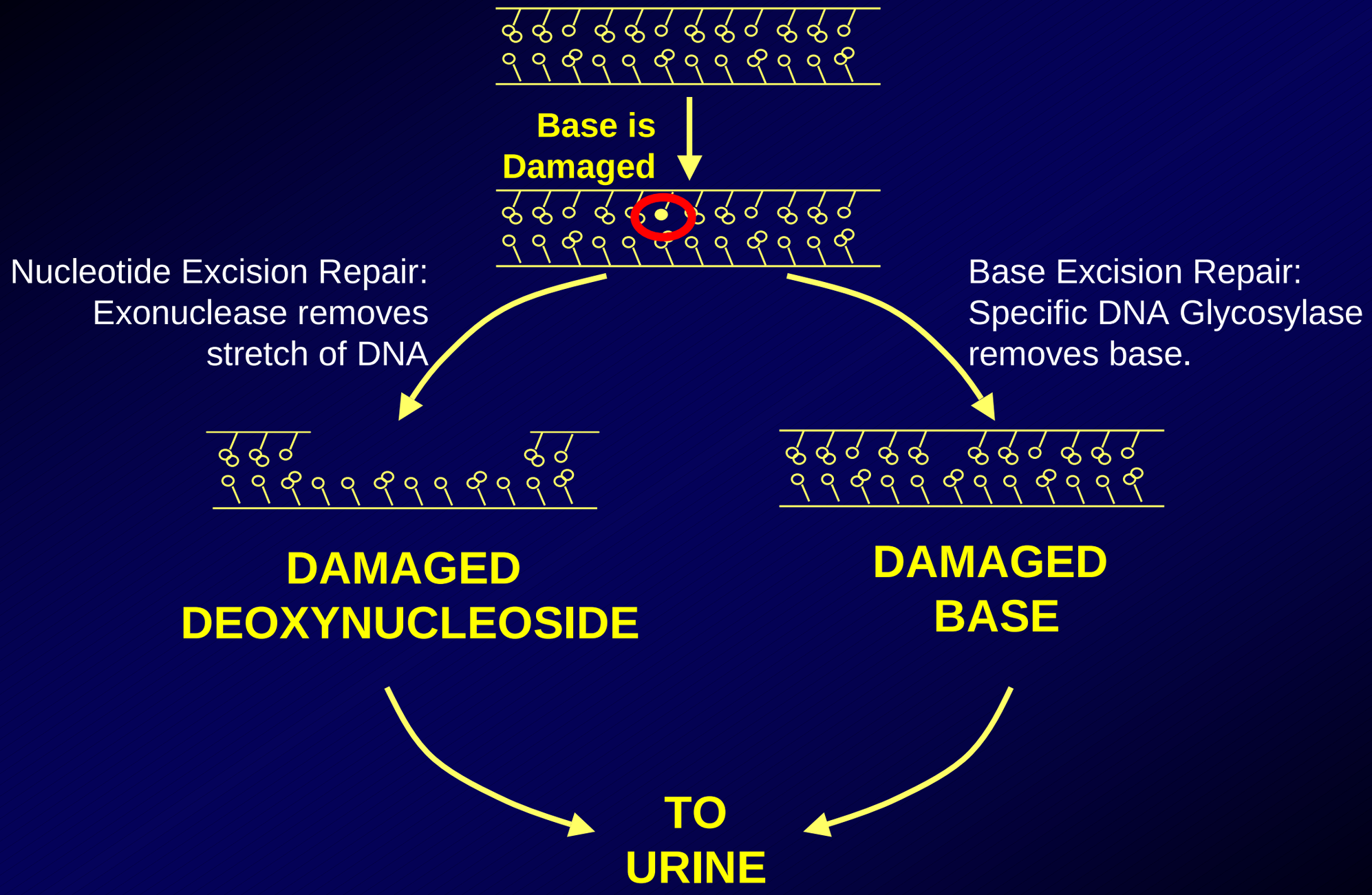
Delaying (or Accelerating) the Degenerative Diseases of Aging

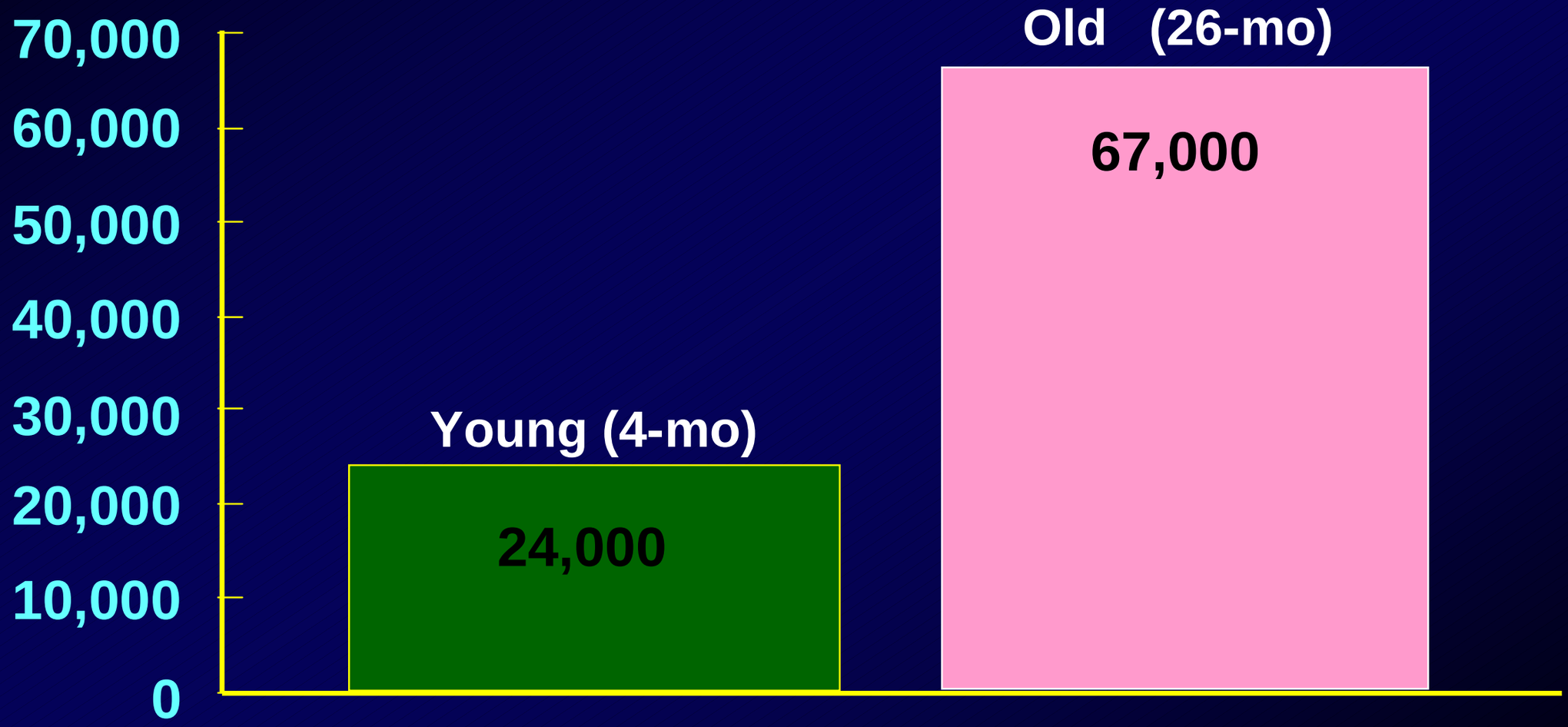
Bruce N. Ames

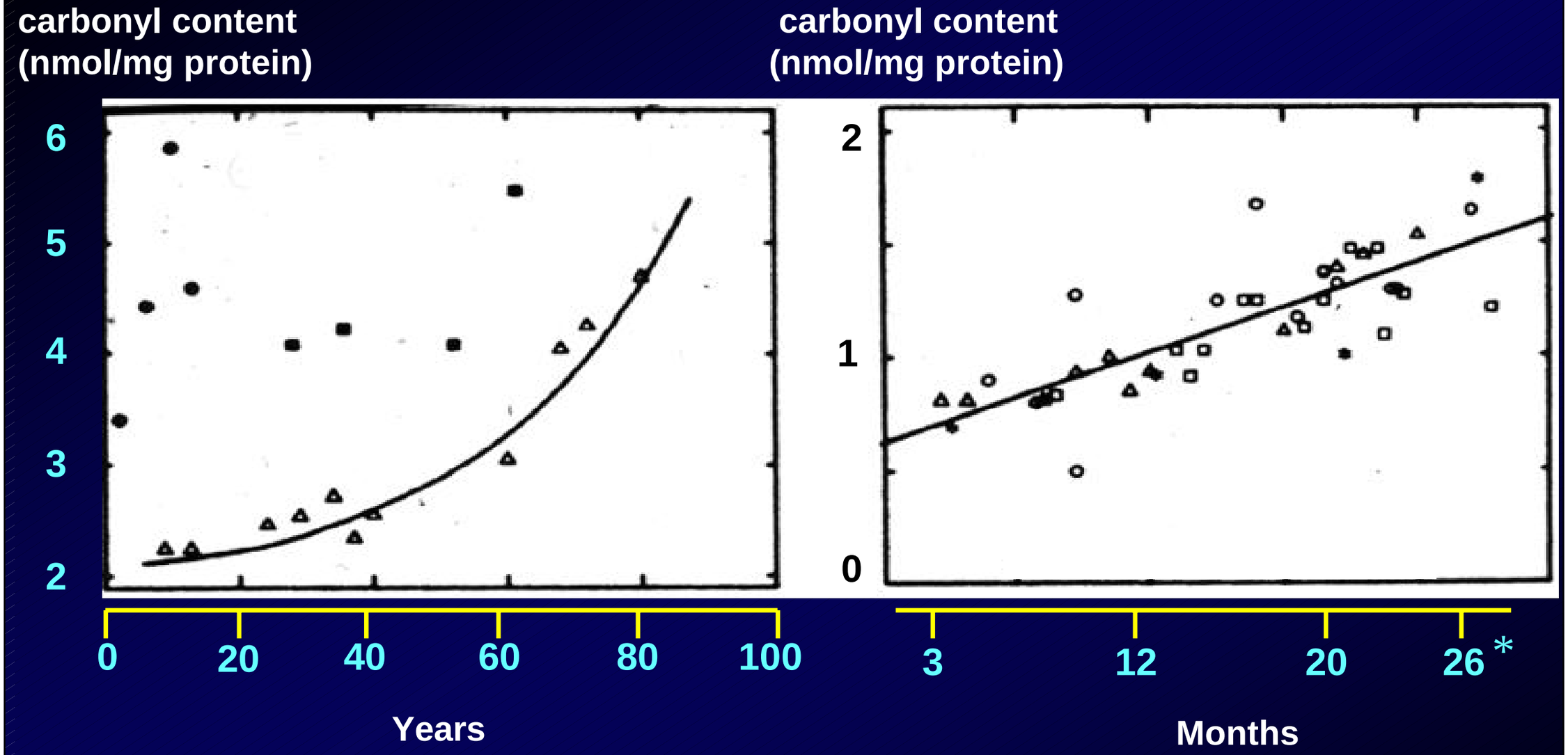
Children's Hospital Oakland Research Institute
Professor, University of California, Berkeley

'06AU6
Portland
DDP

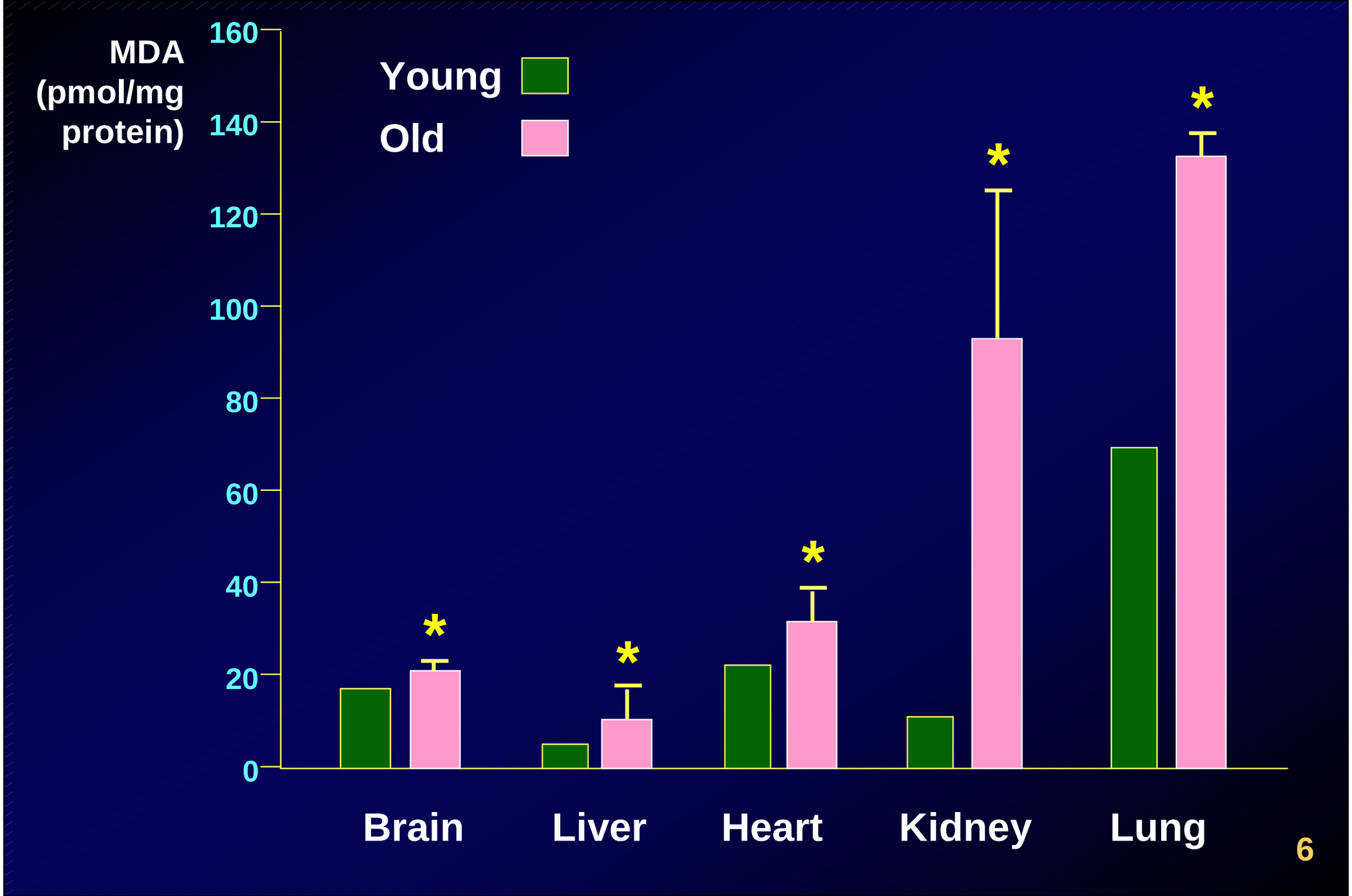

Base is Damaged
Nucleotide Excision Repair: Exonuclease removes stretch of DNA
Base Excision Repair: Specific DNA Glycosylase removes base.
DAMAGED DEOXYNUCLEOSIDE
DAMAGED BASE
TO URINE

Estimated oxidative DNA adducts per rat liver cell





Source: E. Stadtman, *Science* 257, 1220-1224 (1992)


MDA (pmol/mg protein)
160
140
120
100
80
60
40
20
0
Young
Old
*
*
*
*
*
Brain
Liver
Heart
Kidney
Lung

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 10771-10778, November 1994

Review

Oxidative damage and mitochondrial decay in aging

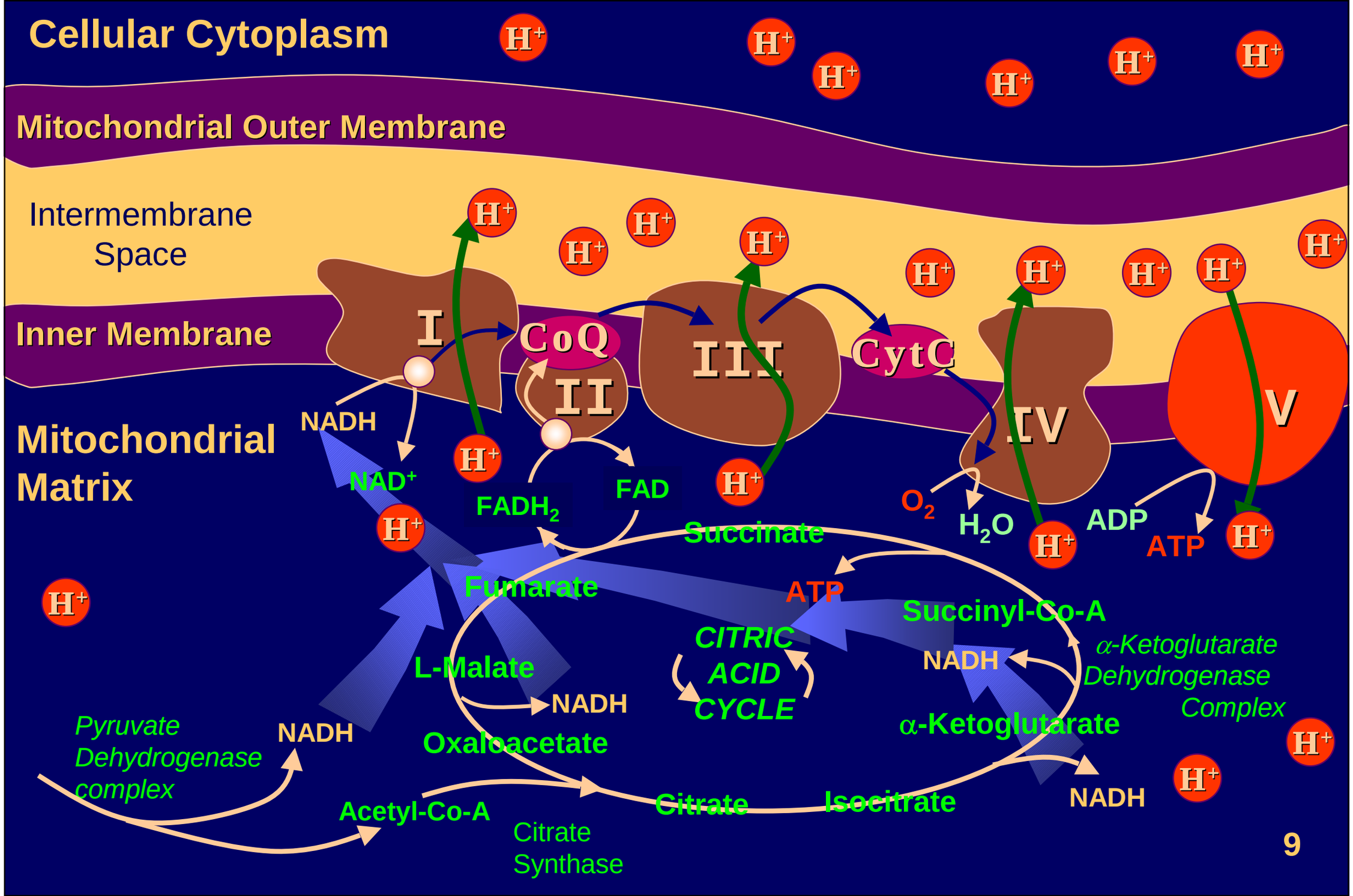
(bioenergetics / mitochondrial DNA / cardiolipin / acetyl-L-carnitine / neurodegeneration)

*Mark K. Shigenaga, Tory M. Hagen, and Bruce N. Ames**
Division of Biochemistry and Molecular Biology,
401 Barker Hall, University of California, Berkeley, CA 94720

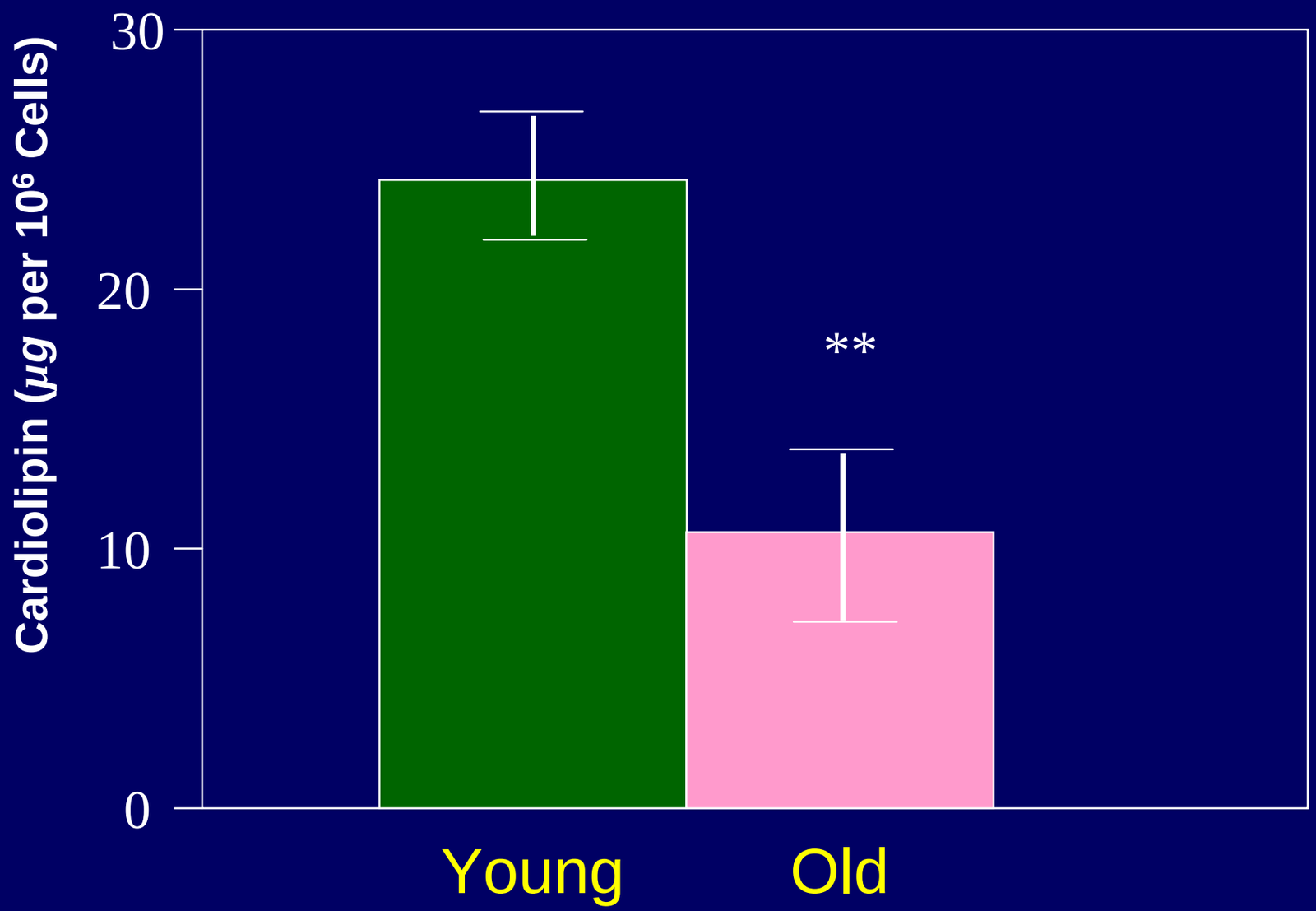
Contributed by Bruce N. Ames, July 27, 1994

