FLAVONOIDS AS DIETARY ANTIOXIDANTS: WHAT ARE THEY? DO WE NEED THEM?

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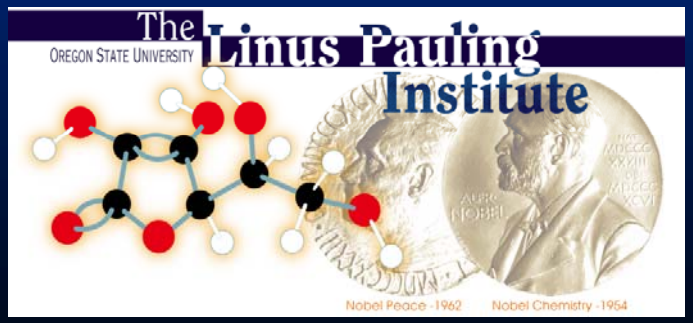
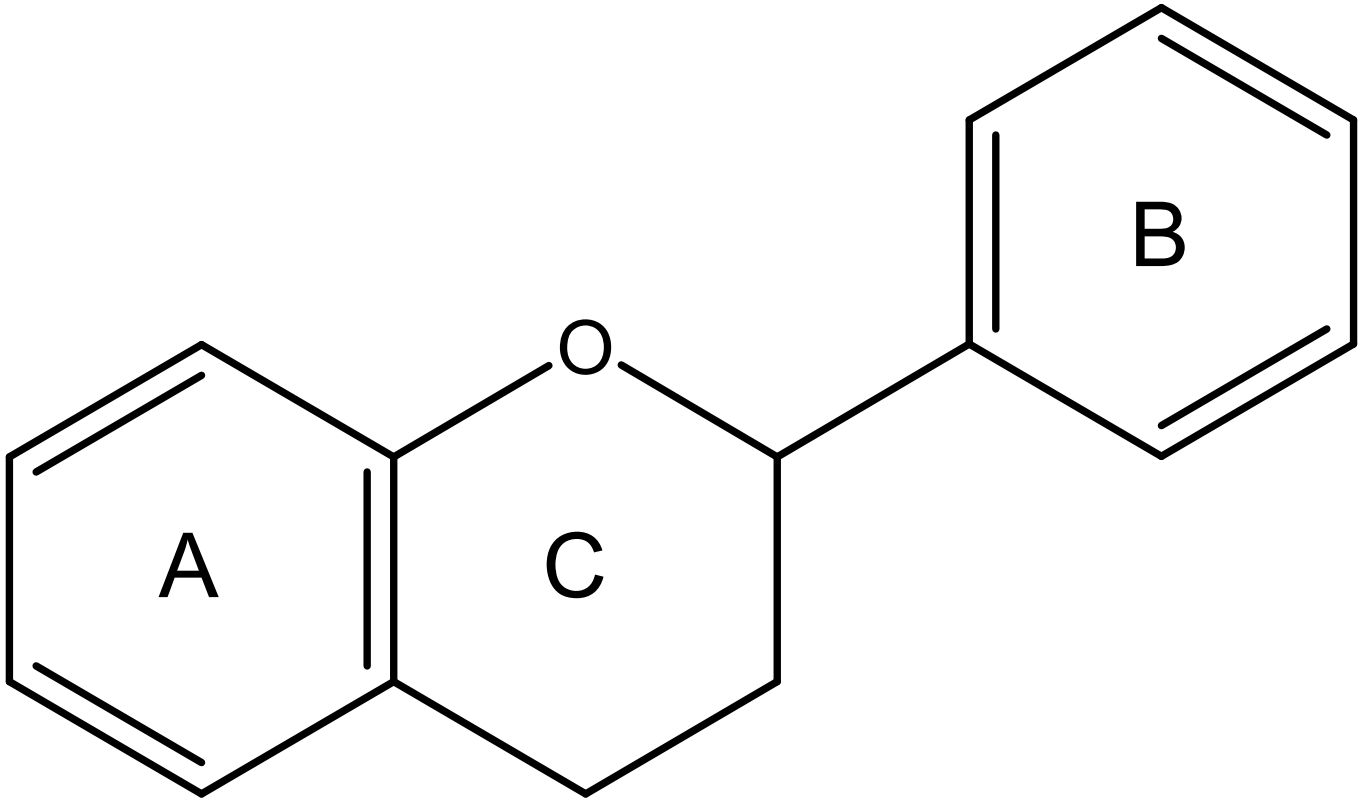


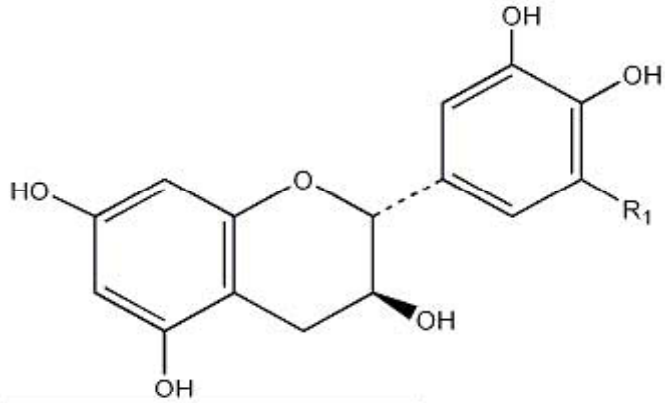
Flavonoids

- Flavonoids are a large family of polyphenolic compounds synthesized by plants that have a common chemical structure.
- Flavonoids may be further divided into subclasses:
  - Anthocyanidins
  - Flavan-3-ols
  - Flavanones
  - Flavonols
  - Flavones
  - Isoflavones

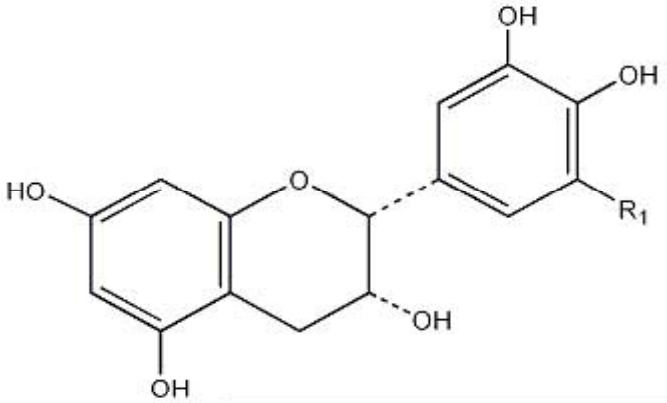
Basic Chemical Structure of a Flavonoid



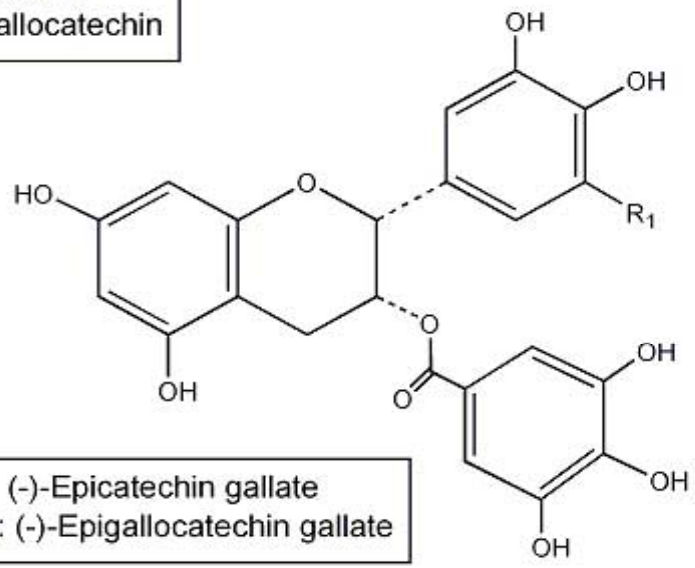
Catechins (Flavan-3-ol Monomers)



R_1 = H: (+)-Catechin
R_1 = OH: (+)-Gallocatechin



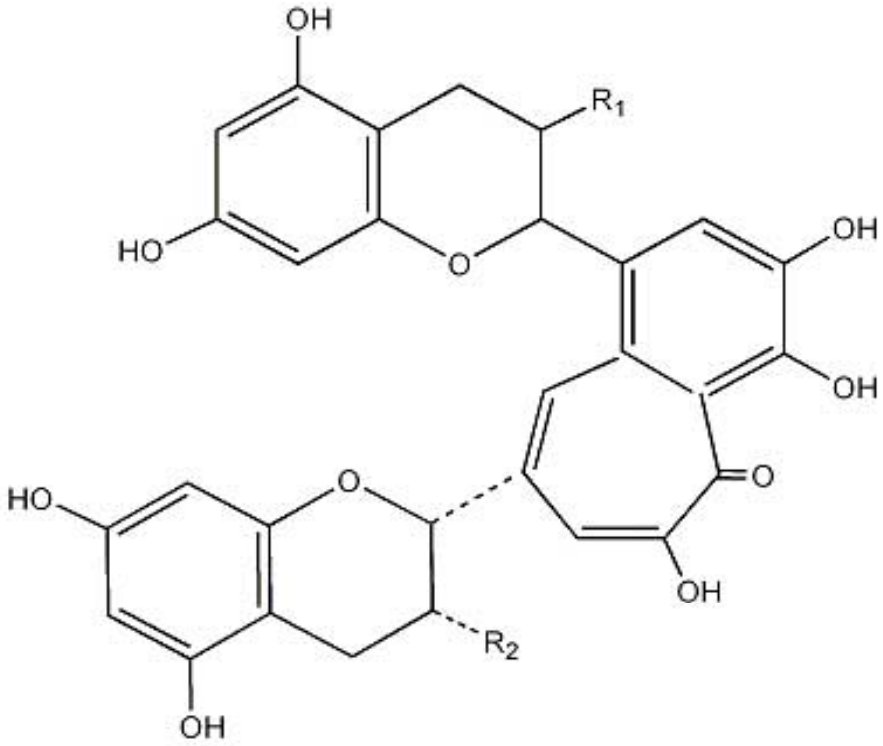
R_1 = H: (-)-Epicatechin
R_1 = OH: (-)-Epigallocatechin



R_1 = H: (-)-Epicatechin gallate
R_1 = OH: (-)-Epigallocatechin gallate

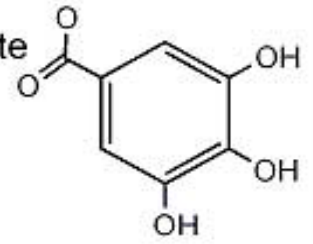
Common Food Sources: Teas (particularly green and white), chocolate, grapes, berries, apples

Theaflavins (Flavan-3-ol Dimers)



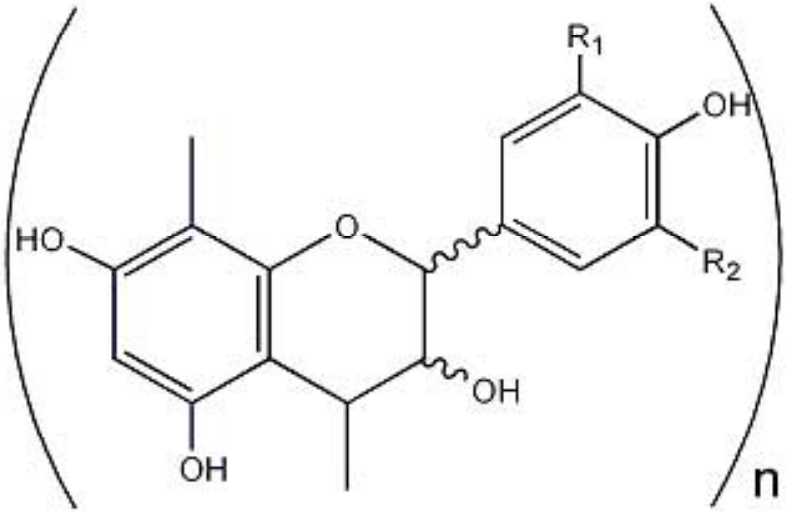
R_1 = OH; R_2 = OH: Theaflavin
R_1 = OH; R_2 = *Gallate: Theaflavin 3-gallate
R_1 = Gallate; R_2 =OH: Theaflavin 3'-gallate
R_1 = Gallate; R_2 = *Gallate: Theaflavin 3,3'-digallate

*Gallate

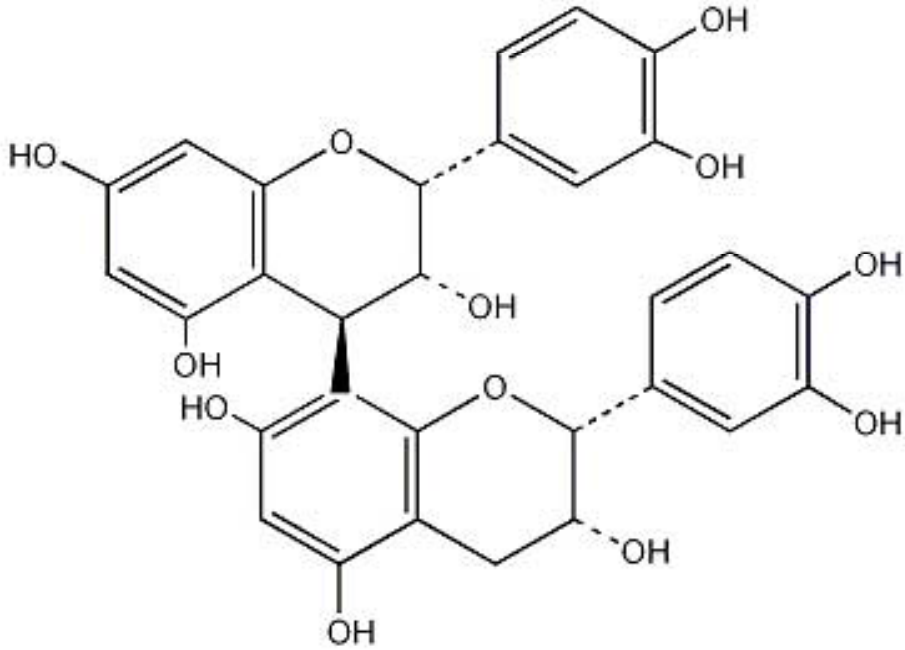


Common Food Sources: Black tea, Oolong teas

Proanthocyanidins (Flavan-3-ol Dimers and Polymers)



R_1 = H; R_2 = OH: Procyanidins
R_1 = OH; R_2 = OH: Prodelphinidins
R_1 = H; R_2 = H: Propelargonidins

