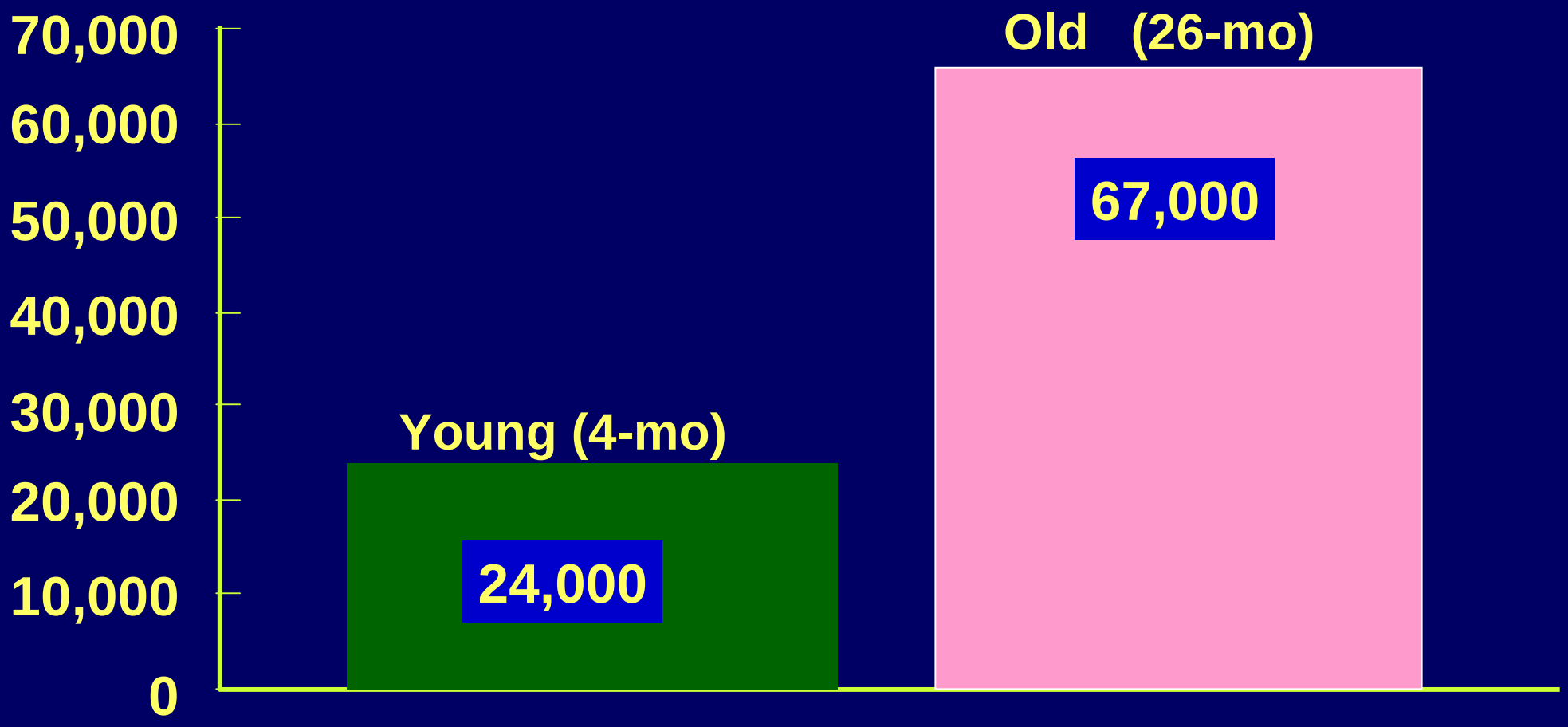
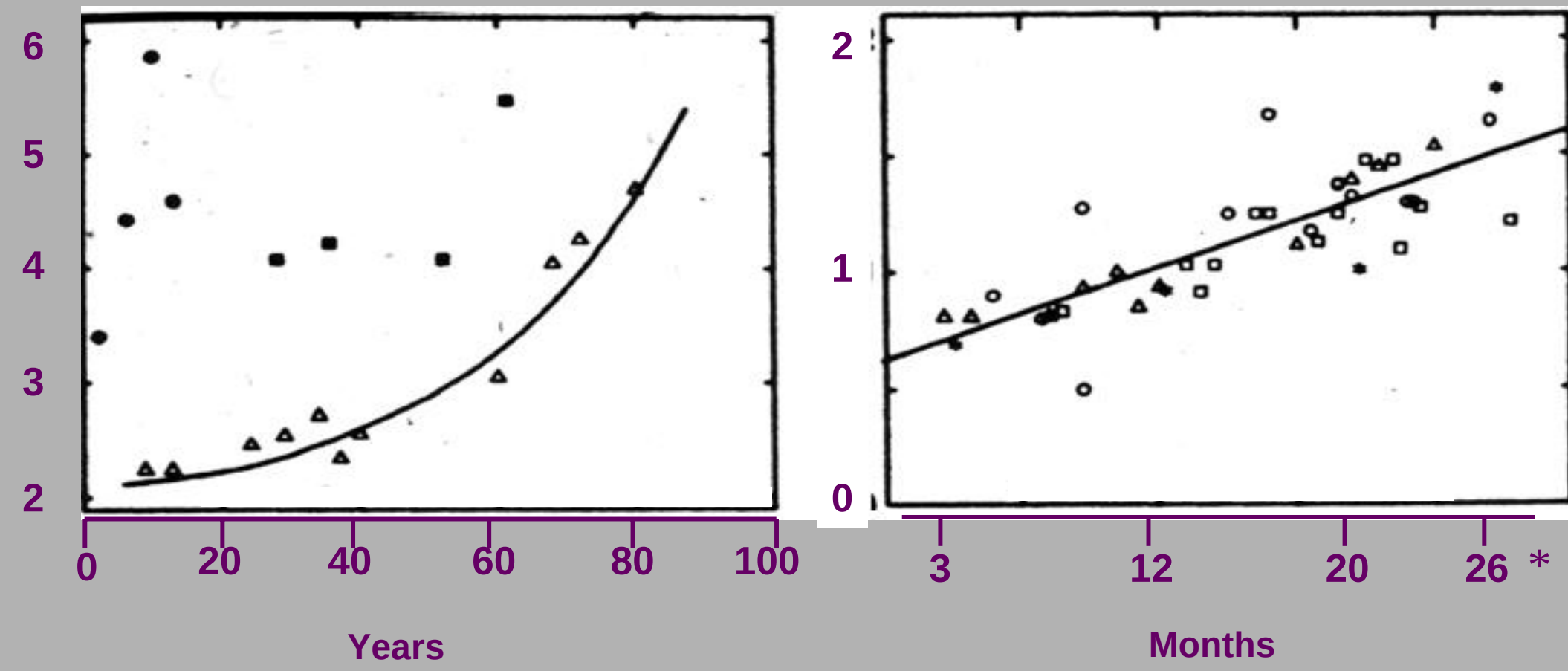
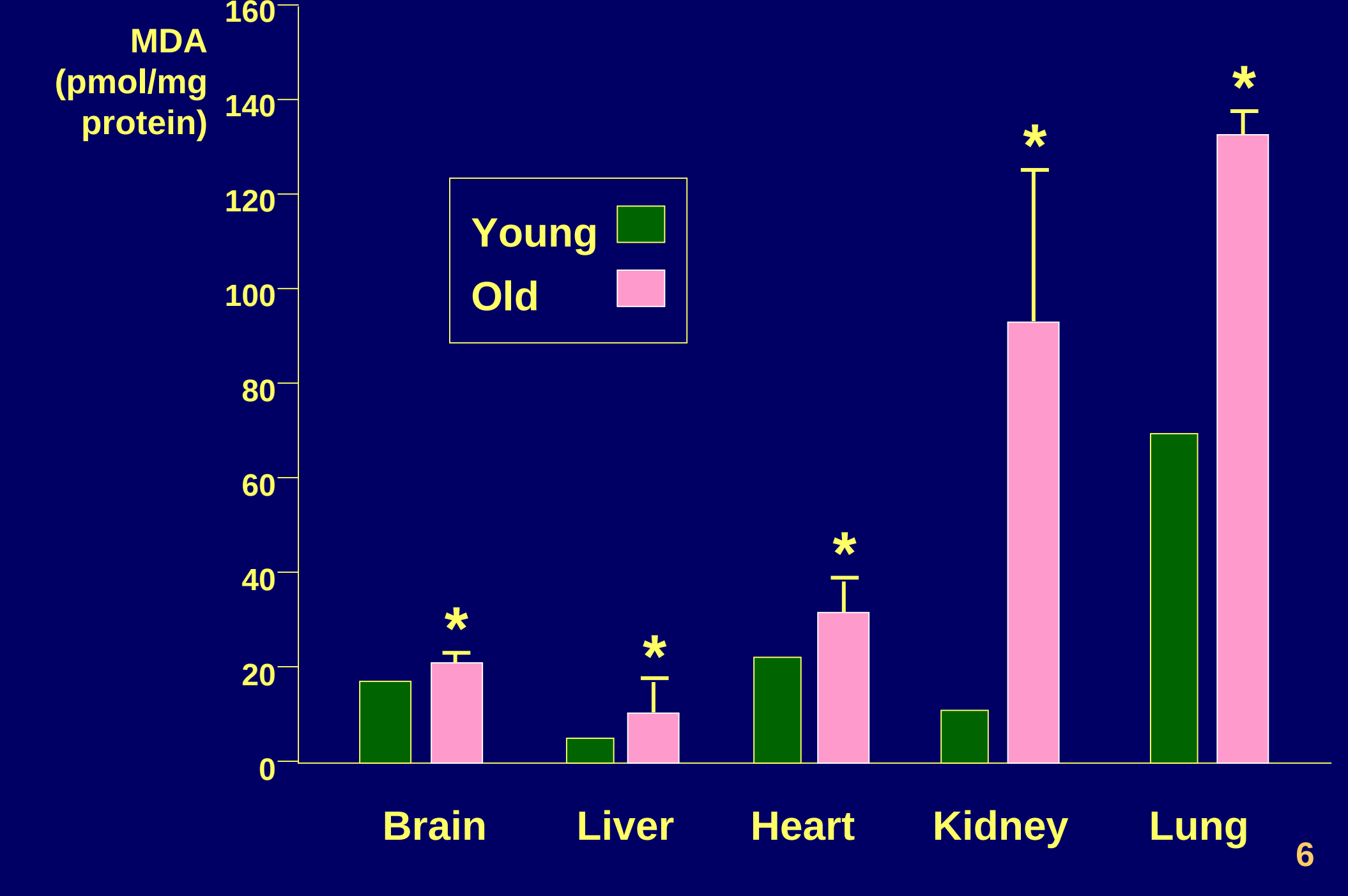

