- ✓ Endogenous Antioxidant network
- ✓ The lesson from clinical trials
- ▪
- ✓ “Functional” antioxidants
- ✓ Antioxidant network and disease prevention

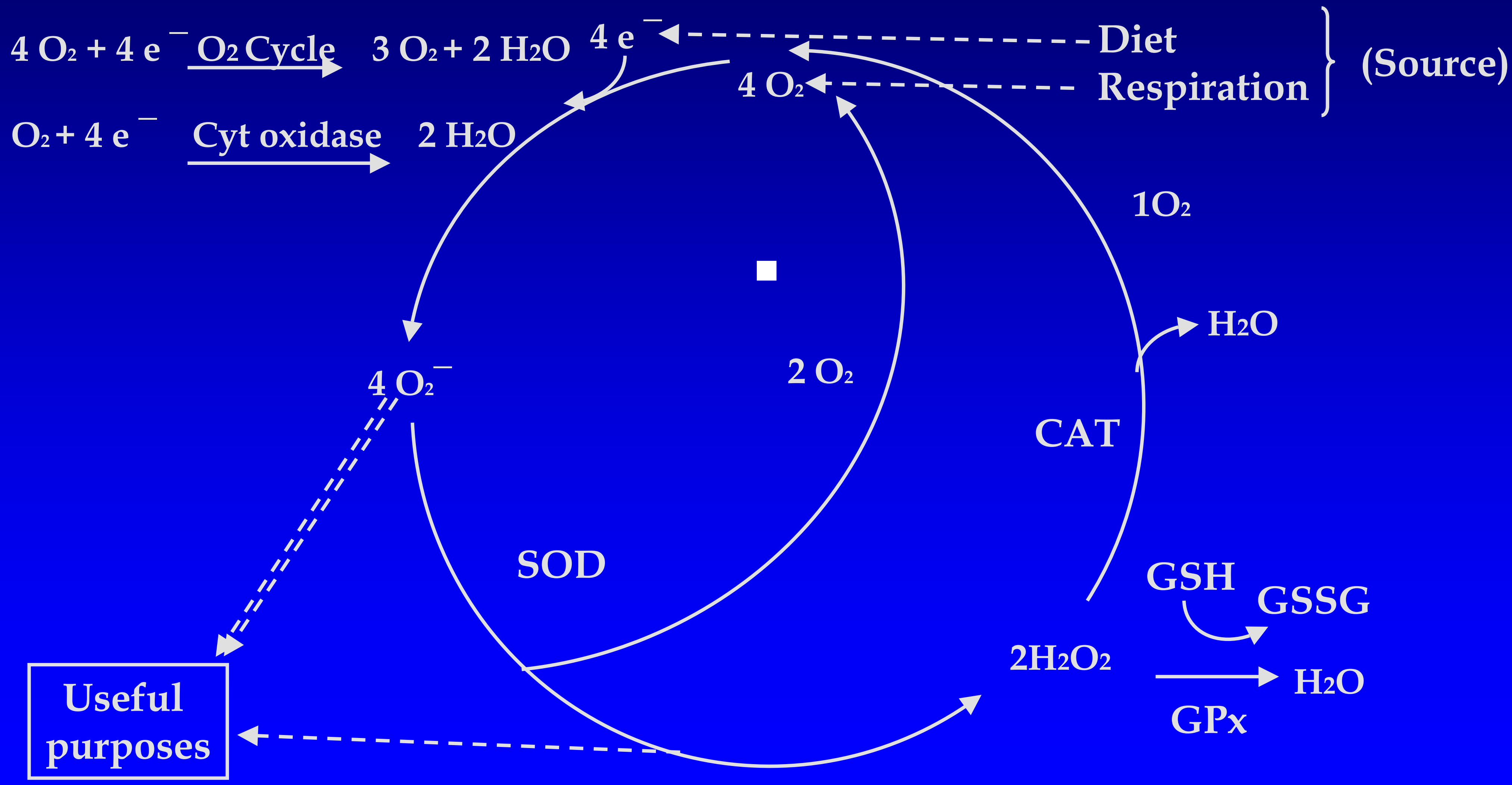
Oxygen Poisoning and X-irradiation: A Mechanism in Common[1]

Rebeca Gerschman, Daniel L. Gilbert, Sylvanus W. Nye, Peter Dwyer, and Wallace O. Fenn[2]

Department of Physiology and Vital Economics, The University of Rochester School of Medicine and Dentistry, Rochester, New York

Science 1954, 119:623-626

THE OXYGEN RADICAL CYCLE



ROS: REACTIVE OXYGEN SPECIES

Radicaliche e non radicaliche

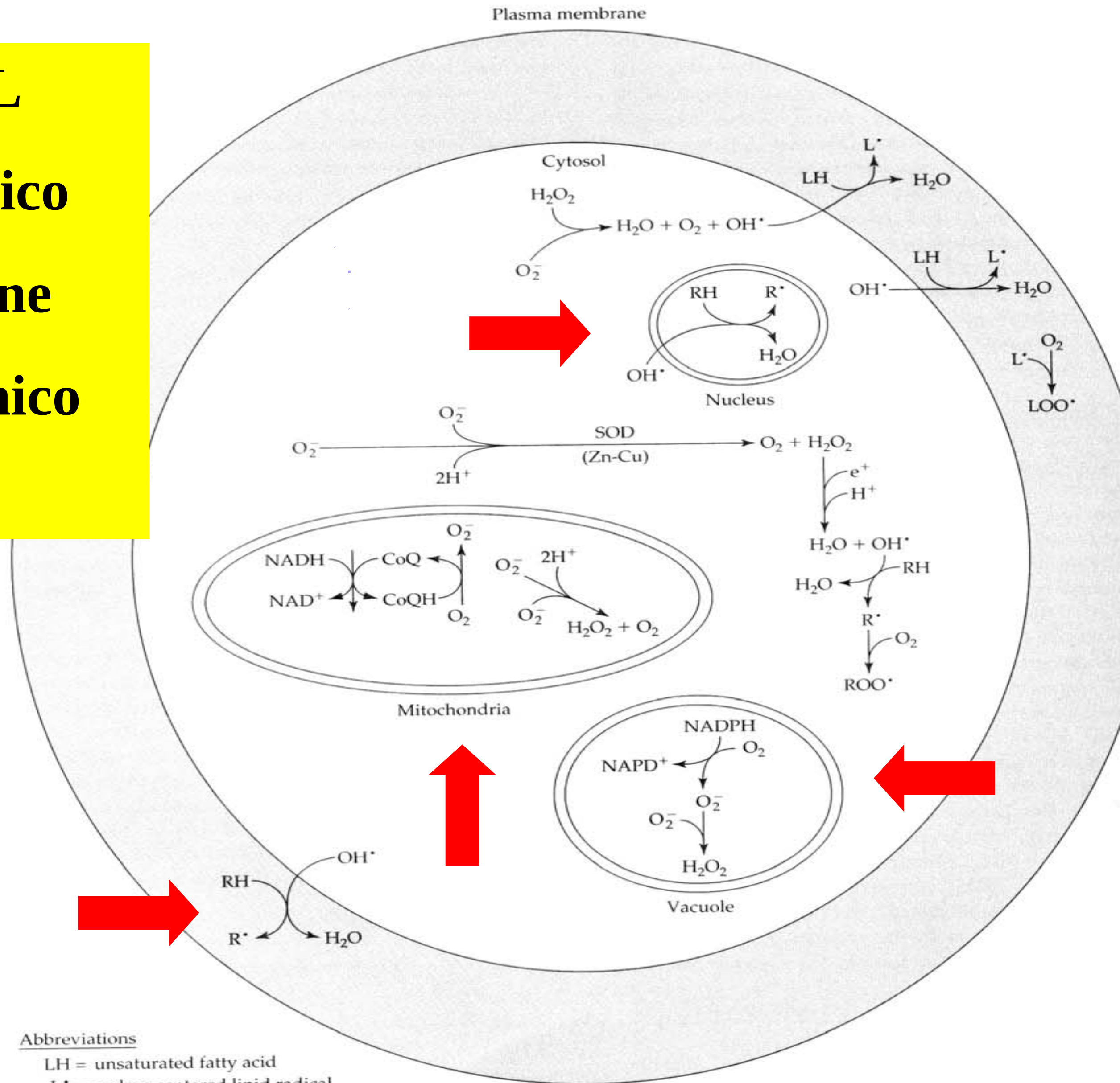
		ORBITALI π		ORBITALI σ	radicale	ossidante
OSSIGENO TRIPLETTO	O_2	↑	↑		SI	NO
ANIONE SUPEROSSIDO	$O_2^{-\bullet}$	↑↓	↑		SI	SI
PEROSSIDO D'IDROGENO	H_2O_2	↑↓	↑↓		NO	SI
RADICALE IDROSSILICO	$HO^{\bullet}$	↑↓	↑↓	↑	SI	SI
ACQUA	H_2O	↑↓	↑↓	↑↓	NO	NO
OSSIGENO SINGOLETTO	$^1O_2$	↑↓			NO	SI

FONTI ENDOGENE DI RL

- **Respirazione ed Esercizio fisico**
- **Meccanismi di detossificazione**
- **Cascata dell'acido arachidonico**
- **Fagocitosi**

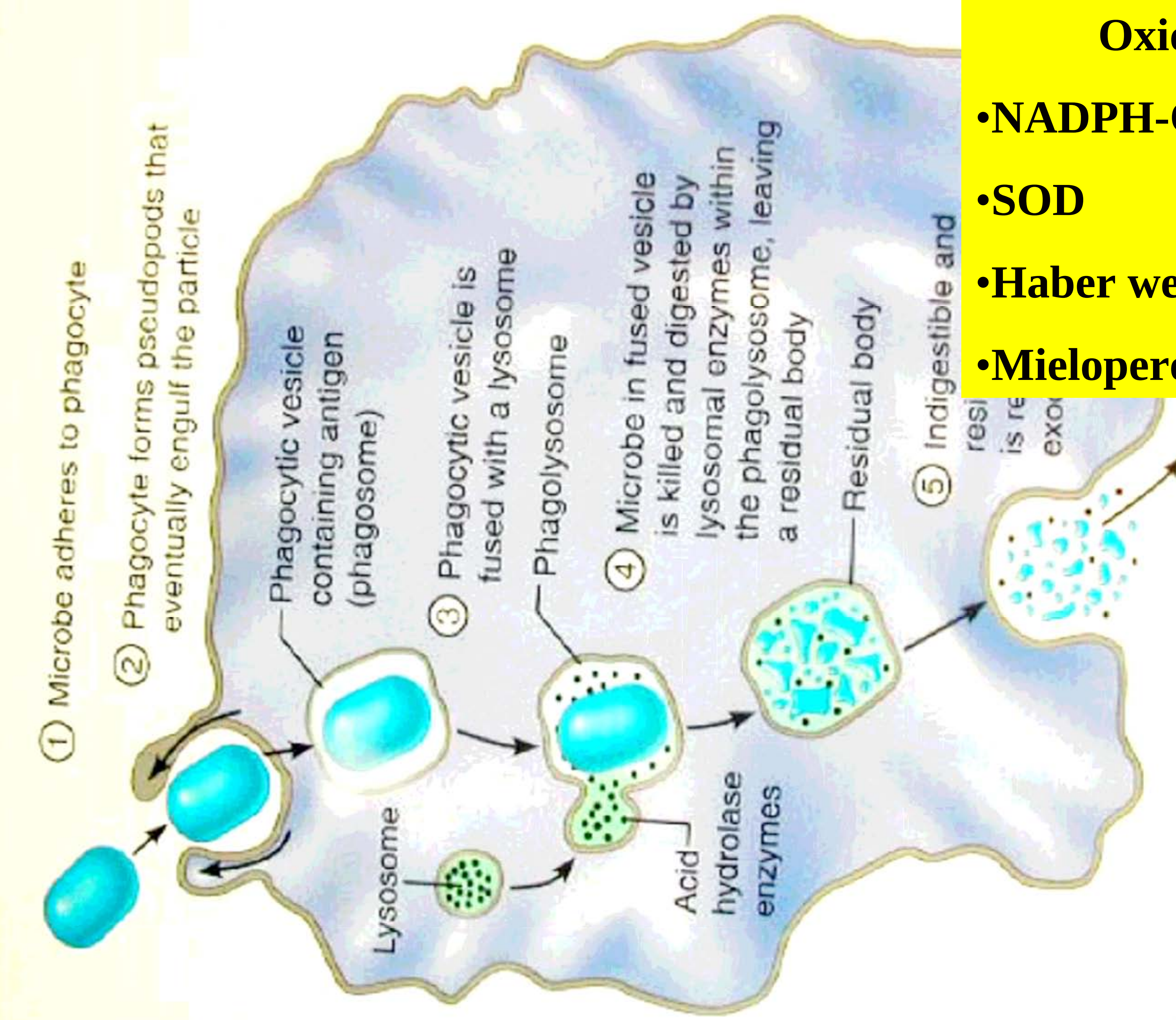
FONTI ESOGENE DI RL

- **Luce ultravioletta**
- **Inquinamento ambientale**
- **Fumo di sigaretta**
- **Radiazioni elettromagnetiche**
- **Alimenti**



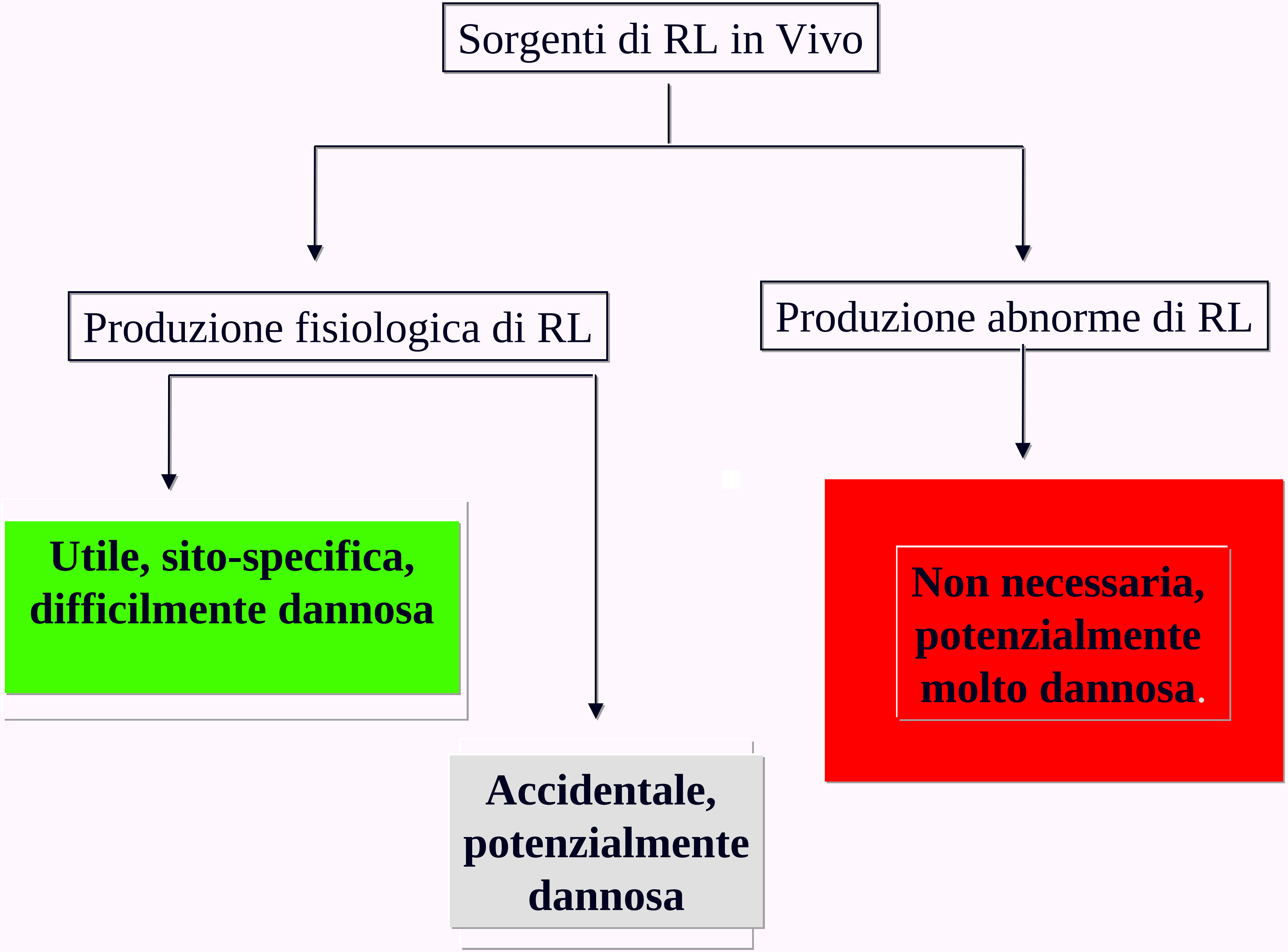
Abbreviations
LH = unsaturated fatty acid
$L^\bullet$ = carbon centered lipid radical
RH = organic compound (e.g., amino acid, nucleic acid)
O_2^- = superoxide radical
$OH^\bullet$ = hydroxy radical
$R^\bullet$ = carbon centered nonlipid radical
H_2O_2 = hydrogen peroxide
$ROO^\bullet$ = nonlipid peroxy radical
$LOO^\bullet$ = peroxy radical

Figure 1 Generation of reactive species.



Oxidative Burst

- •NADPH-Oxidase
- •SOD
- •Haber weiss
- •Mieloperoxidase


Sorgenti di RL in Vivo
Produzione fisiologica di RL
Produzione abnorme di RL
Utile, sito-specifica, difficilmente dannosa
Accidentale, potenzialmente dannosa
Non necessaria, potenzialmente molto dannosa.

MODALITA' DI AZIONE DEGLI ANTIOSSIDANTI

PRIMARI

- Prevengono la formazione di specie radicaliche
- Sequestrano i metalli di transizione

SECONDARI

Reagiscono con radicali formati e li convertono in forme meno reattive interrompendo la reazione a catena

CLASSIFICAZIONE DEGLI ANTIOSSIDANTI

ENDOGENI

ENZIMI: SOD, catalasi, glutatione perossidasi

PROTEINE: proteine-SH, leganti metalli (Fe, Cu)

ALTRE MOLECOLE: acido urico, bilirubina ...