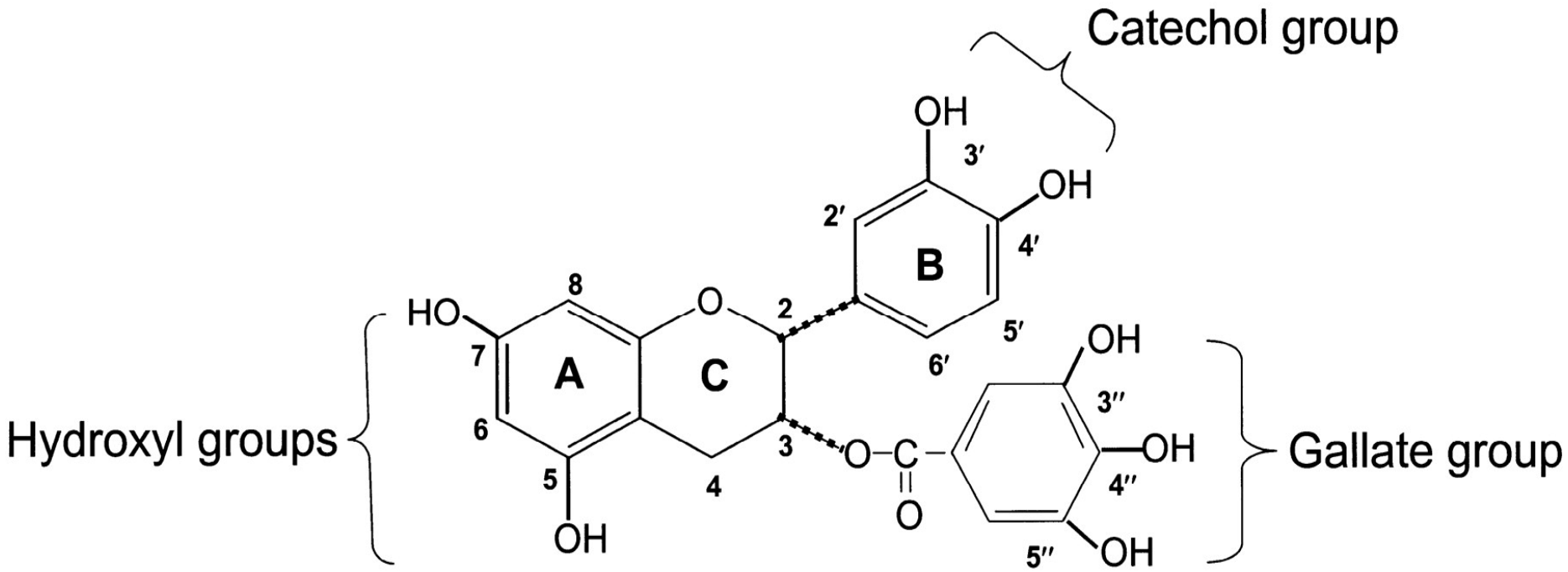


Procyanidin Dimer

Common Food Sources: Chocolate, apples, berries, red grapes, red wine

Functional groups important for the antioxidant activity of flavan-3-ols (epicatechin gallate)

JN THE JOURNAL OF NUTRITION



Frei, B. et al. J. Nutr. 2003;133:3275S-3284S

(Total) Antioxidant Capacity Assays

- *Pure Compounds (e.g., Flavan-3-ols)*
- *Plant Extracts, Beverages (e.g., Tea)*
- *Fruit or Vegetable Extracts*
- *Human Plasma*

Popular Antioxidant Capacity Assays

TEAC: Trolox-Equivalent Antioxidant Capacity

Reduction of $ABTS^{\bullet+}$ (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) (loss of A_{734})

FRAP: Ferric-Reducing Antioxidant Potential

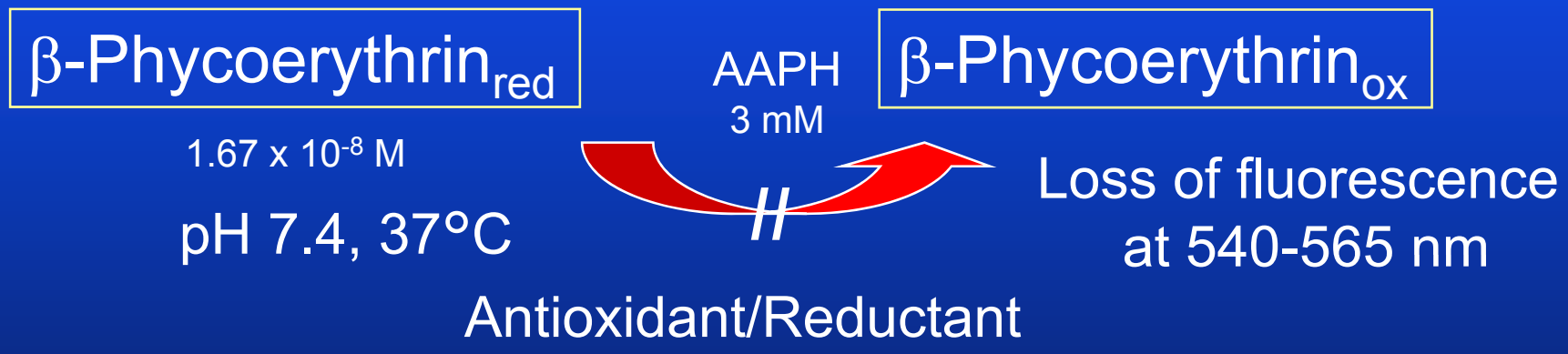
Reduction of Fe^{3+} tripyridyltriazine complex (increase in A_{593})

ORAC: Oxygen-Radical Absorbance Capacity

Inhibition of AAPH-induced oxidation of β-phycoerythrin (loss of fluorescence at 540-565 nm)

Measurement of Antioxidant Capacity

Oxygen Radical Absorbance Capacity (ORAC)



Cao et al. (1993) *Free Radic. Biol. Med*. 14, 303-311

Measurement of Antioxidant Capacity

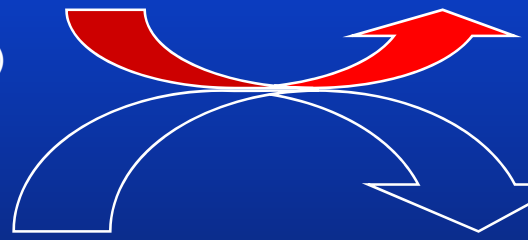
Ferric Reducing Antioxidant Potential (FRAP)

Fe^{3+} - TPTZ

20 mM

10 mM
(in 40 mM HCl)

pH 3.6, 37°C



Fe^{2+} - TPTZ

Absorbance at 593 nm

Antioxidant/Reductant

Oxidized Antioxidant/Oxidant

Benzie & Strain (1996) *Anal. Biochem*. 239, 70-76

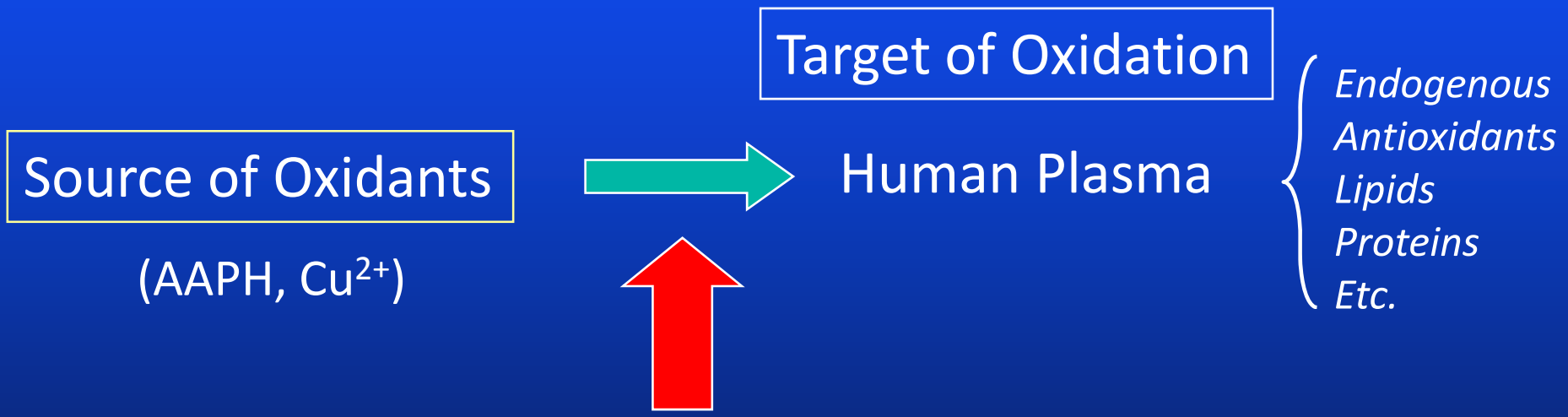
Antioxidant Capacity and Reduction Potentials of Tea Catechins and Antioxidant Vitamins

Antioxidant	TEAC[a]	Reduction potential (mV)[b]
(-)-Epicatechin	2.4 ± 0.02	570
(-)-Epigallocatechin	3.8 ± 0.06	430
(-)-Epicatechin gallate	4.9 ± 0.02	550
(-)-Epigallocatechin gallate	4.8 ± 0.06	430
Theaflavin	2.9 ± 0.08	510
Theaflavin digallate	6.2 ± 0.43	540
Green tea (1,000 ppm)	3.8 ± 0.03	n/a
Black tea (1,000 ppm)	3.5 ± 0.03	n/a
Vitamin E	1.0 ± 0.03	480
Vitamin C	1.0 ± 0.02	280/-175

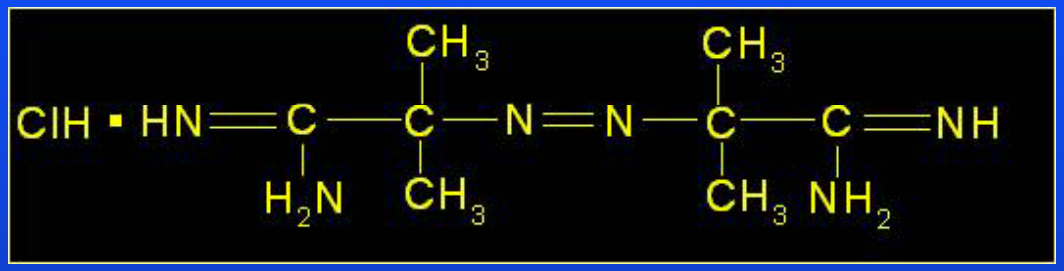
[a] Trolox-Equivalent Antioxidant Capacity
[b] Reduction potential at pH 7, 20° C.