O_2
e^-
O_2^-
e^-
H_2O_2
e^-
•OH
e^-
H_2O

Estimated oxidative DNA adducts per rat liver cell

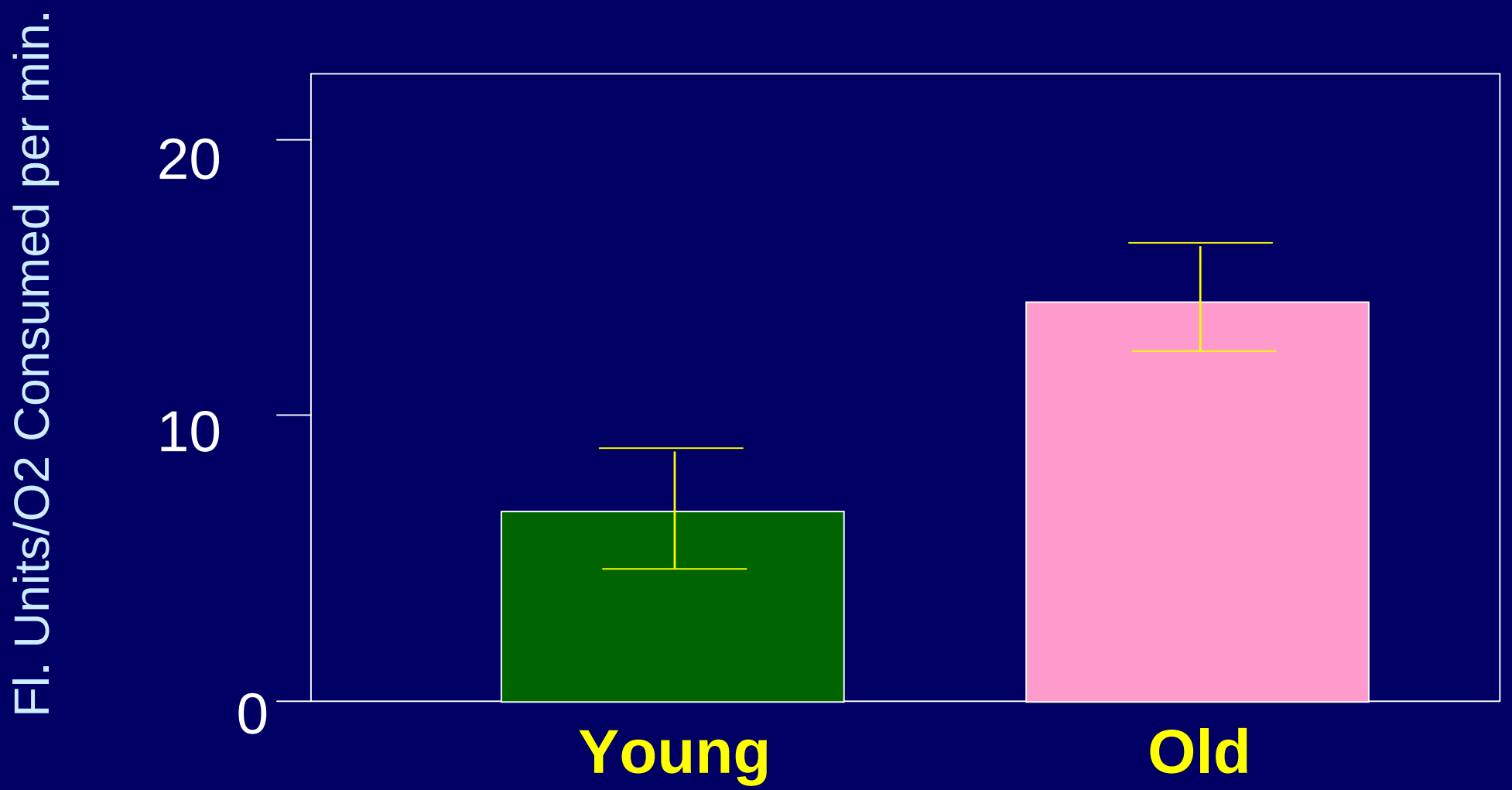




E. Stadtman, Science 257, 1220-1224 (1992)


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

Oxidants in Hepatocytes from Young and Old Rats



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

Review

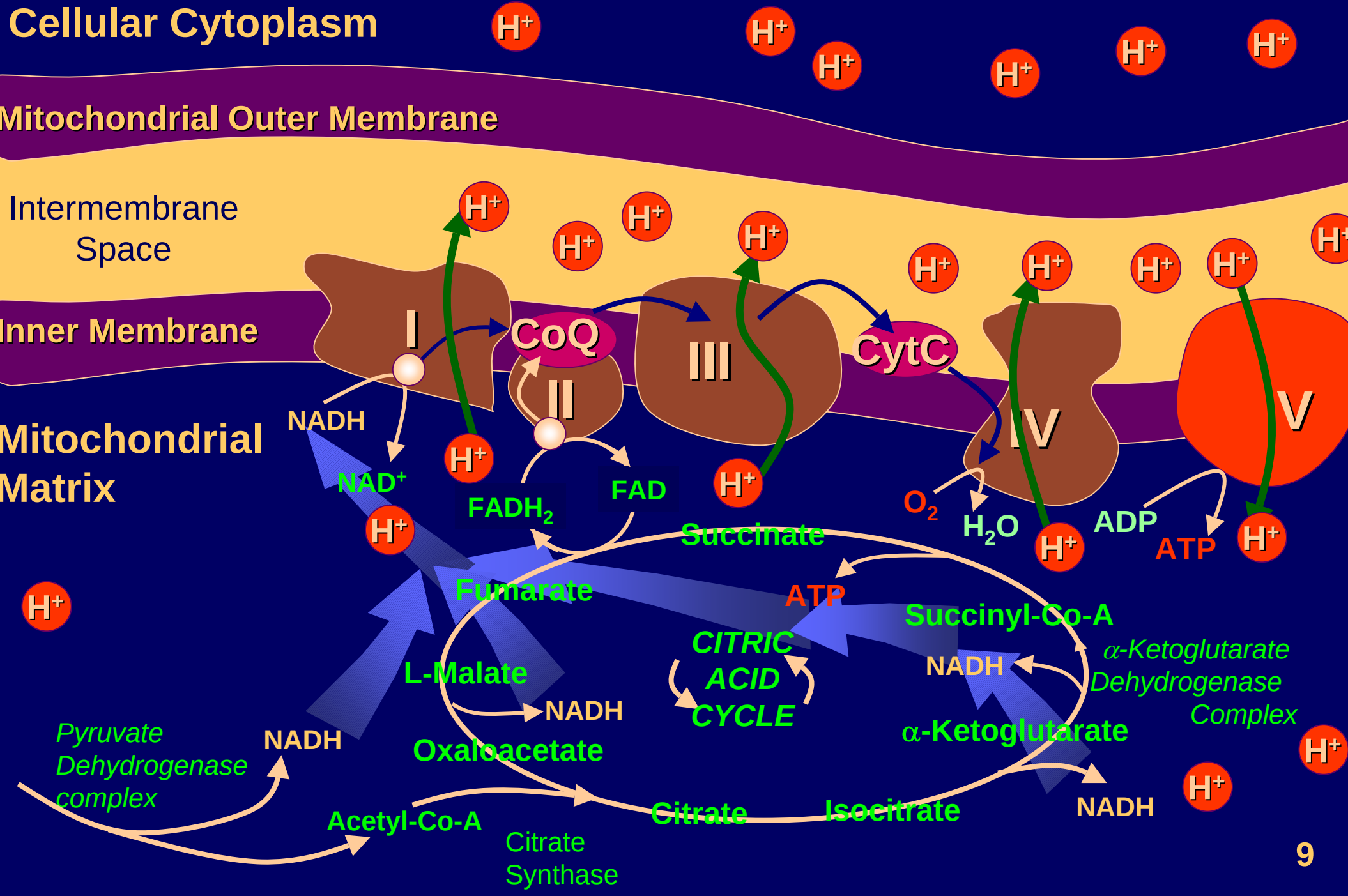
Oxidative damage and mitochondrial decay in aging

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

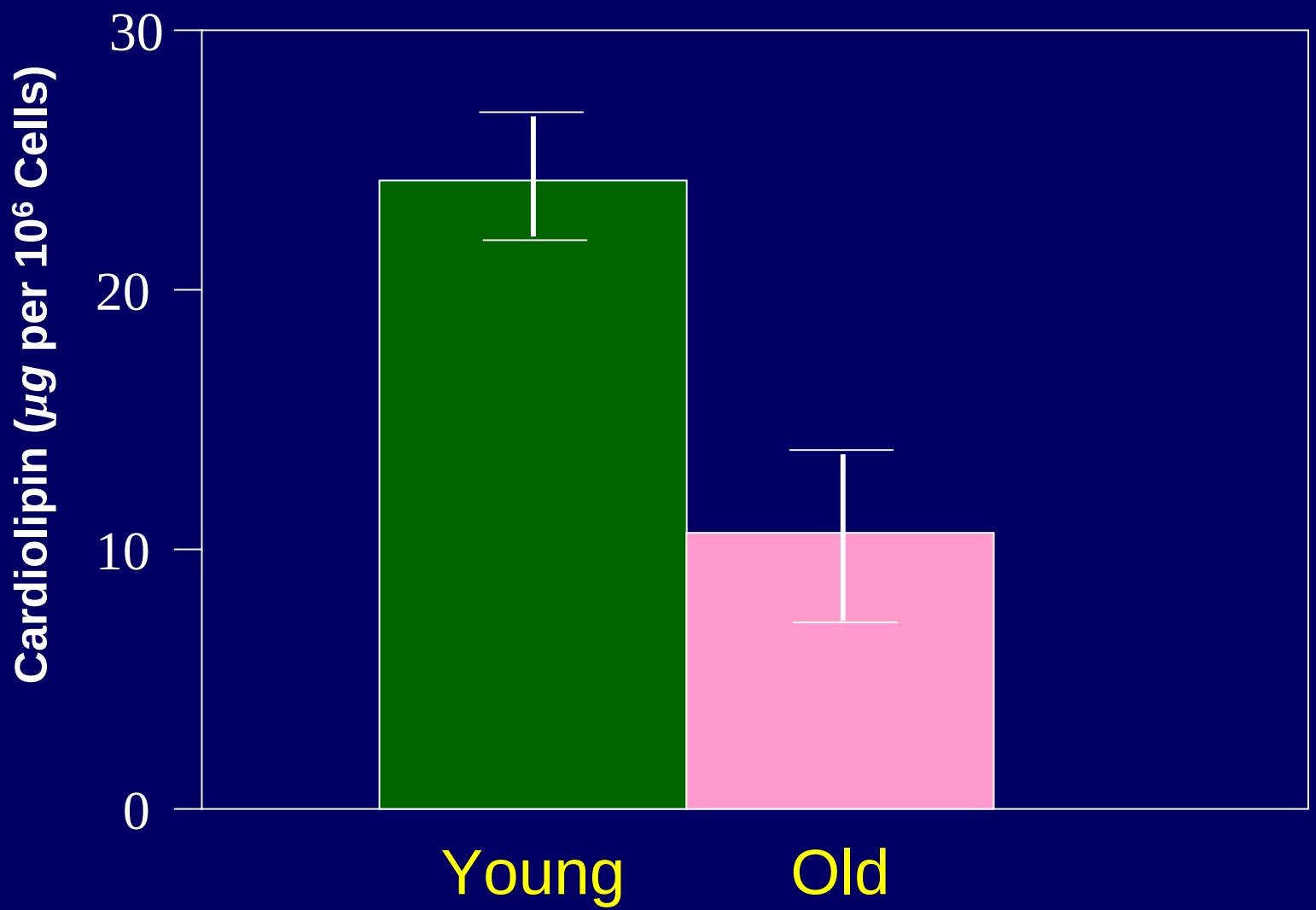
*Mark K. Shigenaga, Tory M. Hagen, and Bruce N. Ames**

Division of Biochemistry and Molecular Biology, 401 Barker Hall, University of California, Berkeley, CA 94720

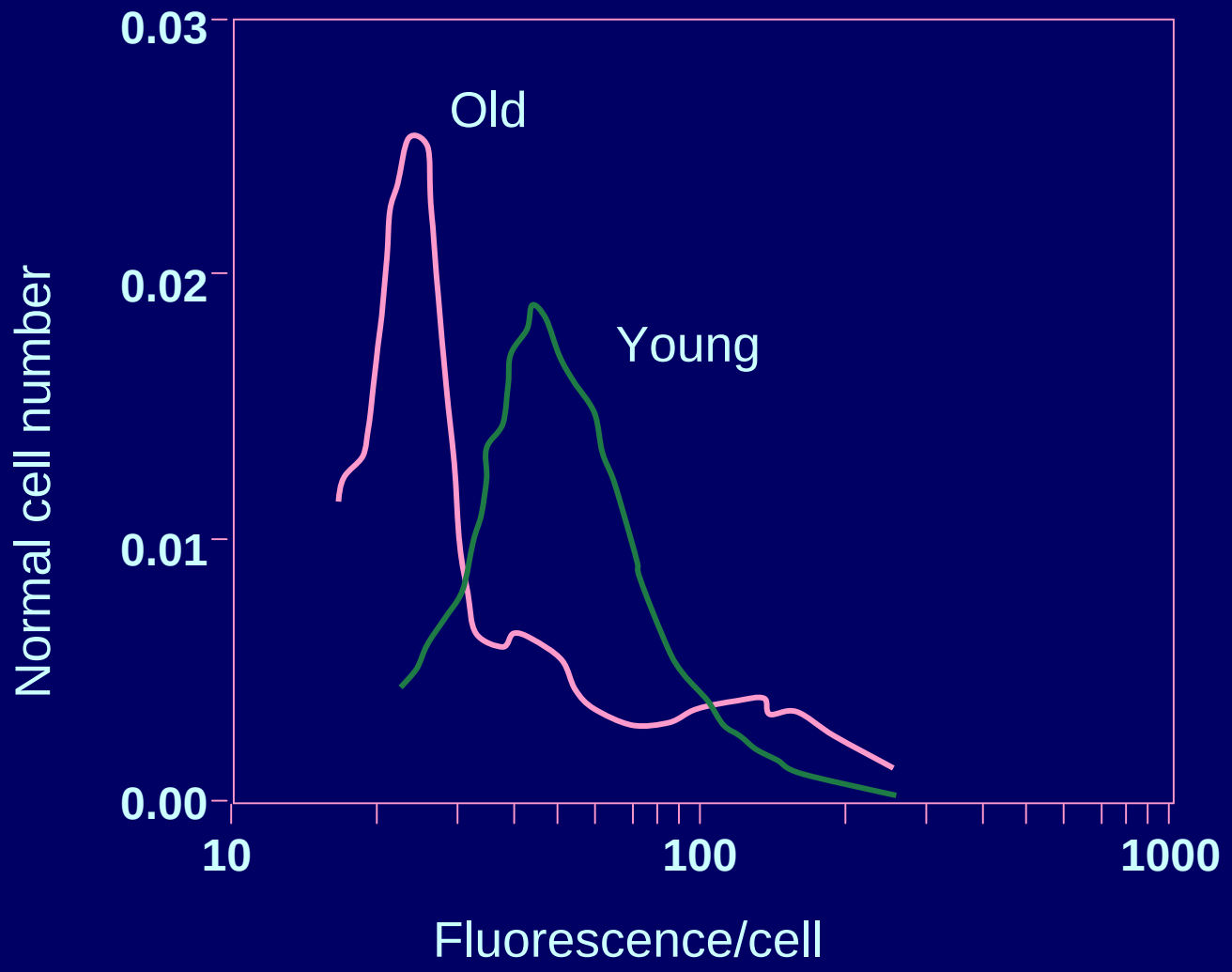
Contributed by Bruce N. Ames, July 27, 1994

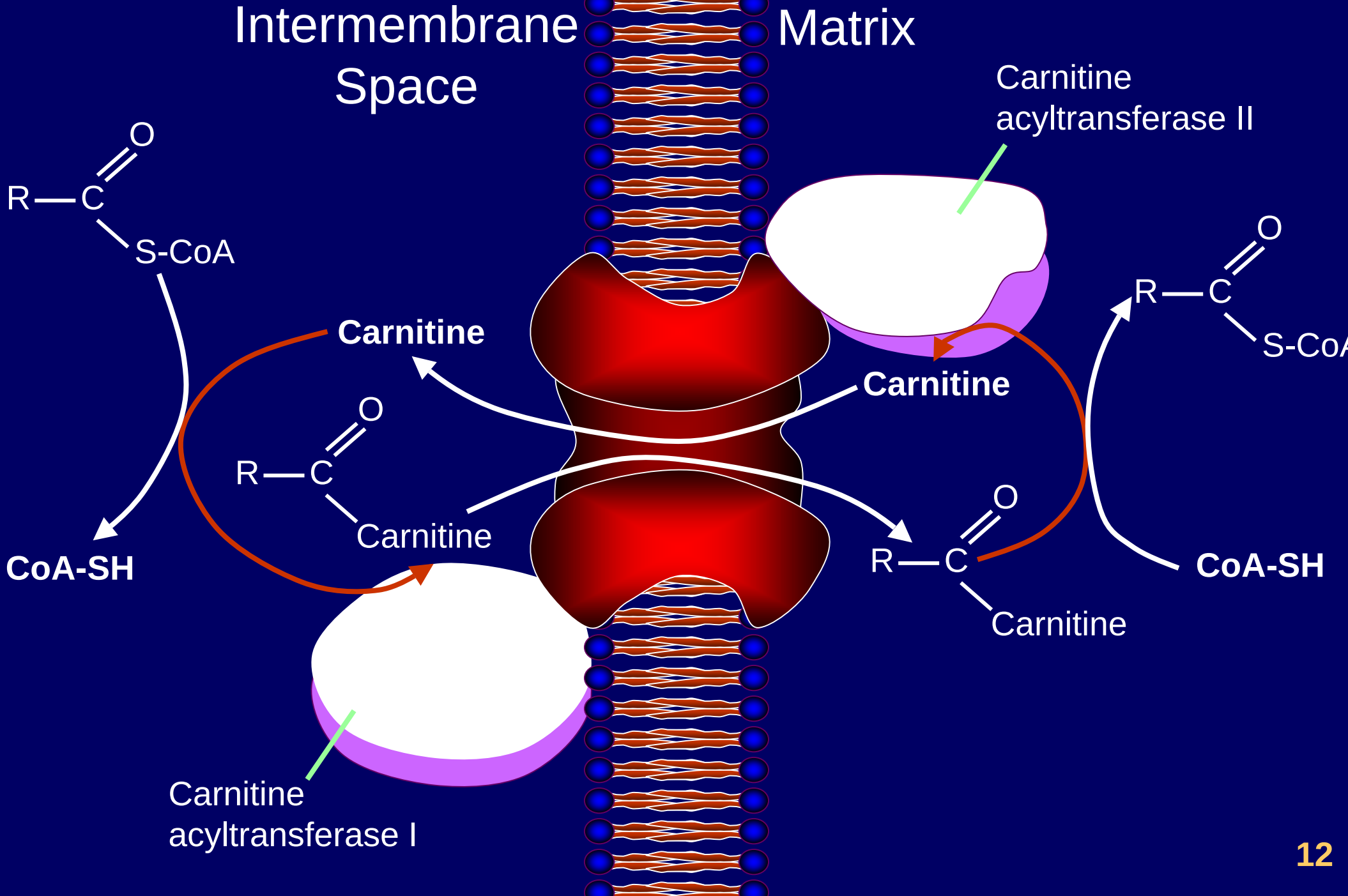

Cellular Cytoplasm
Mitochondrial Outer Membrane
Intermembrane Space
Inner Membrane
Mitochondrial Matrix
H+
I
II
III
IV
V
CoQ
CytC
NADH
NAD+
FADH2
FAD
O2
H2O
ADP
ATP
Succinate
Fumarate
L-Malate
Oxaloacetate
Citrate
Isocitrate
CITRIC ACID CYCLE
α-Ketoglutarate
Succinyl-Co-A
α-Ketoglutarate Dehydrogenase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

Cardiolipin Levels in 3 and 24 Month Old Rat Hepatocytes



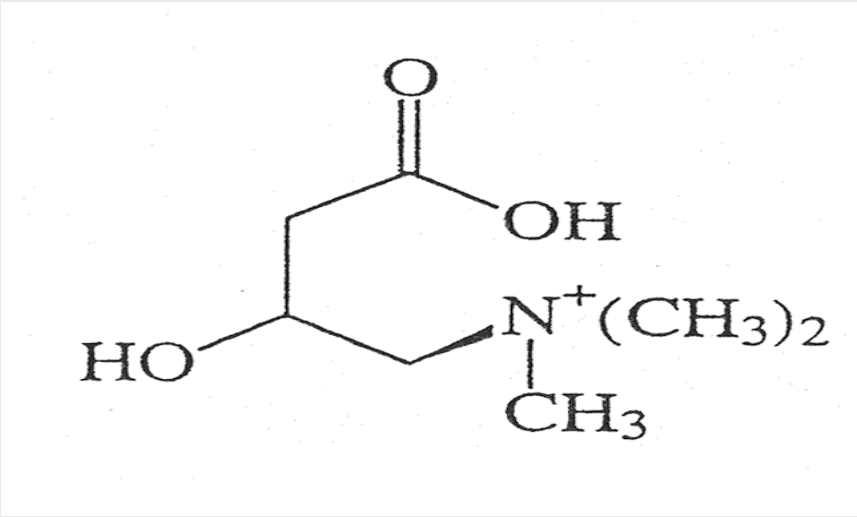
R123 Fluorescence in old and young rat hepatocytes

