New Insights into the Mechanism of Action of Antioxidants

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Dietary Antioxidant

A substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans.

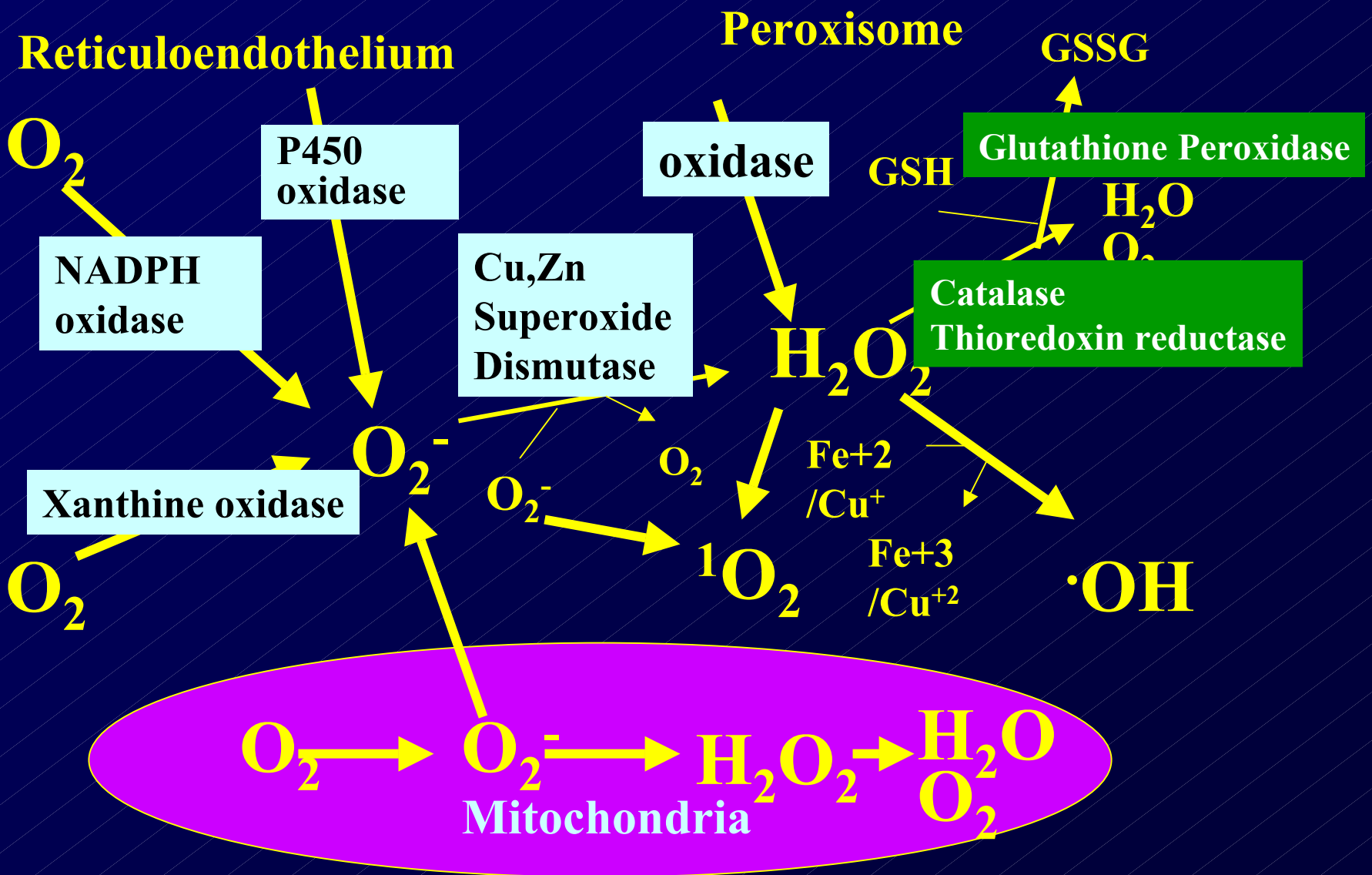
Dietary Reference Intakes, Foods and Nutrition Board
2000 Natl Acad Press

Reactive Species

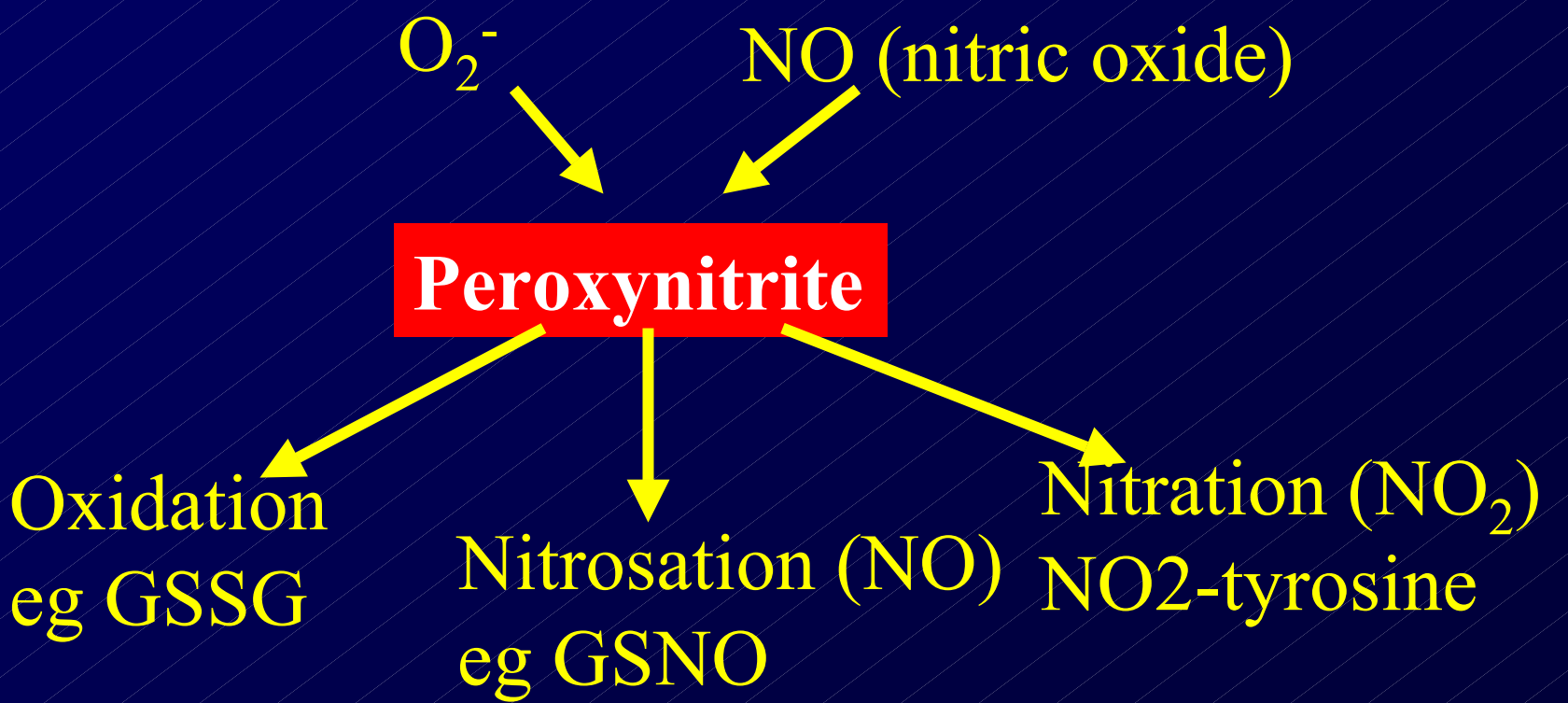
Includes:

- hydroxyl radicals ($^{\bullet}OH$)
- superoxide anions (O_2^-)
- singlet oxygen ($^1O_2$)
- hydrogen peroxides (H_2O_2)
- organic peroxides (R-OOH)
- nitric oxide
- peroxynitrite

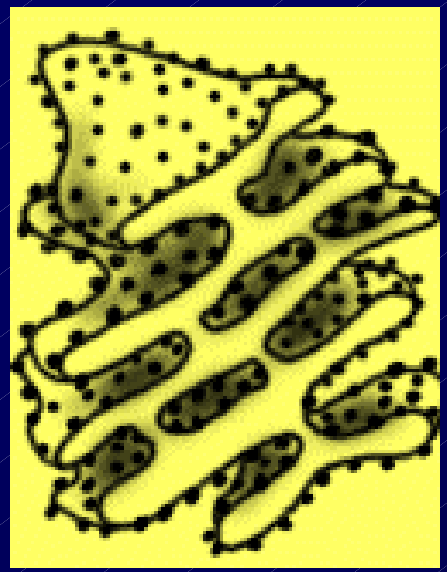
Generation of Reactive Oxygen Species



Nitric Oxide Dependent Reactions



Reactive Oxygen Species



Endoplasmic Reticulum

O_2 → H_2O_2 → •OH

Mitochondrion

- •Damage DNA, RNA
- •Oxidize Proteins (enzymes, histones)
- •Oxidize Lipids
- •Activate Cell Suicide

Diversity in Dietary Antioxidants

Essential

- Vitamin E (tocopherol)
- Vitamin C (ascorbic acid)
- Vitamin A (retinol and carotenoids)
- Numerous minerals- Cu, Mn, Zn, Se, Fe

Non-essential

- glutathione, small peptides
- host of phytochemicals (thousands in food supply)

In Vitro Measures of Antioxidant Capacity

- **ABTS Assay for Antioxidant Activity** (Miller et al. 1997).
- **Total Peroxyl Radical-trapping Potential (TRAP) Assay**. (Wilson (2001).
- **FOX3 (Lipid Peroxidation) Assay.** (Hermes-Lima et al. 1995)
- **Total Phenolics.** (Spanos and Wrolstad, 1990).
- **ORAC** (Ou et al, 2001)

Relative ORAC Efficacy (FL Units)

Compound	Units	Fluids	Units
Caffeic Acid	4.37	Serum	347
Quercetrin	7.28	Urine	1542
Genistein	5.93	Blueberry Juice	23748
Glutathione	0.62	Grape Juice	31441
Catechin	6.76	Raspberry Juice	54034
Vitamin C	0.95	Black Tea	17267

Ou et al, J Agric Food Chem 2001;49:4619-26

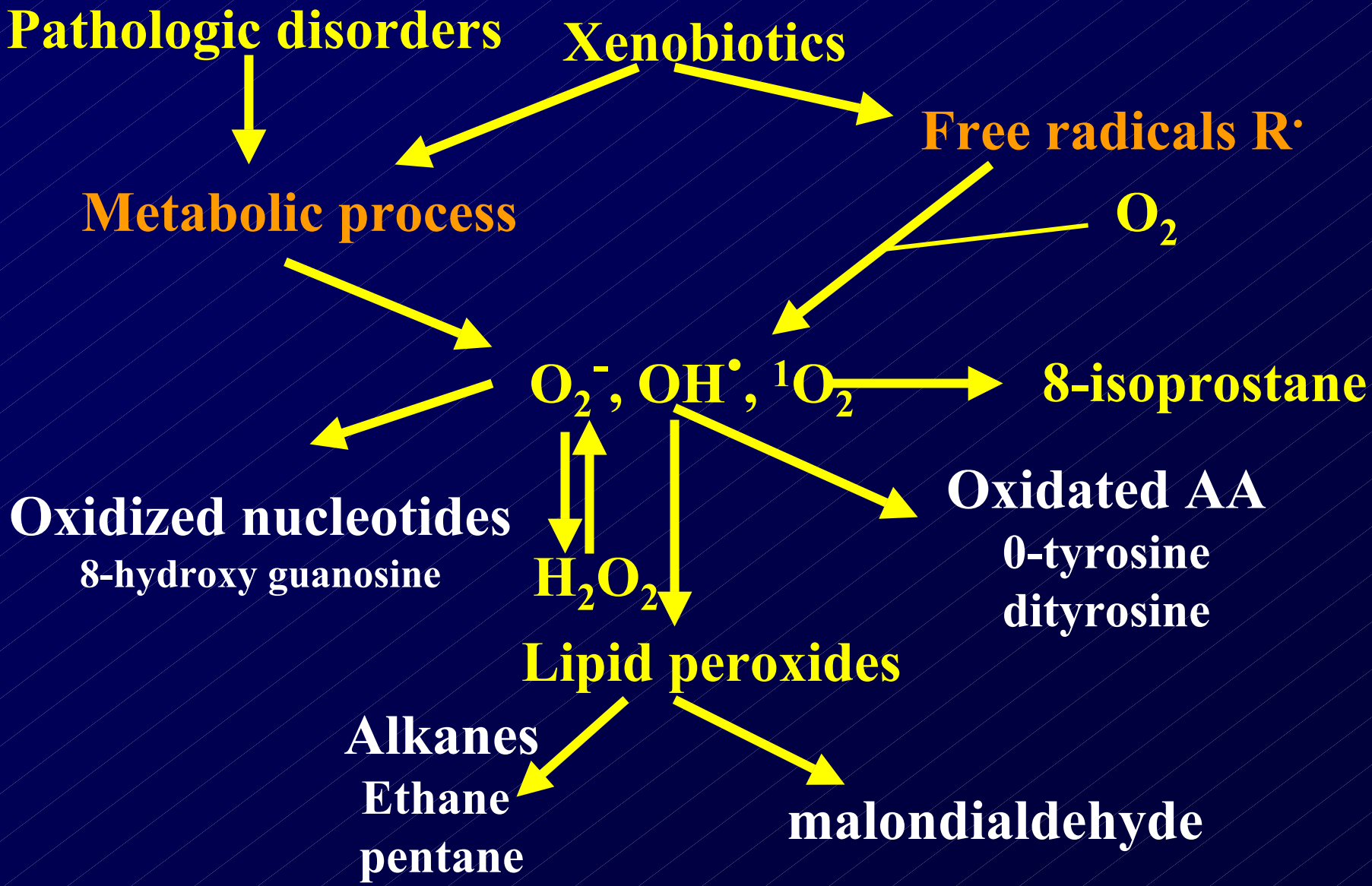
In Vitro Antioxidants Measurement Interpretation