Frei, B. et al. J. Nutr. 2003;133:3275S-3284S

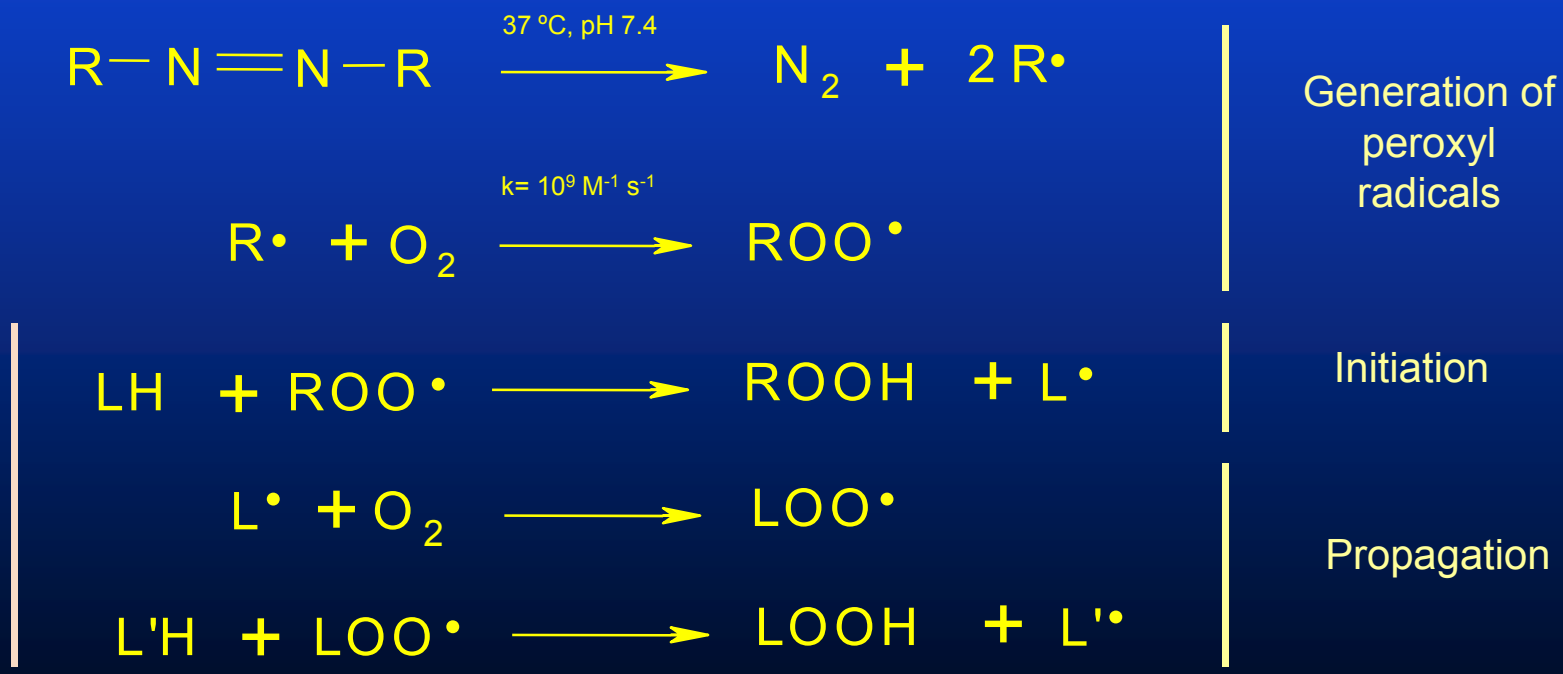
Resistance of Plasma to Oxidation



AAPH-induced Lipid Peroxidation in Plasma



AAPH
2,2'-Azobis(2-Amidino)Propane
Hydrochloride



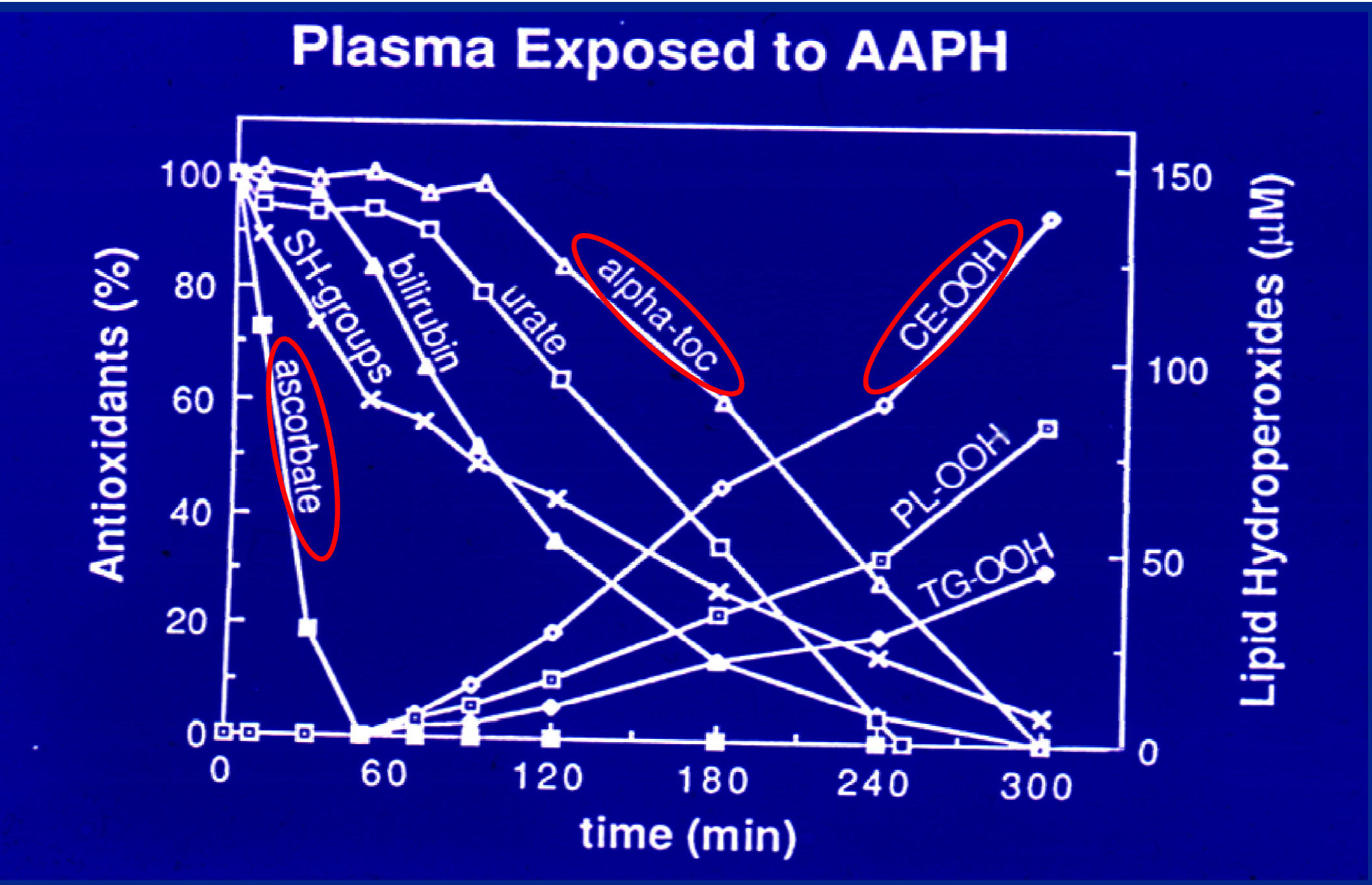
ANTIOXIDANT DEFENSES IN HUMAN PLASMA AND LDL

Antioxidant Proteins

- Fe- and Cu-Binding Proteins (Albumin, Transferrin, Ceruloplasmin, etc.), but basically no antioxidant enzymes (SOD, catalase, etc.)

Small Molecule Antioxidants

	Typical Plasma Concentrations	
• Water-Soluble:	μM	
Uric Acid	300	
Ascorbic Acid (Vitamin C)	50	
Albumin-Bound Bilirubin	15	
Glutathione (GSH)	< 2	
• Lipid-Soluble (Lipoprotein-Associated):		mol/mol LDL
α–Tocopherol (Vitamin E)	25	10
Ubiquinol-10 (Coenzyme Q_{10})	1.0	0.4
β–Carotene (Pro-Vitamin A)	0.5	0.2
Lycopene	0.5	0.2

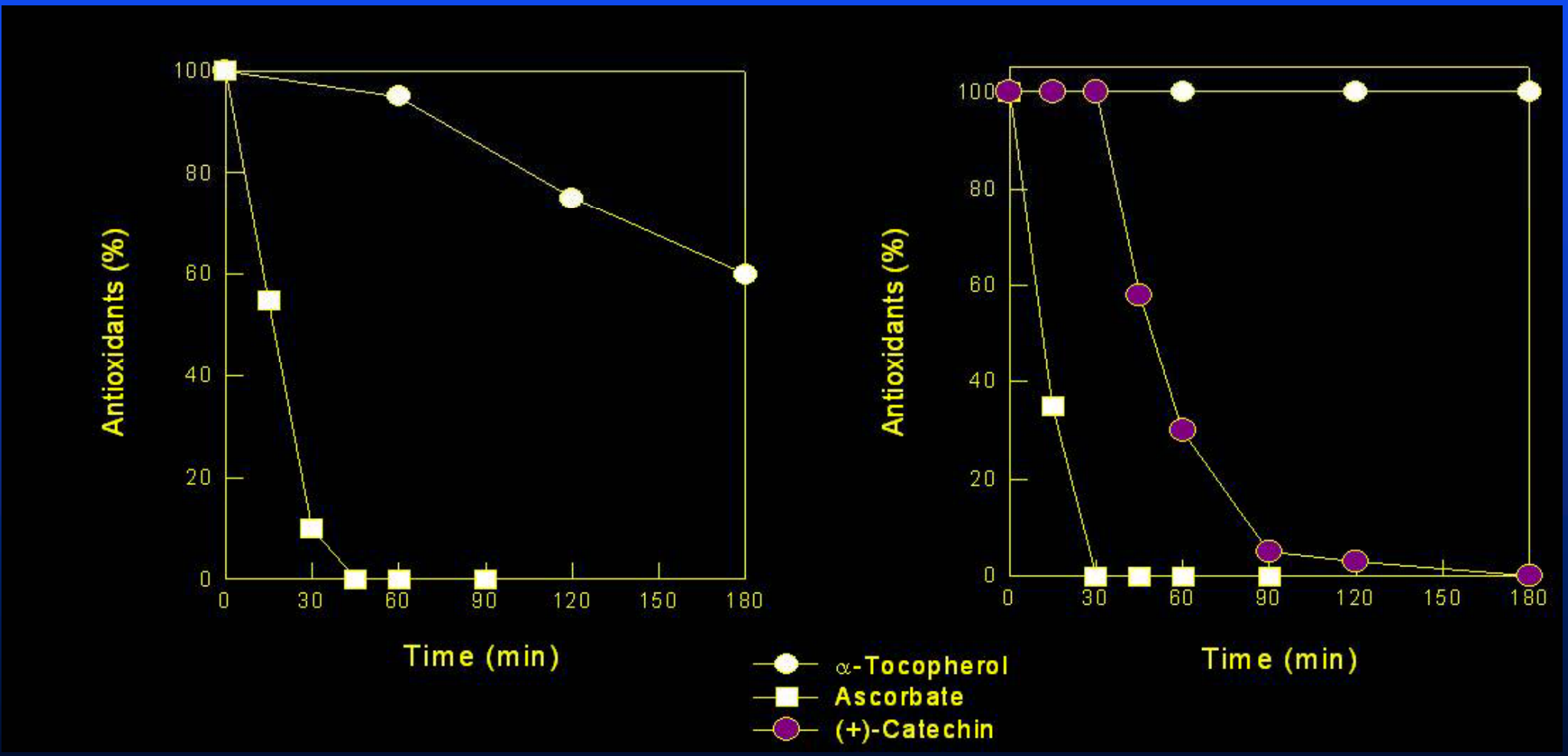


Frei et al. *Proc Natl Acad Sci* 85: 9748-9752; 1988

Effect of (+)-catechin addition on oxidation of ascorbate and α-tocopherol in human plasma exposed to AAPH

Without added (+)-Catechin (Control)

With added (+)-Catechin (100 μM)



Lotito & Fraga (1999) *Biofactors* **10**:125-30

Black Tea Flavonoids (BTF) Lower the Rate of Lipid Peroxidation, but not Ascorbate Oxidation, in Human Plasma *In Vitro*

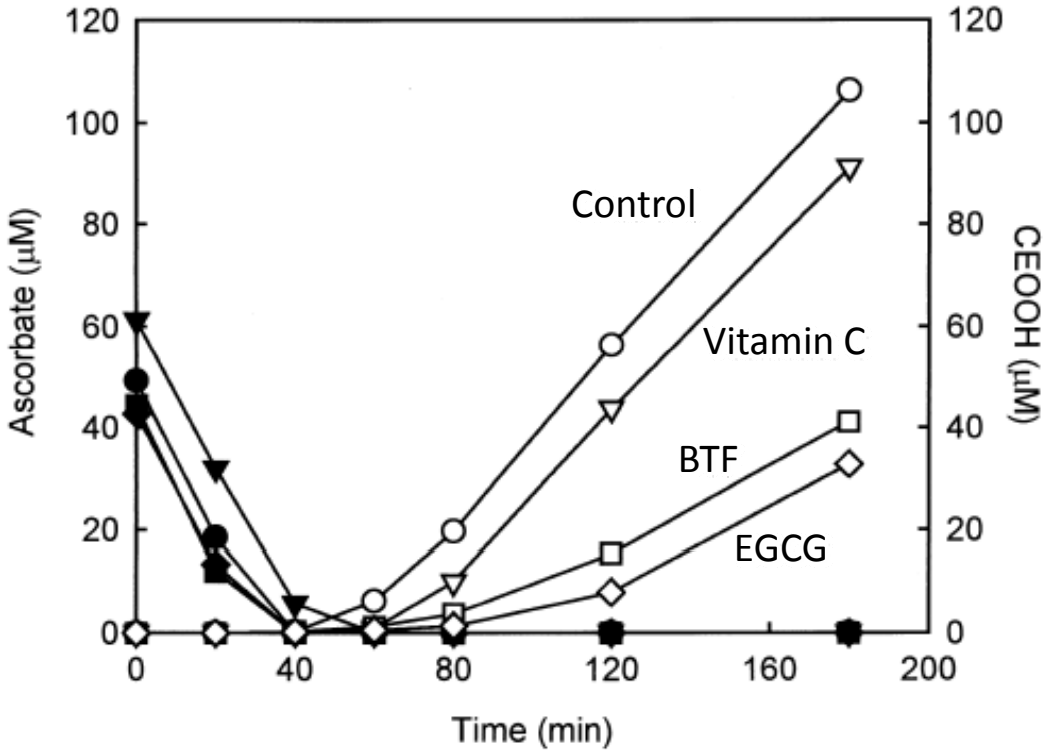


Fig. 1. Vitamin C (ascorbate) consumption and cholesteryl ester hydroperoxide (CEOOH) formation in human plasma incubated at 37°C with 50 mM AAPH in the absence (●, ○) or presence of 25 μM black tea polyphenols (■, □), epigallocatechin gallate (◆, ◇), or ascorbate (▼, ▽). Filled symbols indicate ascorbate measurements, and open symbols CEOOH. One experiment representative of two is shown.

Cherubini, A.; Beal, M. F.; Frei, B. *Free Radic. Biol. Med.* **27**:381-7; 1999

Black Tea Flavonoids (BTF) Dose-dependently Inhibit Lipid Peroxidation in Human Plasma *In Vitro*

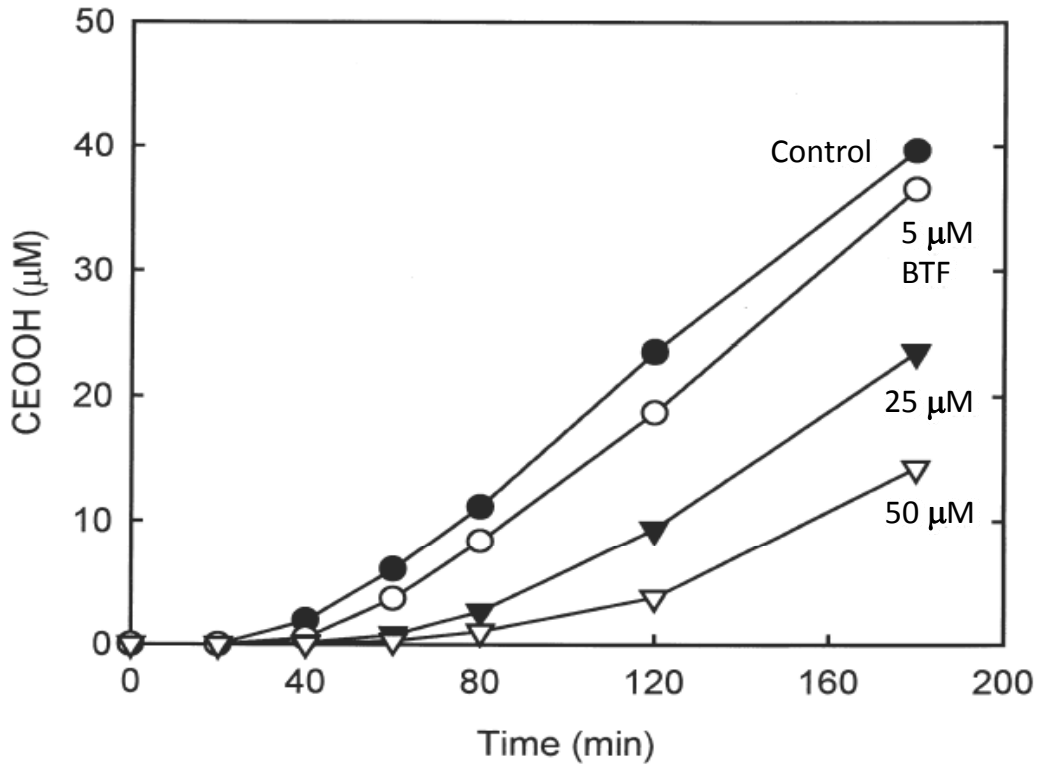


Fig. 2. Cholesteryl ester hydroperoxide (CEOOH) formation in human plasma incubated at 37°C with 50 mM AAPH in the absence (●) or presence of 5 μM (○), 25 μM (▼), and 50 μM (▽) black tea polyphenols. Results shown are the mean of four experiments. Standard deviations were <22% for each data point and are omitted for the sake of clarity.