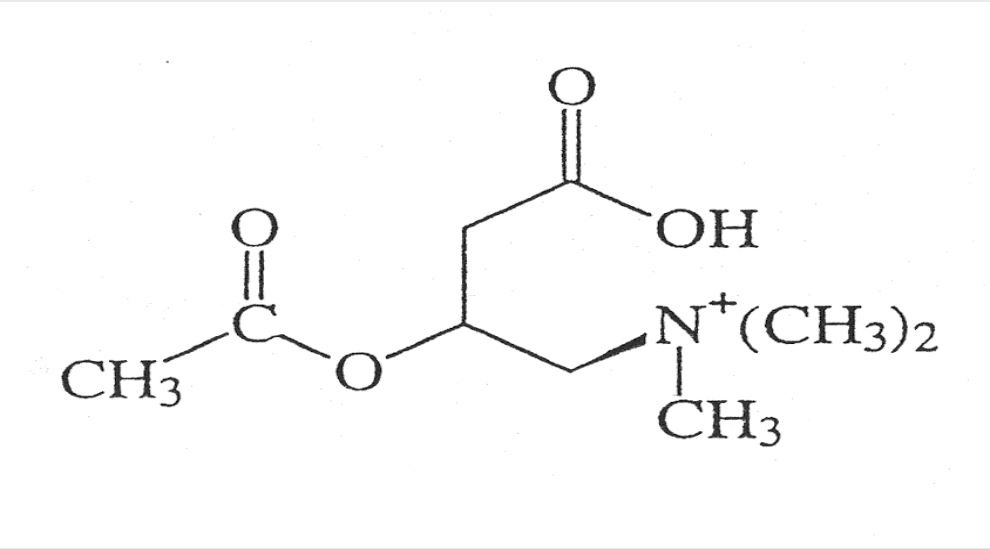



Intermembrane Space
Matrix
Carnitine acyltransferase II
Carnitine acyltransferase I
Carnitine
Carnitine
Carnitine
Carnitine
CoA-SH
CoA-SH
R—C(=O)—S-CoA
R—C(=O)—Carnitine
R—C(=O)—Carnitine
R—C(=O)—S-CoA

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

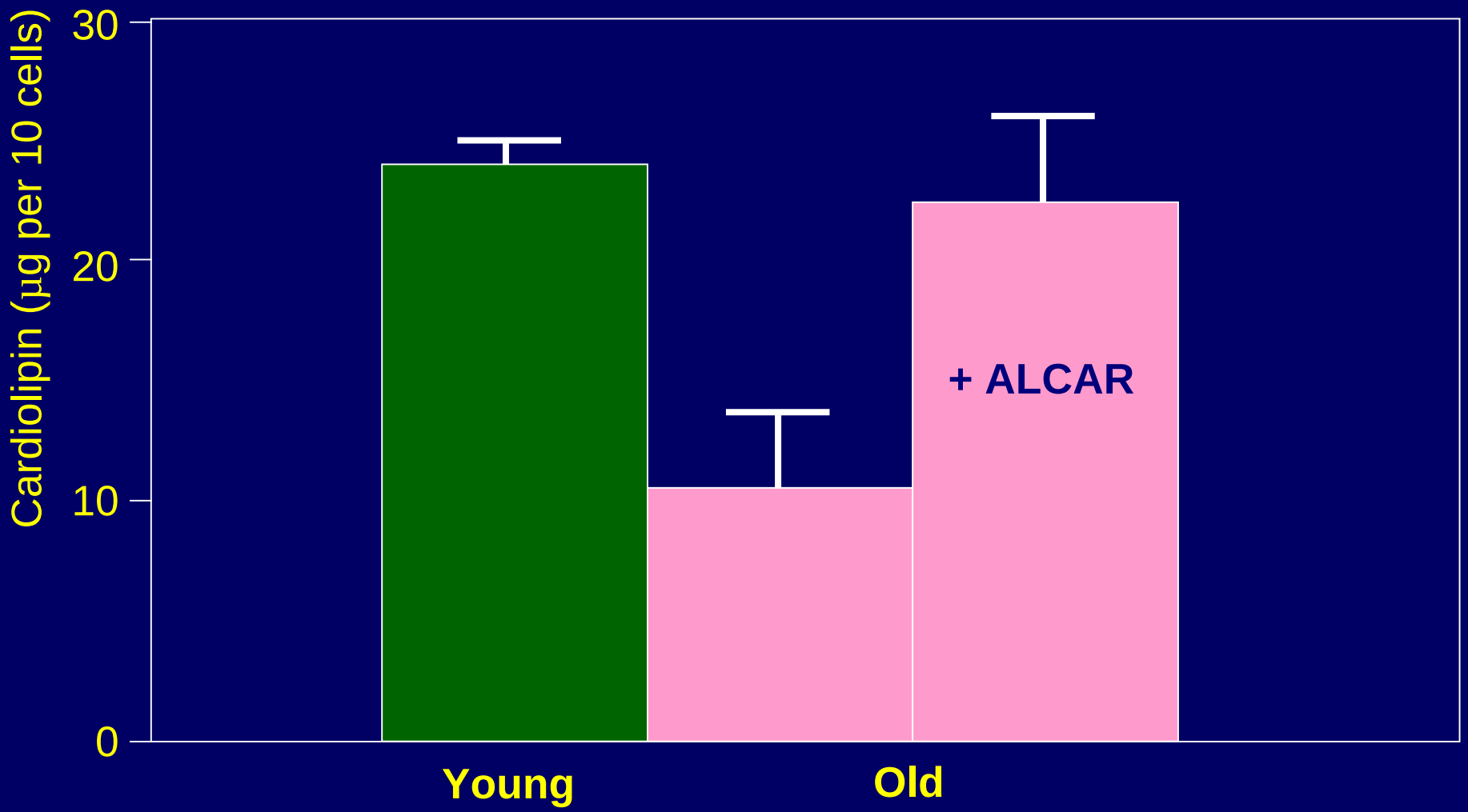
- Transports long-chain fatty acids into mitochondria
- Removes short- and medium-chain fatty acids that accumulate



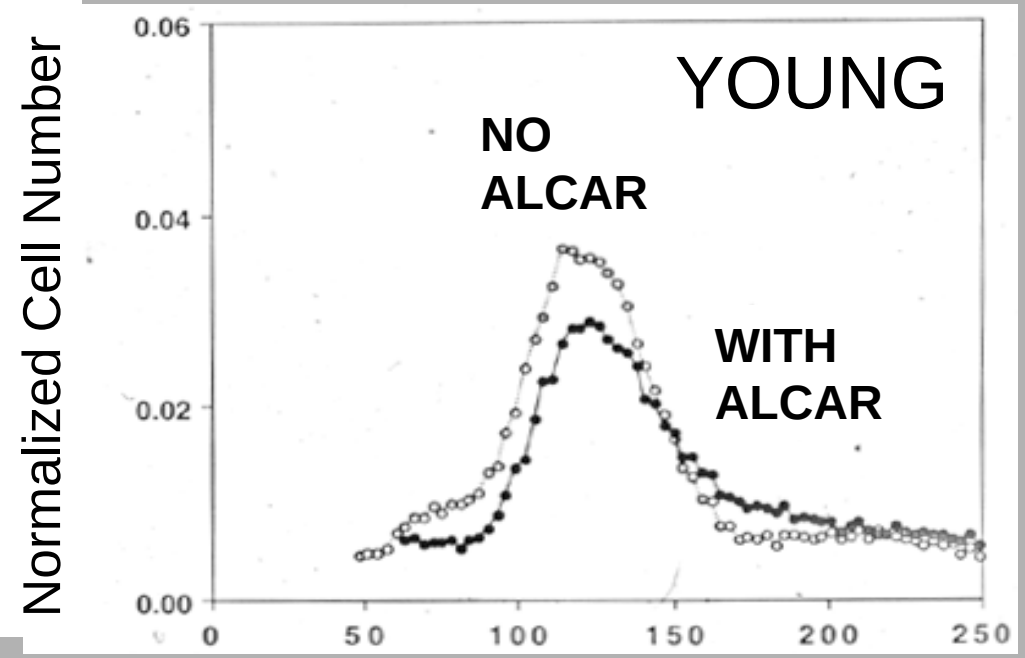


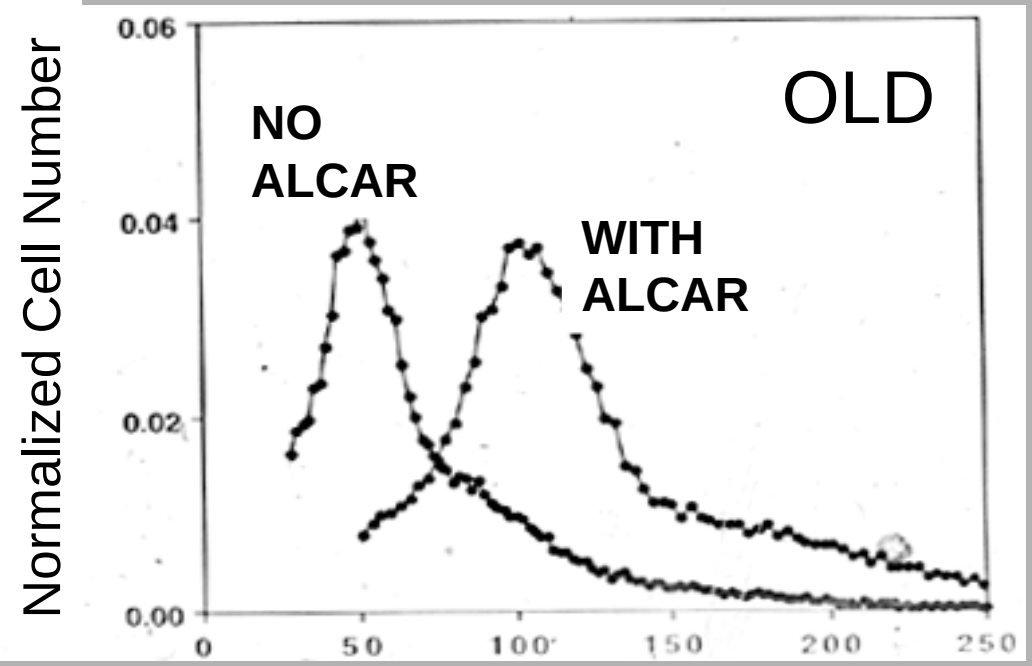
- Mediates the ratio of acetyl-CoA/CoA
- Decreases with age in plasma and in brain
- Improves cognitive function in rats

Effect of ALCAR Supplementation on Cardiolipin Levels



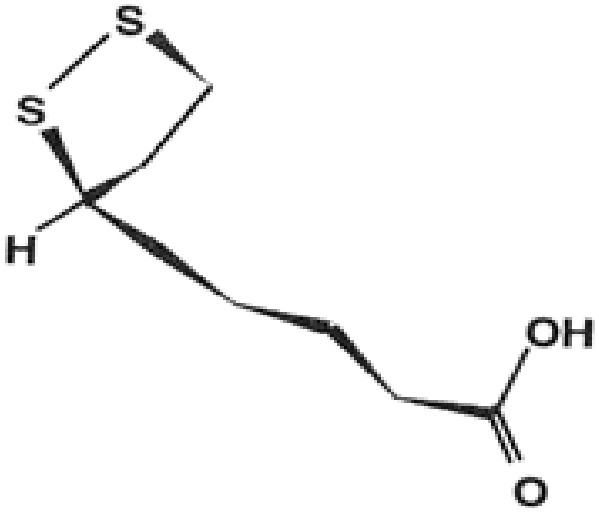
R123 Fluorescence in Young and Old Rat Hepatocytes