Mitochondria in hippocampal neurons

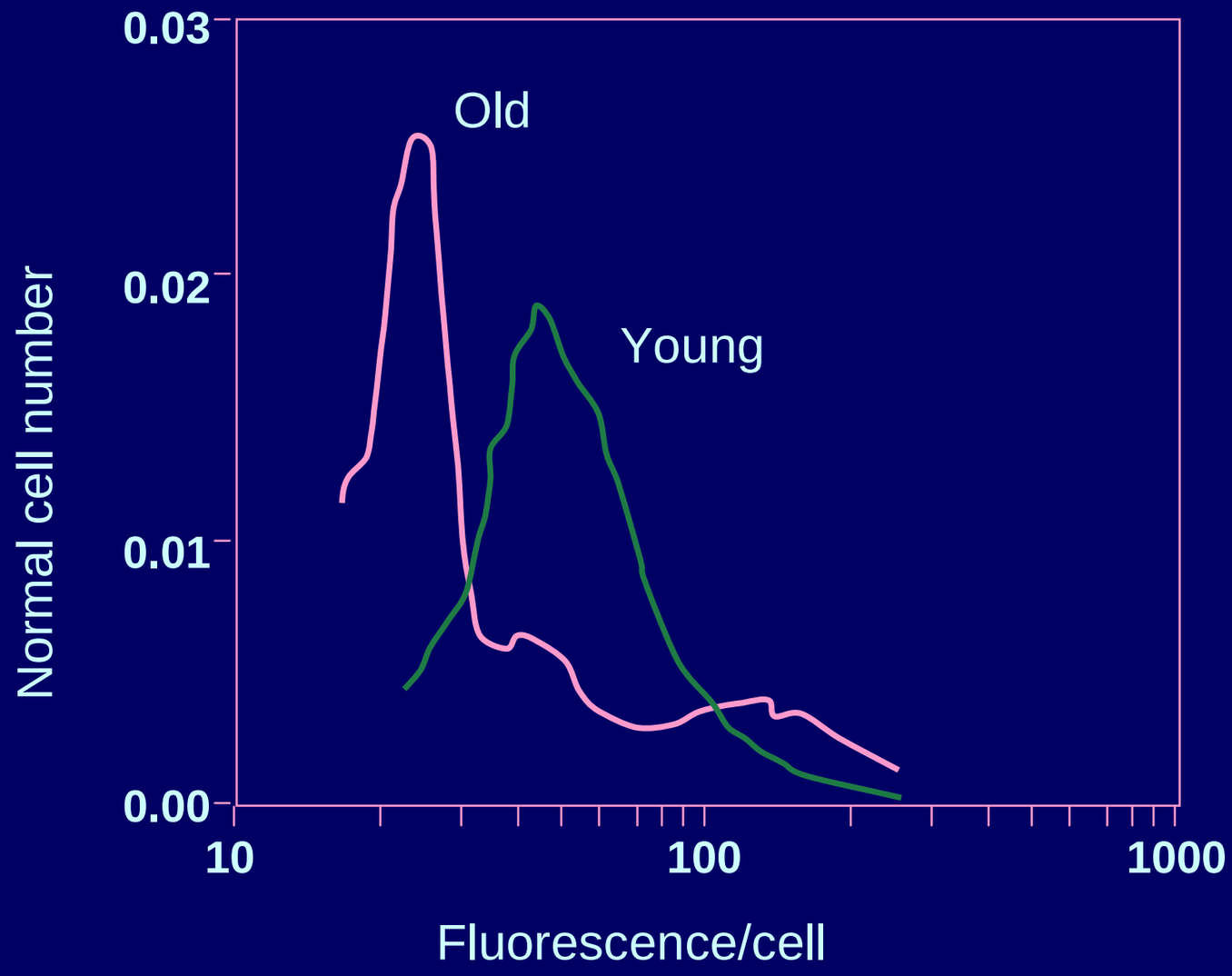
Electron Microscopy Images


Cellular Cytoplasm
Mitochondrial Outer Membrane
Intermembrane Space
Inner Membrane
Mitochondrial Matrix
I
II
III
IV
V
CoQ
CytC
NADH
NAD+
FADH2
FAD
O2
H2O
ADP
ATP
Succinate
Fumarate
L-Malate
Oxaloacetate
Citrate
Isocitrate
CITRIC ACID CYCLE
Succinyl-Co-A
α-Ketoglutarate
α-Ketoglutarate Dehydrogenase Complex
Pyruvate Dehydrogenase complex
Acetyl-Co-A
Citrate Synthase
H+
9

Cardiolipin Levels in 3 and 24 Month Old Rat Hepatocytes



R123 Fluorescence in old and young rat hepatocytes



Mitochondria from old rats compared to those from young rats:

1) Lower Cardiolipin

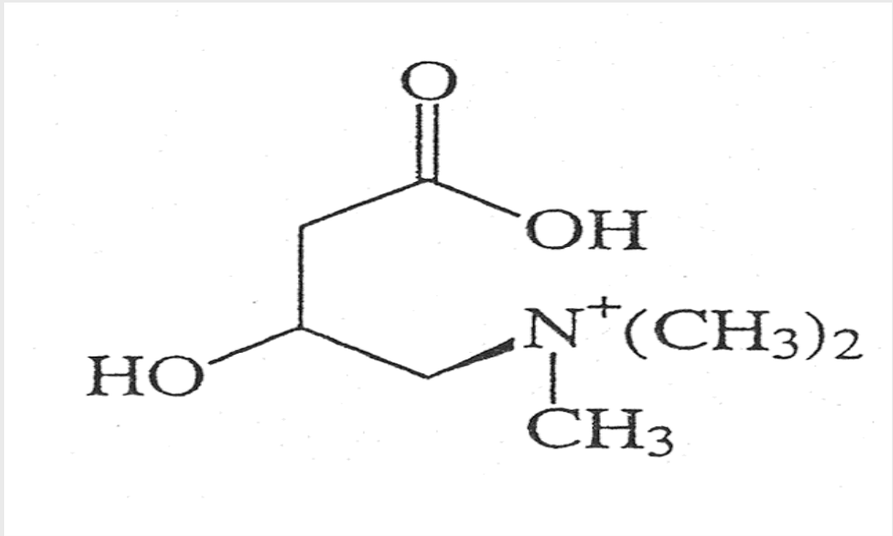
2) Lower Membrane Potential

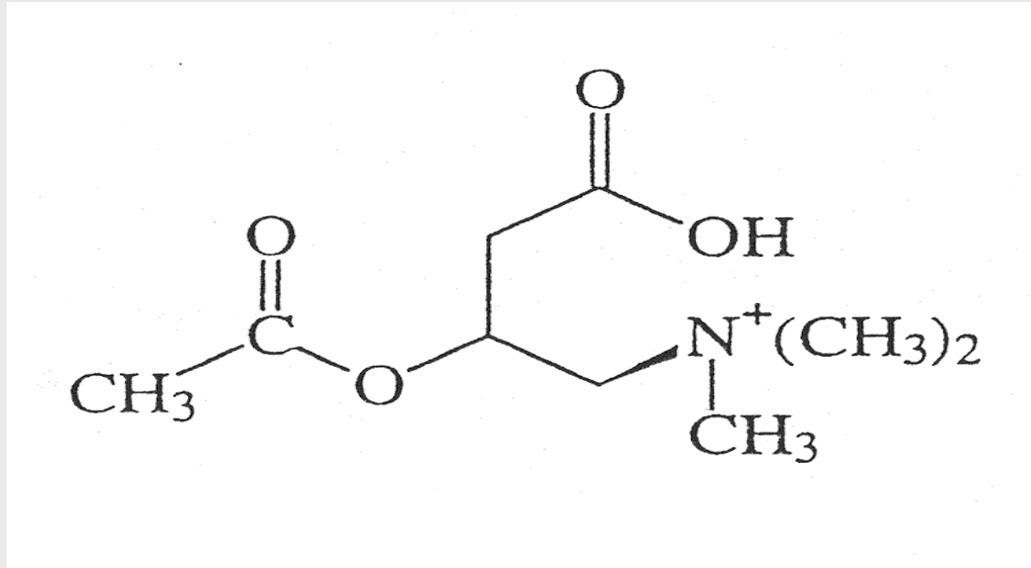
3) Lower Oxygen Utilization

4) Increased Oxidant Leakage

L-Carnitine/Acetyl-L-Carnitine (ALCAR)

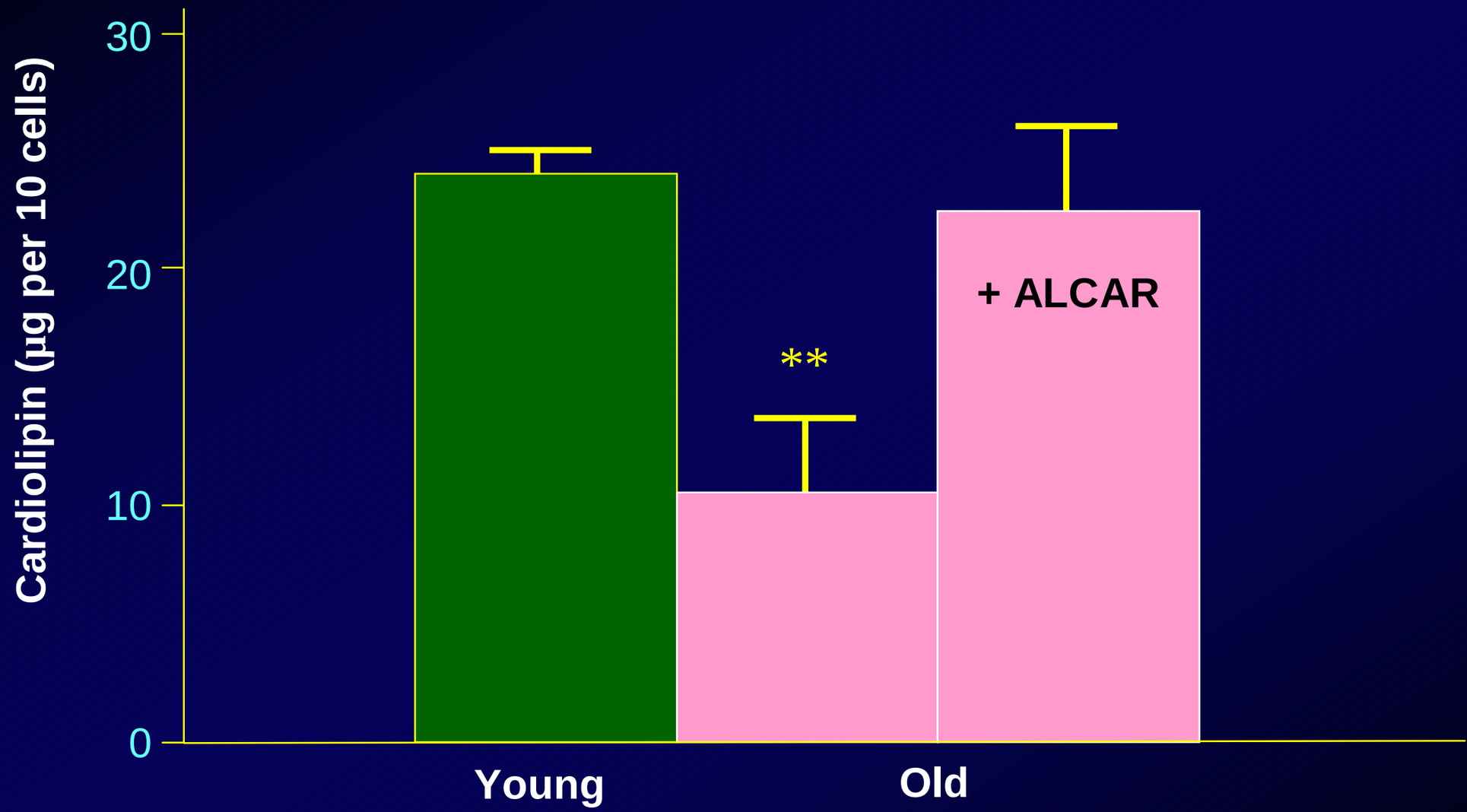
- Transports long-chain fatty acids into mitochondria
- Removes short- and medium-chain fatty acids that accumulate



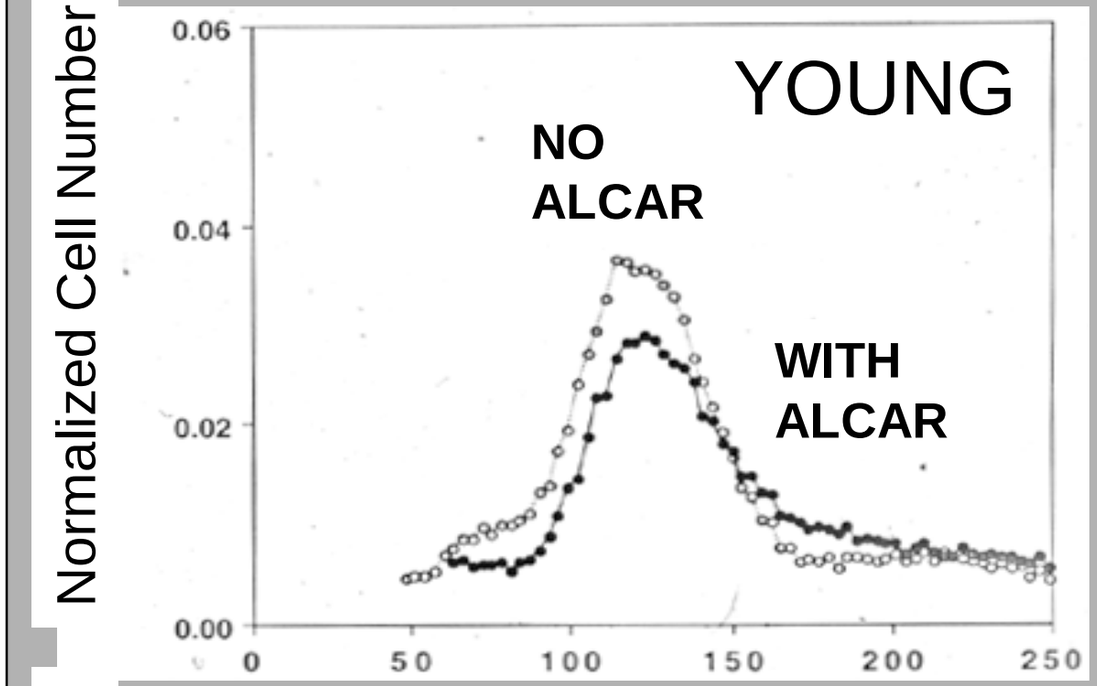


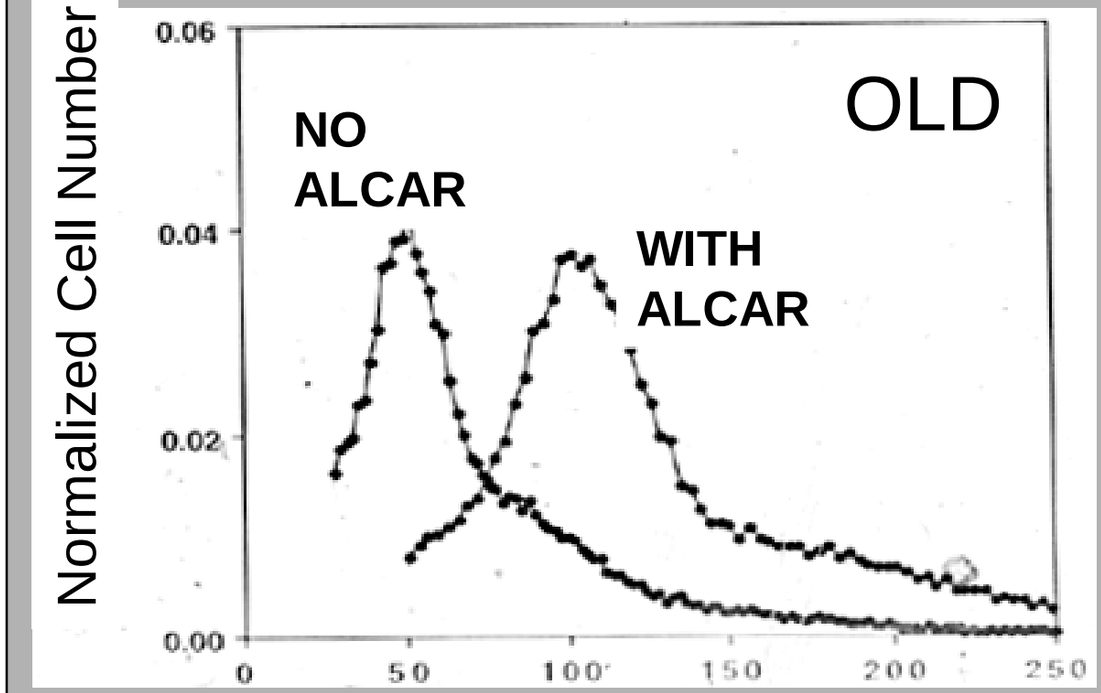
- Mediates the ratio of acetyl-CoA/CoA
- Decreases with age in plasma and in brain
- Improves cognitive function in rats

Effect of ALCAR Supplementation on Cardiolipin Levels



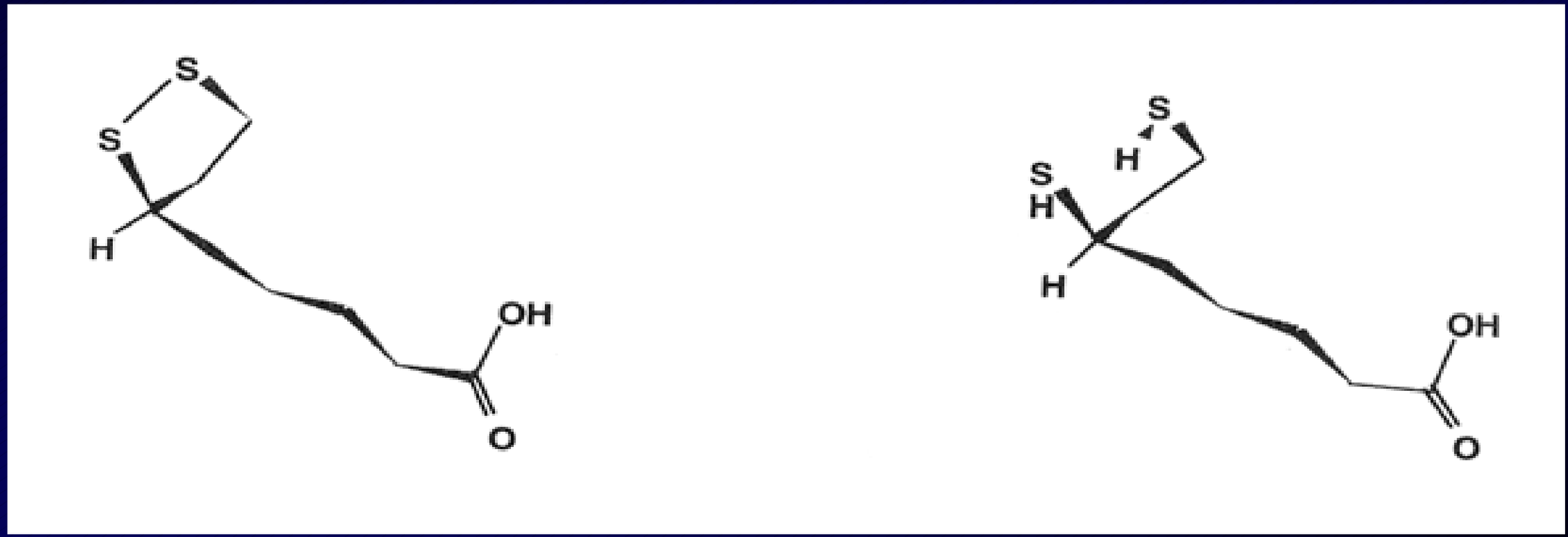
R123 Fluorescence in Young and Old Rat Hepatocytes