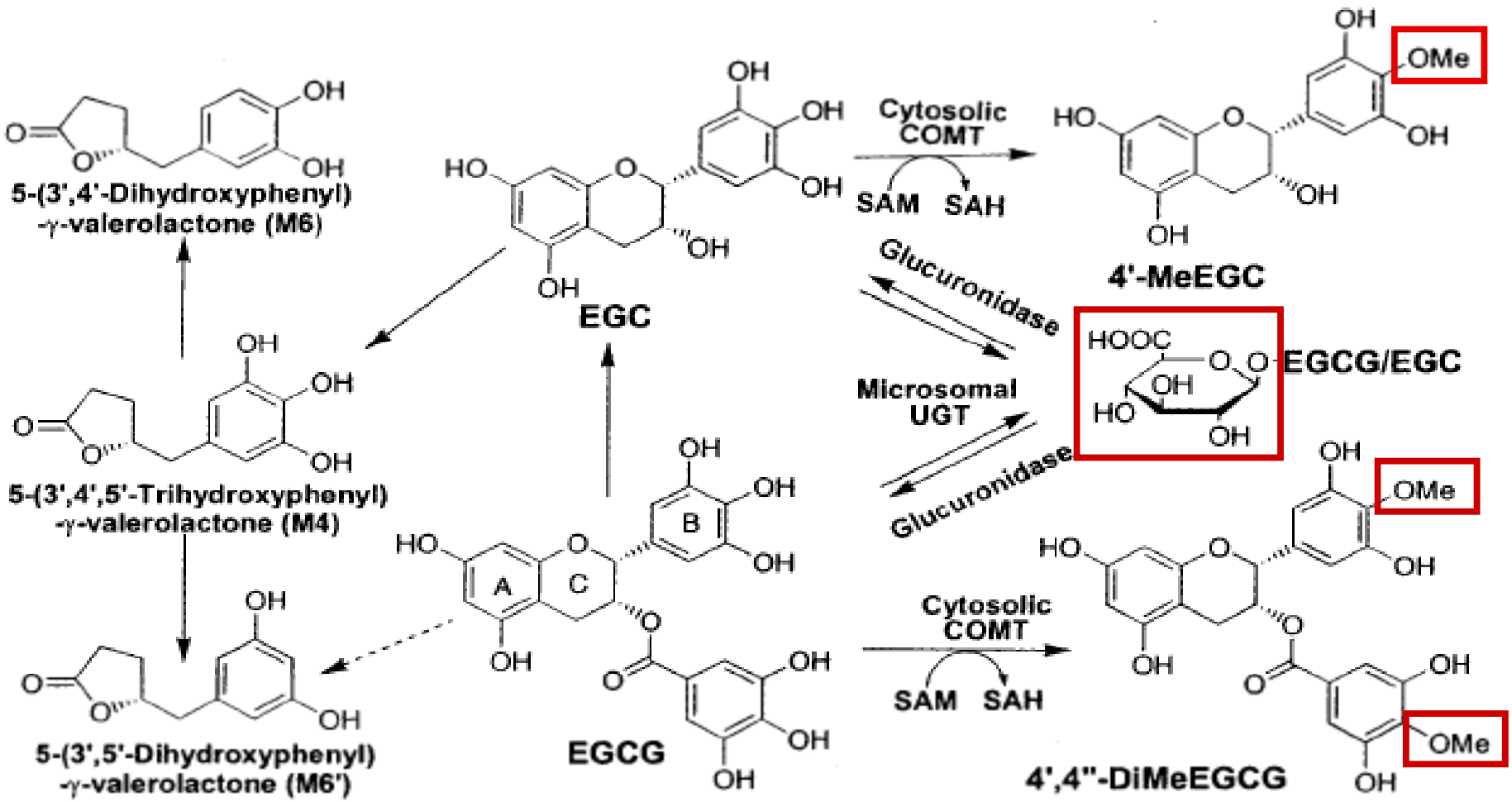
Cherubini, A.; Beal, M. F.; Frei, B. *Free Radic. Biol. Med.* **27**:381-7; 1999

Important Considerations for *In Vivo* Studies

- Low absorption of flavonoids in humans
- Extensive phase II metabolism (glucuronidation, sulfation, methylation) and rapid excretion from the body

Flavonoid metabolites may have lower antioxidant capacity than their parent compounds found in foods and are present in human plasma and tissues at (very) low concentrations!

Metabolic Fate of EGCG



UGT: UDP-glucuronosyltransferase; COMT: catechol-*O*-methyltransferase; SAM: S-adenosyl-L-methionine; SAH: S-adenosyl-L-homocysteine.

Meng et al., (2002) Chem Res Toxicol. 15:1042-50.

Maximal flavonoid concentrations in human plasma after consumption of flavonoid-rich foods are in the low μM range

Subclass	Flavonoids	Maximal Flavonoid Levels in Human Plasma (μM)
Flavonols	Quercetin Myricetin Kaempferol	0.65 (fried onions) 0.74-7.60 (onions) 0.30 (apples)
Flavan-3-ols	(-)-Epicatechin (+)-Catechin (-)-Epigallocatechin gallate	1.00-1.80 (green tea) 0.09-0.34 (black tea) 0.08-0.09 (red wine) 0.26-4.77 (chocolate) 4.92-5.92 (cocoa)
Flavanones	Naringenin Hesperetin Eriodictyol	5.99 (grapefruit juice) 0.06-0.64 (orange juice)
Anthocyanidins	Cyanidin Malvidin Delphinidin Pelargonidin	0.11 (black currant juice) 0.12 (black currant concentrate) 0.10 (elderberry extract) 0.01 (red wine)

Lotito & Frei (2006) *Free Radic Biol Med* 41:1727-1746

Typical plasma and intracellular concentrations of selected water-soluble antioxidants

Antioxidant	Plasma concentrations	Intracellular concentrations
Ascorbate (Vitamin C)	30–110 µmol/L	1–5 mmol/L
Uric acid	120–420 µmol/L	<200 µmol/L
Glutathione	<2 µmol/L	3–7 mmol/L
(-)-Epigallocatechin gallate	<2 µmol/L	<1 µmol/L

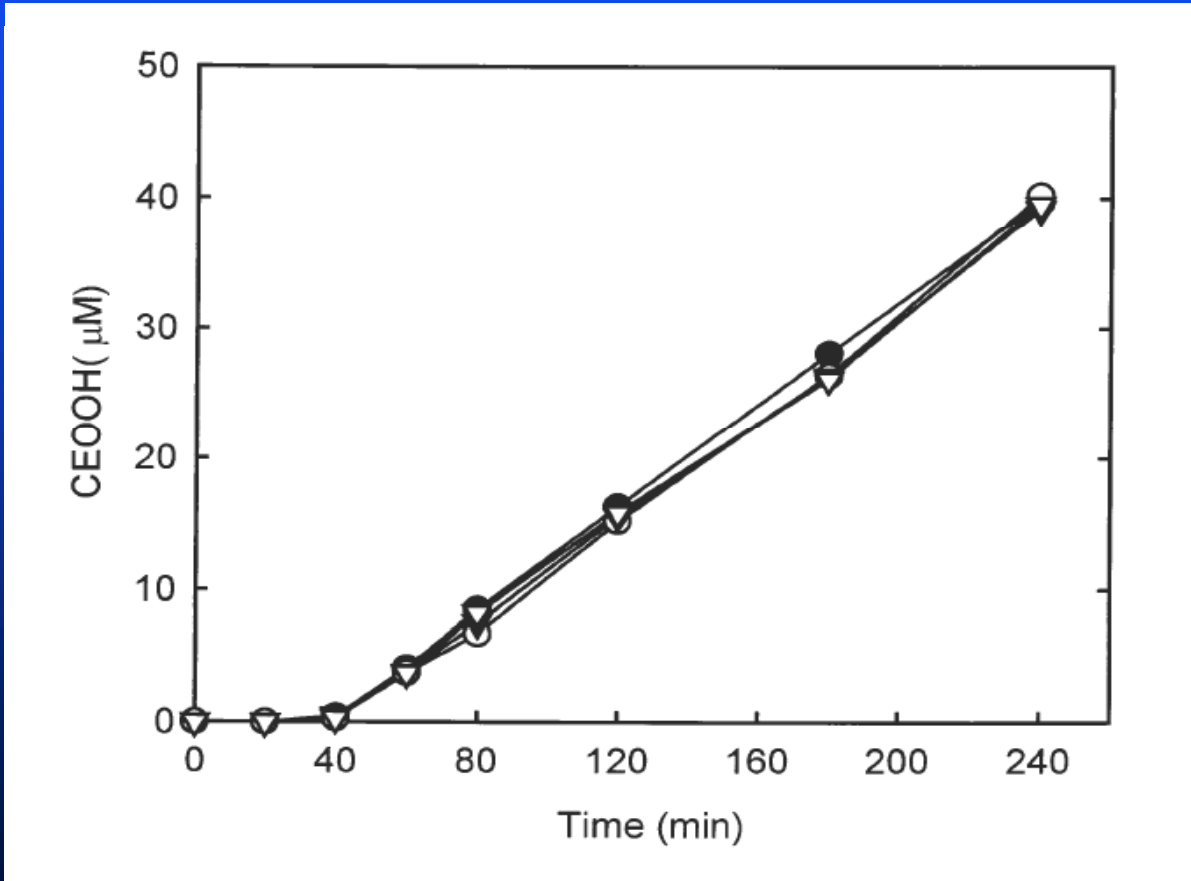
Frei, B. et al. J. Nutr. 2003;133:3275S-3284S

In Vivo Antioxidant Activity of Flavonoids

- *"Ex vivo"* oxidation of plasma or assessment of total antioxidant capacity (TAC) of plasma before and *shortly* (hrs) after consumption of flavonoid-rich foods
- Biomarkers of oxidative lipid, DNA, or protein damage before and after *long-term* consumption of flavonoid-rich foods

Consumption of Black Tea Flavonoids Does NOT Inhibit Lipid Peroxidation in Human Plasma *Ex Vivo*

- Eight healthy subjects
- 500 ml of a solution containing 7.2 mg/ml black tea flavonoids
- Lipid peroxidation measured before and 1, 2, and 3 hours after consumption
- No effect!



Cherubini, A.; Beal, M. F.; Frei, B. *Free Radic. Biol. Med.* **27**:381-7; 1999

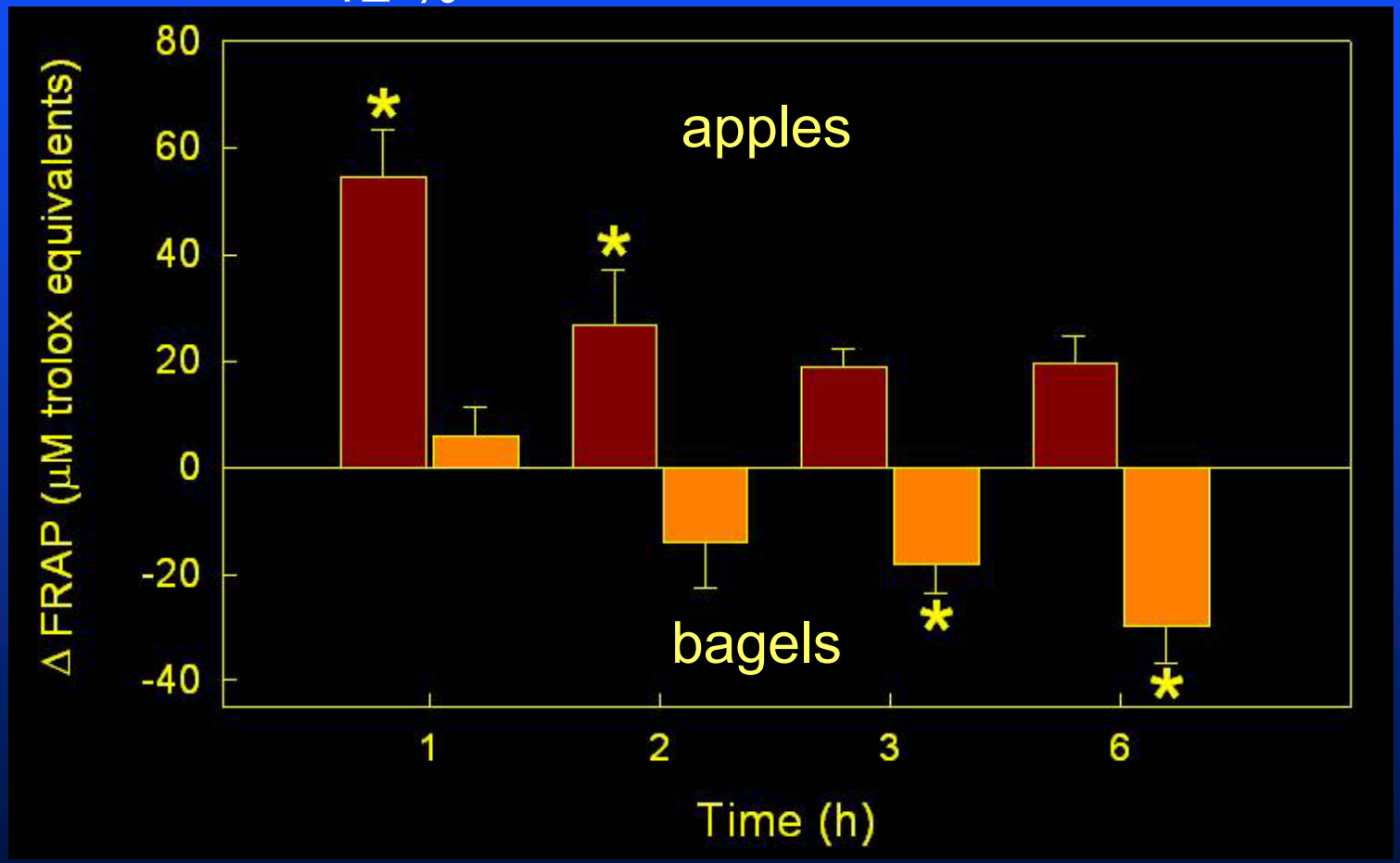
Plasma or serum total antioxidant capacity (TAC) after consumption of flavonoid-rich foods

Food	Study	Method	TAC
Fruit and vegetables	Cao et al. (1998)	ORAC	↑ 5-15 %
Strawberries, spinach, red wine	Cao et al.(1998b)	ORAC FRAP TEAC	↑ 11-24 % (ORAC) ↑ 7-24% (FRAP) ↑ 1-21% (TEAC)
Cranberry juice	Pedersen et al. (2000)	FRAP	↑ 40 μM
Lettuce	Serafini et al. (2002)	TRAP (FL)	↑ 40-50 %
Blueberries	Kay and Holub (2002)	ORAC TEAC	↑ 8.5 % (ORAC) ↑ 4.5 % (TEAC)
Blueberries	Mazza et al. (2002)	ORAC	↑11-50 %
Grape juice	Day et al. (1997)	FRAP	↑ 8 % (at 1 h) ↑ 11 % (at day 8)
Concord grape juice	O'Byrne et al. (2002)	ORAC	↑ 8 %
Green and black tea	Serafini et al. (1996)	TRAP (FL)	↑ 40-48 %
Green tea	Benzie et al. (1999)	FRAP	↑ 4 %
Wine	Day and Stansbie (1995)	FRAP	↑ 24%
Wine	Serafini et al. (1998)	TRAP (FL)	↑ 14 %
Chocolate	Rein et al. (2000)	TRAP (CL)	↑ 31 %
Chocolate	Serafini et al. (2003)	FRAP	↑ 20 %

Lotito & Frei (2006) *Free Radic Biol Med* 41:1727-1746

Consumption of apples, but not bagels, increases plasma antioxidant capacity (FRAP) in humans

