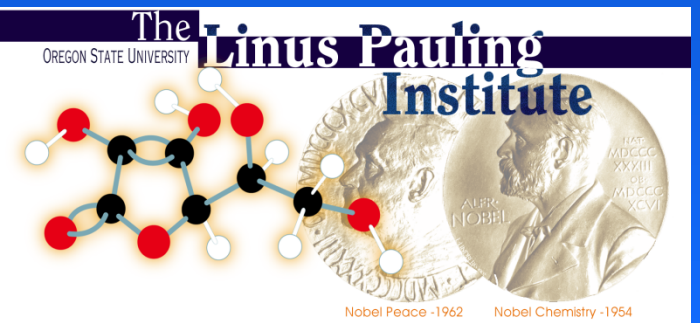
Vitamin C and Chronic Disease: The Right Molecule at the Right Dose

Balz Frei, Ph.D.
Director and Endowed Chair
Linus Pauling Institute
Oregon State University
Corvallis, OR 97331



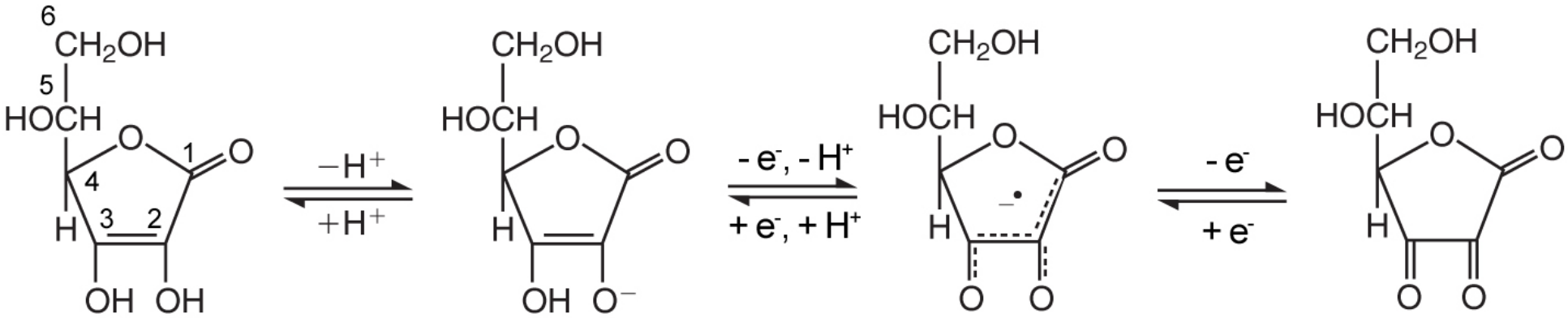
Oregon State University OSU

Ascorbic acid (protonated)

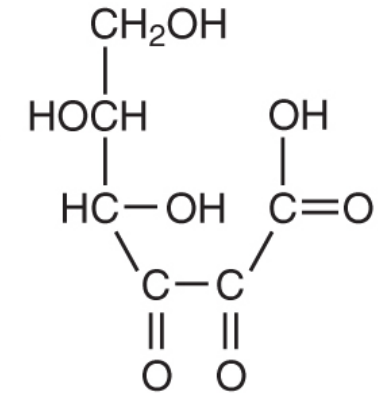
Ascorbic acid (ionized)

Ascorbyl radical

Dehydroascorbic acid



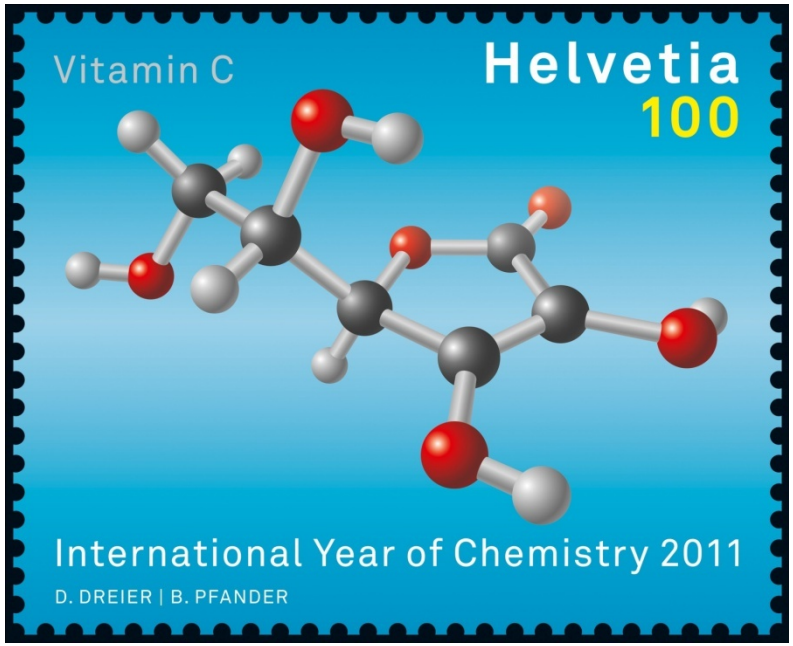
Hydrolytic ring rupture

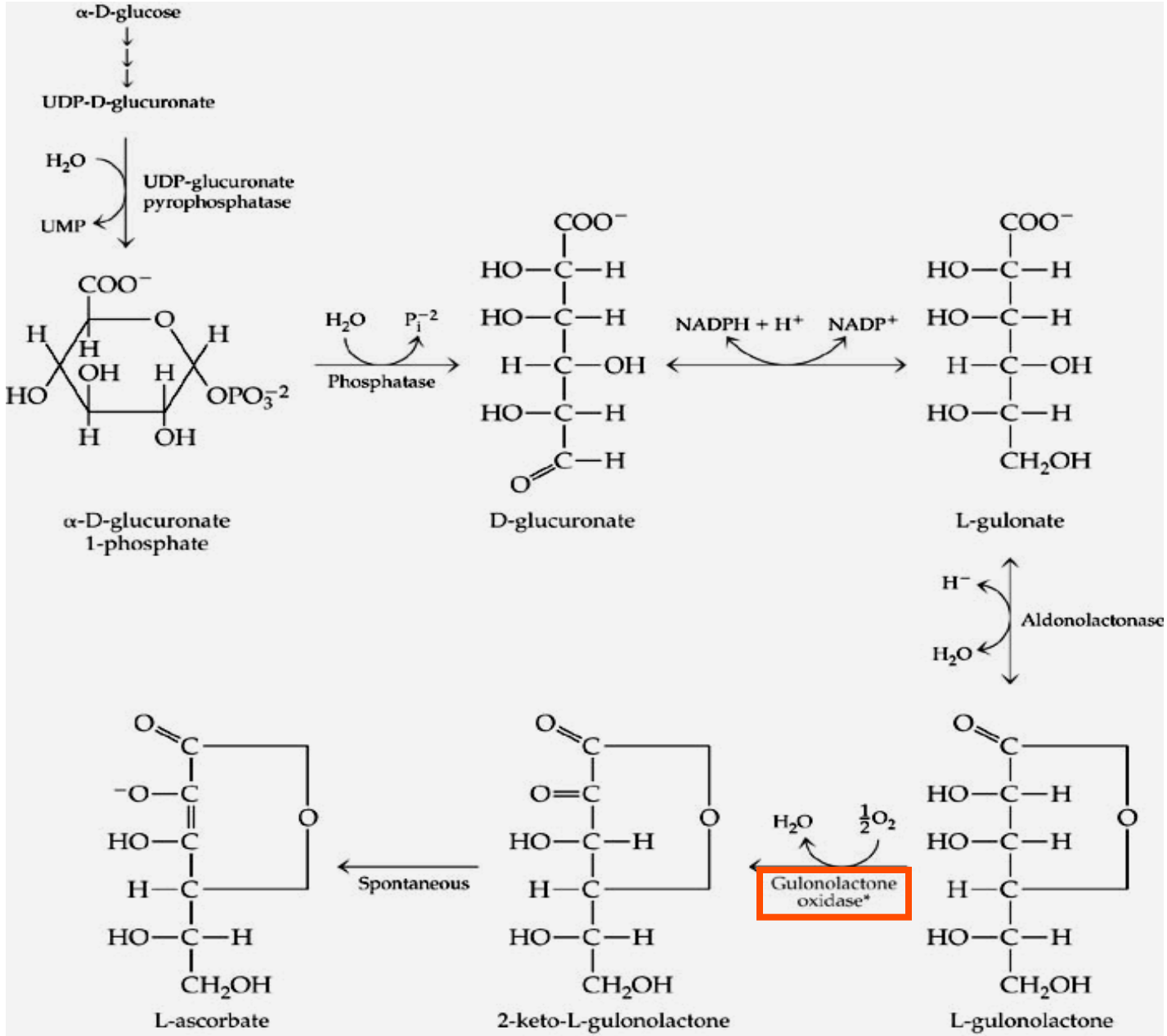


2,3-Diketo-L-gulonic acid

Breakdown Products (Oxalate, Threonate, etc.)