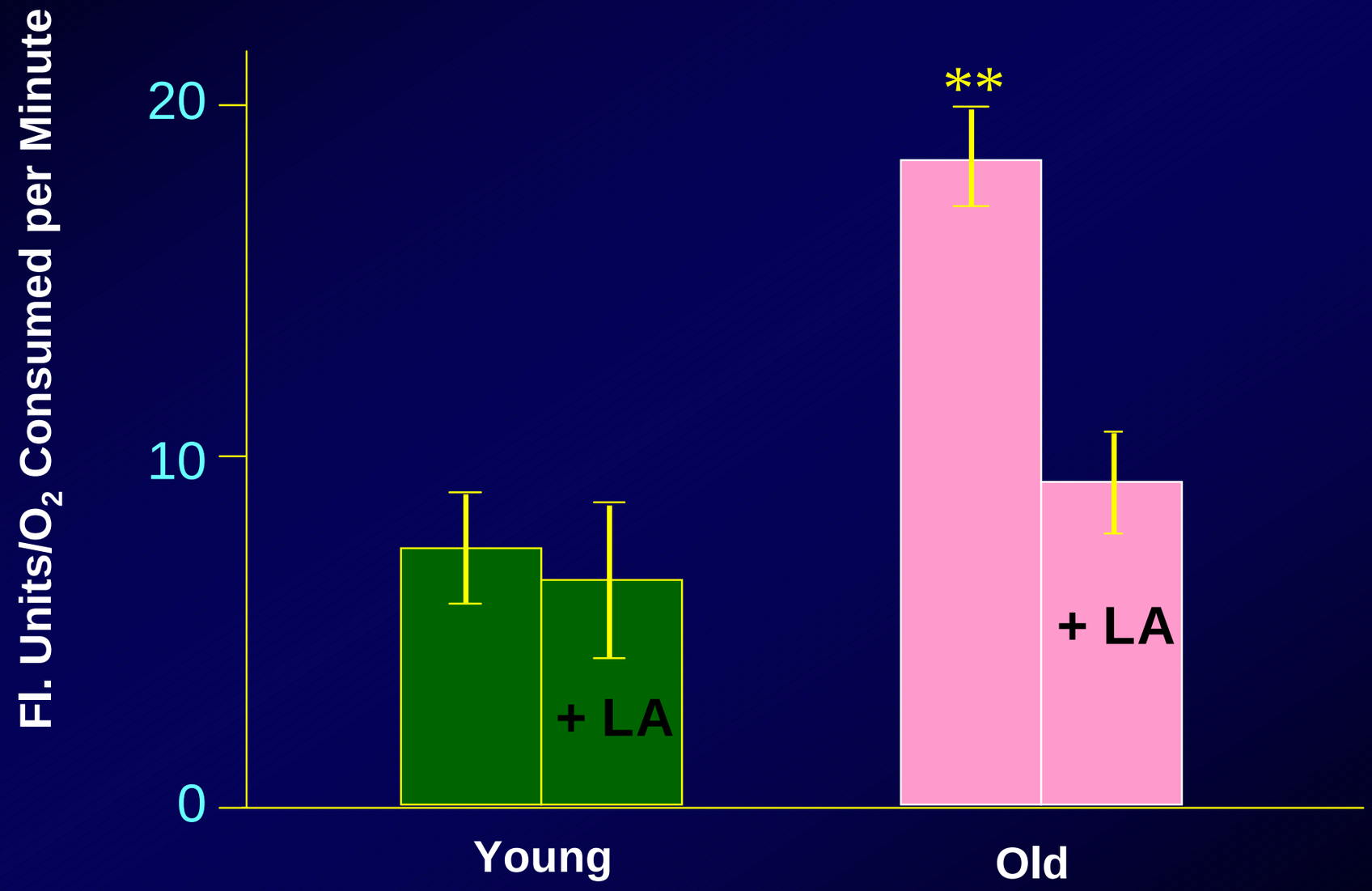


R-α-Lipoic Acid (LA) in mitochondria

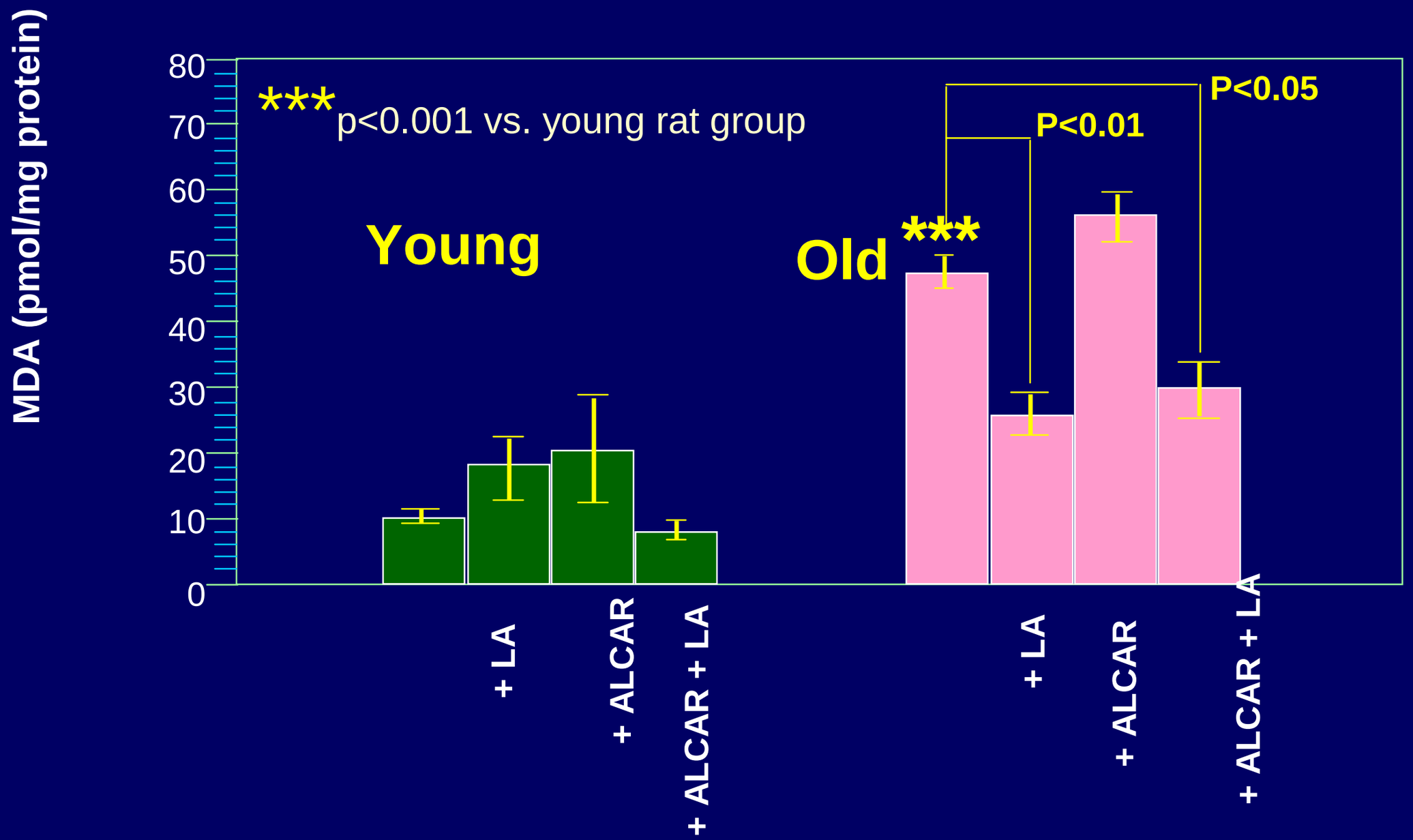
- LA reduced to dihydrolipoic acid, a potent antioxidant, & chelator of Fe & Cu
- Coenzyme of pyruvate and α-ketoglutarate dehydrogenases
- Involved with carbohydrate utilization for ATP production
- Improves cognitive function in aged mice



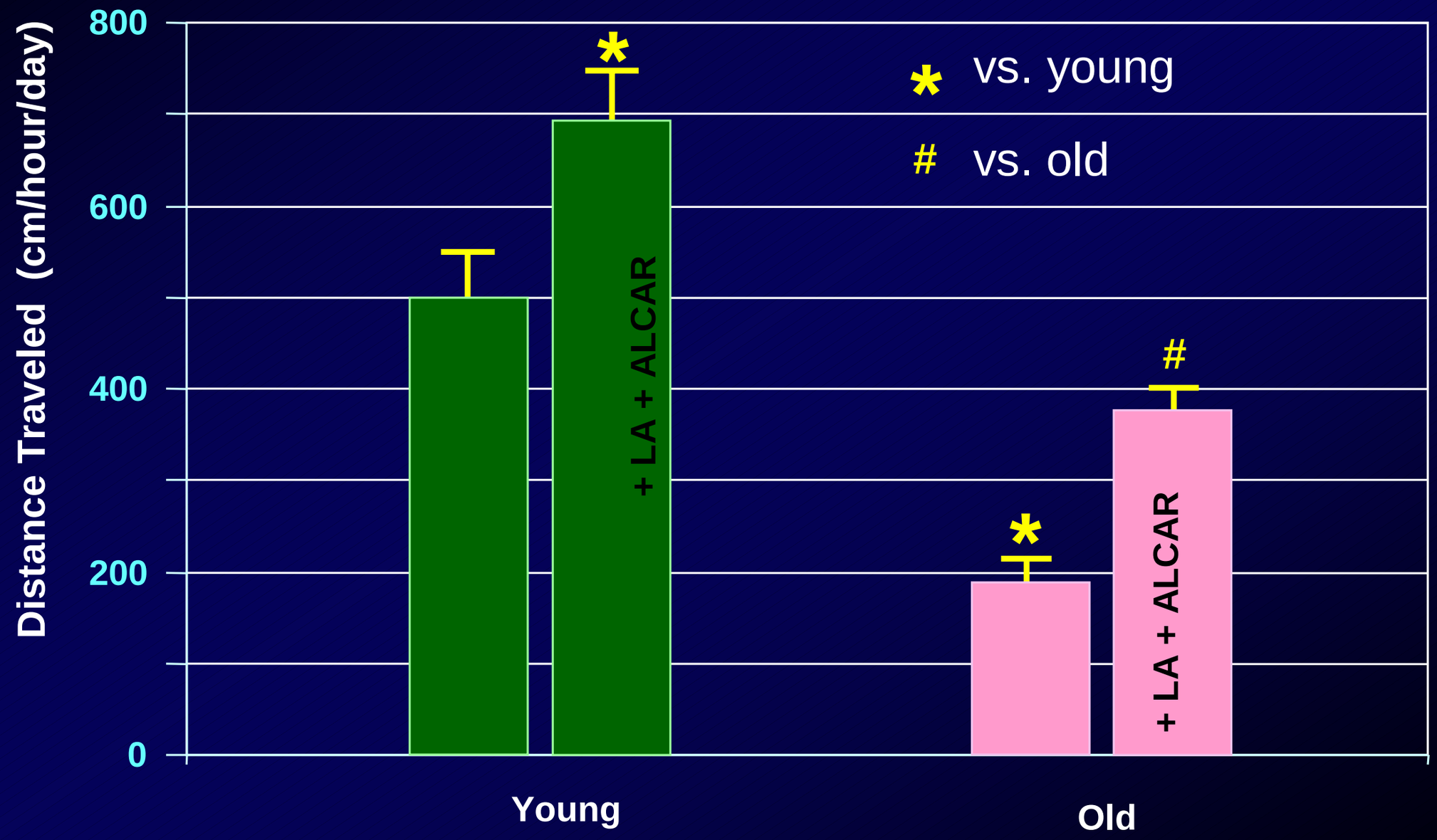
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats



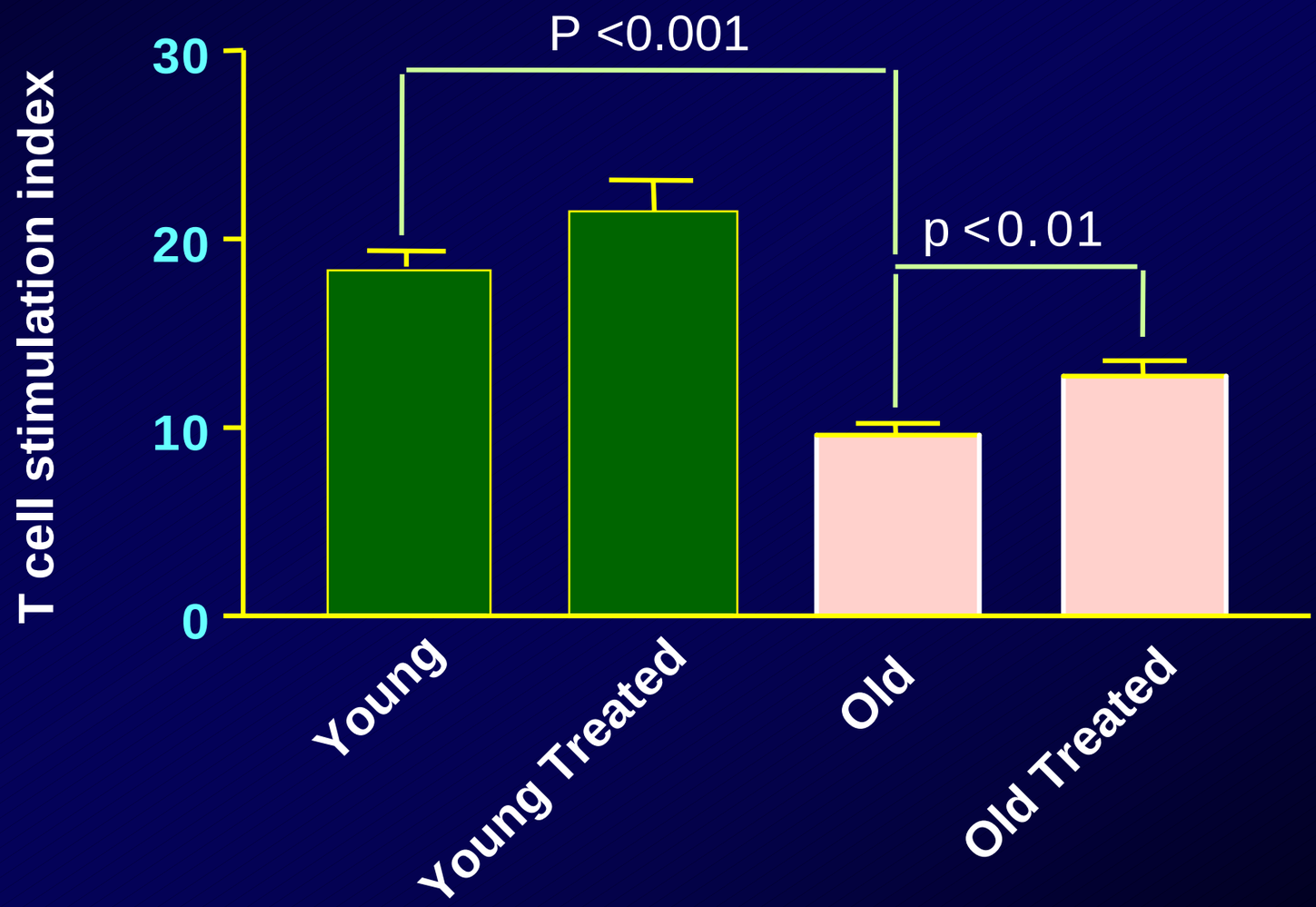
MDA levels in young and old rats with LA, ALCAR, or both



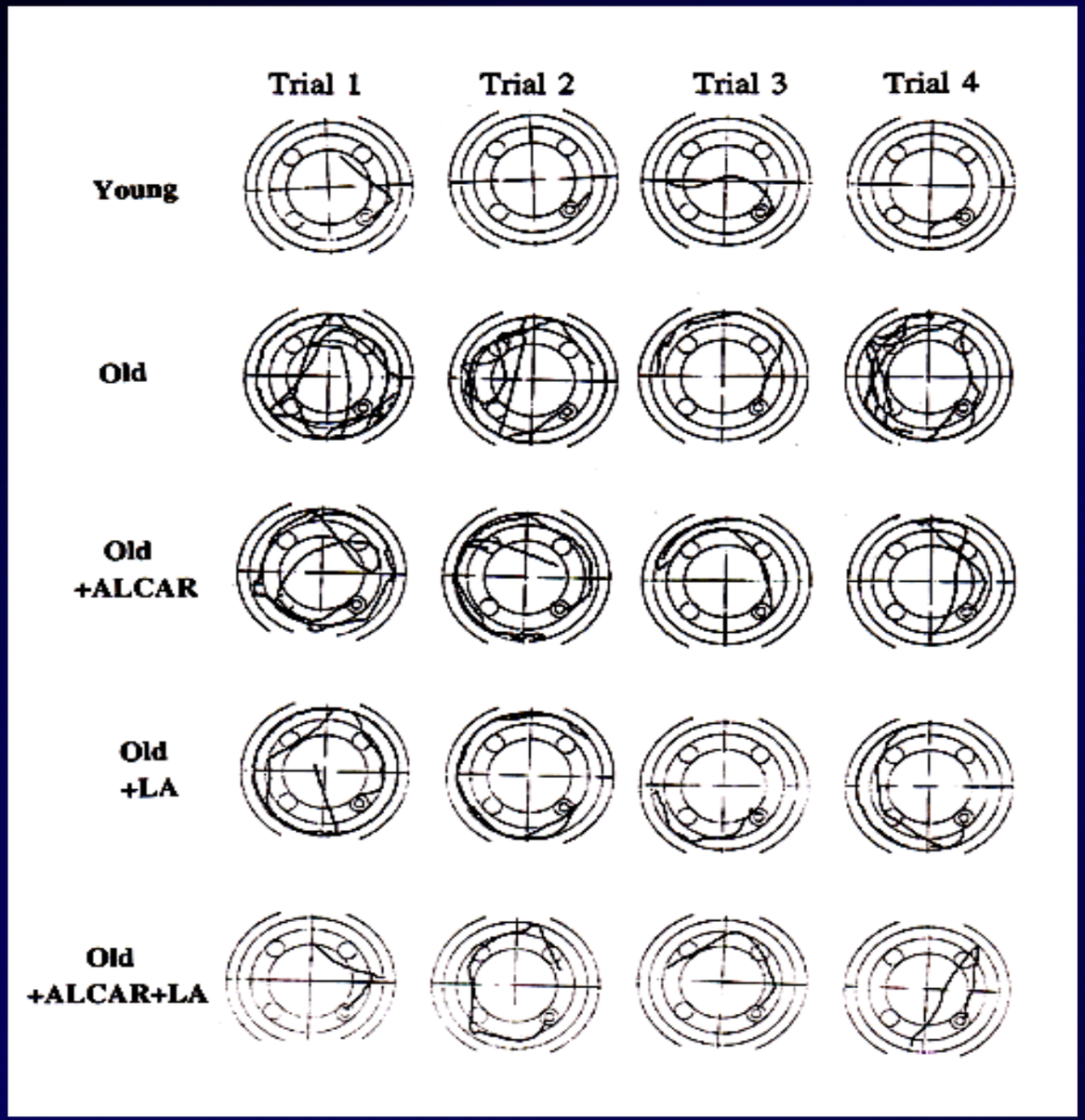
Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)



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.



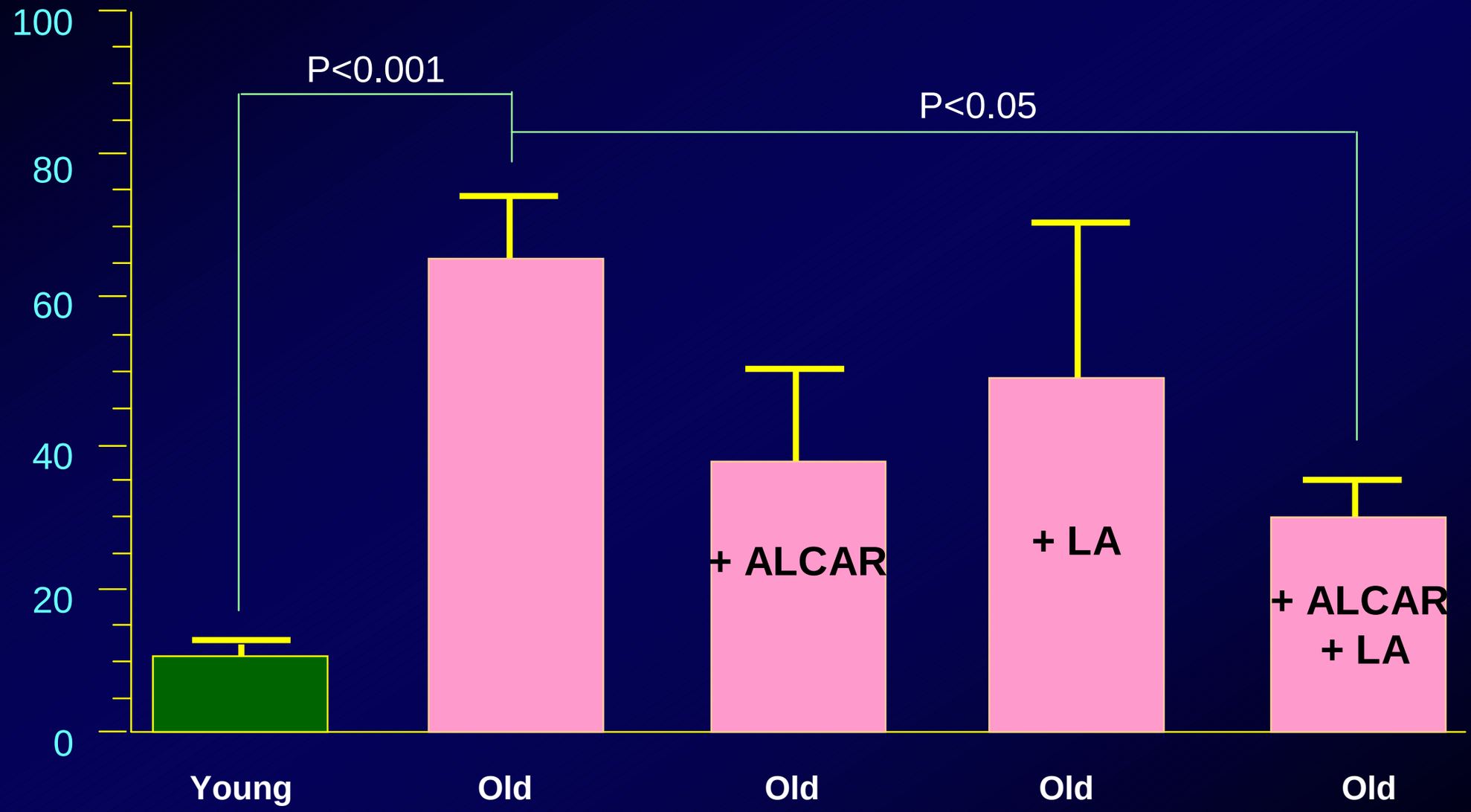
Morris Water Maze for Testing Spatial Memory



Spatial Memory relies on intact hippocampal function.