- Different assays measure the effects of different radical species

- Not clear how to integrate test results for meaningful reflection of in vivo status

- Lack of correlation between assays and oxidative endpoints in vivo **(van den Berg et al J Nutr 2001 Jun;131(6):1714-22).**

Biomarkers


Pathologic disorders
Xenobiotics
Free radicals R·
Metabolic process
O_2
O_2^-, $OH^\bullet$, $^1O_2$
8-isoprostane
Oxidized nucleotides
8-hydroxy guanosine
Oxidated AA
0-tyrosine
dityrosine
H_2O_2
Lipid peroxides
Alkanes
Ethane
pentane
malondialdehyde

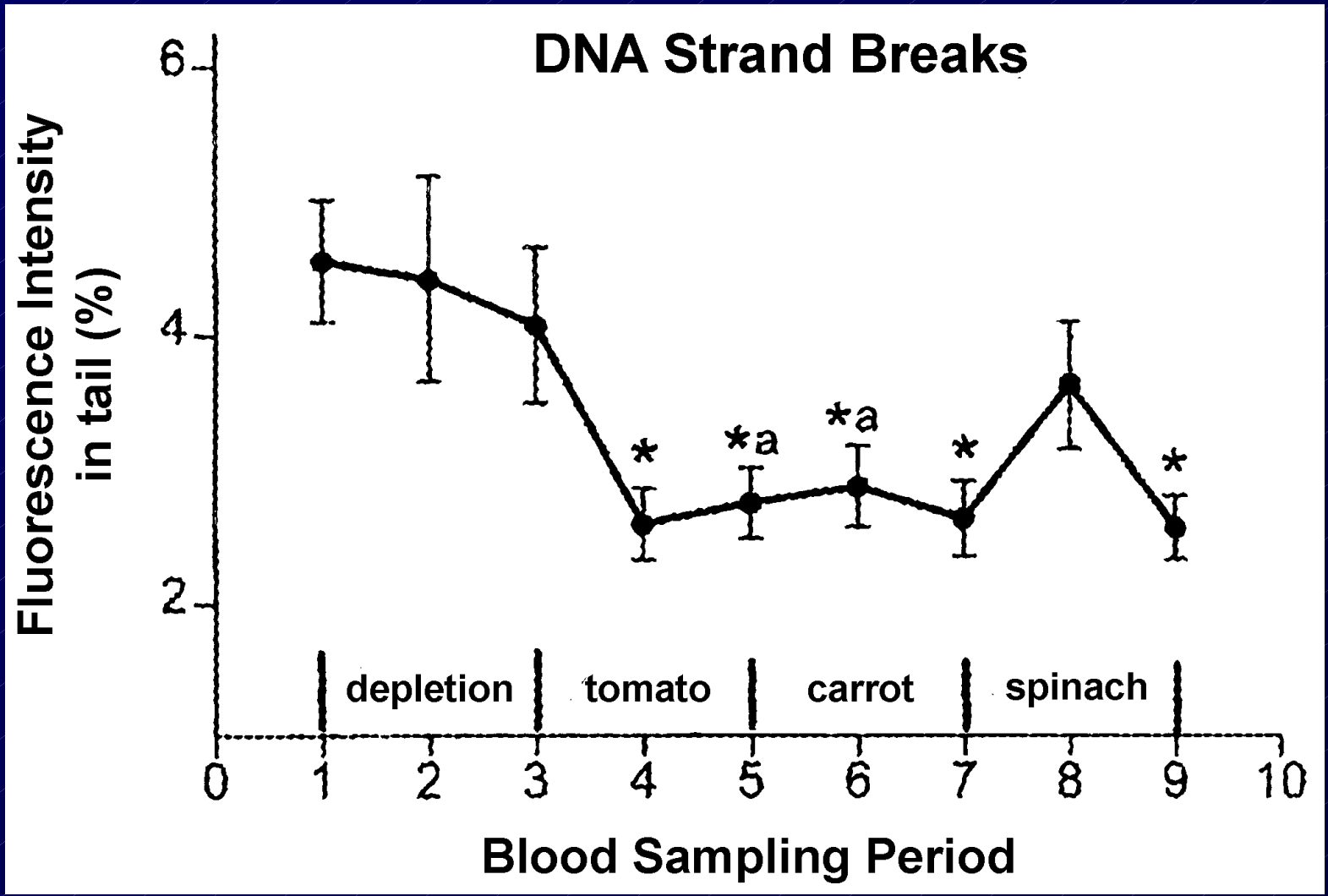
Low or High VF Diets and 8-OHdG in Lymphocyte DNA

Diet[1]	Pre-Intervention residues/ 10^6dG	Post-Intervention residues/ 10^6dG	% Change
Low VF[1]	10.1 ± 1.0 (8.4)	10.0 ± 0.9 (9.4)	-0.5
High VF	9.7± 1.2 (7.8)	8.1 ± 1.0* (5.2)	-16.5

5.8 servings vs 12 for 14 d. Urinary MDA minimal affected

Thompson et al. Carcinogenesis 1999;20:2261.