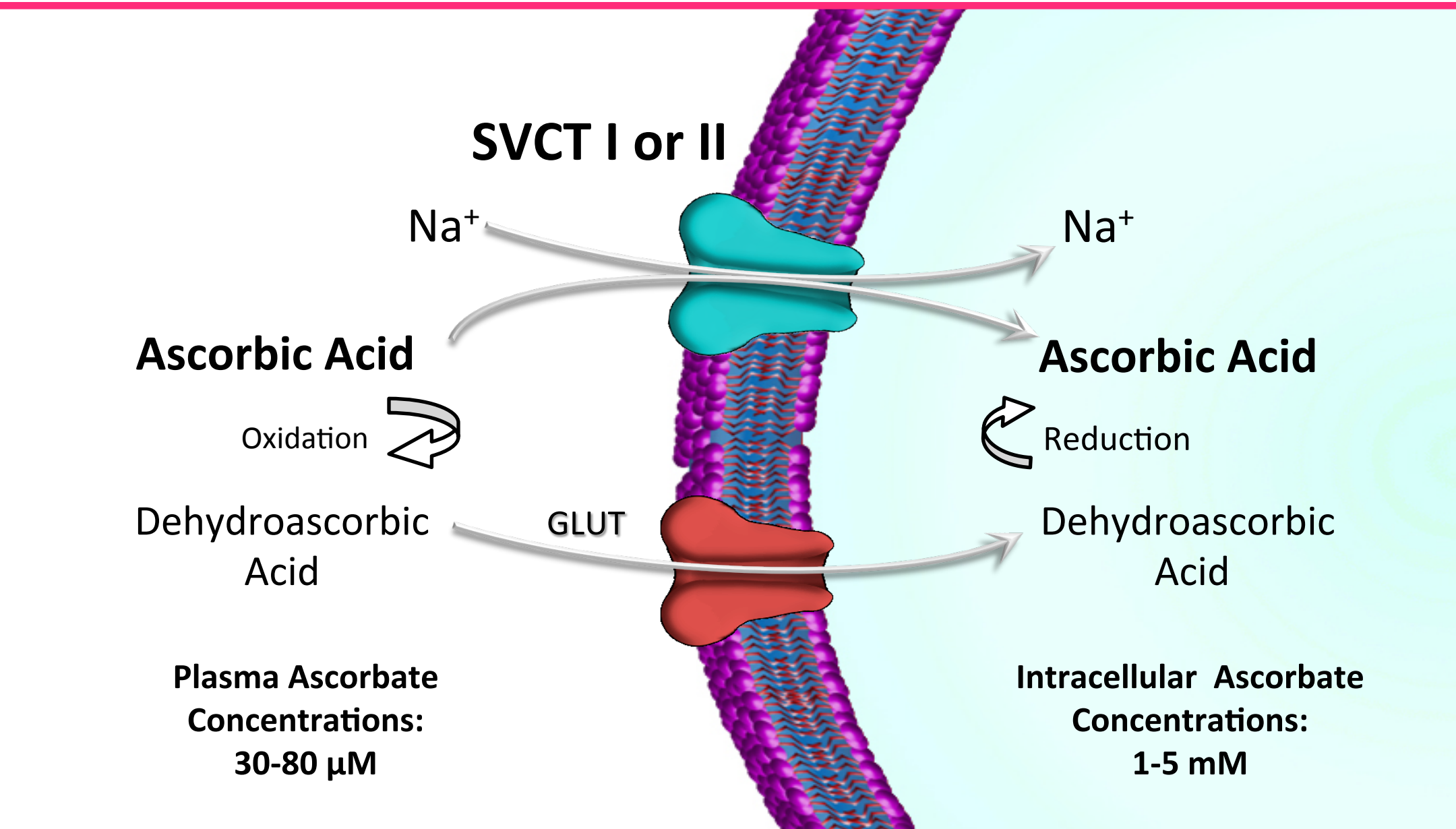
Metabolism





*Lacking in primates

Vitamin C Absorption and Transport: Sodium-dependent Vitamin C Transporters I and II



Enzymatic Functions of Vitamin C

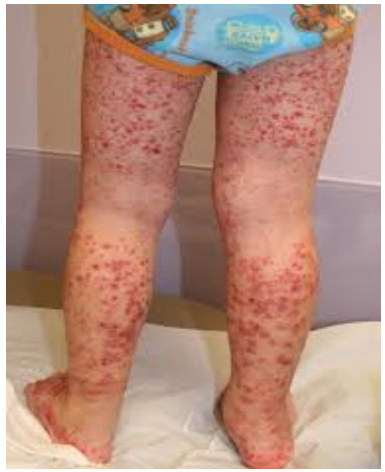
- Monooxygenases (reducing/co-substrate)
  - *Dopamine β-Hydroxylase*
  - *Peptidylglycine α-Amidating Monooxygenase*
- Dioxygenases
  - *Prolyl 4-Hydroxylase, Prolyl 3-Hydroxylase, and Lysyl Hydroxylase*
  - *HIF Hydroxylases*
  - *Trimethyllysine Hydroxylase and γ-Butyrobetaine Hydroxylase*
  - *4-Hydroxyphenylpyruvate Dioxygenase*

Vitamin C Deficiency: Scurvy

- Symptoms:
  - Lassitude and fatigue (covert)
  - Bleeding and bruising, impaired wound healing
  - Hair and tooth loss
  - Joint pain and swelling
  - Death