ESOGENI

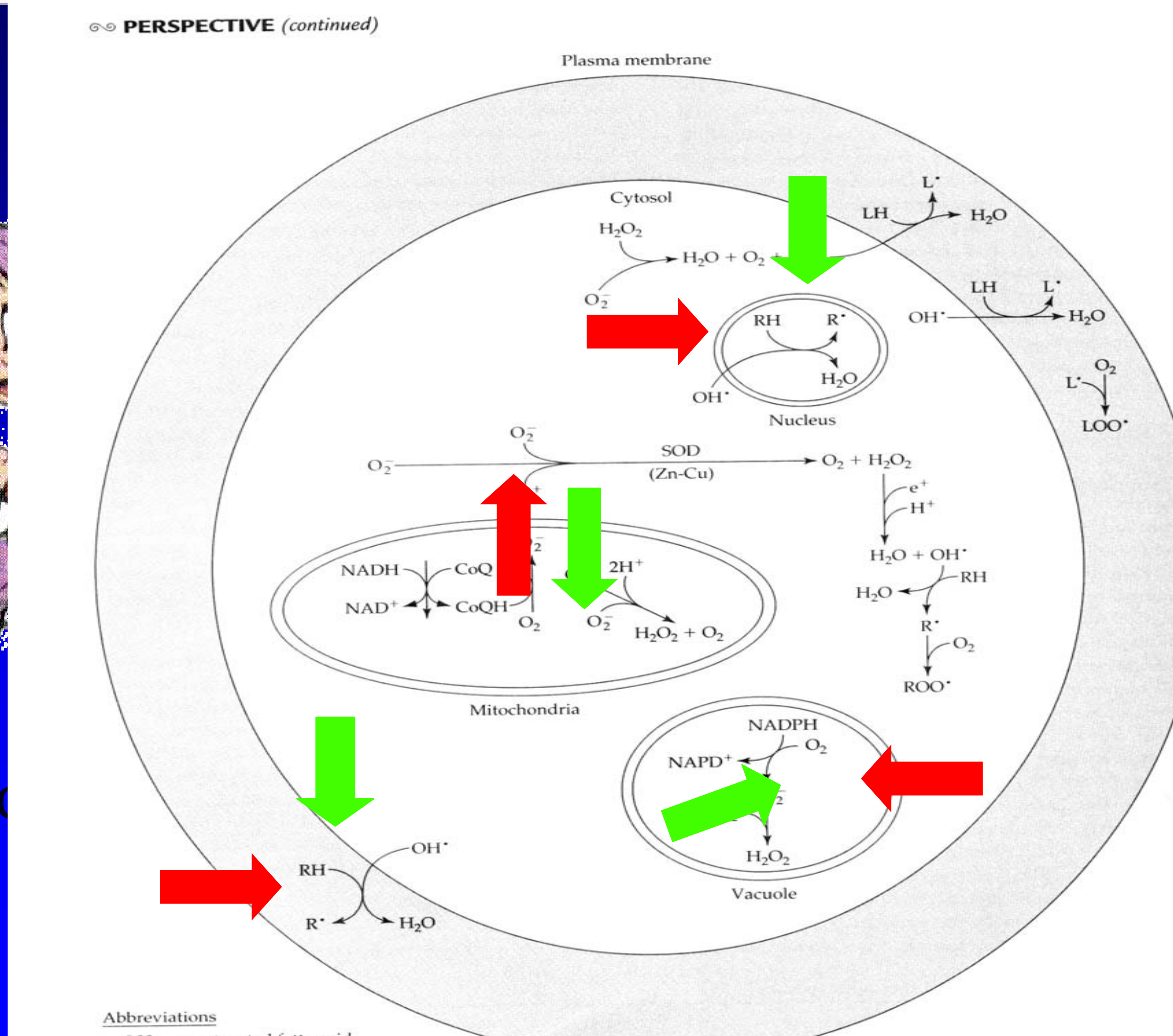
VITAMINICI:

- Vitamina C
- Vitamina E
- Carotenoidi (con funzione di pro-vitamina A)

NON VITAMINICI:

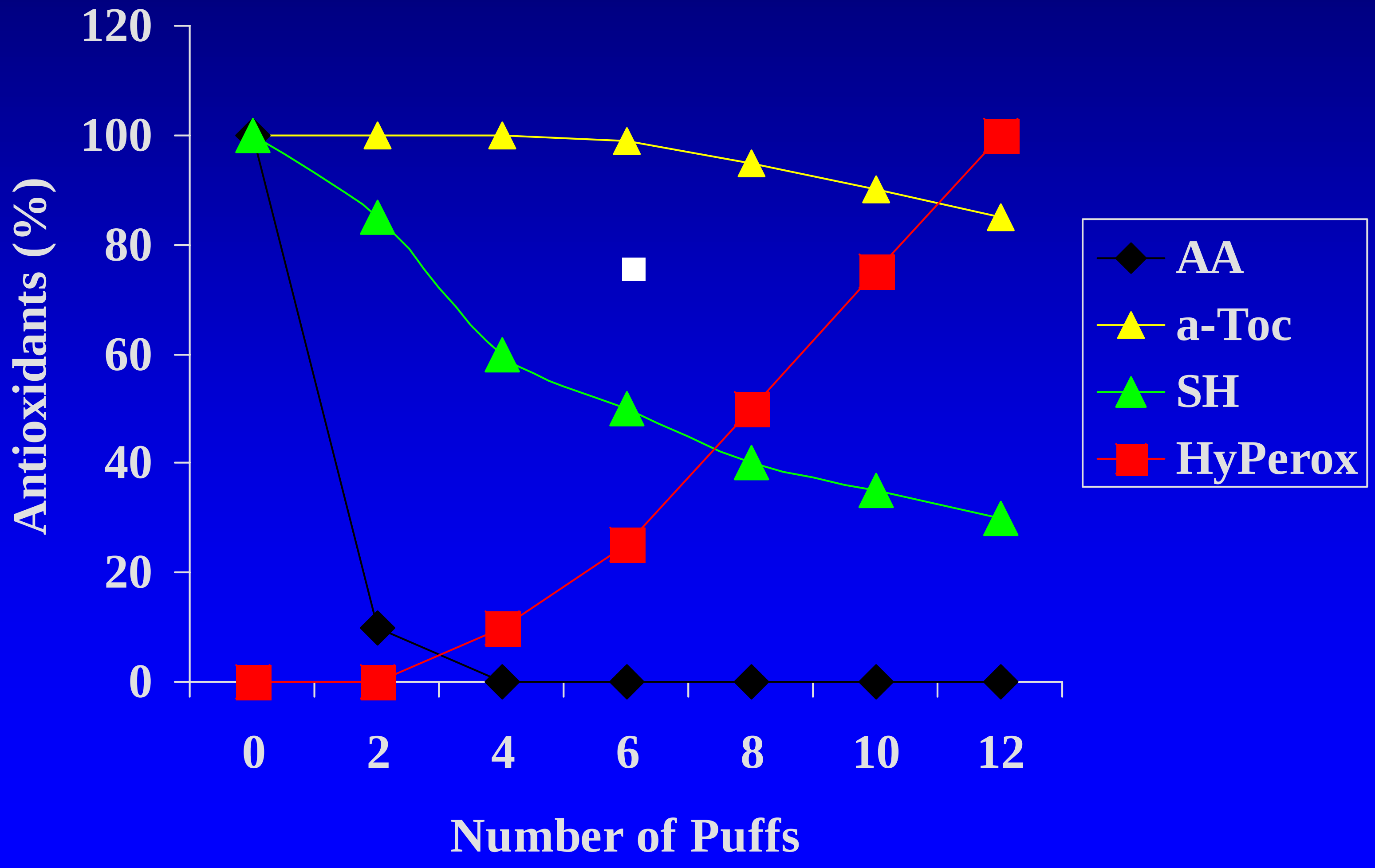
- Carotenoidi
- Polifenoli

THE NON ENZIMATIC ANTIOXIDANT NETWORK

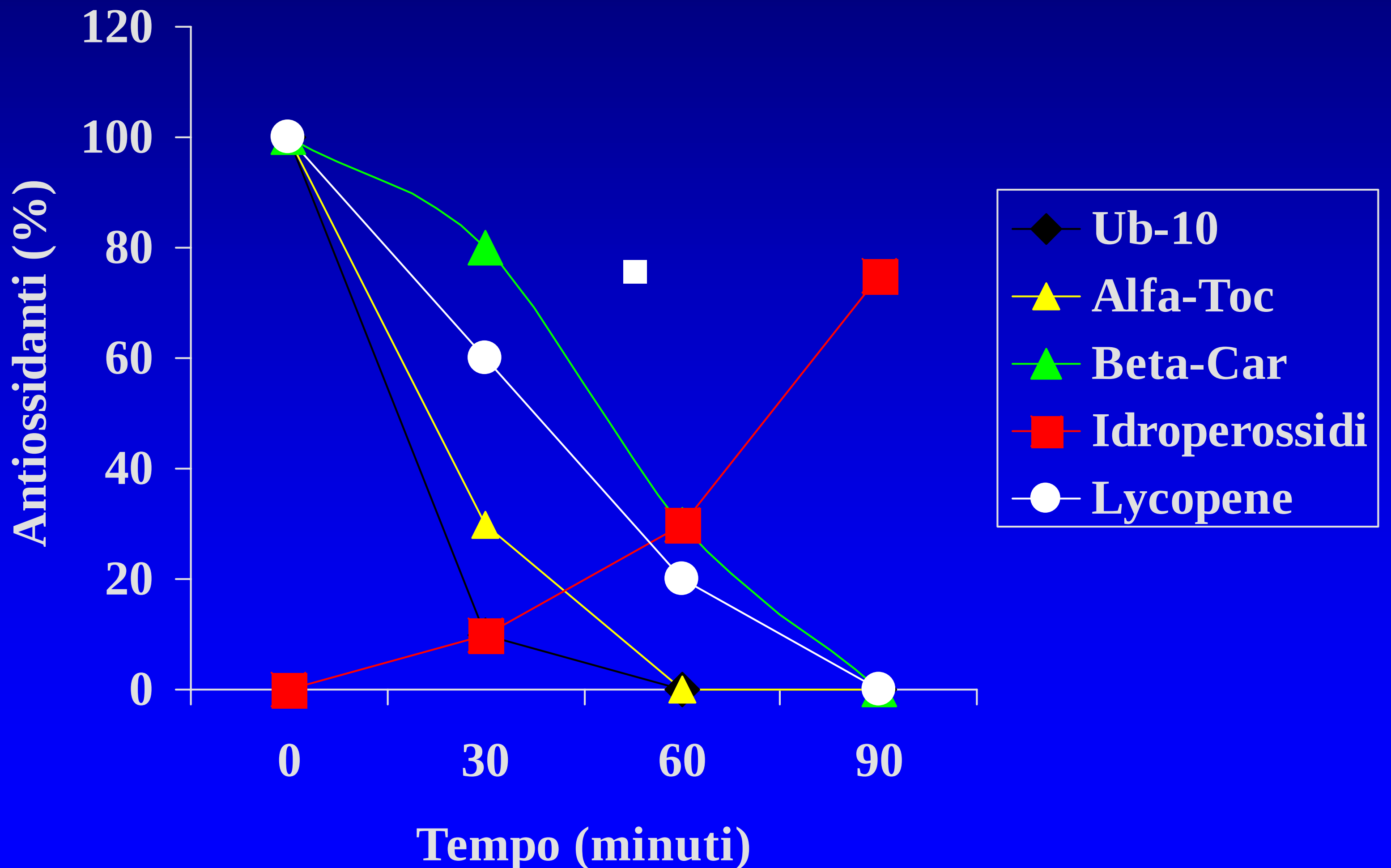


Vit. E Uri … henolics

Antioxidant plasma defence and cigarette smoke



REDOX NETWORK E STRESS OSSIDATIVO LIPOFILO



Frei et al. 1991

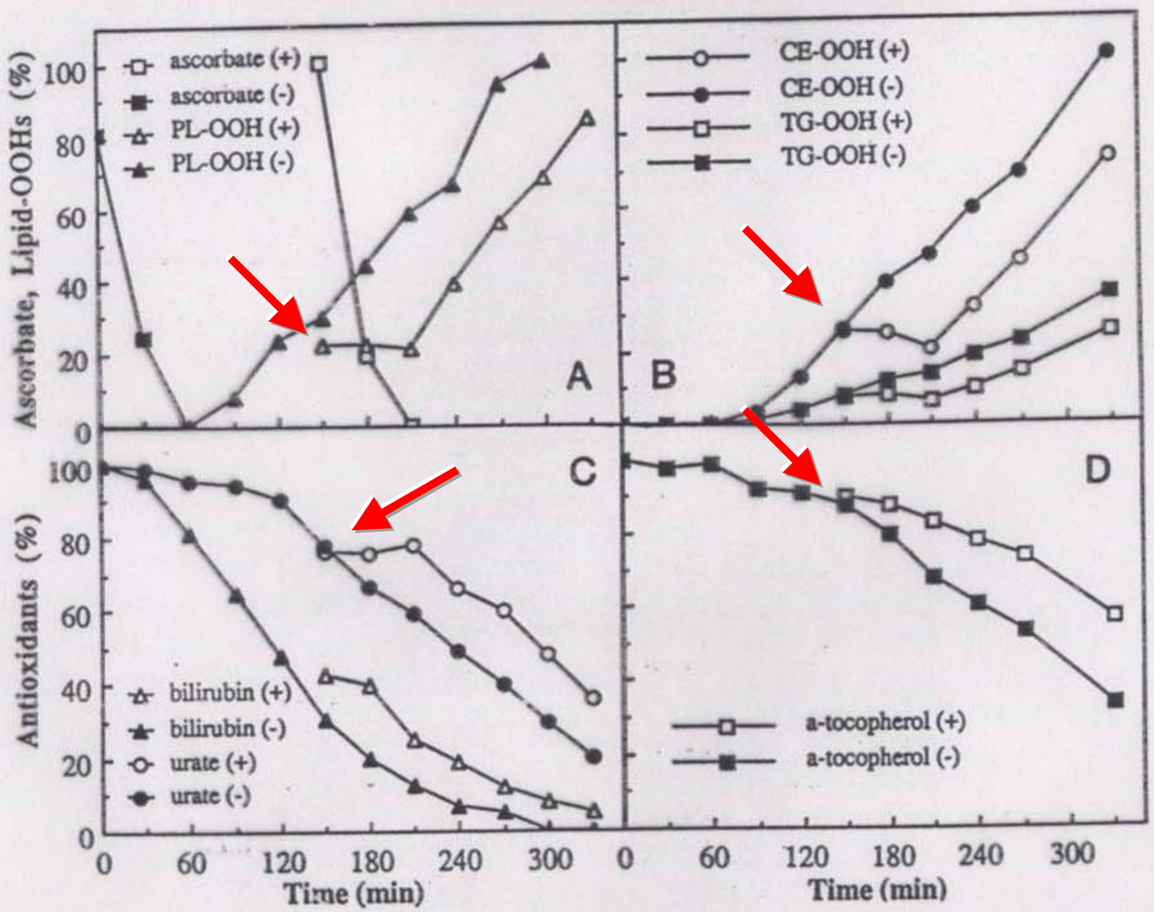
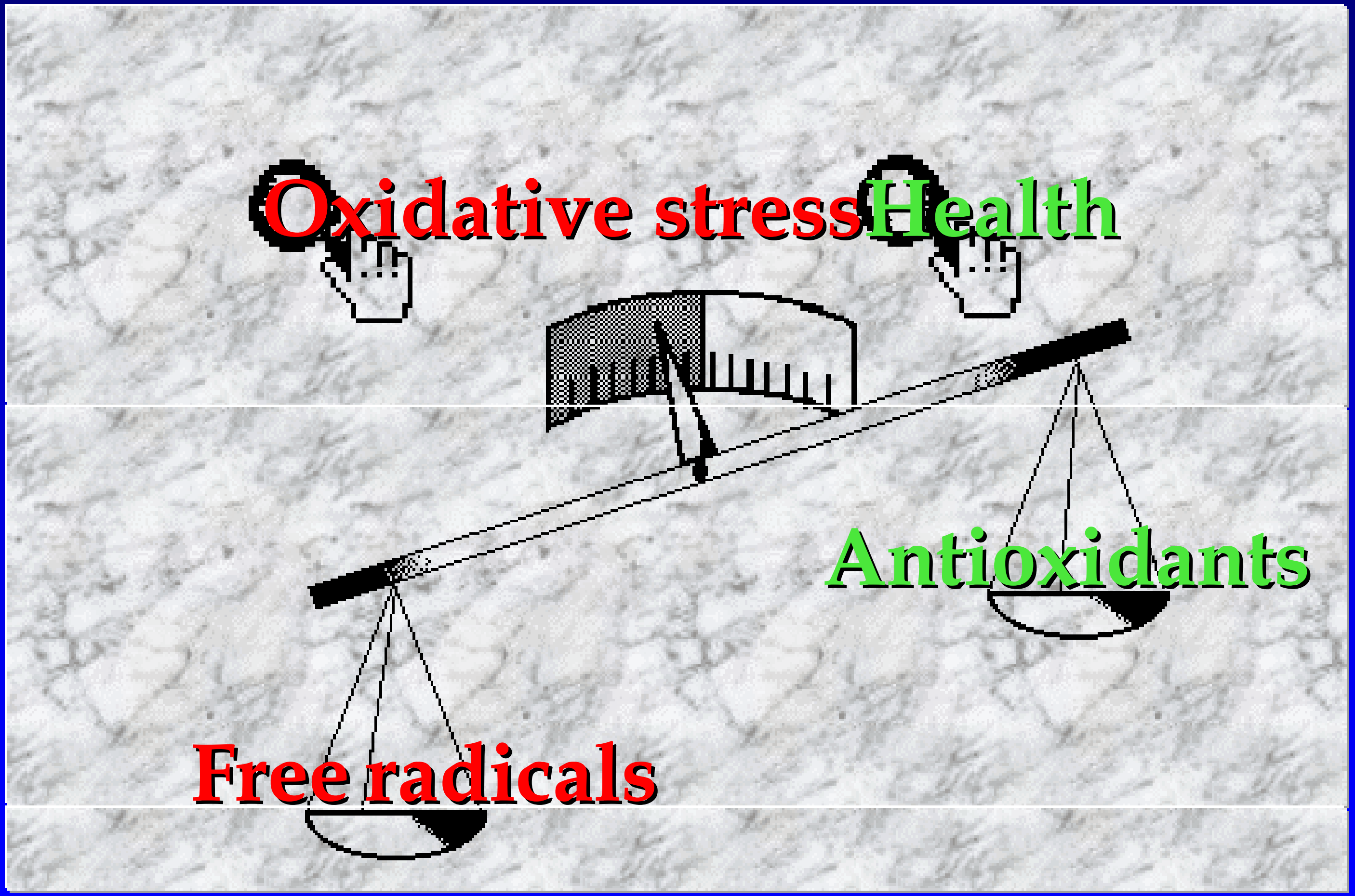
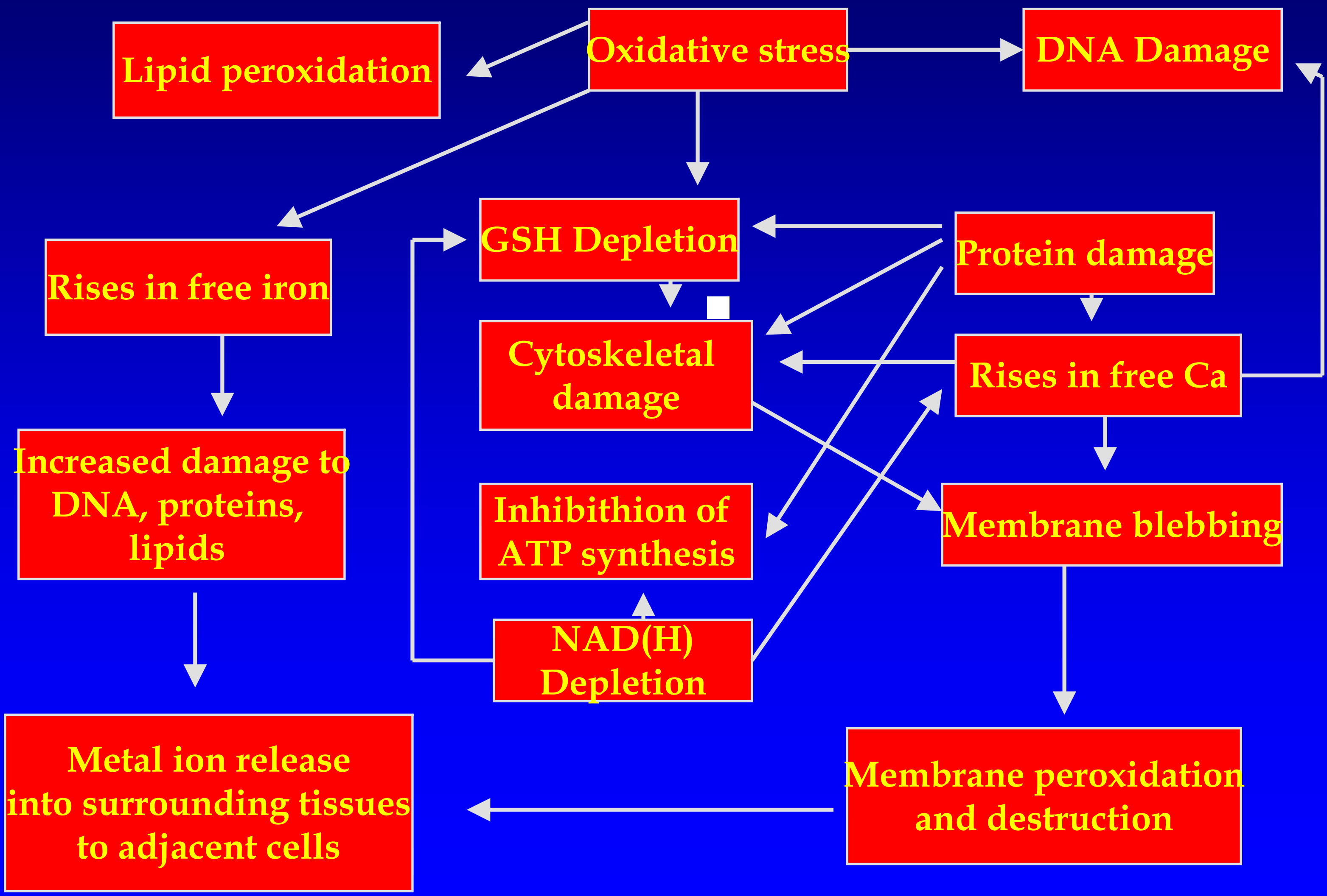


FIG 2. Interruption by ascorbic acid of ongoing lipid peroxidation (panels A and B) and antioxidant depletion (panels C and D) in plasma exposed to aqueous peroxyl radicals. Plasma containing 81 μmol endogenous ascorbic acid/L was incubated at 37 °C with 50 mmol AAPH/L, and after 150 min of incubation 100 μmol ascorbic acid was added (open symbols, +). No ascorbic acid was added to the control incubation (closed symbol, −). Lipid-OOHs, lipid hydroperoxides; PL-OOH, phospholipid hydroperoxides; CE-OOH, cholesterol ester hydroperoxides; TG-OOH, triglyceride hydroperoxides. 100% = 100 μmol ascorbic acid/L; 75 μmol phospholipid hydroperoxides/L; 146 μmol cholesterol ester hydroperoxides or triglyceride hydroperoxides/L; 9.9 μmol bilirubin/L; 334 μmol urate/L; 25.1 μmol α-tocopherol/L. From reference 7.