Treatments improved poor memory in old rats

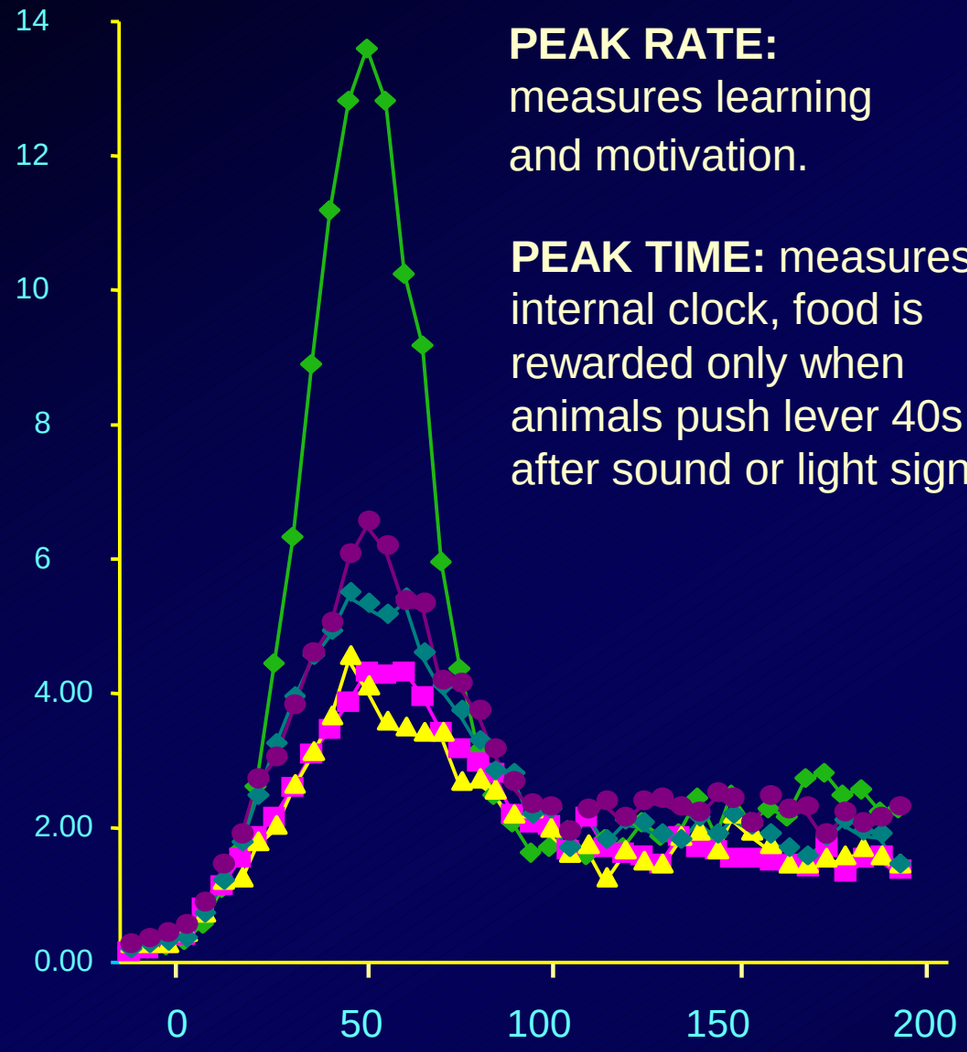
Spatial Memory Tested With Morris Water Maze



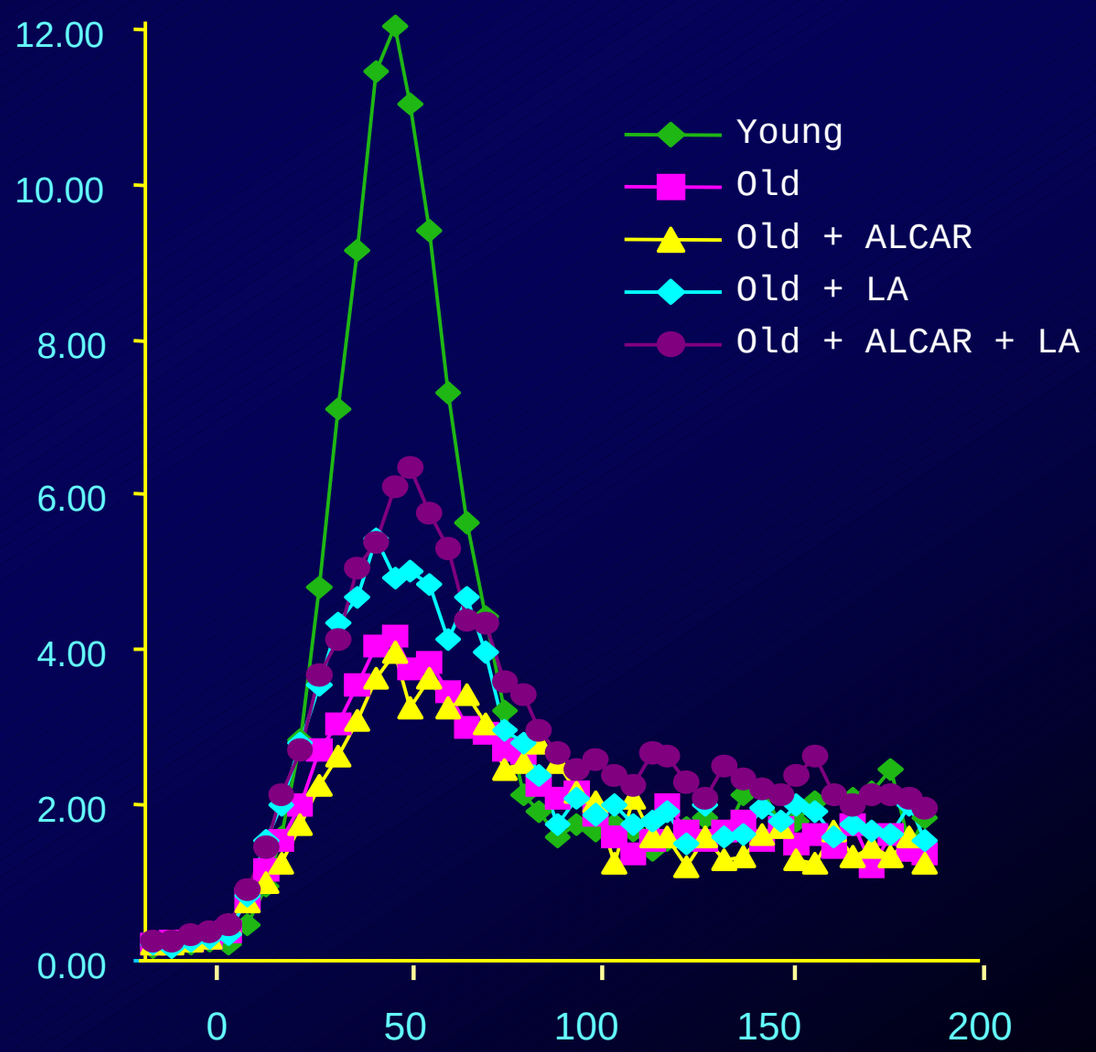
Peak procedure: for measuring temporal memory. Associated with striatum, cerebellum, & hippocampus

PEAK RATE: measures learning and motivation.

PEAK TIME: measures internal clock, food is rewarded only when animals push lever 40s after sound or light signal

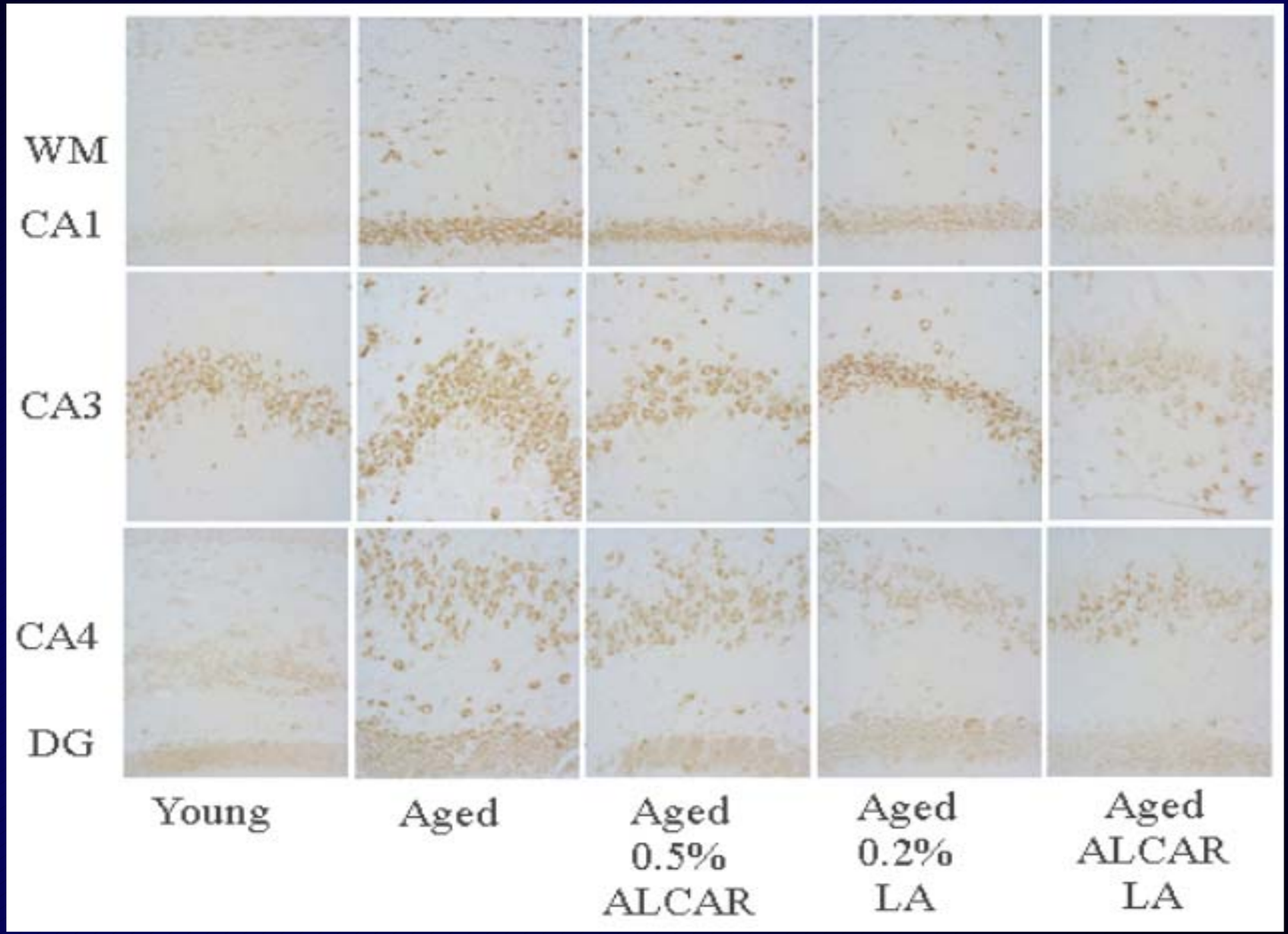


SOUND: Time to Signal



LIGHT: Time to Signal

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

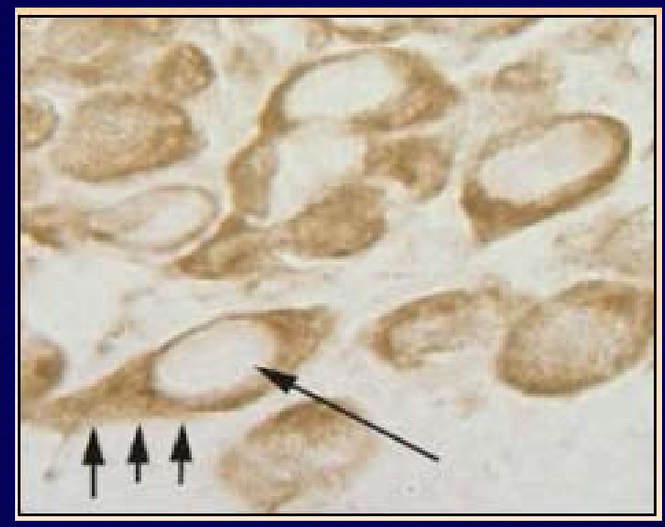


Staining of oxidized nucleic acid in neurons

(mAb to oxo8dG in DNA/oxo8G in R NA)

RNA is Oxidized

(92% is removed by RNase)



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

The Journal of Biological Chemistry
Vol. 278, pp. 8135-8145, March 7, 2003

Modulation of Gene Expression by Cancer Chemopreventive Dithiolethiones through the Keap1-Nrf2 Pathway

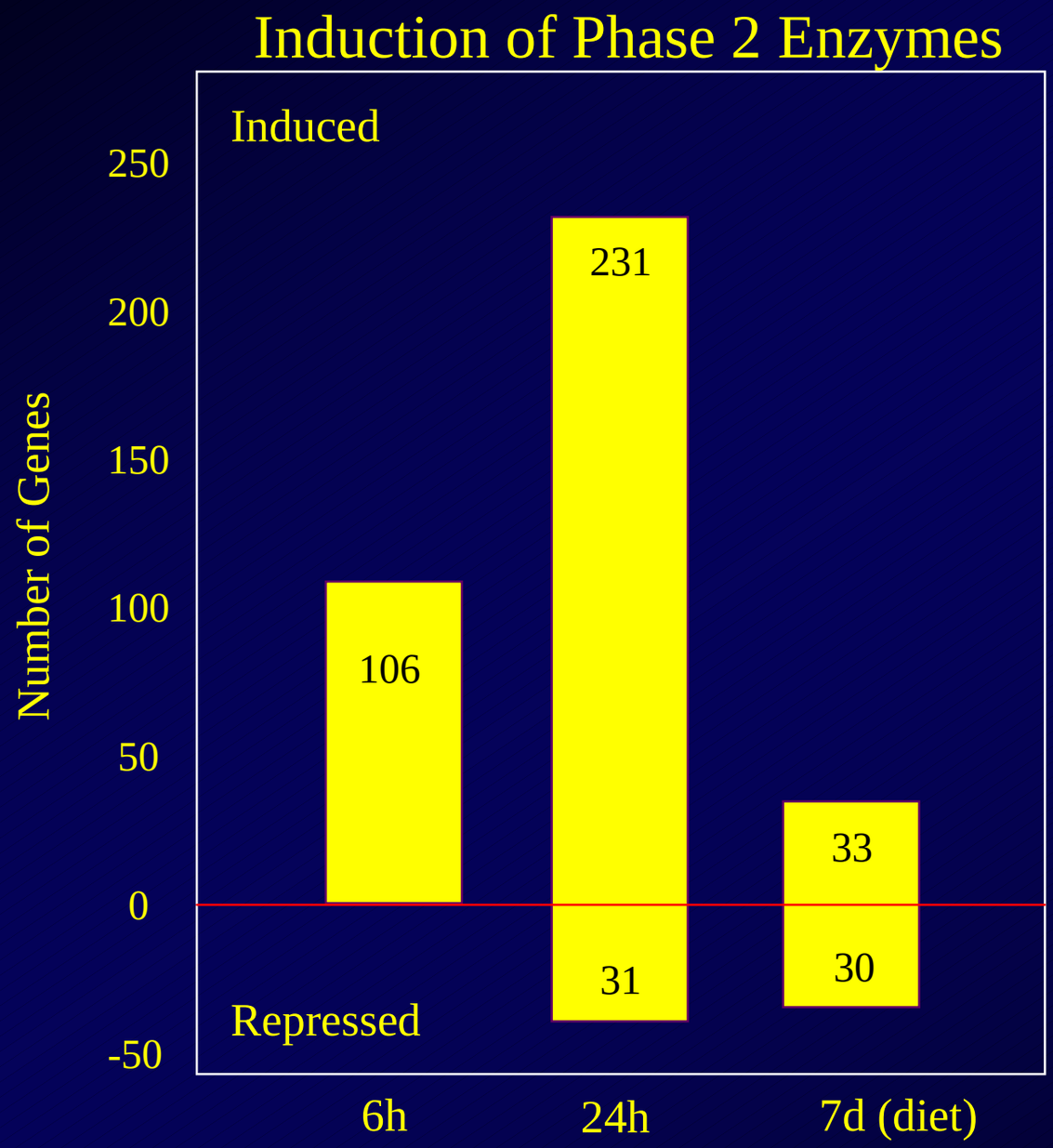
IDENTIFICATION OF NOVEL GENE CLUSTERS FOR CELL SURVIVAL

Mi-Kyoung Kwak, Nobunao Wakabayashi, Ken Itoh, Hozumi Motohashi, Masayuki Yamamoto, and Thomas W. Kensler

Proc. Natl. Acad. Sci. USA
Vol. 101, pp. 3381-3386, March 9, 2004

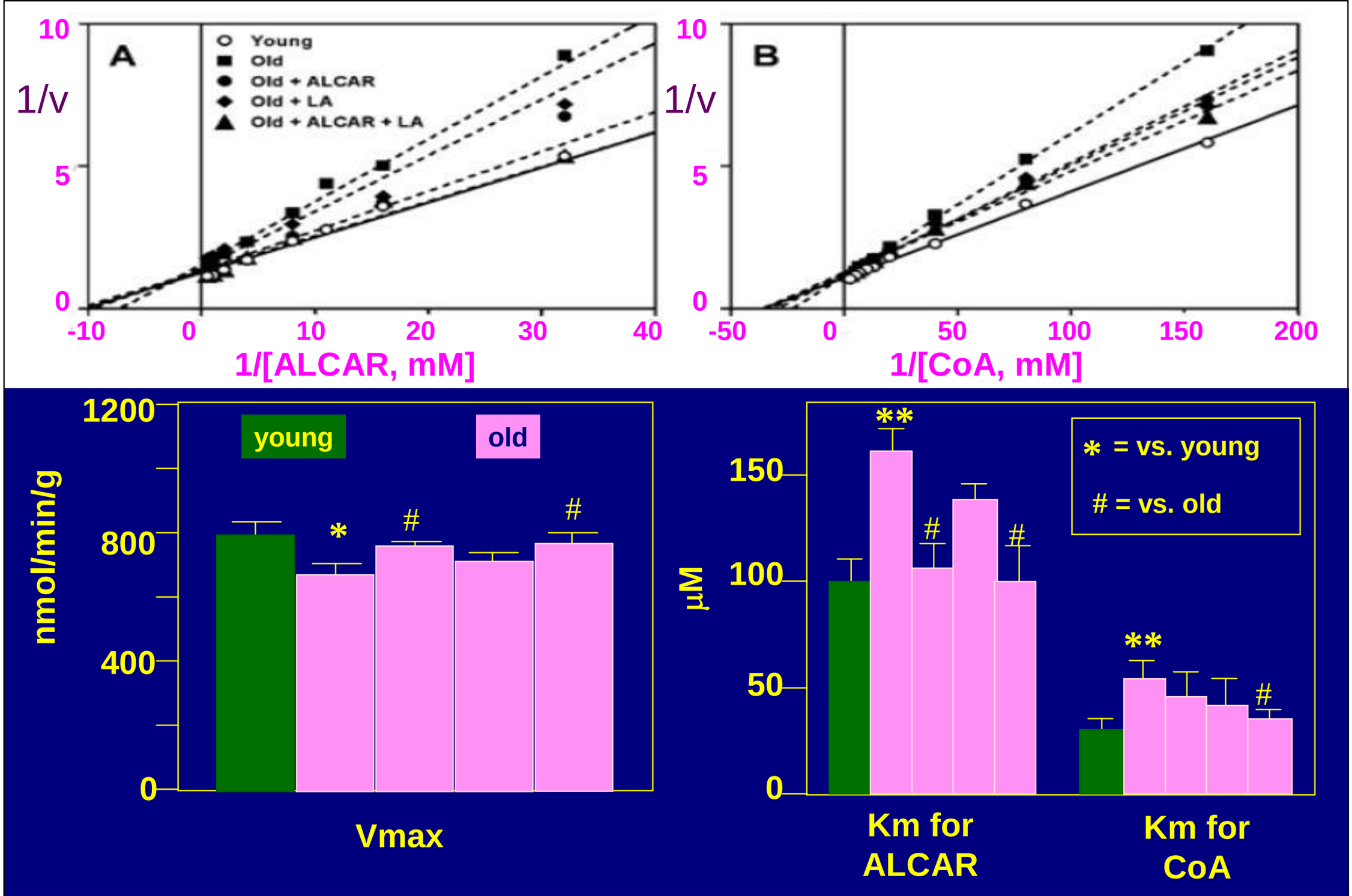
Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid

Jung H. Suh, Swapna V. Shenvi, Brian M. Dixon, Honglei Liu, Anil K. Jaiswal, Rui-Ming Liu, and Tory M. Hagen

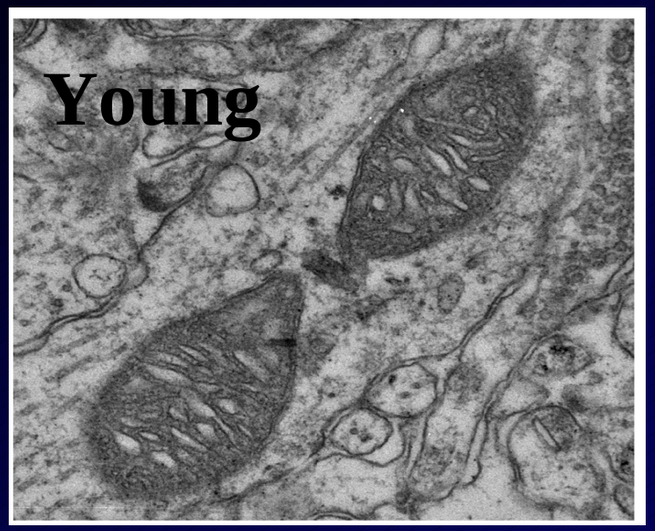


Modulation of Nrf-2-dependent gene expression by D3T in mouse liver.