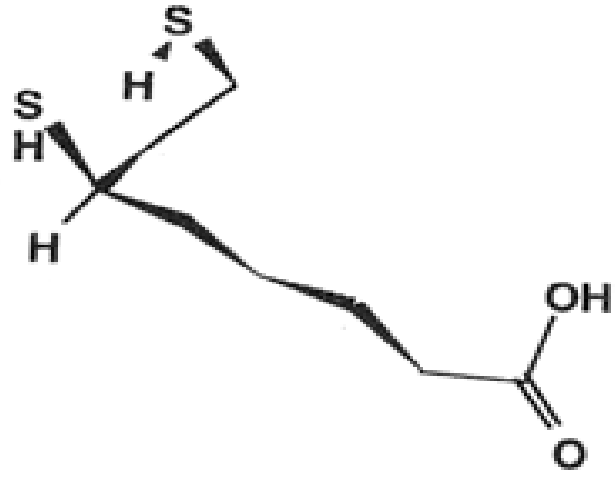




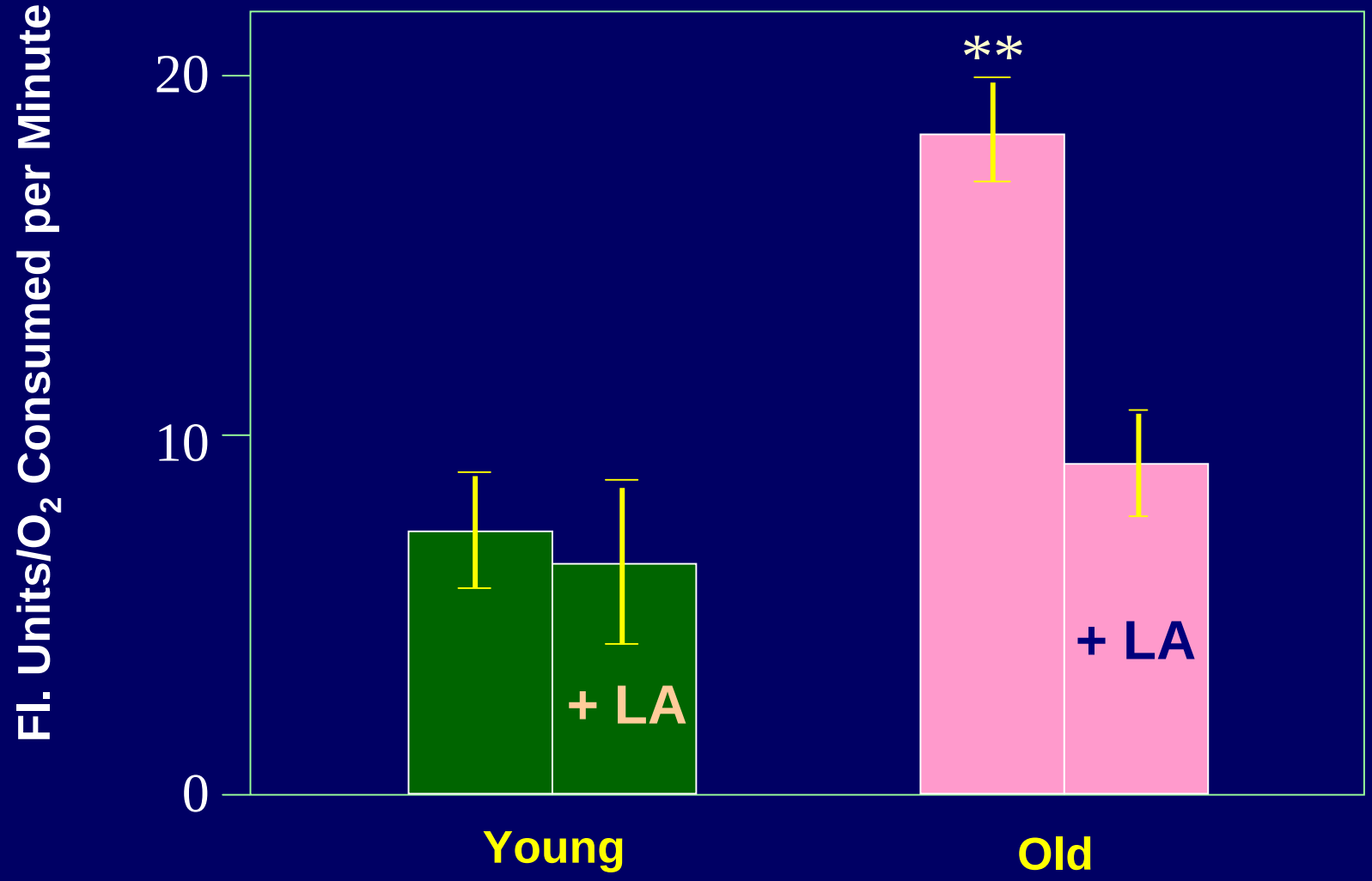
R-α-Lipoic Acid (LA) in mitochondria

- 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

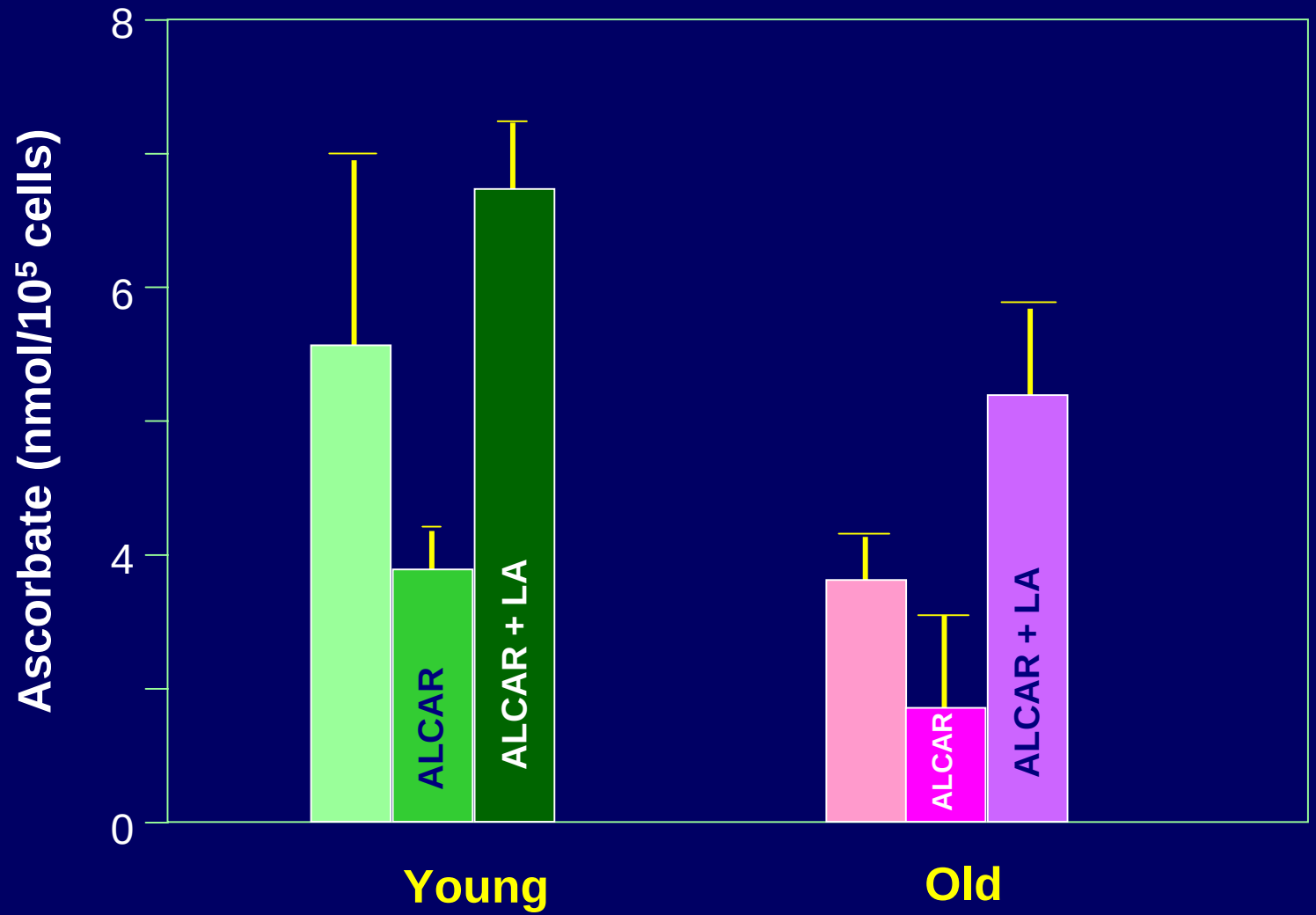




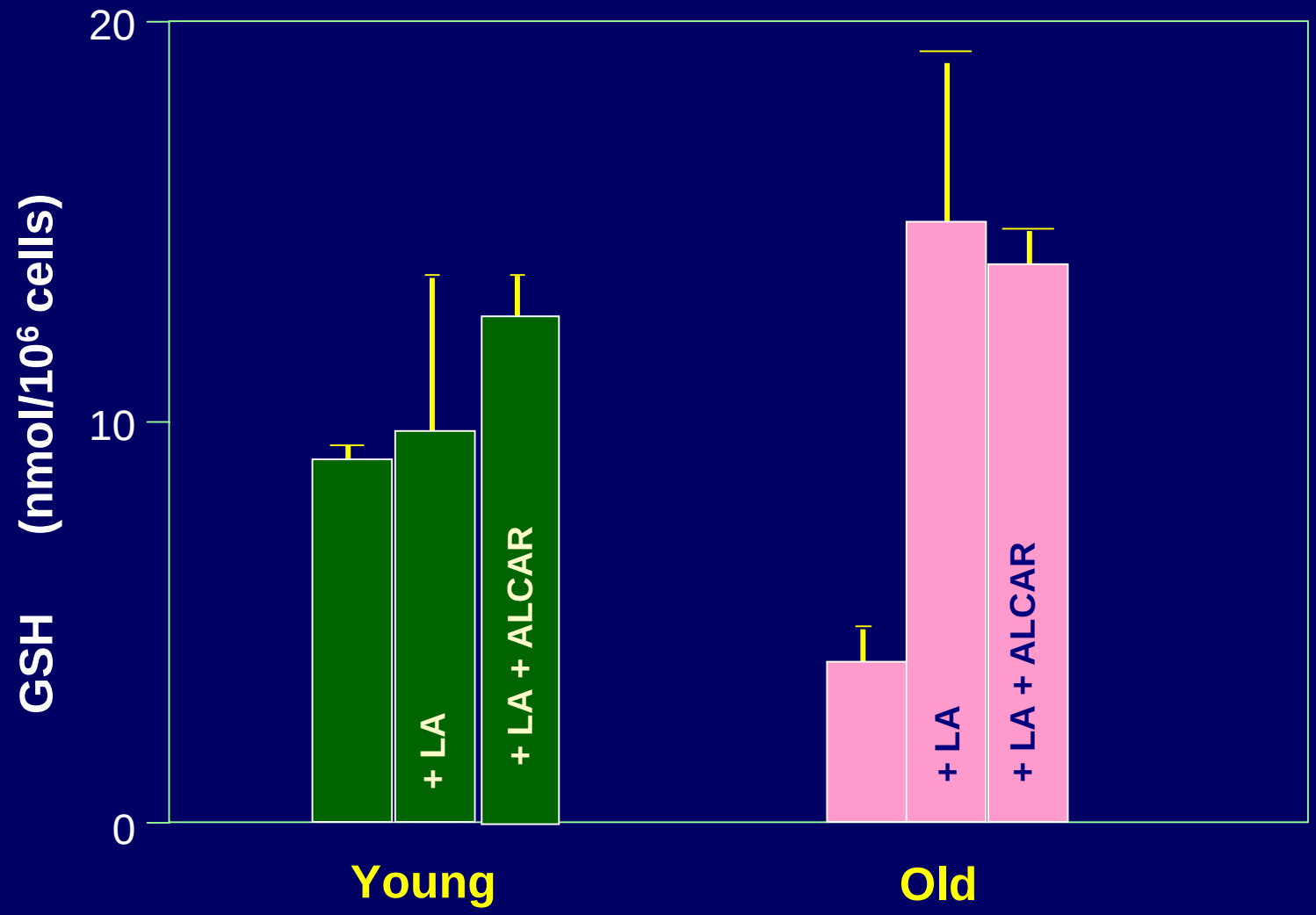
Lipoic Acid Lowers Mitochondrial Oxidants in Old Rats



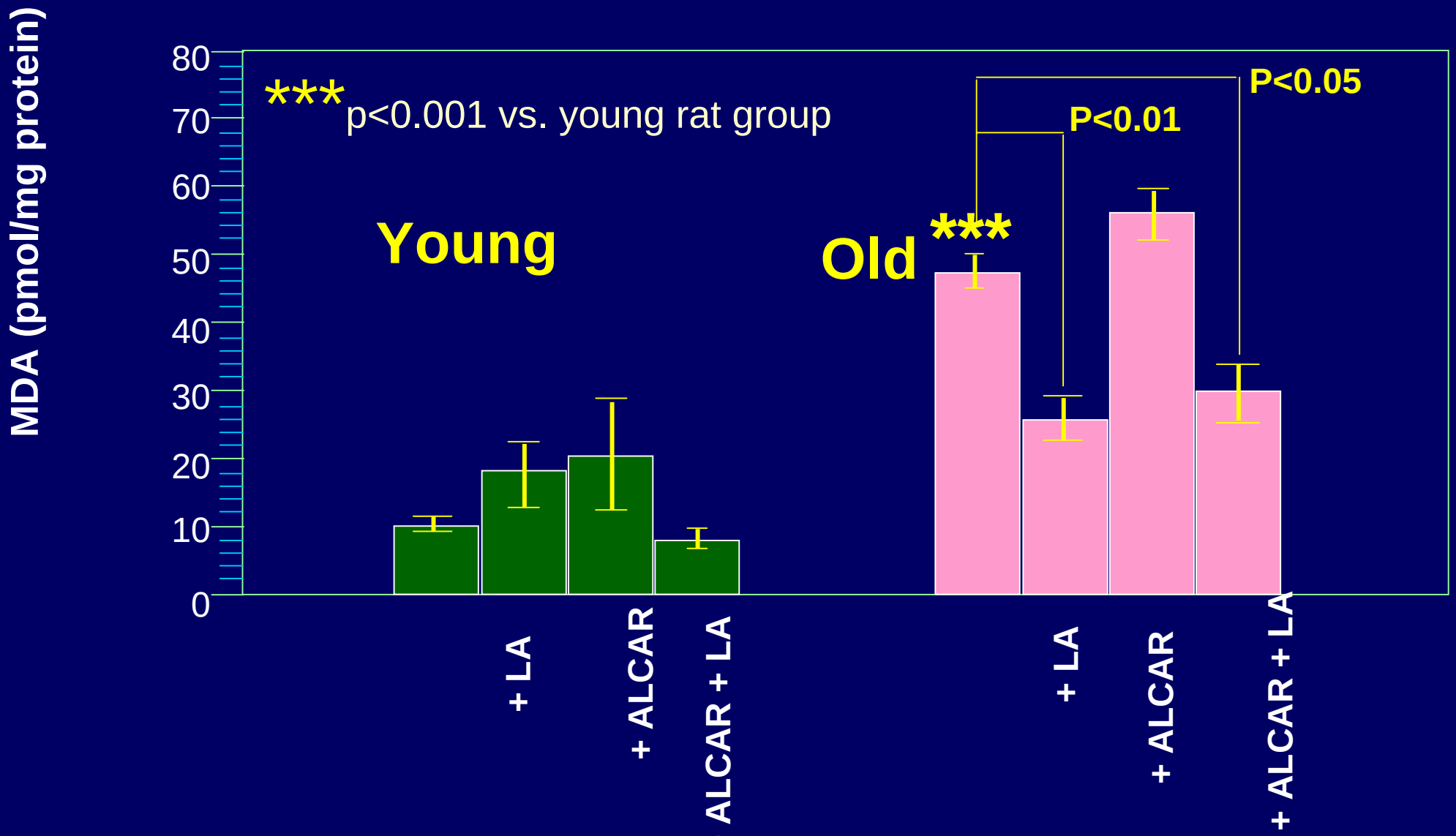
R-Lipoic Acid Restores Cellular Ascorbate



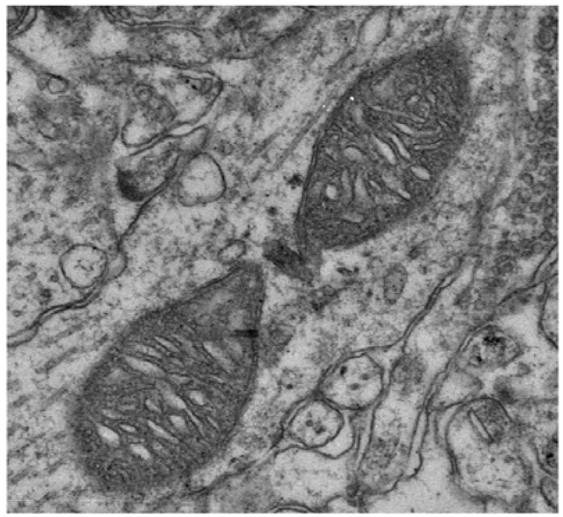
Effect of R-Lipoic Acid [LA] Supplementation on GSH Levels ± ALCAR



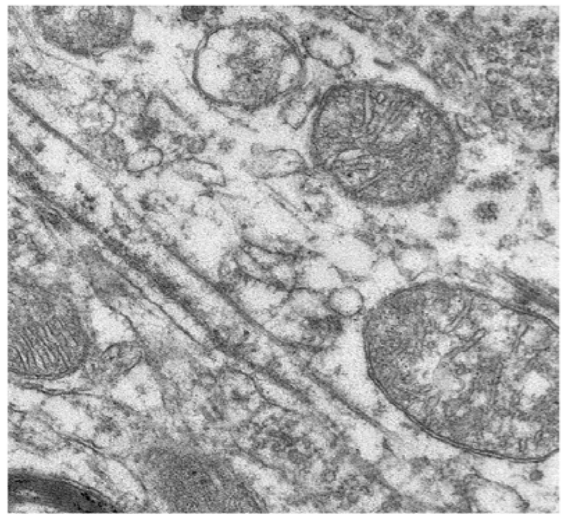
MDA levels in young and old rats with LA, ALCAR, or both