RED-OX EQUILIBRIUM



CELL INJURY



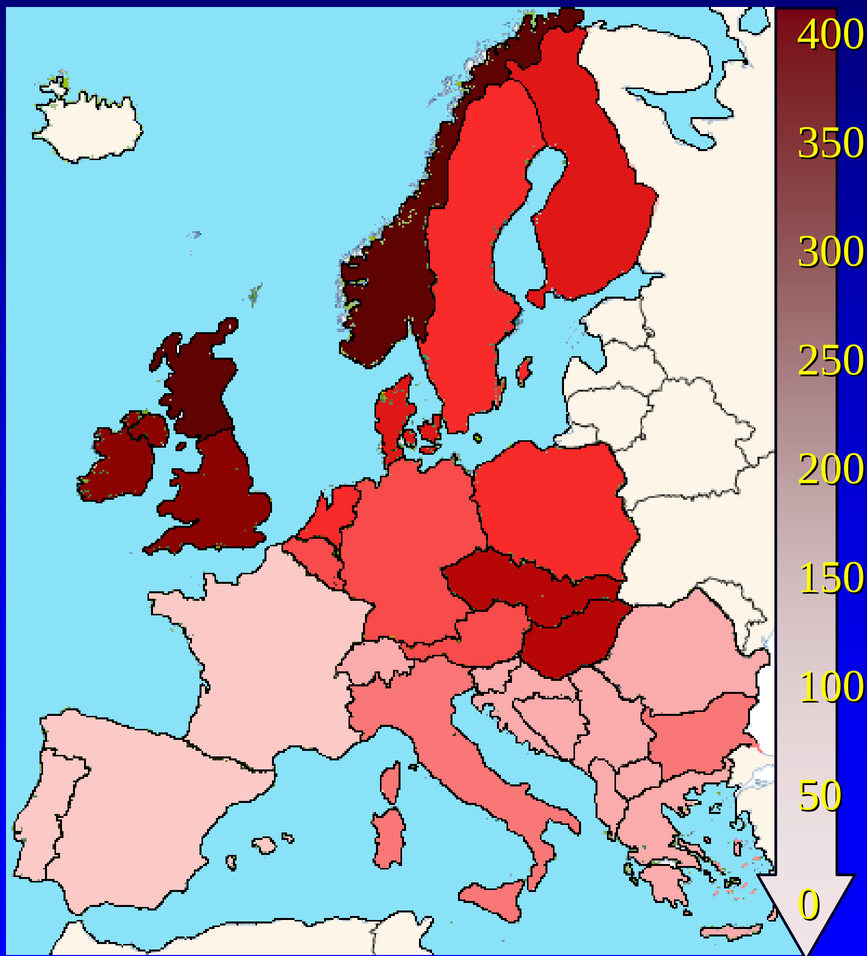
Lipid peroxidation
Oxidative stress
DNA Damage
GSH Depletion
Protein damage
Rises in free iron
Cytoskeletal damage
Rises in free Ca
Increased damage to DNA, proteins, lipids
Inhibithion of ATP synthesis
Membrane blebbing
NAD(H) Depletion
Metal ion release into surrounding tissues to adjacent cells
Membrane peroxidation and destruction

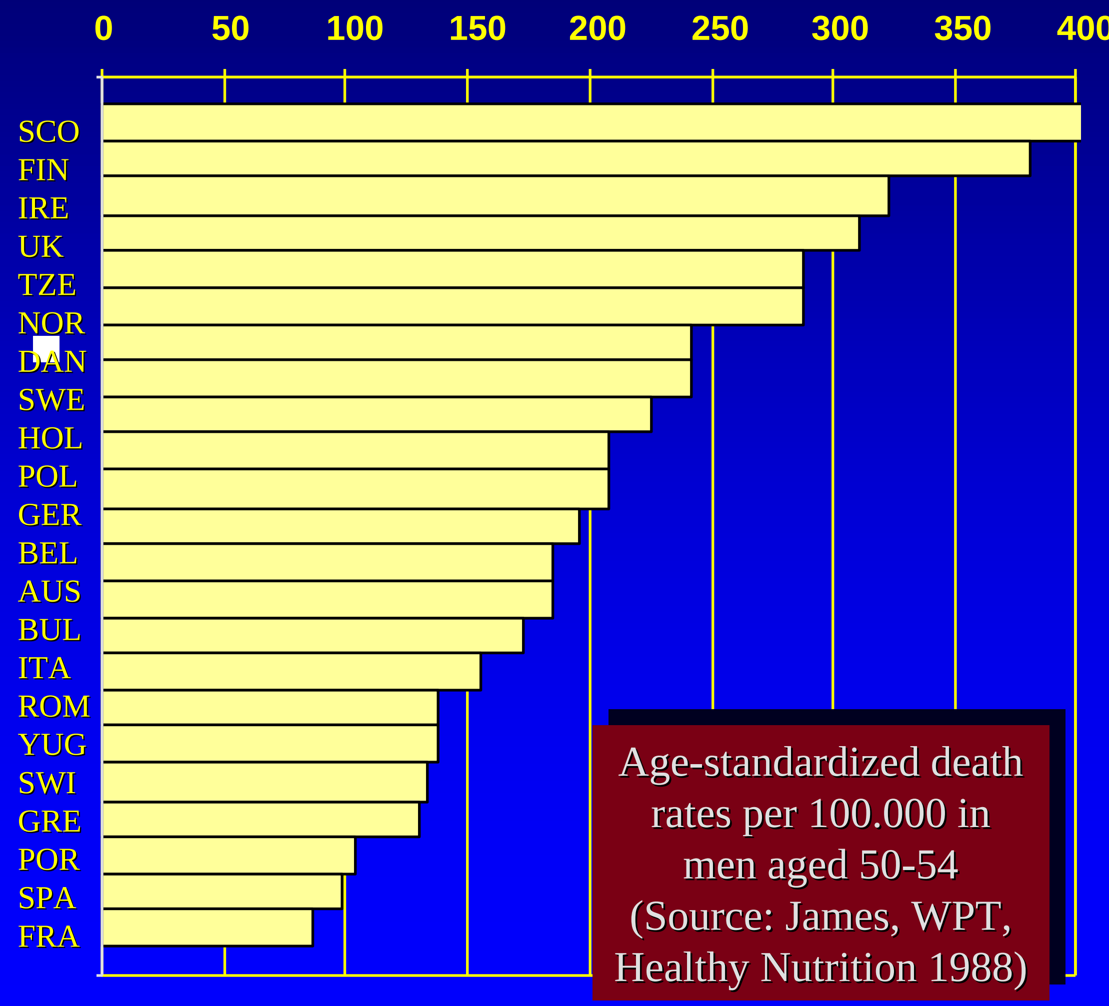
Oxidative stress induced diseases

- ✓ Aging
- ✓ Atherosclerosis
- ✓ Diabetes
- ✓ Cataractogenesis
- ✓ Retinal damage
- ✓ Parkinson’s disease
- ✓ Alzheimer’s disease
- ✓ Muscular dystrophy
- ✓ Multiple sclerosis
- ✓ Lung cancer
- ✓ Emphysema
- ✓ Fanconi’s anemia
- ✓ Thalassemia
- ✓ Autoimmune disease
- ✓ Rheumatoid arthritis
- ✓ Stroke
- ✓ Myocardial infarction
- ✓ Alcohol-induced pathology
- ✓ Glomerulonephritis
- ✓ Down’s syndrome

Dietary Recommendations Keep Evolving

Mortality rates for CHD in Europe

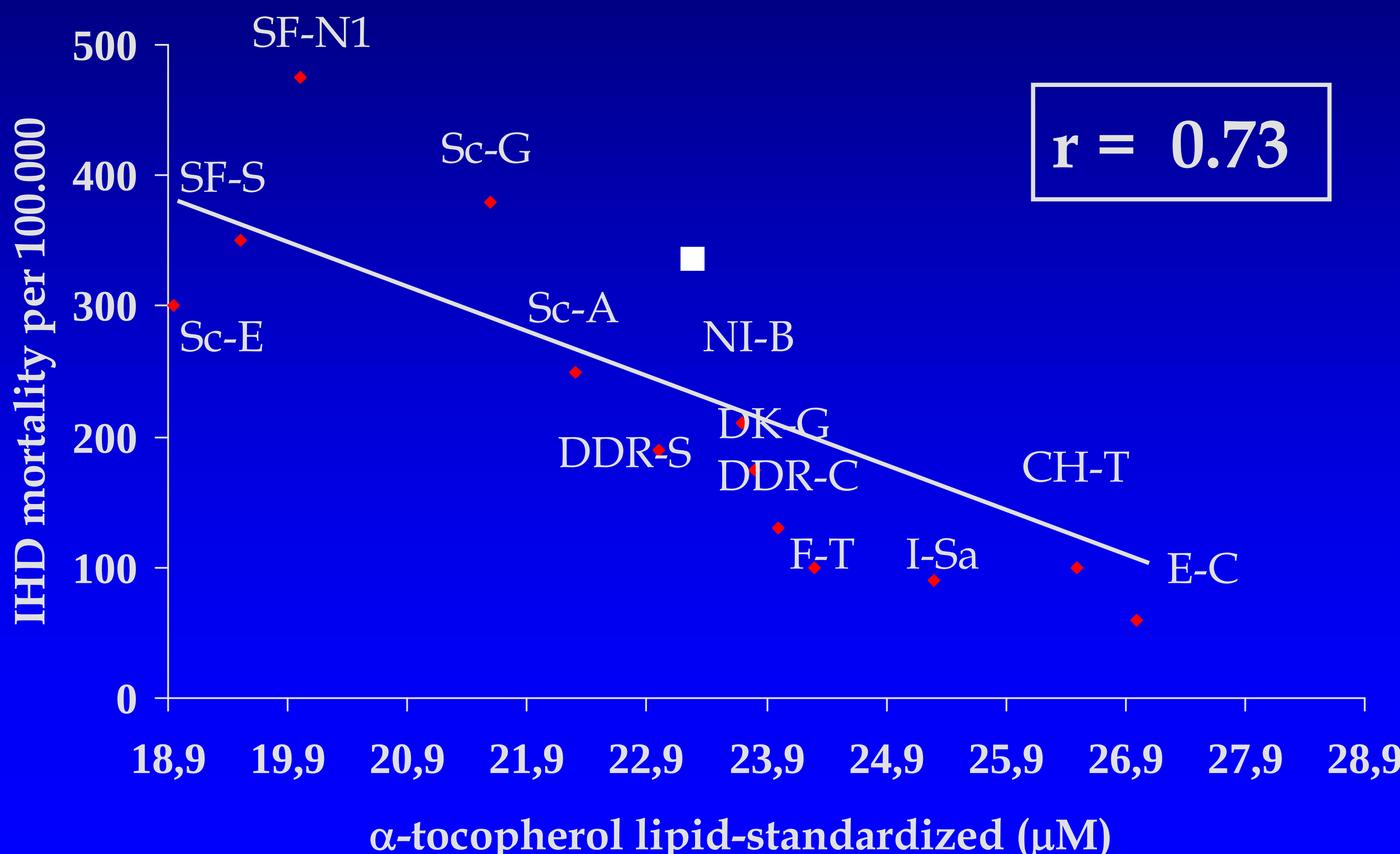




Mediterranean Diet – Common Features

- Olive oil
- Fish and shellfish
- Cereal grains (complex carbohydrates)
- Legumes
- Vegetables
- Fruits
- Wine
- Physical activity

Antioxidant hypothesis of cardiovascular disease



Meta-analysis of randomised trials: β-carotene

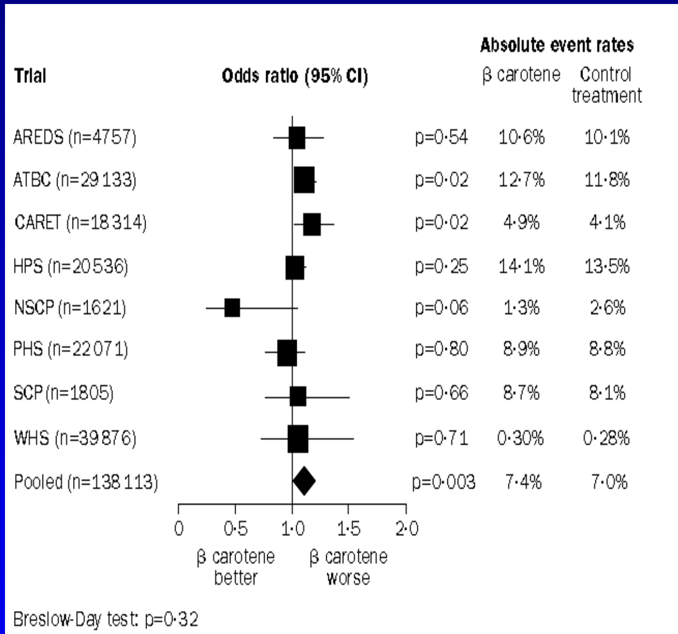


Figure 1: **Odds ratios (95% CI) of all-cause mortality for individuals treated with β carotene or control therapy**

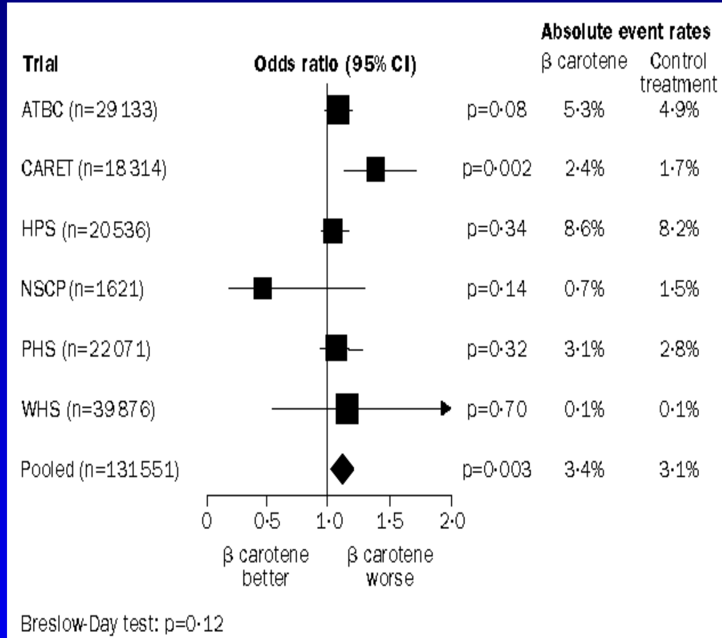


Figure 2: **Odds ratios (95% CI) of cardiovascular death for individuals treated with β carotene or control therapy**

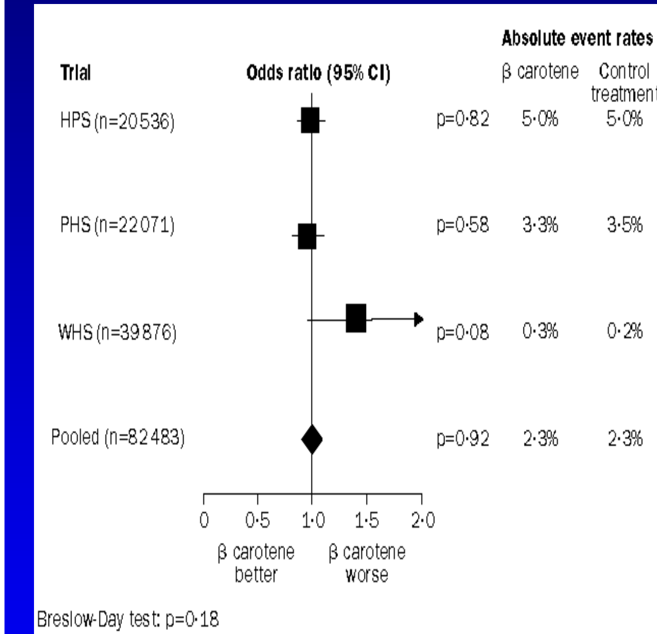


Figure 3: **Odds ratios (95% CI) of all-cause cerebrovascular accident for individuals treated with β carotene or control therapy**

All cause mortality

Cardiovascular death

Cerebrovascular accident

Vivekananthan D.P. et al. The Lancet 2003

Meta-analysis of randomised trials: Vitamin E

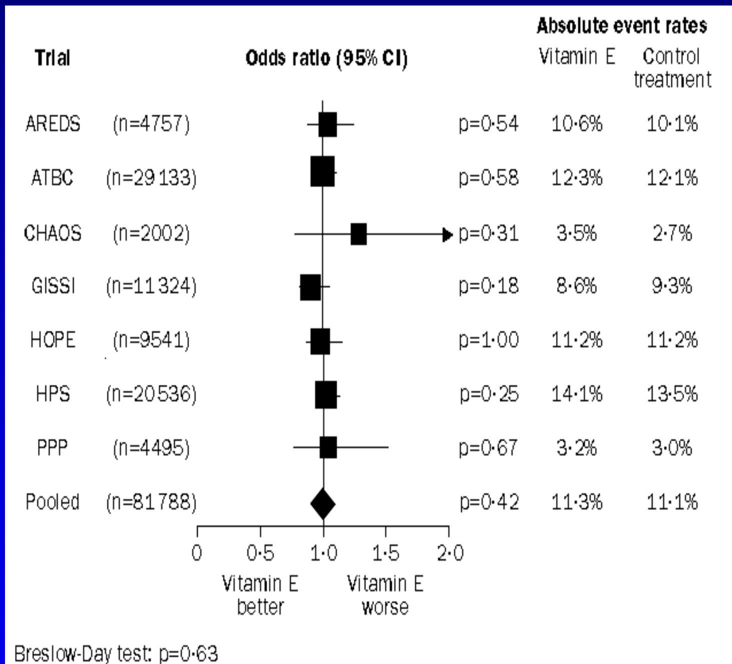


Figure 4: **Odds ratios (95% CI) of all-cause mortality for individuals treated with vitamin E or control therapy**

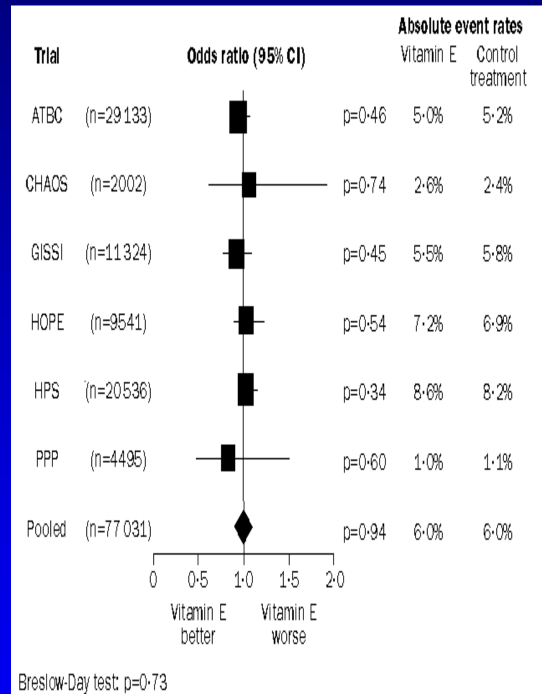


Figure 5: **Odds ratios (95% CI) of cardiovascular death for individuals treated with vitamin E or control therapy**

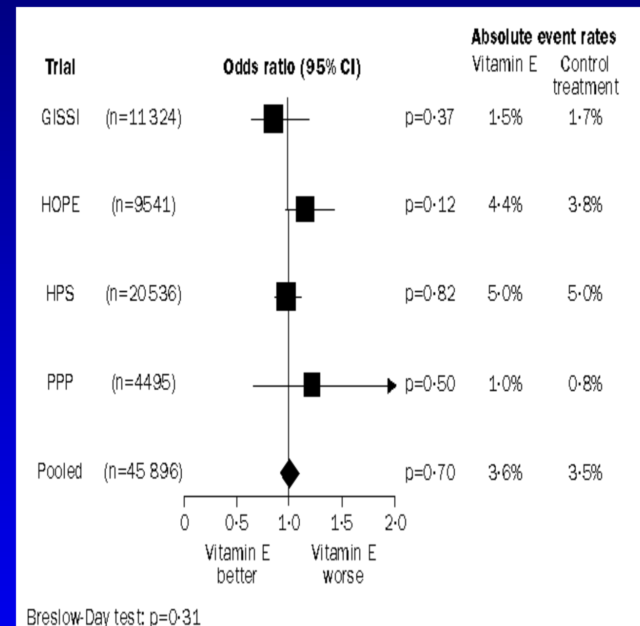


Figure 6: **Odds ratios (95% CI) of all-cause stroke for individuals treated with vitamin E or control therapy**