Insufficient L-carnitine biosynthesis

Defective pro-collagen hydroxylation

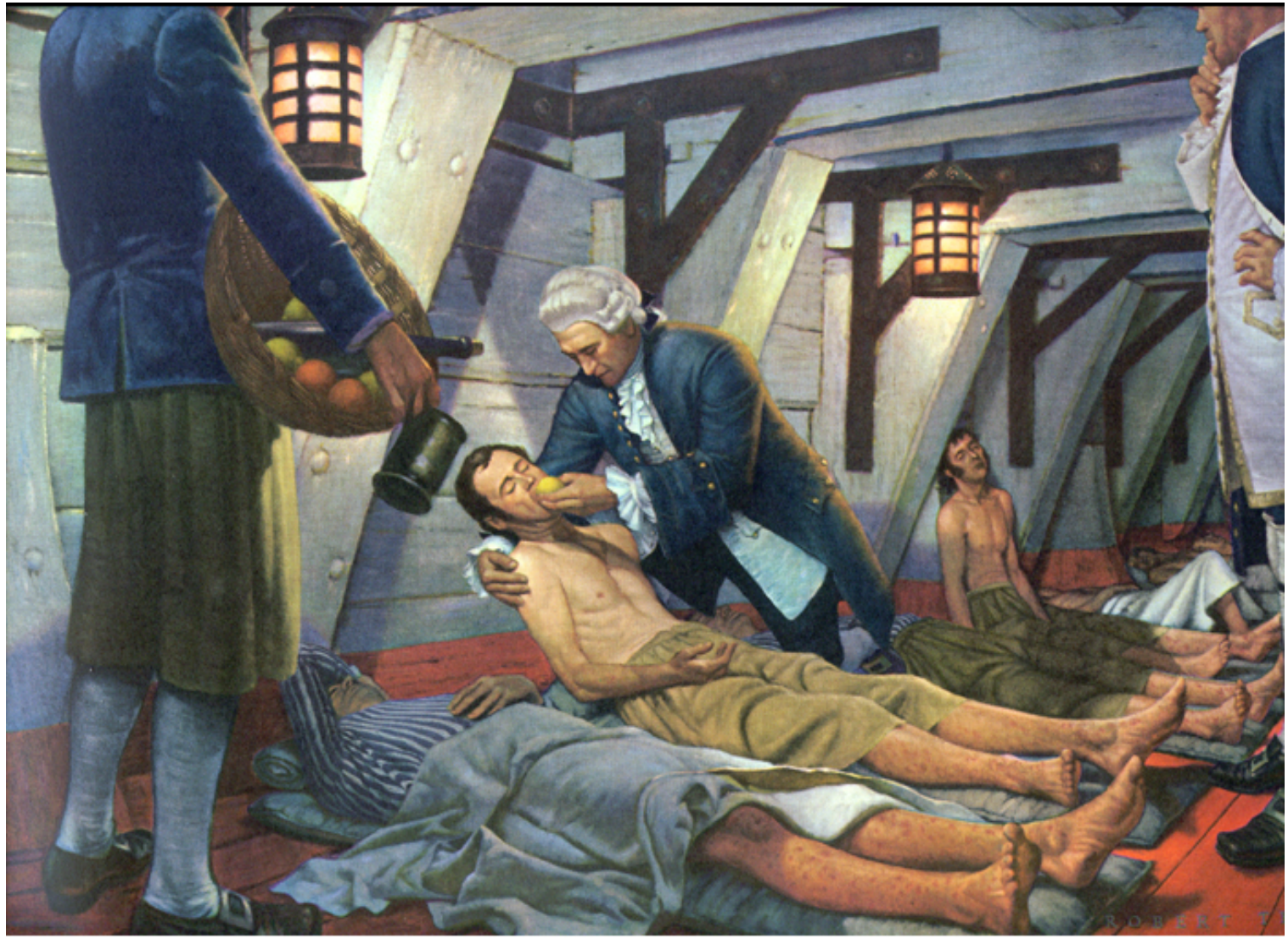


Petechiae
(bleeding under the skin)

Swollen and inflamed gums

The Linus Pauling Institute
Oregon State University

Oregon State University



James Lind, a surgeon in the Royal Navy, conducted clinical tests that proved that citrus fruits and their juices would cure and prevent scurvy, the disease which killed a million seamen between 1600 and 1800. In this painting he is shown aboard HMS *Salisbury* in 1747.

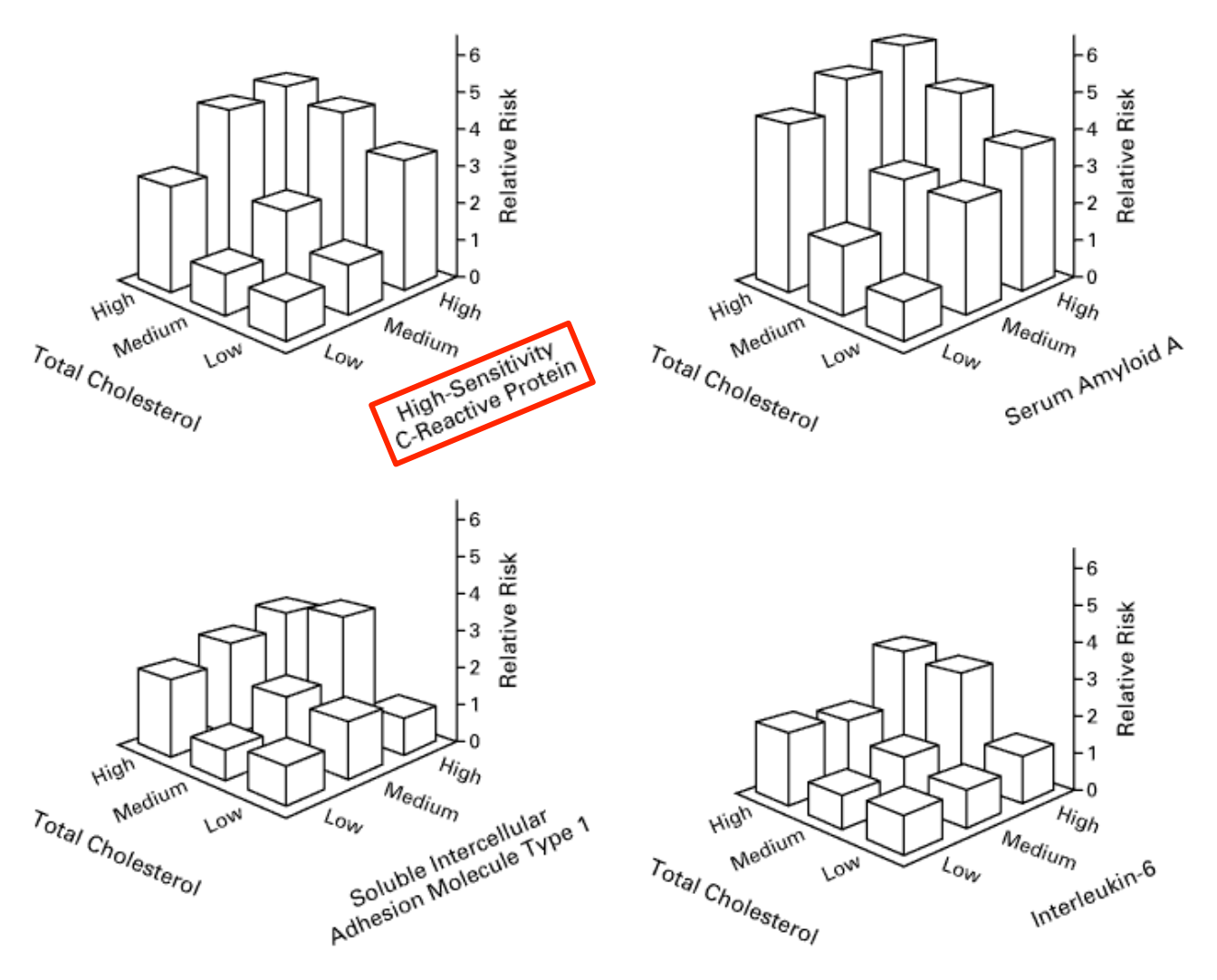
Recommended Dietary Allowance (RDA) in the U.S.

- Men: 90 mg/day
  - Based on the vitamin C intake required for 80% neutrophil saturation with little urinary loss in healthy men
- Women: 75 mg/day
  - Extrapolated from men on the basis of body mass
  - Higher RDA for pregnant and lactating women
- Smokers: plus 35 mg/day
  - Ascorbate turnover is approximately 35 mg/day greater in smokers due to smoking-induced oxidative stress

Vitamin C in Chronic Disease Prevention

- Is there biological plausibility (mechanisms of action) for a role of vitamin C in chronic disease prevention (nonindex disease), in particular cardiovascular diseases (CVD):
  - Hypertension
  - Coronary Heart Disease (CHD)
  - Stroke
- What is the human/epidemiological evidence that vitamin C contributes to the prevention of CVD?
- What is the optimal intake of vitamin C in humans?

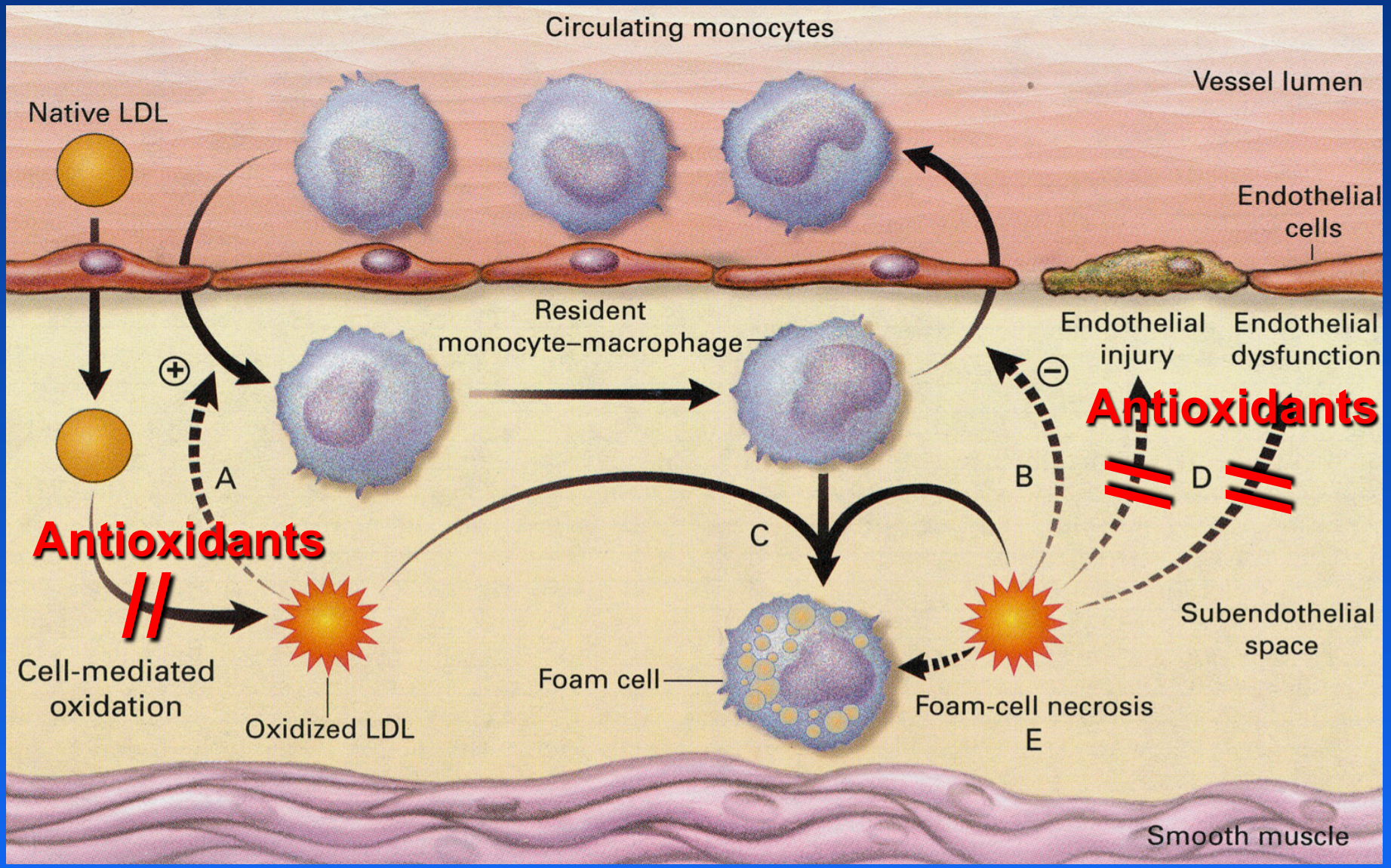
Coronary heart disease (CHD) is an inflammatory disease