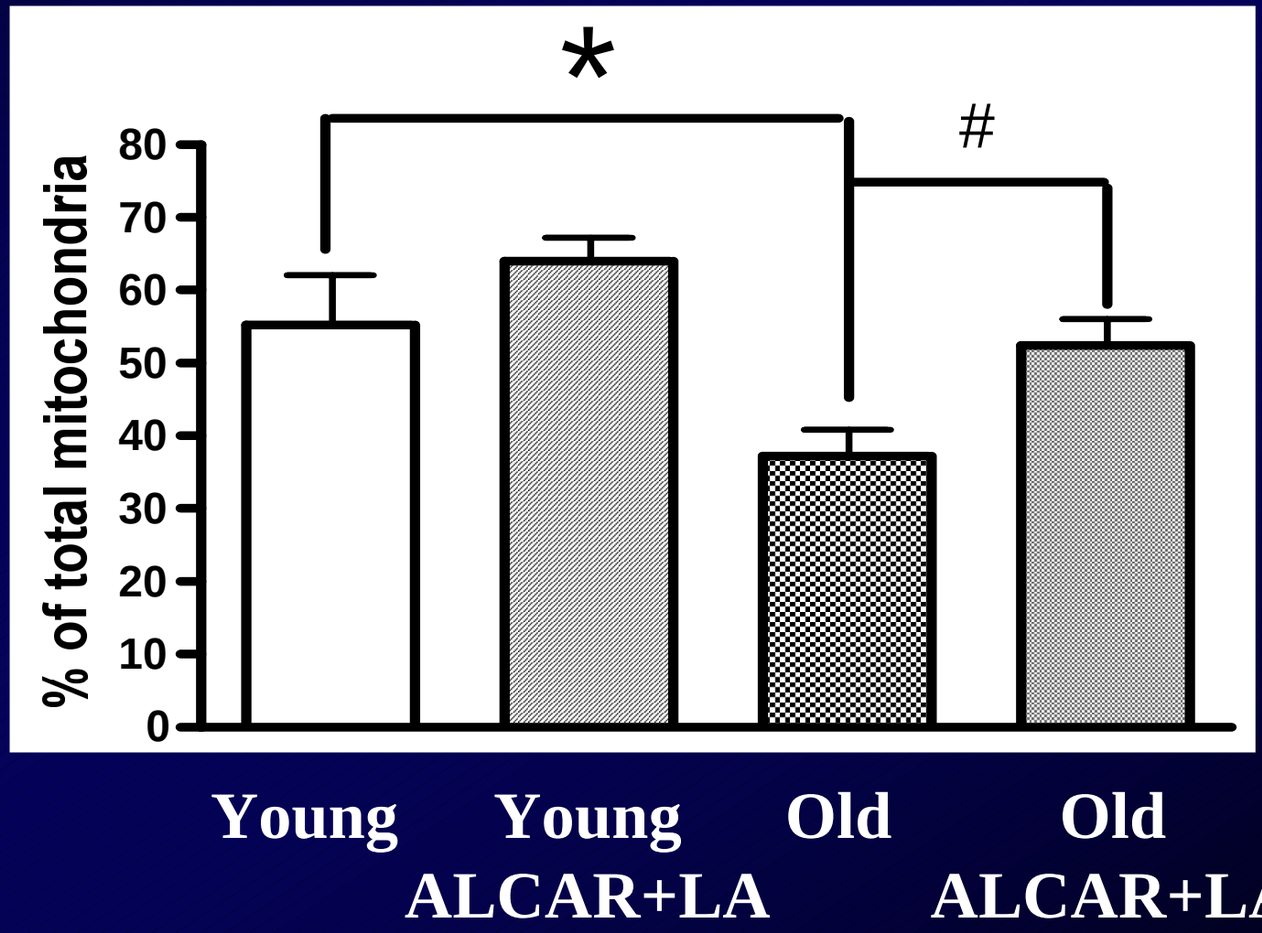
Kwak, et al. J bio Chem, 2003

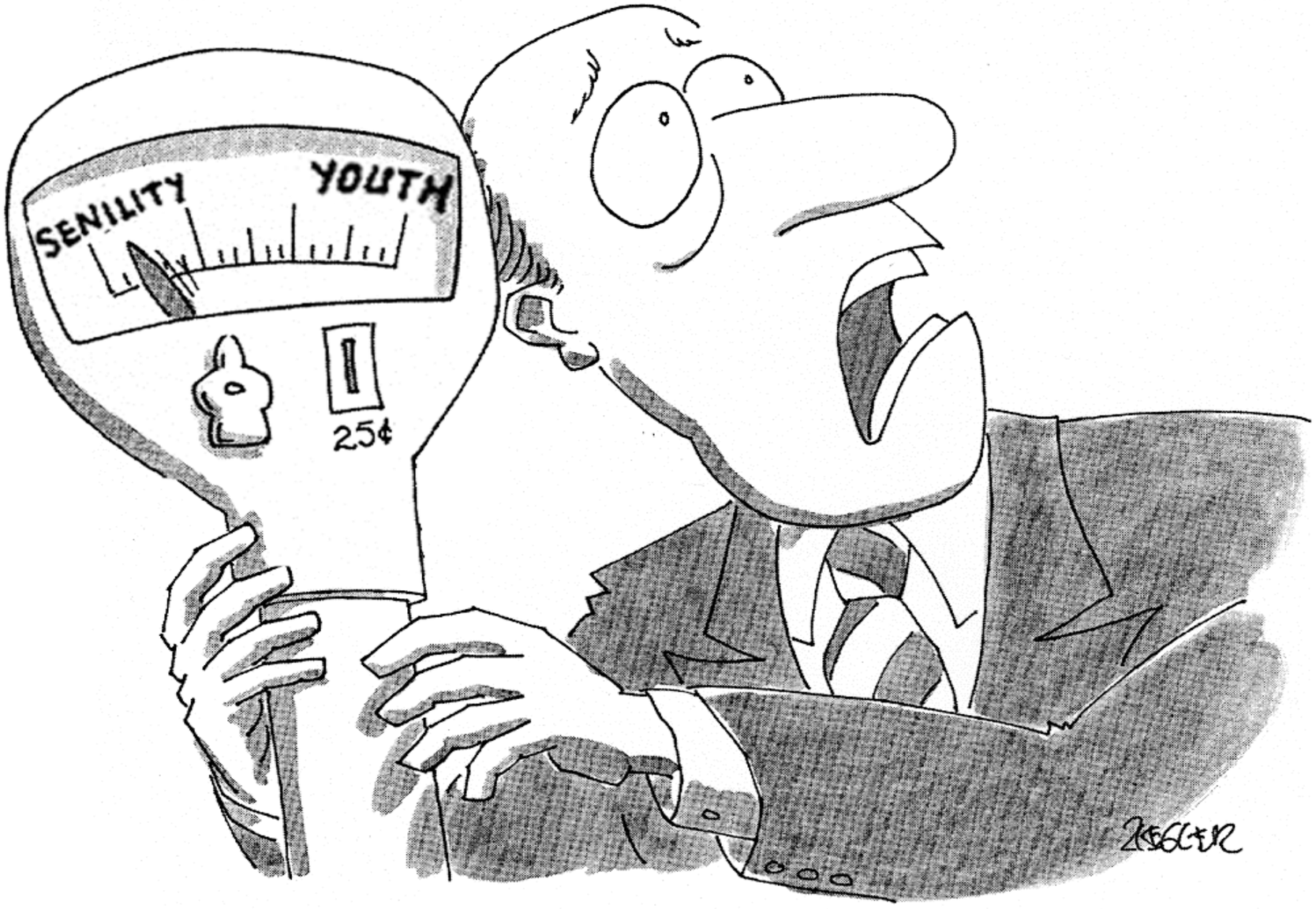

A
Young
Old
Old + ALCAR
Old + LA
Old + ALCAR + LA
1/v
10
5
0
-10
0
10
20
30
40
1/[ALCAR, mM]
B
1/v
10
5
0
-50
0
50
100
150
200
1/[CoA, mM]
young
old
nmol/min/g
1200
800
400
0
*
#
#
Vmax
** = vs. young
# = vs. old
µM
150
100
50
0
**
#
#
**
#
Km for ALCAR
Km for CoA

Age-related loss of undamaged mitochondria in hippocampal neurons



Electron Microscopy Images



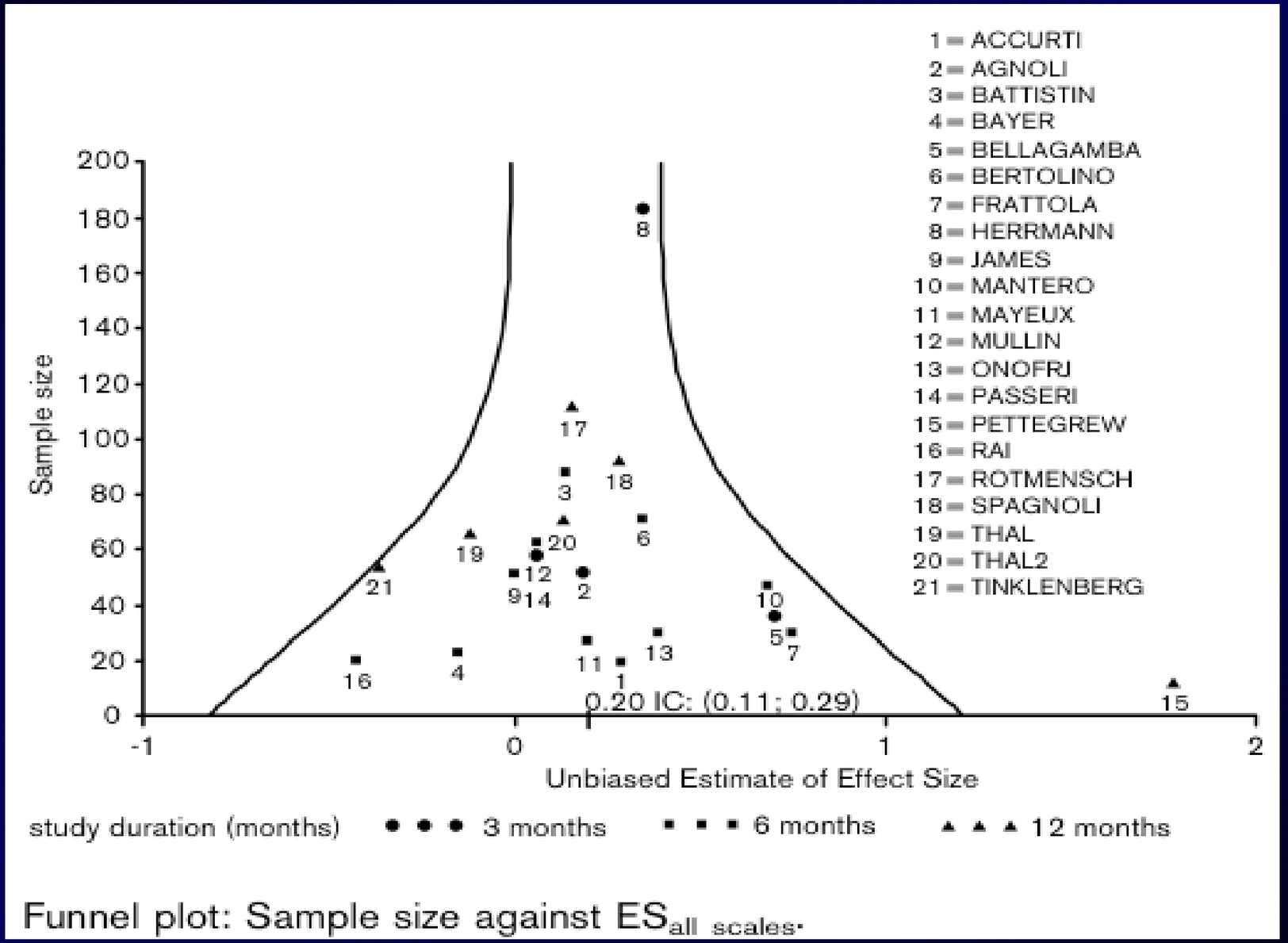


"More quarters! For God's sake, more quarters!"

"You're fifty-seven years old.
I'd like to get that down a bit."

New Yorker, June 6, 2005

Meta-analysis of acetyl-L-carnitine versus placebo for mild cognitive impairment and mild Alzheimer's disease

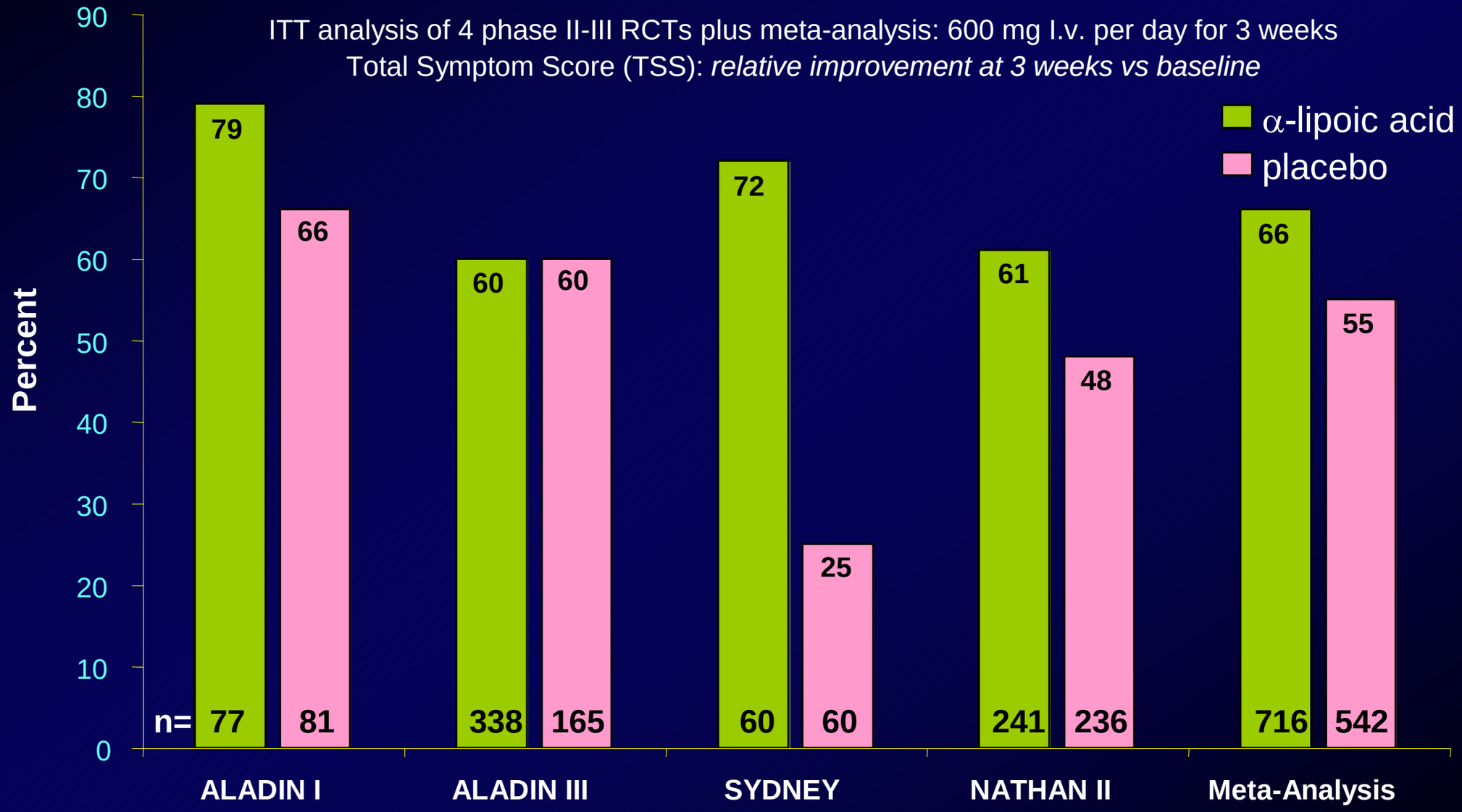


Funnel plot: Sample size against $ES_{\text{all scales}}$.

Montgomery, S.A., Thal, L.J., and Amrein, R., *Int. Clin. Psychopharmacol* 18:61-71 (2003)

Treatment with alpha-lipoic acid significantly improves both neuropathic symptoms and deficits in diabetic patients with symptomatic diabetic neuropathy

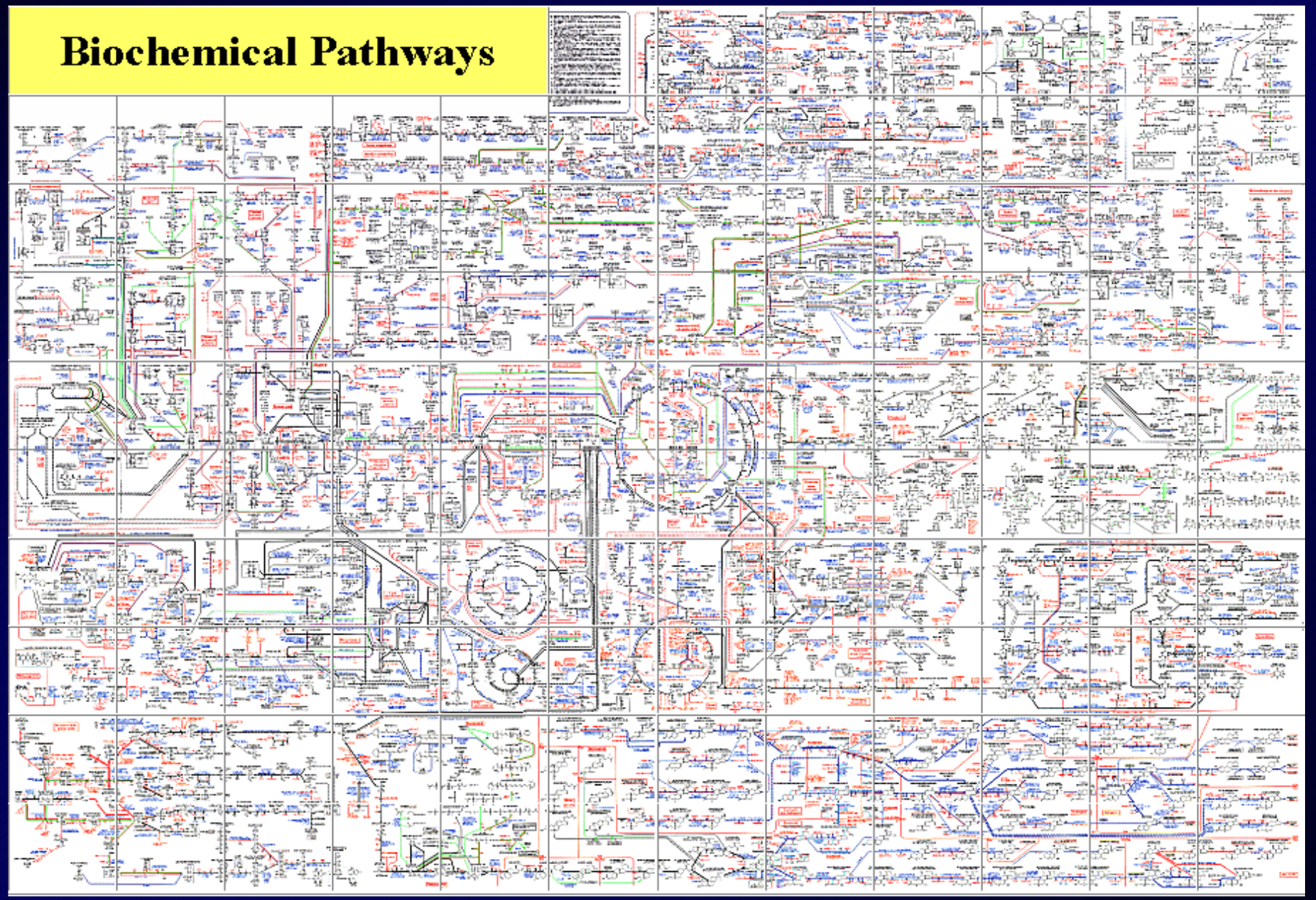
ITT analysis of 4 phase II-III RCTs plus meta-analysis: 600 mg I.v. per day for 3 weeks
Total Symptom Score (TSS): *relative improvement at 3 weeks vs baseline*



* p<0.05 vs Placebo

Source: Professor Daniel Ziegler of the Diabetes Research Institute, Düsseldorf, Germany: Meta-Analysis Provides Highest Level of Evidence, *Diabetes Monitor* (*2002, p6*)