All cause mortality

Cardiovascular death

Stroke

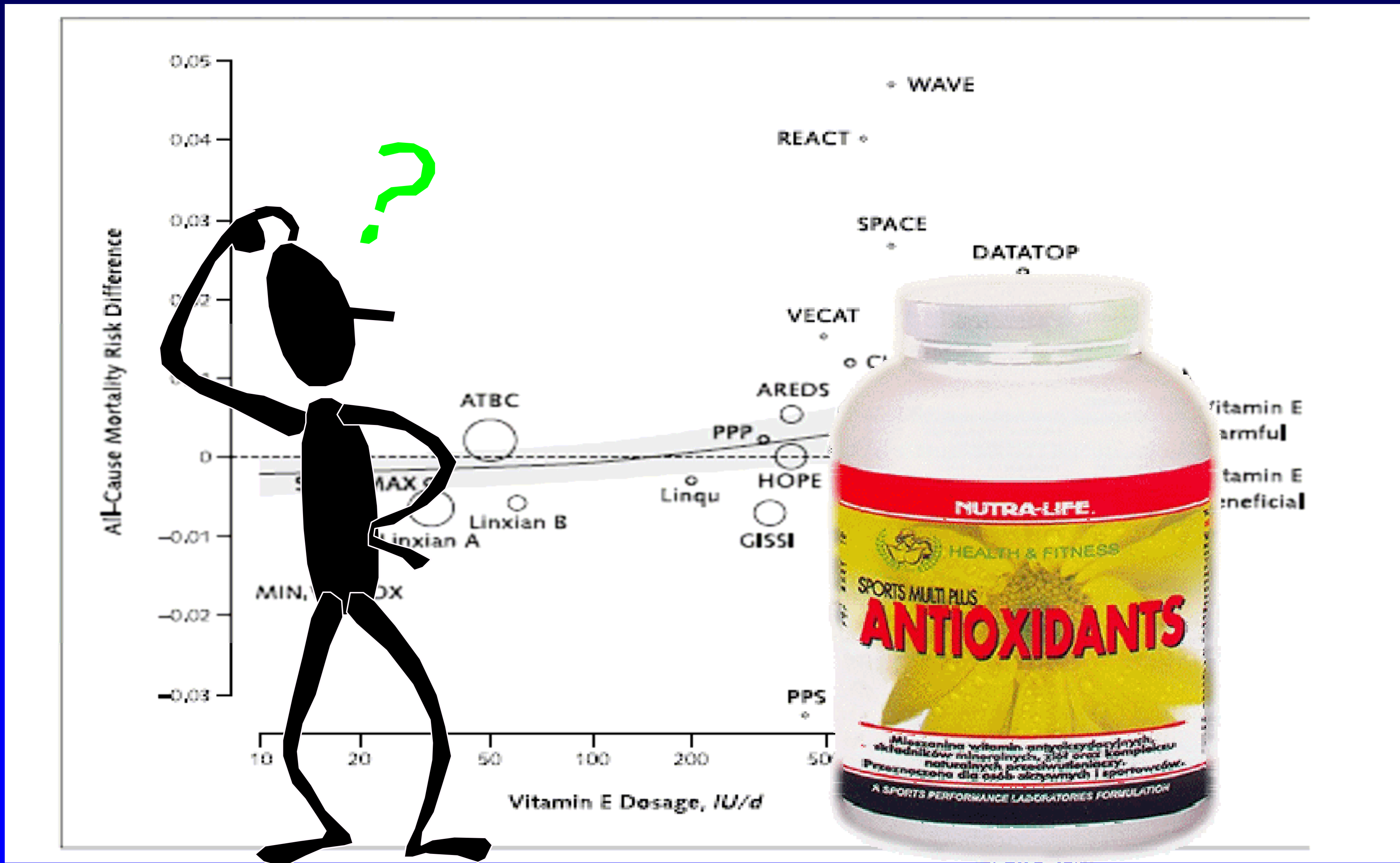
Vivekananthan D.P. et al. The Lancet 2003

Study	Country	Subjects	Intervention	Outcome
Blot et al, 1993	China	29584 subjects, 40-69 years	5-year supplementation 15 mg/d β-carotene + 30 mg/d vitamin E + 50 µg Se	Decrease in total cancer rates (RR = 0.87) Decrease in stomach cancer rates (RR = 0.79)
ATBC	Finland	29133 chronic heavy smokers (M) 50-69 years	8-year supplementation 50 mg/d α-tocopherol 20 mg/d β-carotene combination of both	β-carotene group: Significant increase (18 %) in lung cancer incidence
CARET	USA	18 314 subjects, smokers, former smokers and asbestos-exposed workers 45-74 years	4-year supplementation 30 mg/d β-carotene 25000 IU retinol	Significant increase in lung cancer incidence (28 %; RR = 1.36)
Physician's Health Study,	USA	11000 healthy physicians (M) 40-84 years	12-year supplementation 50 mg/d β-carotene	No changes in lung cancer incidence and mortality
Clark et al, 1996	USA	1312 patients with lesions on skin, mean age 63 years 18-80 years	4.5-year supplementation 200 µg/d Se	No significant changes on incidence of skin cancer No significant reduction in all-cause mortality Significant reduction in total cancer mortality (RR = 0.50) and incidence (RR = 0.63) Significant reduction in prostate cancer (RR = 0.37), colorectal cancer (RR = 0.42) and lung cancer (RR = 0.54)

Serafini et al. Nutr & Cancer 2007

Women's Health)	**USA**	**39876 female health professionals ≥ 45 years**	**2-year supplementation 50 mg/d β-carotene**	**No significant differences in cancer risk and cardiovascular death**
Heart Protection Study	**UK**	**20000 patients with coronary disease,cardiovascular events, diabetes 40-80 years**	**5-year supplementation 600 mg/d vitamin E,250 mg/d vitamin C, 20 mg/d β-carotene**	**No changes in cancer incidence**
SUVIMAX Study	**France**	**12741subjects (M,W) 45-60 years**	**7.5-year supplementation vitamin C, vitamin E β-carotene, Se, zinc**	**Women: no changes in cancer incidence Men: significant decrease in prostate cancer incidence (RR = 0.52)**
HOPE trial	**Multi center**	**patients with cardiovascular disease or diabetes mellitus ≥ 55 years**	**6-year supplementation (9541 subjects) 400 IU/d Vitamin E**	**No differences in cancer incidence and mortality Higher risk of heart failure (RR = 1.21)**

TO PILLS OR NOT TO PILLS…



Miller E.R. et al. 2005

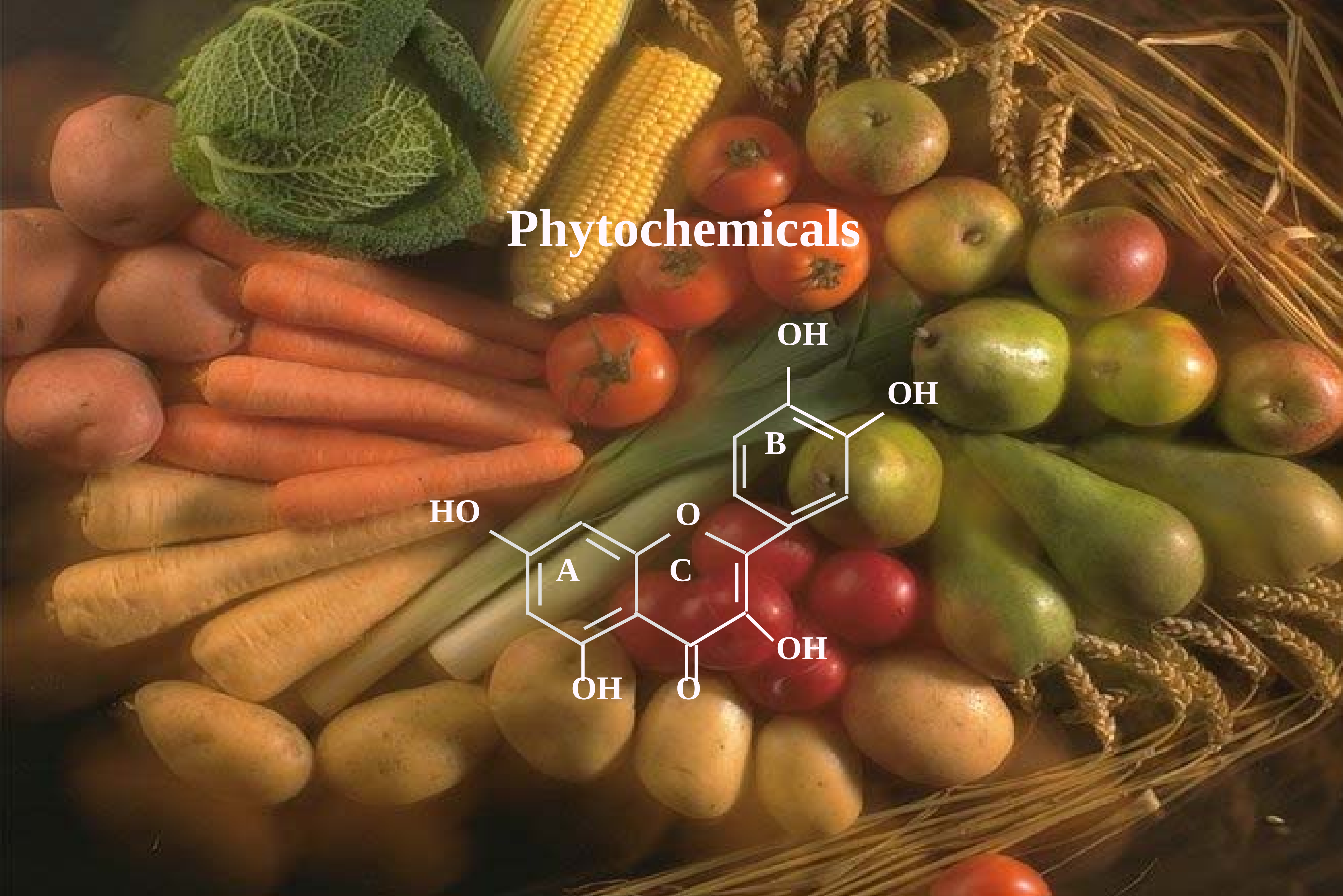
Journal of the Science of Food and Agriculture *J Sci Food Agric* **86**:1989–1991 (2006)

Perspective

Back to the origin of the 'antioxidant hypothesis': the lost role of the antioxidant network in disease prevention

Scotland. In his pioneer study, Gey showed that an index representative of the pro- and antioxidant factors – cholesterol level divided by an arbitrary

that high intakes of dietary antioxidants prevent oxidation of plasma and thereby provide protection against diseases induced by oxidative stress. The importance of synergistic interactions between dietary antioxidants playing a key role in a multilevel defence system against radical attack was recognized by Gey,[1]


Phytochemicals
OH
OH
B
HO
O
A
C
OH
OH
O

Antioxidants Phytochemicals

- Important chemicals found in foods
- Not currently known if they are required
- Play an important role in prevention of dz
- May be up to 10,000 in a single food
- Over 600 currently identified
- Like nutrients, also found in foods
- Many different methods of identifying, many foods not yet tested for their content
- Numbers currently reported not yet complete

Polyphenolics:

Swain and Bate-Smith

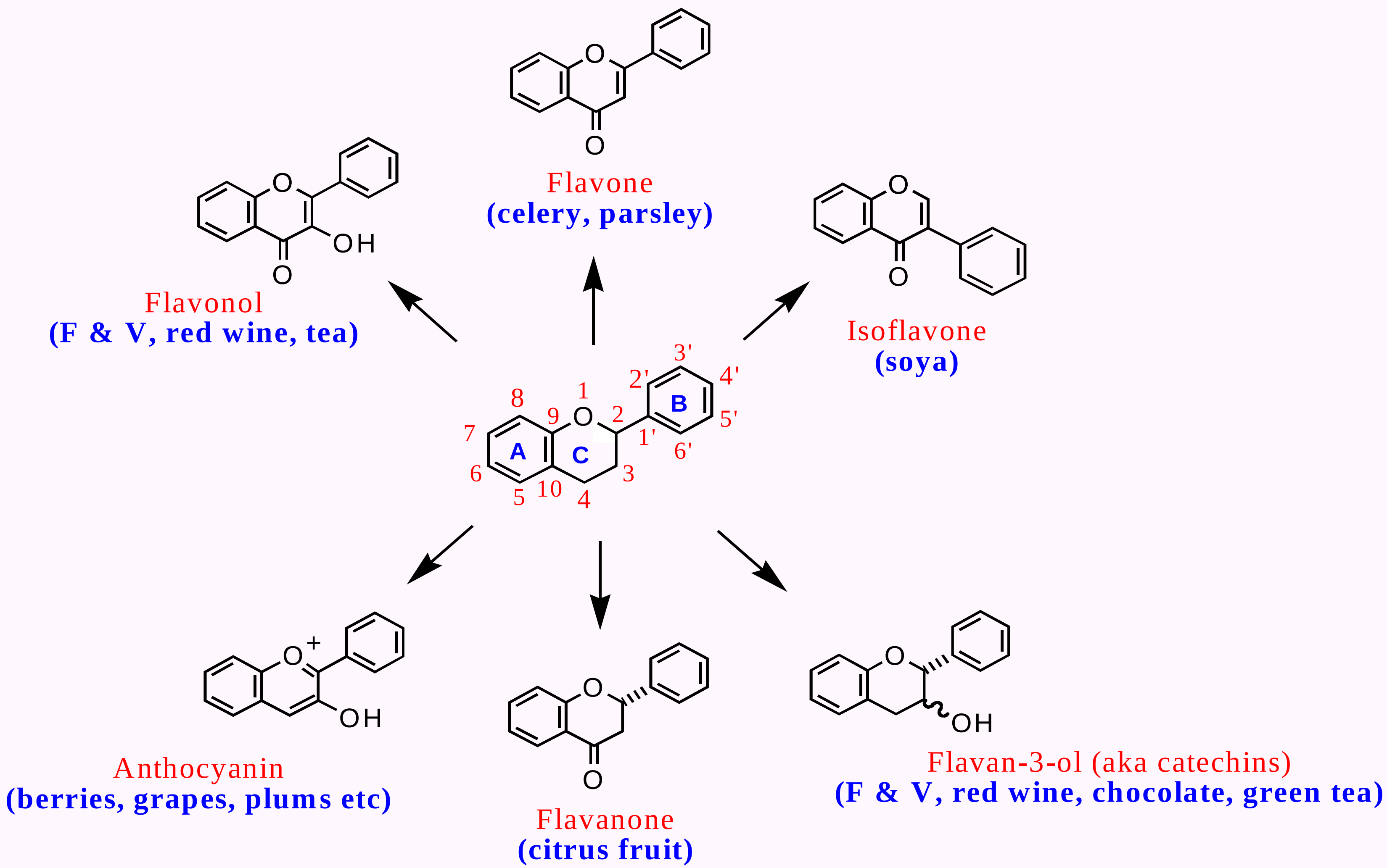
“Phenolic compounds vary not only in the number and orientation of phenolic hydroxyl groups but also in the vast number of basic skeletons which they possess. Therefore, their physical parameters or their response to any given analytical reagent is almost as varied as the number of compounds themselves.”

Comparative Biochemistry, vol III, p 755 (1962)

POLYPHENOLS

- **Monomer**
  - Flavonoid
    - Flavone
    - Flavonol
    - Flavonone
    - Flavononol
    - Isoflavone
    - Anthocyanine
    - Chalcone
    - Aurone
  - Chlorogenic acid
  - Gallic acid
  - Ellagic acid
- **Polymer (Tannin)**
  - Condensed type
    - Proanthocyanidine
  - Hydrolysable type
    - Gallotannin
    - Ellagtannin

Common C_6-C_3-C_6 Flavonoid Structures



Phenolics content of foods

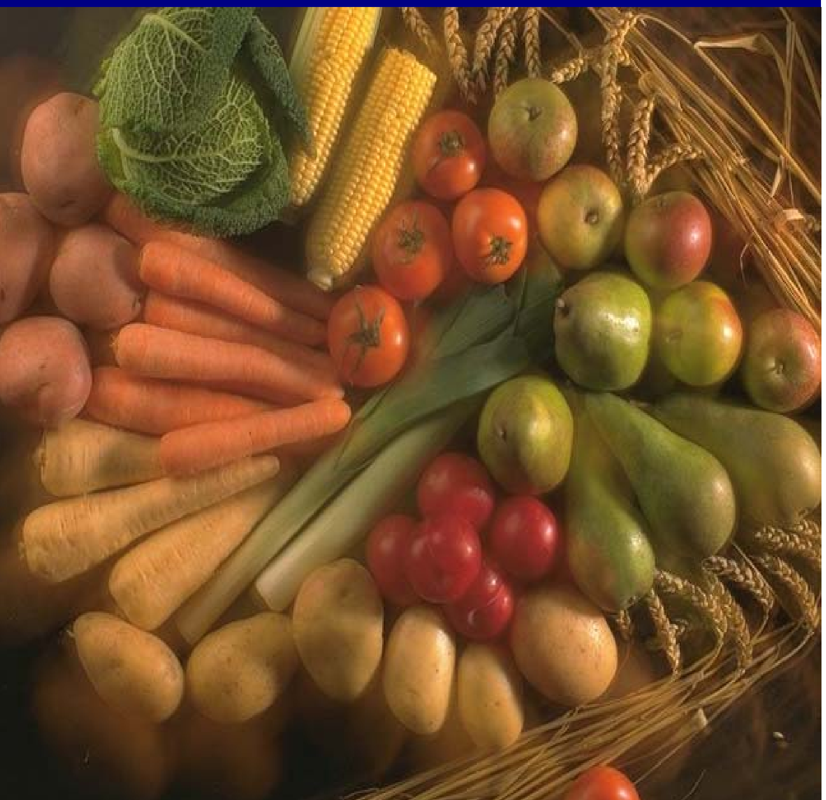


TABLE 1
Polyphenols in foods

	Source (serving size)	Polyphenol content: By wt or vol	Polyphenol content: By serving
		mg/kg fresh wt (or mg/L)	*mg/serving*
Hydroxybenzoic acids (2, 6)	Blackberry (100 g)	80–270	8–27
Protocatechuic acid	Raspberry (100 g)	60–100	6–10
Gallic acid	Black currant (100 g)	40–130	4–13
p-Hydroxybenzoic acid	Strawberry (200 g)	20–90	4–18
Hydroxycinnamic acids (2, 5–7)	Blueberry (100 g)	2000–2200	200–220
Caffeic acid	Kiwi (100 g)	600–1000	60–100
Chlorogenic acid	Cherry (200 g)	180–1150	36–230
Coumaric acid	Plum (200 g)	140–1150	28–230
Ferulic acid	Aubergine (200 g)	600–660	120–132
Sinapic acid	Apple (200 g)	50–600	10–120
	Pear (200 g)	15–600	3–120
	Chicory (200 g)	200–500	40–100
	Artichoke (100 g)	450	45
	Potato (200 g)	100–190	20–38
	Corn flour (75 g)	310	23
	Flour: wheat, rice, oat (75 g)	70–90	5–7
	Cider (200 mL)	10–500	2–100
	Coffee (200 mL)	350–1750	70–350
Anthocyanins (8–10)	Aubergine (200 g)	7500	1500
Cyanidin	Blackberry (100 g)	1000–4000	100–400
Pelargonidin	Black currant (100 g)	1300–4000	130–400
Peonidin	Blueberry (100 g)	250–5000	25–500
Delphinidin	Black grape (200 g)	300–7500	60–1500
Malvidin	Cherry (200 g)	350–4500	70–900
	Rhubarb (100 g)	2000	200
	Strawberry (200 g)	150–750	30–150
	Red wine (100 mL)	200–350	20–35
	Plum (200 g)	20–250	4–50
	Red cabbage (200 g)	250	50
Flavonols (11–18)	Yellow onion (100 g)	350–1200	35–120
Quercetin	Curly kale (200 g)	300–600	60–120
Kaempferol	Leek (200 g)	30–225	6–45
Myricetin	Cherry tomato (200 g)	15–200	3–40
	Broccoli (200 g)	40–100	8–20
	Blueberry (100 g)	30–160	3–16
	Black currant (100 g)	30–70	3–7
	Apricot (200 g)	25–50	5–10
	Apple (200 g)	20–40	4–8
	Beans, green or white (200 g)	10–50	2–10
	Black grape (200 g)	15–40	3–8
	Tomato (200 g)	2–15	0.4–3.0
	Black tea infusion (200 mL)	30–45	6–9
	Green tea infusion (200 mL)	20–35	4–7
	Red wine (100 mL)	2–30	0.2–3
Flavones (11–12, 14, 18)	Parsley (5 g)	240–1850	1.2–9.2
Apigenin	Celery (200 g)	20–140	4–28
Luteolin	Capsicum pepper (100 g)	5–10	0.5–1
Flavanones (19–21)	Orange juice (200 mL)	215–685	40–140
Hesperetin	Grapefruit juice (200 mL)	100–650	20–130
Naringenin	Lemon juice (200 mL)	50–300	10–60
Eriodictyol			
Isoflavones (22–25)	Soy flour (75 g)	800–1800	60–135
Daidzein	Soybeans, boiled (200 g)	200–900	40–180
Genistein	Miso (100 g)	250–900	25–90
Glycitein	Tofu (100 g)	80–700	8–70
	Tempeh (100 g)	430–530	43–53
	Soy milk (200 mL)	30–175	6–35
Monomeric flavanols (6, 17, 26, 27)	Chocolate (50 g)	460–610	23–30
Catechin	Beans (200 g)	350–550	70–110
Epicatechin	Apricot (200 g)	100–250	20–50
	Cherry (200 g)	50–220	10–44
	Grape (200 g)	30–175	6–35
	Peach (200 g)	50–140	10–28
	Blackberry (100 g)	130	13
	Apple (200 g)	20–120	4–24
	Green tea (200 mL)	100–800	20–160
	Black tea (200 mL)	60–500	12–100
	Red wine (100 mL)	80–300	8–30
	Cider (200 mL)	40	8