DNA Strand Breaks



Pool-Zobel et al. Carcinogenesis 1997;18:1847-50

Low or High VF Diets and Urinary 8-EPG

Diet[1]	Pre-Intervention	Post-Intervention	% Change
	ng/mg creatinine		
Low VF[1]	4.2 ± 0.3 (3.9)	3.7 ± 0.3 (3.81)	-10.9
High VF	3.7 ± 0.2 (3.3)	2.5 ± 0.1* (2.3)	-30.7

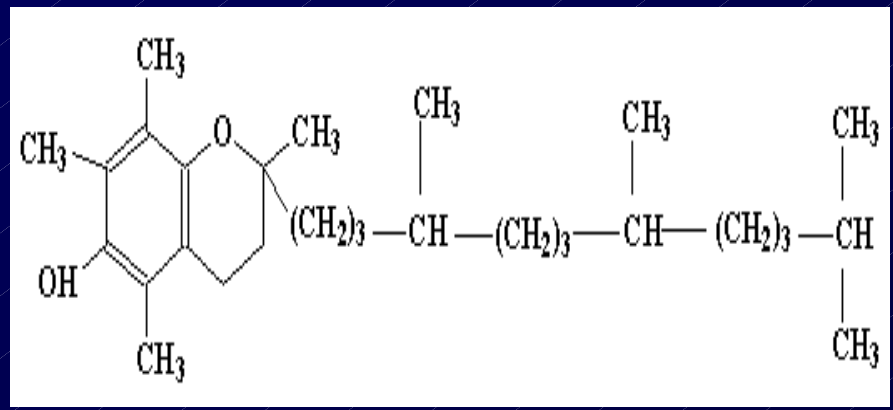
5.8 vs 12 servings.

Thompson et al. Carcinogenesis 1999;20:2261.

Vitamin E

O_2- → H_2O_2 → •OH

Mitochondrion



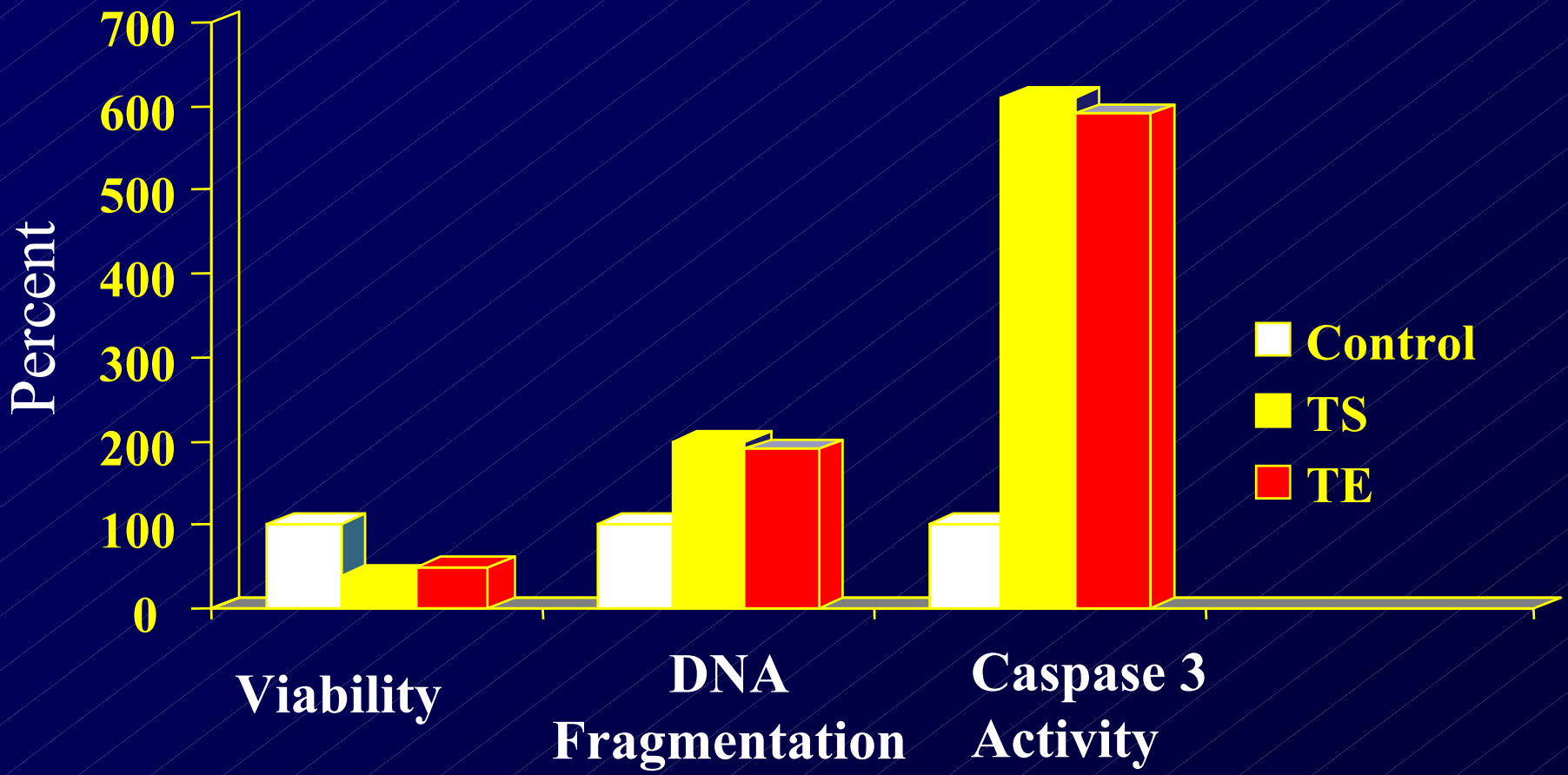
Vitamin E

- tocopherol a free radical (peroxyl) scavenger within membranes
  - -TH + LOO° → -T° +LOOH
  - -T° + ascorbate → -TH + ascorbate°

Effects of Dietary Supplements on Oxidative Damage Markers

- In several studies, vitamin E and diet supplement mixtures have been shown to favorably influence indicators of oxidative status, such as susceptibility of LDL to oxidation
- Randomized clinical trials have generally not confirmed a beneficial effect of supplements (e.g., vitamin E) on disease risk or outcome (Blumberg Nutr Clin Care 2002;5:50-5)