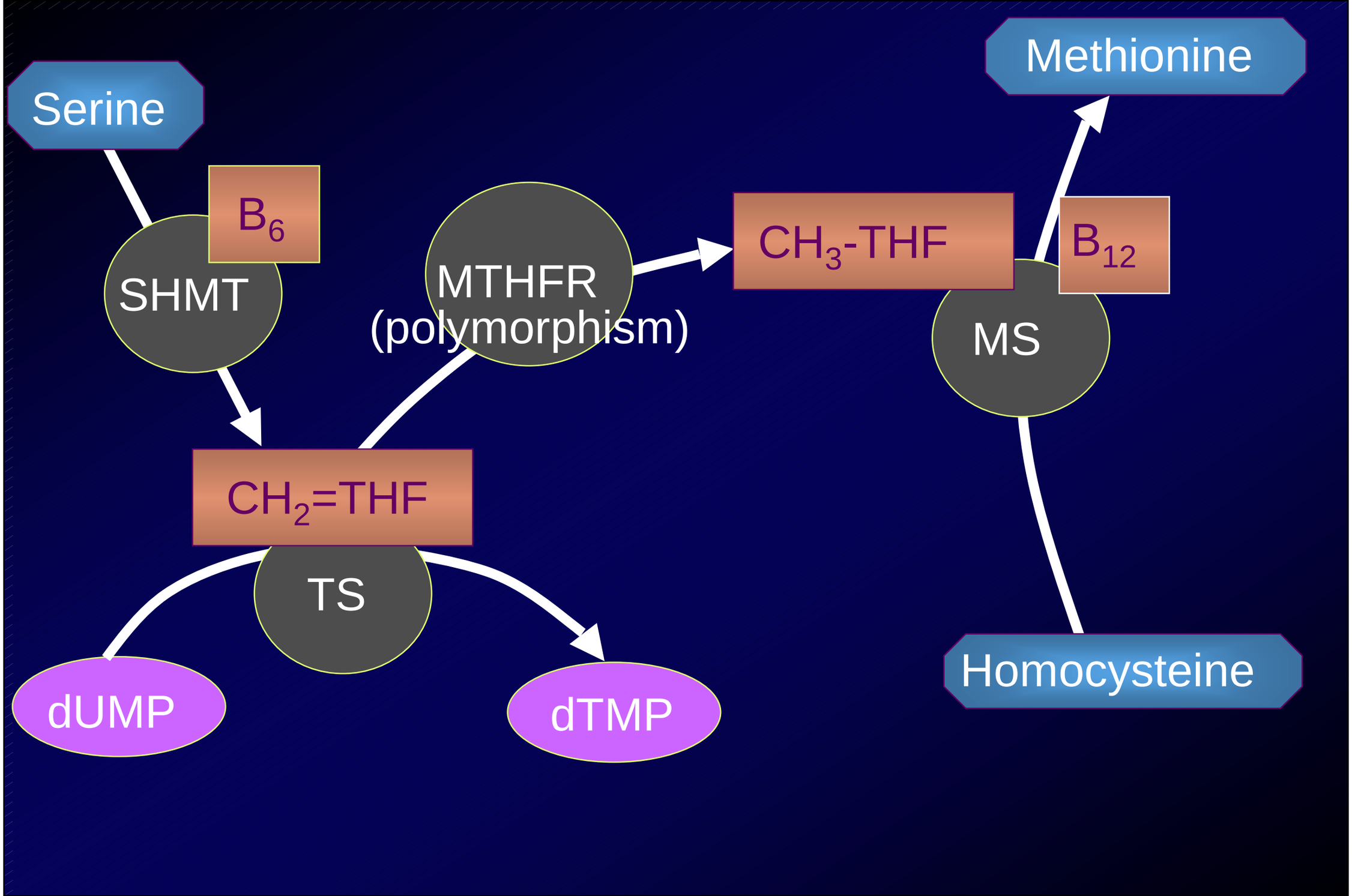
Biochemical Pathways

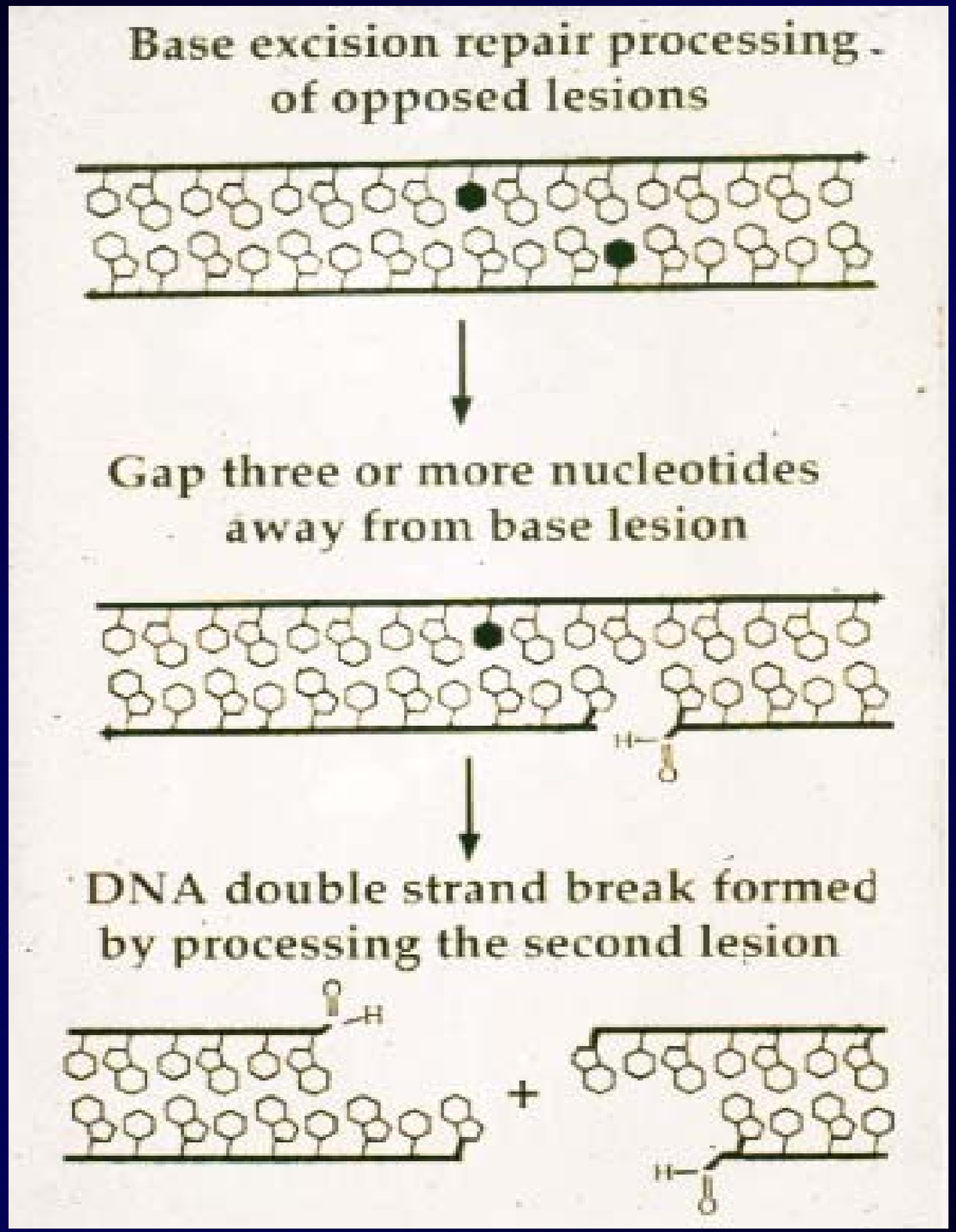


Micronutrient Undernutrition in Americans

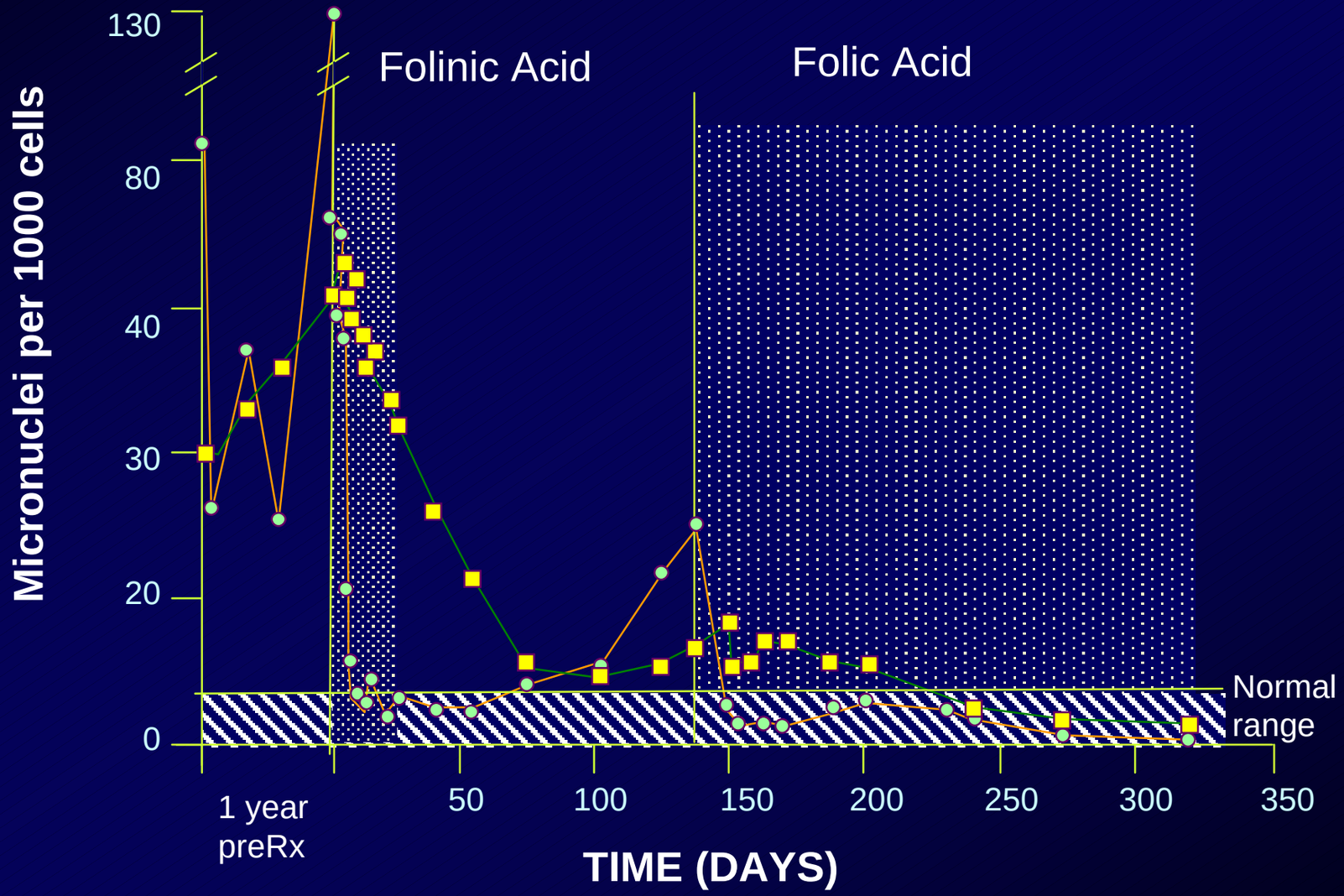
Nutrient	Population Group	% Ingesting < EAR * From Food
Minerals		
Iron	Women 14 - 50 years	16 %
Magnesium	All	56 %
Zinc	All	12 %
Vitamins		
B6	Women > 70 years	49 %
Folate	Adult Women	16 %
E	All	93 %
C	All	31 %

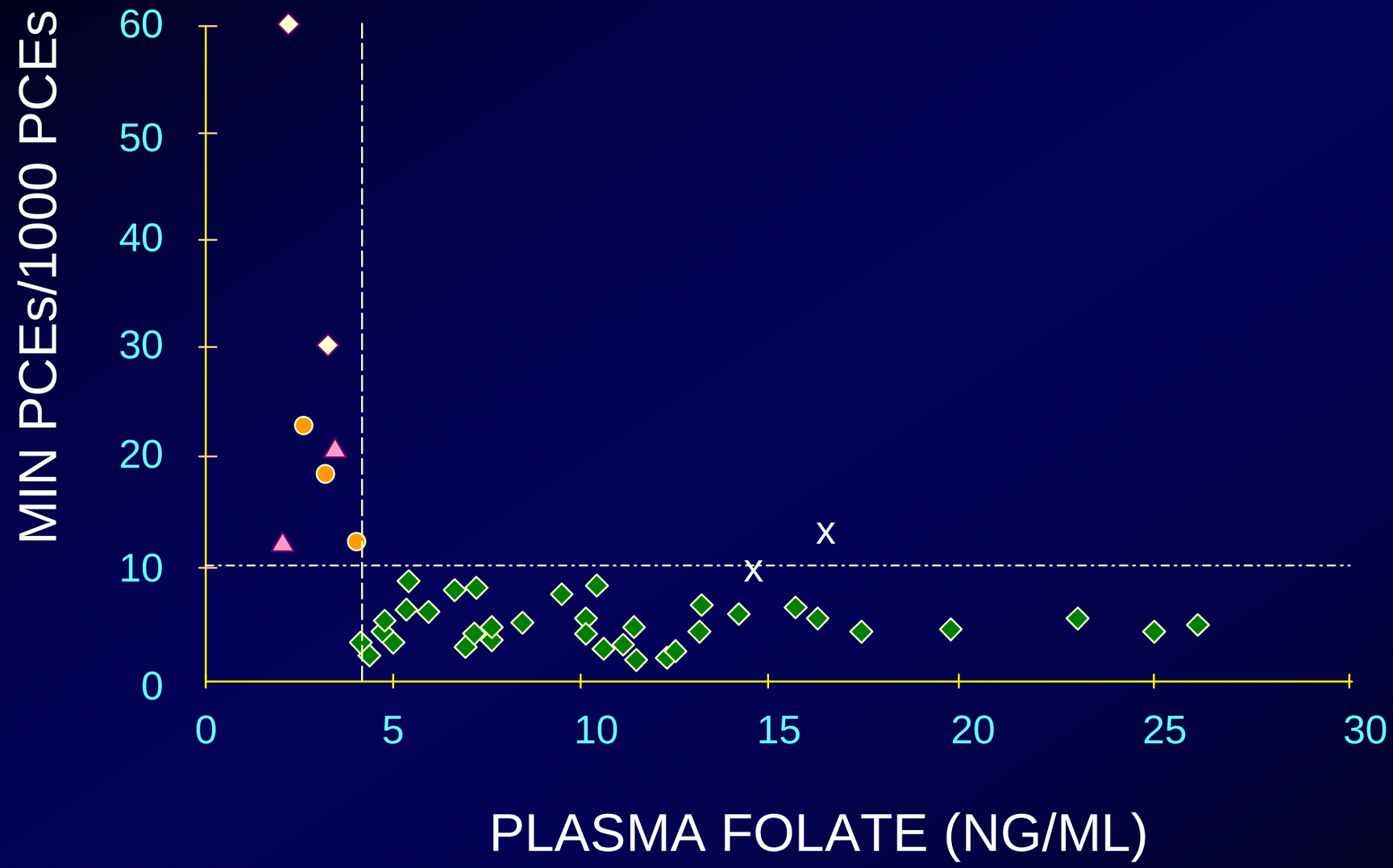
* USDA What we Eat in America (NHANES 2001-2002) Sept. 2005


Serine
B6
SHMT
MTHFR
(polymorphism)
CH3-THF
Methionine
B12
MS
CH2=THF
TS
dUMP
dTMP
Homocysteine


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
+

Micronuclei in: RNA positive erythrocytes
RNA negative erythrocytes




MIN PCEs/1000 PCEs
60
50
40
30
20
10
0
0
5
10
15
20
25
30
PLASMA FOLATE (NG/ML)

Folate, Vitamin B12, Homocysteine Status and Chromosome Damage Rate in Lymphocytes of Older Men

Michael Fenech, Ivor Dreostl, and Josephine Rinaldi, *Carcinogenesis* **13**:1329-1336, **1997**

Folate, Vitamin B12, Homocysteine Status and DNA Damage in Young Australian Adults

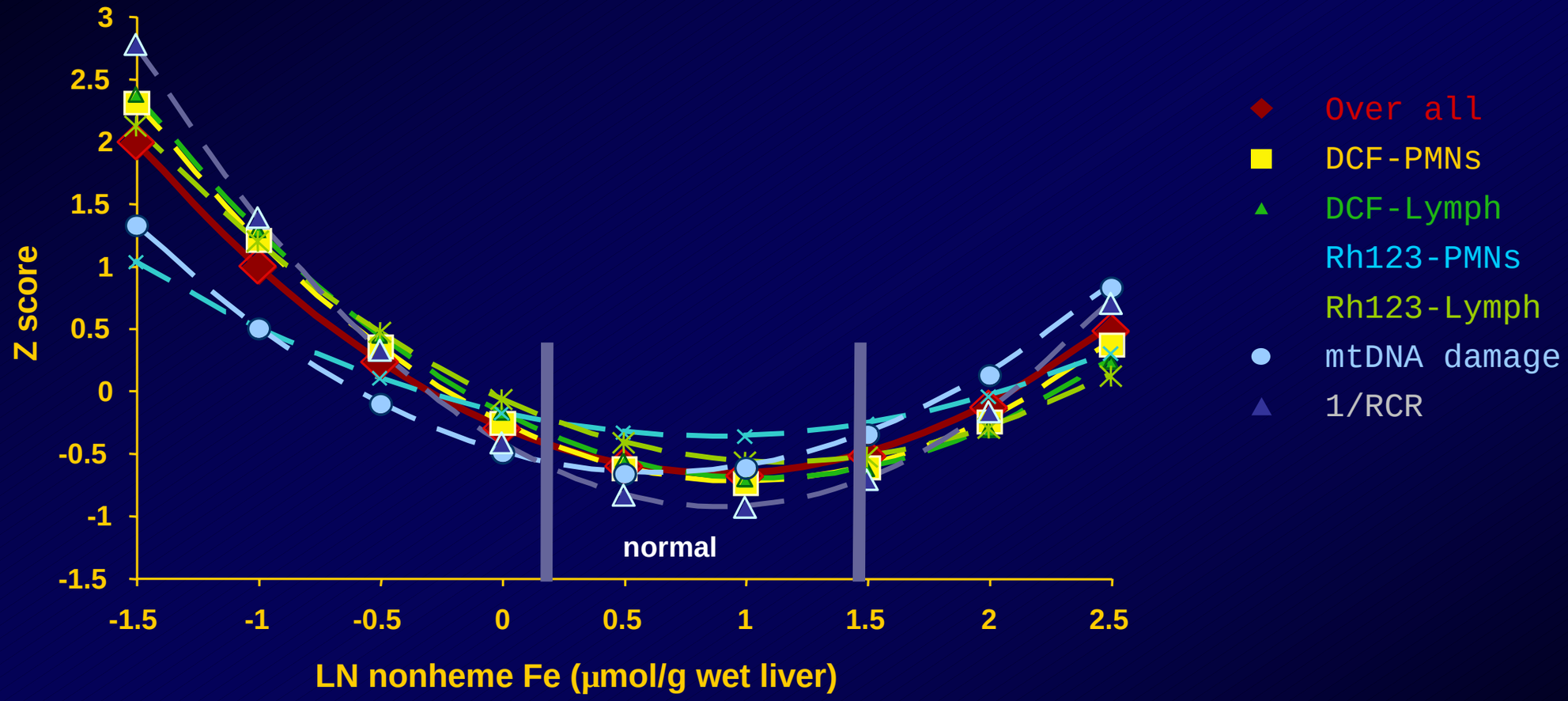
Michael Fenech, Claire Aitken, and Josephine Rinaldi, *Carcinogenesis* **19**:1163 - 1173, **1998**

Micronucleus Frequency in Human Lymphocytes is Related to Plasma Vitamin B12 and Homocysteine

Michael Fenech, *Mutation Research* **42:** 299 - 304, **1999**

In a series of studies, we have been able to confirm that the micronucleus index in cytokinesis-blocked lymphocytes is significantly negatively correlated with plasma vitamin B12 (B12) concentration and significantly positively correlated with plasma homocysteine (HC). Furthermore we have shown in a randomized double-blind placebo-controlled dietary intervention study that intake of 3.5 times the RDI of folic acid and B12 significantly reduces the micronucleus index only in those with above average levels of micronucleus frequency. Micronucleus frequency is minimized when plasma HC is below 7.5 µmol/l and plasma B12 is above 300 pmol/l. Therefore, it is important to take account of the effect of B12 and HC when using the micronucleus assay for human biomonitoring studies.

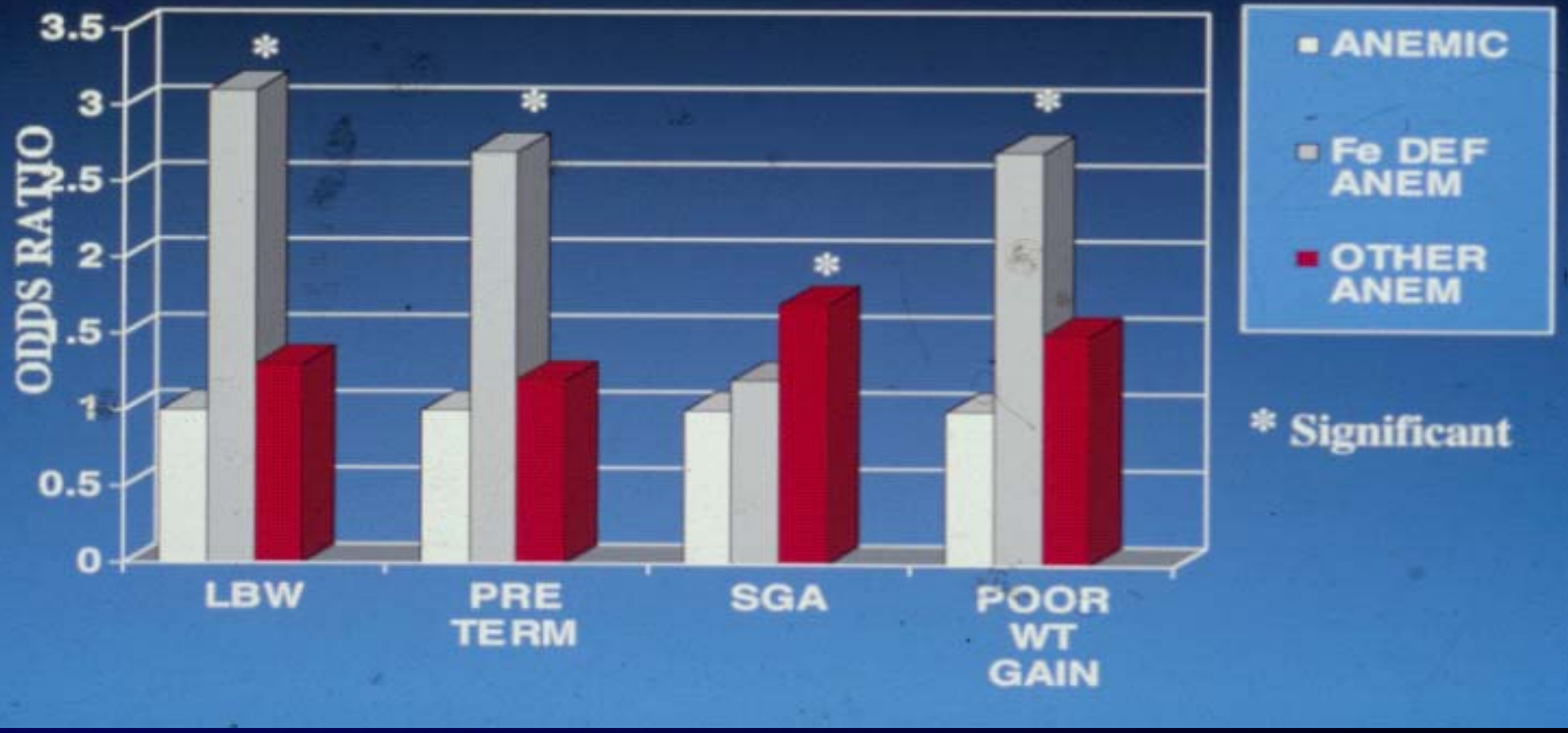
Analysis of nonlinear regression models: comparison of an overall model and individual models of Z-transformed values vs. ln- nonheme liver iron



. 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.

ADJUSTED ODDS RATIOS FOR INADEQUATE PREGNANCY OUTCOME AMONG ANEMIC PREGNANT WOMEN.

(Source: Scholl et al., AJCN 1992)



An overview of evidence for a causal relationship between iron deficiency during development and cognitive or behavioral function in children

Joyce C McCann and Bruce N Ames
(2006) Submitted

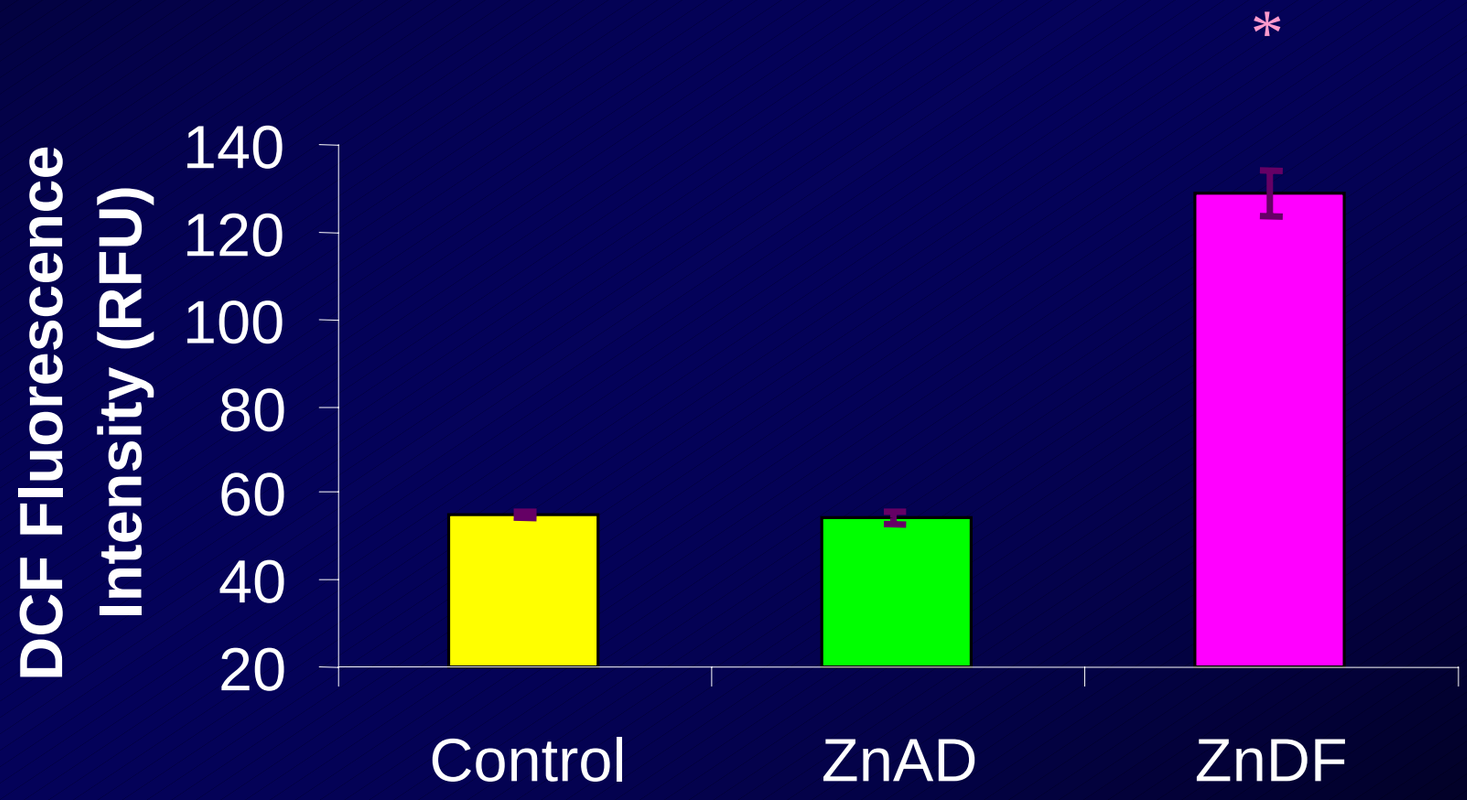
Is docosahexaenoic acid, an n3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals

Joyce C McCann and Bruce N Ames
American Journal of Clinical Nutrition (2005) 82:281-95

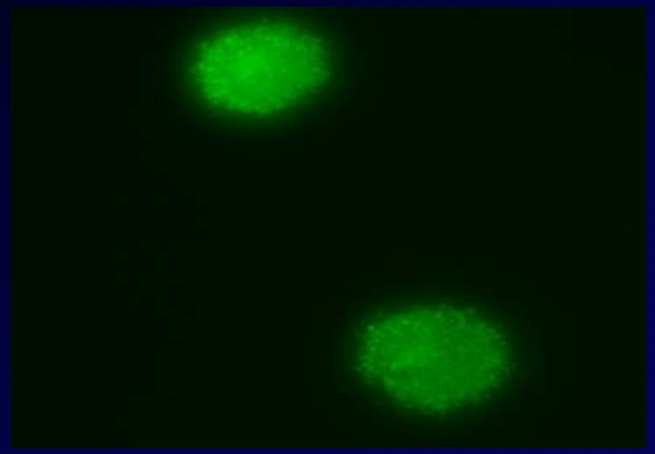
An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring

Joyce C McCann, Mark Hudes, and Bruce N Ames
Neuroscience & Biobehavioral Reviews, (2006) 30:696-712.