Source: Manach et al. AJCN 2004

II. The role of phenolic antioxidants in plants

- Phenolic antioxidants are secondary metabolites that function primarily in the defense of the plants
- *Functions are varied*:
  - UV screens / antioxidants
  - Protection from certain types of herbivory (anti-feedants)
  - Protection from pathogen attack
  - Function in wound healing
  - Plant pigments (attract pollinators)

Levels will reflect these dynamics

Old Polyphenols vs New Polyphenols

- Antinutrients
- Polyphenol Oxidase Substrates
- Astringe Taste Source

\+

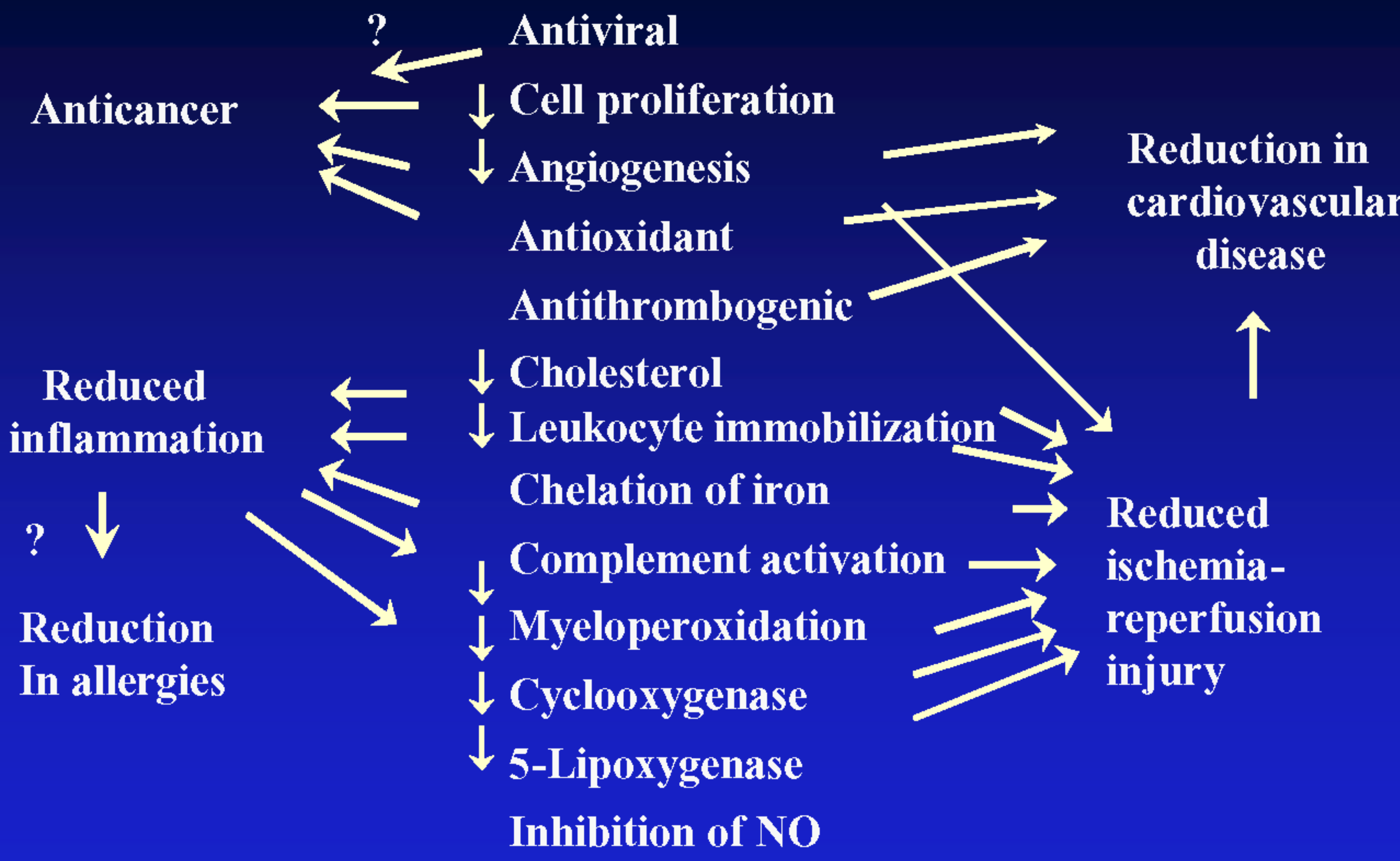
- Antioxidants
- Anticancer agents
- Other biological activities

Total Phenolics Analysis = Total Phenolics Analysis

Folin-Ciocalteu Method

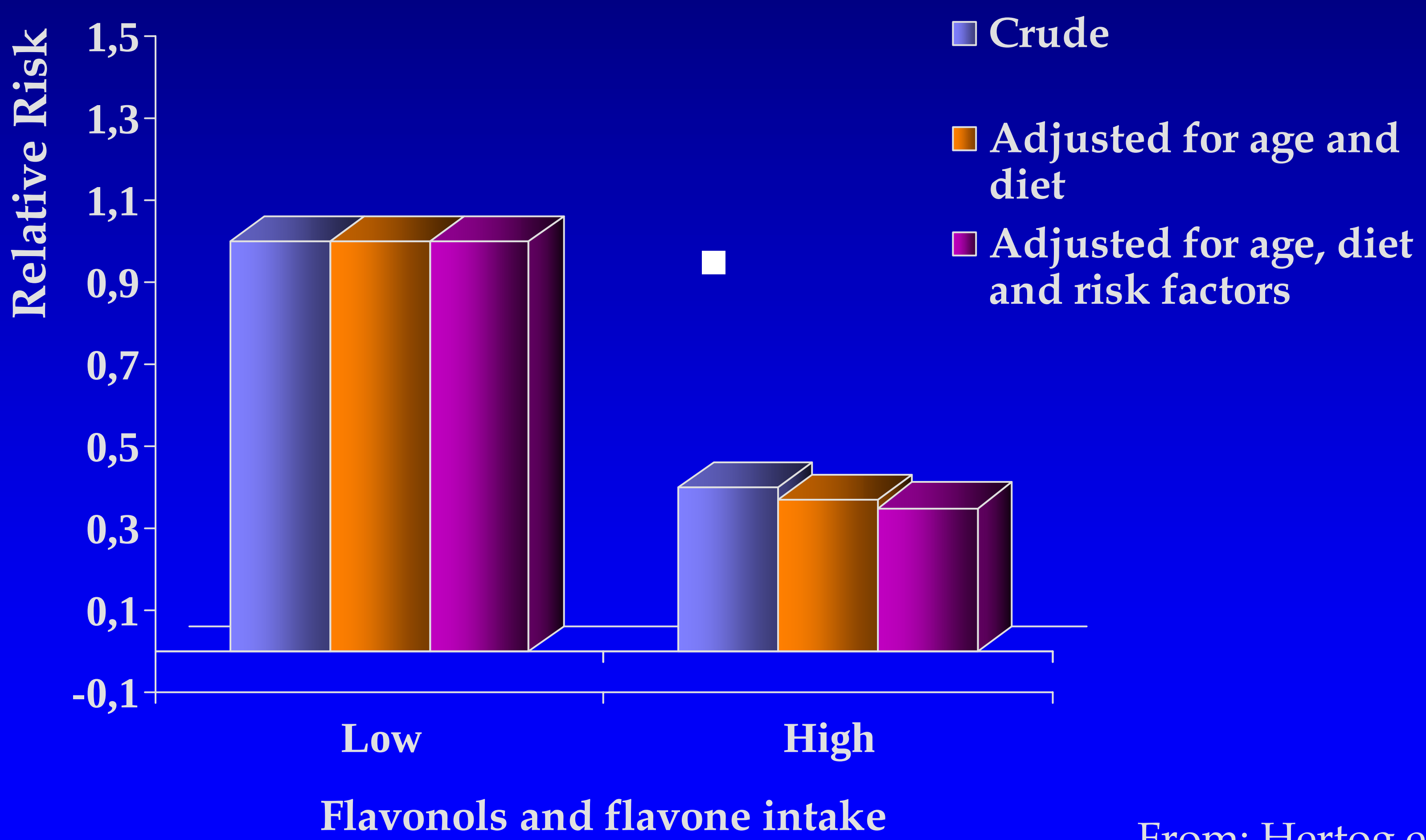
* Of 159 articles on total phenolics, 54 are on Folin-Ciocalteu method

Potential Flavonoid Health Mechanisms



Nijveldt et al. Am J Clin Nutr 2001

Flavonoids and CHD



From: Hertog et al. 1993

Flavonoidi e cardiovascolare

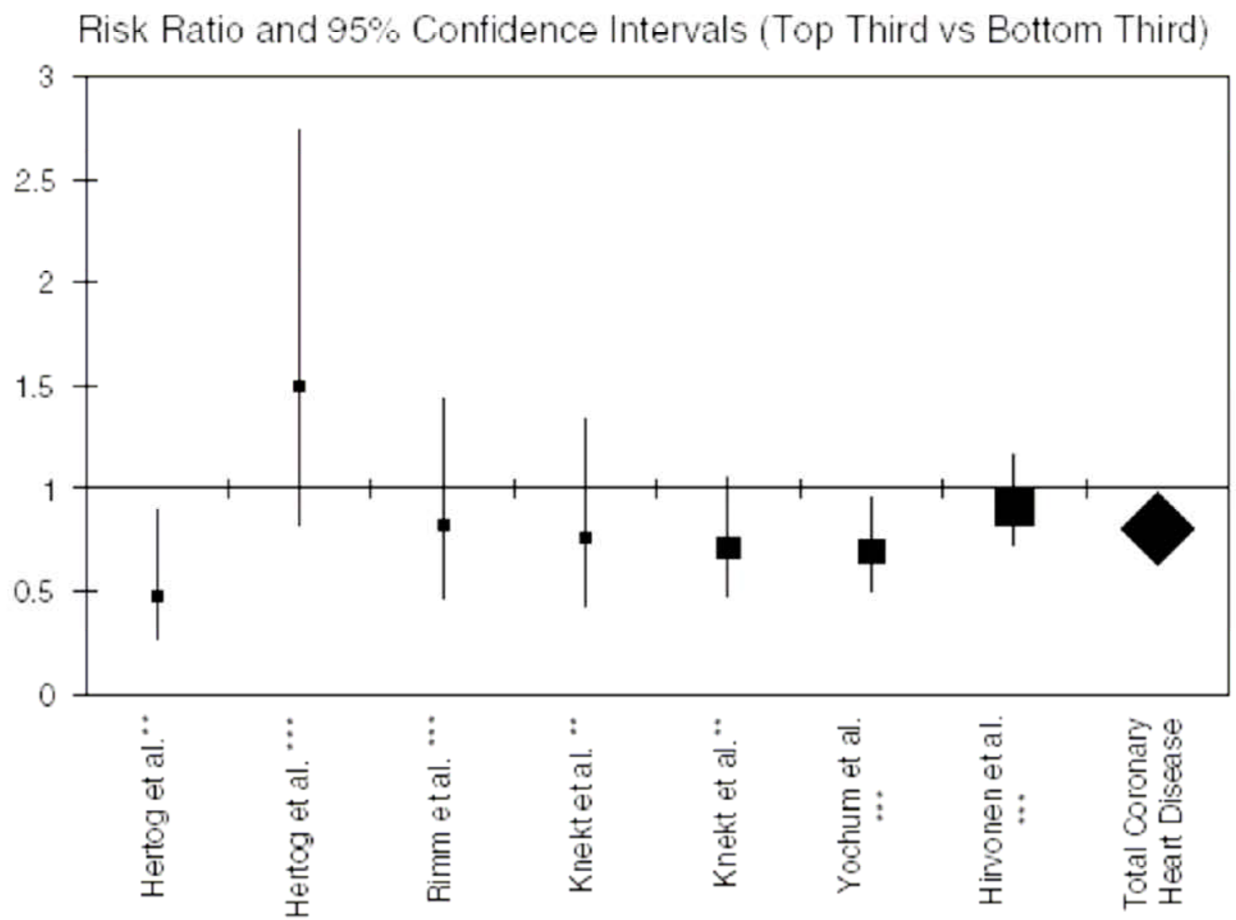
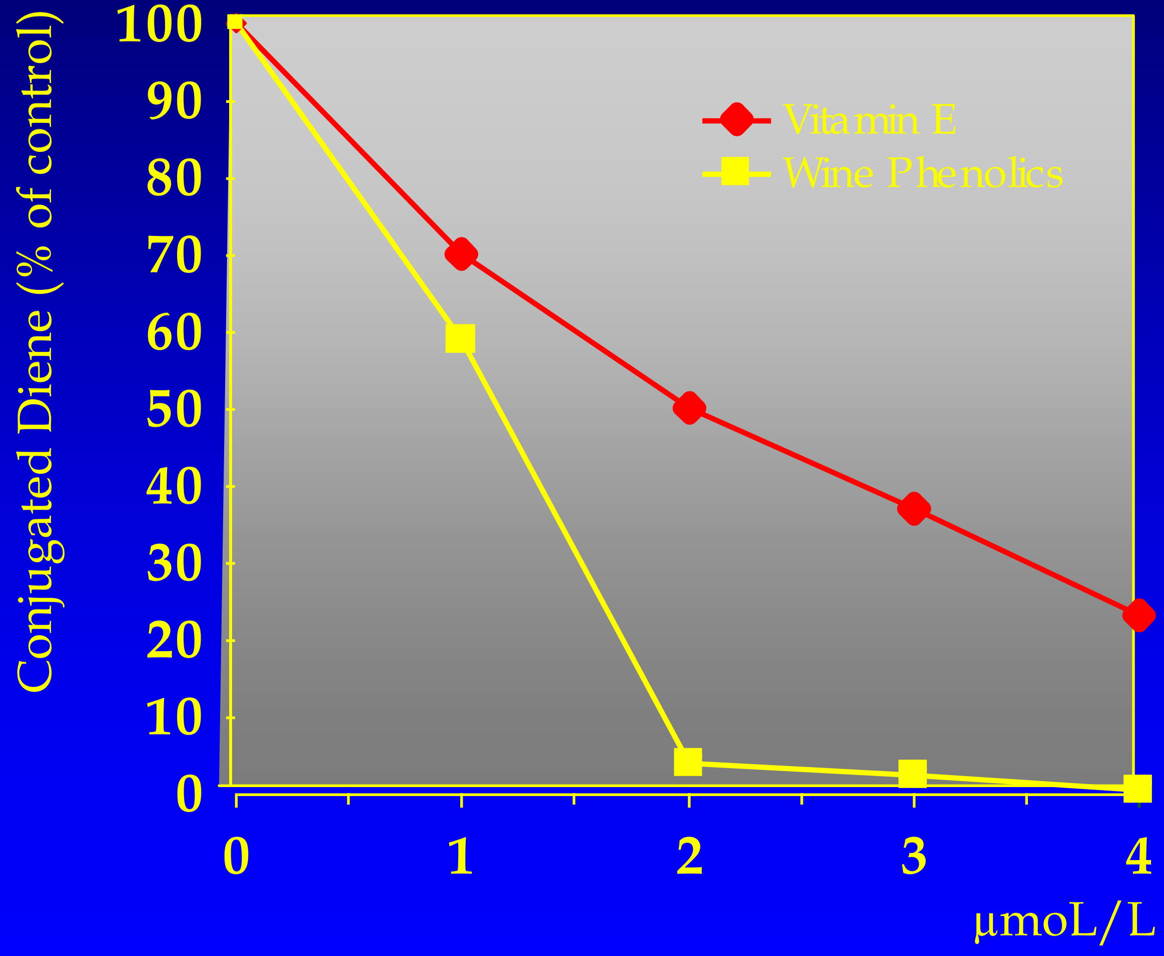


Figure 1 Prospective cohort studies of flavonoid and CHD. Risk ratios compare top and bottom thirds of baseline measurements. Black squares indicate the risk ratio in each study, with the square size proportional to the number of CHD fatal events and the horizontal lines representing 95% CI. The combined risk ratio and its 95% CI is denoted by the black diamond. * indicates level of adjustment; **, adjustment for age sex, smoking, BMI, blood pressure, dietary cholesterol and fat intake; ***, for these plus some measure of social class.

RR = 0.80, (CI 0.69-0.93)

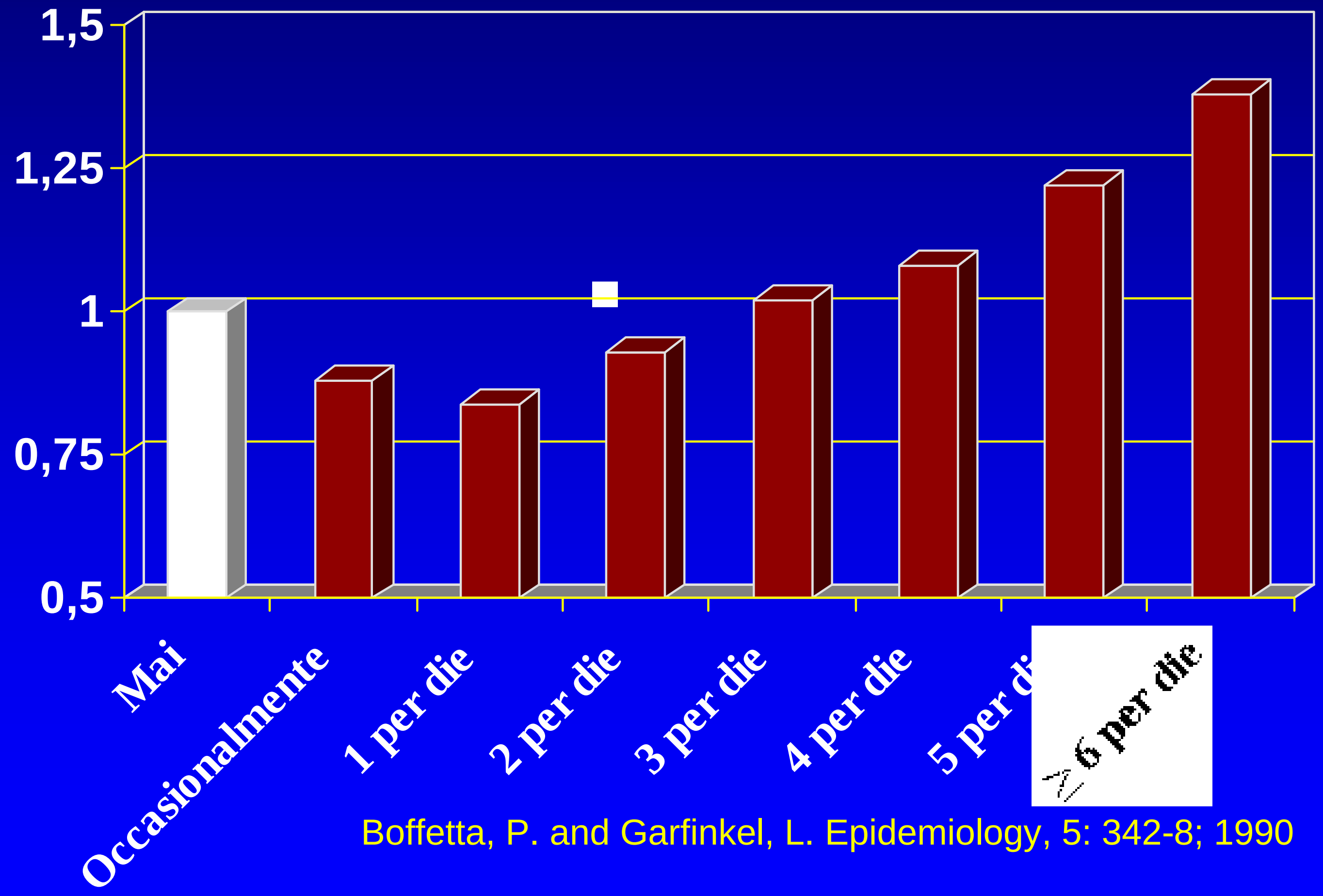
Huxley and Neil, Eur J Clin Nutr 2003; 57: 904-8.