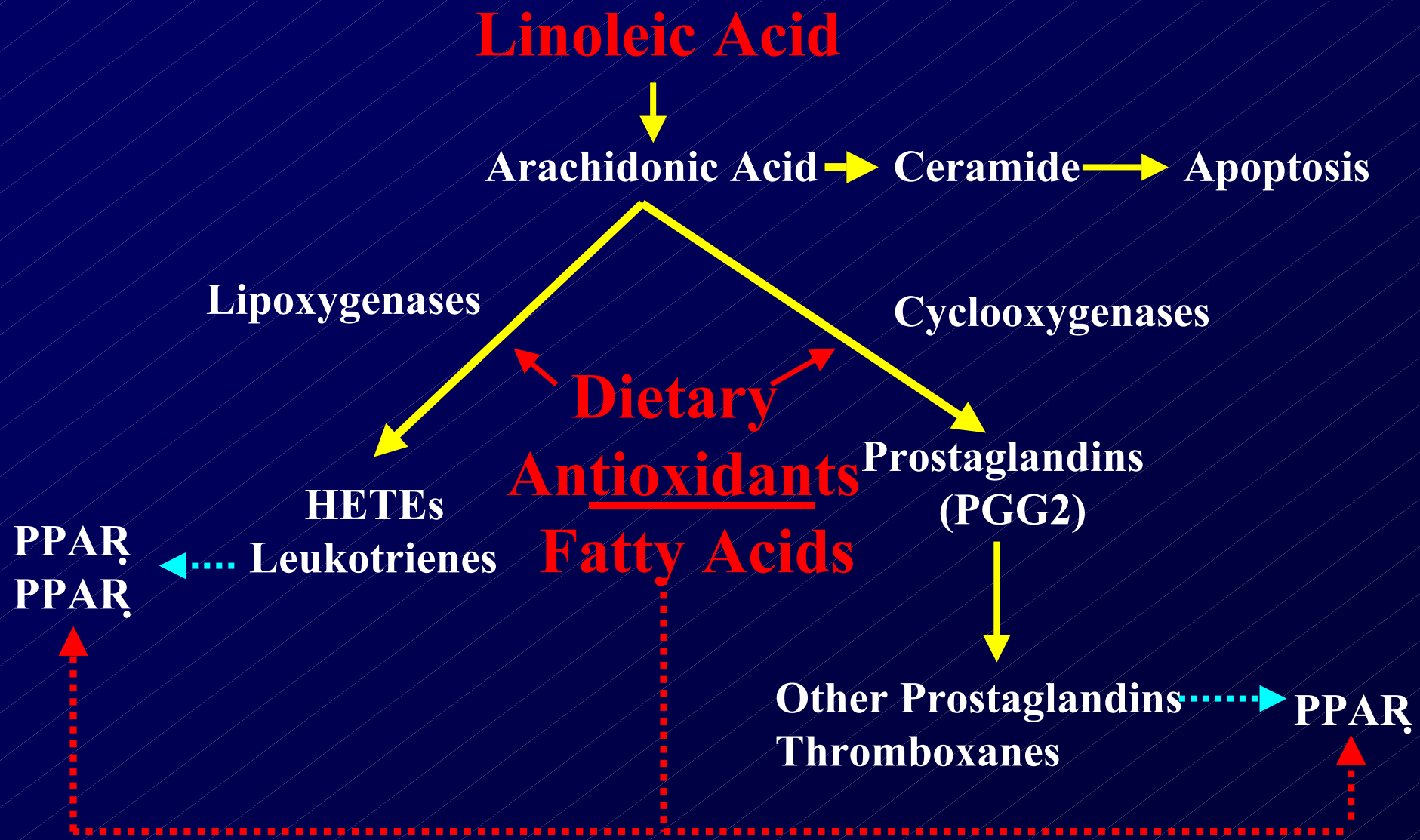
α-tocopheryloxybutyric Acid (TE) and Cell Apoptosis (% Control)



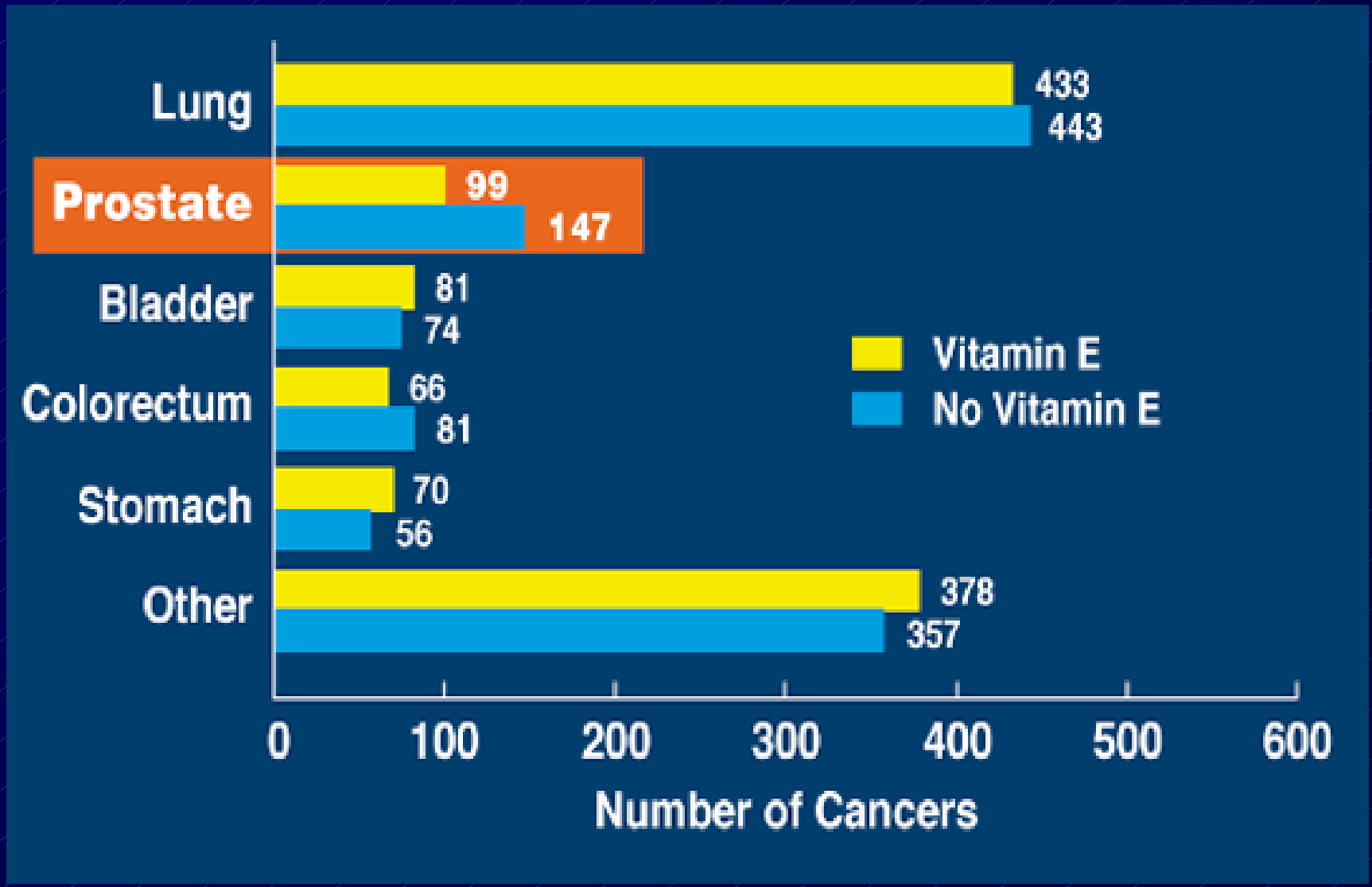
TE = modified vitamin E with no antioxidant properties

Akazawa 2002 Jpn J Pharm 89: 417.