12 %



ANOVA

$p < 0.0001$

$p < 0.001$

* = significantly different from baseline by Tukey-Kramer post-hoc analysis

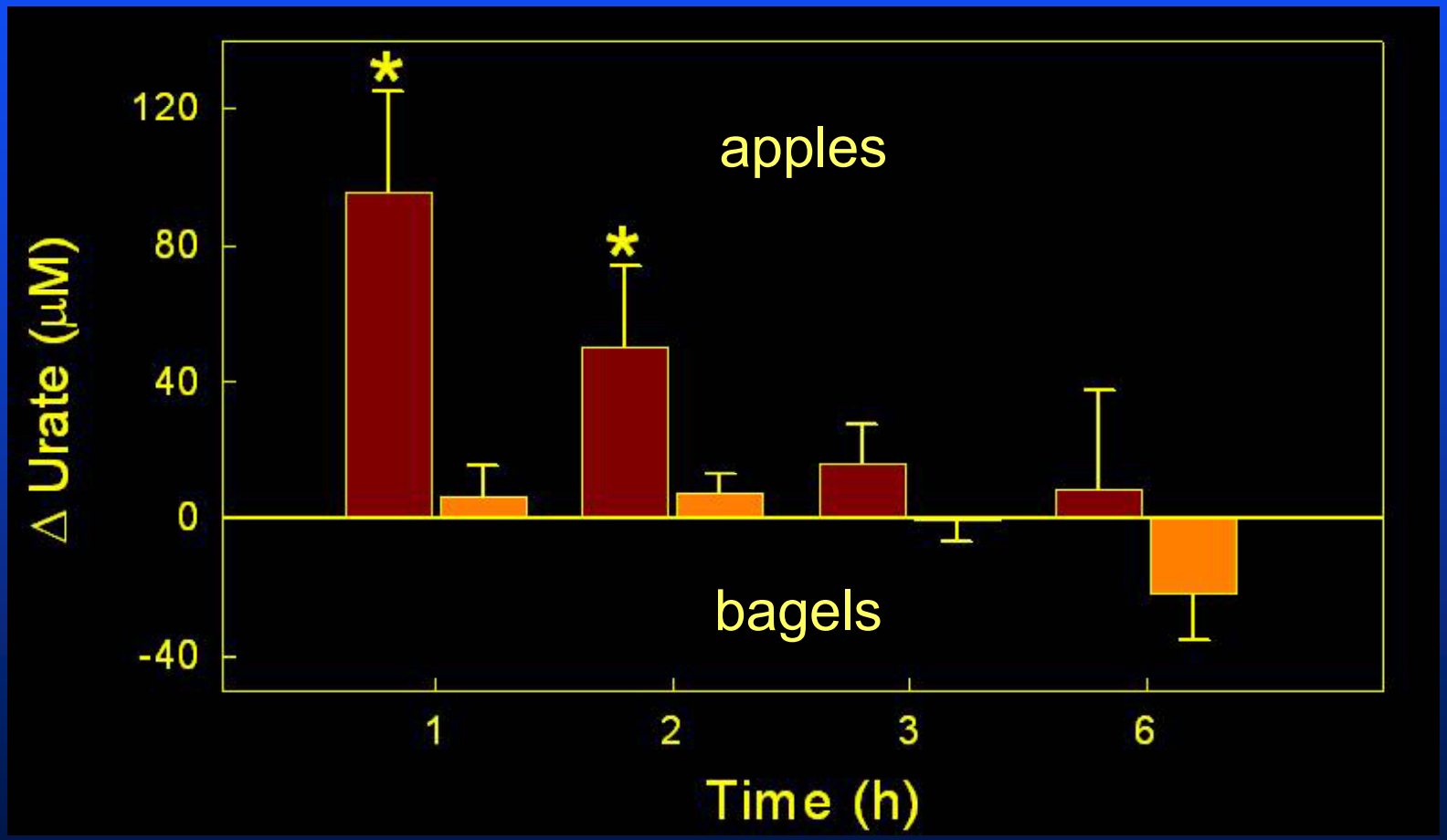
Contribution of different antioxidants to the total antioxidant capacity of human plasma measured as FRAP

Human plasma

Ascorbic acid (50 μM)	15 %
Uric acid (300 μM)	60 %
α-Tocopherol (30 μM)	5 %
Bilirubin (15 μM)	5 %
Proteins	10 %
Others	5 %

Benzie & Strain (1996) Anal. Biochem. 239, 70-76

Consumption of apples, but not bagels, increases plasma urate levels in humans



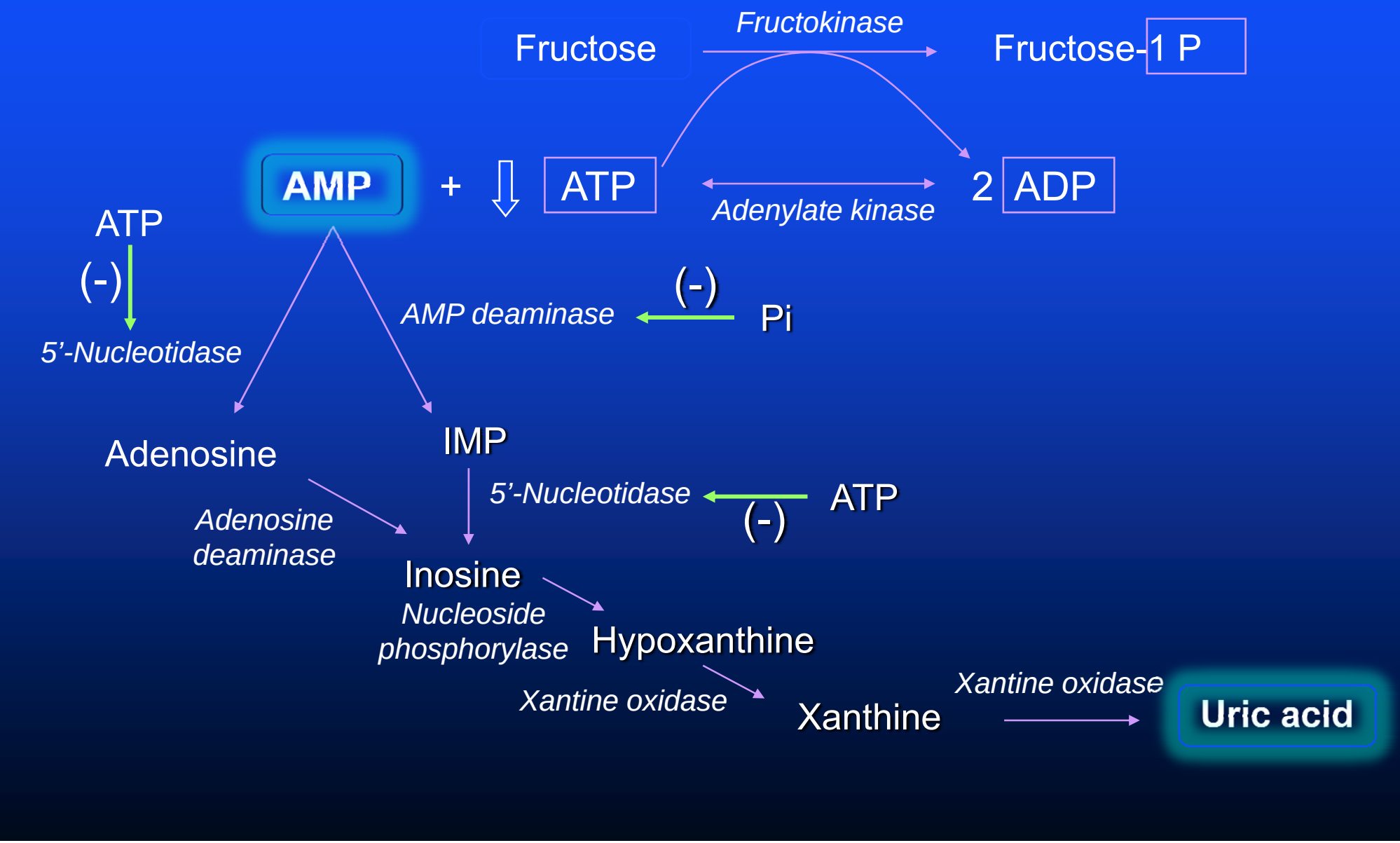
ANOVA

$p < 0.01$

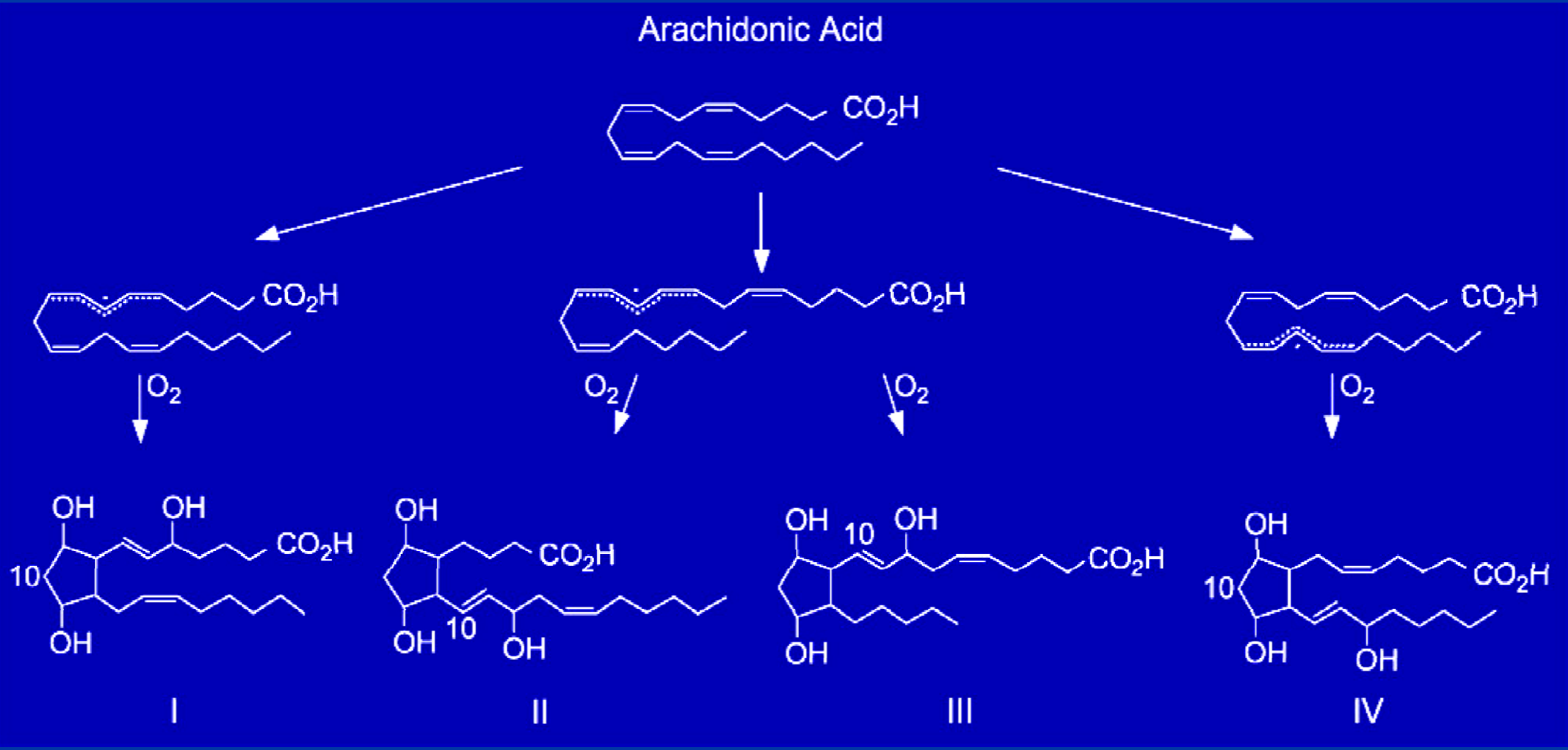
$p < 0.05$

* = significantly different from baseline by Tukey-Kramer post-hoc analysis

The Metabolic Effect of Fructose on Urate



F_2-Isoprostanes: A validated *in vivo* biomarker of lipid peroxidation



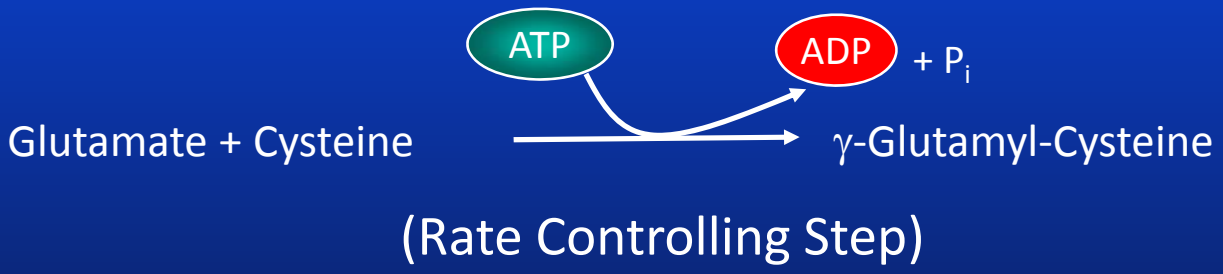
Morrow *et al.* (1995) New Engl. J. Med. **332**, 1198

F_2-Isoprostanes in Humans Consuming Tea (Extract or High Flavonoid Diets: No Effect

Reference	Design (N)	Treatment	Results
Freese et al. (1999)	Parallel Design (10/group) Healthy women	3 g/d Green Tea Extract (GTE) Placebo x 4 wk	Urine F_2-isoP: **NS difference** GTE vs. placebo
O'Reilly et al. (2001)	Crossover Design (32) Healthy men and women	Onions + 300 ml/day Black Tea - High Flavonoid Diet (HF) Low Flavonoid Diet (LF) x 4 wk	Plasma F_2-isoP: **NS difference** HF vs. LF
Hodgson et al. (2002) Trial #1	Crossover Design (13) Men and women w/ mild systolic HTN	1,000 ml/d Green Tea (GT) 1,000 ml/d Black Tea (BT) 1,000 ml/d Hot Water + Caffeine (HWC) x 7d	Urine F_2-isoP: **NS difference** BT or GT vs. HWC
Hodgson et al. (2002) Trial # 2	Crossover Design (22) Hyperlipidemic men and women	1,200 ml/d Black Tea (BT) 1,200 ml/d Hot Water + Caffeine (HWC) x 4 wk	Urine F_2-isoP **NS difference** BT vs. HWC
Baer et al. (2006)	Crossover Design (19) Male smokers	Black Tea (BT), Placebo (P), Placebo + Caffeine (PC) 7 servings per day x 7 wk	Plasma F_2-isoP **NS difference** BT vs. P or PC

Glutathione

- Tripeptide: γ-Glu-Cys-Gly
- Important Cellular Antioxidant & Detoxicant
- Co-Substrate for Detoxification Enzymes
- Buffers Thiol Redox State

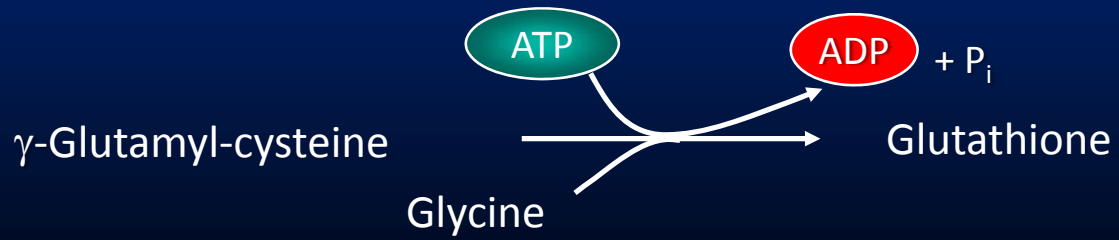


(Rate Controlling Step)

