Delaying the Degenerative Diseases of Aging

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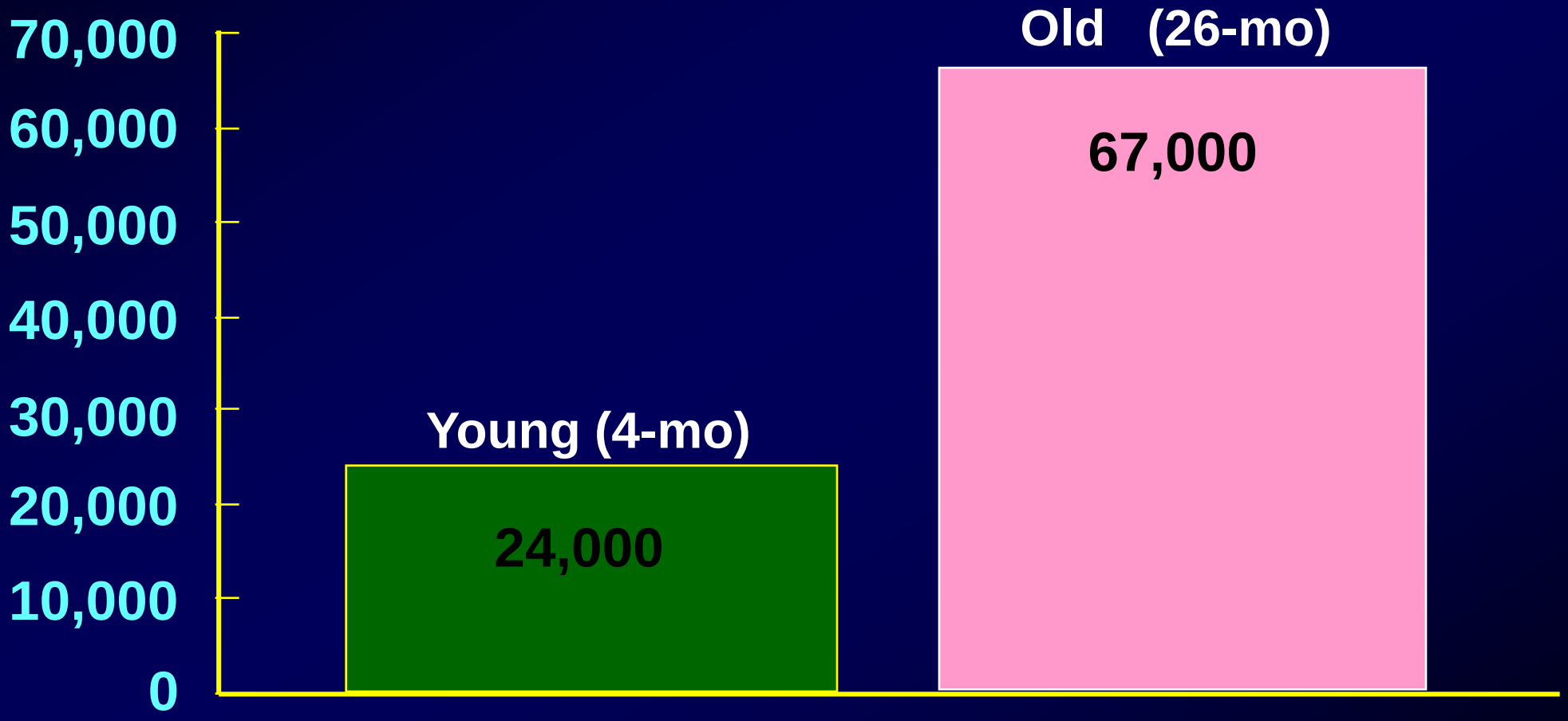
Children's Hospital Oakland Research Institute

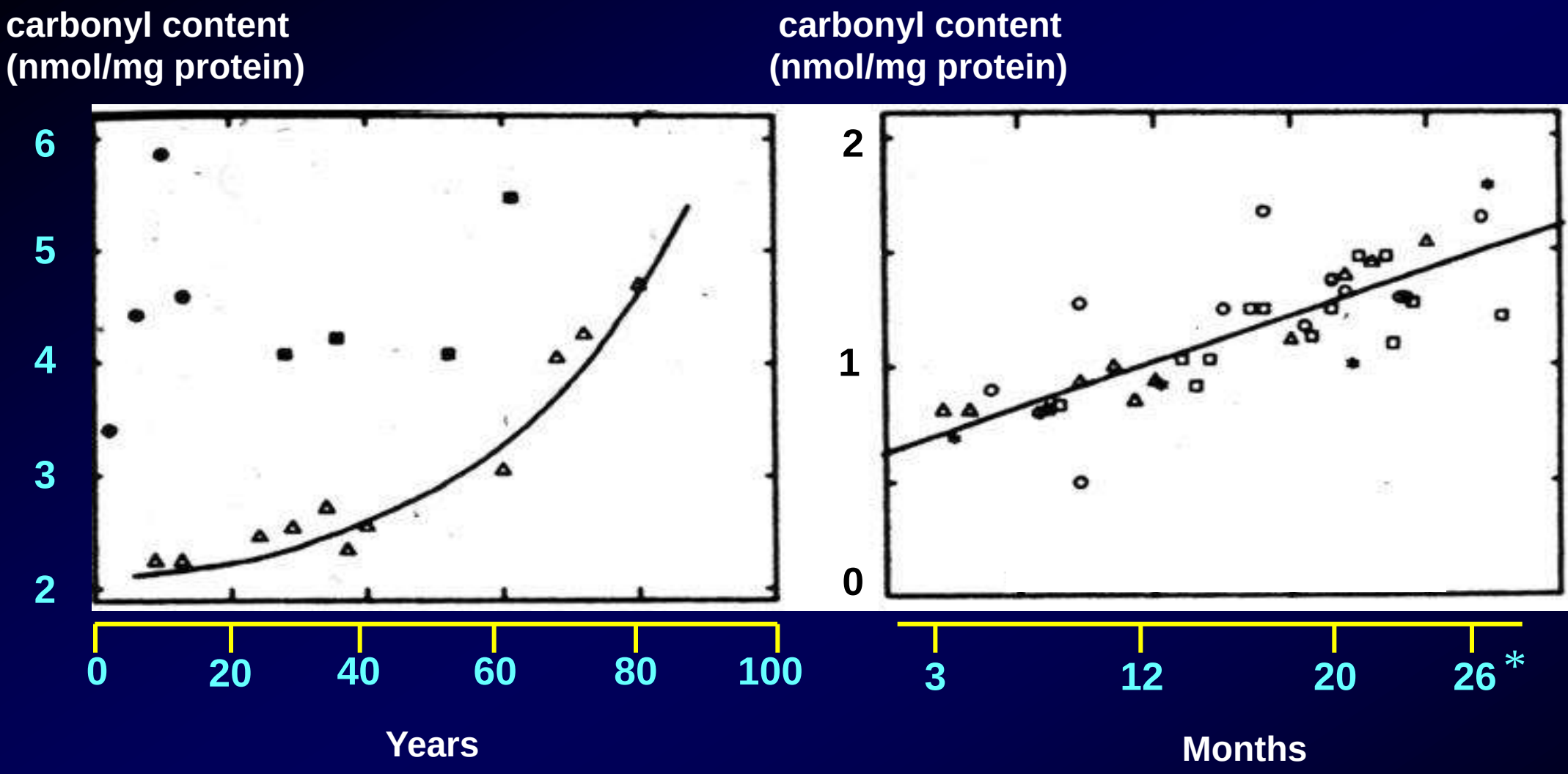
Professor, University of California, Berkeley

5 August '07

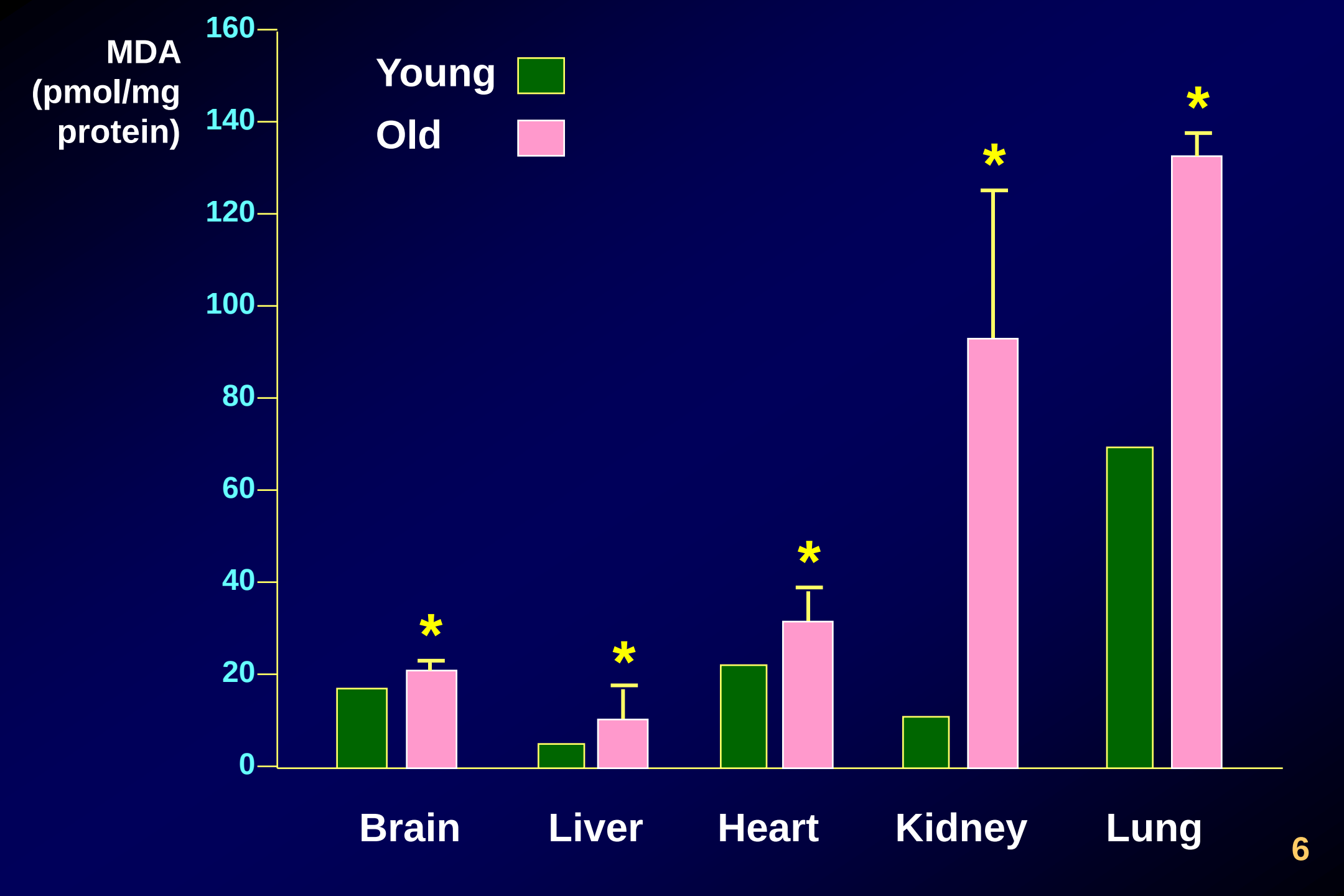
DDP Oakland

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

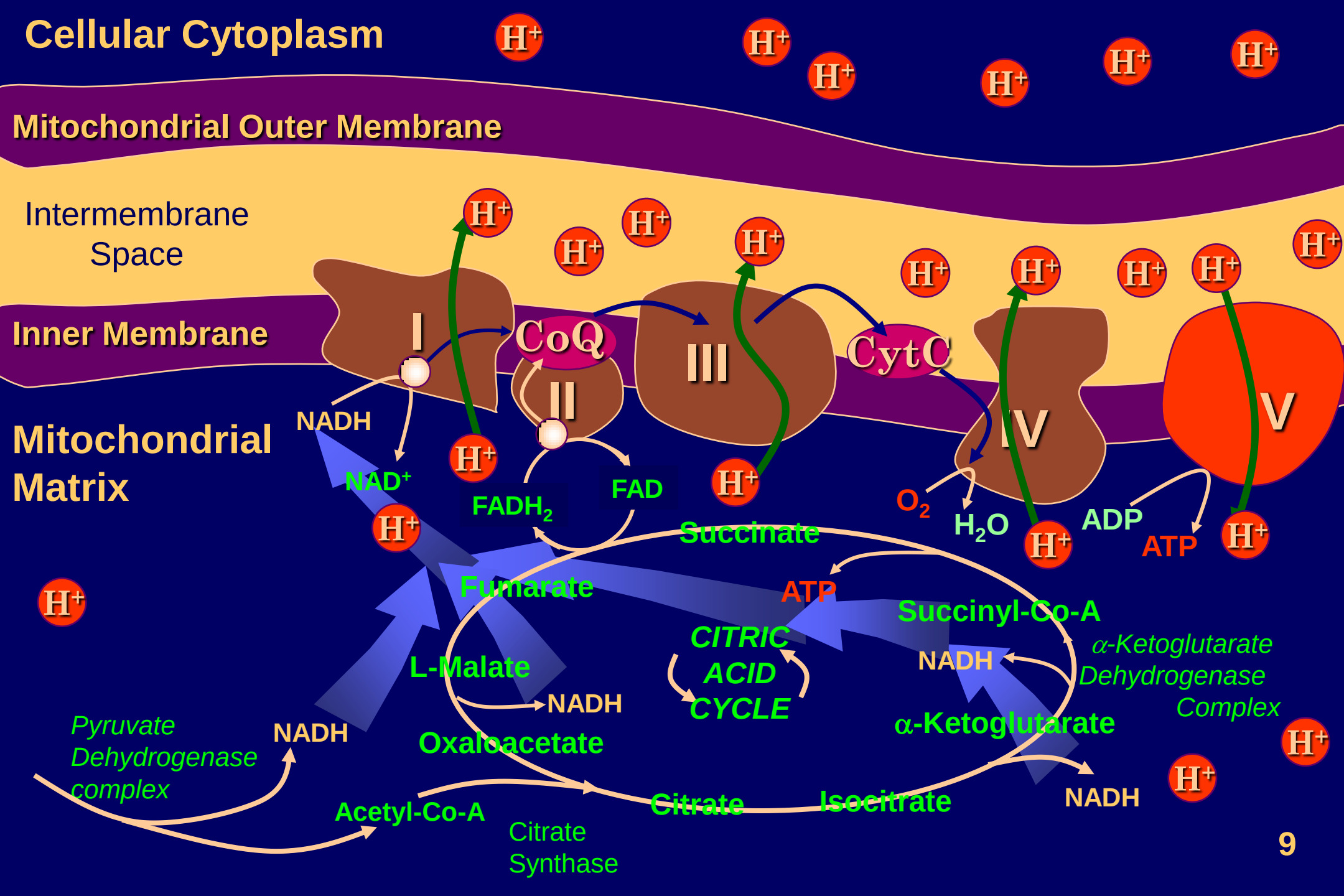
Review

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


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

Mitochondria from old rats compared to those from young rats:

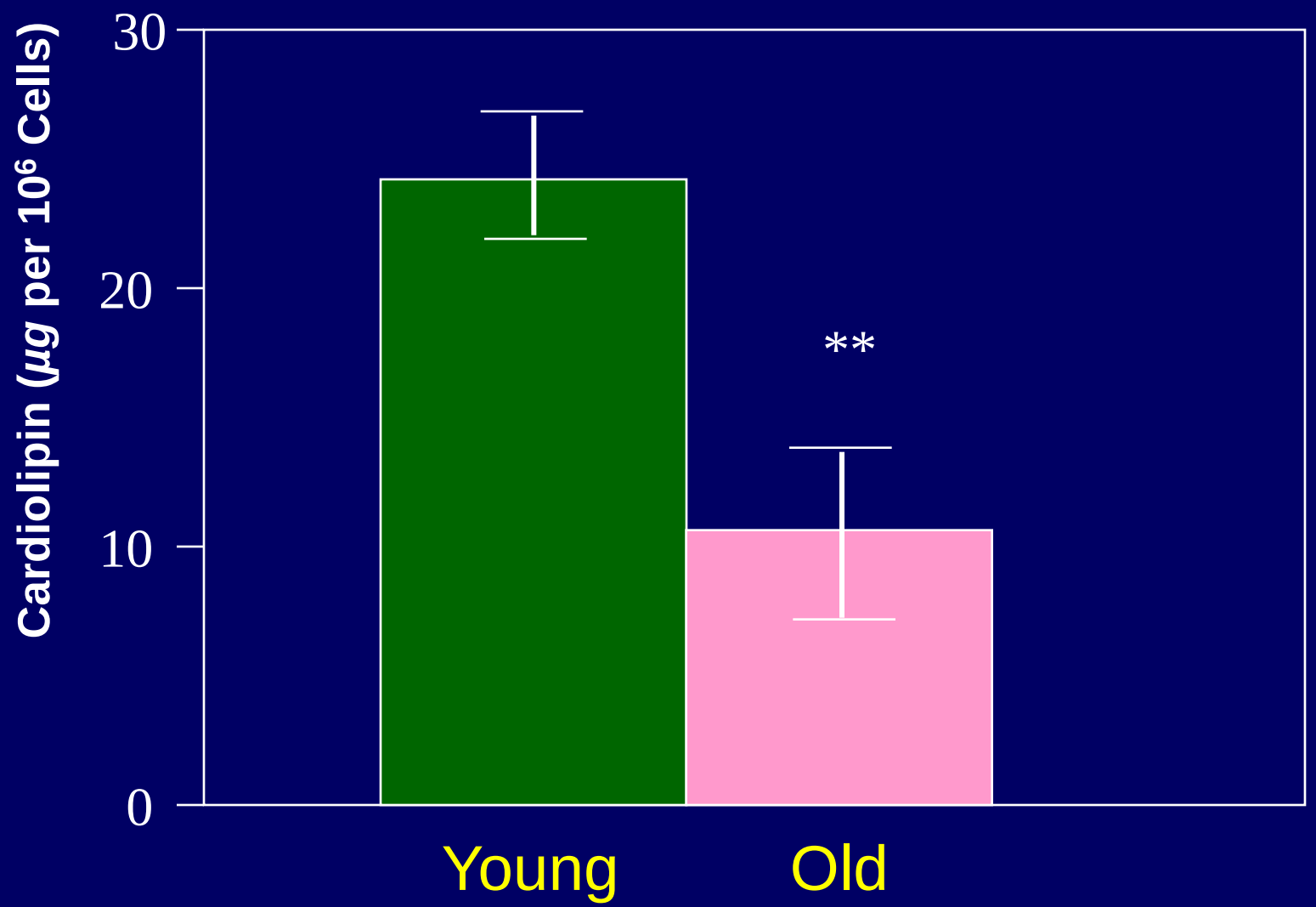
1) Lower Cardiolipin

2) Lower Membrane Potential

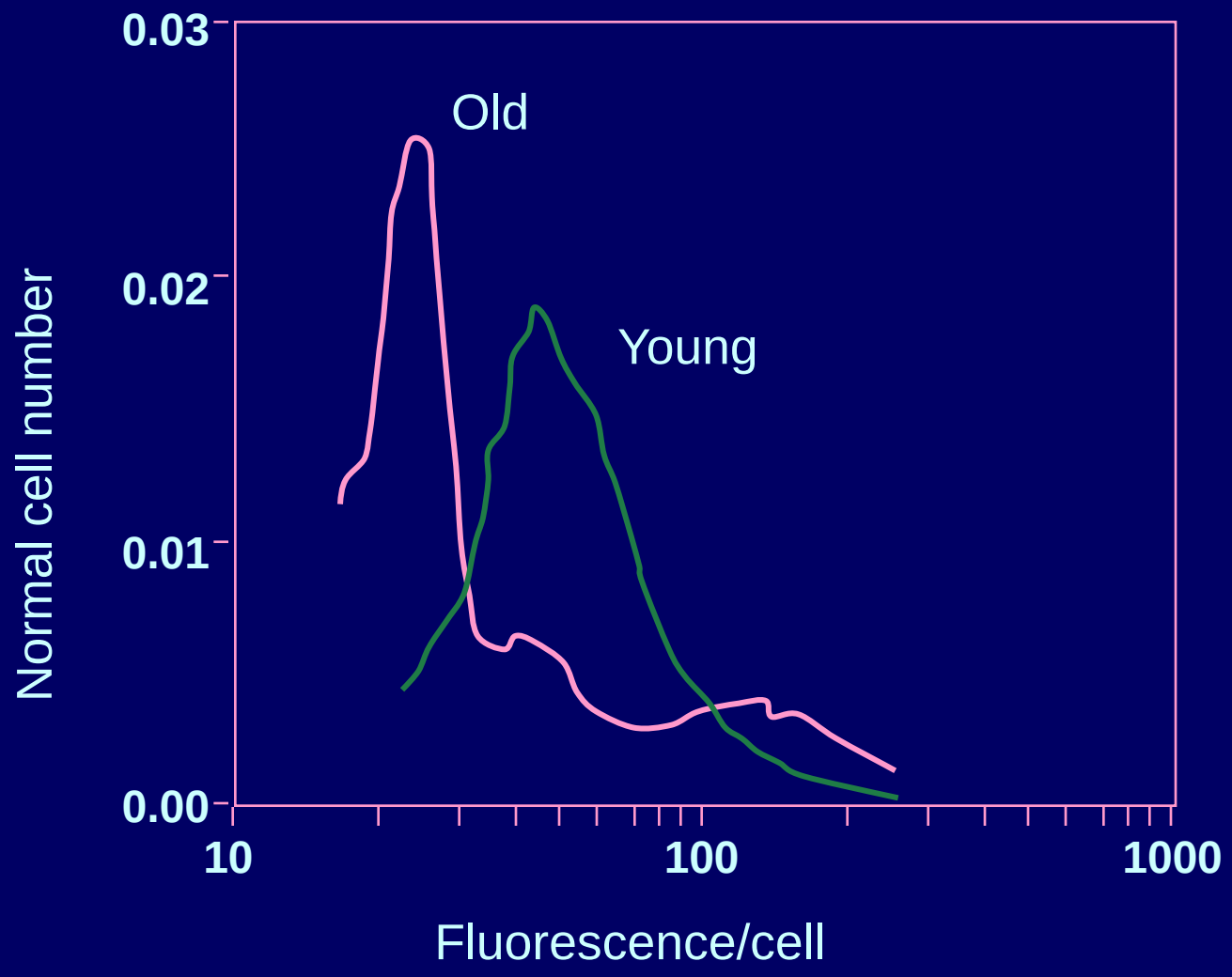
3) Lower Oxygen Utilization

4) Increased Oxidant Leakage

Cardiolipin Levels in 3 and 24 Month Old Rat Hepatocytes

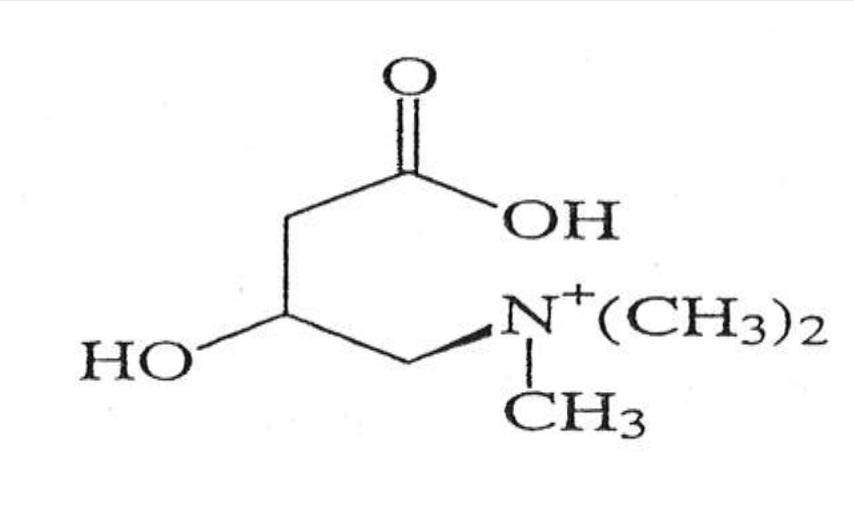


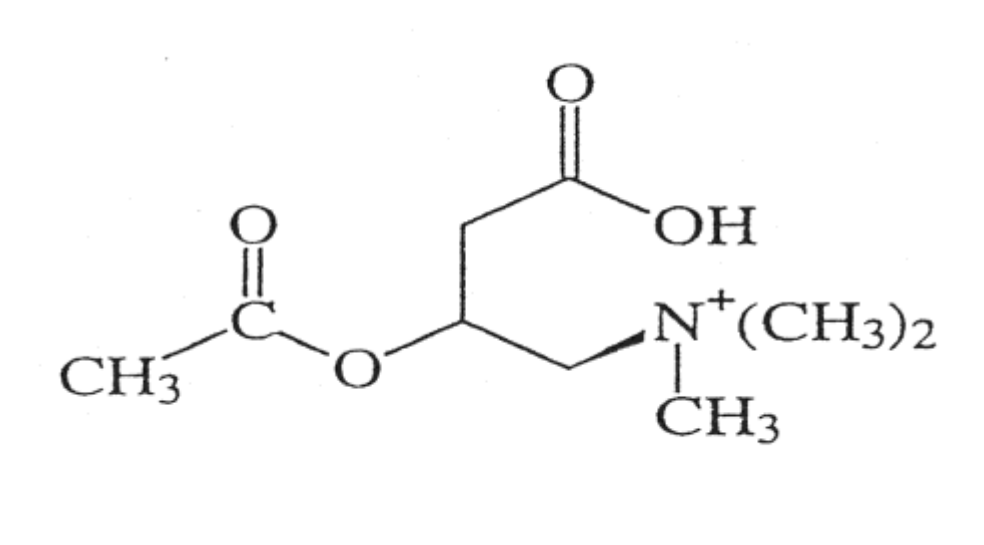
R123 Fluorescence in old and young rat hepatocytes



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

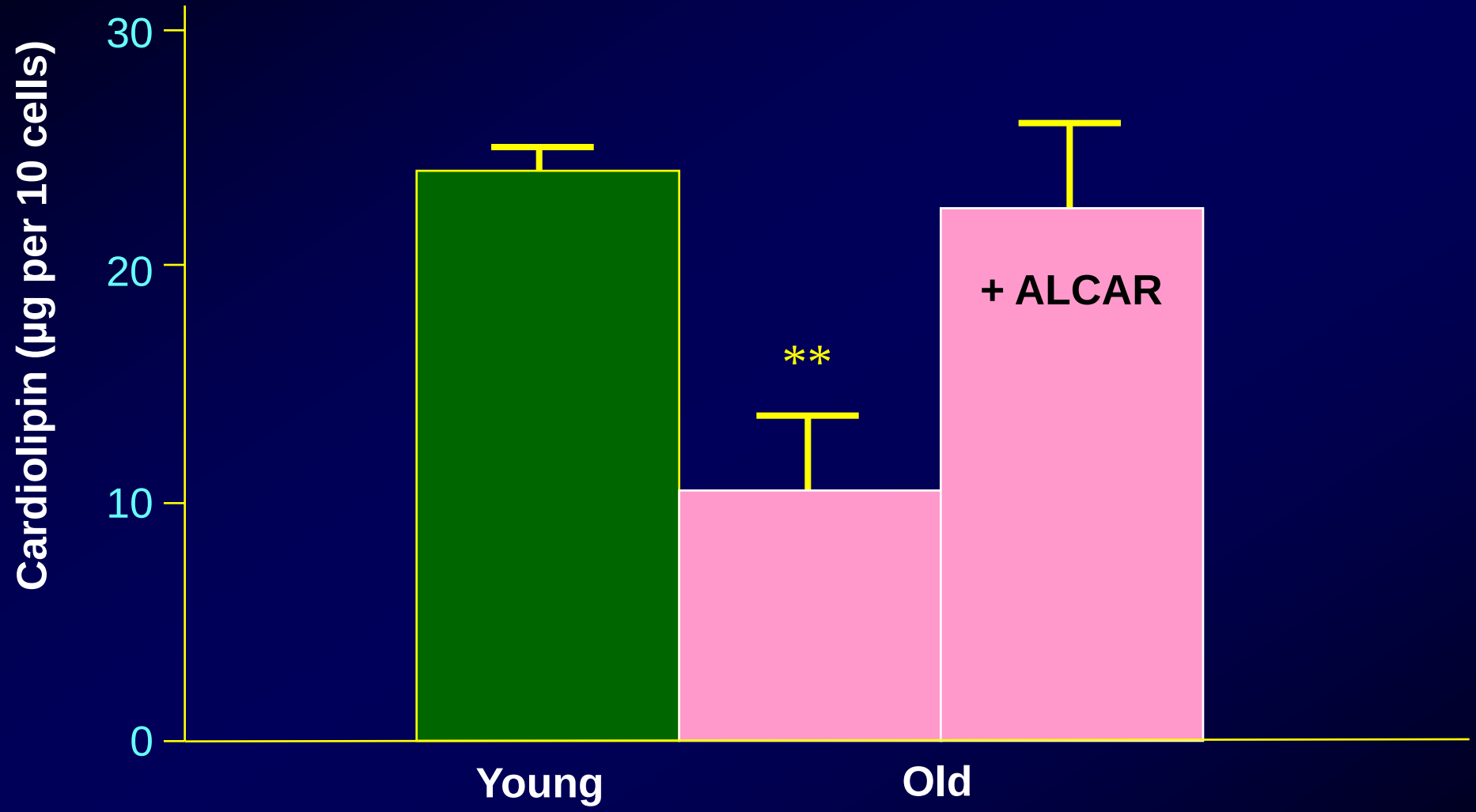
- Transports long-chain fatty acids into mitochondria
- Removes short- and medium-chain fatty acids that accumulate



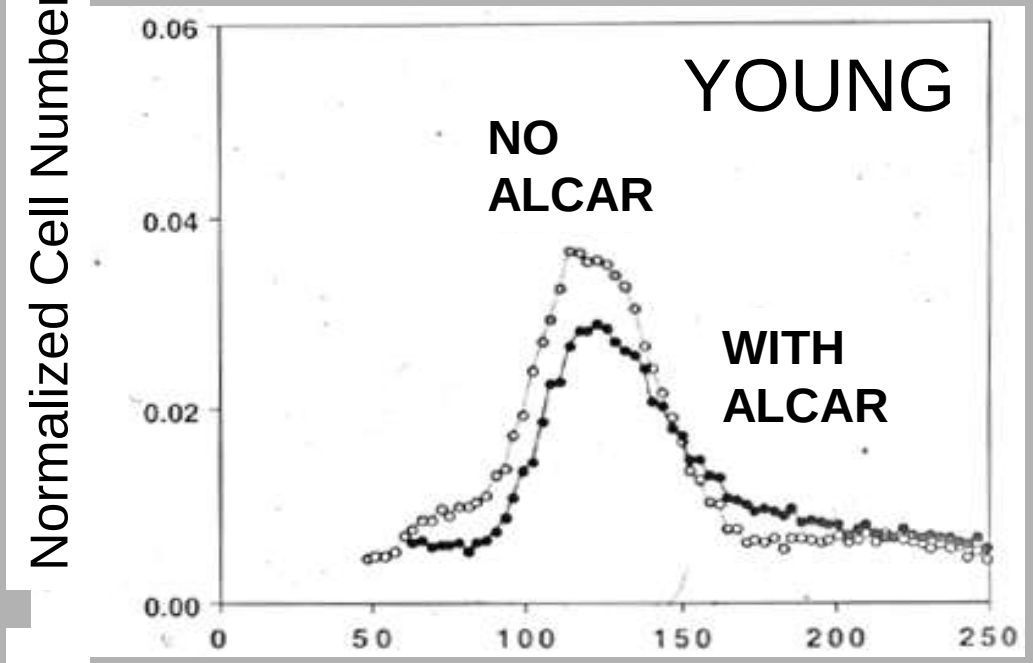


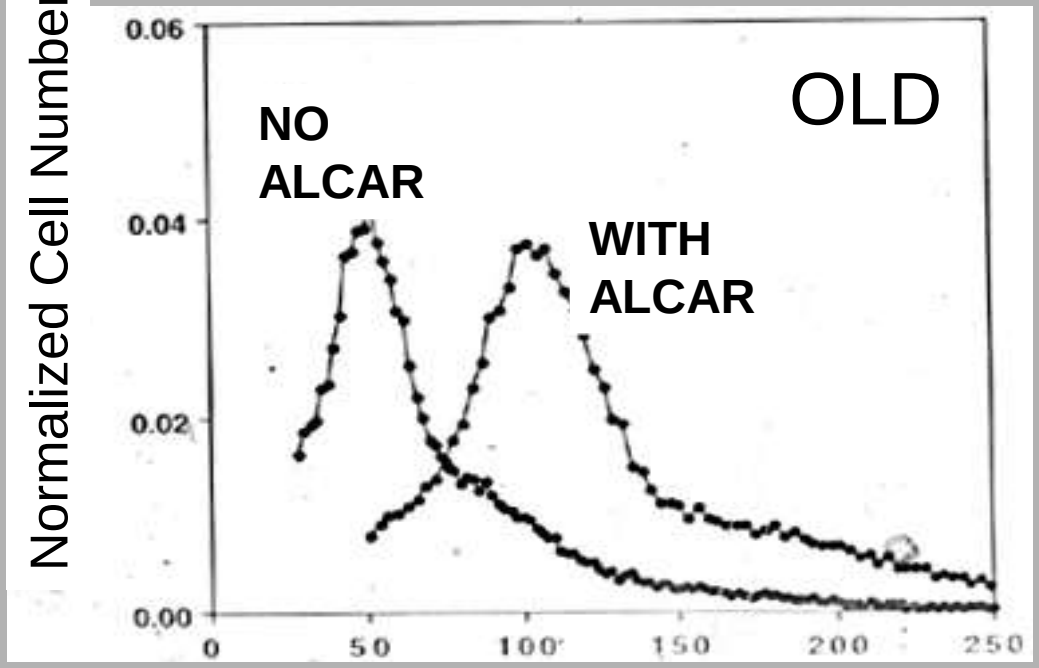
- Mediates the ratio of acetyl-CoA/CoA
- Decreases with age in plasma and in brain
- Improves cognitive function in rats

Effect of ALCAR Supplementation on Cardiolipin Levels



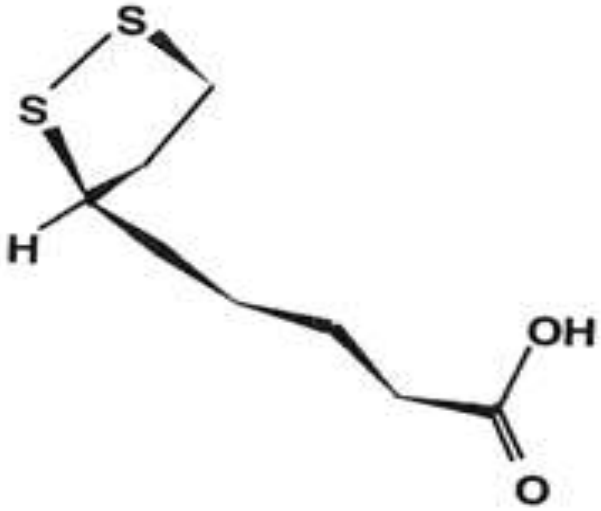
R123 Fluorescence in Young and Old Rat Hepatocytes