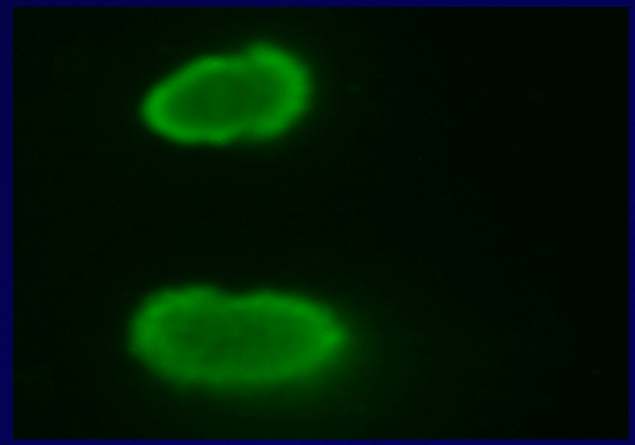
Zinc Deficiency Induces Increased Oxidative Stress in C6 Glioma Cells



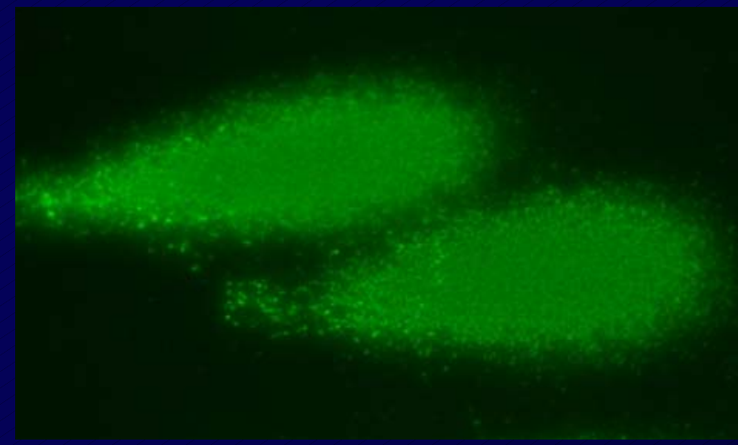
Zinc Deficiency Induces Fapy Glycosylase (Fpg)-sensitive Single Strand Breaks in Human Lung Fibroblasts



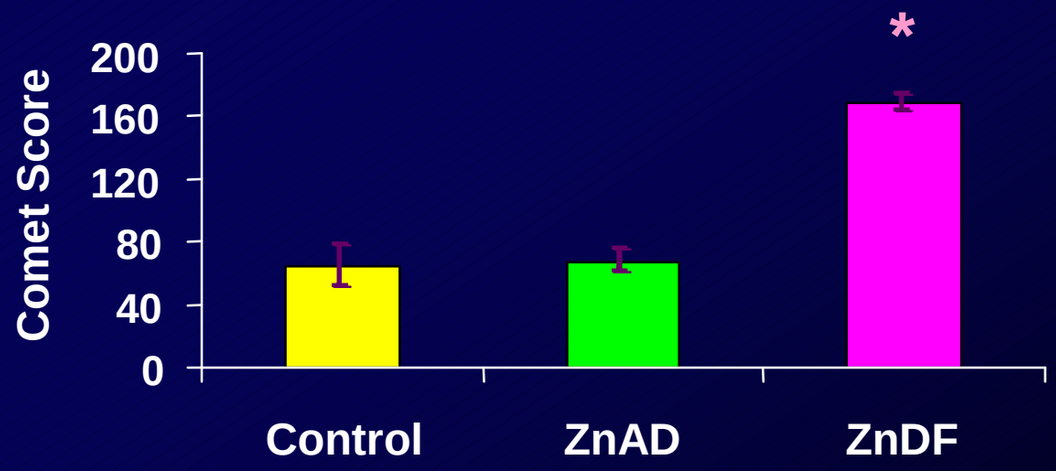
Control (+Fpg)

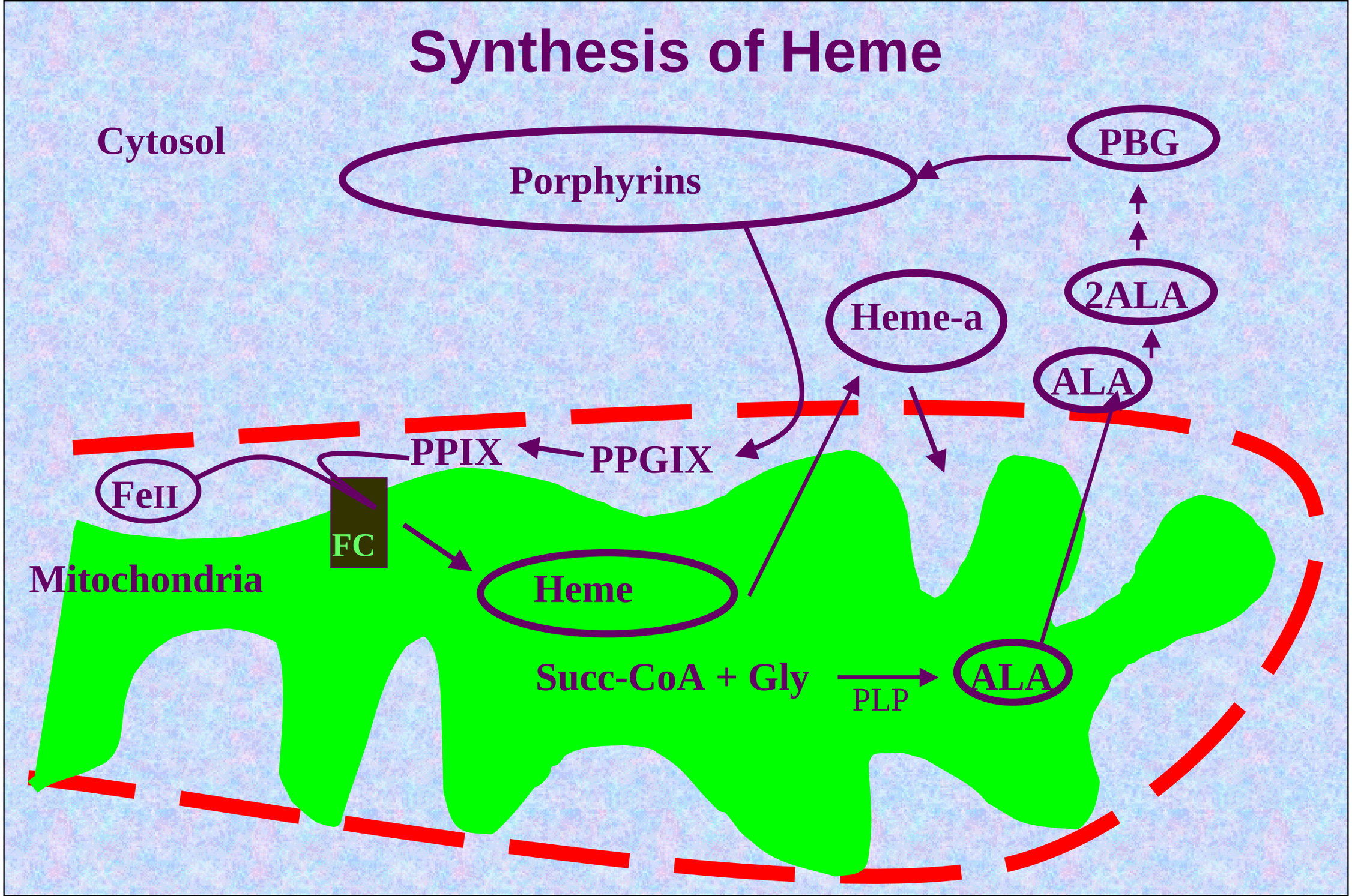


ZnAD (+Fpg)

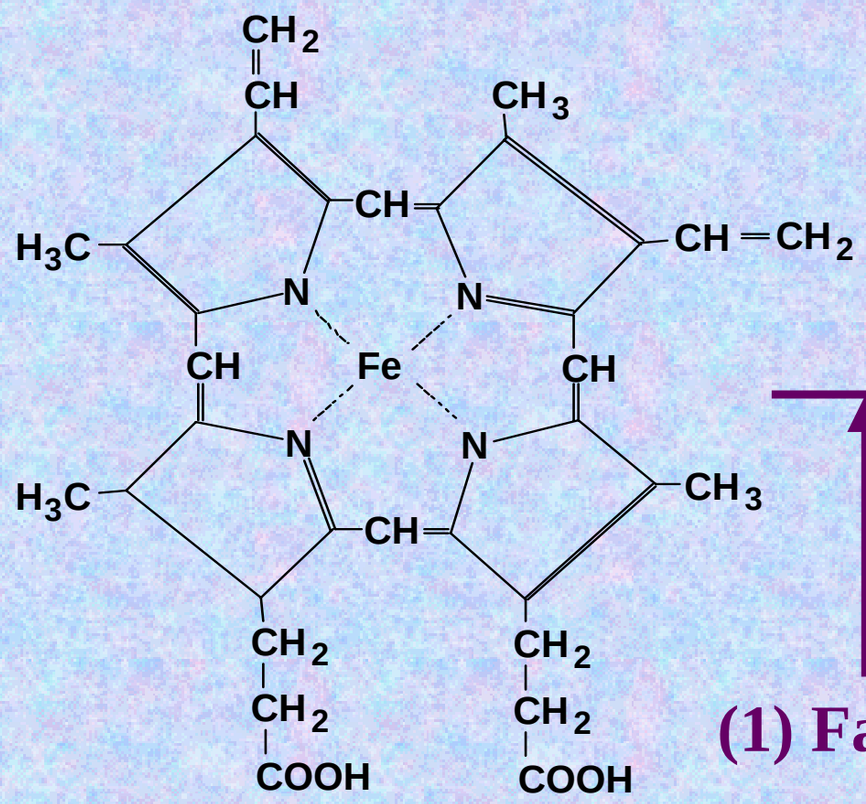


ZnDF (+Fpg)




Synthesis of Heme
Cytosol
Porphyrins
PBG
2ALA
ALA
Heme-a
PPIX
PPGIX
FeII
FC
Mitochondria
Heme
Succ-CoA + Gly
PLP
ALA

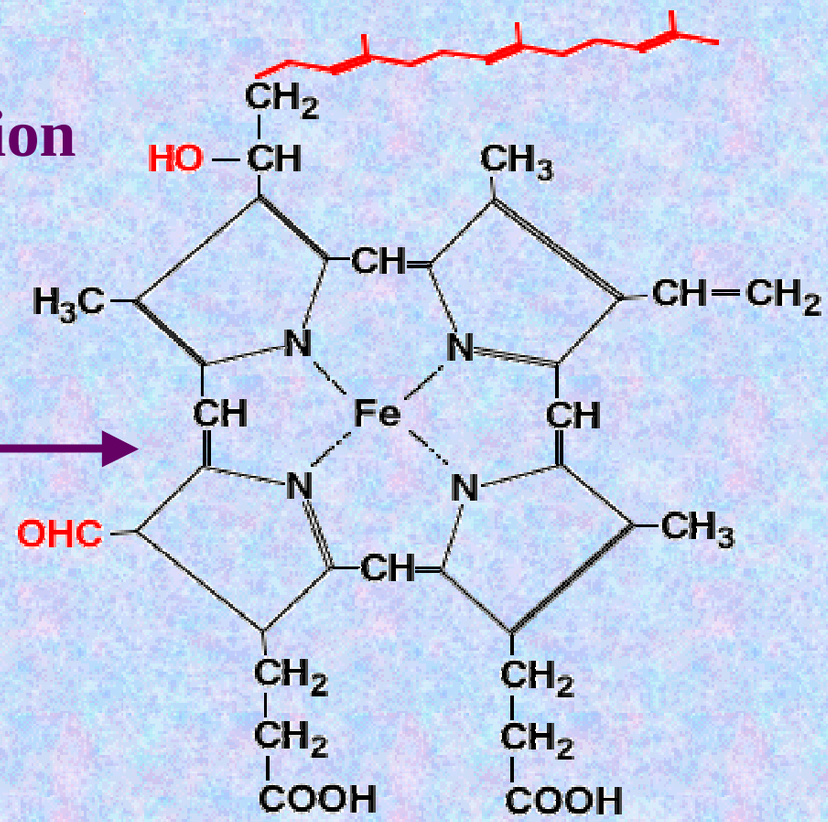
Maturation of heme-*b* to heme-*a* is rate limiting for the assembly of complex IV



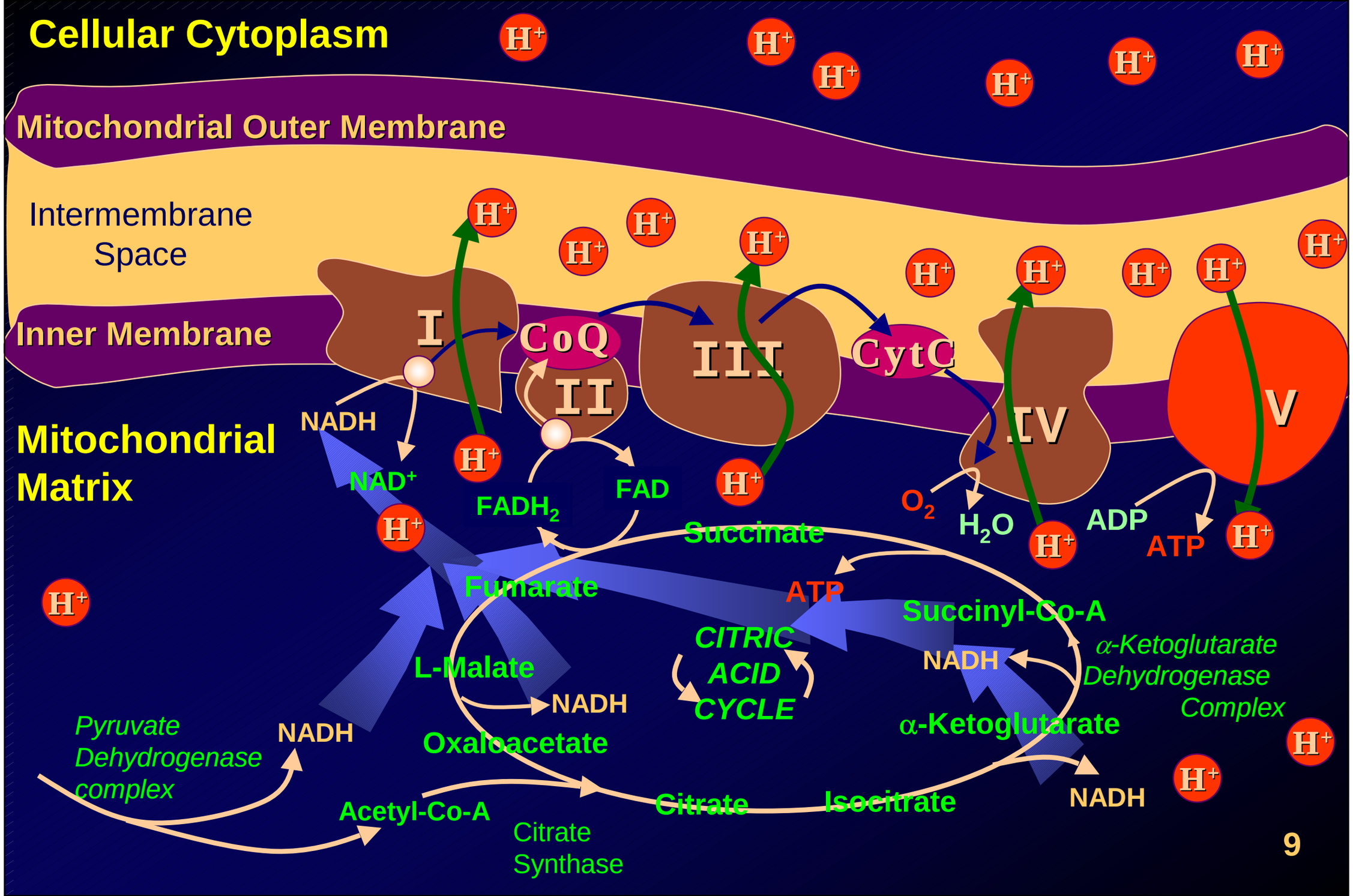
Heme-*b*

(2) Oxidation

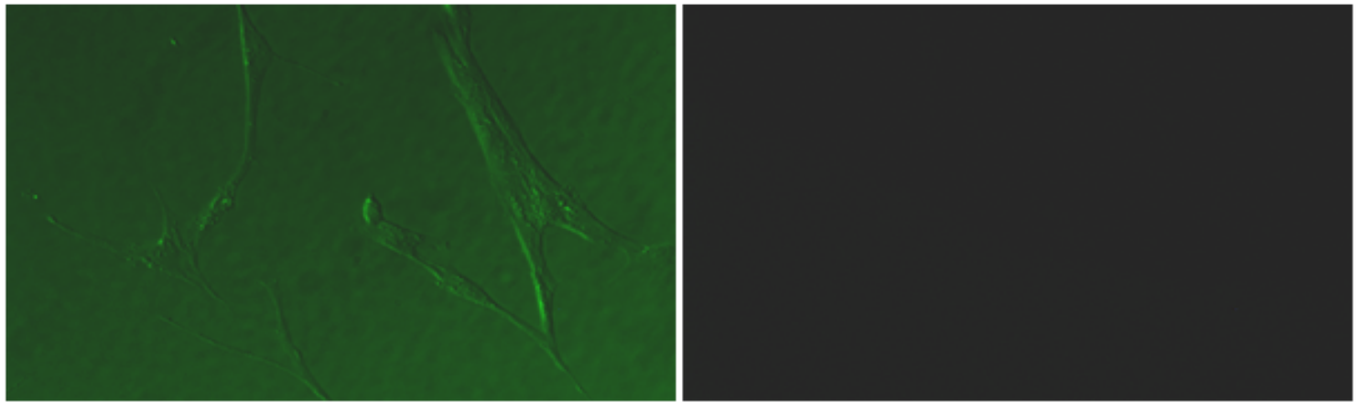
(1) Farnesylation



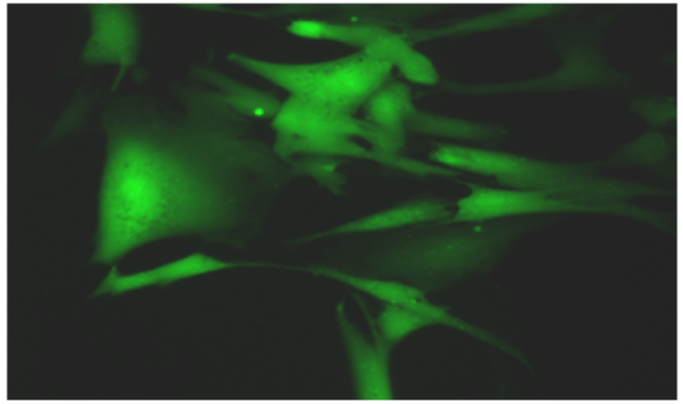
Heme-*a*
(Only in complex IV)


Cellular Cytoplasm
Mitochondrial Outer Membrane
Intermembrane Space
Inner Membrane
Mitochondrial Matrix
I
II
III
IV
V
CoQ
CytC
NADH
NAD+
FADH2
FAD
O2
H2O
ADP
ATP
Succinate
Fumarate
L-Malate
Oxaloacetate
Citrate
Isocitrate
CITRIC ACID CYCLE
α-Ketoglutarate
Succinyl-Co-A
α-Ketoglutarate Dehydrogenase Complex
Pyruvate Dehydrogenase complex
Acetyl-Co-A
Citrate Synthase
H+
9

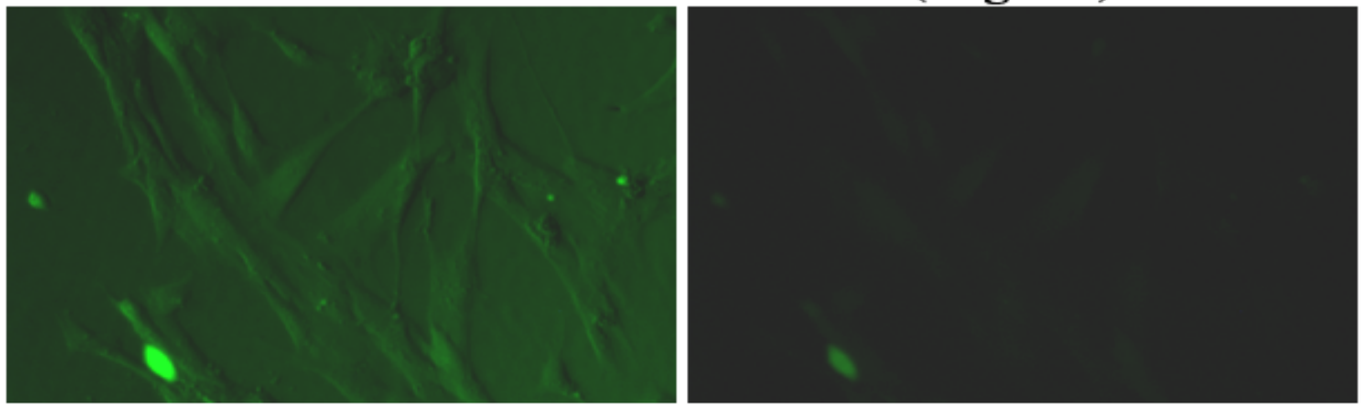
Biotin Sufficient



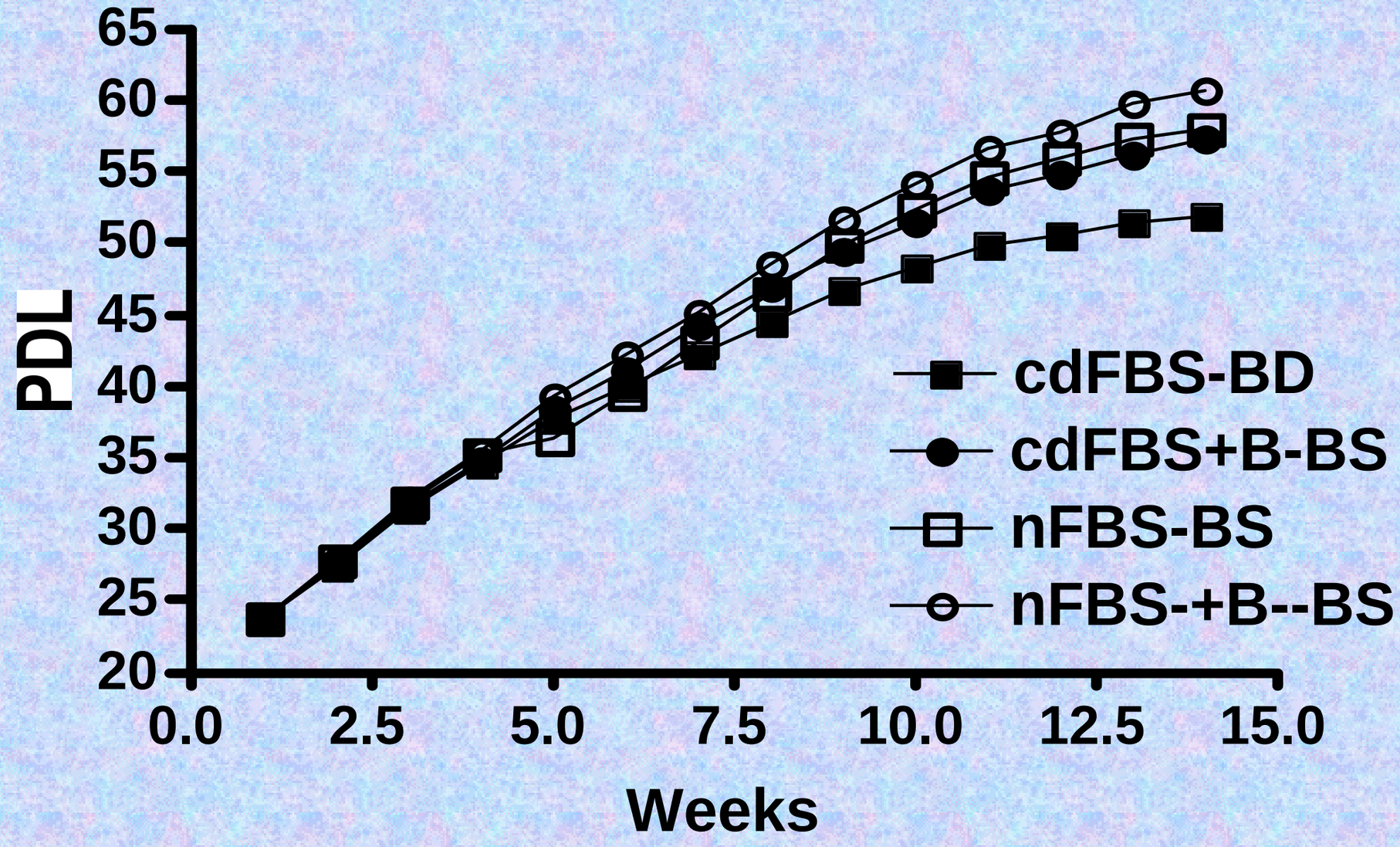
Biotin Deficient



Biotin deficient + Biotin (5ng/ ml)