INHIBITION OF LDL OXIDATION BY RED WINE PHENOLIC COMPOUNDS



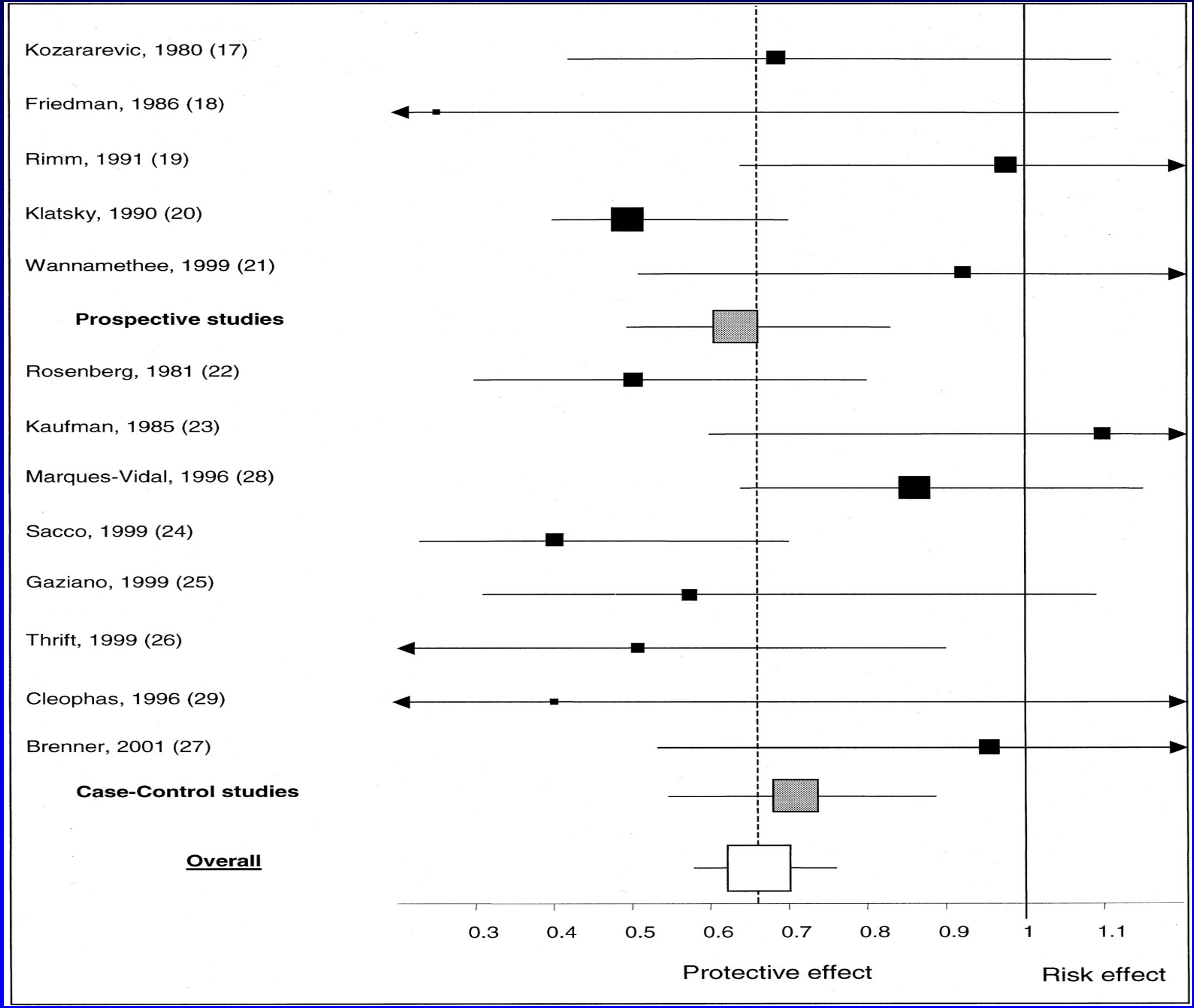
From: Frankel et al. Lancet, 341:454-57;1993

Mortalità e consumo di etanolo



Boffetta, P. and Garfinkel, L. Epidemiology, 5: 342-8; 1990

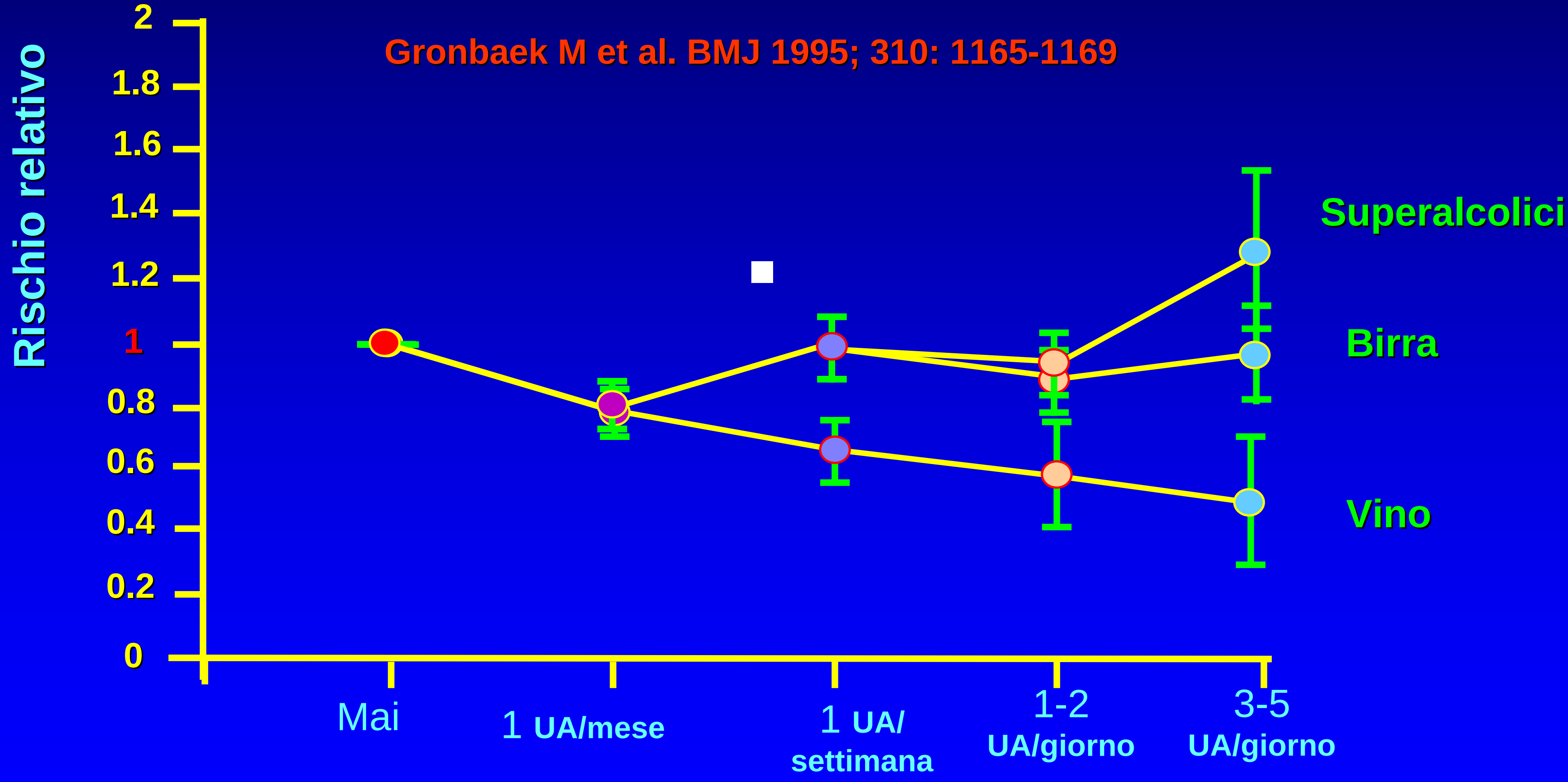
Odds ratios for vascular disease comparing wine intake versus no wine intake



Di Castelnuovo, A. et al. Circulation 2002;105:2836-2844

American Heart Association. Learn and Live.

Consumo di bevande alcoliche e mortalità per tutte le cause

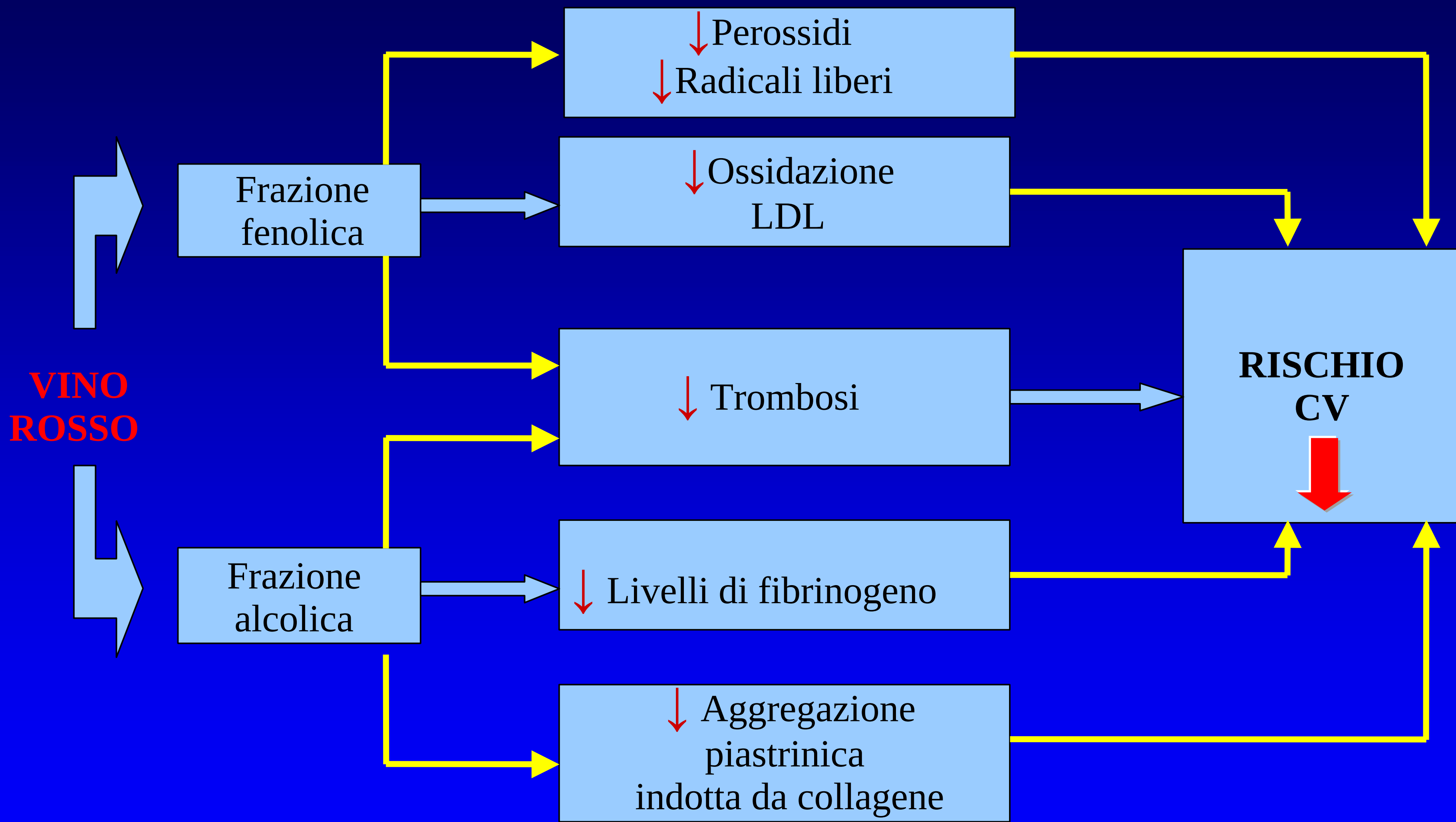


Wine and beer are bought in association with..

Wine drinkers choices

Beer drinkers choices:

Johansen, D et al BMJ, 20 January 2006


VINO ROSSO
Frazione fenolica
Frazione alcolica
↓Perossidi
↓Radicali liberi
↓Ossidazione LDL
↓ Trombosi
↓ Livelli di fibrinogeno
↓ Aggregazione piastrinica indotta da collagene
RISCHIO CV

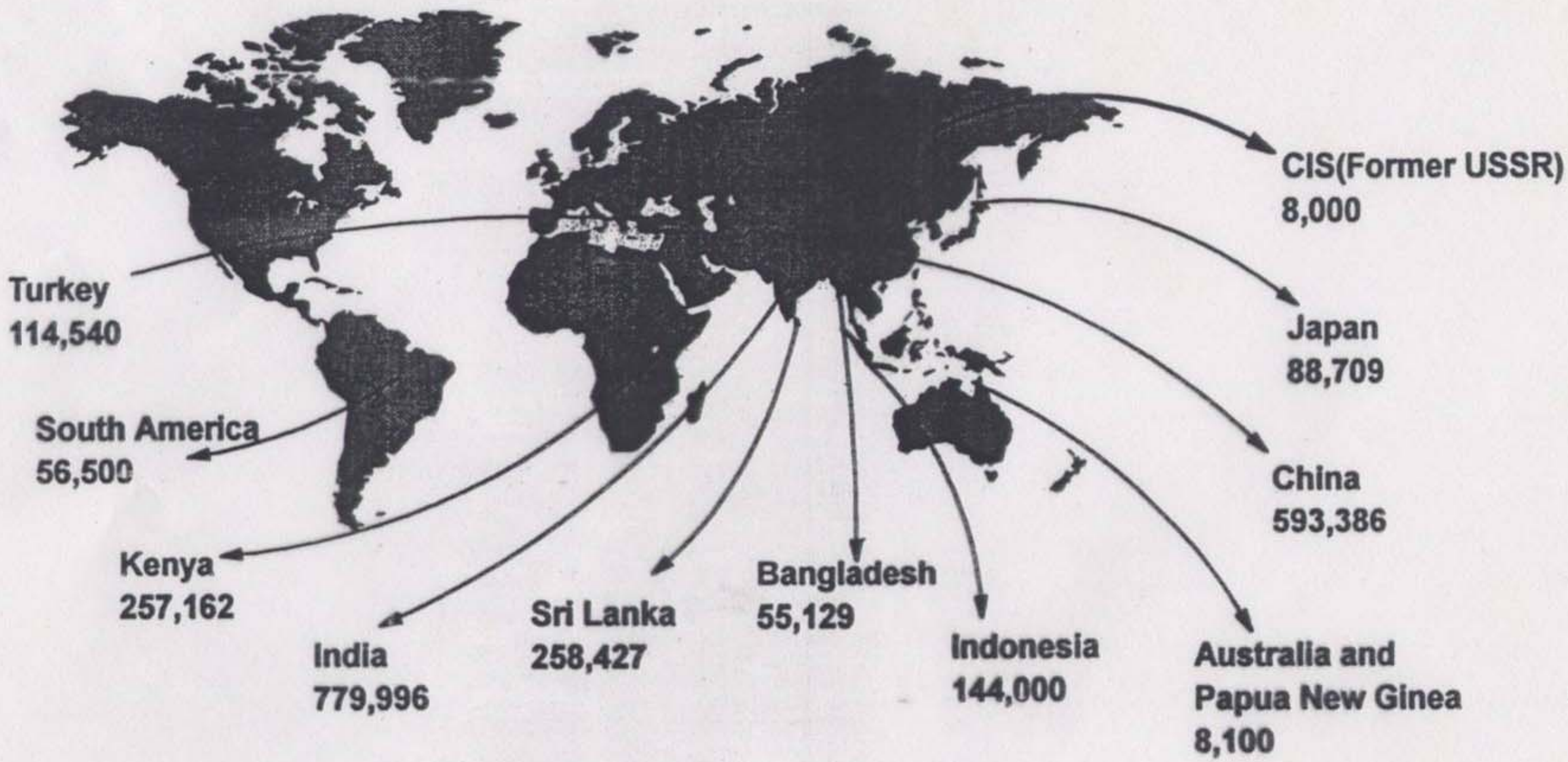


FIGURE 1. World tea production 1996 (metric tons).

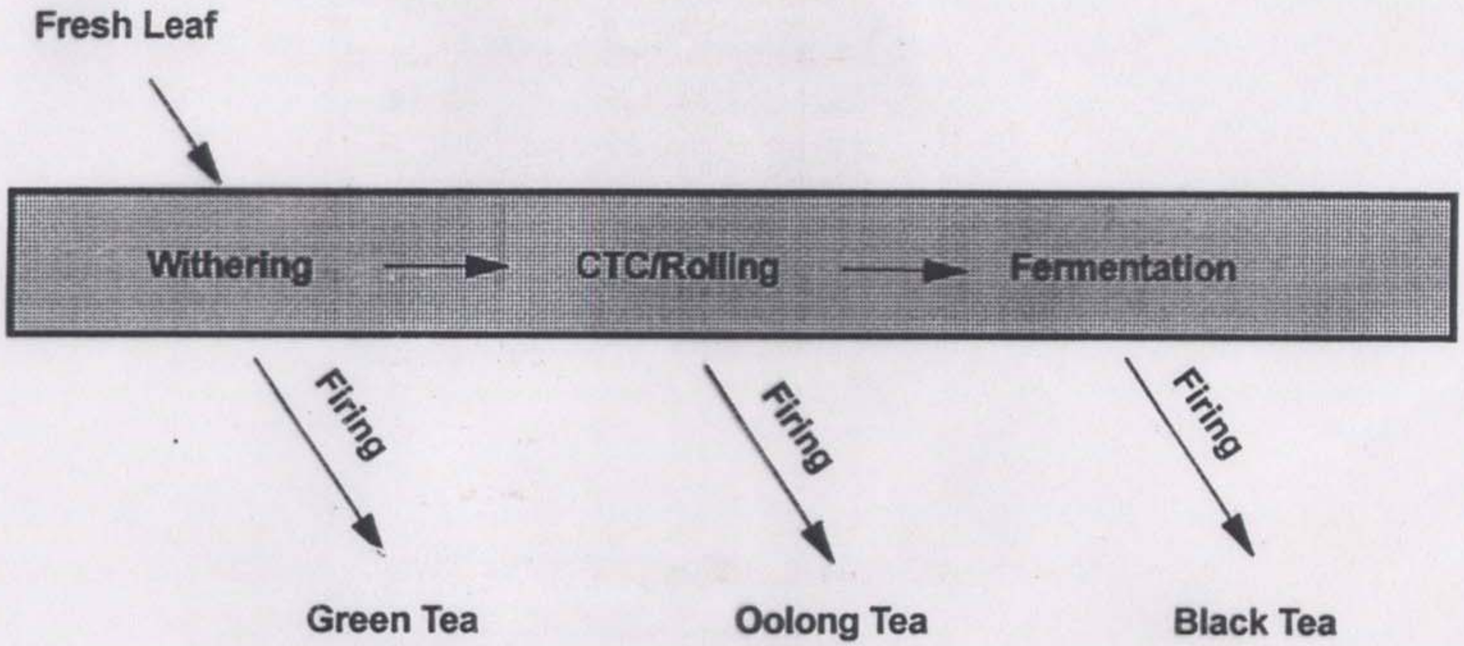


FIGURE 5. Tea manufacturing process.