Arachidonic Acid Metabolic Pathway



ATBC Study
Cancers According to Vitamin E Treatment



Prostate Cancer Prevention Selenium and Vitamin E Cancer Prevention Trail (SELECT) ($175M)

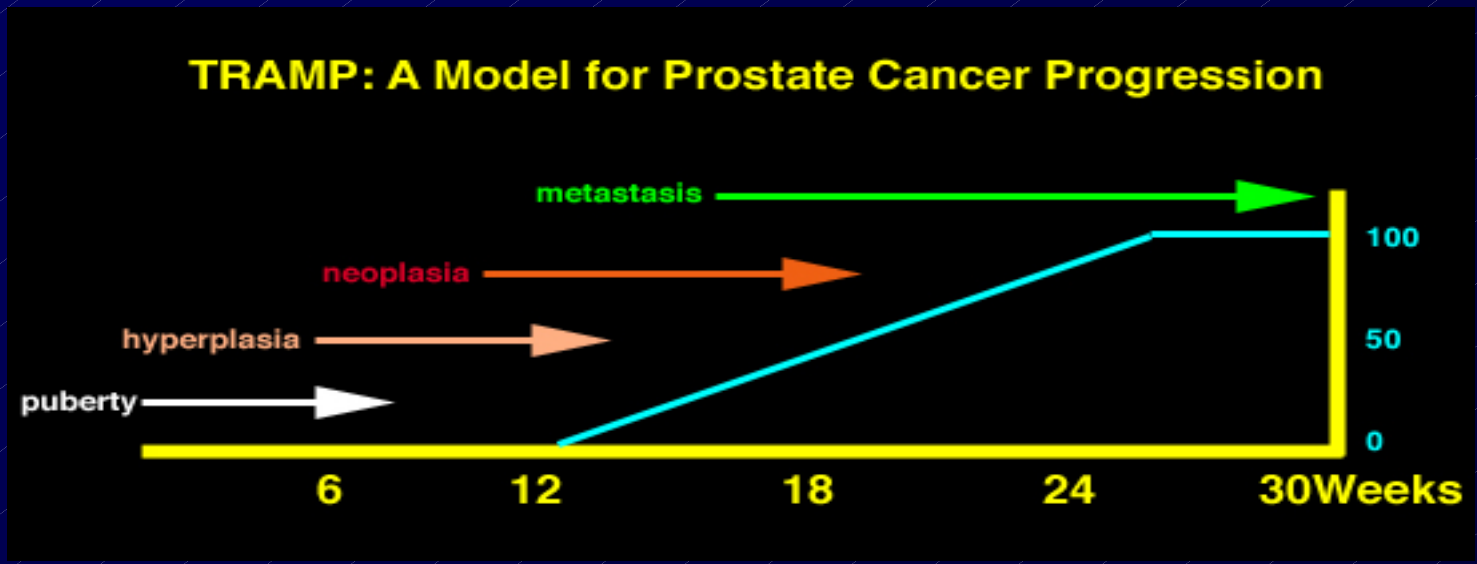
32,000 men, age ≥55 (≥ 50 for Black Men)

7 + Years Treatment
Endpoint Prostate Cancer Incidence

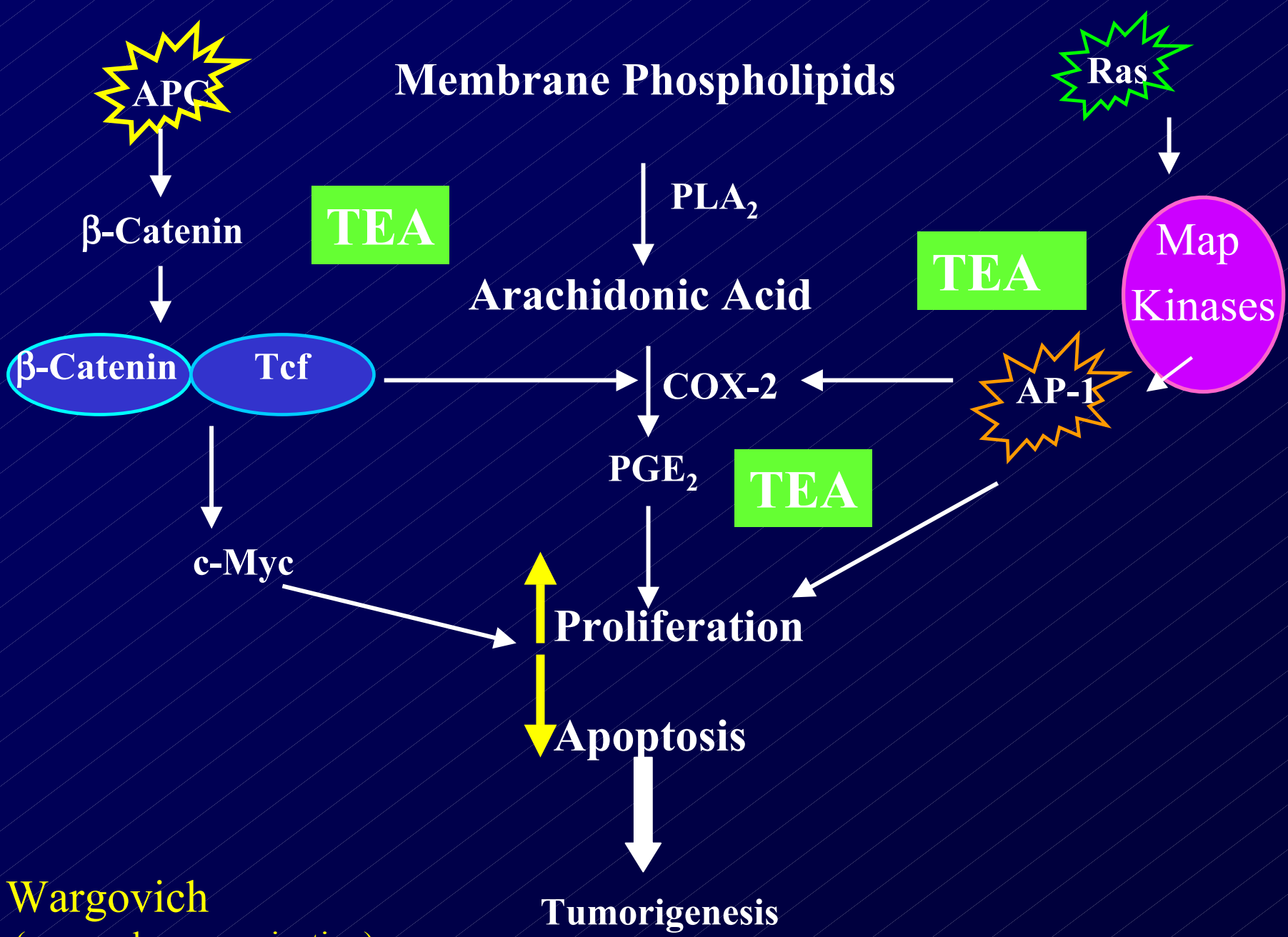
The TRAMP Mouse

- **Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) animal model that expresses the oncogene SV40 T antigen specifically in the epithelium of the prostate.**