Relative Risk of Cardiovascular Events among Apparently Healthy Postmenopausal Women According to Base-Line Levels of Total Cholesterol and Markers of Inflammation. Each marker of inflammation improved risk-prediction models based on lipid testing alone, an effect that was strongest for hs-CRP and serum amyloid A.

Ridker et al. (2000) *N Engl J Med* 342:836–843

Role of inflammation and oxidative stress in atherosclerosis



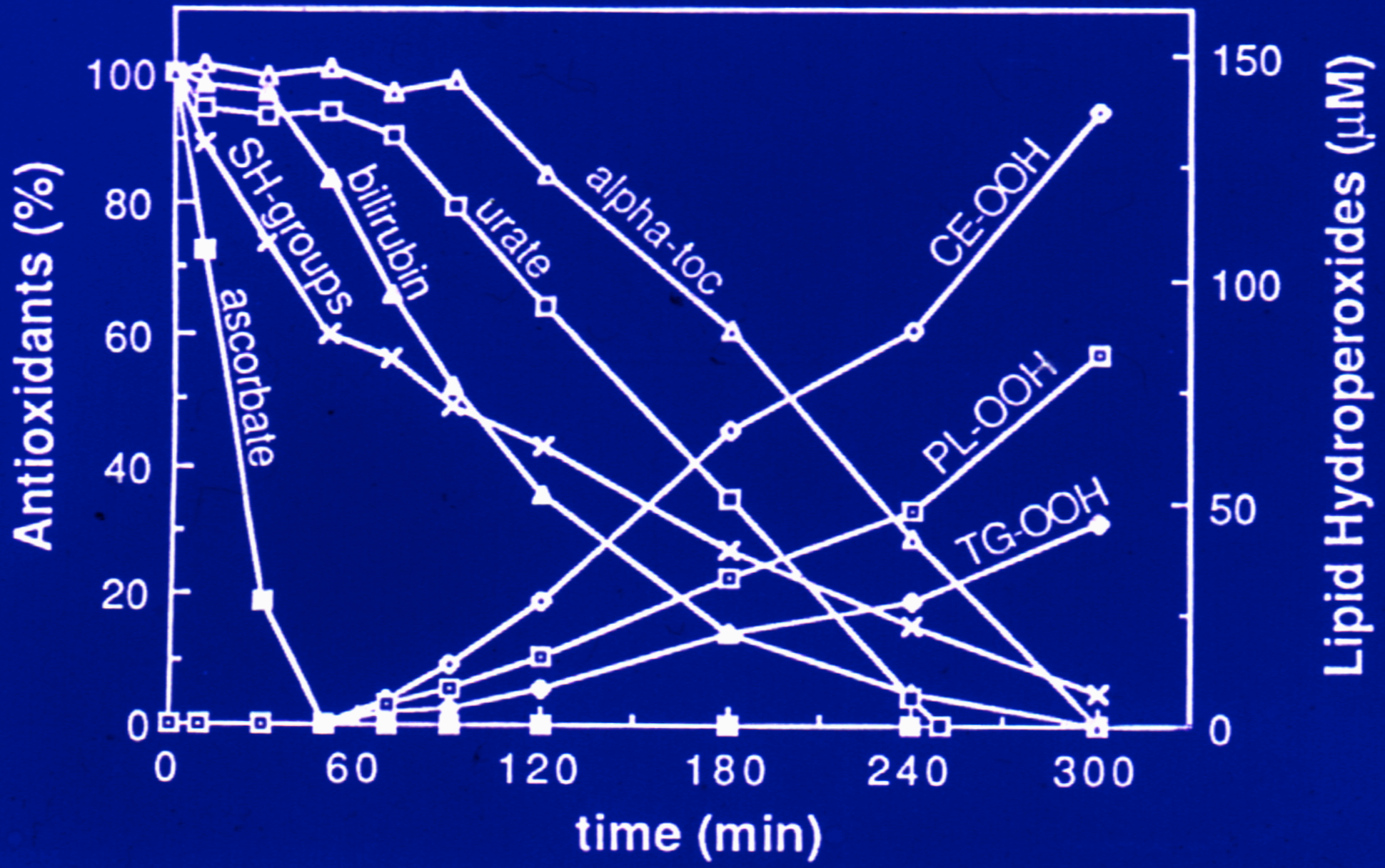
Diaz, Frei *et al. New Engl. J. Med*. 1997;337:408-416

Antioxidant Defenses in Human Plasma and LDL

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

How effectively do the endogenous antioxidants in human plasma (vitamin C, uric acid, bilirubin) and LDL (vitamin E) prevent lipid peroxidation and oxidative (atherogenic) modification of LDL?

Plasma Exposed to AAPH



Frei B, Stocker R, Ames BN. *Proc Natl Acad Sci USA*. 1988;85:9748-52

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6377–6381, August 1989
Medical Sciences

Ascorbate is an outstanding antioxidant in human blood plasma

(oxidant stress/lipid peroxidation/protein thiols/α-tocopherol)

BALZ FREI, LAURA ENGLAND, AND BRUCE N. AMES*

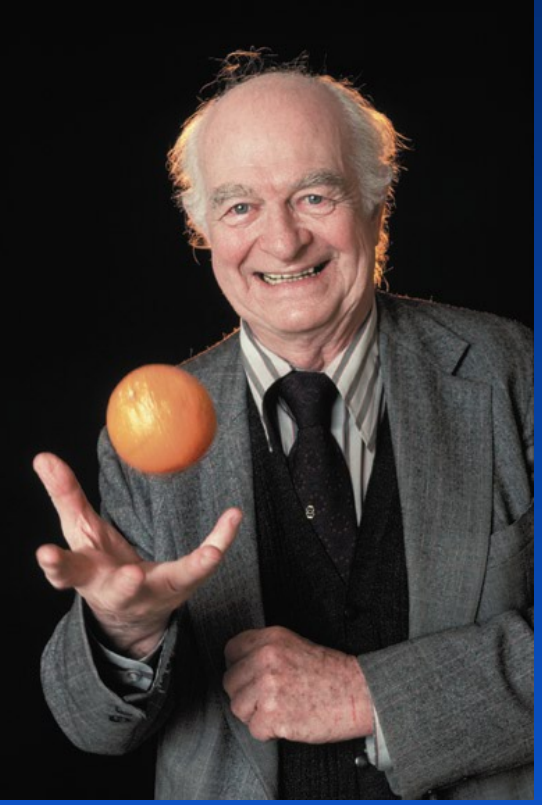
Department of Biochemistry, University of California, Berkeley, CA 94720