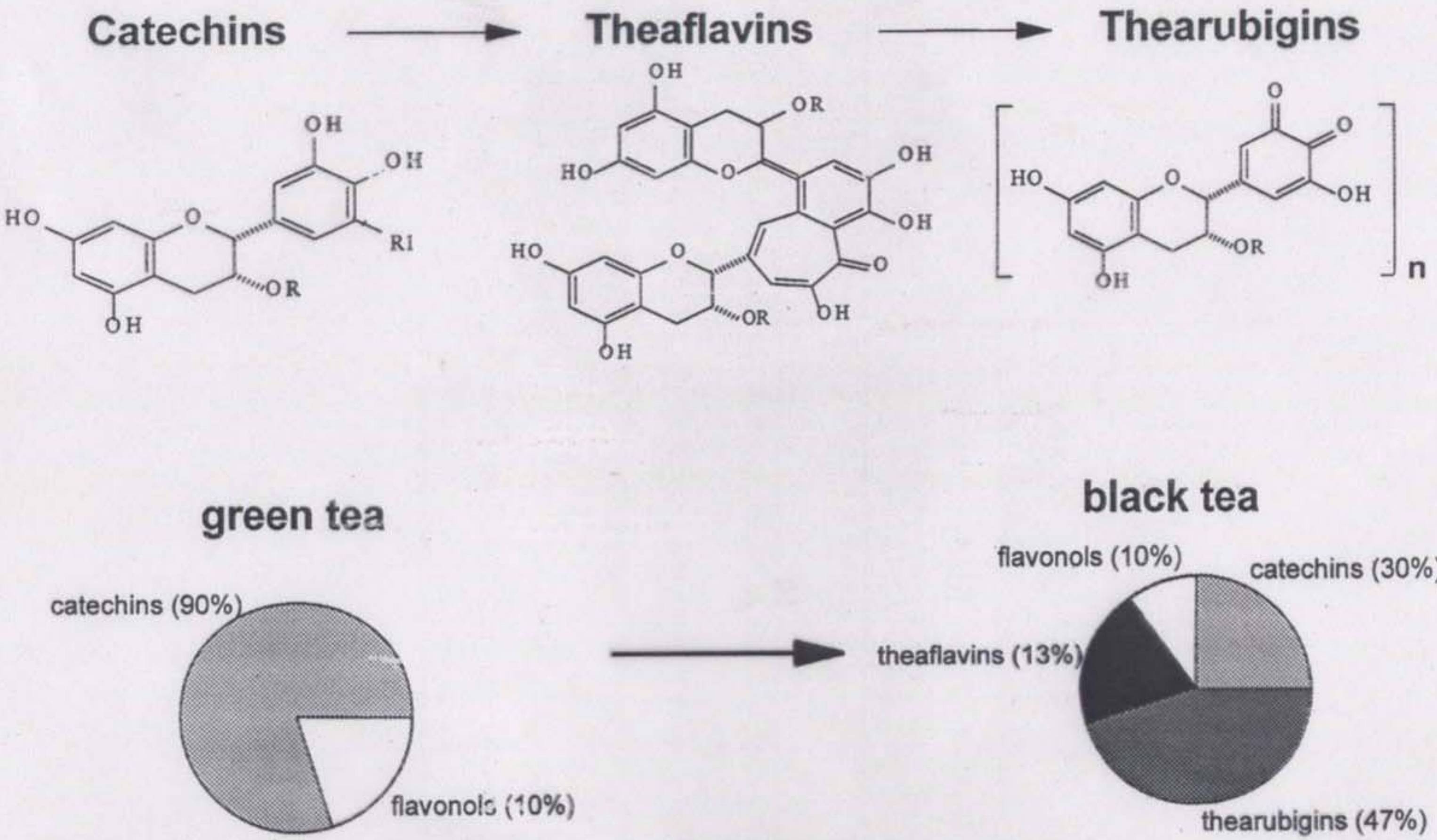
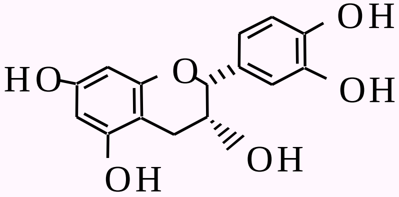
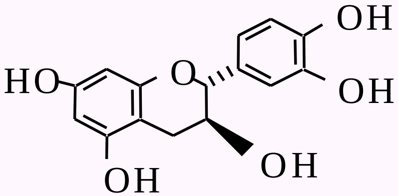


FIGURE 1. Composition of flavonoids in green and black tea. The distribution flavonoids in green and black tea is expressed as percentage of total flavonoids. Catechins: R, R1 = H: EC; R1 = OH, R = H: EGC; R1 = H, R = gallate: ECG and R1 = OH, R = gallate: EGCG. Theaflavins, R = H or gallate. Thearubigins: hypothesized structure.

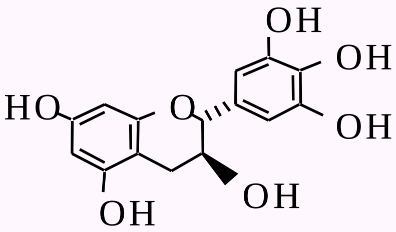
Principle flavan-3-ols in tea leaves



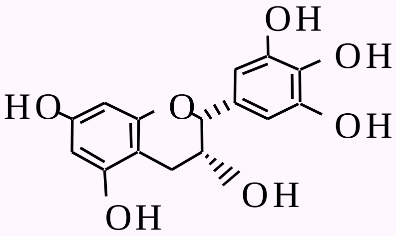
(-)-Epicatechin



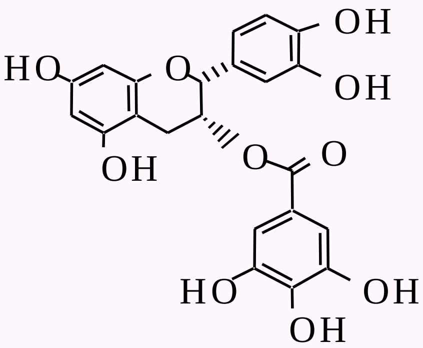
(+)-Catechin



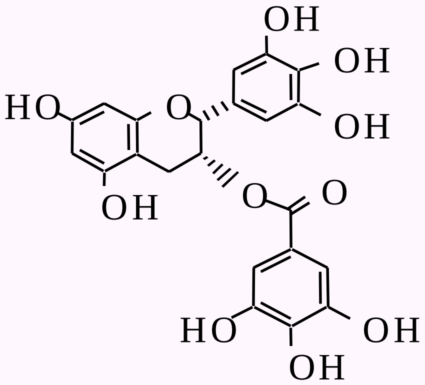
(+)-Gallocatechin



(-)-Epigallocatechin

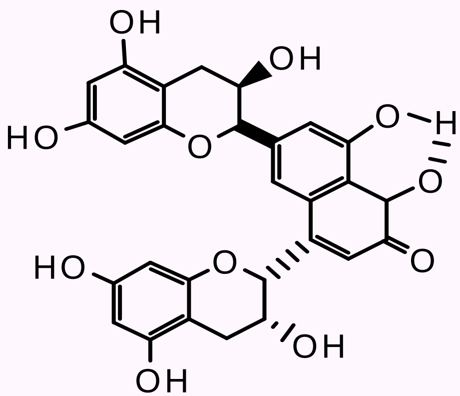


(-)-Epicatechin gallate

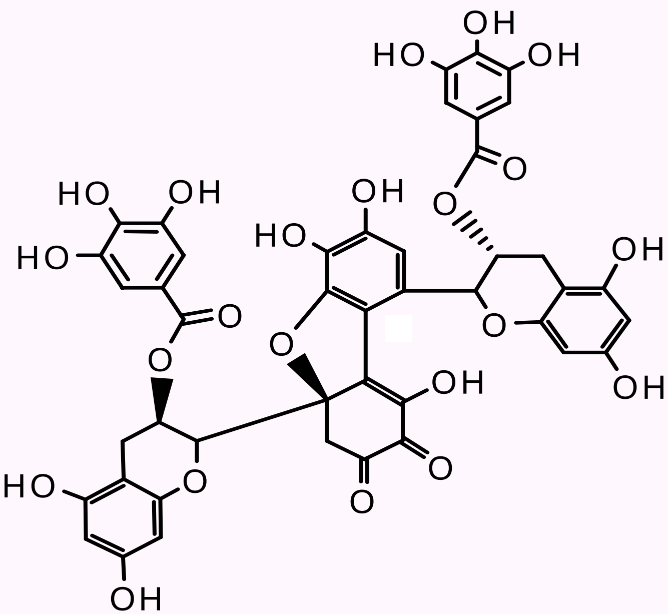


(-)-Epigallocatechin gallate

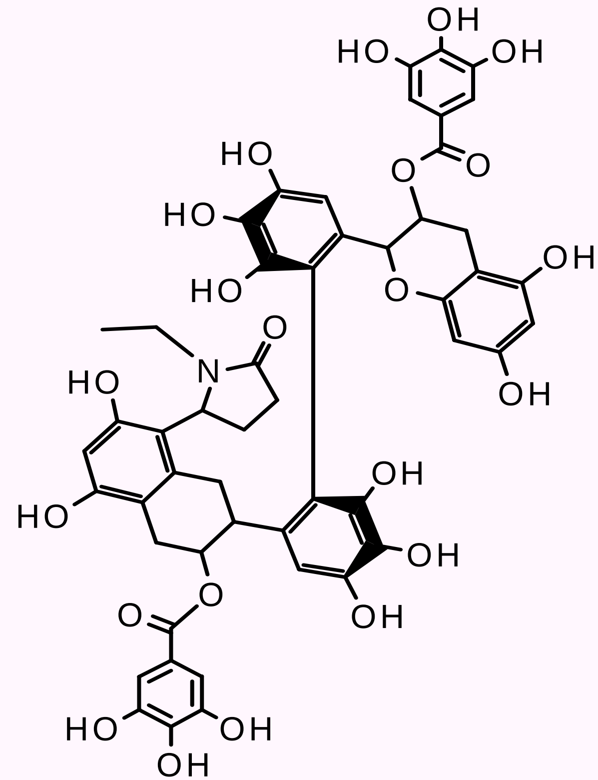
Black Tea Phenolics Associated With Thearubigin Production



Theanapthoquinone



Dehydrotheasinensin AQ



8'-Ethylpyrrolidinonyl-theasinensin A

TABLE 3

Relative risk (RR) of a first myocardial infarction in 4807 Dutch participants in the Rotterdam Study, by categories of tea intake

	Tea intake		
	0 mL/d	1–375 mL/d	>375 mL/d
Median tea intake (mL/d)	0.0	250.0	500.0
Number of subjects	583	2340	1884
Follow-up time (person-years)	3277	12990	10588
All incident myocardial infarctions			
Number of events	24	77	45
RR, age- and sex-adjusted model	1	0.72 (0.49, 1.13)[1]	0.51 (0.30, 0.84)
RR, multivariate model[2]	1	0.77 (0.48, 1.25)	0.57 (0.33, 0.98)
Nonfatal myocardial infarctions			
Number of events	18	61	57
RR, age- and sex-adjusted model	1	0.80 (0.47, 1.36)	0.62 (0.35, 1.11)
RR, multivariate model[2]	1	0.84 (0.49, 1.47)	0.68 (0.37, 1.26)
Fatal myocardial infarction			
Number of events	6	16	8
RR, age- and sex-adjusted model	1	0.47 (0.18, 1.23)	0.24 (0.08, 0.72)
RR, multivariate model[2]	1	0.58 (0.22, 1.57)	0.30 (0.09, 0.94)

[1] 95% CI in parentheses. Values obtained with a Cox proportional hazards analysis.

[2] Model adjusted for age, sex, BMI, smoking status, pack-years of cigarette smoking, education level, and daily intakes of alcohol, coffee, polyunsaturated fat, saturated fat, fiber, vitamin E, and total energy.

TABLE 4

Relative risk (RR) of a first myocardial infarction in 4807 Dutch participants in the Rotterdam Study, by tertiles of flavonoid intake

	Tertile of flavonoid intake		
	Lower (<22.8 mg/d)	Middle (22.8–32.9 mg/d)	Upper (>32.9 mg/d)
Median flavonoid intake (mg/d)	16.8	27.5	40.0
Number of subjects	1602	1603	1602
Follow-up time (person-years)	8851	8909	8973
All incident myocardial infarctions			
Number of events	62	43	41
RR, age- and sex-adjusted model	1	0.69 (0.47, 1.02)[1]	0.68 (0.46, 1.02)
RR, multivariate model[2]	1	0.74 (0.49, 1.11)	0.76 (0.49, 1.18)
Nonfatal myocardial infarctions			
Number of events	45	36	35
RR, age- and sex-adjusted model	1	0.81 (0.52, 1.26)	0.83 (0.53, 1.30)
RR, multivariate model[2]	1	0.85 (0.54, 1.34)	0.93 (0.57, 1.52)
Fatal myocardial infarction			
Number of events	17	7	6
RR, age- and sex-adjusted model	1	0.38 (0.16, 0.93)	0.33 (0.13, 0.83)
RR, multivariate model[2]	1	0.42 (0.17, 1.06)	0.35 (0.13, 0.98)

[1] 95% CI in parentheses. Values obtained with a Cox proportional hazards analysis.

[2] Model adjusted for age, sex, BMI, smoking status, pack-years of cigarette smoking, education level, and daily intakes of alcohol, coffee, polyunsaturated fat, saturated fat, fiber, vitamin E, and total energy.

SUBJECTS

- ✓ Fifteeen healthy volunteers

  (29-45 y, 7 W 8 M)

- ✓ Non smokers, normolipidemic
- ✓ No drug or vitamin supplements

STUDY DESIGN

H_2O (250 mL) (n = 5)

GT (250 mL) (n = 5)

BT (250 mL) (n = 5)

Blood sampling (0, 30, 50 and 80 min.)

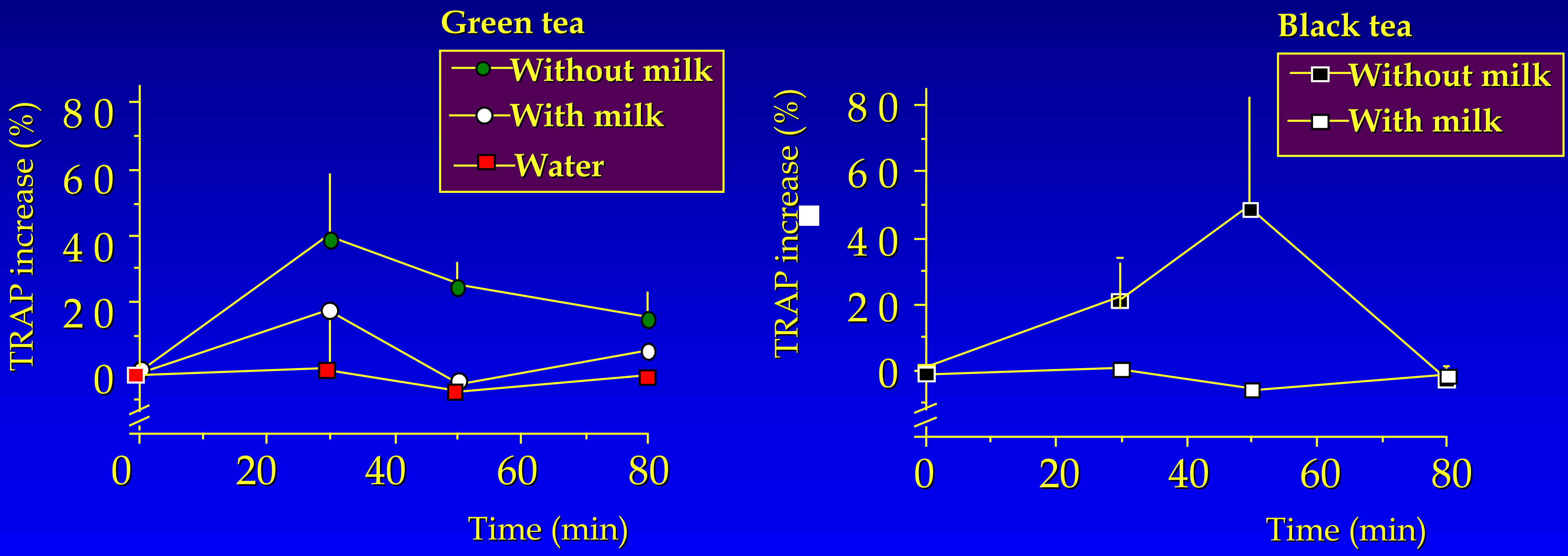
7 Days wash-out

GT + milk (1:3)

BT + milk (1:3)

Blood sampling (0, 30, 50 and 80 min.)

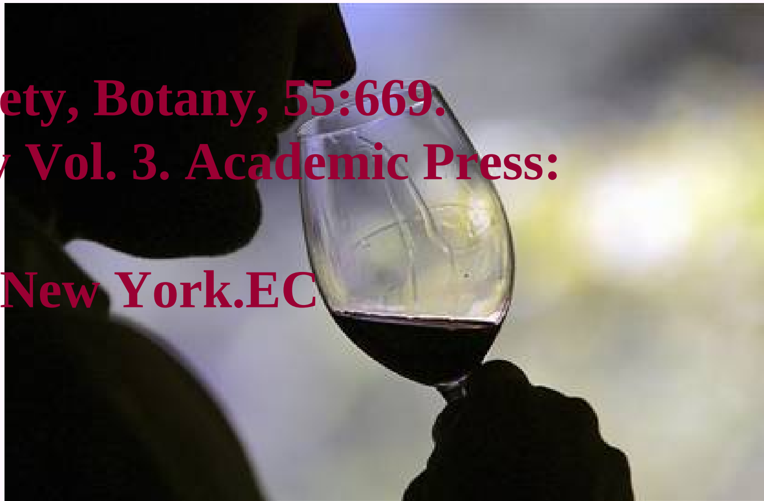
PLASMA ANTIOXIDANT CAPACITY



Serafini M et al. Lancet 344: 626; 1994; Serafini et al. Eur. J. Clin. Nutr. 50:28-32; 1996

Polyphenols and protein binding

"Polyphenols bind protein making them insoluble and inducing precipitation. The activity binding with protein is considered to be mainly due to formation of multi-hydrogen bonds. The precipitate formation with protein is a characteristic property of regular tannins, but there are also some PP which show potent binding activity in spite of their smaller MW"



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Bate-Smith E.C., Swain T. (1962) In Comparative Chemistry Vol. 3. Academic Press: New York.
Okuda T. (1997) In: Food and Free Radicals. Plenum Press, New York.EC

Epicatechin in Human Plasma: In Vivo Determination and Effect of Chocolate Consumption on Plasma Oxidation Status[1,2]

Dietrich Rein,* Silvina Lotito,† Roberta R. Holt,* Carl L. Keen,* Harold H. Schmitz** and Cesar G. Fraga†3

*Department of Nutrition, University of California, Davis, California 95616; †Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, 1113 Buenos Aires, Argentina and **Analytical and Applied Sciences, Mars Incorporated, Hackettstown, New Jersey 07840

TABLE 2

Oxidative stress parameters evaluated before and after the consumption of a procyanidin-rich or a low-procyanidin food[1]

Parameter	Time after chocolate consumption: 0	2	6
		h	
Epicatechin, *nmol/L*			
Procyanidin-rich meal	22 ± 4	257 ± 66[2]	153 ± 69
Low-procyanidin meal	11 ± 10	12 ± 11	11 ± 9
Vitamin E, *µmol/L*			
Procyanidin-rich meal	38 ± 5	38 ± 5	36 ± 5
Low-procyanidin meal	ND	ND	ND
Vitamin C, *µmol/L*			
Procyanidin-rich meal	62 ± 6	60 ± 6	61 ± 7
Low-procyanidin meal	ND	ND	ND
Uric acid, *µmol/L*			
Procyanidin-rich meal	271 ± 33	303 ± 41	273 ± 39
Low-procyanidin meal	241 ± 40	[illegible]	229 ± 14
Antioxidant capacity, *s*			
Procyanidin-rich meal	389 ± 39	510 ± 43[2]	306 ± 34
Low-procyanidin meal	344 ± 41	291 ± 23	257 ± 30
TBARS, *µmol/mmol* triglycerides			
Procyanidin-rich meal	3.14 ± 0.52	1.87 ± 0.26[2]	2.20 ± 0.38[2]
Low-procyanidin meal	2.03 ± 0.25	2.09 ± 0.20	2.04 ± 0.30

1 Values are means ± SE (n = 10 for the group fed the procyanidin-rich dark chocolate and n = 3 for the control group fed the low-procyanidin vanilla milk chips). Comparison of 2- and 6-h values with 0-h baseline by paired t test.

2 $P < 0.01$.

ND, not determined.

A Dose-Response Effect from Chocolate Consumption on Plasma Epicatechin and Oxidative Damage[1,2]

Janice F. Wang,* Derek D. Schramm,* Roberta R. Holt,* Jodi L. Ensunsa,* Cesar G. Fraga,** Harold H. Schmitz‡ and Carl L. Keen*†3

Departments of *Nutrition and †Internal Medicine, University of California, Davis, California 95616-8669; **Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires, 1113 Buenos Aires, Argentina and ‡Analytical & Applied Sciences, Mars Incorporated, Hackettstown, New Jersey 07840

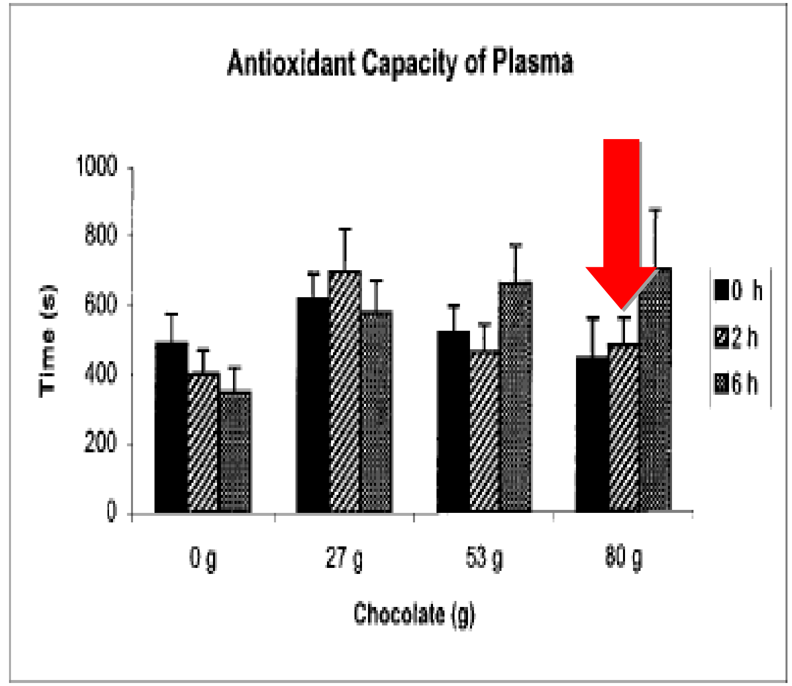

FIGURE 1 Antioxidant capacity of plasma at baseline and 2 and 6 h after the consumption of procyanidin-rich chocolate. Values are expressed as changes in lag time(s) before chemiluminescence is observed. A weak trend (P = 0.5302) was observed for increasing antioxidant capacity with increasing amount of procyanidin-rich chocolate consumed. Variability is represented as SEM. Please refer to Table 1 for the number of subjects in each group.