- **Gupta et al. Proc Natl Acad Sci U S A 2001;98(18):10350-10355. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols.**



Greenberg et al. (Found on TRAMP webpage)



Wargovich
(personal communication)

Candidate Genes Responsive to (-) Epigallocatechin-3-Gallate in Human Prostate Cancer (LNCaP) Cells

GENES INDUCED

- Tyrosine receptor kinase type E mRNA
- Phosphoglycerate kinase
- Adenylate kinase 2A
- CDK8 protein kinase
- Putative serine/threonine protein kinase
- Ribosomal protein kinase B
- Mevalonate kinase
- Protein tyrosine phosphatase
- Prostatic acid phosphatase
- Receptor-type protein tyrosine phosphatase γ
- Protein tyrosine phosphatase lC
- STE-20 related kinase SPAK
- IAR/receptor-like protein tyrosine phosphatase
- Pyrroline 5-carboxylate synthase
- Glomerular epithelial protein 1
- Platelet-derived growth factor A type receptor

GENES REPRESSED

- Protein kinase C-α
- 41 kDa protein kinase related to rat ERK2
- Type 1b cGMP-dependent protein kinase
- Adenosine kinase short form
- Phosphatidylinositol 3-kinase homolog
- Protein tyrosine phosphatase PIR1
- Protein tyrosine phosphatase zeta
- KIAA0369 gene
- Leukocyte common antigen T2OO

Wang and Mukhtar Cancer Lett 2002;182:43

Protein Oxidation

- Proteins are major targets for ROS (Davies et al Free Rad Biol Med 1999)
  - Long half lives, a cumulative indicator
- Measurements:
  - Protein carbonyls: general marker of oxidation in vivo (Stadtman and Berlett Drug Metabol Rev, 1998)
  - Specific Protein Oxidation Products
    - Ease of oxidation of aromatic side-chains
      - Formation of o-Tyr, m-Tyr, di-tyrosine:
  - Protein inactivation (MnSOD)

Protein Oxidation

Amyloid beta-peptide (Abeta) is a 42-43 amino acid peptide known to accumulate in Alzheimer's disease (AD) brain. The neurotoxicity caused by Abeta is a result of its associated free radicals, which can play an important role in generating oxidative stress. Increased protein oxidation, reactive oxygen species (ROS) formation, and neurotoxicity induced by Abeta(1-42) in primary rat embryonic hippocampal neuronal culture are prevented by the free radical scavenger and antioxidant vitamin E.

Yatin et al. J Alzheimers Dis 2000;2:123

ROS and Apoptosis

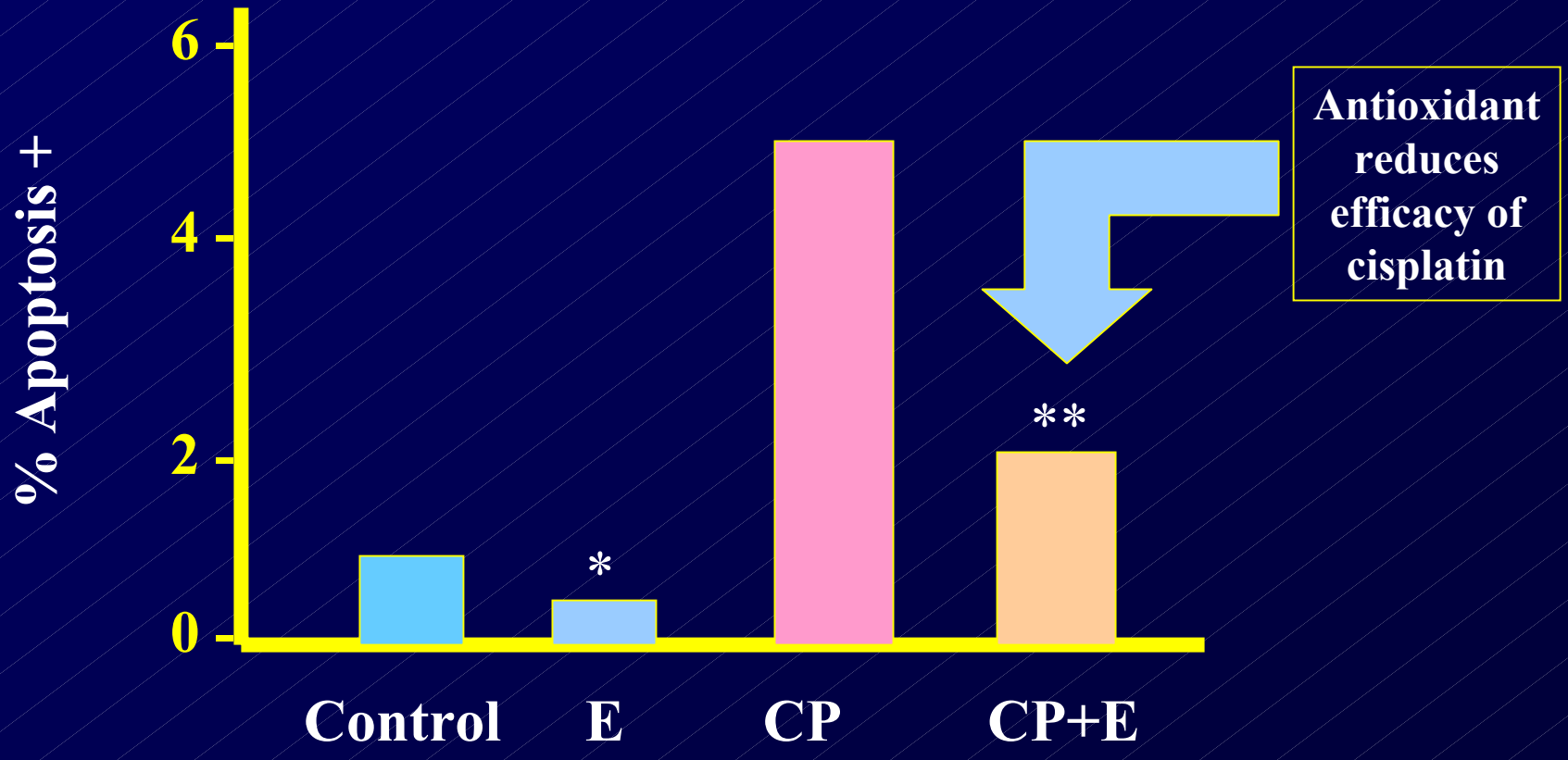
There is a growing body of evidence that ROS may not only regulate apoptotic signal transduction, but also activate apoptotic death pathways.