Contributed by Bruce N. Ames, June 5, 1989

ABSTRACT **We have shown recently that the temporal order of antioxidant consumption in human blood plasma exposed to a constant flux of aqueous peroxyl radicals is ascorbate = protein thiols > bilirubin > urate > α-tocopherol and that detectable lipid peroxidation starts only after ascorbate has been consumed completely. In this paper, we show that it is indeed ascorbate that completely protects plasma lipids against detectable peroxidative damage induced by aqueous peroxyl radicals and that ascorbate is the only plasma antioxidant that can do so. Plasma devoid of ascorbate, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and susceptible to peroxidative damage to lipids. The plasma proteins' thiols, although they become oxidized immediately upon exposure to aqueous peroxyl radicals, are inefficient radical scavengers and appear to be consumed mainly by autoxidation. Our data demonstrate that ascorbate is the most effective aqueous-phase antioxidant in human blood plasma and suggest that in humans ascorbate is a physiological antioxidant of major importance for protection against diseases and degenerative processes caused by oxidant stress.**

nates (9, 10), that ascorbate can have prooxidant activity in the presence of free transition metal catalysts (11). However, these *in vitro* findings are unlikely to be relevant to the *in vivo* situation in healthy organisms (6, 7), where most transition metal ions are not free, but attached to binding proteins, and thus are prevented from participating in free radical reactions outside the protein (2, 12, 13). Only under pathological conditions that cause release of heme or metal ions from their binding proteins—e.g., from hemoglobin (14) or ferritin (15)—could ascorbate act as a prooxidant. Under such conditions, however, not only ascorbate, but also α-tocopherol can be expected to be deleterious, since α-tocopherol, too, displays prooxidant effects in the presence of free iron ions (16).

In this study, we have used human blood plasma as a physiological model system to compare the antioxidant efficacy of ascorbate with that of α-tocopherol and other biological antioxidants. We have shown recently (17) that in plasma exposed to a water-soluble radical initiator or the oxidants generated by polymorphonuclear leukocytes no lipid peroxidation can be detected as long as ascorbate is present. This strongly suggested, yet did not prove, that in



Orthomolecular Medicine

The right molecule at the right concentration

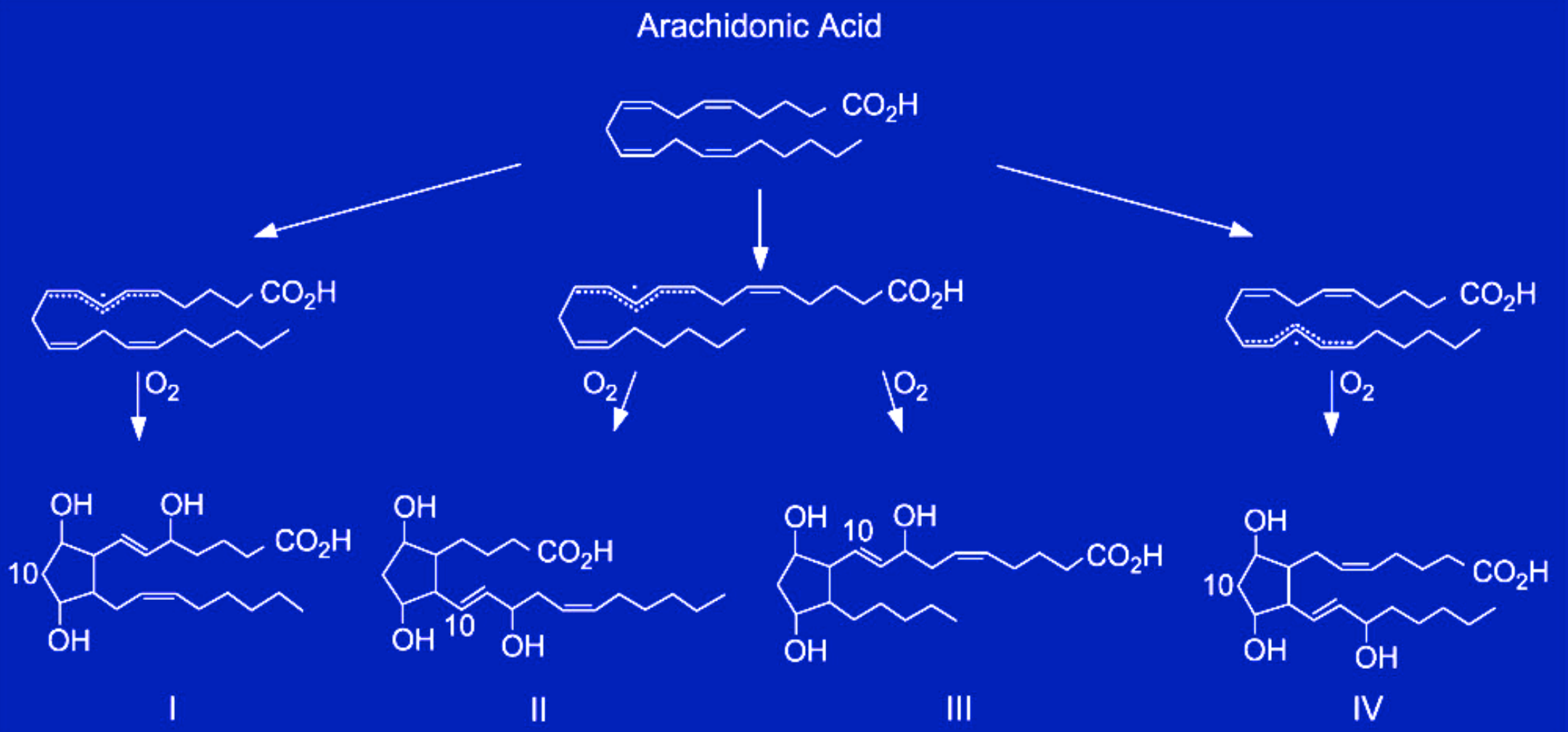
"Orthomolecular medicine is the preservation of good health and the treatment of disease by varying the concentrations in the human body of substances that are normally present in the body and required for health."

Linus Pauling (1986) "How to Live Longer and Feel Better"

Vitamin C effectively inhibits lipid peroxidation in human plasma and isolated LDL induced by:

- Aqueous peroxyl radicals (AAPH) Frei *et al.*, PNAS 1988, 1989; Polidori *et al.*, Arch Biochem Biophys 2004
- The gas-phase of cigarette smoke Frei *et al.*, Biochem J 1991
- Activated polymorphonuclear leukocytes Frei *et al.*, PNAS 1988, Stocker *et al.*, PNAS 1991
- HOCl and MPO/H_2O_2/Cl^- +/- NO_2^- (apoB modification) Carr *et al.*, Biochem J 2000; Carr and Frei, J Biol Chem 2001
- Human aortic endothelial cells Martin and Frei, ATVB 1997
- Superoxide radicals and hydrogen peroxide (X/XO system) Frei *et al.*, Adv Exp Med Biol 1990
- Copper Retsky *et al.*, J Biol Chem 1993, Biochim Biophys Acta 1995, Suh *et al.*, FRBM 2003
- Iron Berger *et al.*, J Biol Chem 1997, Suh *et al.*, FRBM 2003

F_2-Isoprostanes, a validated marker of *in vivo* lipid peroxidation: Formation from arachidonic acid