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Subjects and clinical study design. Thirteen healthy, nonsmoking adults with no history of heart disease or hemostatic disorders participated in the study. Their current health status was evaluated via a questionnaire. All participants gave written informed consent before their participation in the study, which was approved by the Human Subjects Review Committee (University of California, Davis, California).

Ten subjects (four men and six premenopausal women, age range 26–49 years, body mass index 23.2 ± 1.2 kg/m^2) consumed 80 g of procyanidin-rich chocolate in the form of 105 g of M&M's Semi-Sweet Chocolate Mini Baking Bits made with Cocoapro cocoa (Mars Incorporated, Hackettstown, NJ), and three subjects (one man and two women, age range 28–36 years, body mass index 21.1 ± 0.2 kg/m^2) consumed isocaloric amounts of vanilla milk chips (low-procyanidin food) (Guittard Chocolate Company, Burlingame, CA). The procyanidin-rich chocolate provided 557 mg total procyanidins, of which 137 mg (470 µmol) was epicatechin, as determined by Adamson et al. (1999), whereas the vanilla milk chips did not contain detectable levels of procyanidins or epicatechin. The procyanidin-rich chocolate provided 27 g of fat. Participants were instructed to abstain from vitamin supplements, alcoholic beverages and caffeine- or theobromine-containing foods for at least 24 h before and during the test day. Subjects fasted at least 8 h before test food

Study design. Participants were asked to refrain from taking vitamin supplements, nonsteroidal anti-inflammatory medications and foods rich in polyphenols for 24 h and to fast overnight for 12 h before each feeding trial. Blood was collected at baseline (0 h) and 2 and 6 h after the first blood draw. Subjects were instructed to consume the test foods (chocolate and bread, or bread only) within 15 min of the first blood draw. All subjects were asked to consume an

Test foods provided 0, 27, 53 or 80 g of procyanidin-rich chocolate in the form of M&M's Semi-Sweet Chocolate Mini Baking Bits made with Cocoapro cocoa (Mars Incorporated, Hackettstown, NJ) and included a serving of 47 g of bread. The 27-g chocolate portion was in the form of candy-coated M&M's baking bits, which provided 0.732 MJ, 0.021 g caffeine, 0.18 g theobromine. Each 27-g chocolate portion contained [illegible] g epicatechin and a total of 186 mg of procyanidins. The bread ([illegible]) provided 0.544 MJ, 0.75 g total fat, 25 g carbohydrate and 4[illegible] protein in a 47-g serving.

TAC

SUBJECTS

- ✓ Twelve healthy volunteers
  (25-35 y, 7 W 5 M)
- ✓ Non smokers, normolipidemic
- ✓ No drug or vitamin supplements

STUDY DESIGN

CK
(100 g)

Group A (n = 4)

CKM
(200 g)

Group B (n = 4)

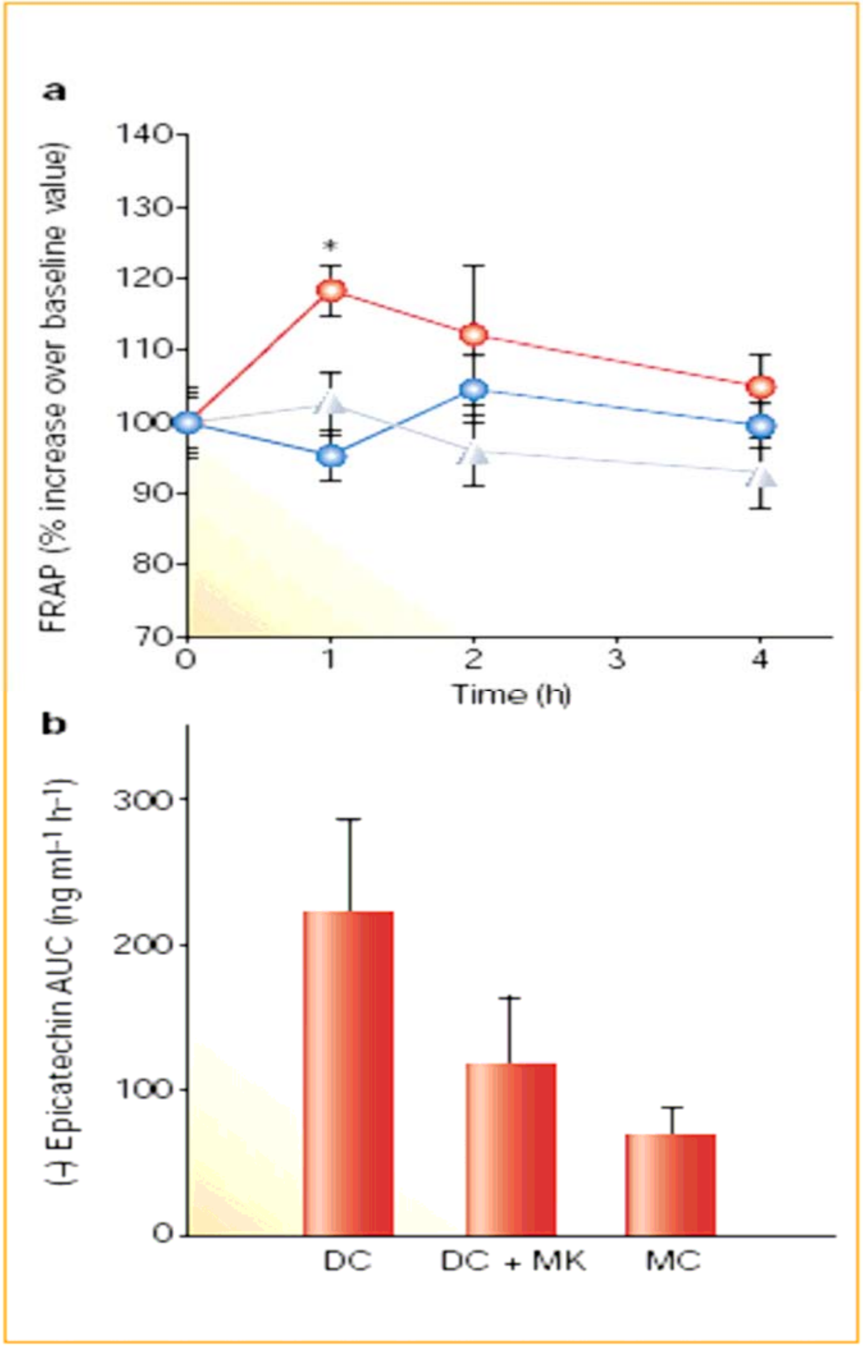
CK + M
(100 g + 200 mL)

Group C (n = 4)

Blood sampling (0, 1, 2 and 4 h.)

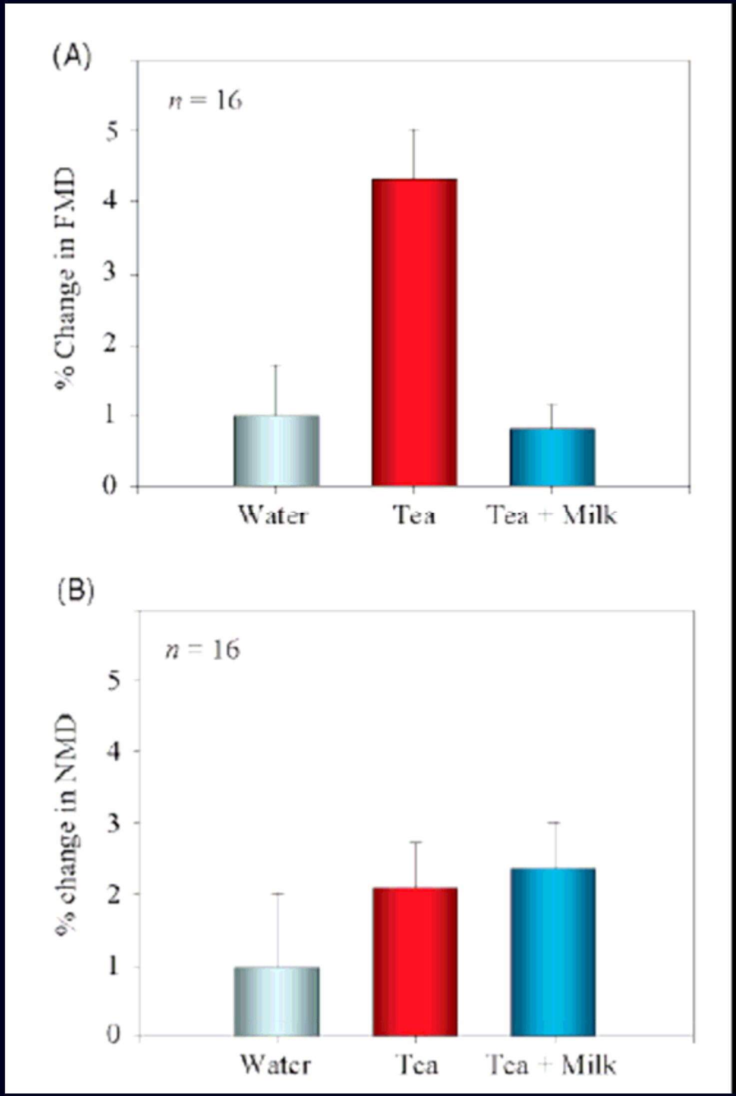
7 Days wash-out

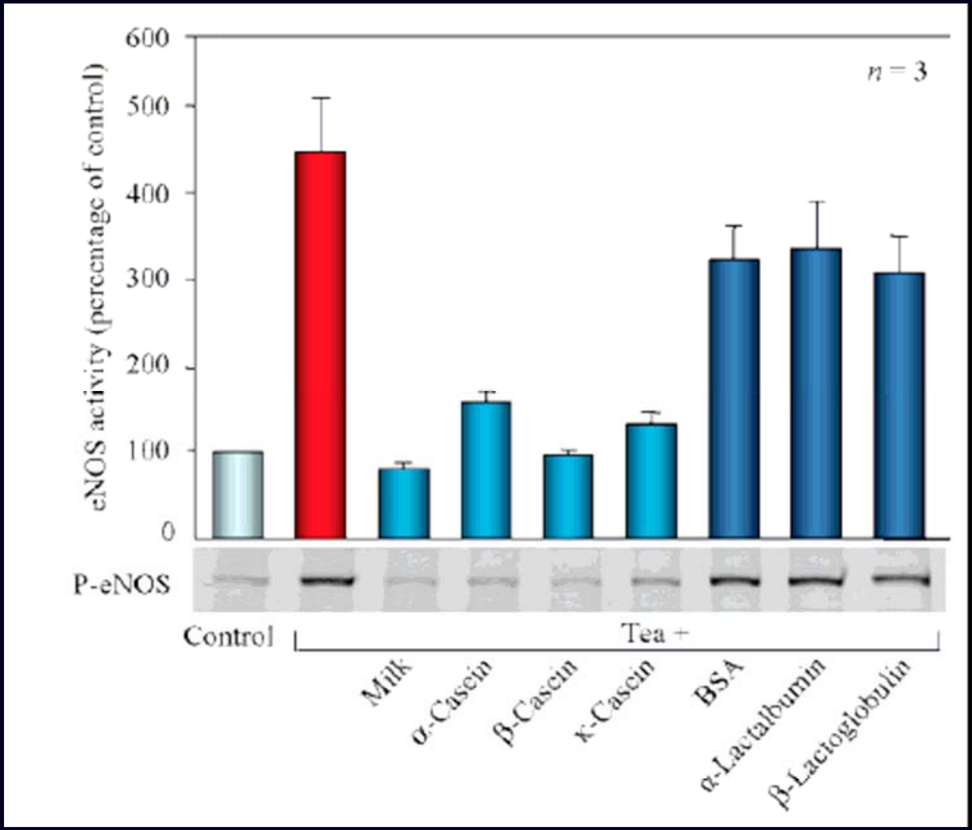
Phases 2 and 3



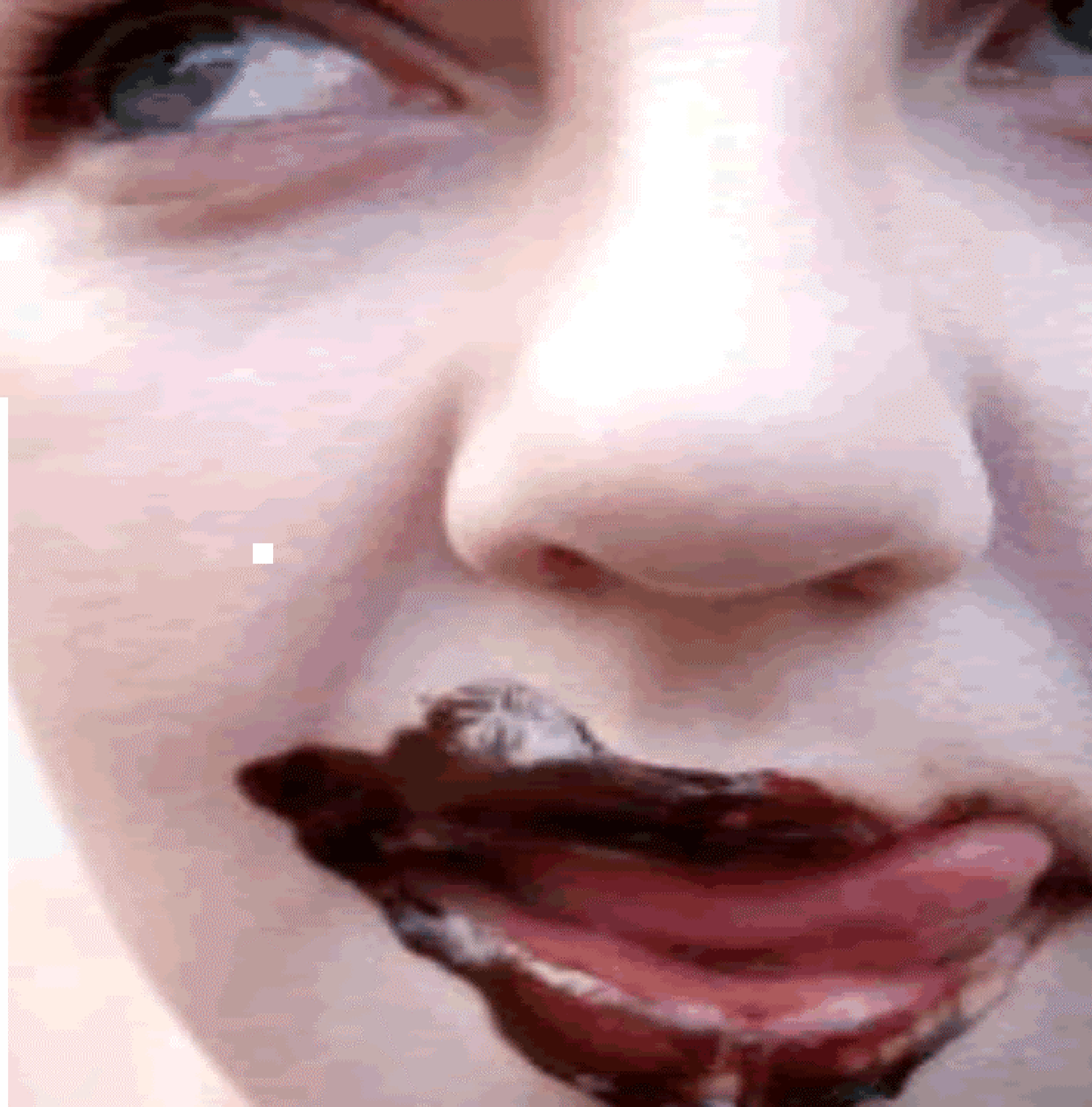
NATURE | VOL 424 | 28 AUGUST 2003 | www.nature.com/natu

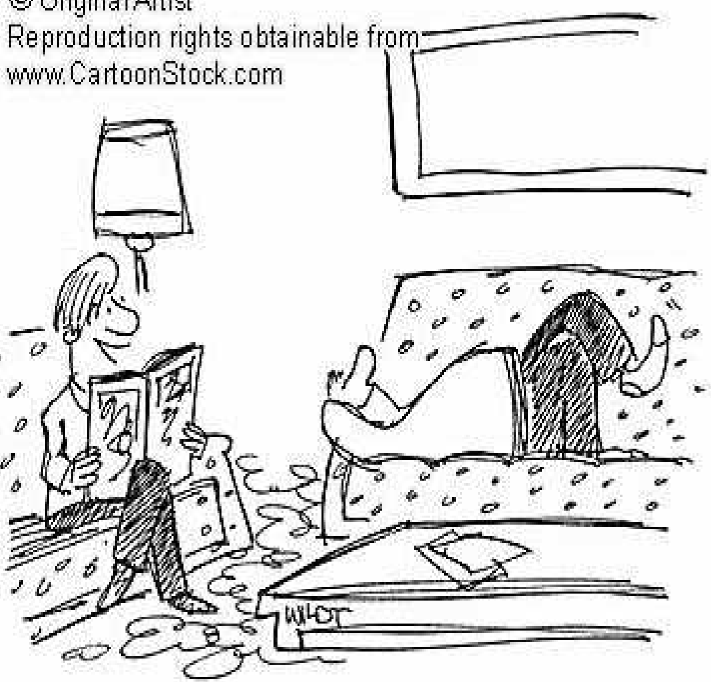
Black tea, milk and cardiovascular function





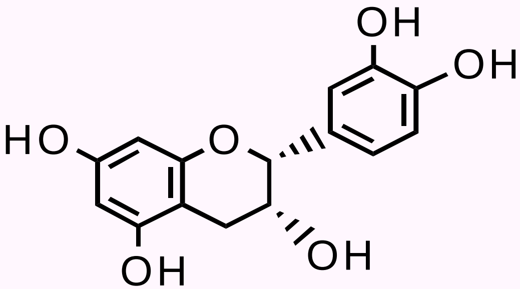
Lopez et al. Eur Heart J, 2007



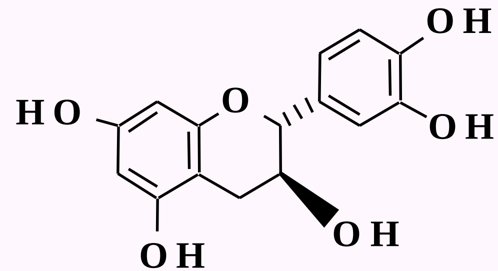


"Medical science continues to provide hope in a troubled world. Now they say chocolate is good for you!"

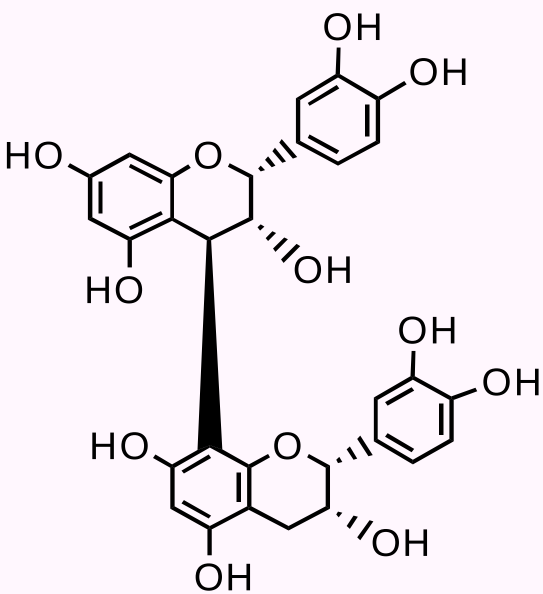
I FLAVONOIDI DEL CACAO



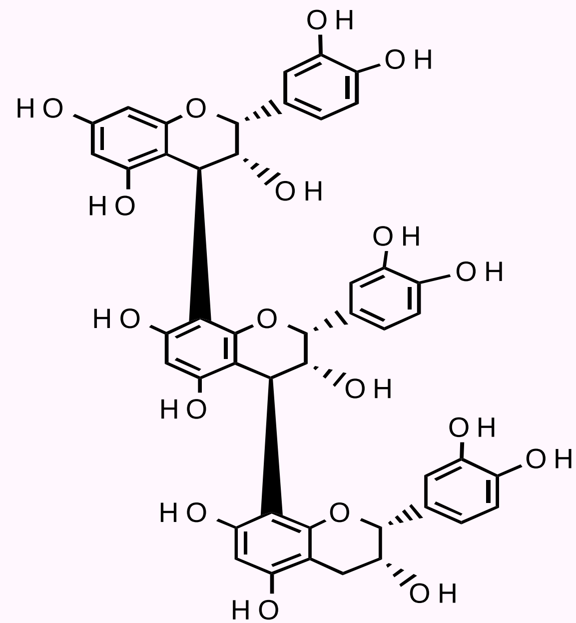
(Š)-Epicatechin



(+)-Catechin



Proanthocyanidin B_2 dimer



Proanthocyanidin C_1 trimer