Biotin deficiency accelerates cell senescence

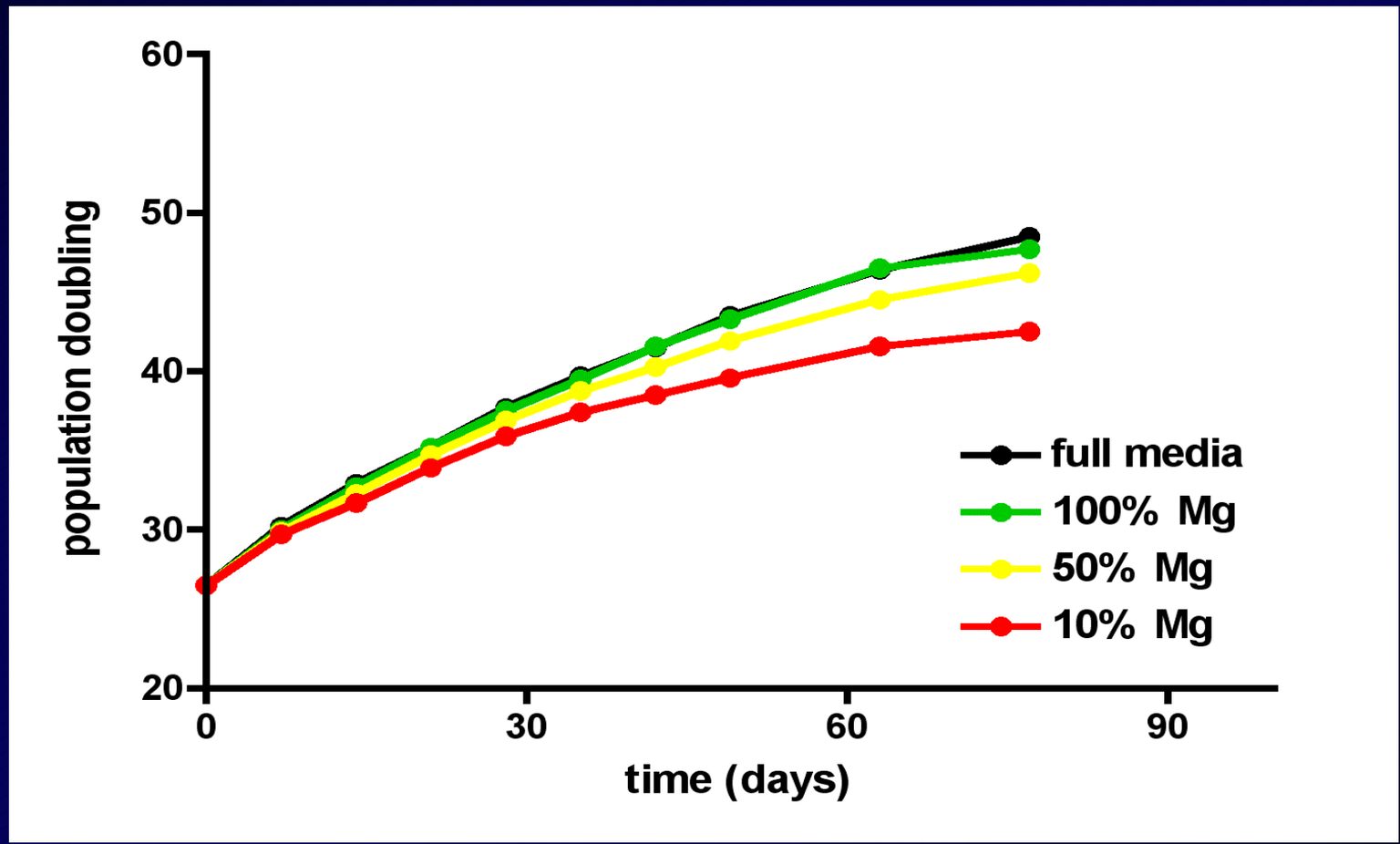


Micronutrient deficiency and heme synthesis in human cell culture

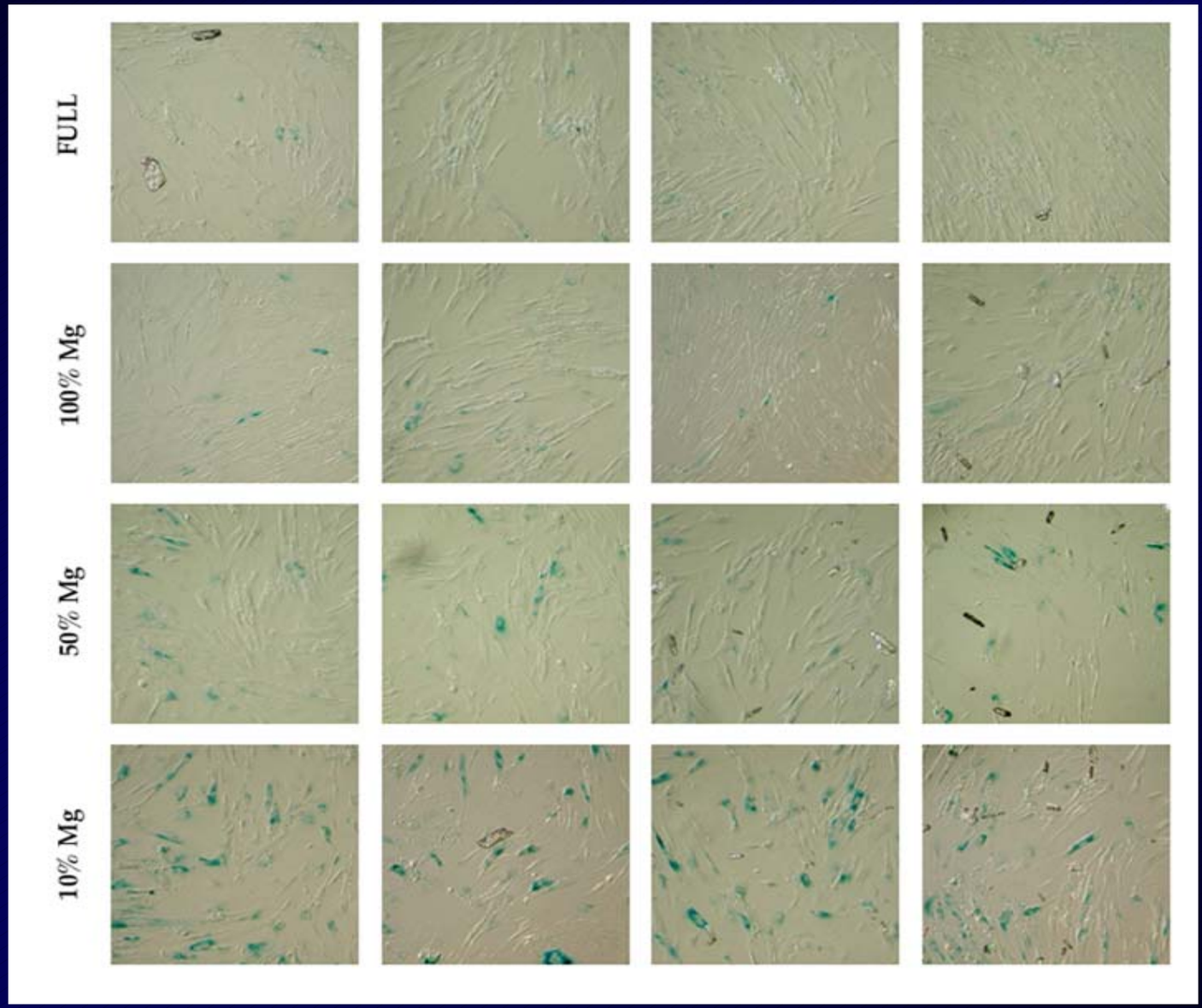
Micronutrient Deficiency	Heme Deficit	Complex IV Deficit	Oxidative Stress	DNA Damage	Early Senescence
Pyridoxine	[+]				
Zinc	+	+	+	+	
Riboflavin					
Iron	+	+	+	+	
Copper	[+]	+	[+]		
Biotin	+	+	+	+	+
Lipoic Acid			[+]		
Pantothenate	[+]	[+]			

+ = Atamna/Ames, [+] Literature

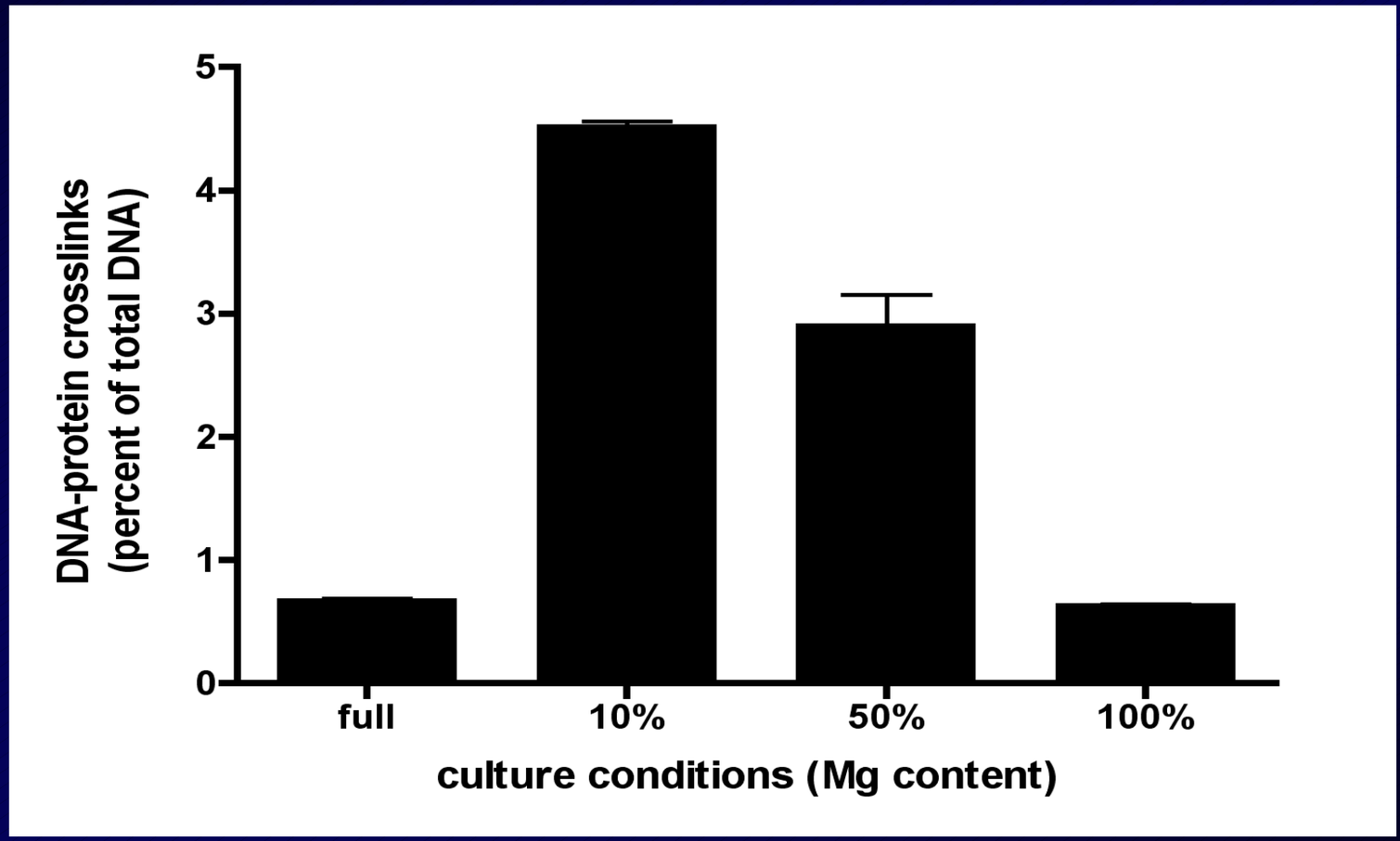
Magnesium Deficiency Shortens Fibroblast Lifespan



Magnesium Deficiency Induces Senescence Marker



Magnesium Deficiency Induces DNA-Protein Crosslinks



Calcium Deficiency

Fenech: chromosome breaks

Lipkin: colon cancer mice

Folate Deficiency

MacGregor/Ames/Fenech: chromosome breaks mice/humans

Willett: epi colon cancer humans

Vitamin D Deficiency

Garland: epi colorectal cancer humans

Magnesium Deficiency

Bell: chromosome breaks humans

Larsson: epi colorectal cancer humans

Zinc Deficiency

Fong: esophageal cancer humans/rodents

Vitamin B12

Fenech: Chromosome breaks

Selenium

Rao: DNA damage

Combs/Trumbo: Cancer humans

Omega-3 FA

Denkins: Cancer

Niacin

Kirkland/Depeint: DNA damage

Choline

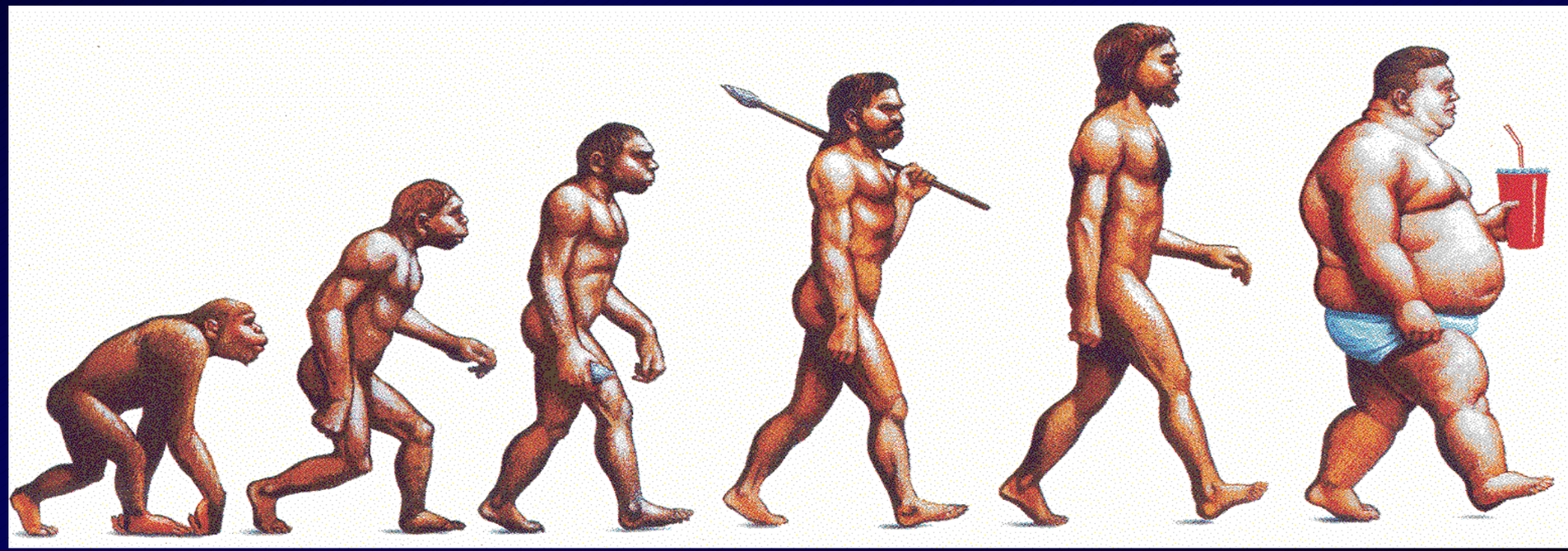
da Costa: DNA damage in humans

Many micronutrient deficiencies are found to cause DNA damage in mice or human cells in culture and, where assayed, earlier senescence.

> ***I hypothesize that: 1) episodic shortage of each micronutrient throughout evolution caused natural selection to favor short-term survival of the organism at the expense of long-term health; 2) this was achieved by allocating scarce micronutrients by enzyme triage through an adjustment of the binding affinity of each enzyme for its required micronutrient.***

The consequences of the triage are evident at all levels. For example, in metabolic reactions, ATP synthesis would be favored over DNA-protecting enzymes; in cells, erythrocytes over leukocytes; and in organs, the heart over the liver.

If this hypothesis is validated, ensuring micronutrient adequacy in humans throughout life is essential for maximizing longevity and minimizing the degenerative diseases of aging.



The Economist, December 13, 2003

Energy Sources - 1999-2000

	Food	Cumulative Percentage
1.	Regular soft drinks	7.1
2.	Cake, sweet rolls, doughnuts, pastries	10.6
3.	Hamburgers, cheeseburgers, meatloaf	13.8
4.	Pizza	16.8
5.	Potato chips, corn chips, popcorn	19.7
6.	Rice	22.4
7.	Rolls, buns, English muffins, bagels	25.0
8.	Cheese or cheese spread	27.6
9.	Beer	30.2
10.	French fries, fried potatoes	32.4

Gladys Block from National Health and Nutrition Examination Survey (NHANES) 2000.

CAUTION: HAZARDOUS WAIST

Visceral fat increases your risk of
heart disease, diabetes, etc.

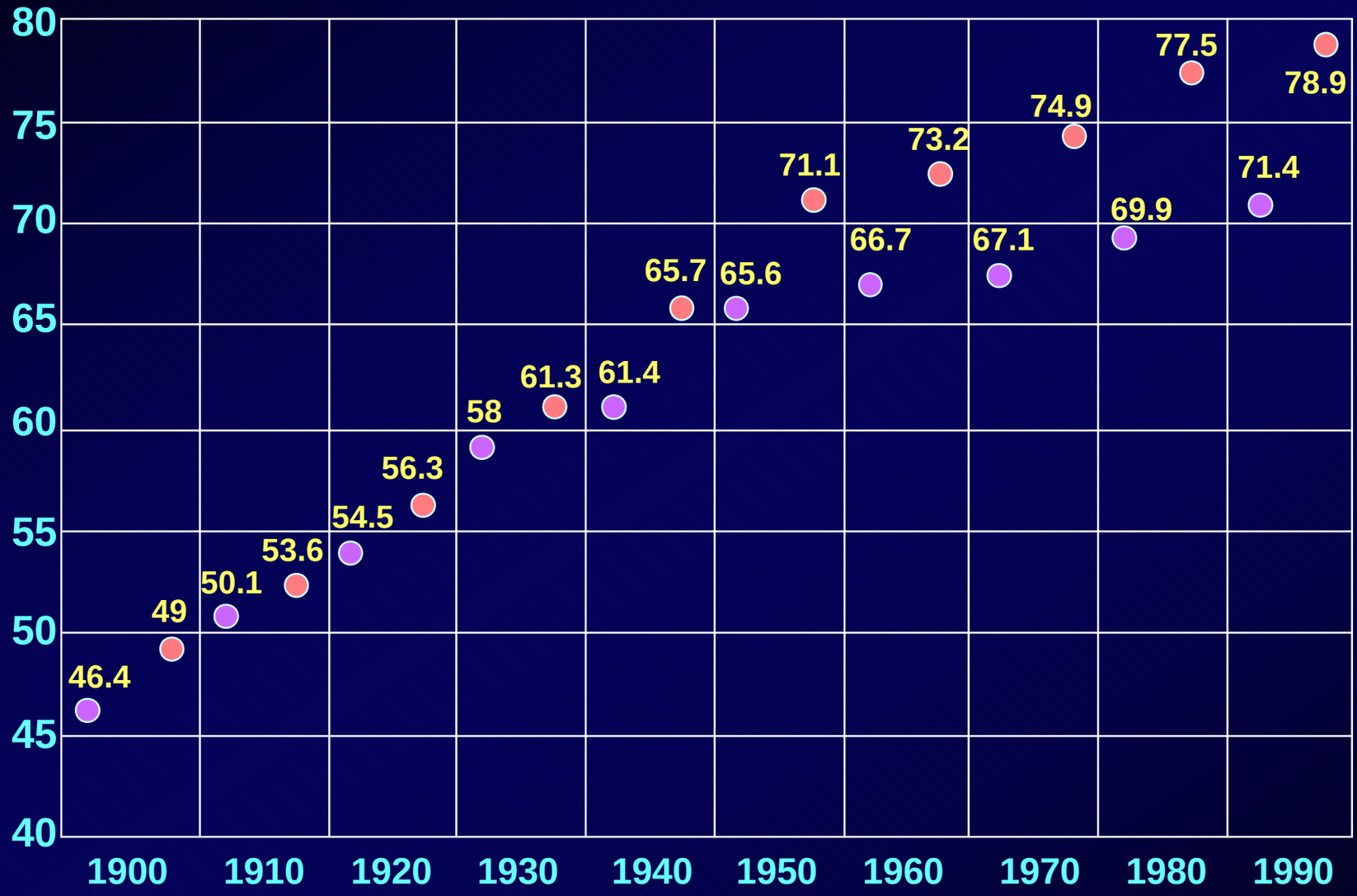
Start a waist disposal program today.

Dr. Allen Spiegel, NIDDK/NIH

“The main distinguishing characteristic between man and the lower animals is the desire to take pills”

Mark Twain

Life Expectancy of Men and Women at Birth



SOURCE: National Institute on Aging

END