γ-Glutamylcysteine Ligase (GCL)

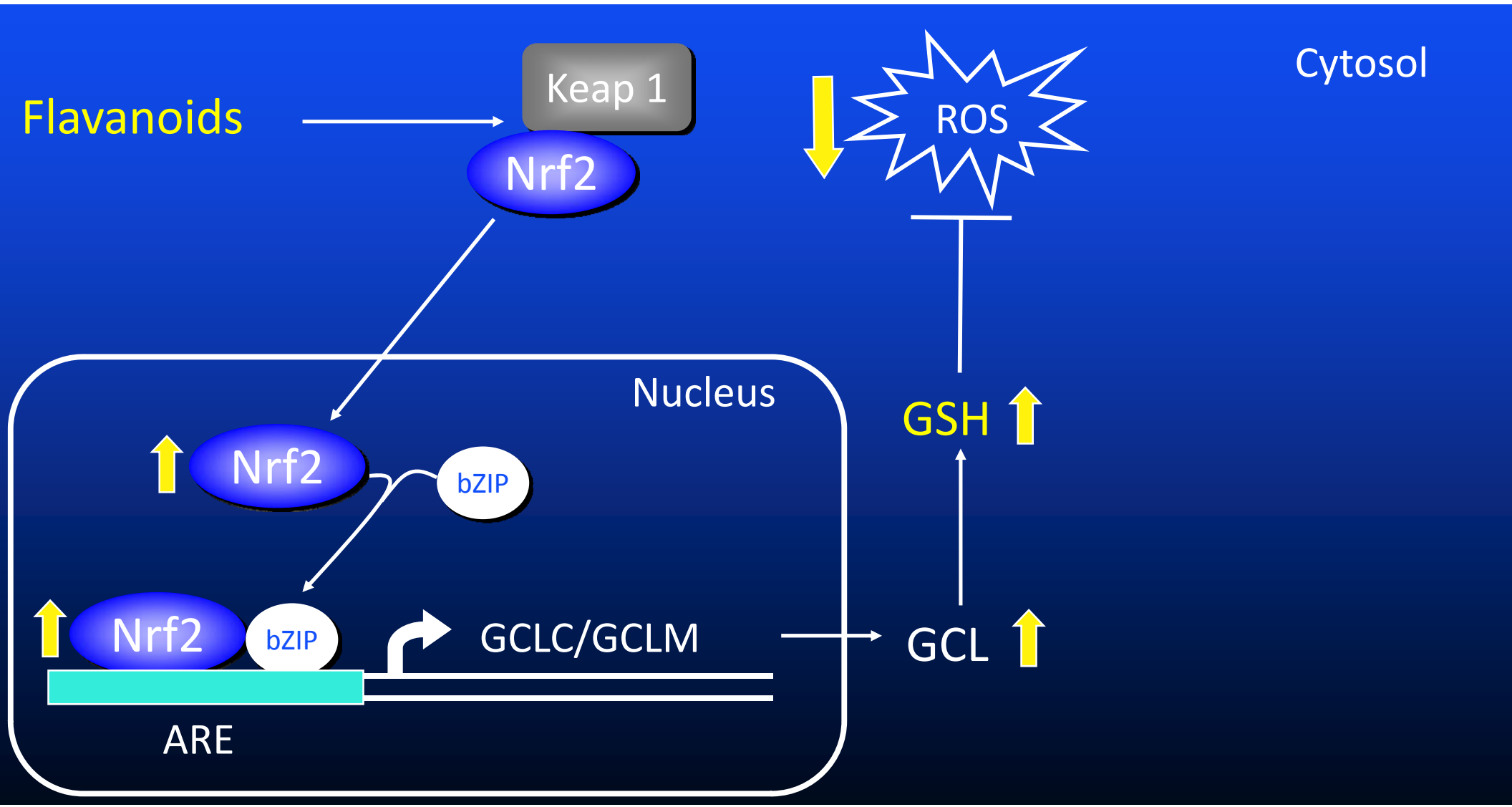
GCLC

GCLM



Glutathione Synthase (GS)

“Indirect” antioxidant effect of flavanoids: Nrf2/AGE-mediated GSH biosynthesis



Good News – Bad News:

- There is no scientific proof that flavonoids exert physiologically relevant antioxidant effects in humans.
- There is good scientific evidence that flavonoids exert (non-antioxidant) physiological effects and potentially exert health benefits in humans.

Inverse relationship between flavonoid intake* and cardiovascular risk

*(mainly from apples, onions, and black tea)

Stensvold 1992: Tea, Norway (CHD death)

Brown 1993: Tea, Scotland (CHD)

Hertog 1995: Flavonoids, Zutphen (CHD death)

Knekt 1996: Flavonoids, Finland (CHD death)
- Men
- Women

Keli 1996: Black tea, Zutphen (Stroke)

Rimm 1996: Flavonoids in HPFS (CHD death)
- Prior CAD
- No Prior CAD

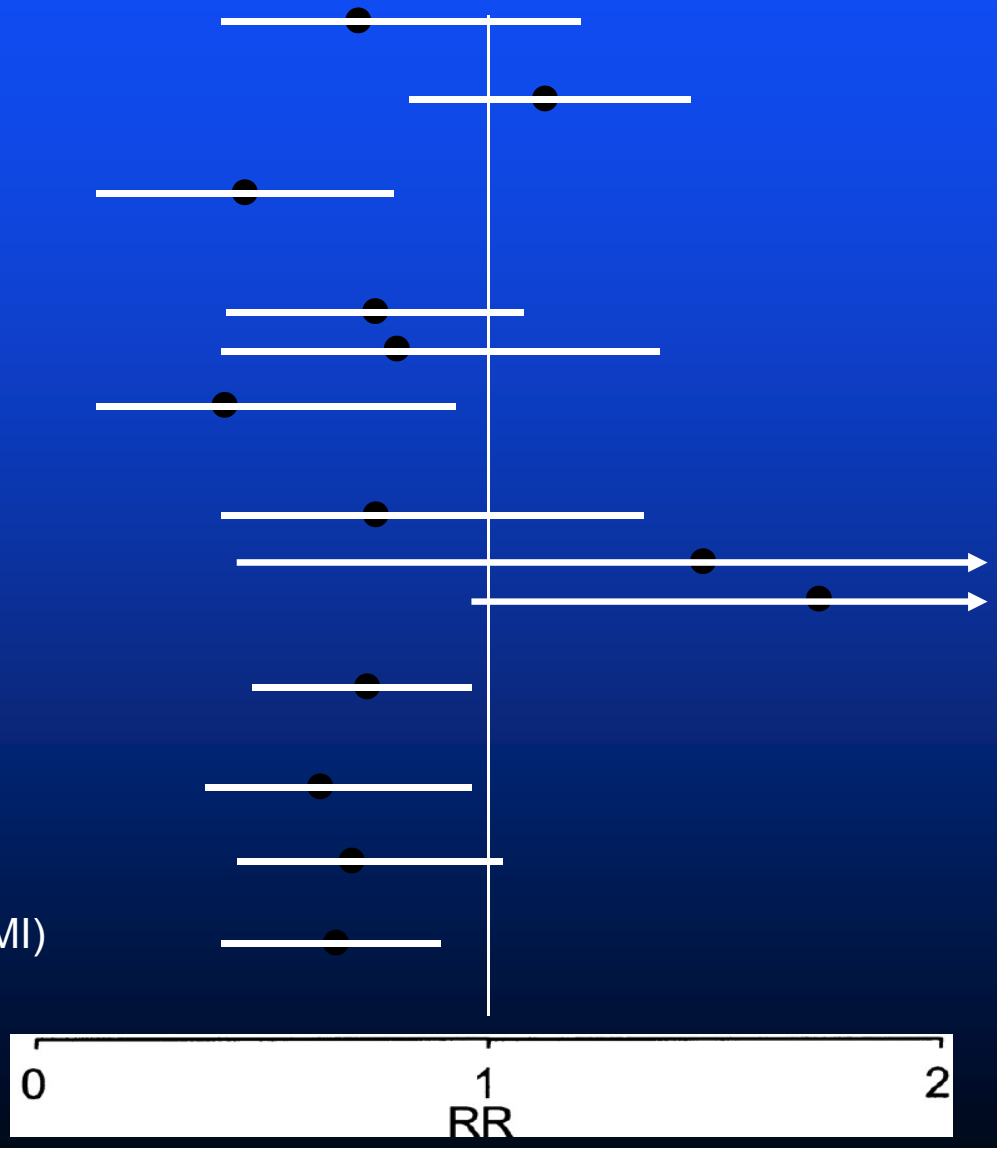
Hertog 1997: Flavonoids, Welch Men (CHD death)

Yochum 1999: Flavonoids, Women in Iowa (CHD)

Sesso 1999: Tea >1 cup/day, USA (MI)

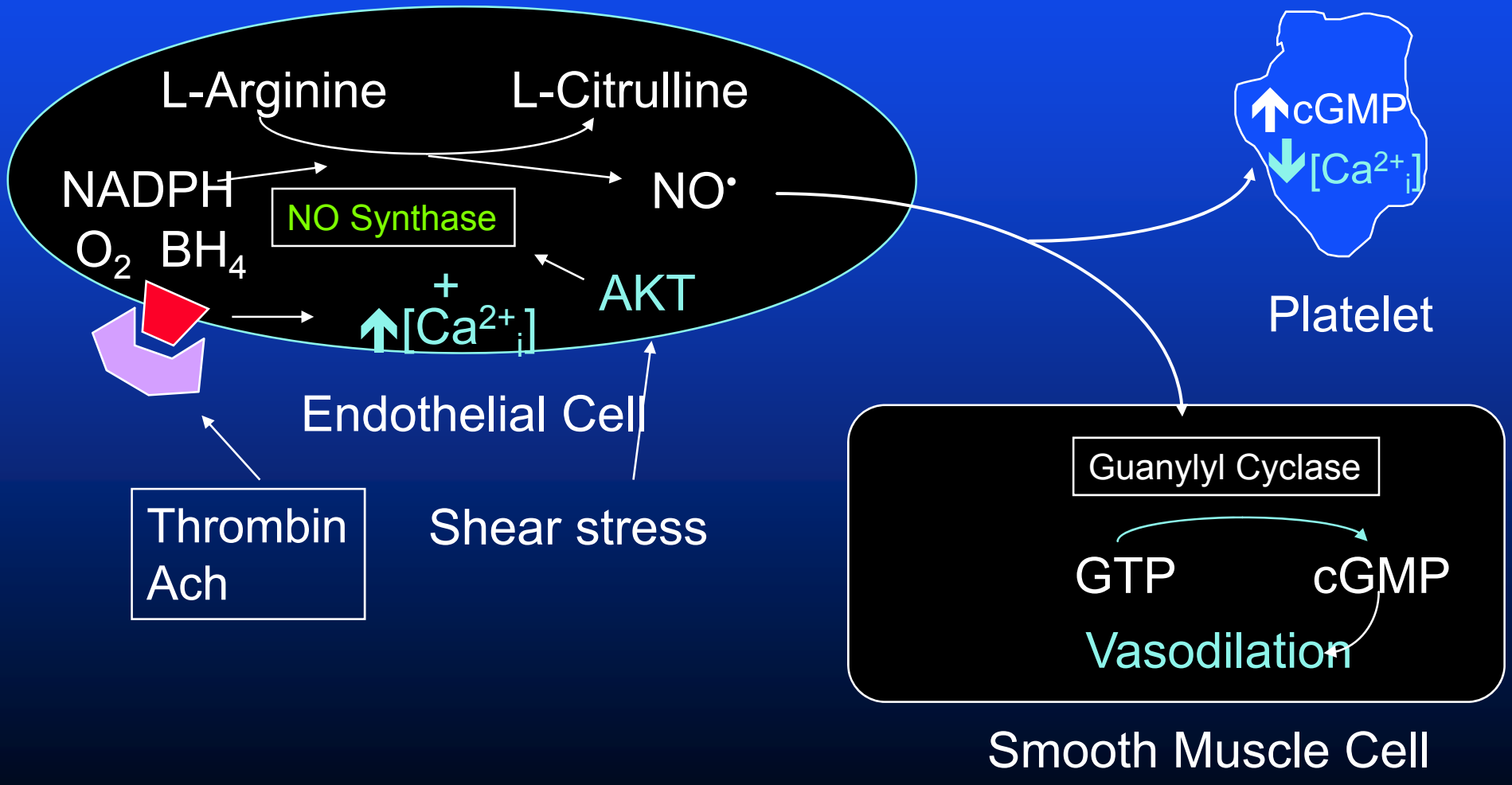
Geleijnse 2002: Tea (>375 ml/d), Rotterdam (MI)

Mukamal 2002: Tea (>2 cups/day), USA (recurrent MI)