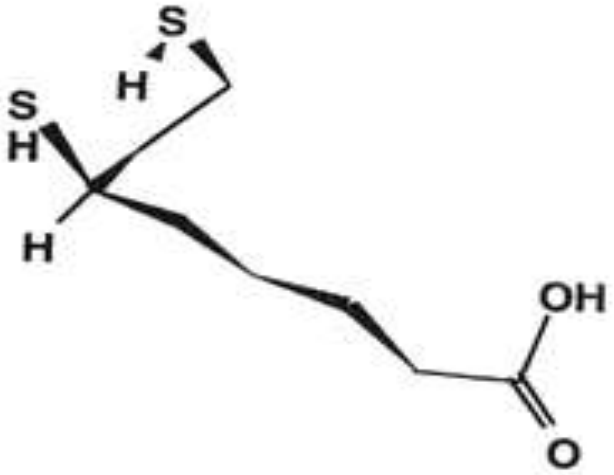




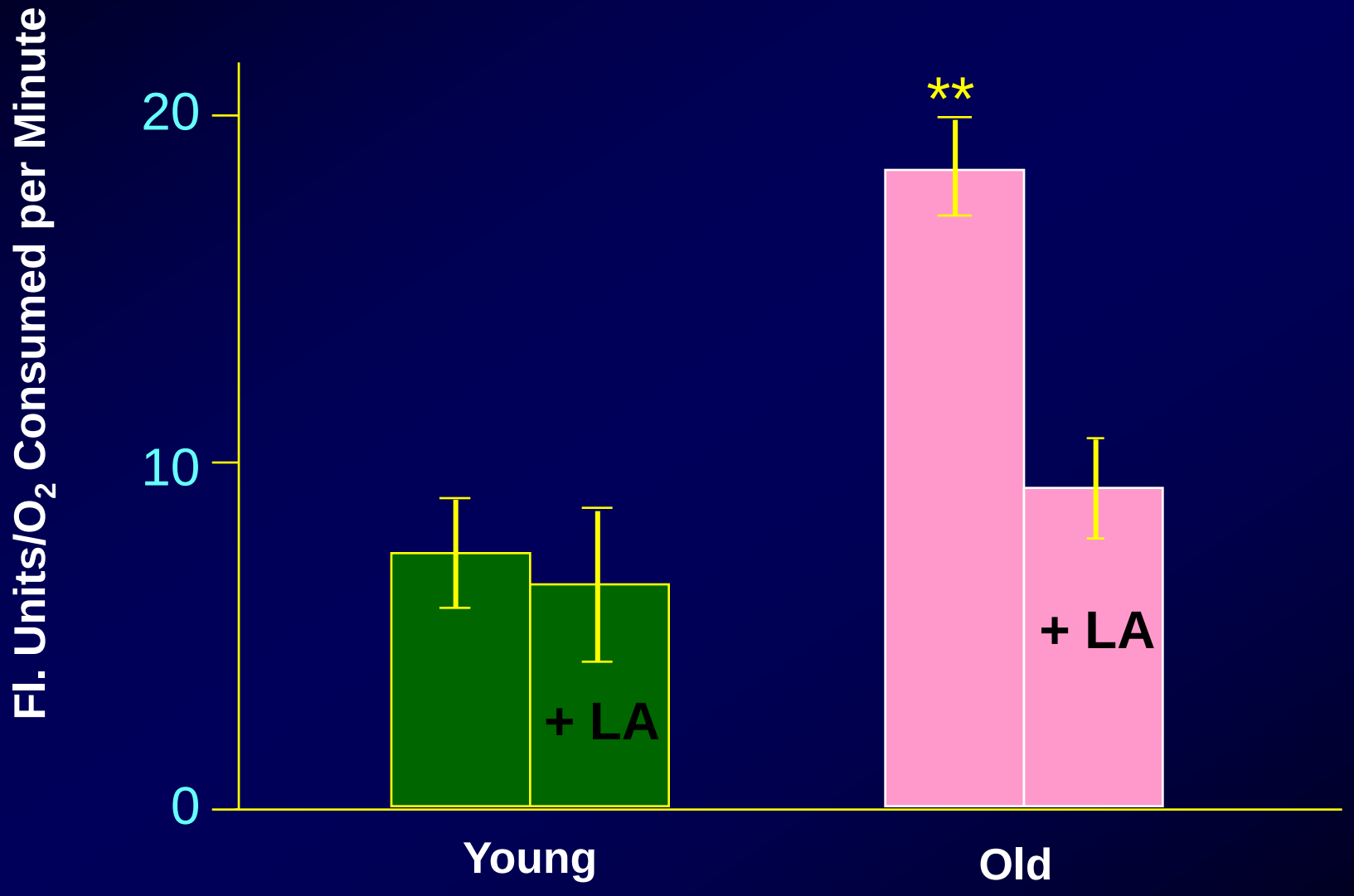
R-α-Lipoic Acid (LA) in mitochondria

- 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

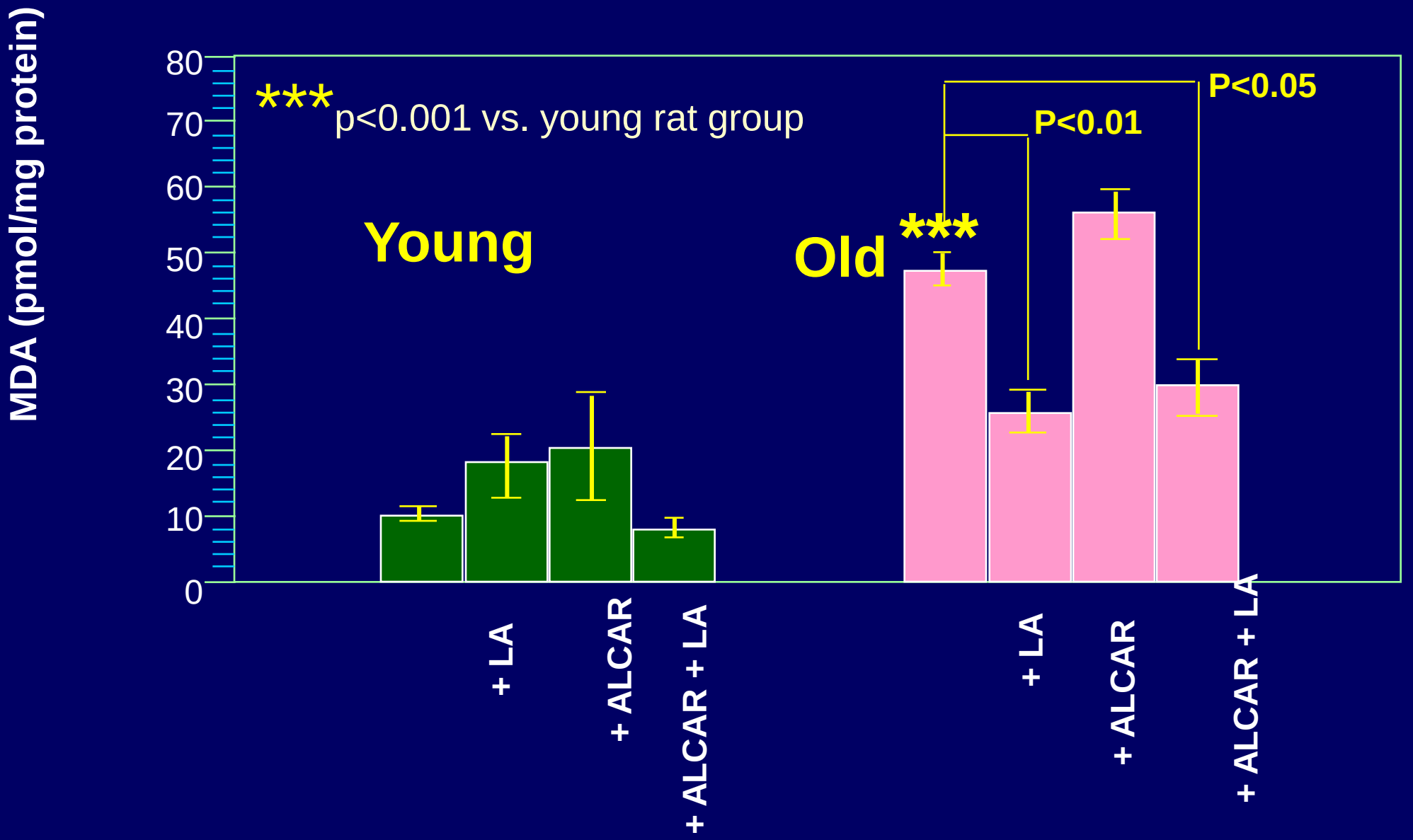




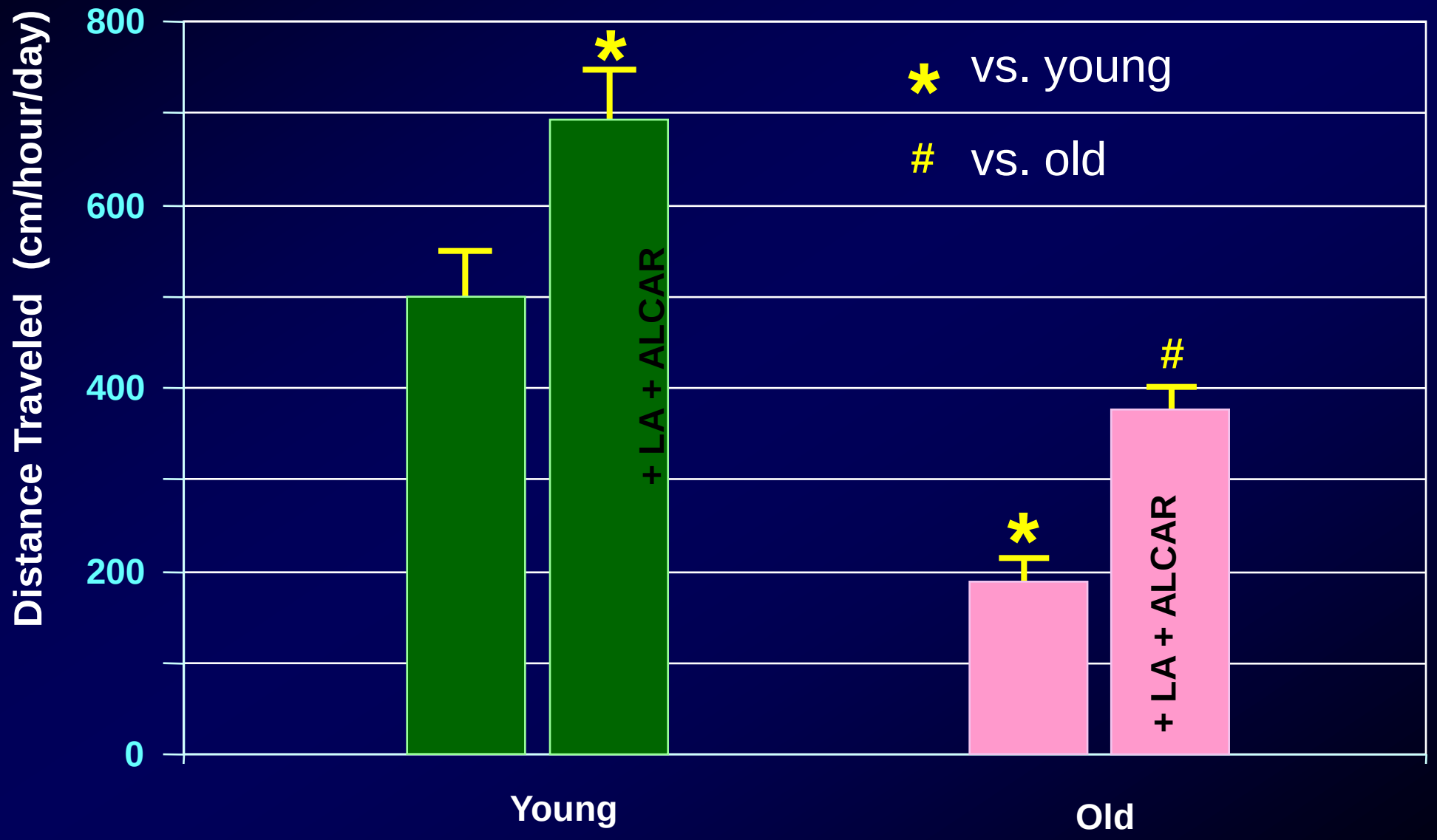
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats



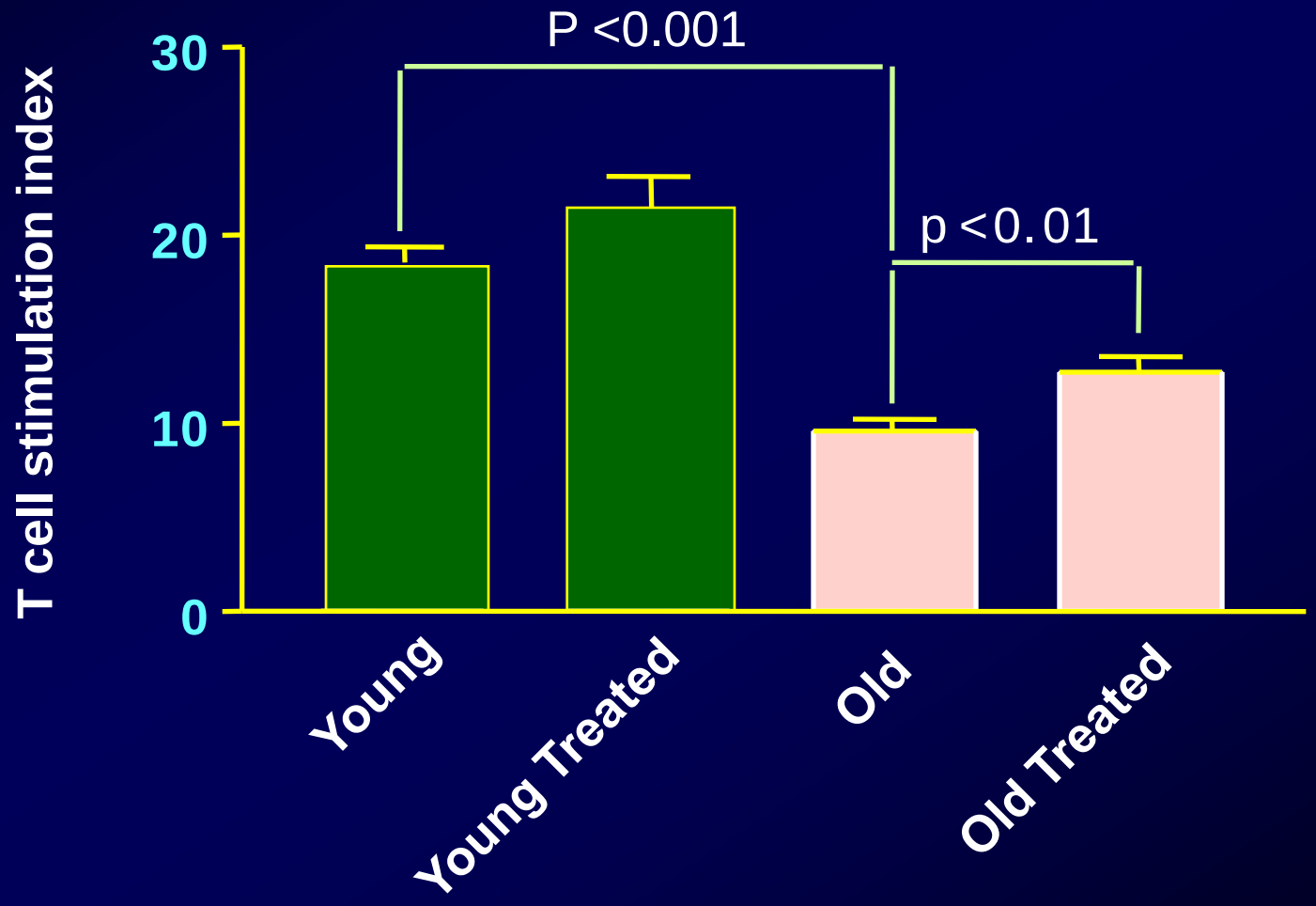
MDA levels in young and old rats with LA, ALCAR, or both



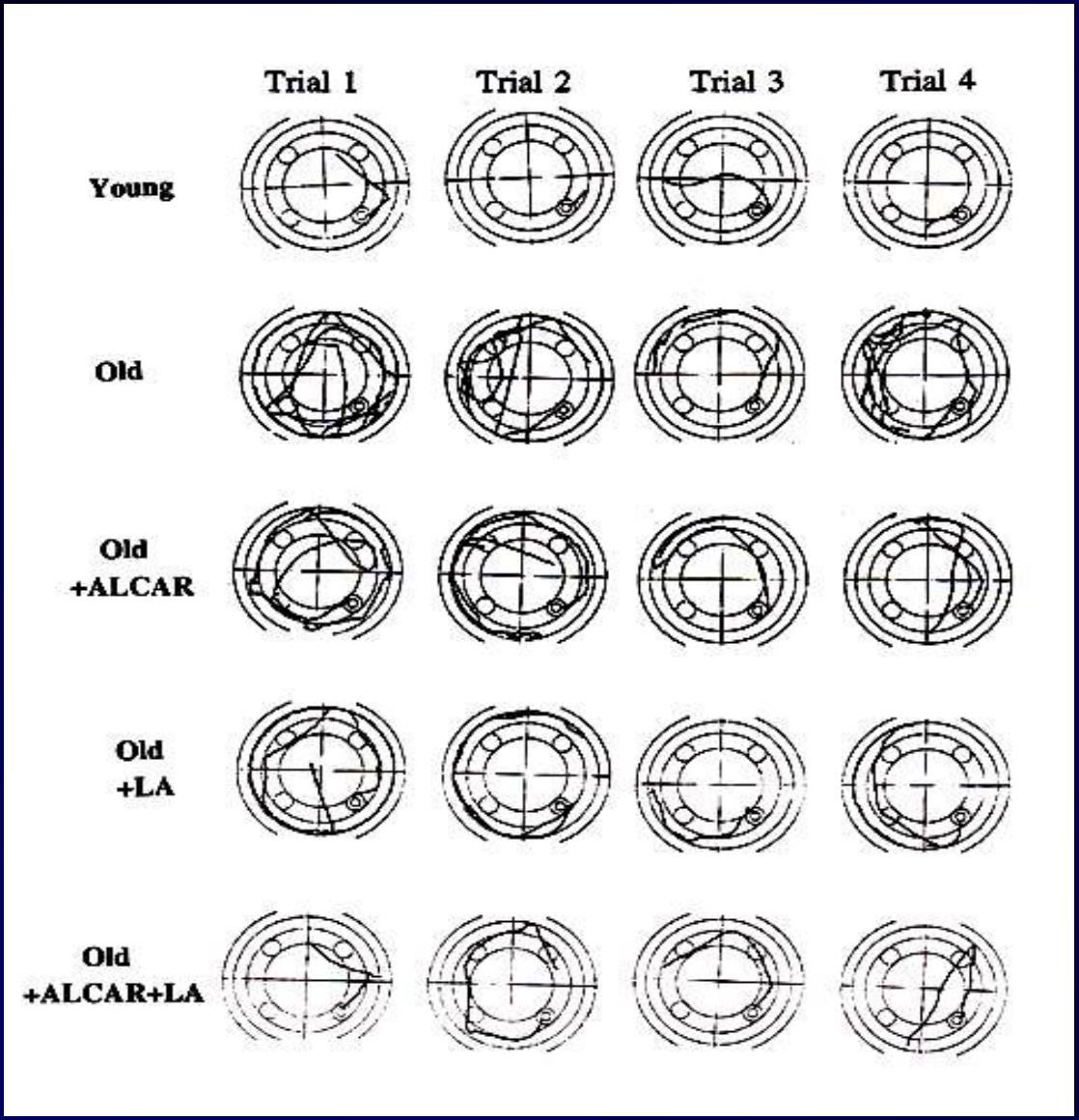
Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)



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.



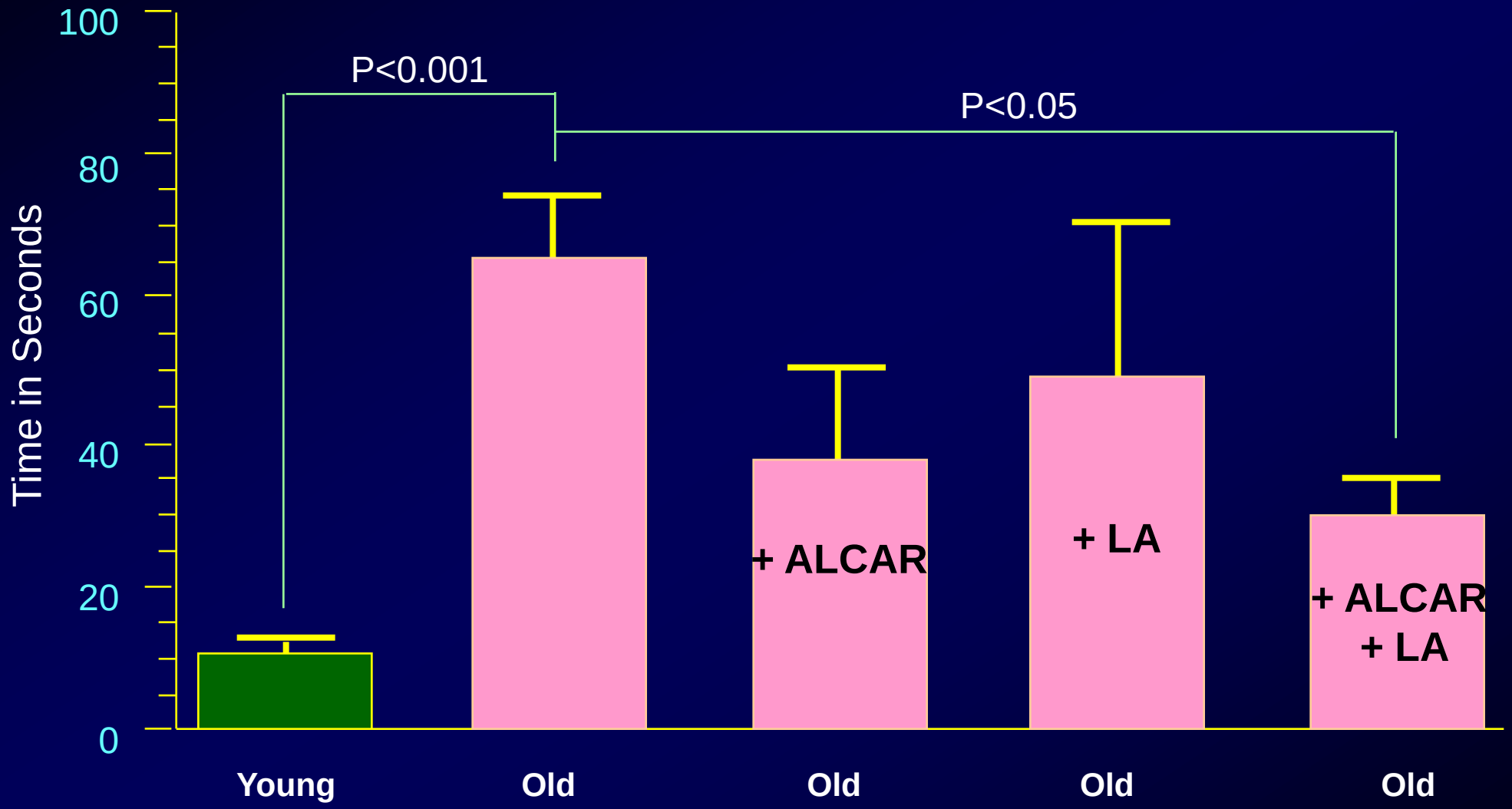
Morris Water Maze for Testing Spatial Memory



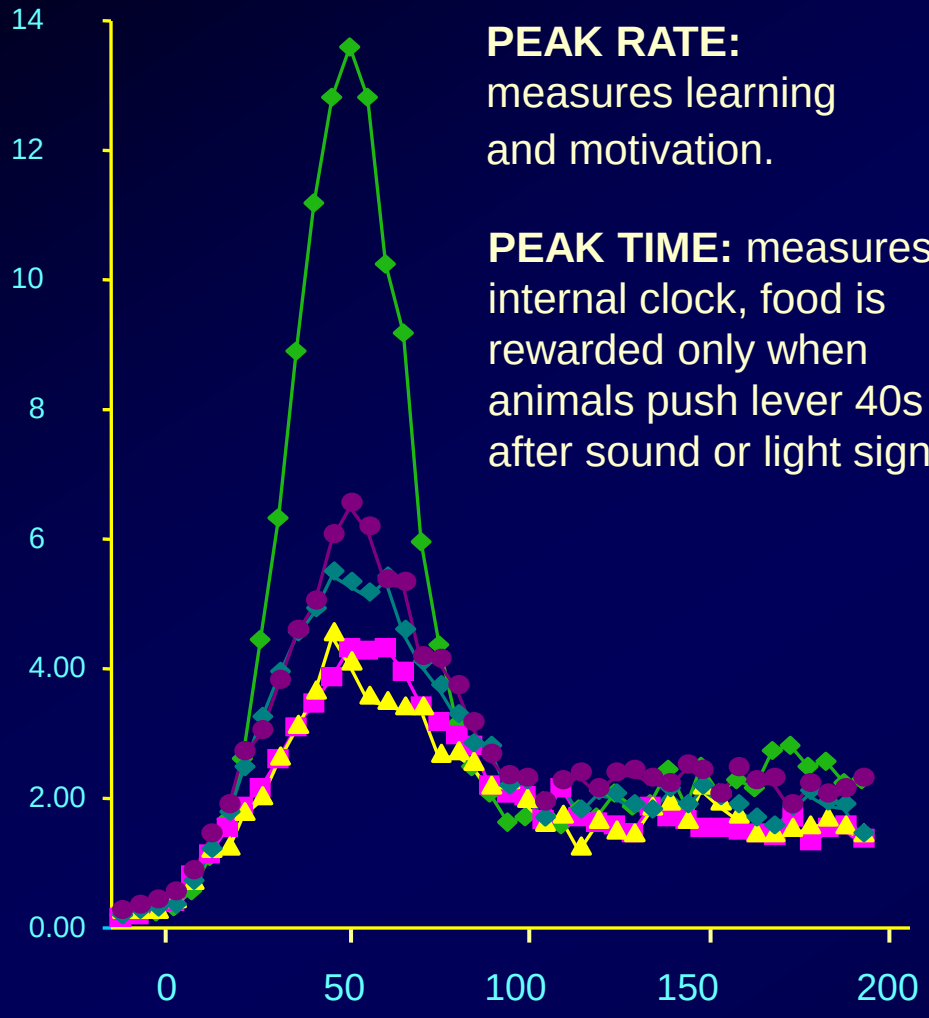
Spatial Memory relies on intact hippocampal function.