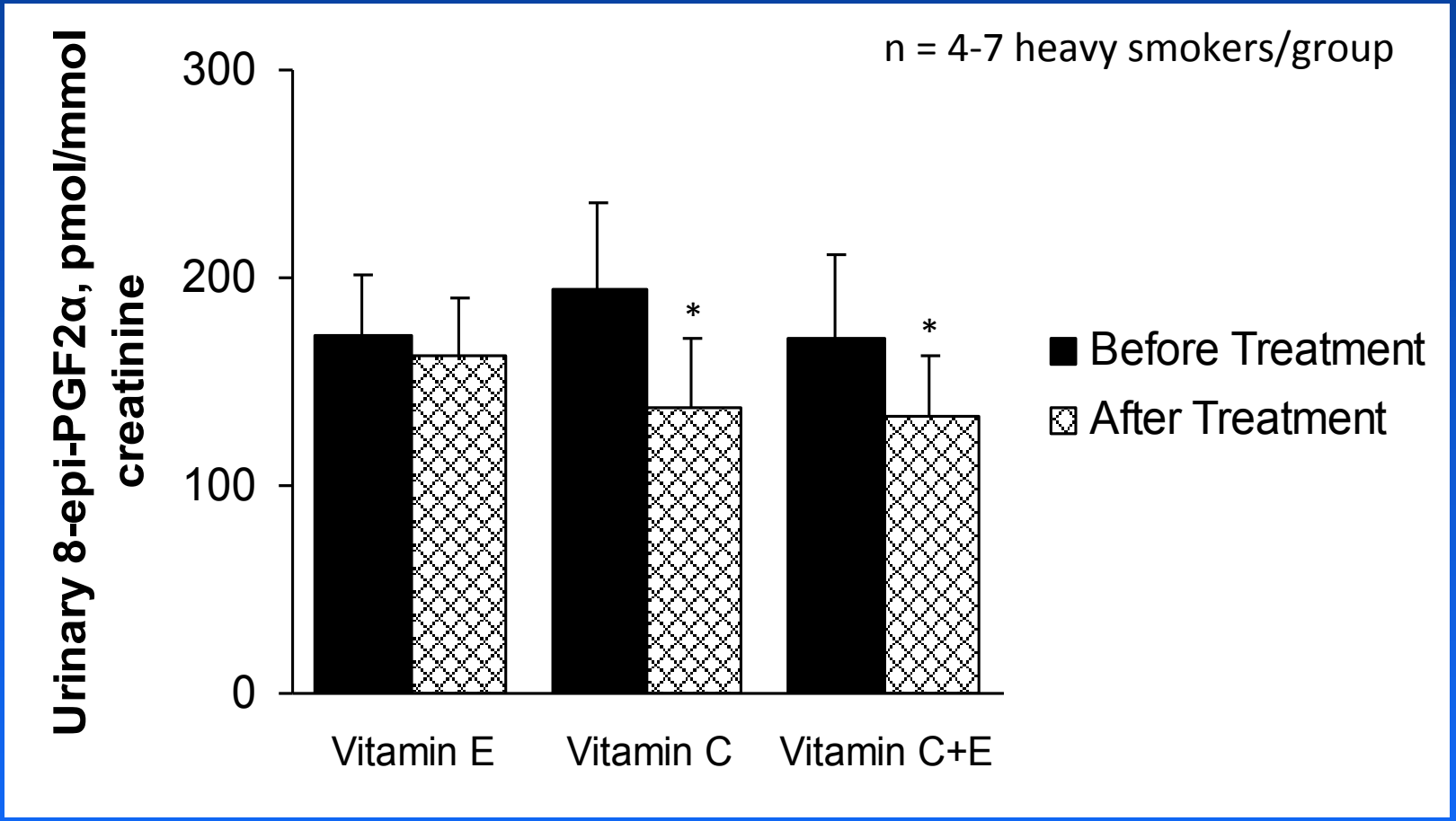
Morrow *et al.* (1995) *New Engl. J. Med.* 332, 1198

Elevated 8-iso-$PGF_{2\alpha}$ Levels in:

- Cigarette smokers
- Human atherosclerotic lesions
- Diabetics
- Hypercholesterolemics
- Alzheimer's disease patients
- Alcoholics/patients with liver cirrhosis
- Obese subjects
- During vascular reperfusion
- Etc.

Supplementation with vitamin C–but not vitamin E–lowers urinary F_2-isoprostane levels in smokers

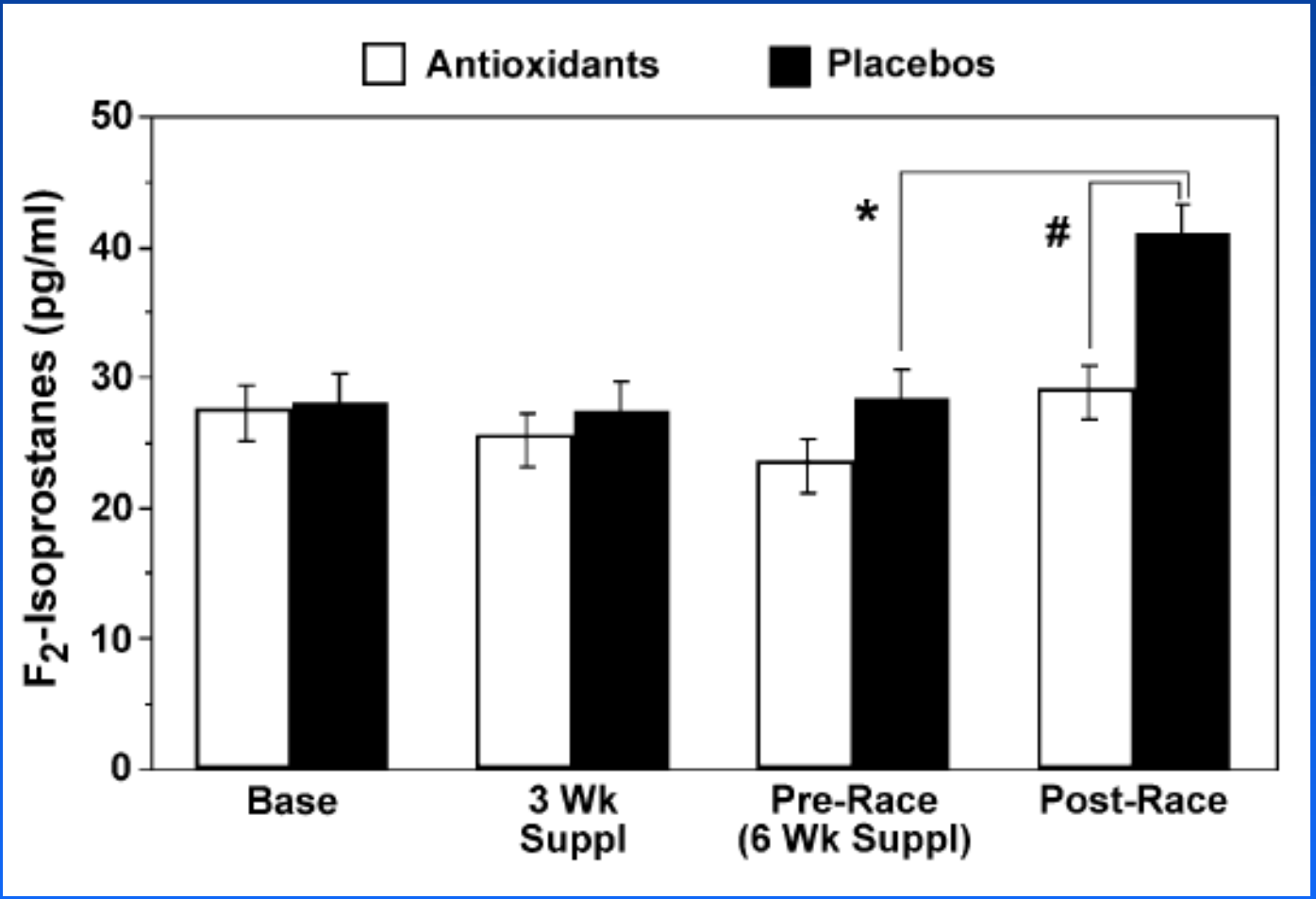
Supplementation with 2 g/d of vitamin C and/or 800 IU/d of vitamin E for 5 days