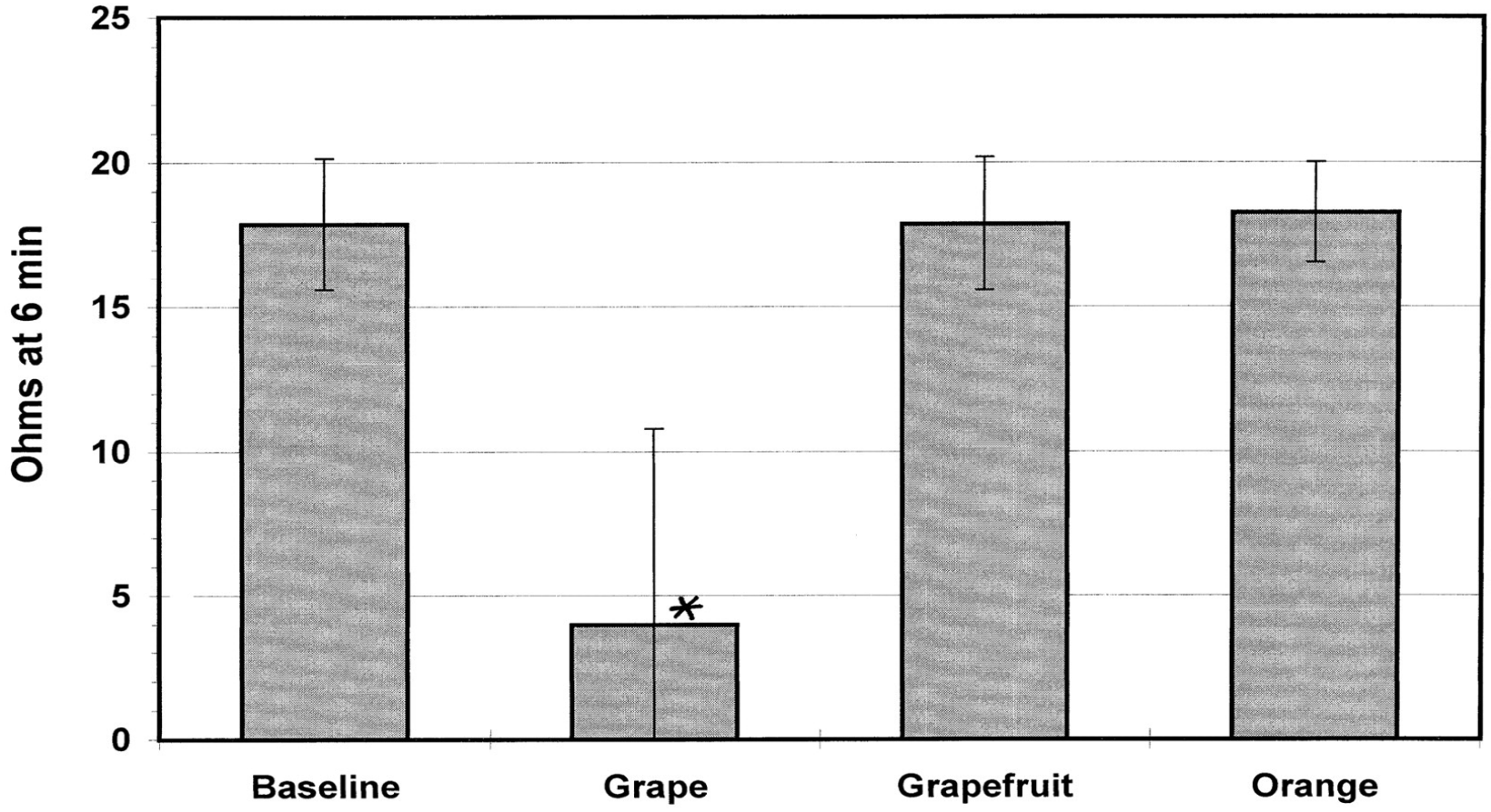


Vita JA (2003) J Nutr 133,3293S-3297S

Synthesis and Action of EDNO

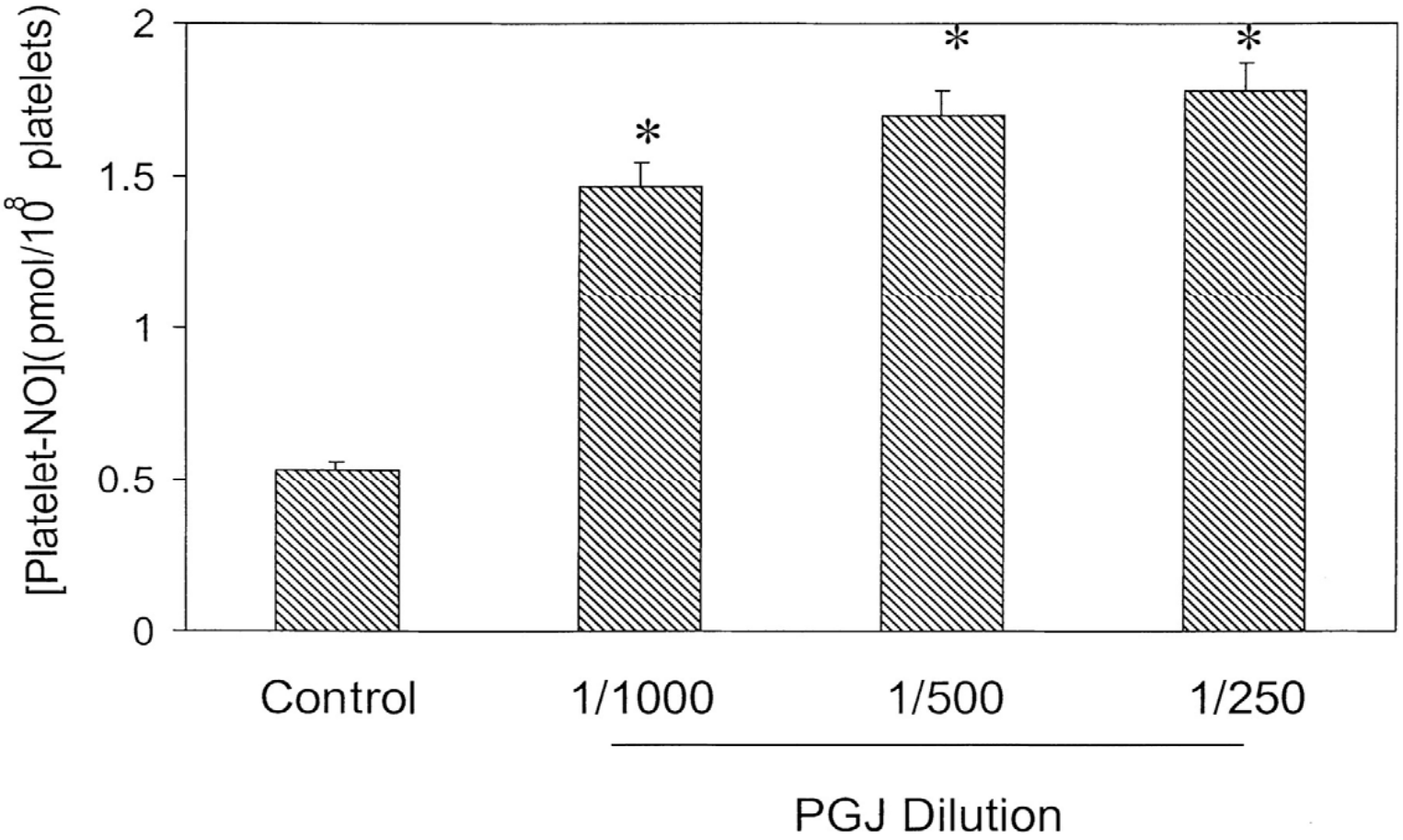


Platelet aggregation response in humans after drinking purple grape, grapefruit, or orange juice for 1 wk



Keevil, J. G. et al. J. Nutr. 2000;130:53-56

Effect of purple grape juice on NO production by platelets

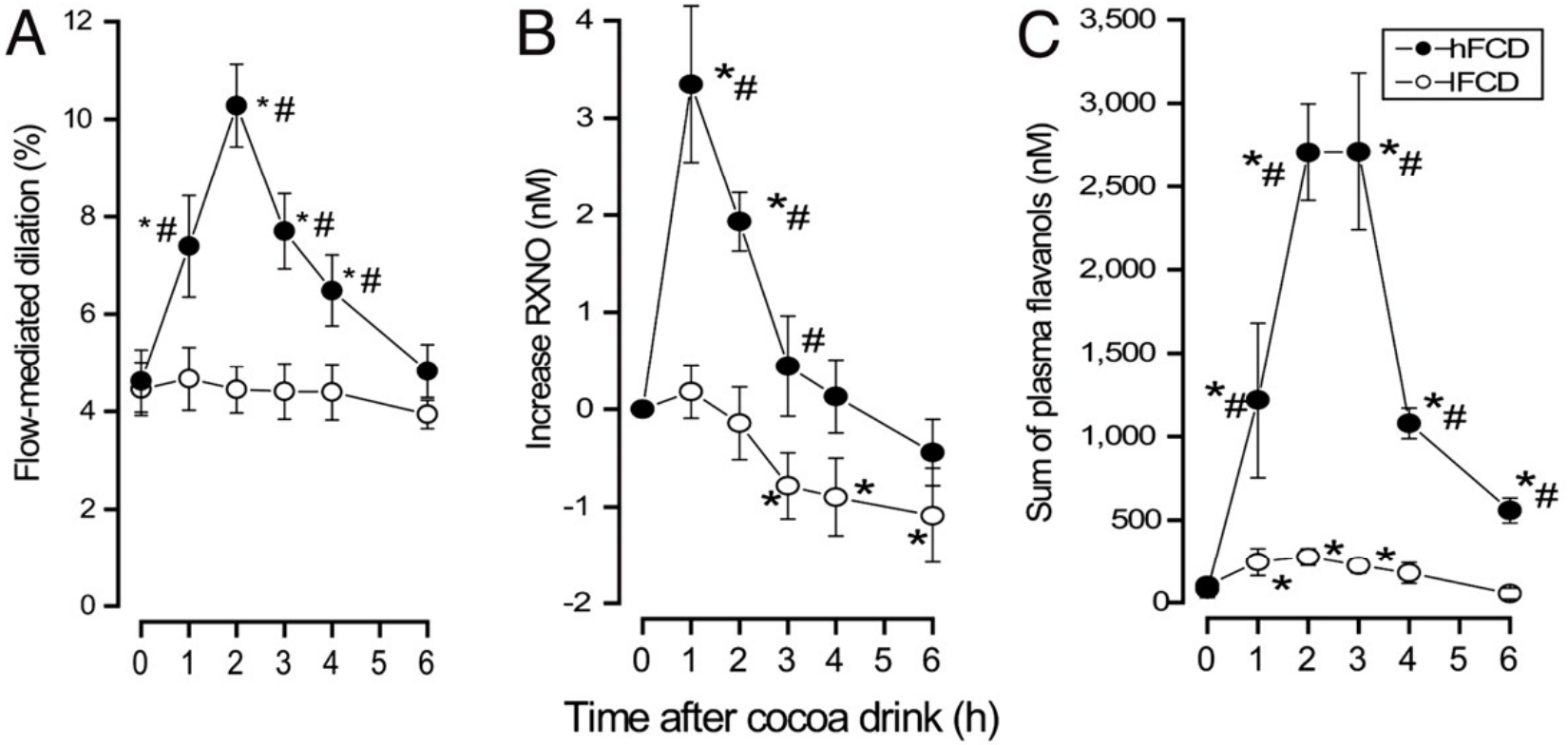


Freedman, J. E. et al. Circulation 2001;103:2792-2798

Circulation

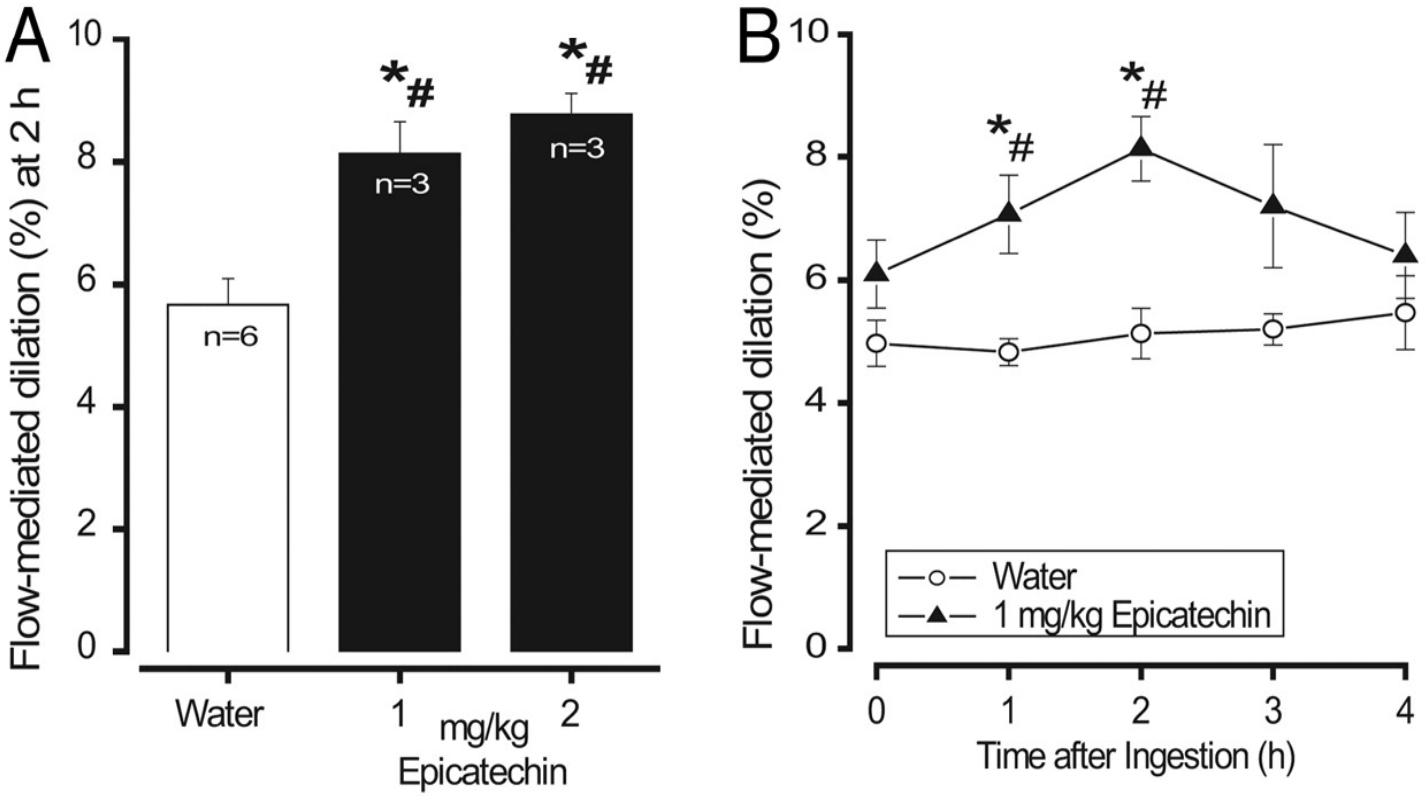
Consumption of flavanol-rich cocoa improves vascular NO production in humans

Time courses of vasodilation, NO levels, and total circulating flavanols



Schroeter H. et.al. PNAS 2006;103:1024-1029

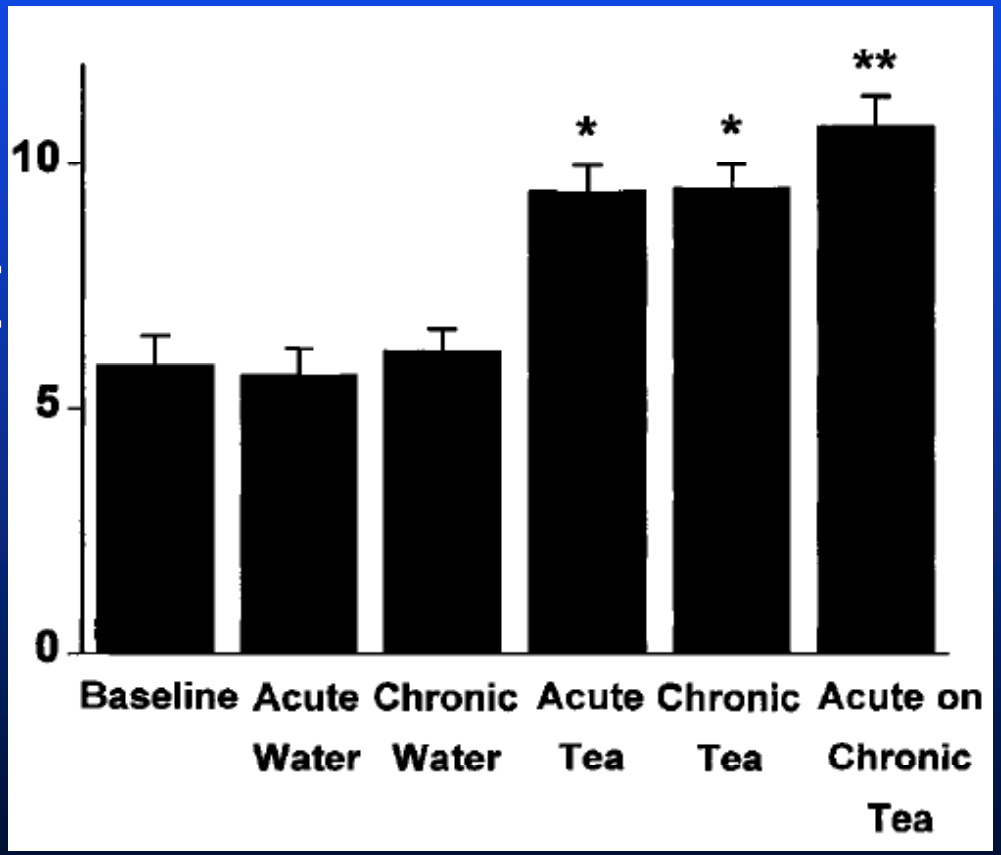
Oral ingestion of the flavanol, epicatechin, improves vascular NO production