Treatments improved poor memory in old rats

Spatial Memory Tested With Morris Water Maze



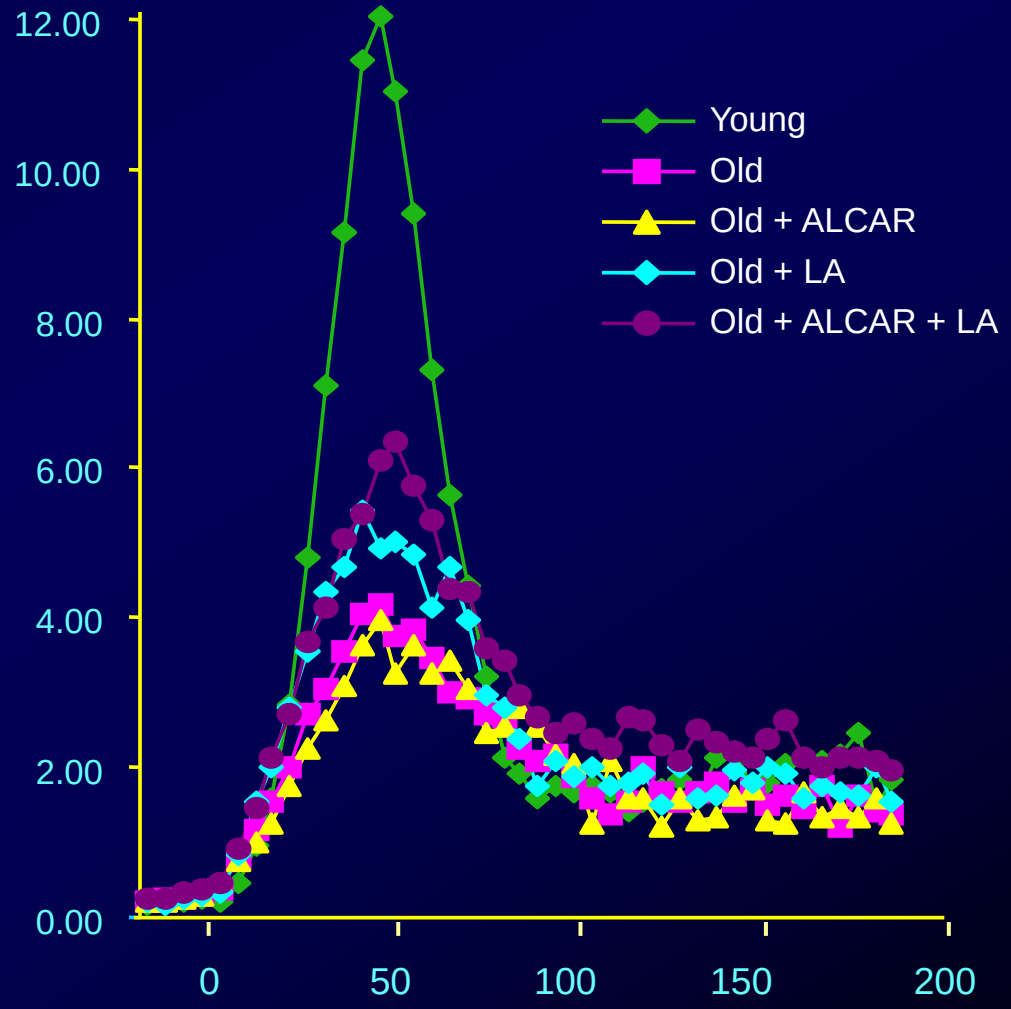
Peak procedure: for measuring temporal memory. Associated with striatum, cerebellum, & hippocampus



PEAK RATE: measures learning and motivation.

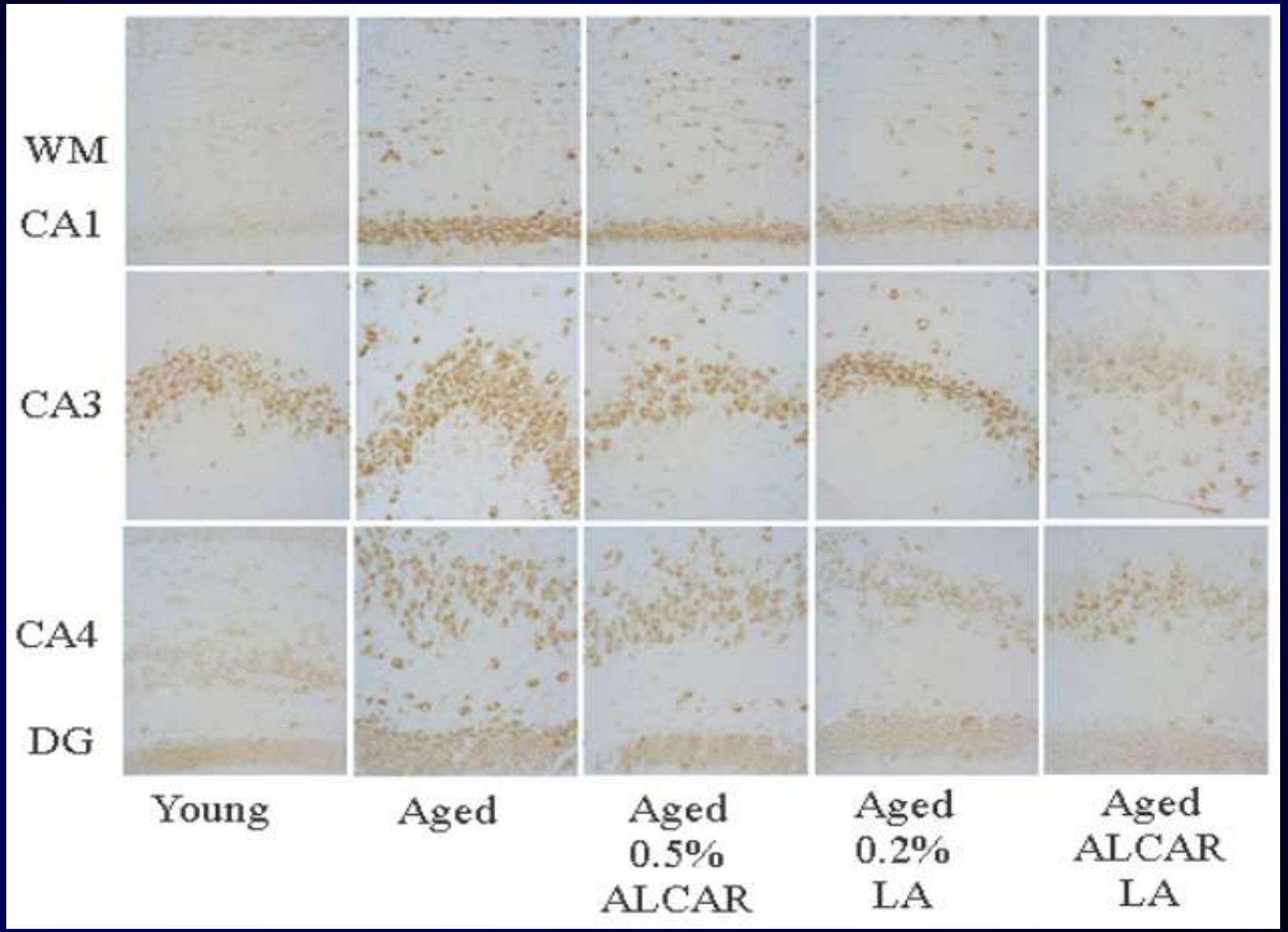
PEAK TIME: measures internal clock, food is rewarded only when animals push lever 40s after sound or light signal

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

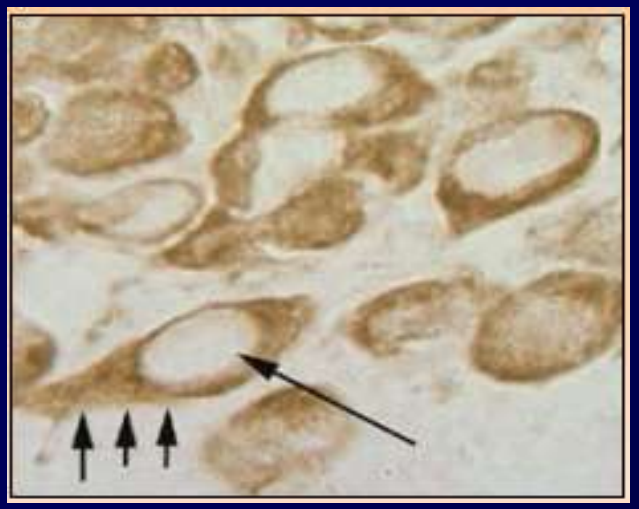


Staining of oxidized nucleic acid in neurons

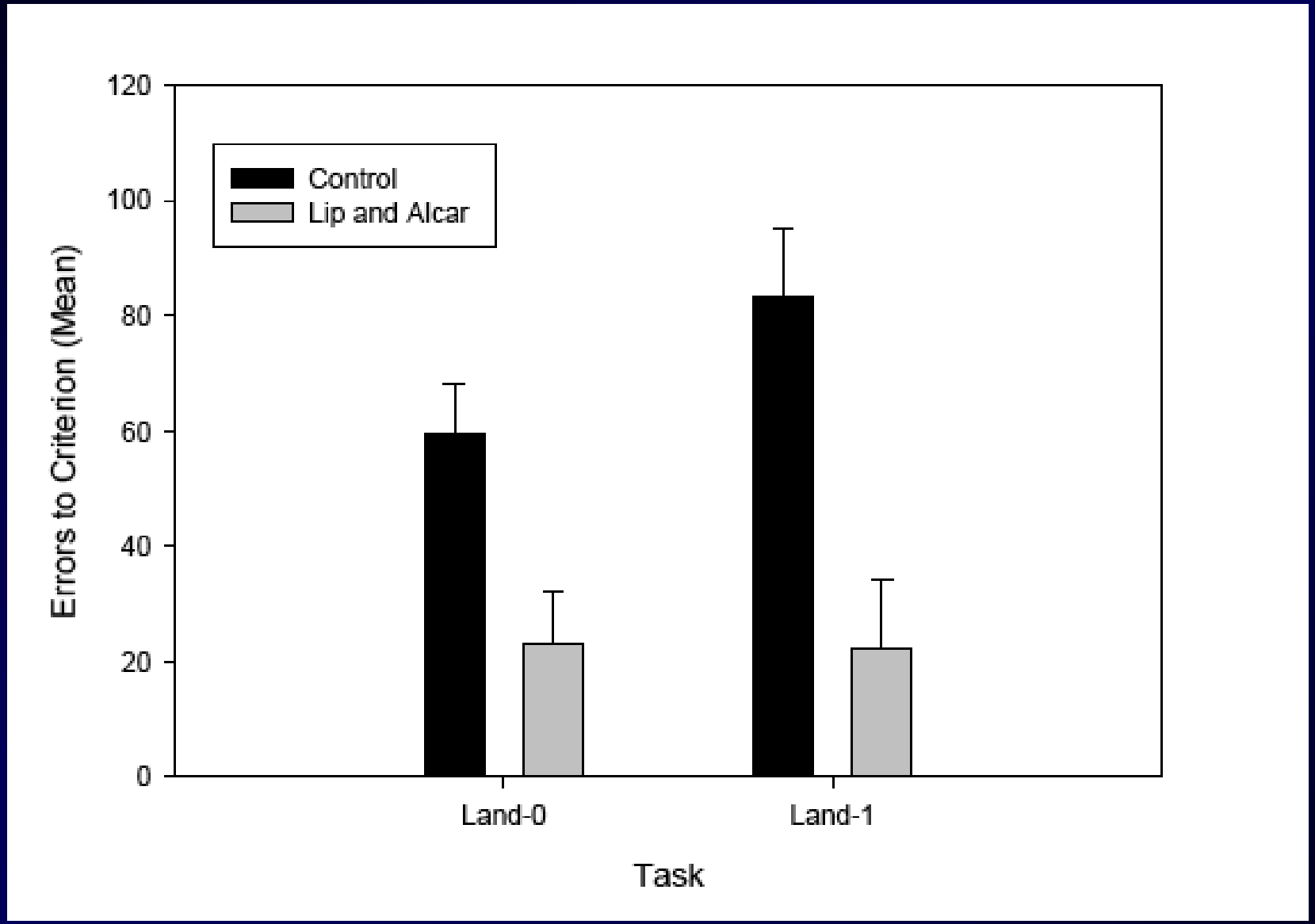
(mAb to oxo8dG in DNA/oxo8G in R□NA)

RNA is Oxidized

(92% is removed by RNase)



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


120
100
80
60
40
20
0
Errors to Criterion (Mean)
Control
Lip and Alcar
Land-0
Land-1
Task

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

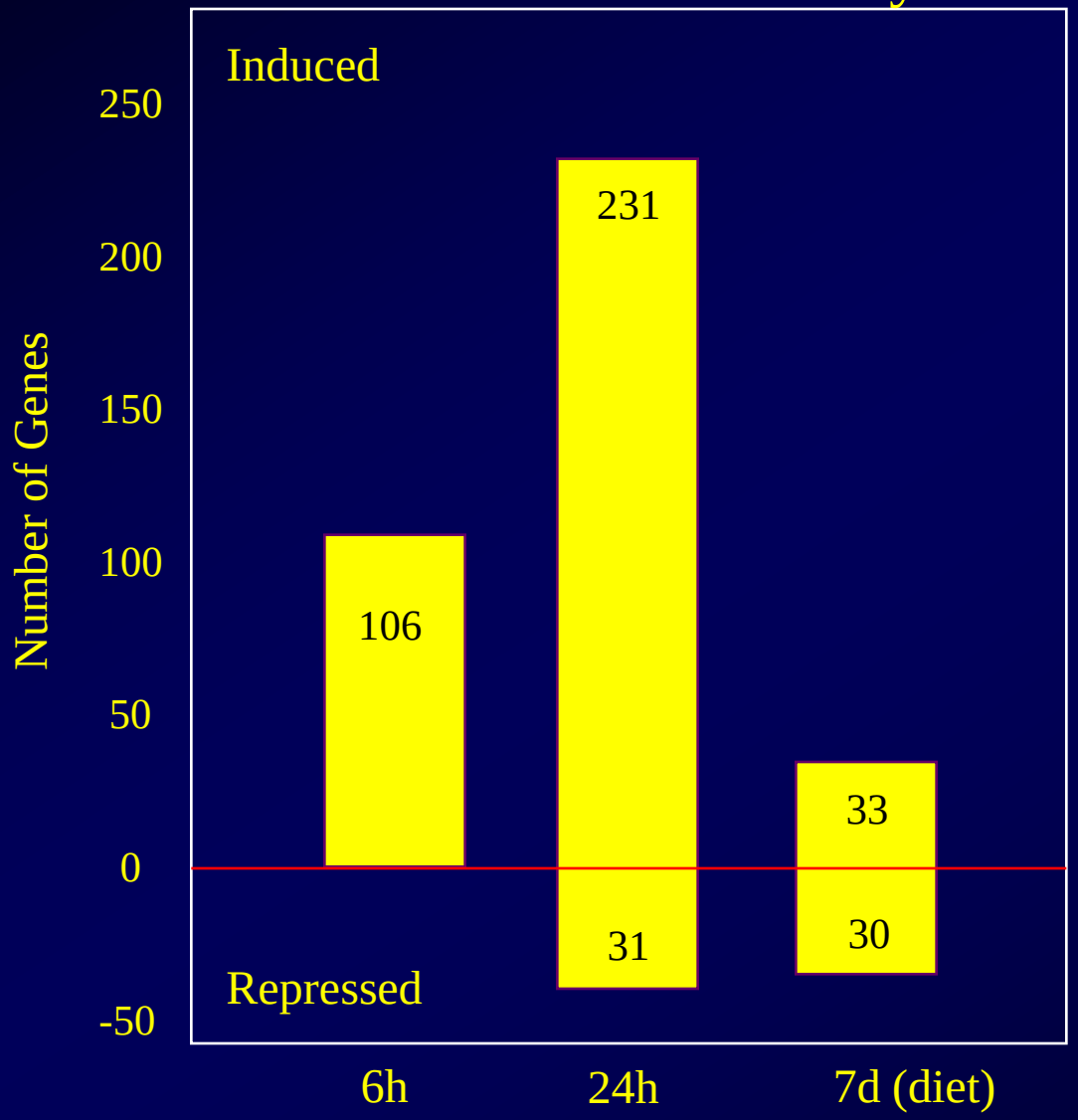
Mi-Kyoung Kwak, Nobunao Wakabayashi, Ken Itoh, Hozumi Motohashi, Masayuki Yamamoto, and Thomas W. Kensler

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

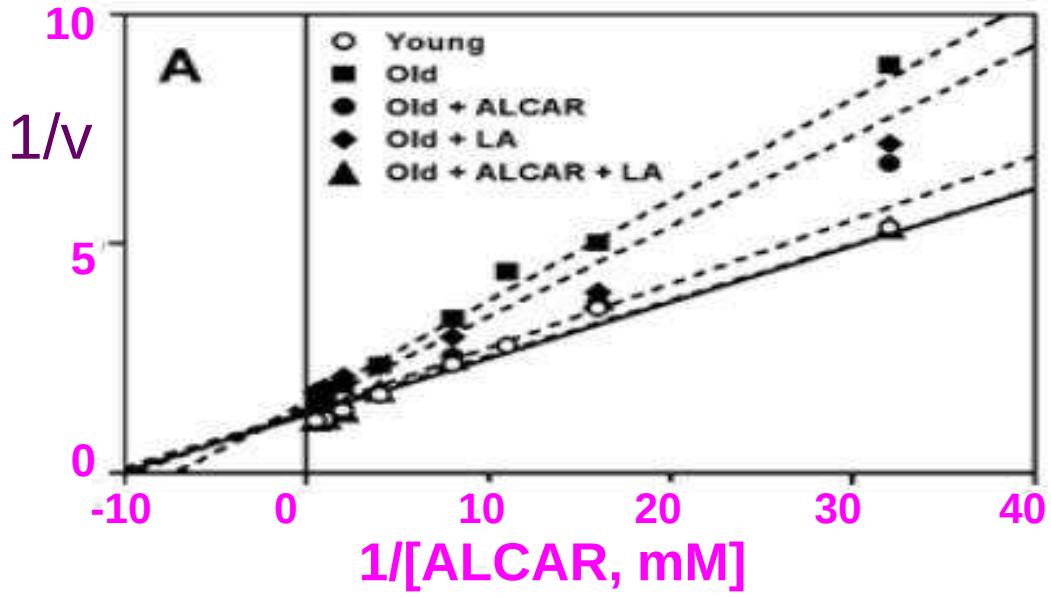
Jung H. Suh, Swapna V. Shenvi, Brian M. Dixon, Honglei Liu, Anil K. Jaiswal, Rui-Ming Liu, and Tory M. Hagen