Mitochondria decay with age



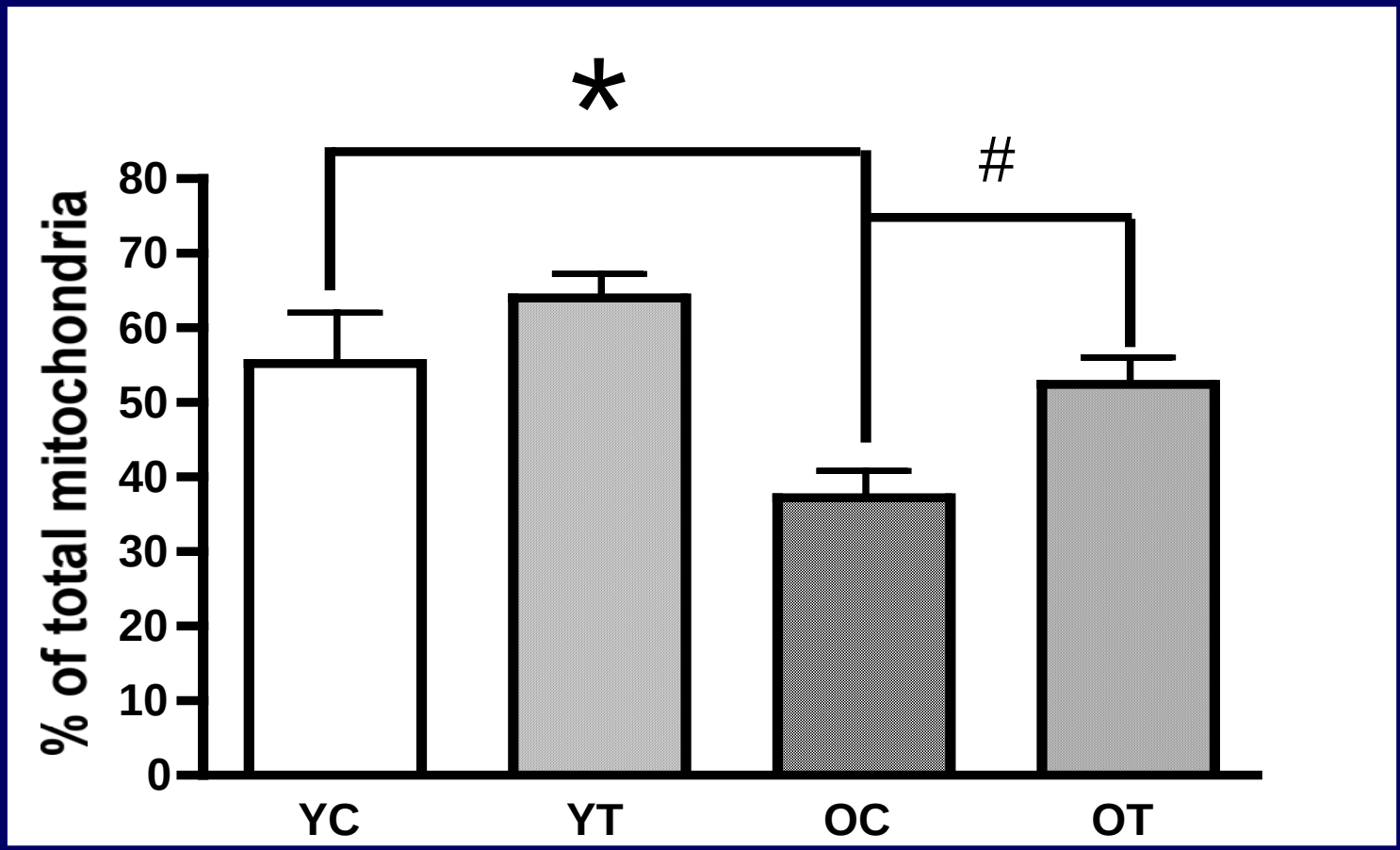
Young



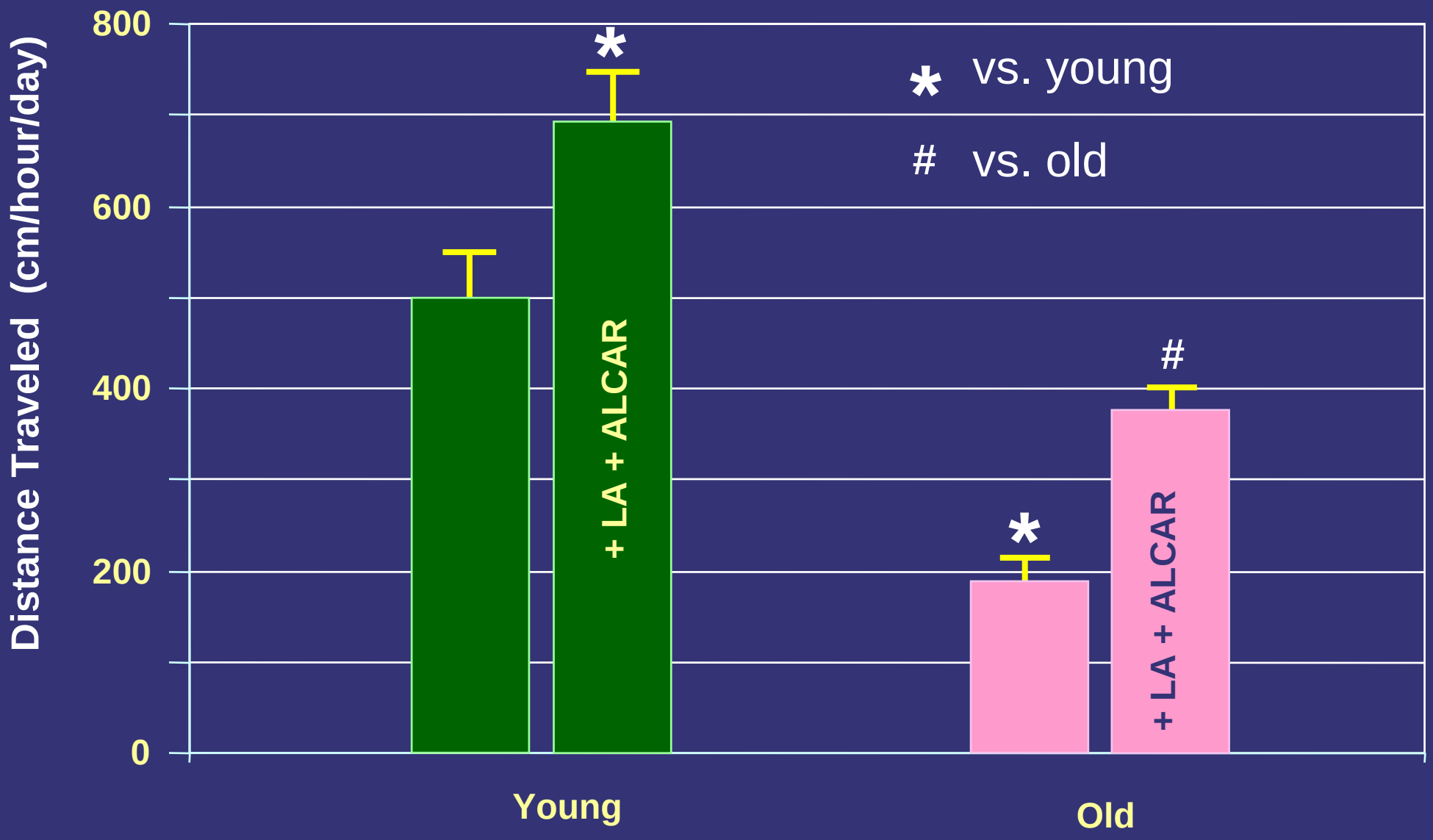
Old

1. Decreased cardiolipin levels and structural deficit;
2. Decreased membrane potential (the driving force for ATP synthesis) and cellular oxygen consumption;
3. Increased oxidation and heterogeneity;
4. Prone to oxidative damage, leading to a vicious cycle.

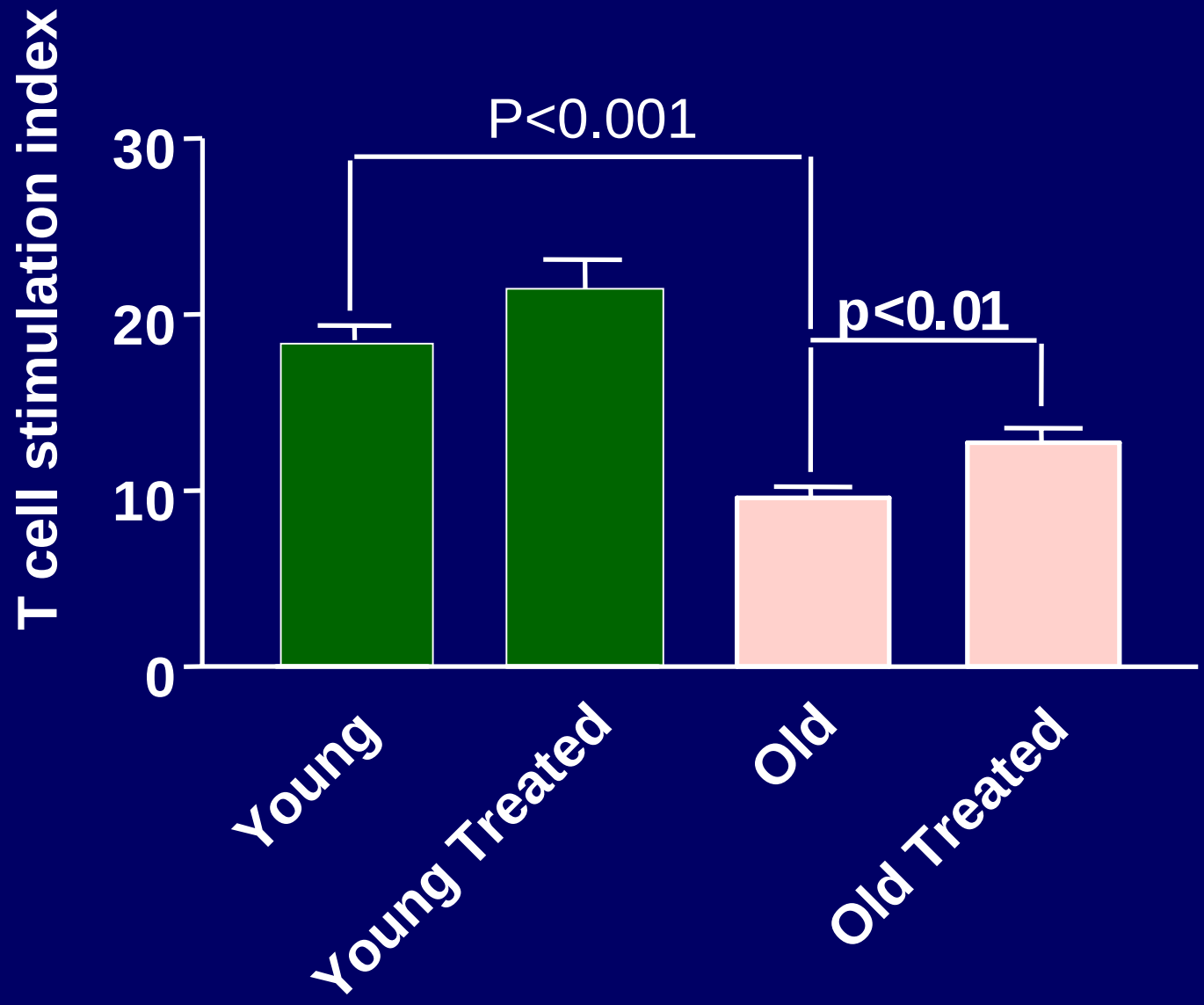
Intact mitochondria in hippocampal neurons by electron microscopy



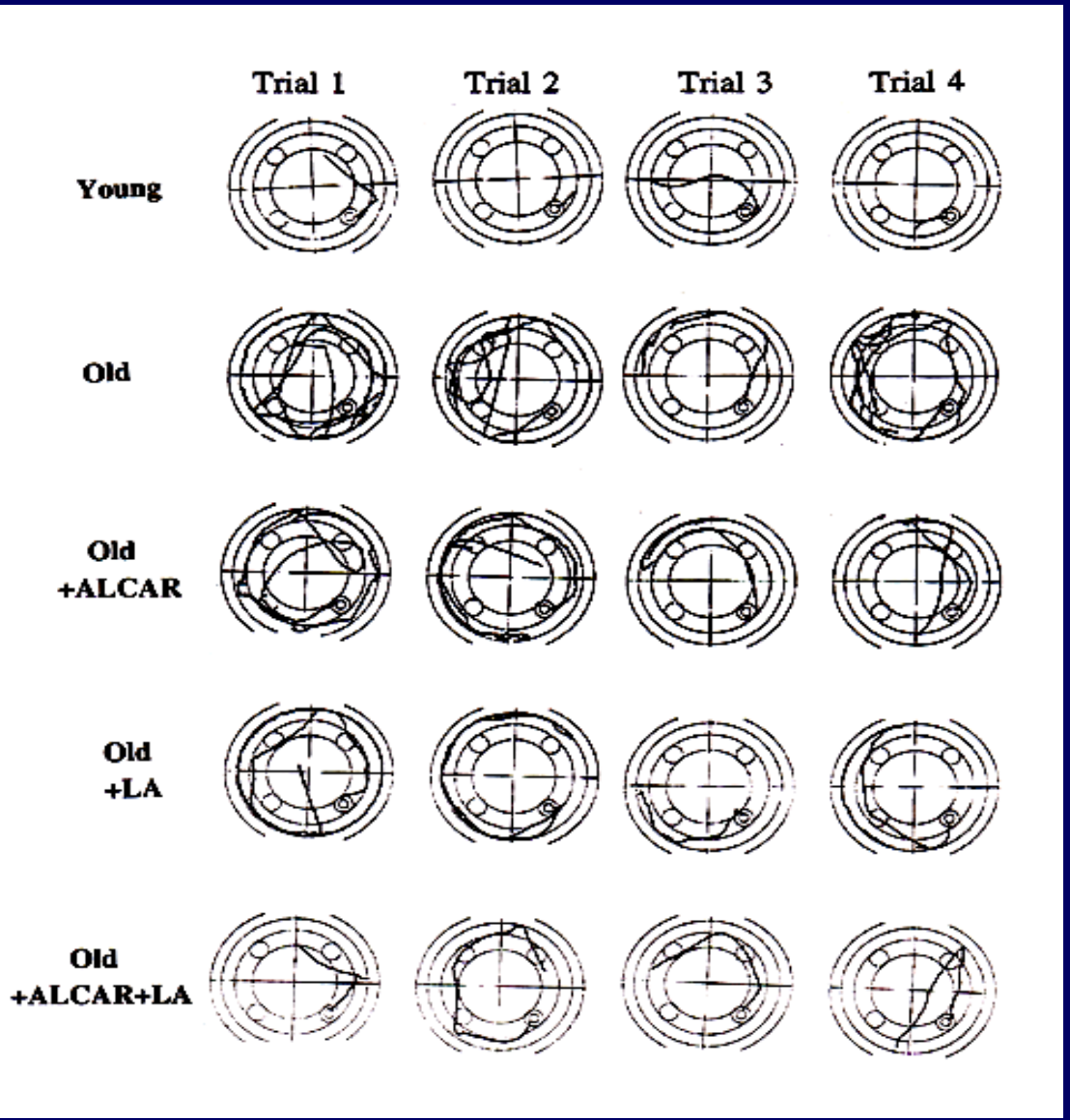
Ambulatory Activity before and After Supplementation with Lipoic Acid (LA) + Acetyl-L-Carnitine (ALCAR)



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.