Reilly et al. *Circulation* 1996:94:19-25

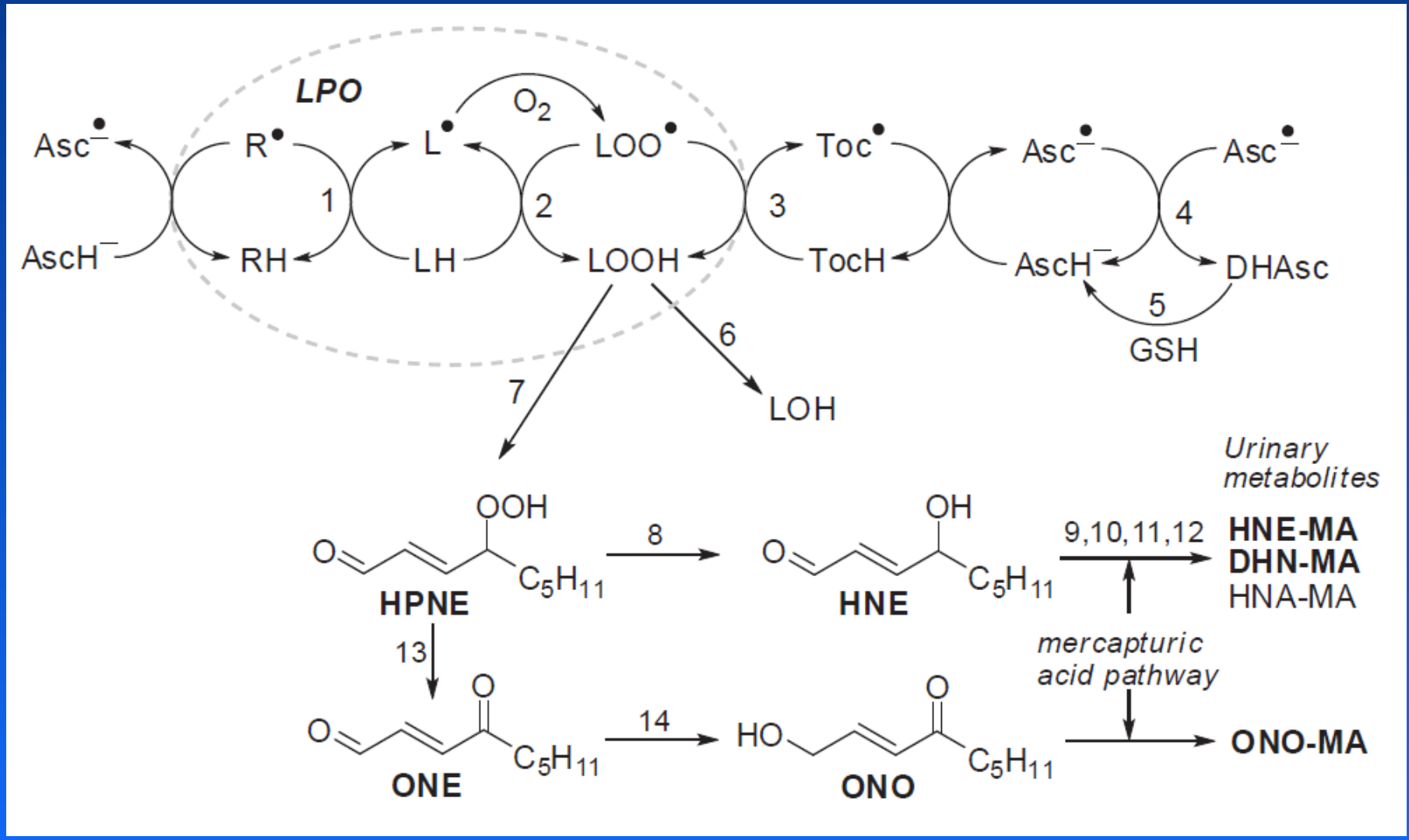
Vitamin C and E supplementation prevents the increase in plasma F_2-isoprostanes following a 50-km ultramarathon

Supplementation with 300 mg *RRR*-α-tocopheryl acetate and 1000 mg vitamin C daily for 21 days



Mastaloudis et al. *Free Radic Biol Med.* 2004;36:1329-41

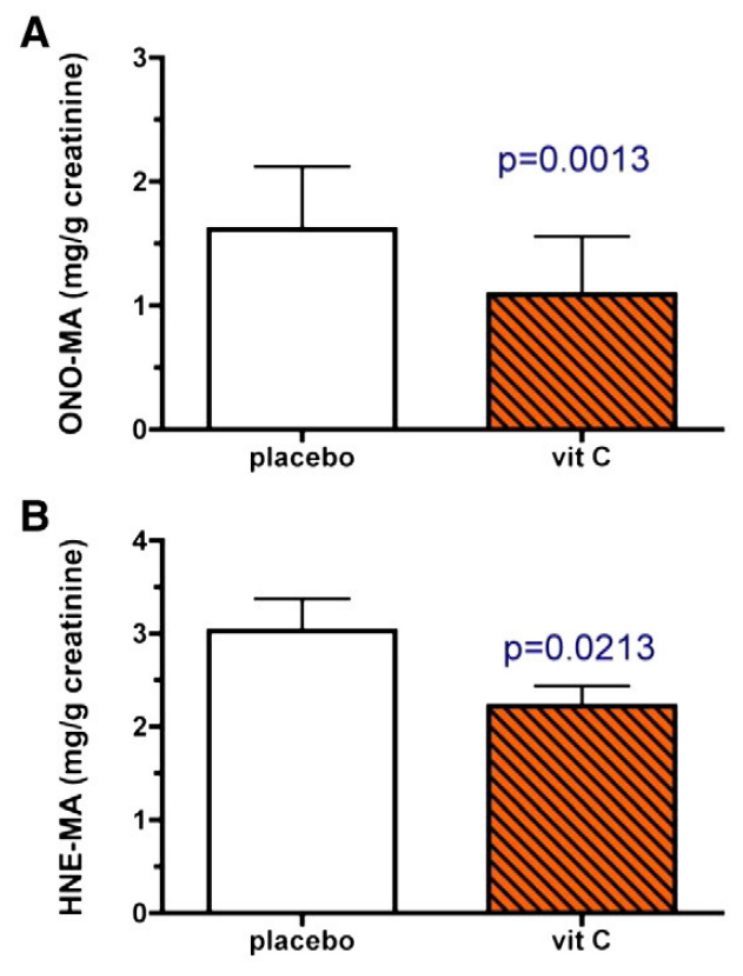
Urinary levels of 4-hydroperoxy-2-nonenal (HPNE) metabolites: Novel markers of *in vivo* lipid peroxidation



Kuiper et al. *Free Radic Biol Med.* 2011;50:848-853

Vitamin C supplementation lowers urinary levels of 4-hydroperoxy-2-nonenal (HPNE) metabolites in humans

Daily supplementation with 2 x 500 mg vitamin C or placebo for 17 days in 12 non-smokers (6M, 6F) and 10 smokers (6M, 4F) (crossover-design)



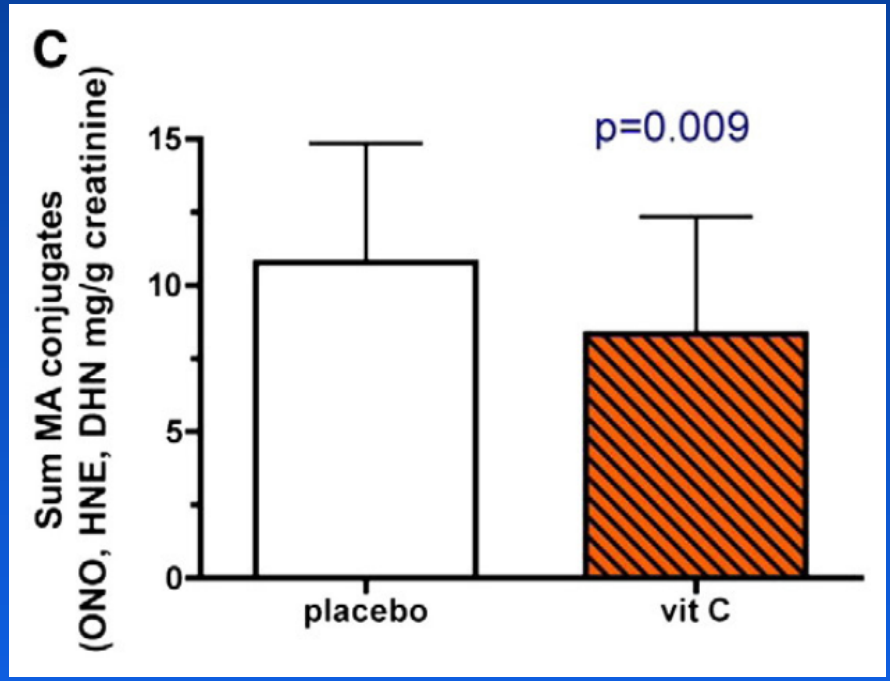


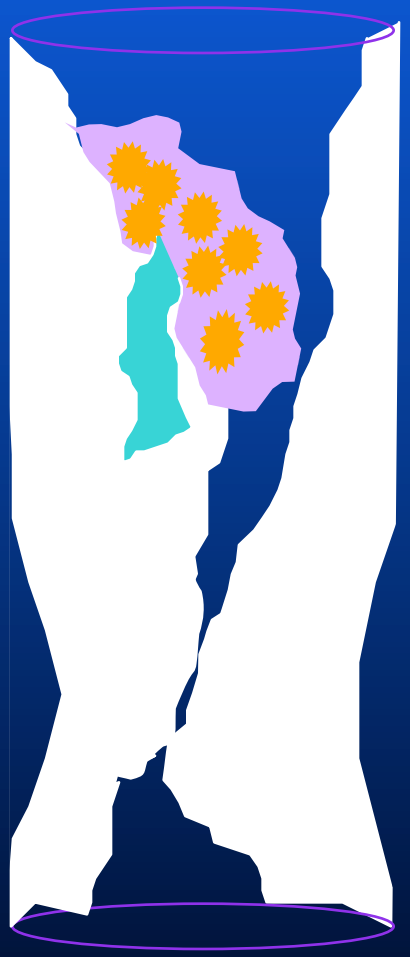
Fig. 4. Modulation of urinary levels of HPNE metabolites (means ± SD) by vitamin C supplements. Urine was collected from all subjects shown in Fig. 3. Vitamin C supplementation decreased urinary levels of (A) ONO-MA, (B) HNE-MA, and (C) the total of ONO-MA, HNE-MA, and DHN-MA.

