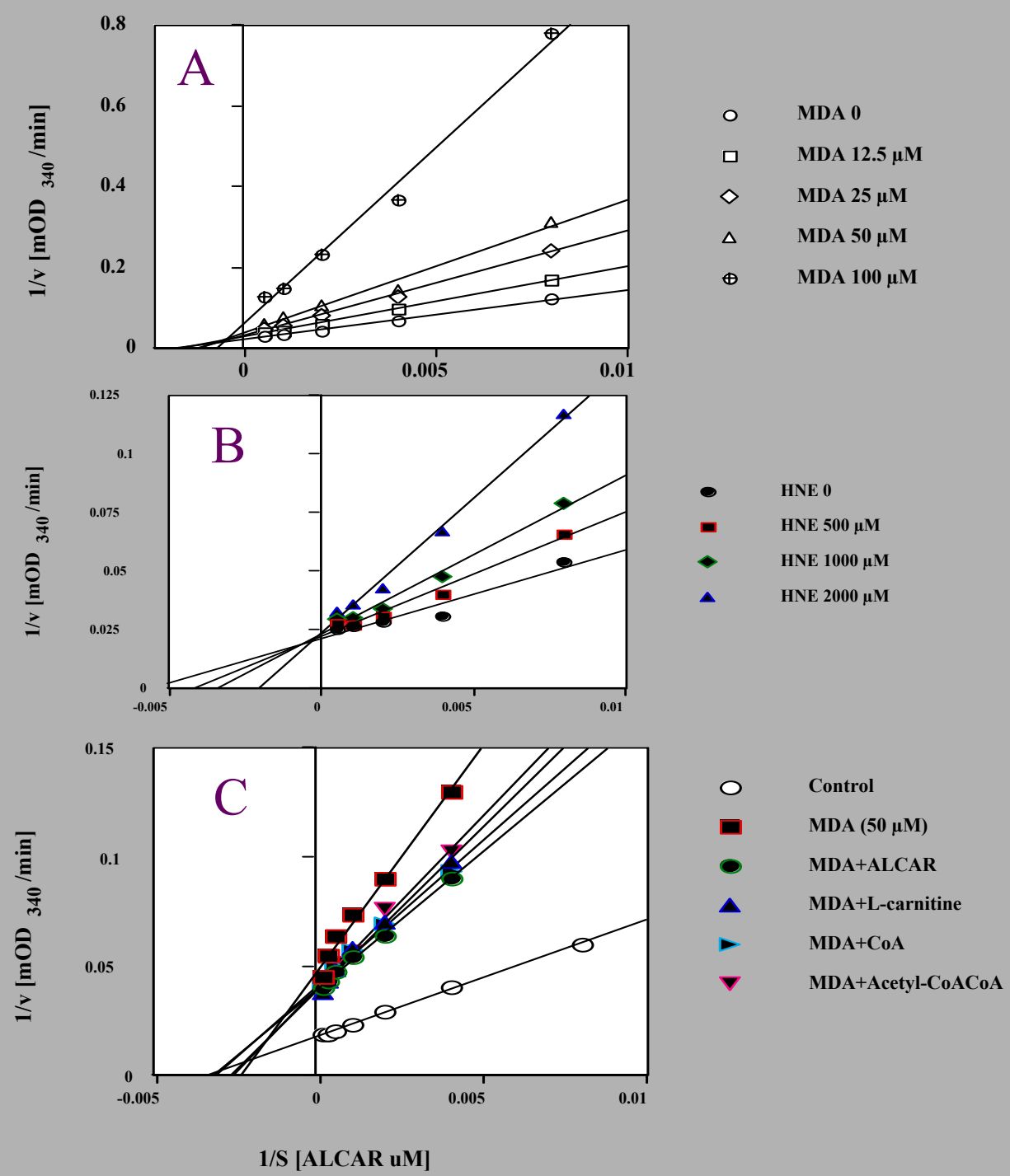
*oxo8G: 8-hydroxyguanosine; oxo8dG: 8-hydroxy-2'-deoxyguanosine

Dendritic spine density of cortical neurons in rat brain with Golgi staining: Effects of 11 month treatment with ALCAR, LA or ALCAR+LA



Liu, Mervis, and Ames, Unpublished data





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