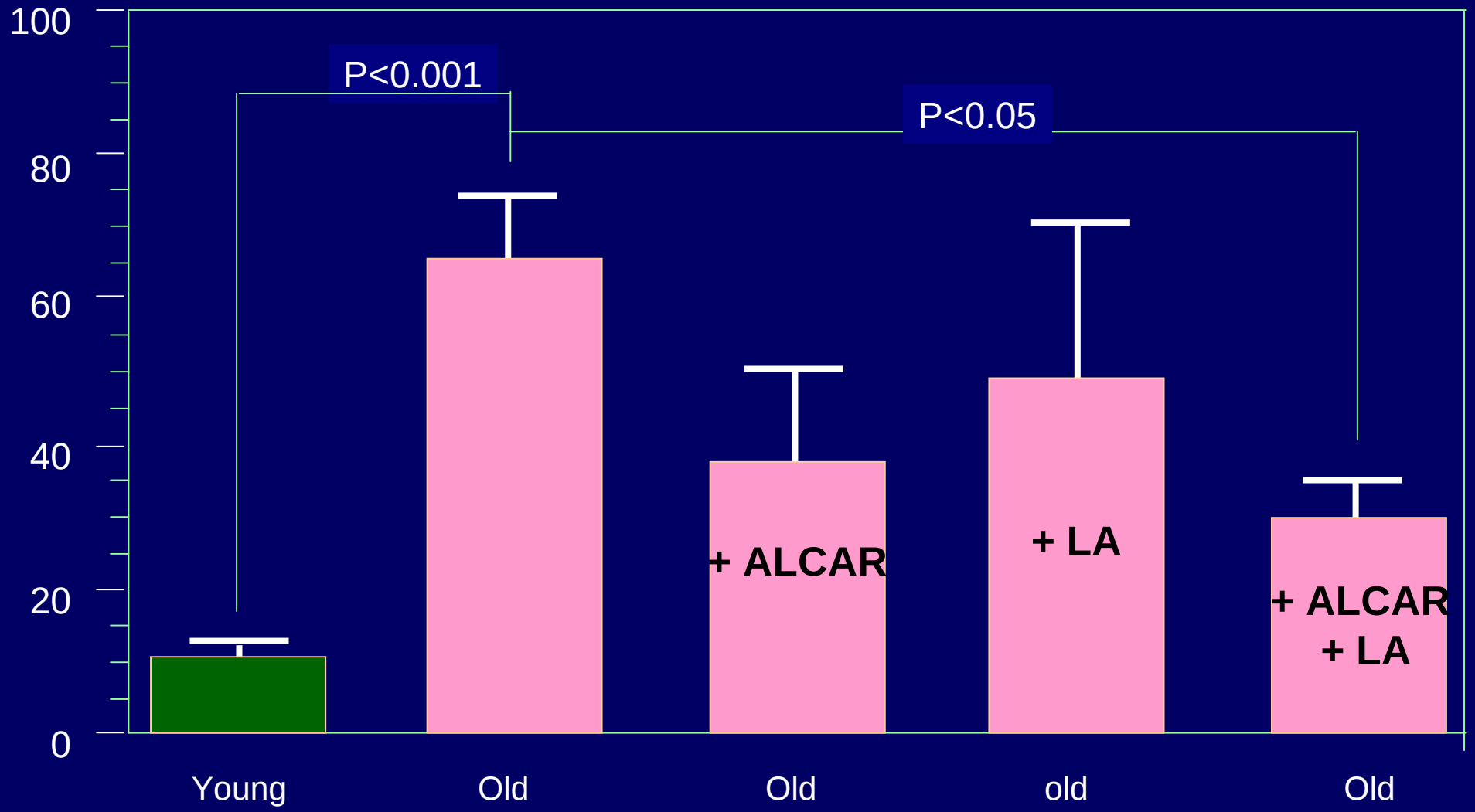
Morris Water Maze for Testing Spatial Memory



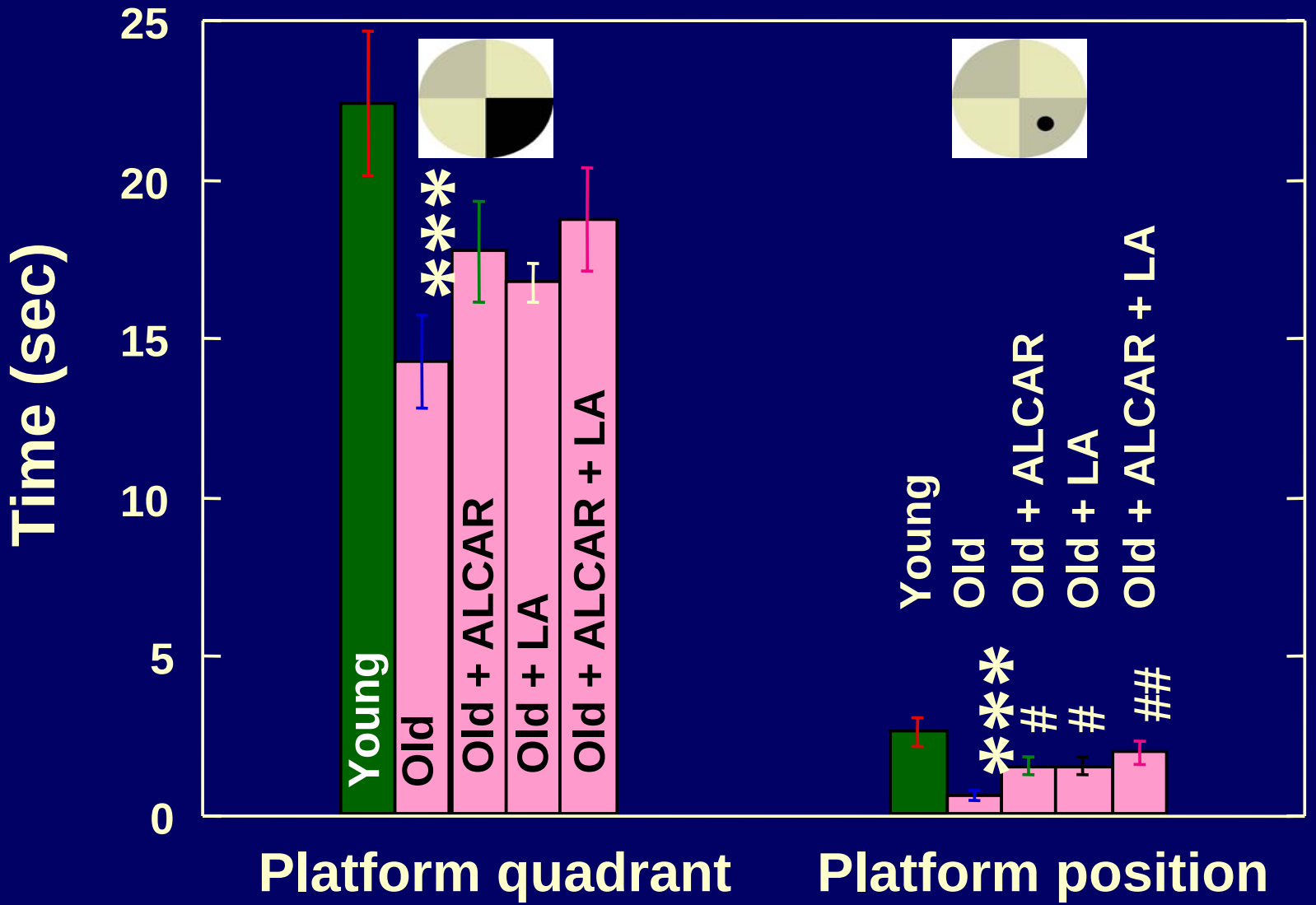
Spatial Memory relies on intact hippocampal function.

Treatments improved poor memory in old rats

Spatial Memory Tested With Morris Water Maze



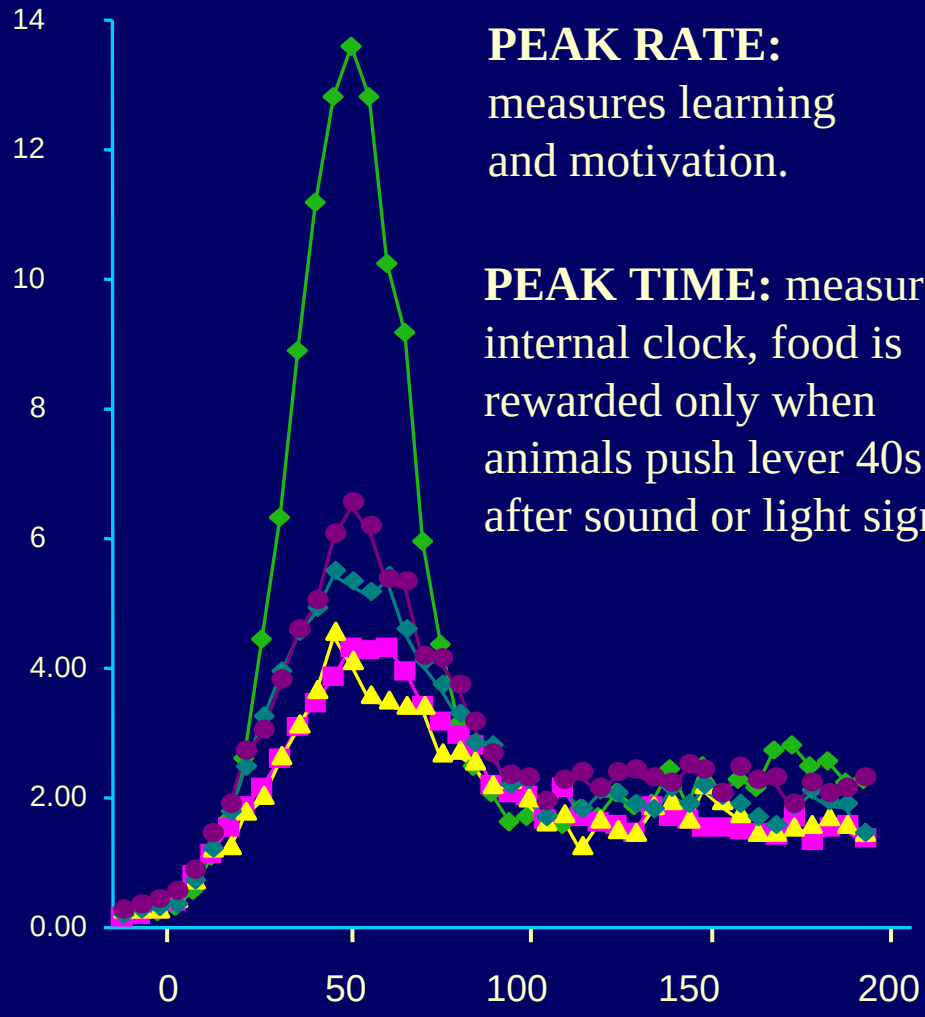
Improvements in Spatial Memory of Old Rats Treated with ALCAR, LA, or Both



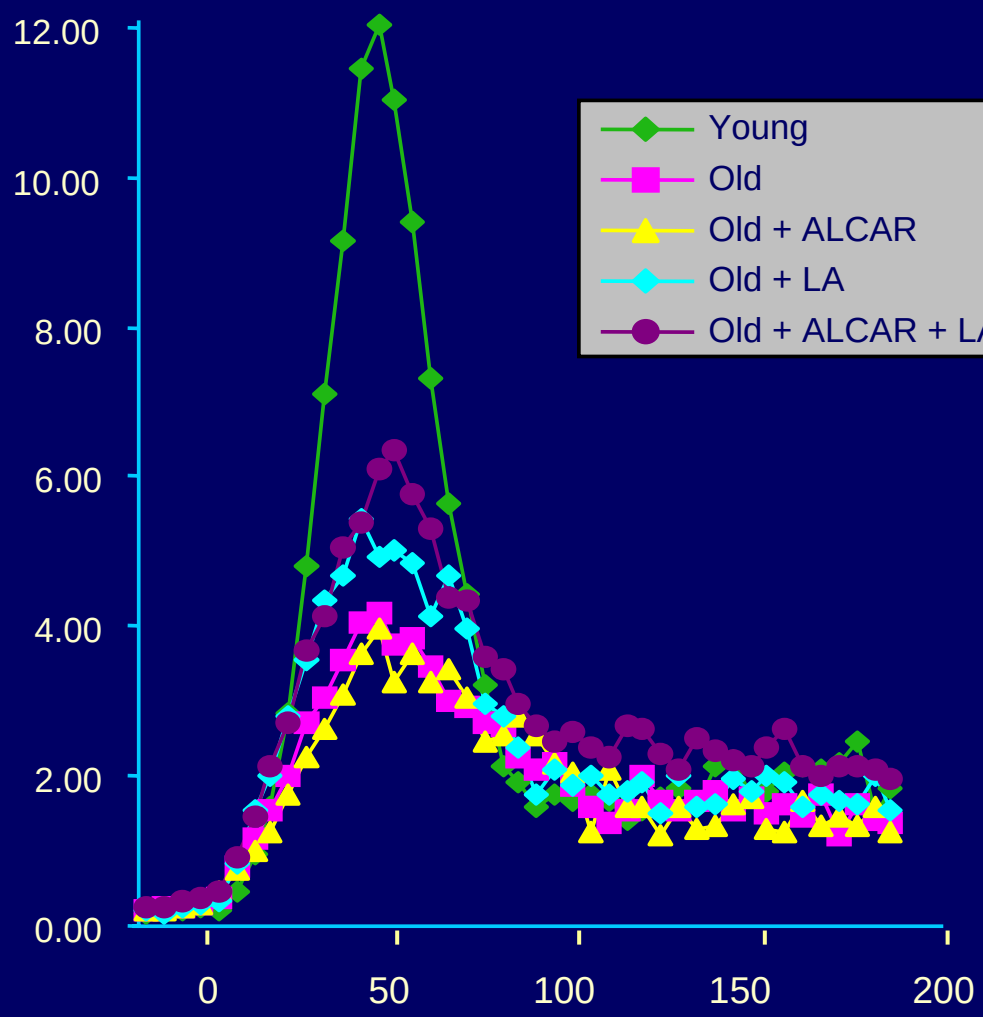
Peak procedure: for measuring temporal memory. Associated with striatum, cerebellum, & hippocampus