Slater et al. Biochem. Biophys. Acta 1271:59, 1995.

Johnson et al. Proc. Natl. Acad. Sci. 93: 11848, 1996.

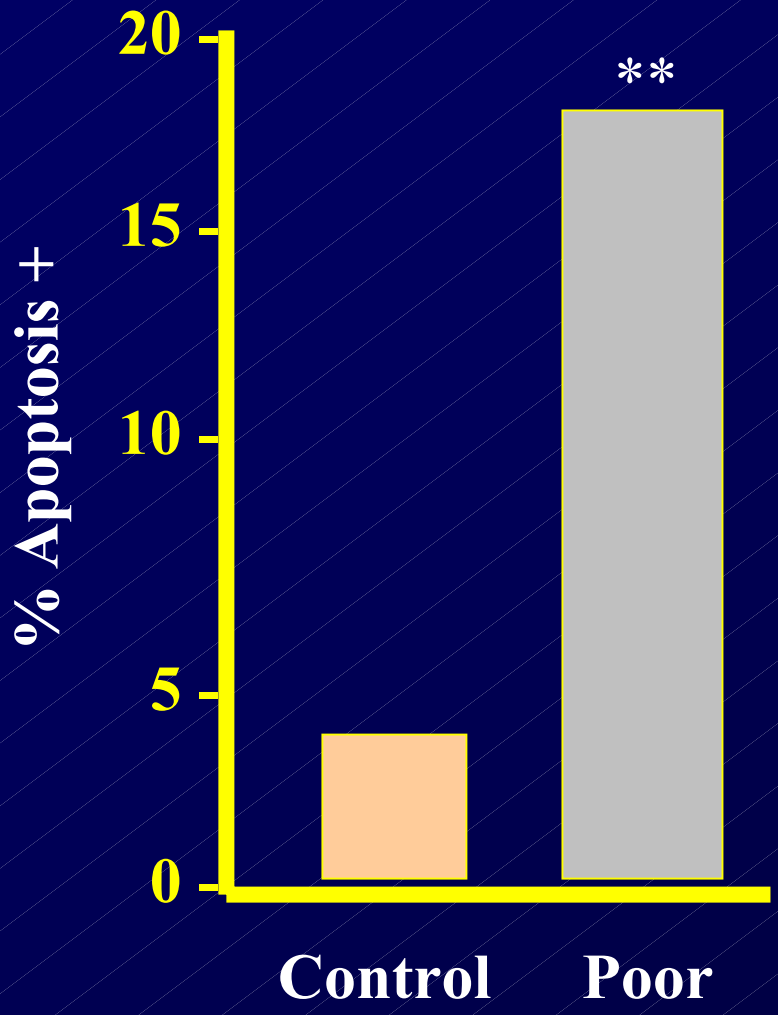
Jabs, T. Biochem. Pharmacol. 57:231, 1998.

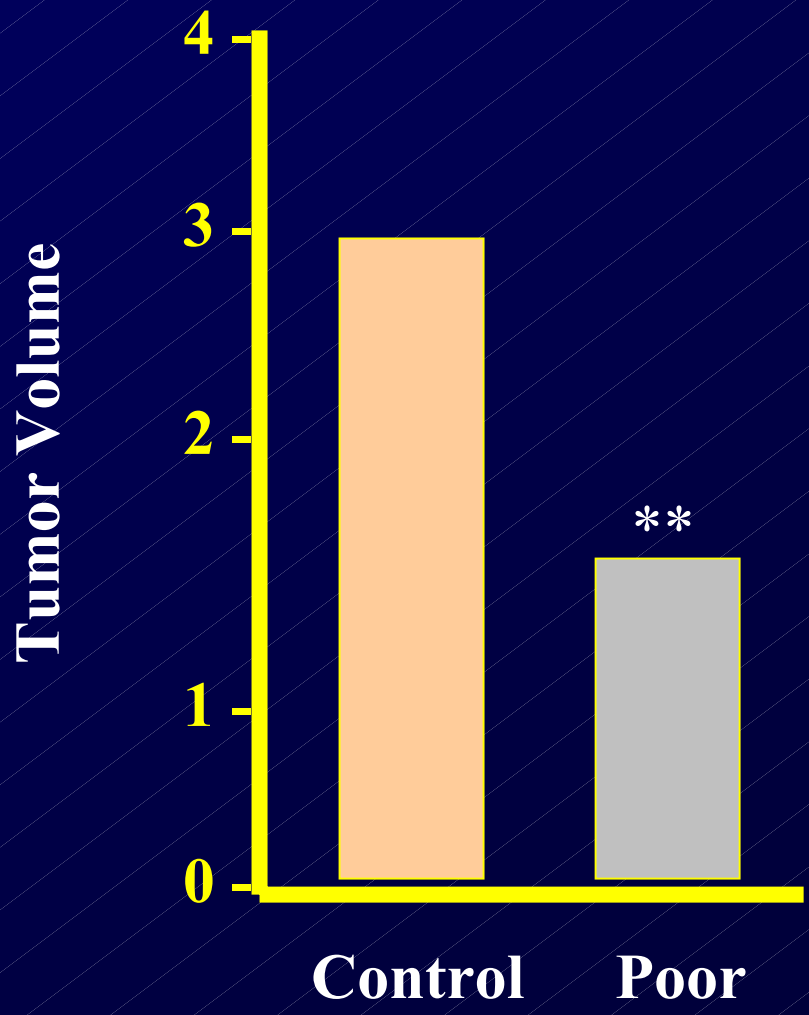
Vitamin E Inhibits Cisplatin-Induced Apoptosis in MCF-7 Breast Cancer Cells.



Salganik, et al. Carcinogenesis 21: 909, 2000.

Antioxidant Depletion Inhibits Brain Tumor Growth.





Salganik, et al. Carcinogenesis 21: 909, 2000.

One Size Does Not Fit All!


Just when I knew all of life's answers,
they changed all the questions.