Schroeter H. et.al. PNAS 2006;103:1024-1029

Acute and Chronic Black Tea Consumption Reverses Endothelial Dysfunction in CAD Patients

- 50 subjects with CAD
- No antioxidant supplements
- Standard meds including ASA and lipid lowering (77%)
- FMD at baseline, 2 hours, and 4 weeks after tea and water in crossover design



Duffy et al. Circulation 2001

Lack of Effect of Chronic Black Tea Consumption on Markers of Oxidative Stress and Inflammation

Marker	Baseline	Water	Tea
Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) (µg/mmol creatinine) (n = 53)	0.61 ± 0.19	0.59 ± 0.15	0.60 ± 0.17
Urinary 8-isoprostanes (ng/mmol creatinine) (n = 53)	67.6 ± 44.7	76.5 ± 67.8	62.6 ± 37.8
Plasma ORAC (µmol Trolox activity/L) (n = 44)	5086 ± 1308	6240 ± 1296	5839 ± 1585
Protein-Free Plasma ORAC (µmol Trolox activity/L) (n = 44)	975 ± 368	1002 ± 250	1061 ± 338
Plasma FRAP (µmol Trolox activity/L) (n = 44)	750 ± 227	723 ± 183	786 ± 165
CRP (mg/L)* (n = 29)	3.2 ± 2.7	2.0 ± 1.9*	2.9 ± 3.6

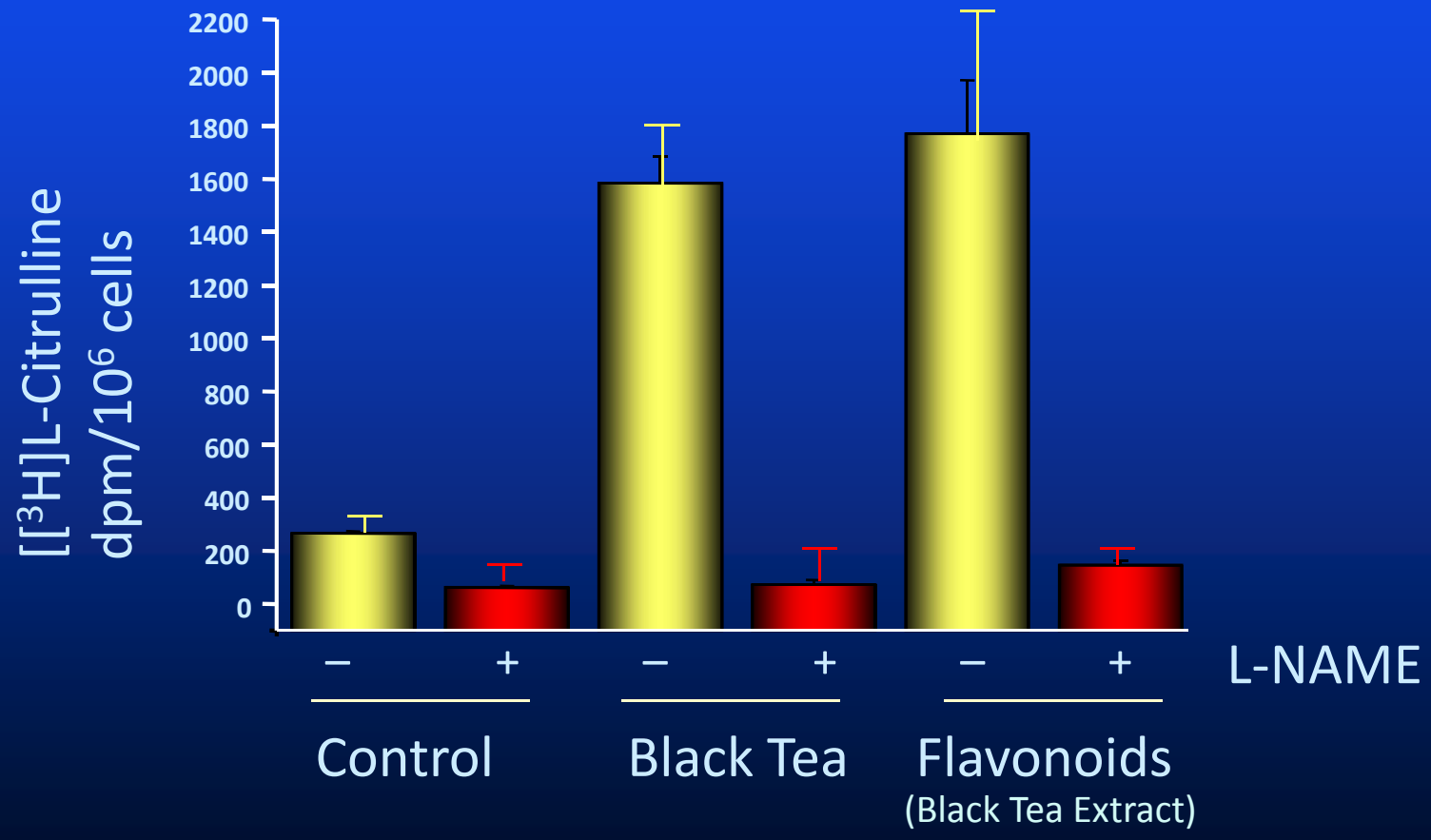
* $P = 0.02$ by two-way repeated-measures ANOVA, with C-reactive protein after 4 weeks of water significantly lower than either baseline ($P = 0.02$) or chronic tea ingestion ($P = 0.03$).

Widlansky ME, et al., Free Radic Biol Med. 2005; 38:499-506.

How Do Tea, Cocoa, Epicatechin, etc. Improve Vascular NO Production?

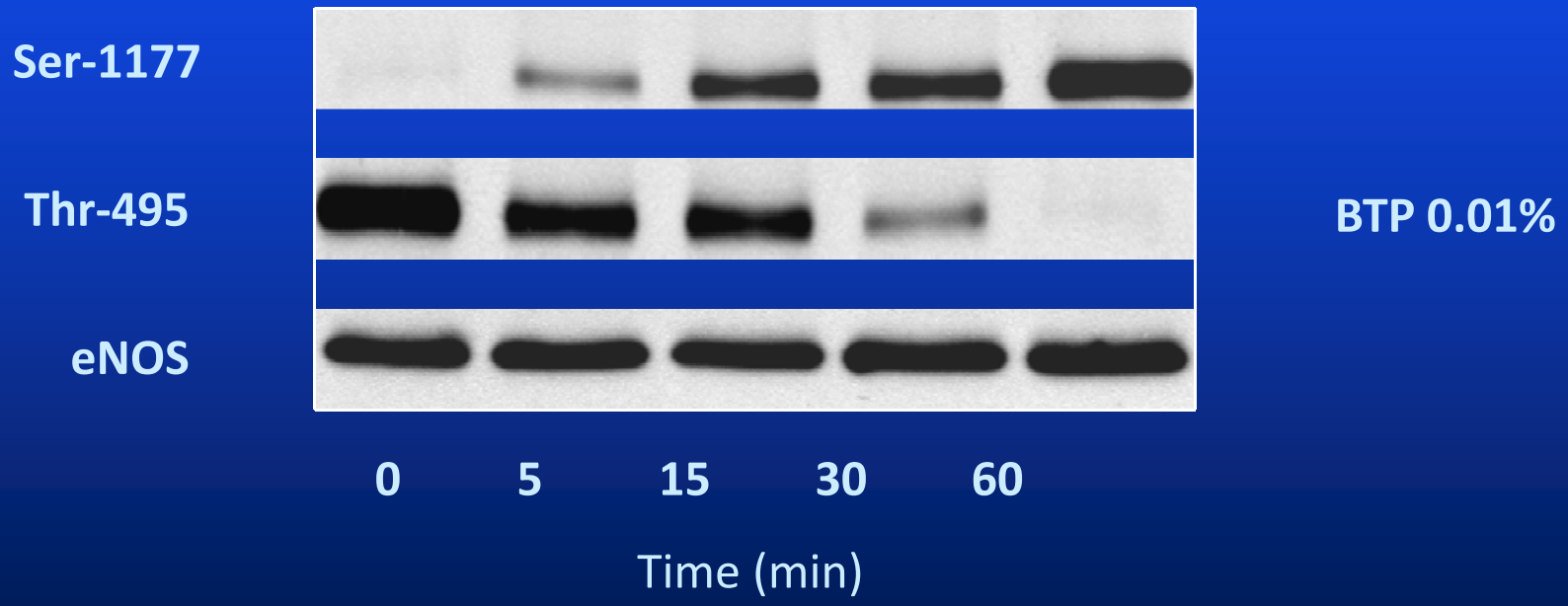
- No effect on systemic markers of oxidative stress (F_2-Isoprostanes)
- No effect on plasma antioxidant capacity (ORAC, FRAP)
- No effect on CRP
- Not attributable to caffeine
- No effect on blood pressure
- No effect on serum lipids

Black Tea Flavonoids Activate Endothelial Nitric Oxide Synthase (eNOS)

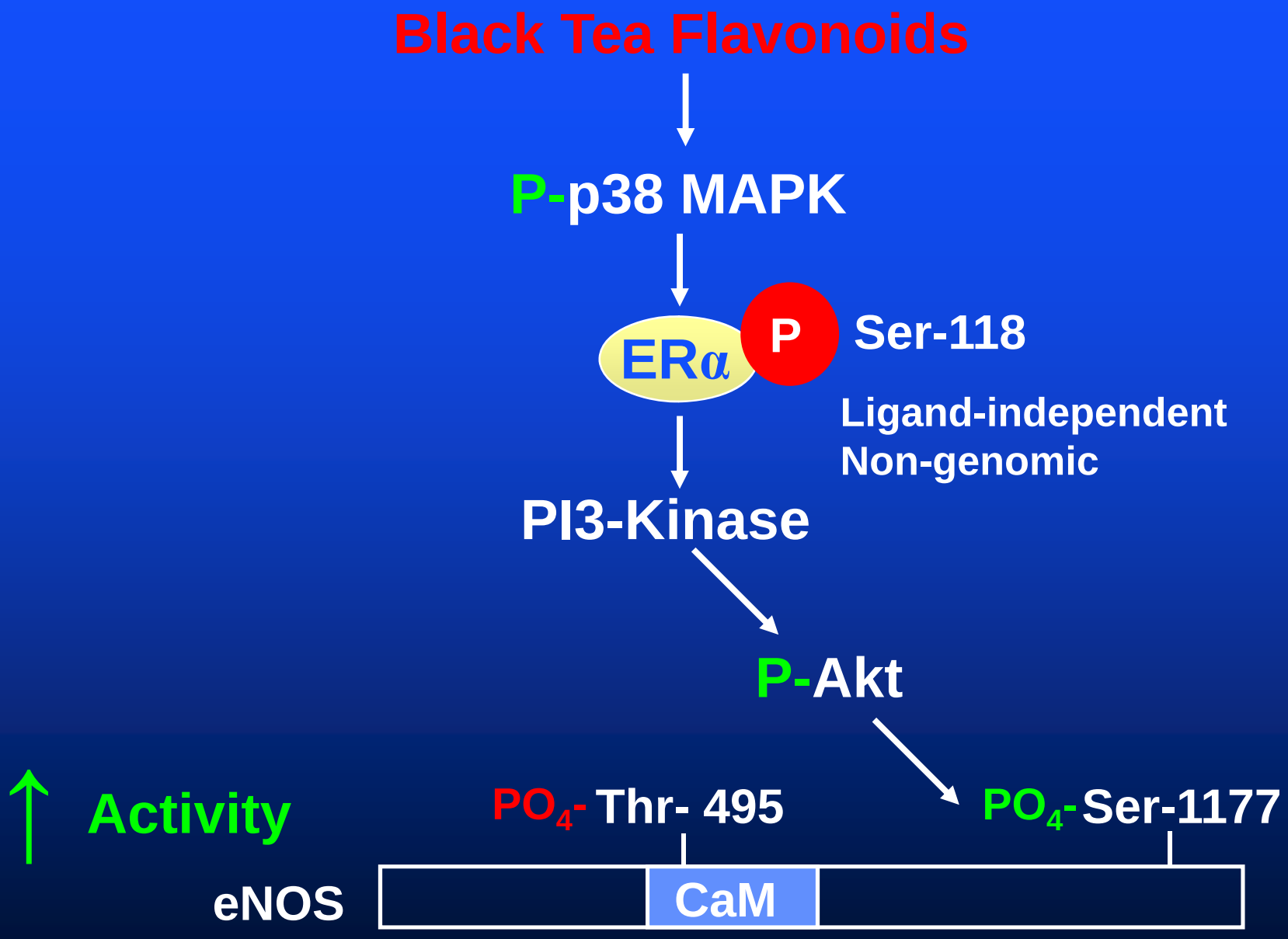


Anter and Keaney *J. Biol. Chem.* 2004;279:46637

Black Tea Flavonoids Phosphorylate eNOS in Endothelial Cells



Anter and Keaney *J. Biol. Chem.* 2004;279:46637



Anter et al. *J. Biol. Chem.* 2004;279:46637
Anter et al. *Circ. Res.* 2005;96:1072

Biological Activities of Flavonoids

- Modulation of cell-signalling pathways
  - Could provide protection from cardiovascular disease by:
    - Increasing eNOS activity and NO production
    - Decreasing platelet aggregation
    - Decreasing inflammation
    - Decreasing monocyte adhesion to the vascular wall

Biological Activities of Flavonoids

- Modulation of cell-signalling pathways
  - Could provide protection from cancer by:
    - Stimulating phase II (detoxification) enzyme activity
    - Inhibiting proliferation and inducing apoptosis of cancer cells
    - Inhibiting tumor invasion and new blood vessel formation (angiogenesis)
    - Decreasing inflammation

Take Home Messages

- There is no scientific proof that flavonoids exert physiologically relevant antioxidant effects in humans.

- Low concentrations of flavonoids can modulate cell-signalling pathways and exert physiological effects, independent of their antioxidant capacity.

- Flavan-3-ols – found in tea, purple grape juice, red wine, cocoa, apples, and berries – can increase NO production, inhibit platelet aggregation, and reduce inflammation, all factors associated with a lower coronary risk in humans.