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

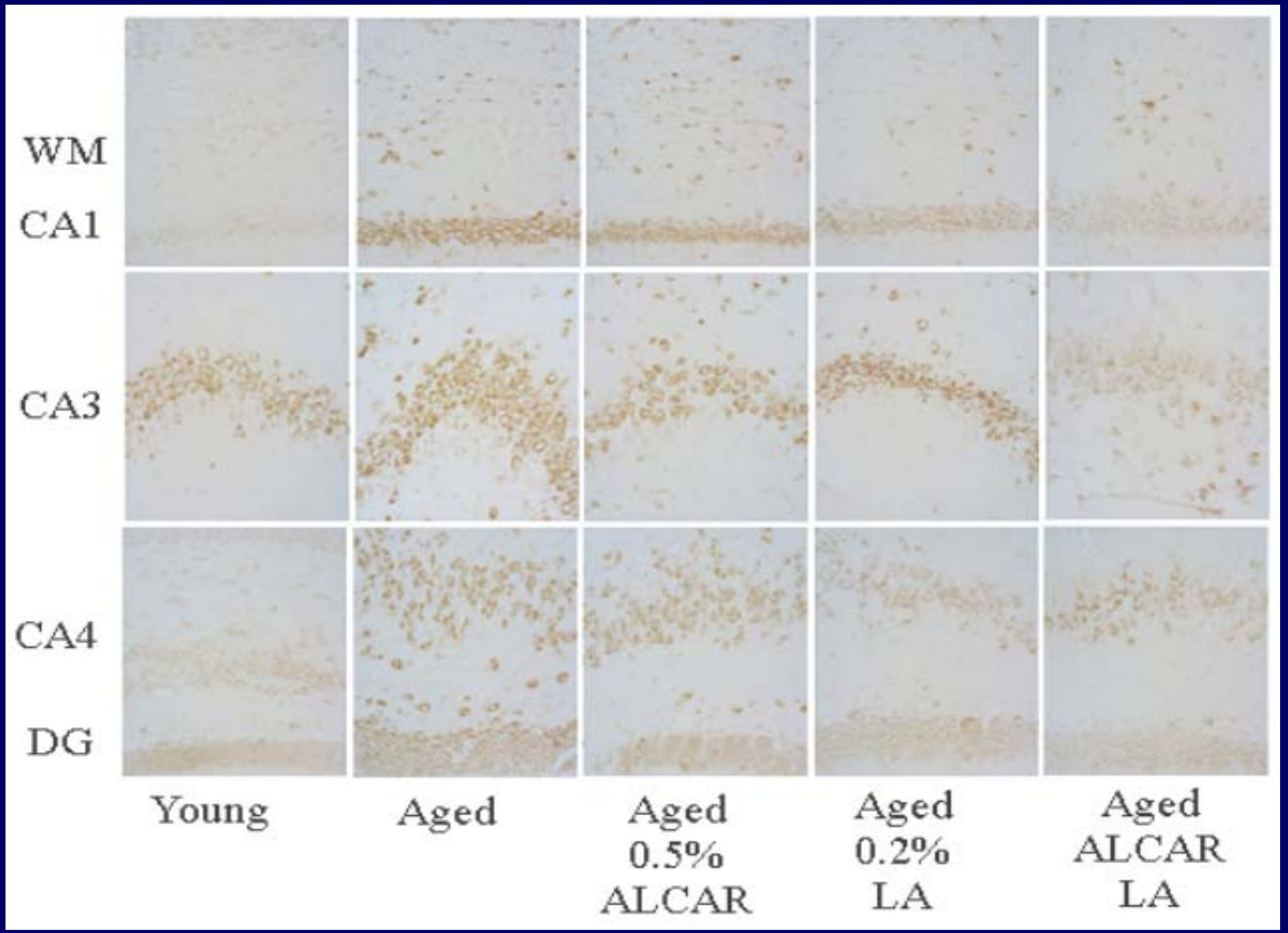


SOUND: Time to Signal



LIGHT: Time to Signal

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

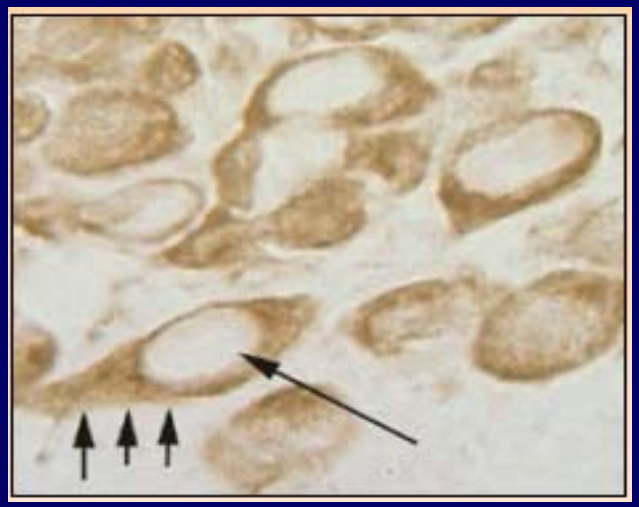


Staining of oxidized nucleic acid in neurons

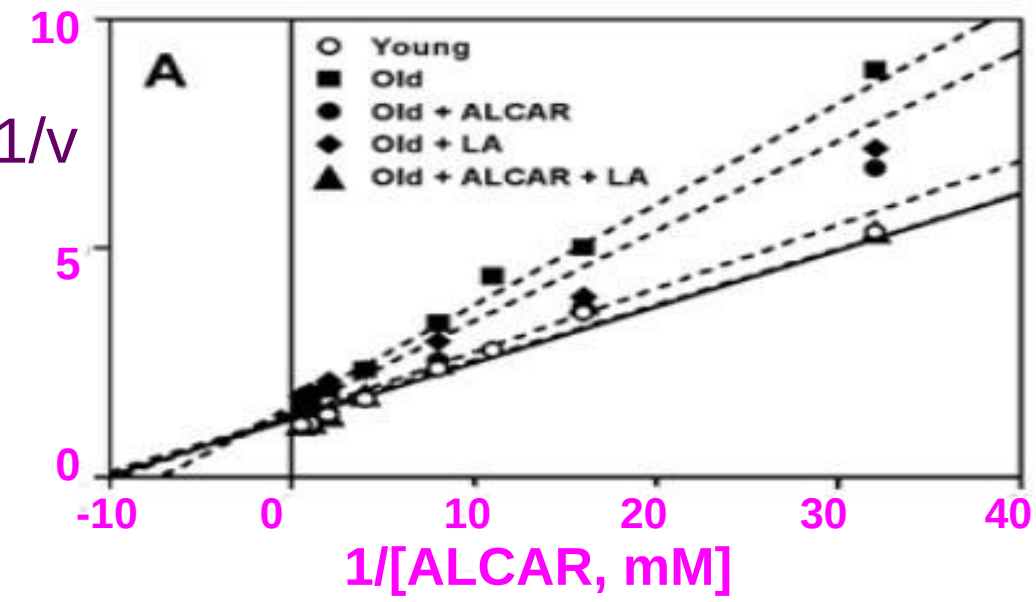
(mAb to oxo8dG in DNA/oxo8G in R NA)

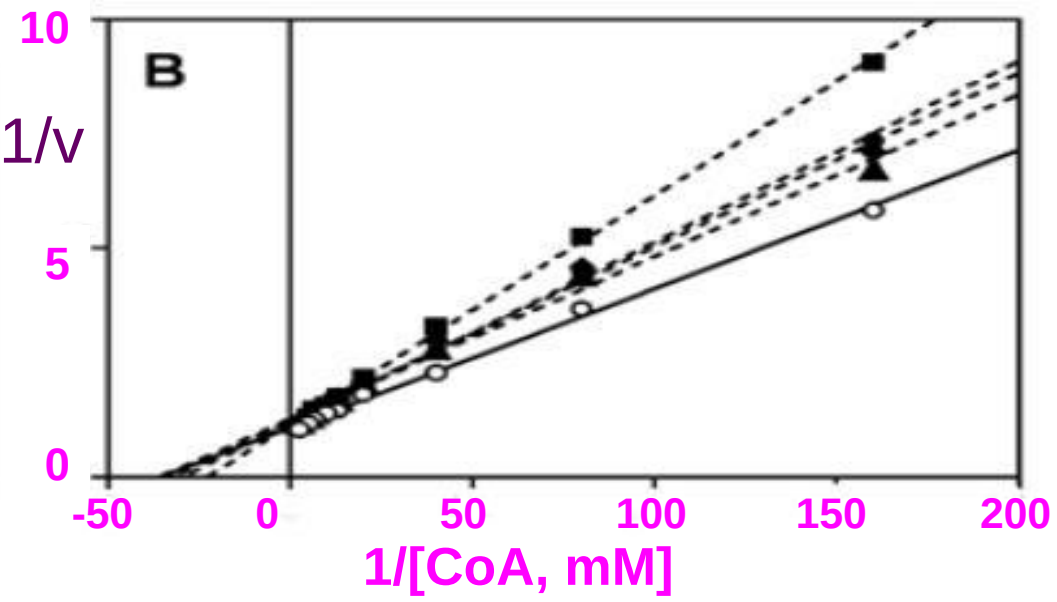
RNA is Oxidized

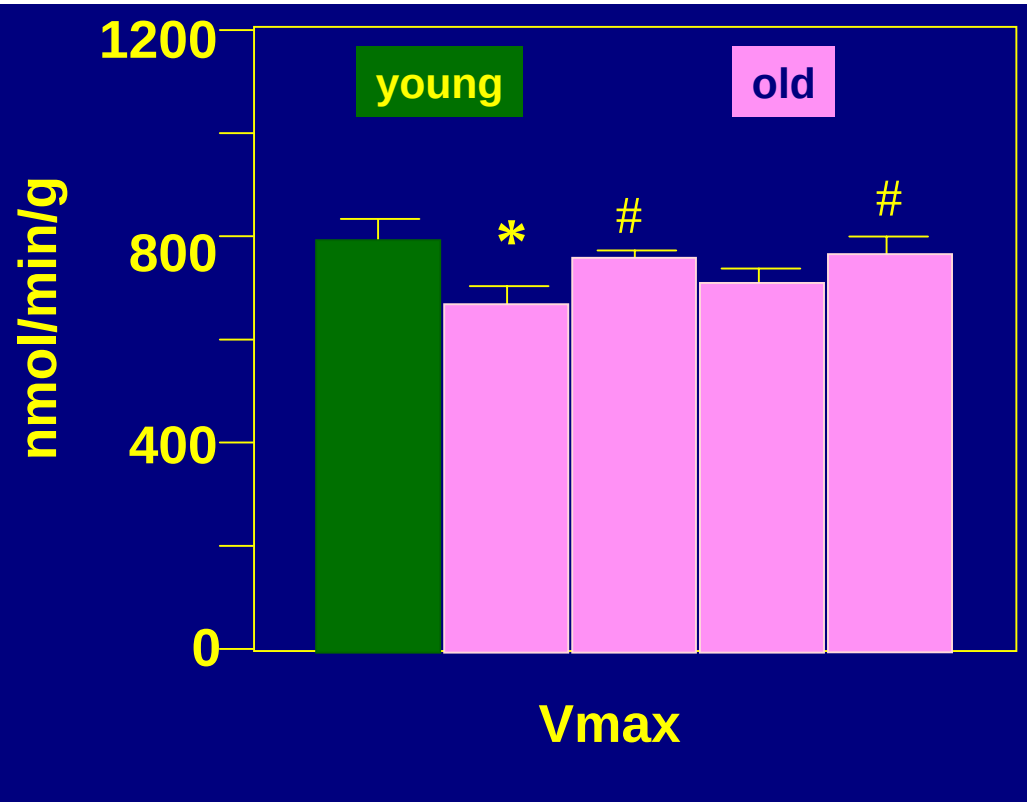
(92% is removed by RNase)

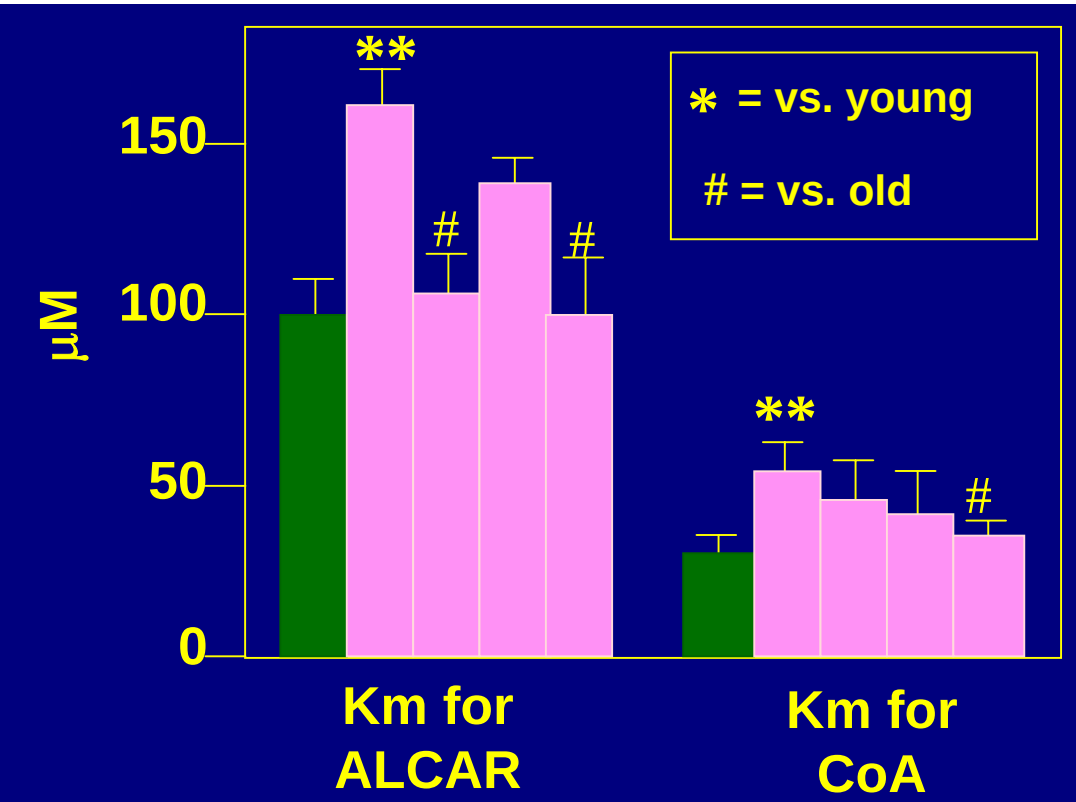


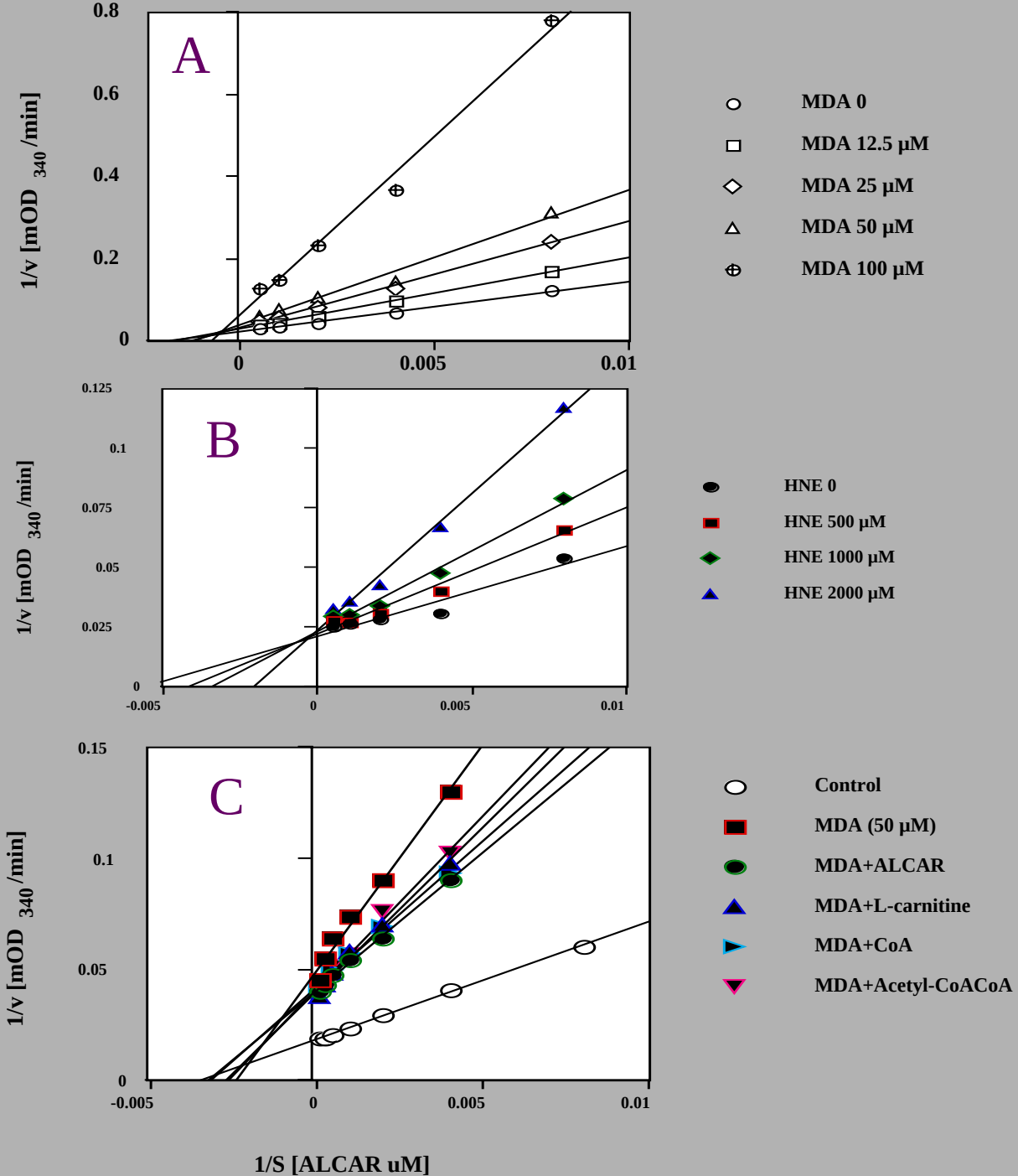
*oxo8G: 8-hydroxyguanosine; oxo8dG: 8-hydroxy-2'-deoxyguanosine