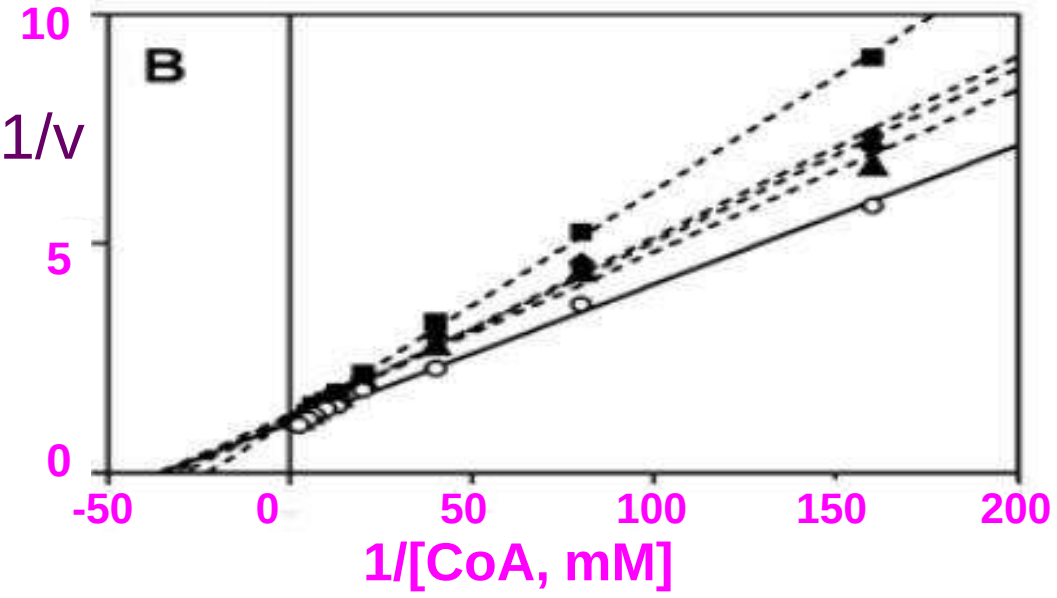
Induction of Phase 2 Enzymes

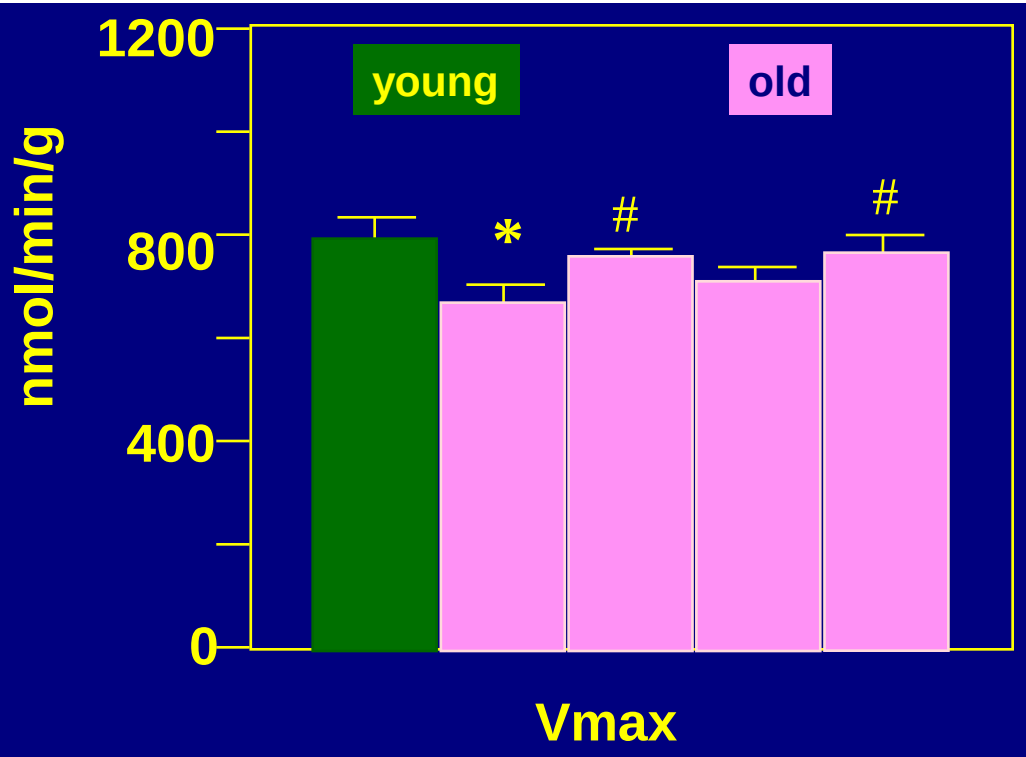


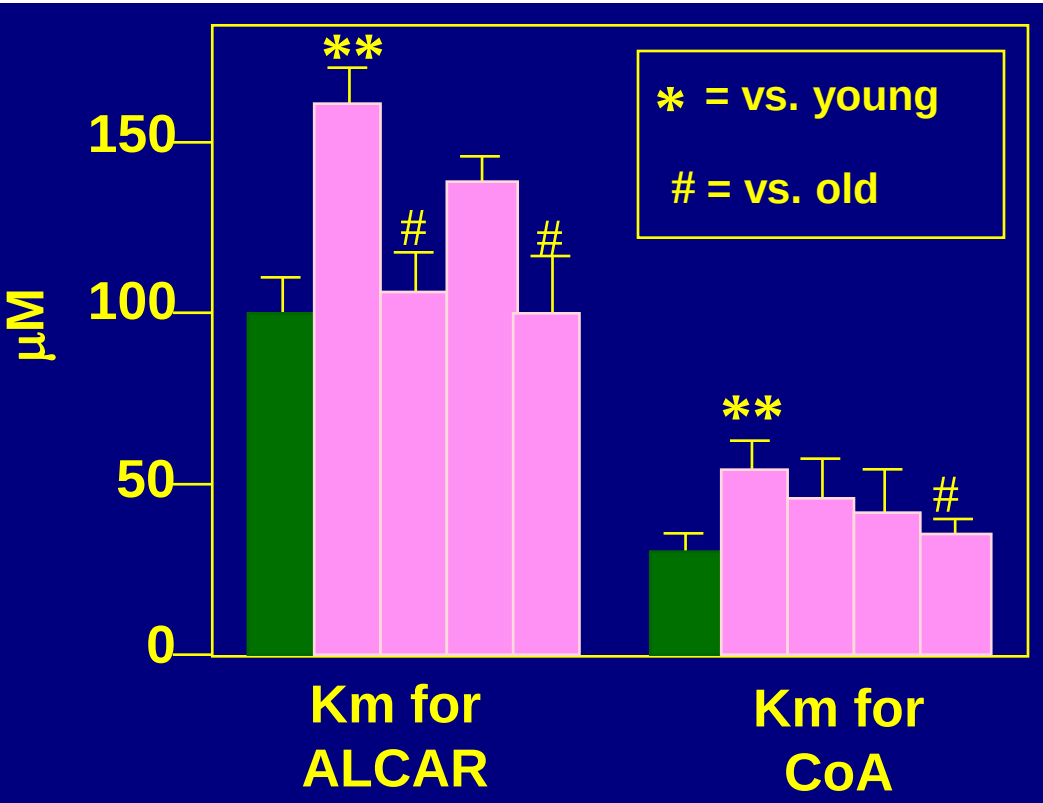
Modulation of Nrf-2-dependent gene expression by D3T in mouse liver.

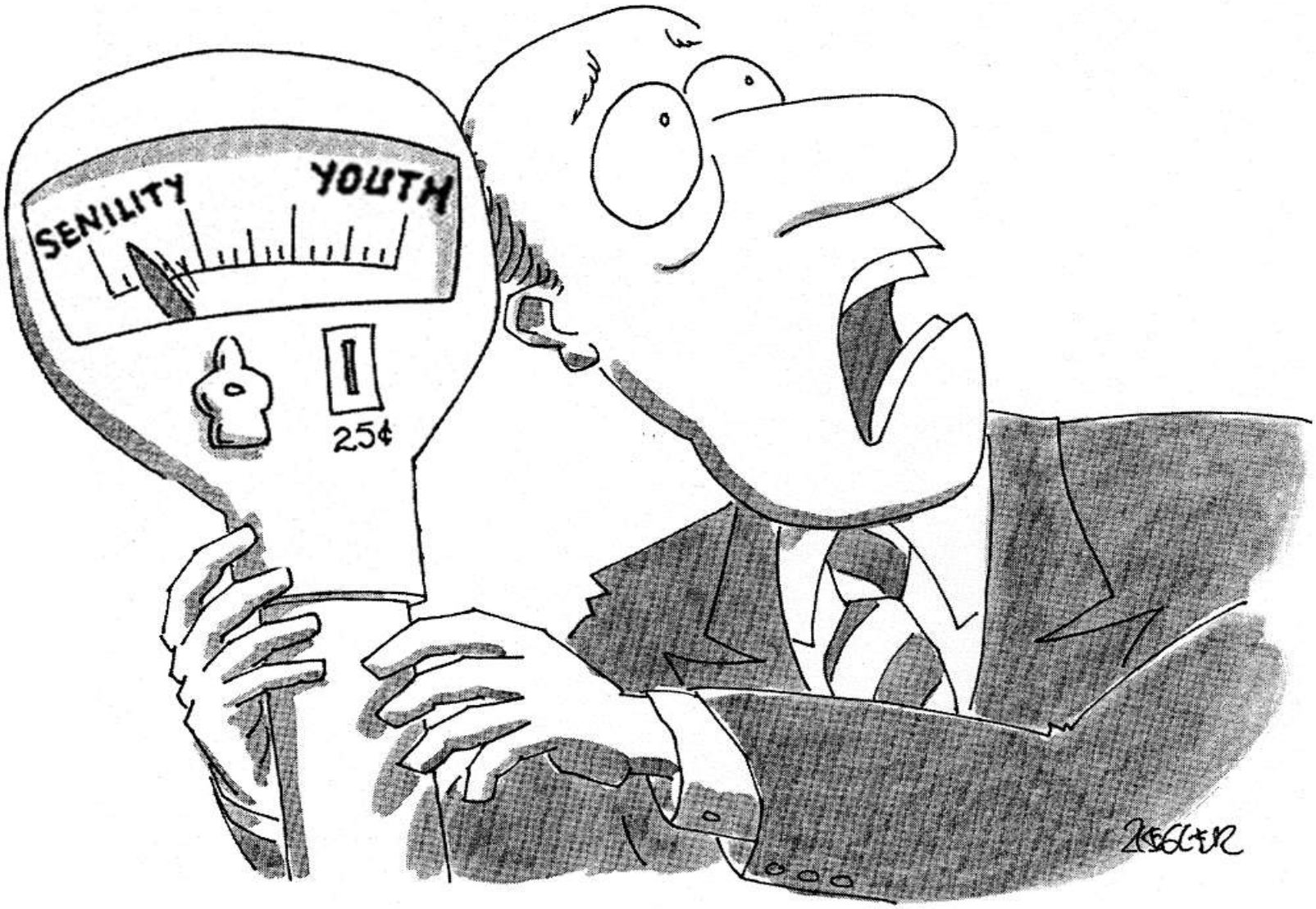
Kwak, et al. J bio Chem, 2003


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


µM
150
100
50
0
**
#
#
**
#
* = vs. young
# = vs. old
Km for ALCAR
Km for CoA

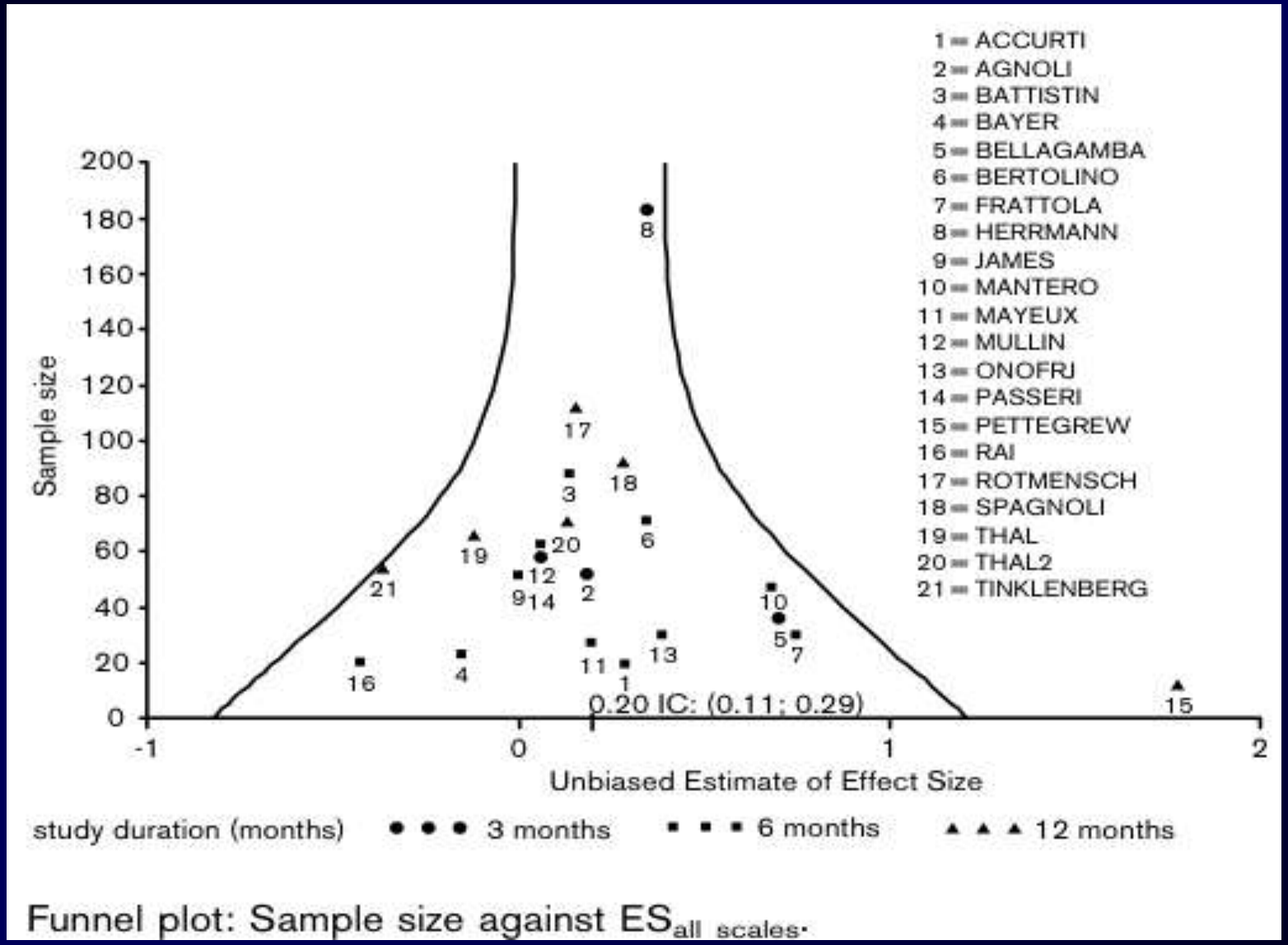


"More quarters! For God's sake, more quarters!"

“You’re fifty-seven years old.
I’d like to get that down a bit.”

New Yorker, June 6, 2005

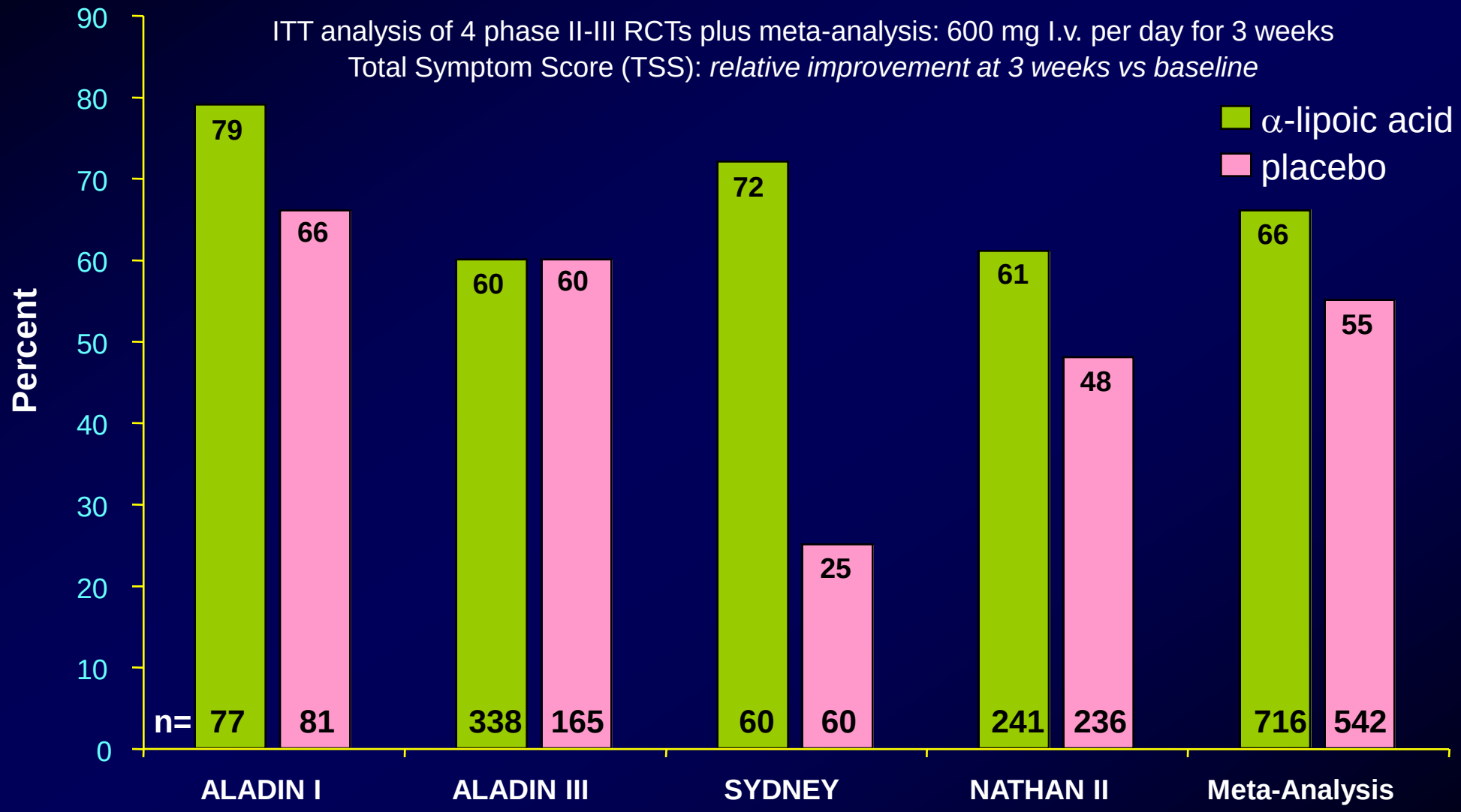
Meta-analysis of acetyl-L-carnitine versus placebo for mild cognitive impairment and mild Alzheimer's disease



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)

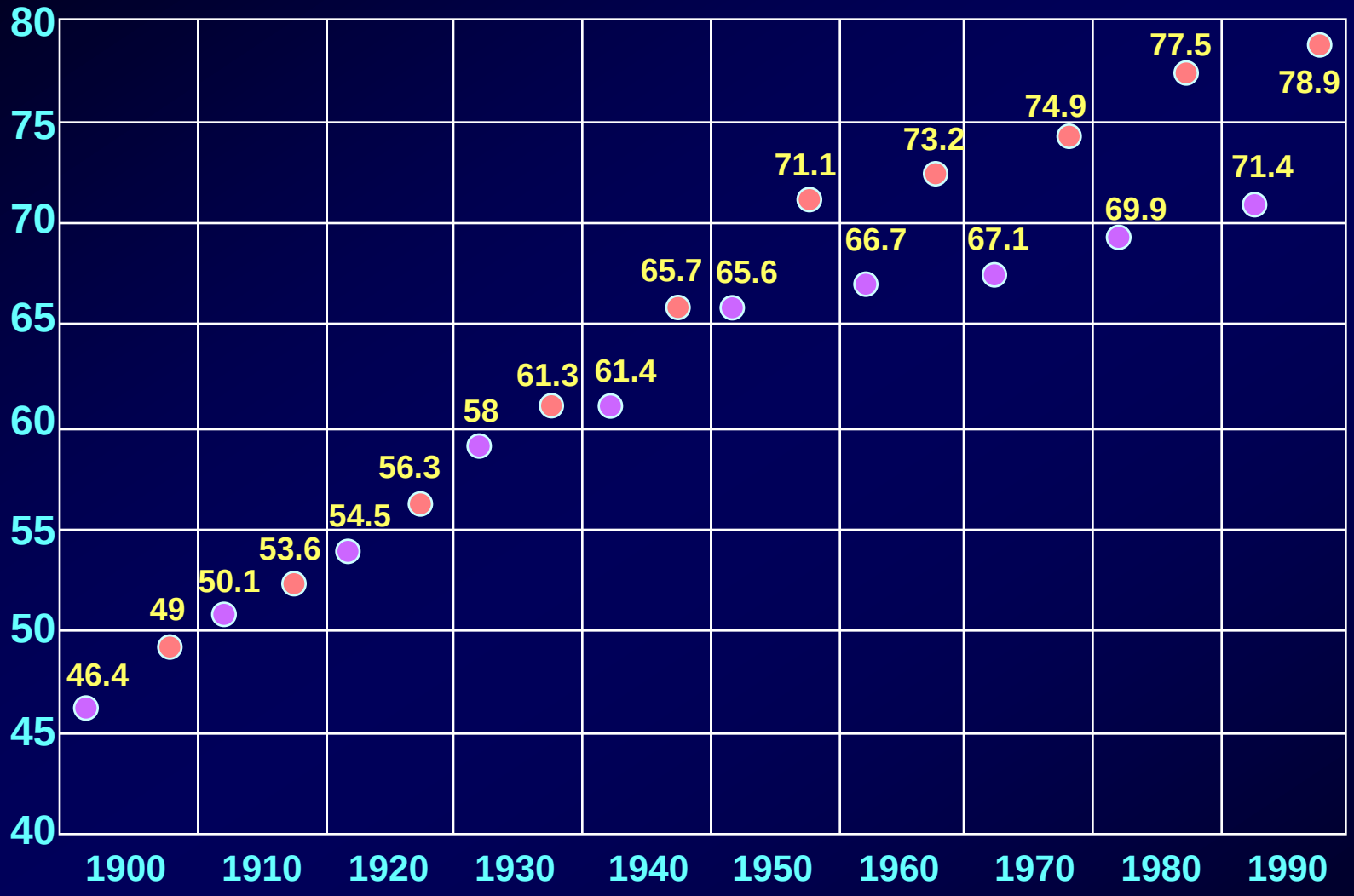
Treatment with alpha-lipoic acid significantly improves both neuropathic symptoms and deficits in diabetic patients with symptomatic diabetic neuropathy



* 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*)

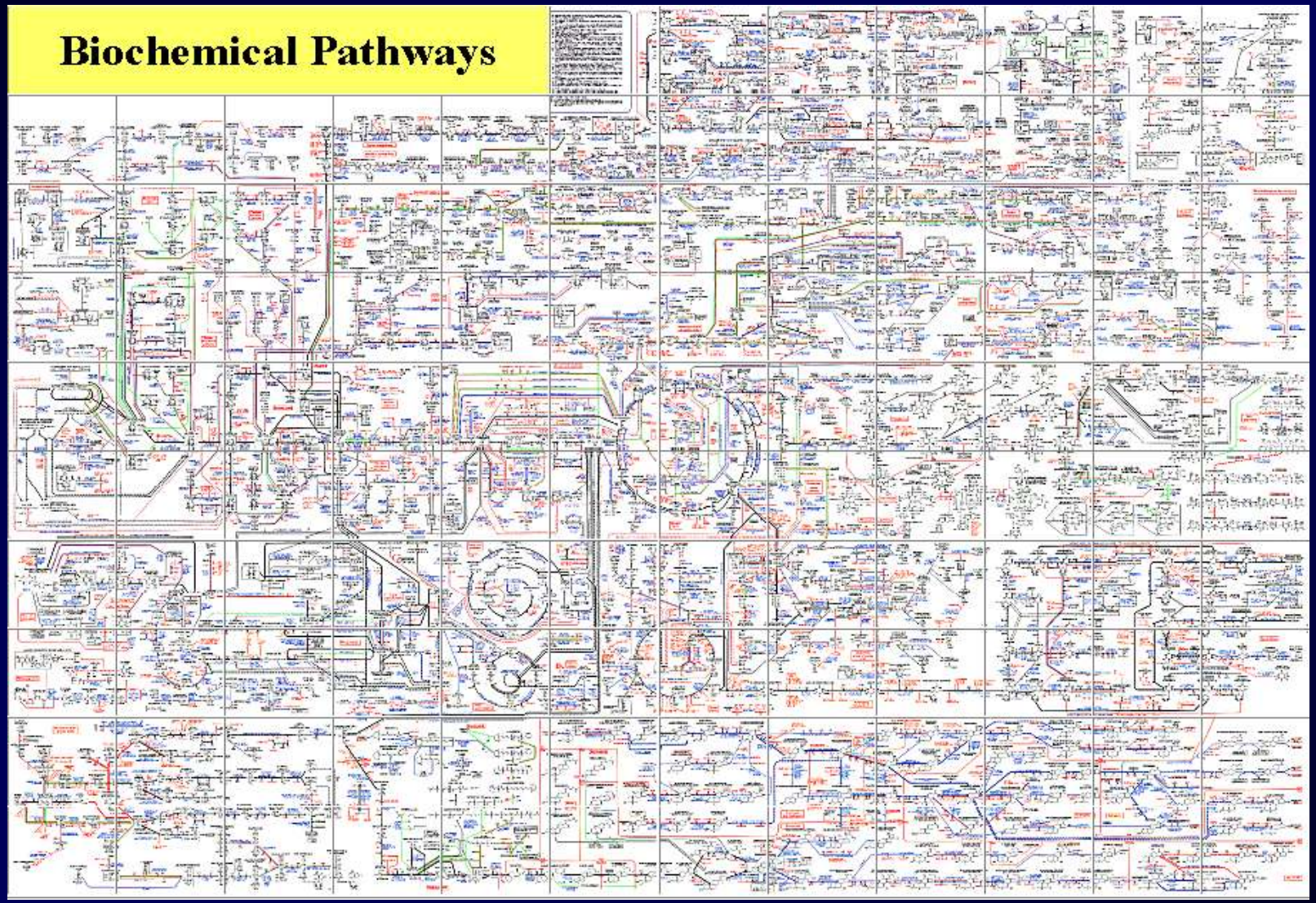
Life Expectancy of Men and Women at Birth