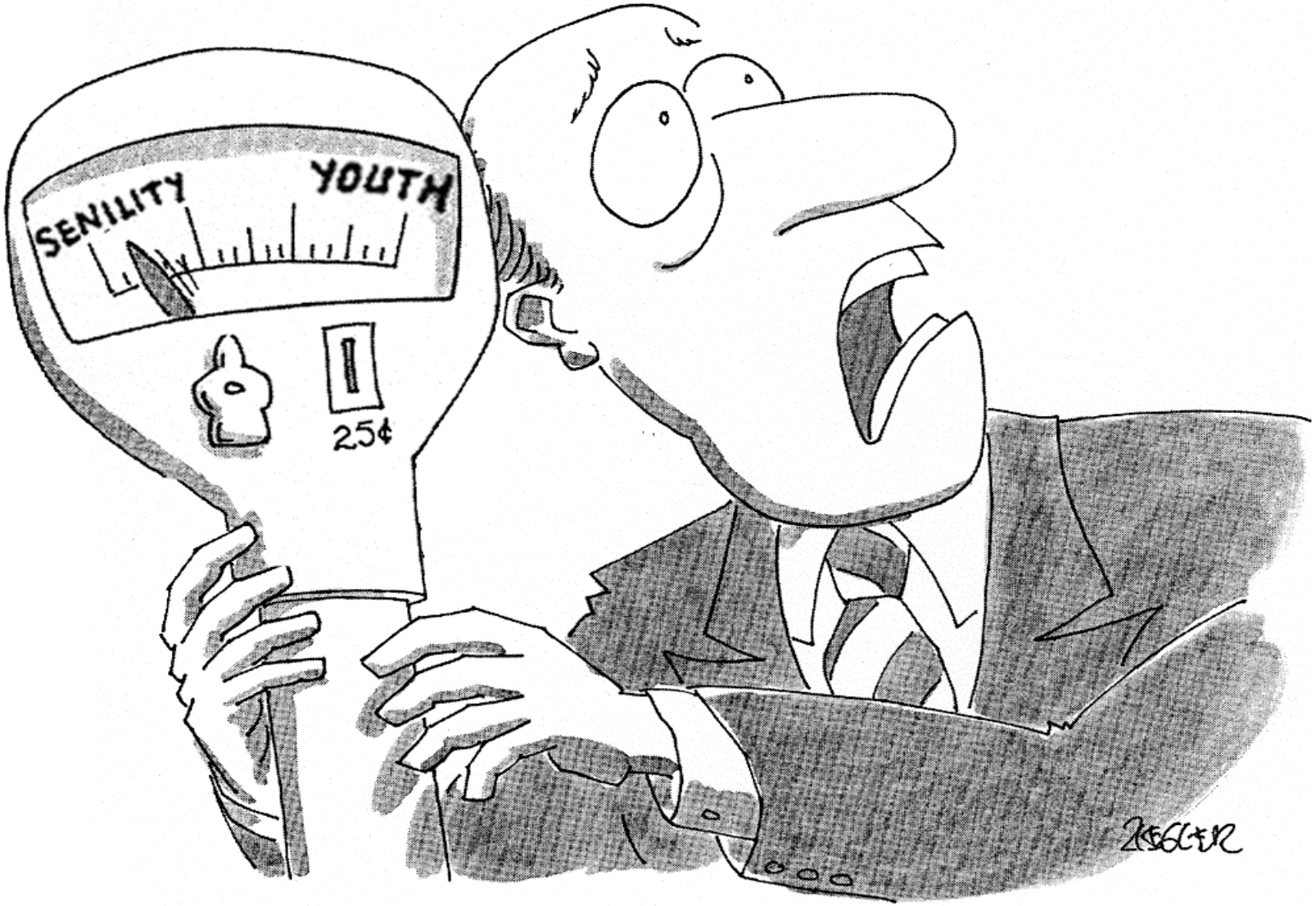

A
Young
Old
Old + ALCAR
Old + LA
Old + ALCAR + LA
1/v
10
5
0
-10
0
10
20
30
40
1/[ALCAR, mM]


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

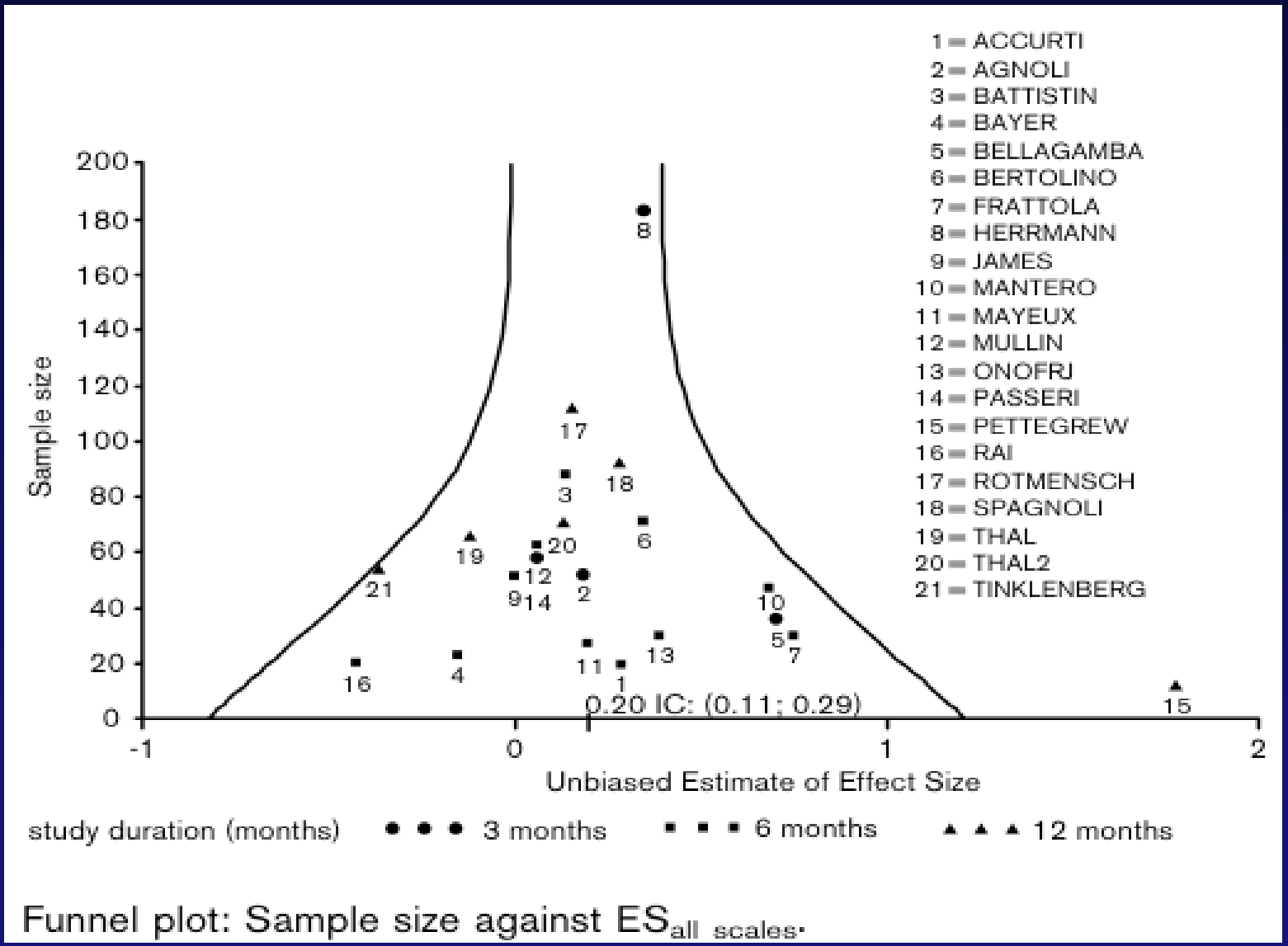

* = vs. young
# = vs. old
µM
150
100
50
0
**
#
#
**
#
Km for ALCAR
Km for CoA


A
1/v [mOD 340 /min]
0.8
0.6
0.4
0.2
0
0
0.005
0.01
MDA 0
MDA 12.5 µM
MDA 25 µM
MDA 50 µM
MDA 100 µM
B
1/v [mOD 340 /min]
0.125
0.1
0.075
0.05
0.025
0
-0.005
0
0.005
0.01
HNE 0
HNE 500 µM
HNE 1000 µM
HNE 2000 µM
C
1/v [mOD 340 /min]
0.15
0.1
0.05
0
-0.005
0
0.005
0.01
Control
MDA (50 µM)
MDA+ALCAR
MDA+L-carnitine
MDA+CoA
MDA+Acetyl-CoACoA
1/S [ALCAR uM]



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

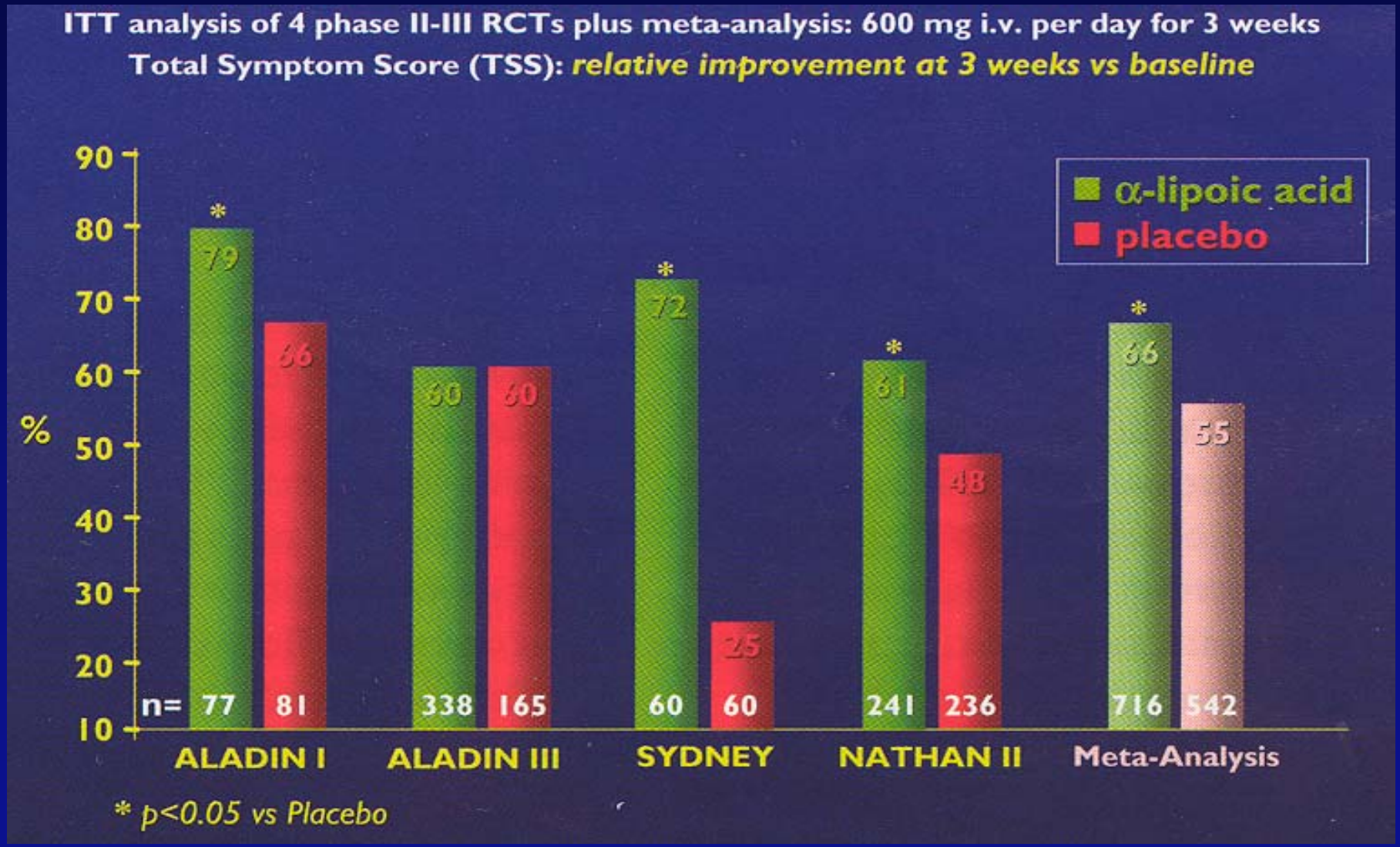
Meta-analysis of acetyl-L-carnitine versus placebo for mild cognitive impairment and mild Alzheimer's disease



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

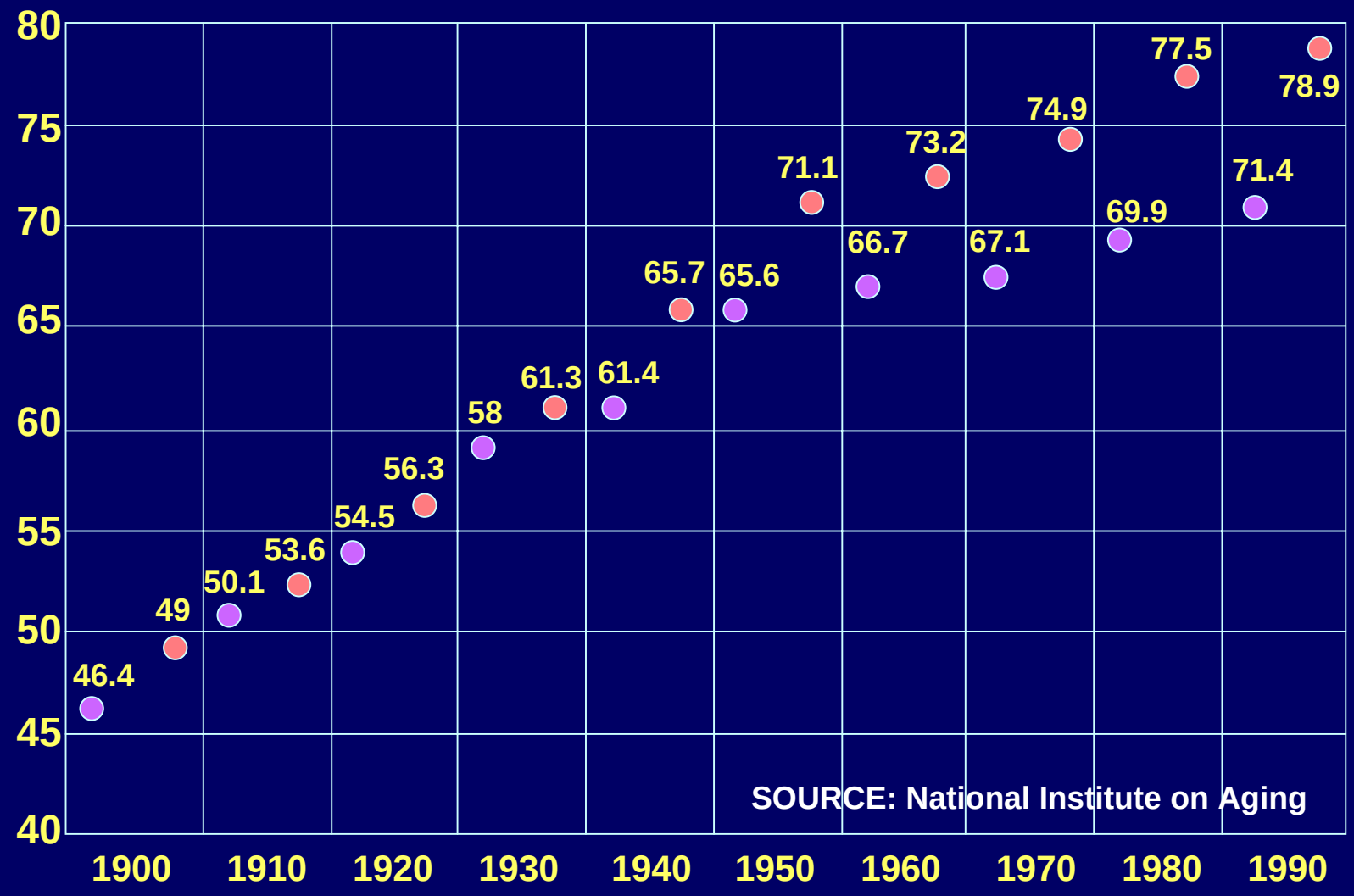
Montgomery, S.A., Thal, L.J., and Amrein, R., *Int. Clin. Psychopharmacol* 18:61-71 (2003)

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



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



ACKNOWLEDGEMENTS

Children's Hospital Oakland Research Institute
University of California at Berkeley
University of California at Irvine
Linus Pauling Institute, Oregon State University

Dr. Jiankang Liu
Dr. Tory Hagen
Dr. Afshin Gharib
Dr. David Killilea
Dr. Patrick Walter
Dr. Hani Atamna
Dr. Emily Ho
Dr. Elizabeth Head
Dr. Carl W. Cotman