SOURCE: National Institute on Aging

Accelerating the Degenerative Diseases of Aging

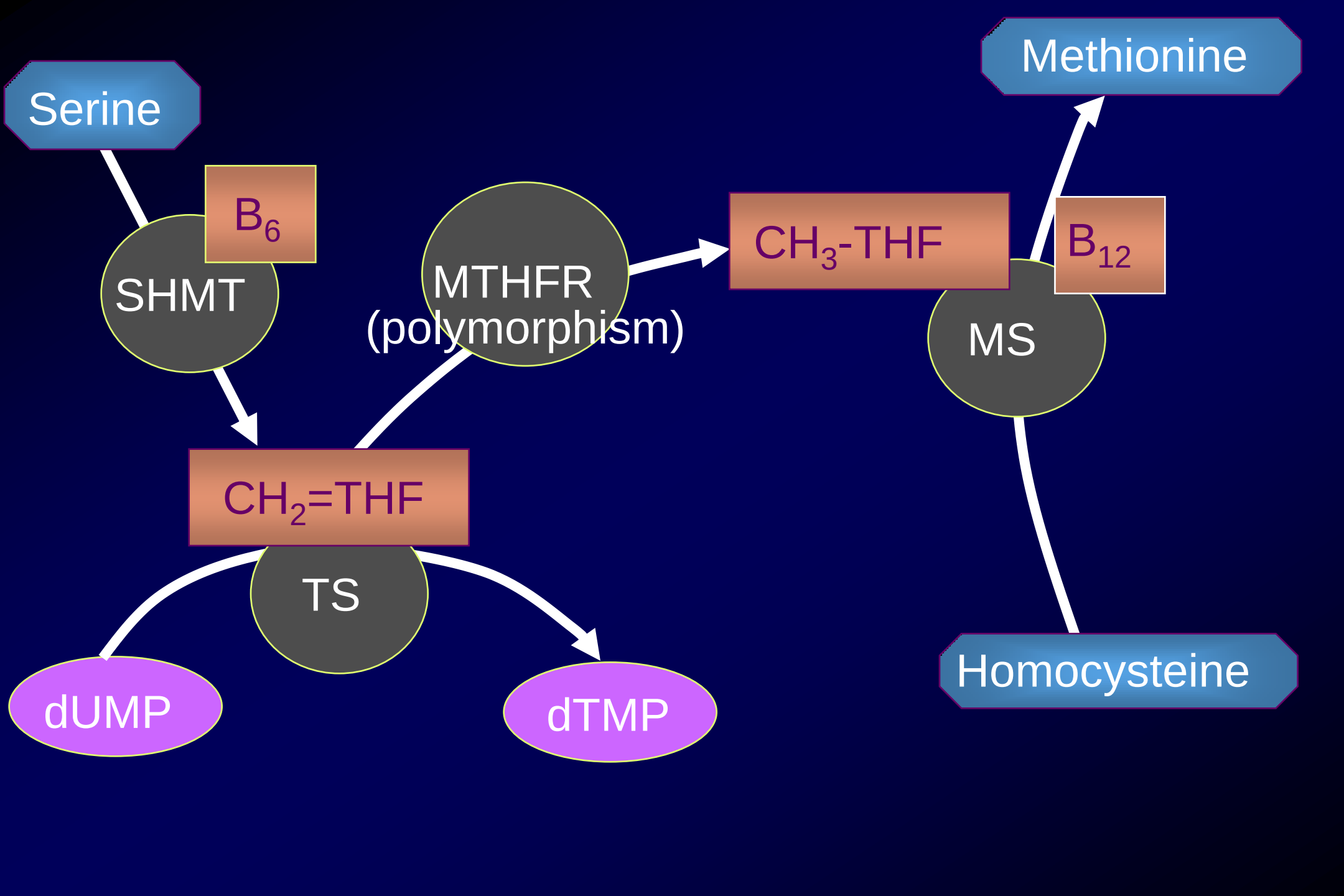
Biochemical Pathways



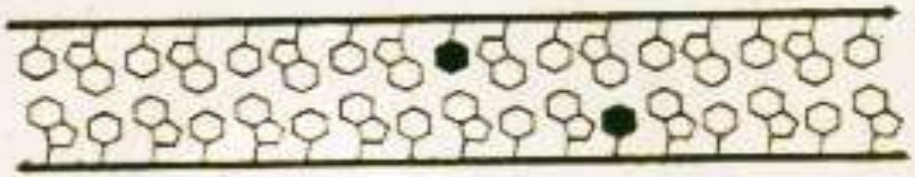
Micronutrient Undernutrition in Americans

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 %

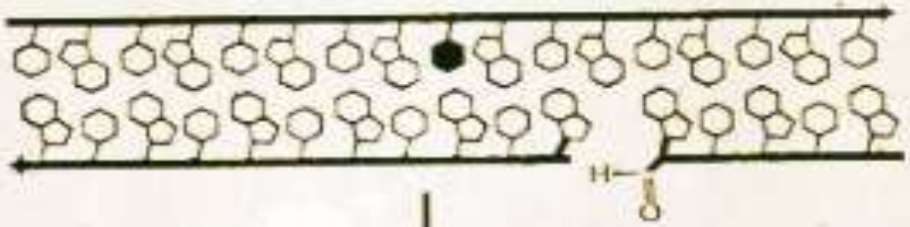
* USDA What we Eat in America (NHANES 2001-2002) Sept. 2005


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

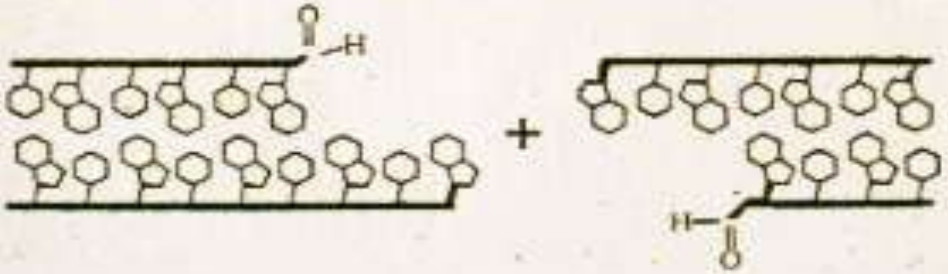
Base excision repair processing of opposed lesions



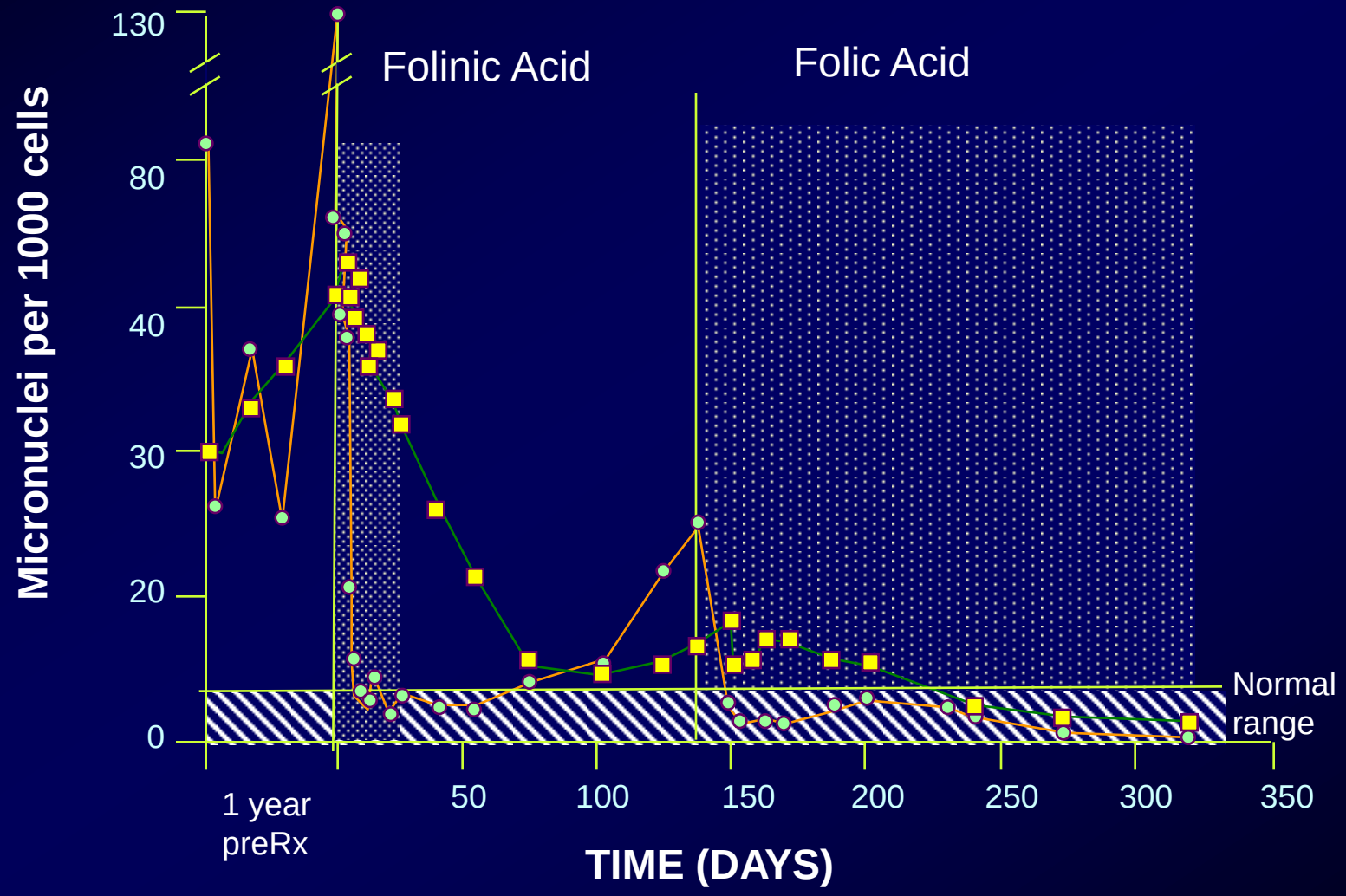
Gap three or more nucleotides away from base lesion

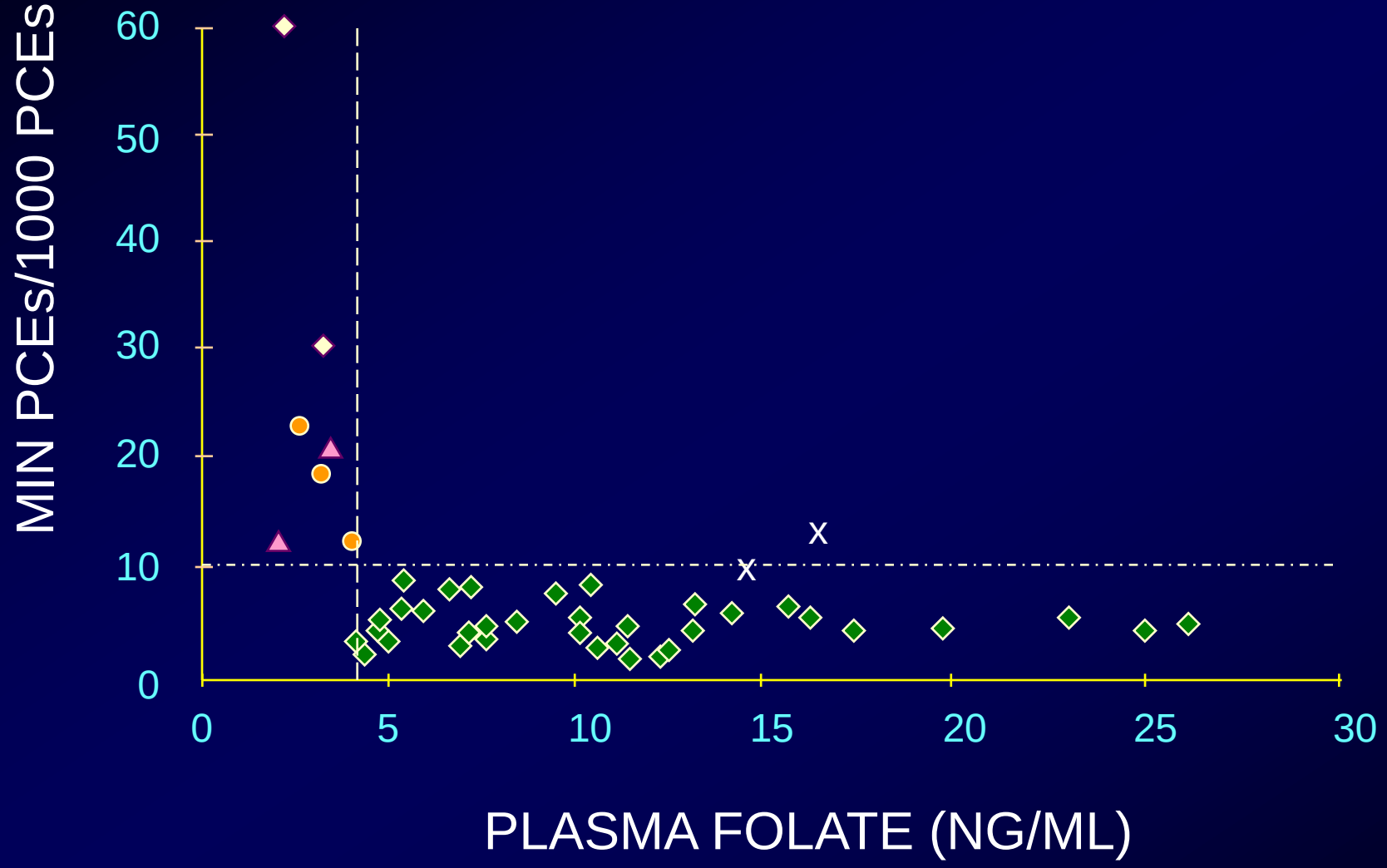


DNA double strand break formed by processing the second lesion



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

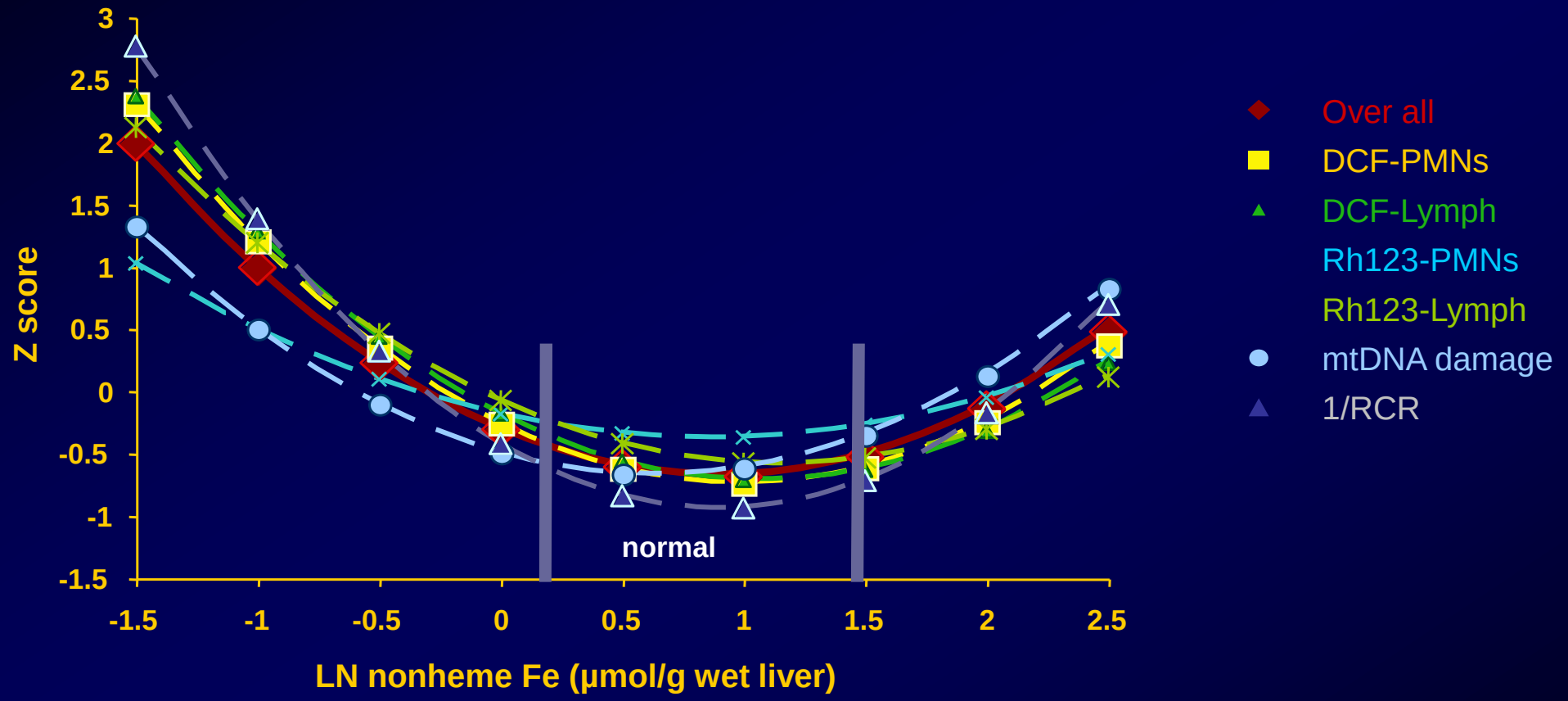
Michael Fenech, Claire Aitken, and Josephine Rinaldi, *Carcinogenesis* **19**:1163 - 1173, **1998**

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.

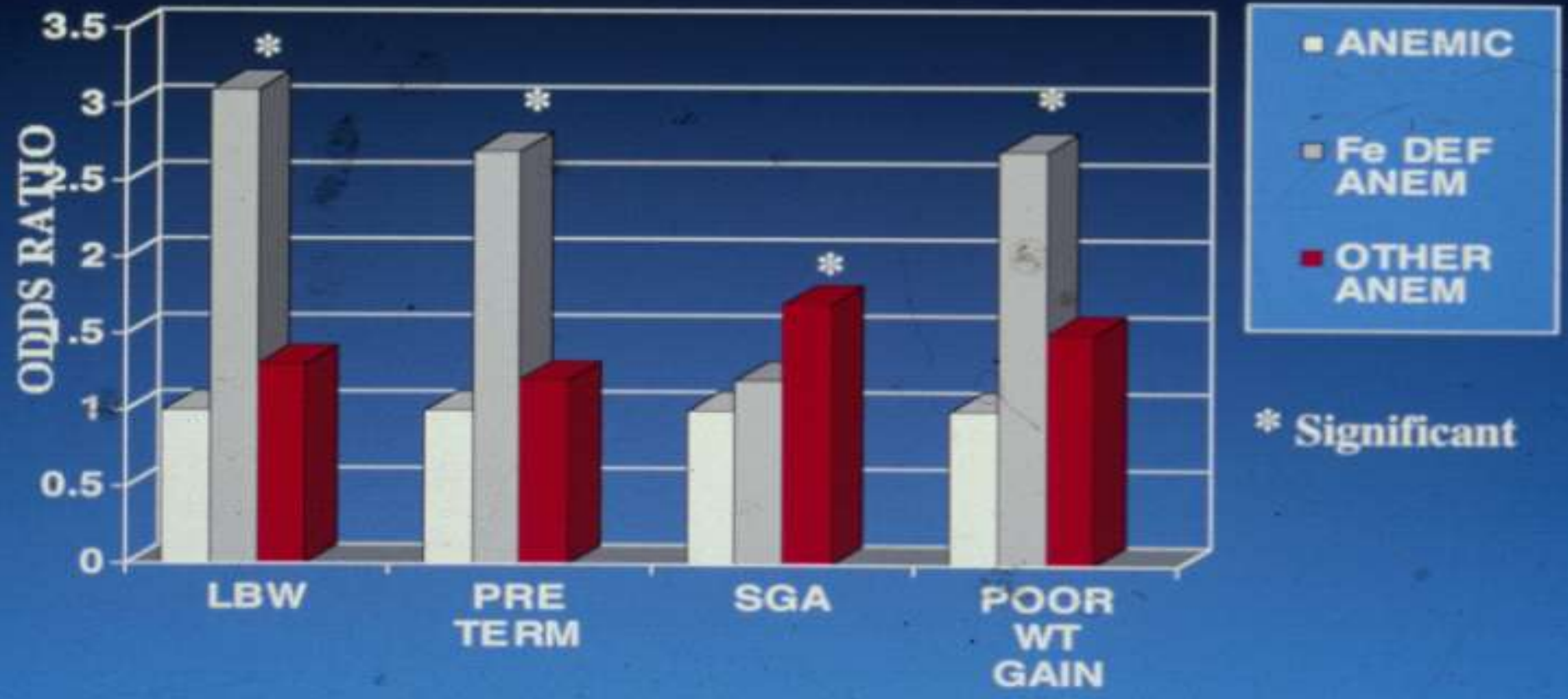
Analysis of nonlinear regression models: comparison of an overall model and individual models of Z-transformed values vs. ln- nonheme liver iron



. 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
(2007) AJCN in press

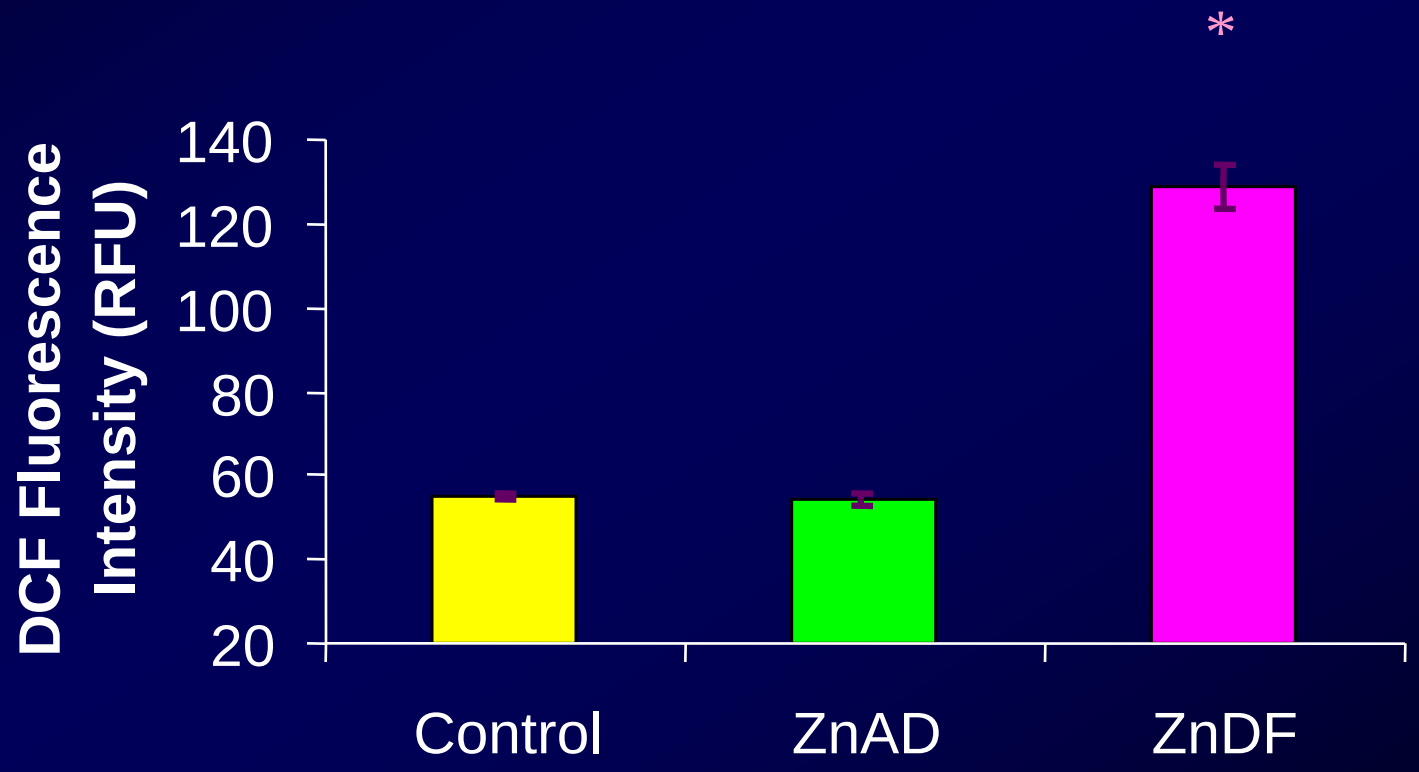
Is docosahexaenoic acid, an n3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals

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

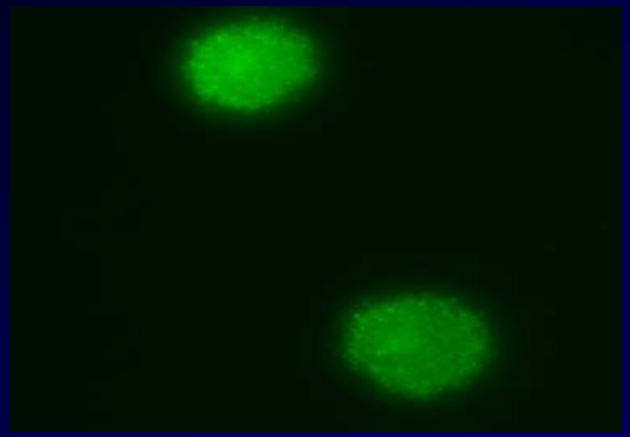
An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring

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

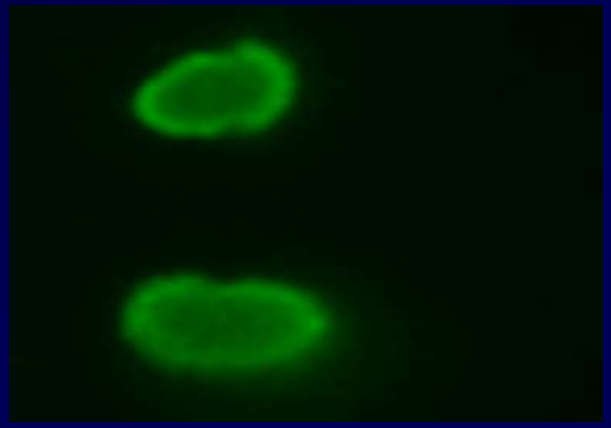
Zinc Deficiency Induces Increased Oxidative Stress in C6 Glioma Cells



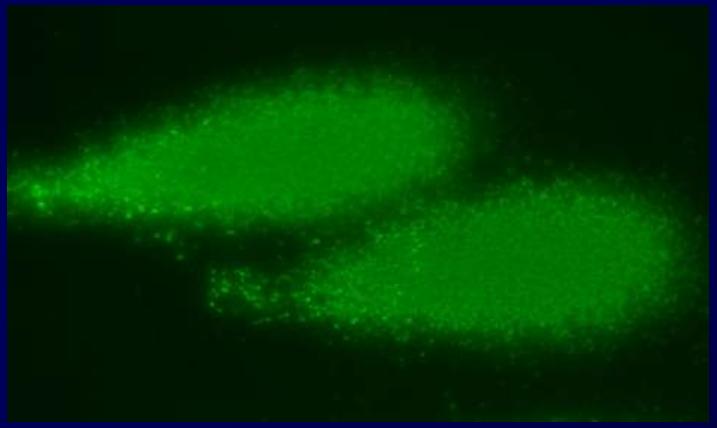
Zinc Deficiency Induces Fapy Glycosylase (Fpg)-sensitive Single Strand Breaks in Human Lung Fibroblasts



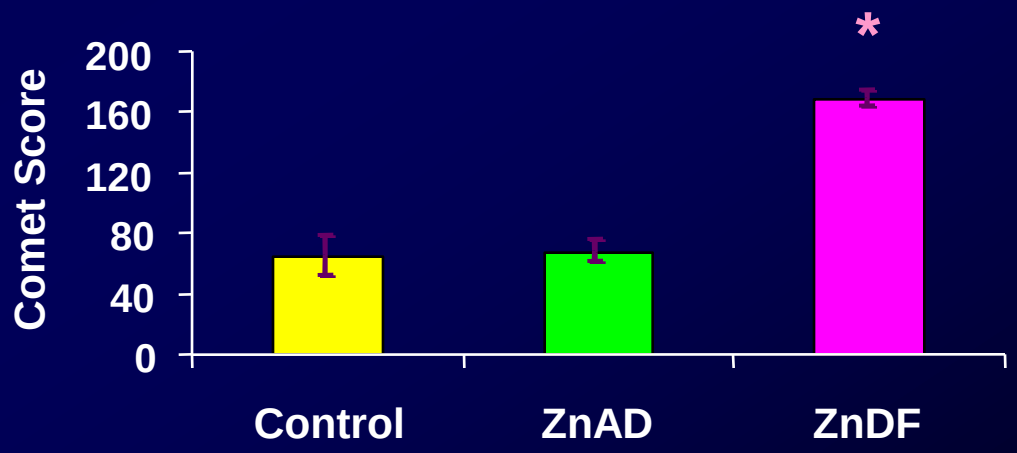
Control (+Fpg)



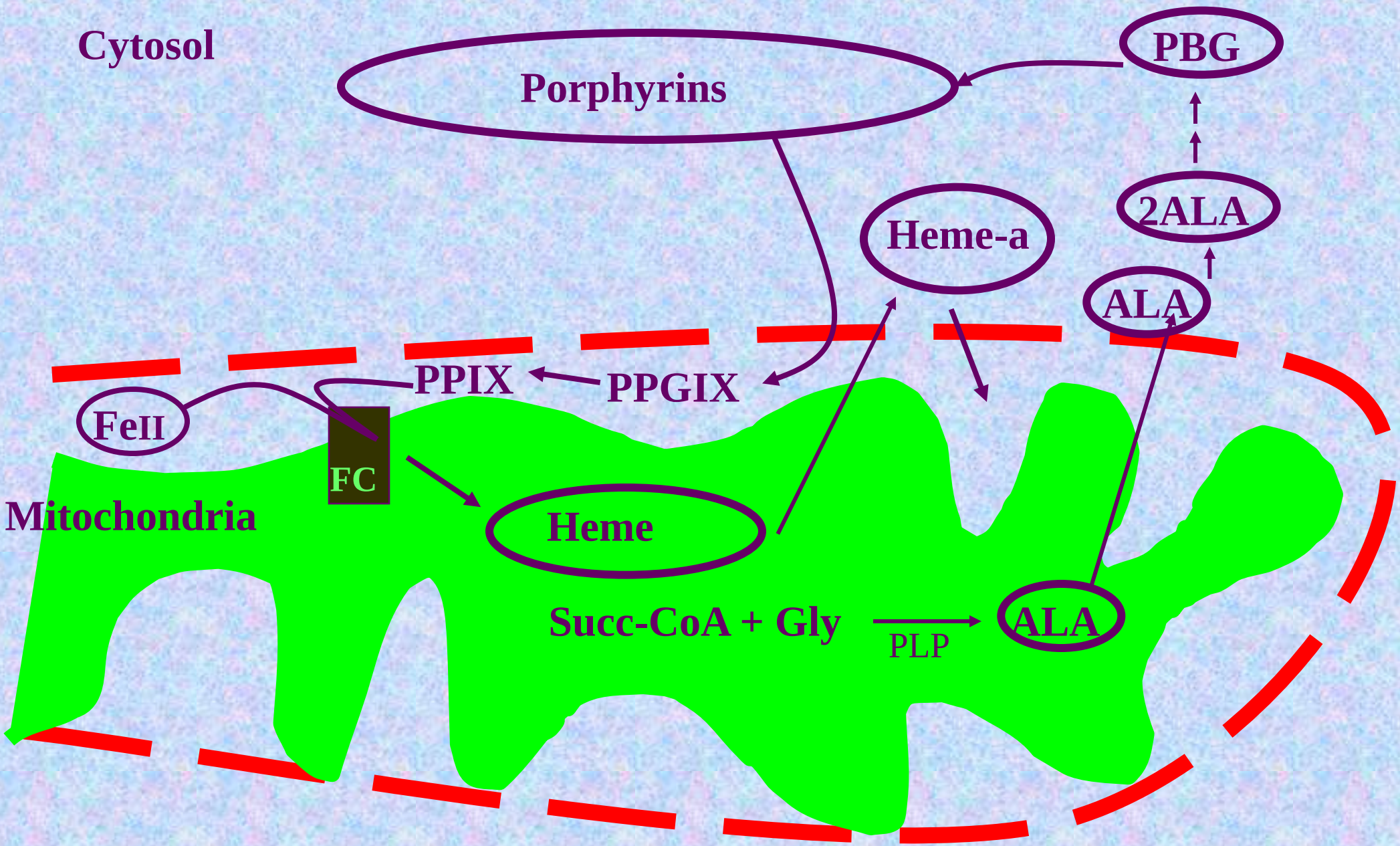
ZnAD (+Fpg)

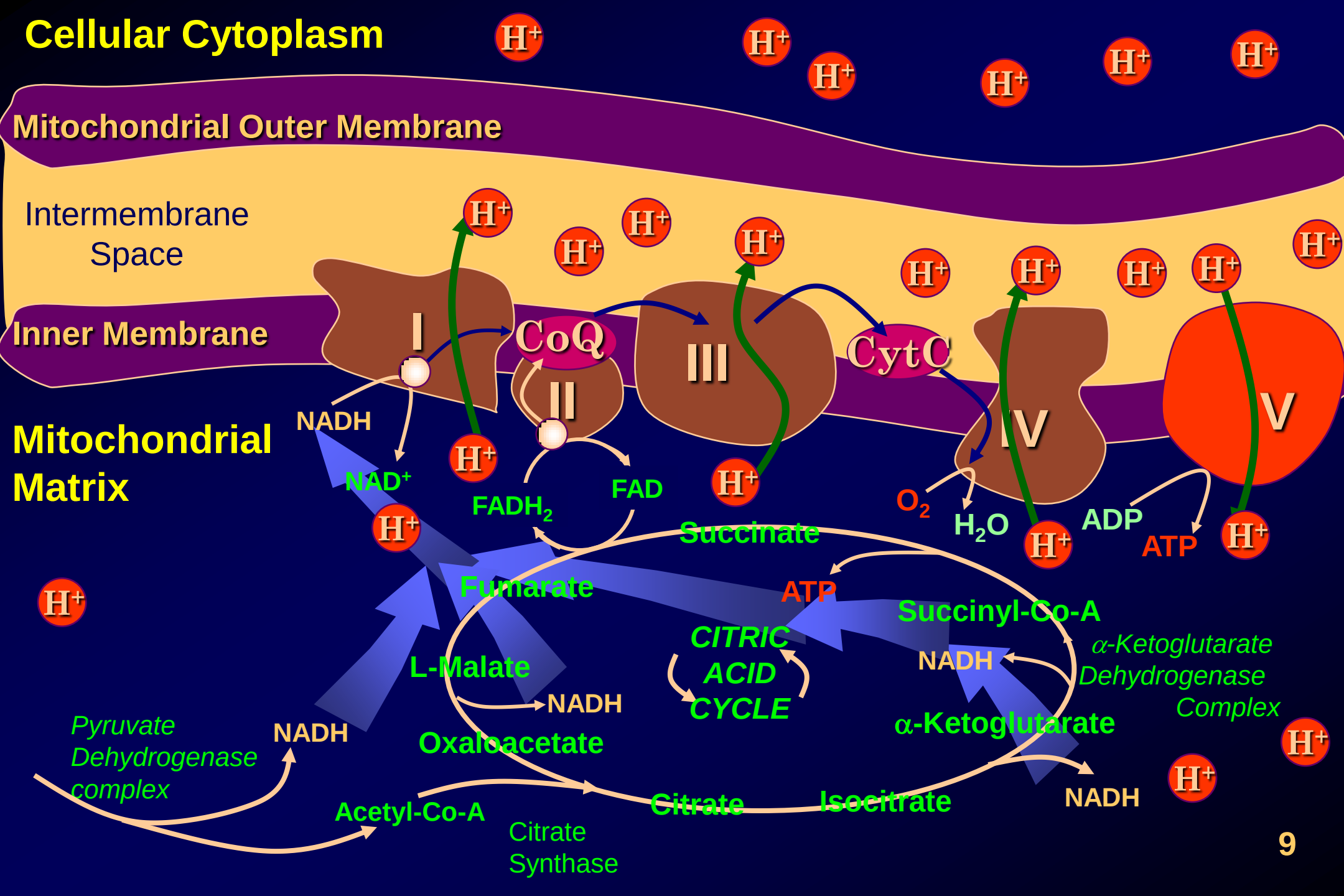


ZnDF (+Fpg)

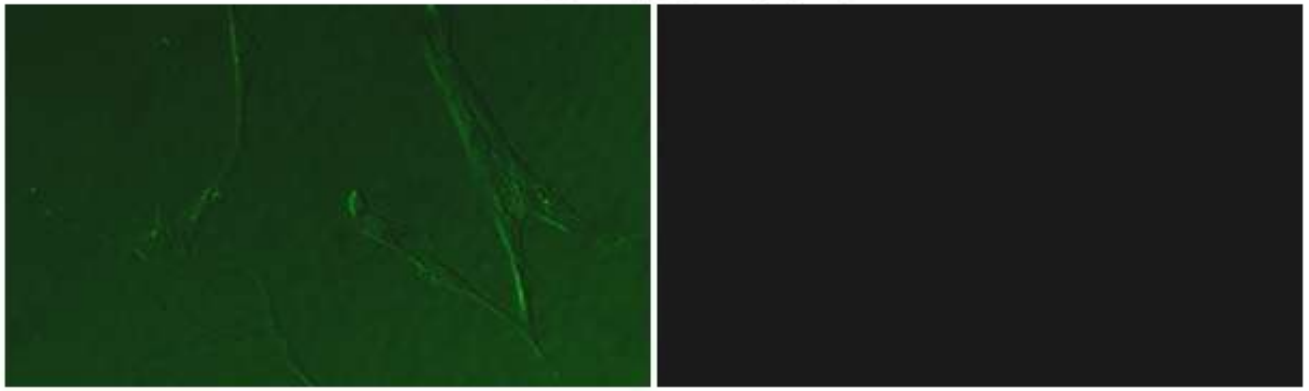


Synthesis of Heme

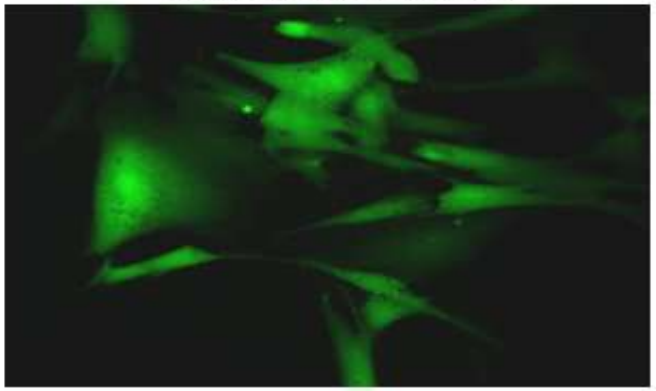



Cellular Cytoplasm
Mitochondrial Outer Membrane
Intermembrane Space
Inner Membrane
Mitochondrial Matrix
I
II
III
IV
V
CoQ
CytC
NADH
NAD+
FADH2
FAD
O2
H2O
ADP
ATP
Succinate
Fumarate
L-Malate
Oxaloacetate
CITRIC ACID CYCLE
Citrate
Isocitrate
α-Ketoglutarate
Succinyl-Co-A
α-Ketoglutarate Dehydrogenase Complex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

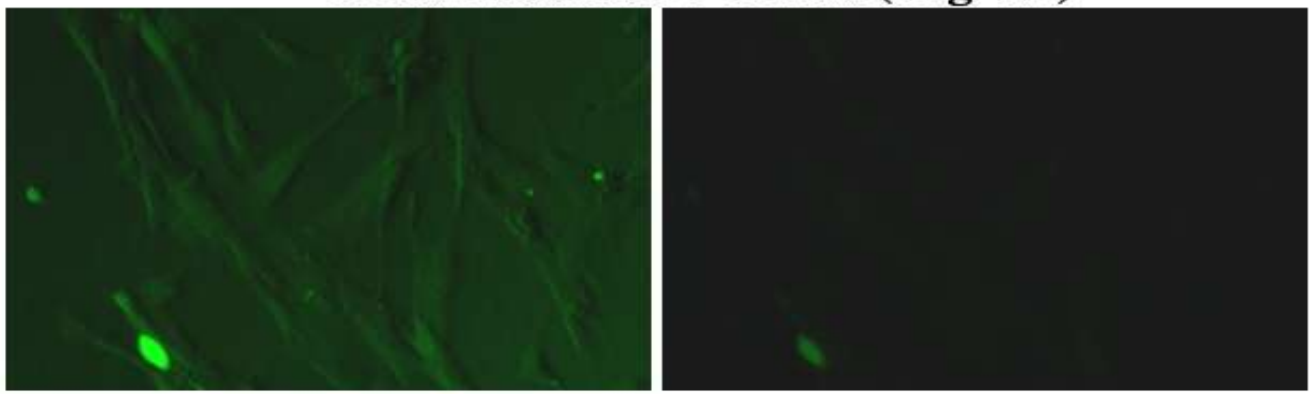
Biotin Sufficient



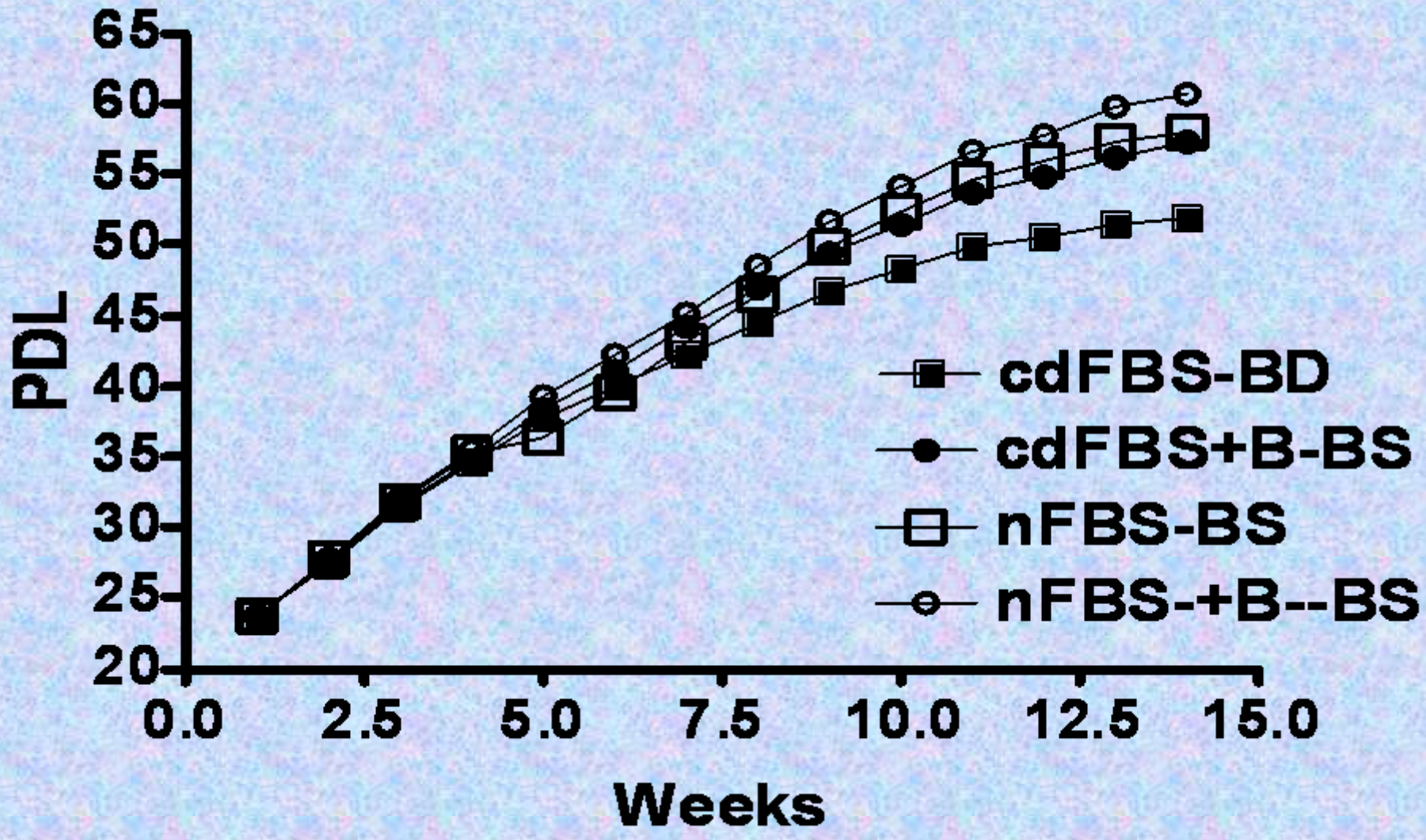
Biotin Deficient



Biotin deficient + Biotin (5ng/ ml)



Biotin deficiency accelerates cell senescence

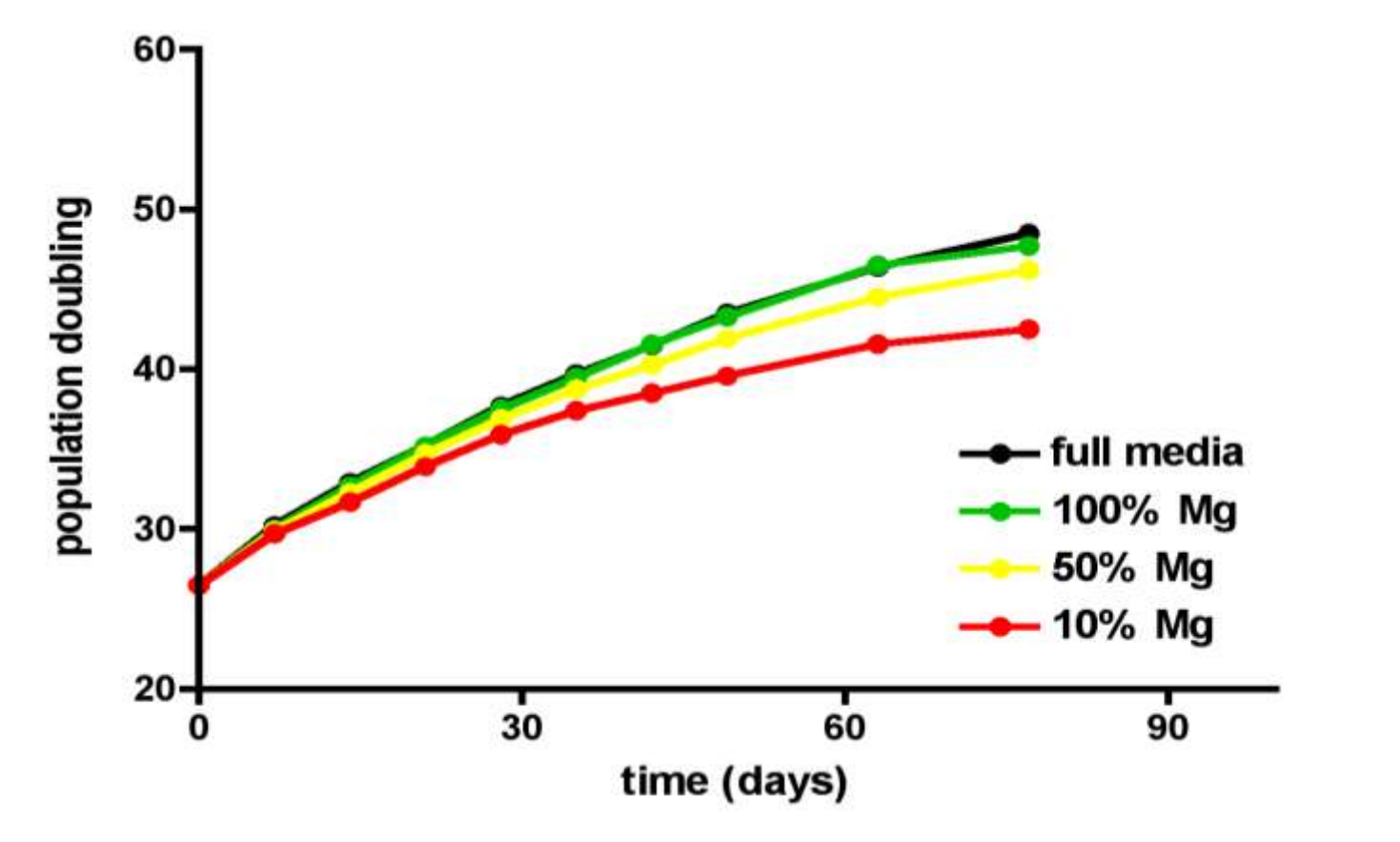


Micronutrient deficiency and heme synthesis in human cell culture

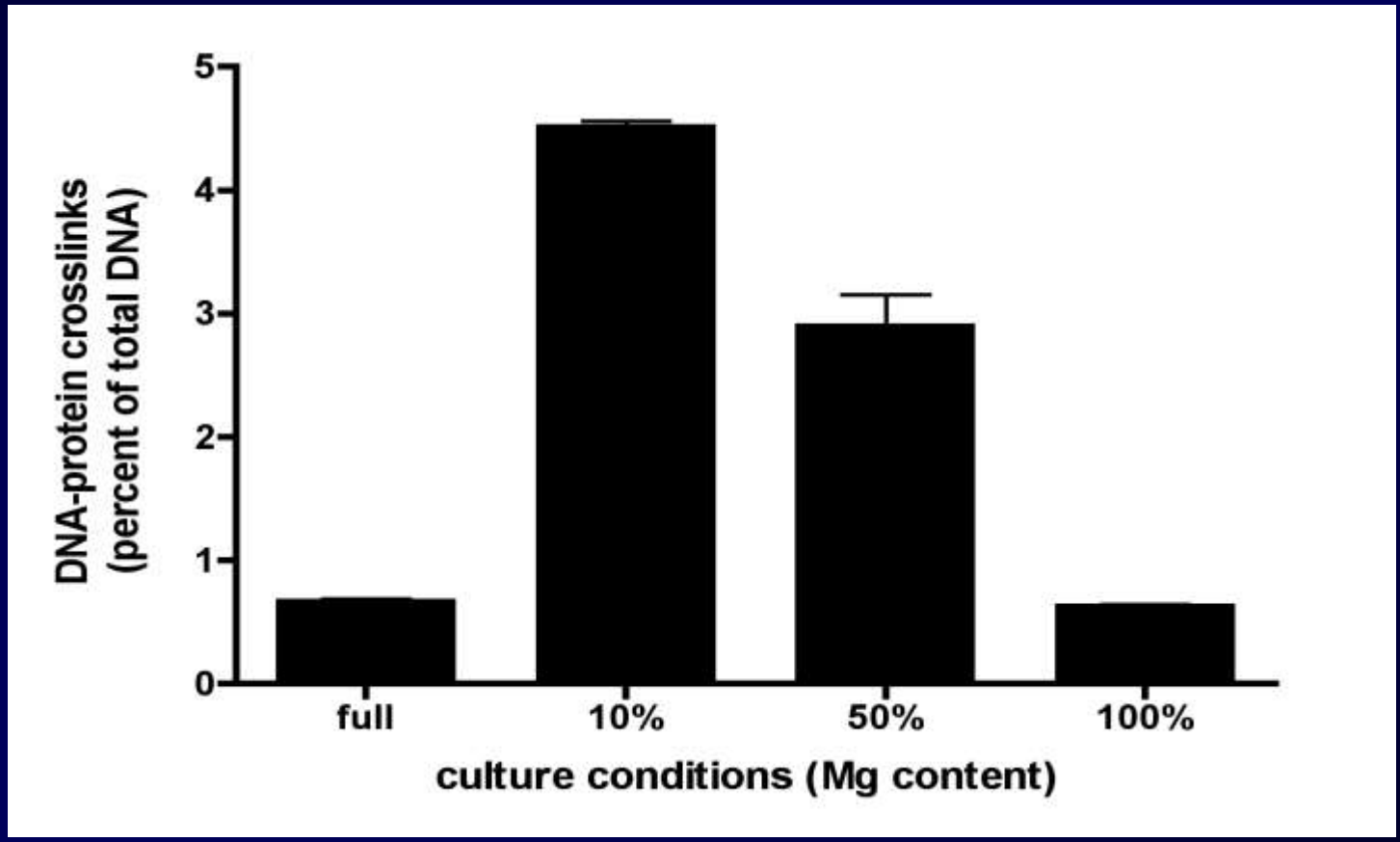
Micronutrient Deficiency	Heme Deficit	Complex IV Deficit	Oxidative Stress	DNA Damage	Early Senescence
Pyridoxine	[+]			++	++
Zinc		+	#	#	
Riboflavin					
Iron	+	+	[+]	[+]	
Copper	[+]	[+]	[+]		
Biotin	+	+	+	+	+
Lipoic Acid			[+]		
Pantothenate	[+]	[+]			

+ = Atamna/Ames, ++Askree /Ames, #Ho/Ames [+] Literature

Magnesium Deficiency Shortens Fibroblast Lifespan



Magnesium Deficiency Induces DNA-Protein Crosslinks



Calcium Deficiency

Fenech: chromosome breaks breaks

Lipkin: colon cancer mice

Folate Deficiency

MacGregor/Ames/Fenech: chromosome breaks mice/humans humans

Willett: epi colon cancer humans

Vitamin D Deficiency

Garland: epi colorectal cancer humans

Magnesium Deficiency

Bell: chromosome breaks humans damage

Larsson: epi colorectal cancer humans

Zinc Deficiency

Fong: esophageal cancer humans/rodents humans

Vitamin B12

Fenech: Chromosome

Selenium

Rao: DNA damage

Combs/Trumbo: Cancer

Omega-3 FA

Denkins: Cancer

Niacin

Kirkland/Depeint: DNA

Choline

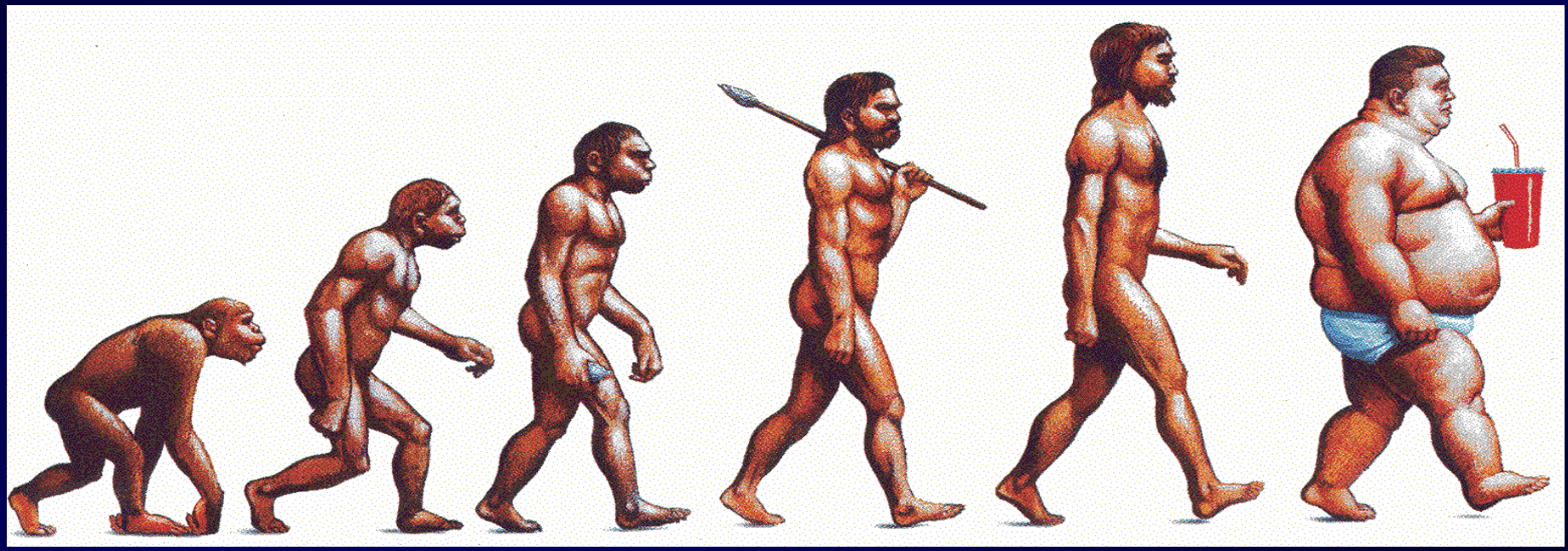
da Costa: DNA damage in

Proc. Natl. Acad. Sci. USA
Vol. 103, pp. 17589-17594, November 2006

Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage

Bruce N. Ames
Children's Hospital of Oakland Research Institute, Nutrition and Metabolism Center,
5700 Martin Luther King Jr. Way, Oakland, CA 94609

Inadequate dietary intakes of vitamins and minerals are widespread, most likely due to excessive consumption of energy-rich, micronutrient-poor, refined food. Inadequate intakes may result in chronic metabolic disruption, including mitochondrial decay. Deficiencies in many micronutrients cause DNA damage, such as chromosome breaks, in cultured human cells or *in vivo*. Some of these deficiencies also cause mitochondrial decay with oxidant leakage and cellular aging, and are associated with late onset diseases such as cancer**. I propose DNA damage and late onset disease are consequences of a triage allocation response to micronutrient scarcity. Episodic shortages of micronutrients were common during evolution. Natural selection favors short-term survival at the expense of long-term health. I hypothesize that short-term survival was achieved by allocating scarce micronutrients by triage, in part through an adjustment of the binding affinity of each protein for its required micronutrient.** If this hypothesis is correct, micronutrient deficiencies that trigger the triage response would accelerate cancer, aging, and neural decay but would leave critical metabolic functions, such as ATP production, intact. Evidence that micronutrient malnutrition increases late onset diseases, such as cancer, is discussed. A multivitamin-mineral supplement is one low-cost way to ensure intake of the Recommended Dietary Allowance of micronutrients throughout life.



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.

Dr. Allen Spiegel, NIDDK/NIH

CAUTION: HAZARDOUS WAIST

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

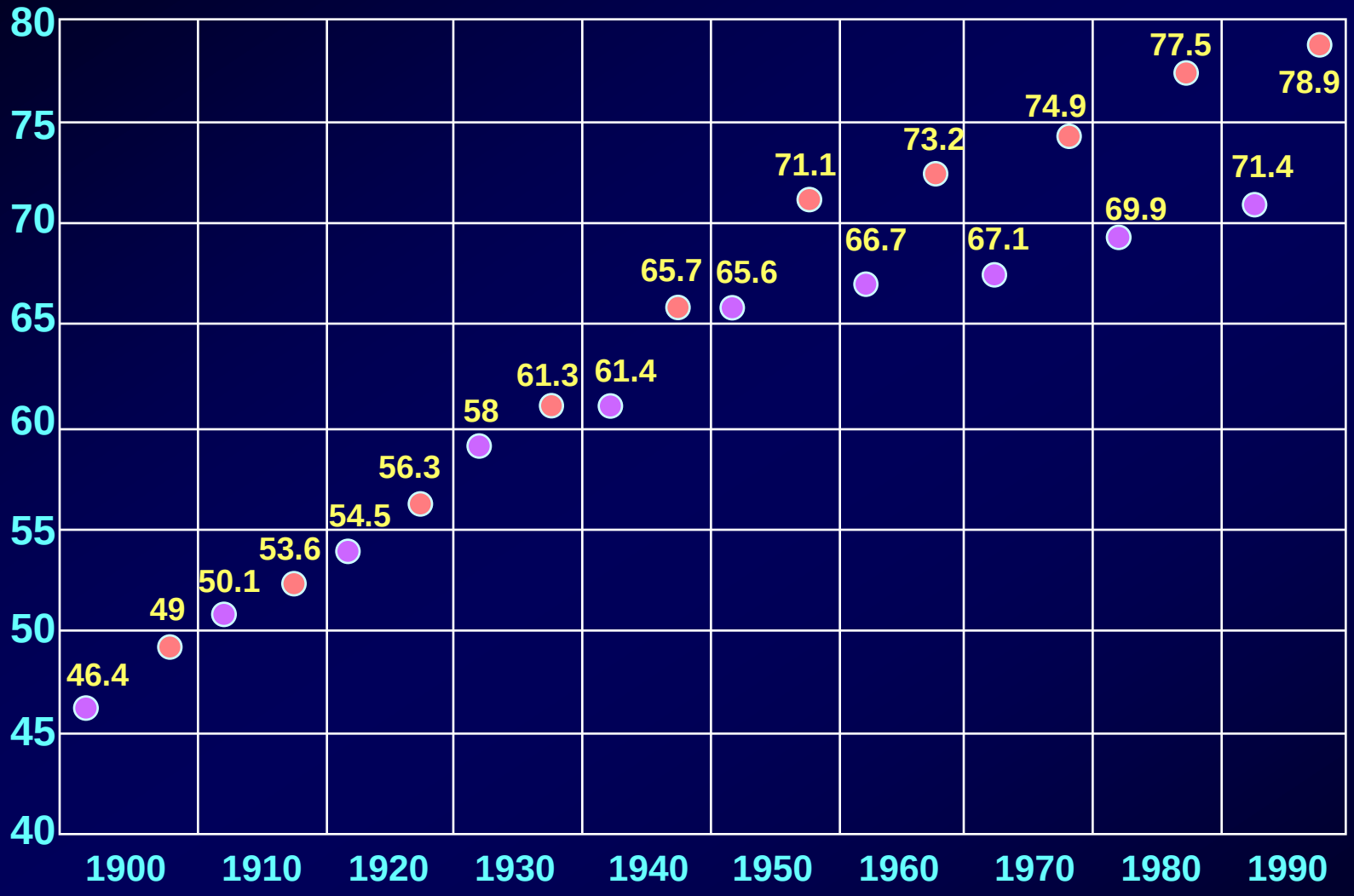
Start a waist disposal program today.

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

Mark Twain

If you want fiber, Madame, I suggest you eat the menu.

Life Expectancy of Men and Women at Birth



SOURCE: National Institute on Aging

END