Kuiper et al. *Free Radic Biol Med.* 2011;50:848-853

The Causes of Most Heart Attacks and Strokes:
Plaque Rupture, Thrombus Formation, and Vasoconstriction

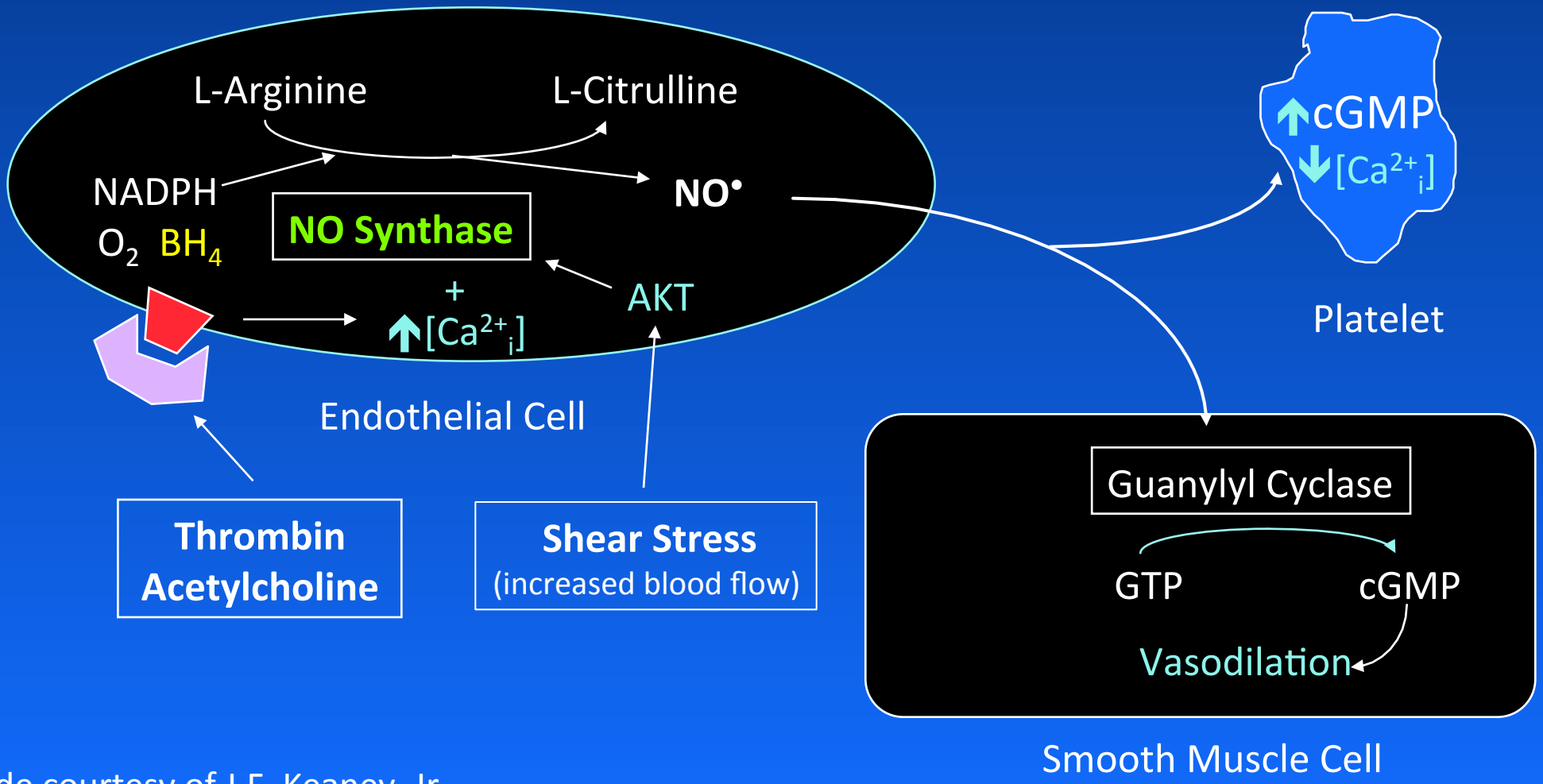
- Narrowing of Arterial Lumen due to Atherosclerosis
- Atherosclerotic Plaque Rupture
- Platelet Adhesion and Aggregation
- Thrombus Formation
- Vasoconstriction

Inhibited by Nitric Oxide (NO)



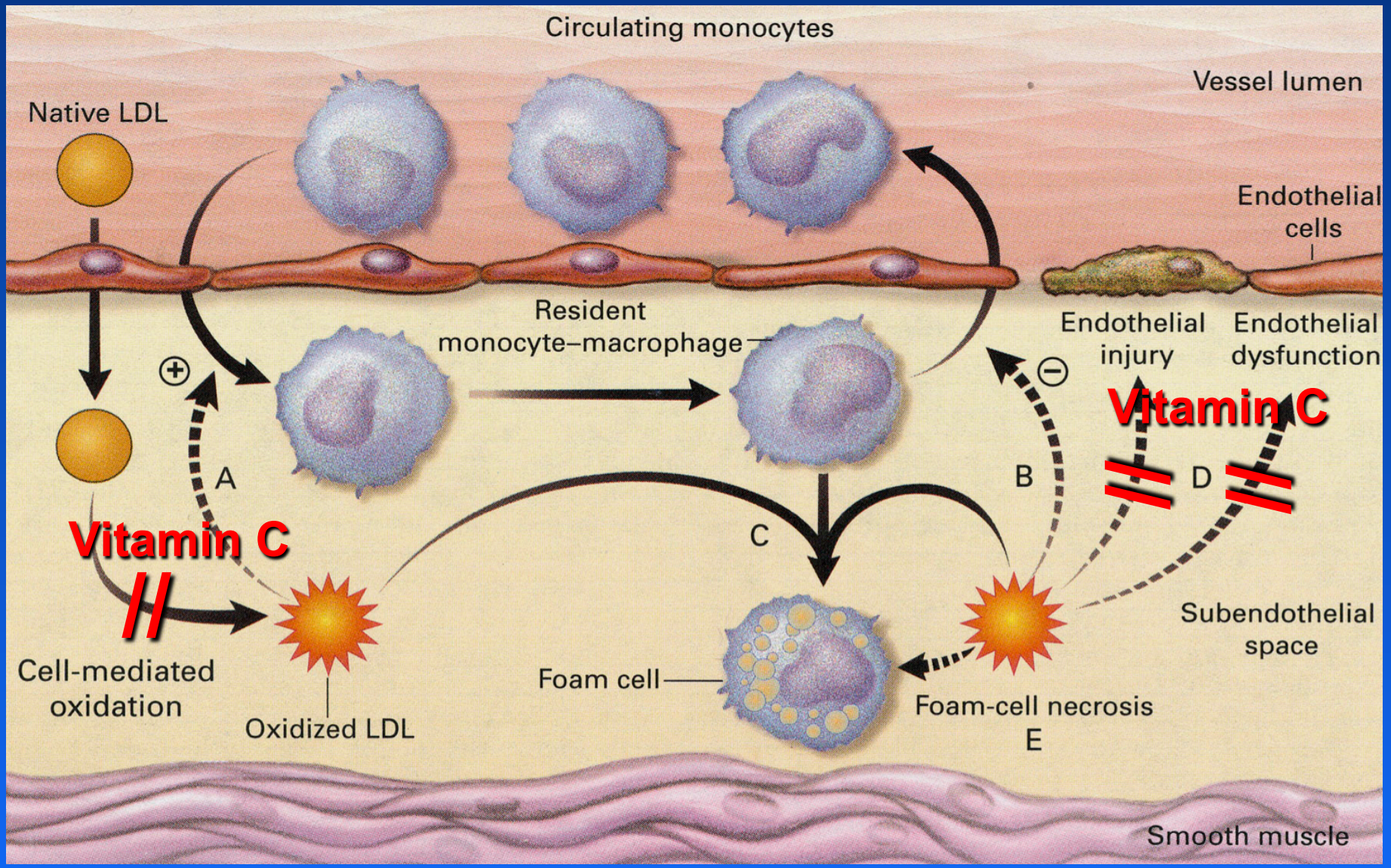
Slide courtesy of John F. Keaney, Jr.

Synthesis and Action of EDNO



Slide courtesy of J.F. Keaney, Jr.

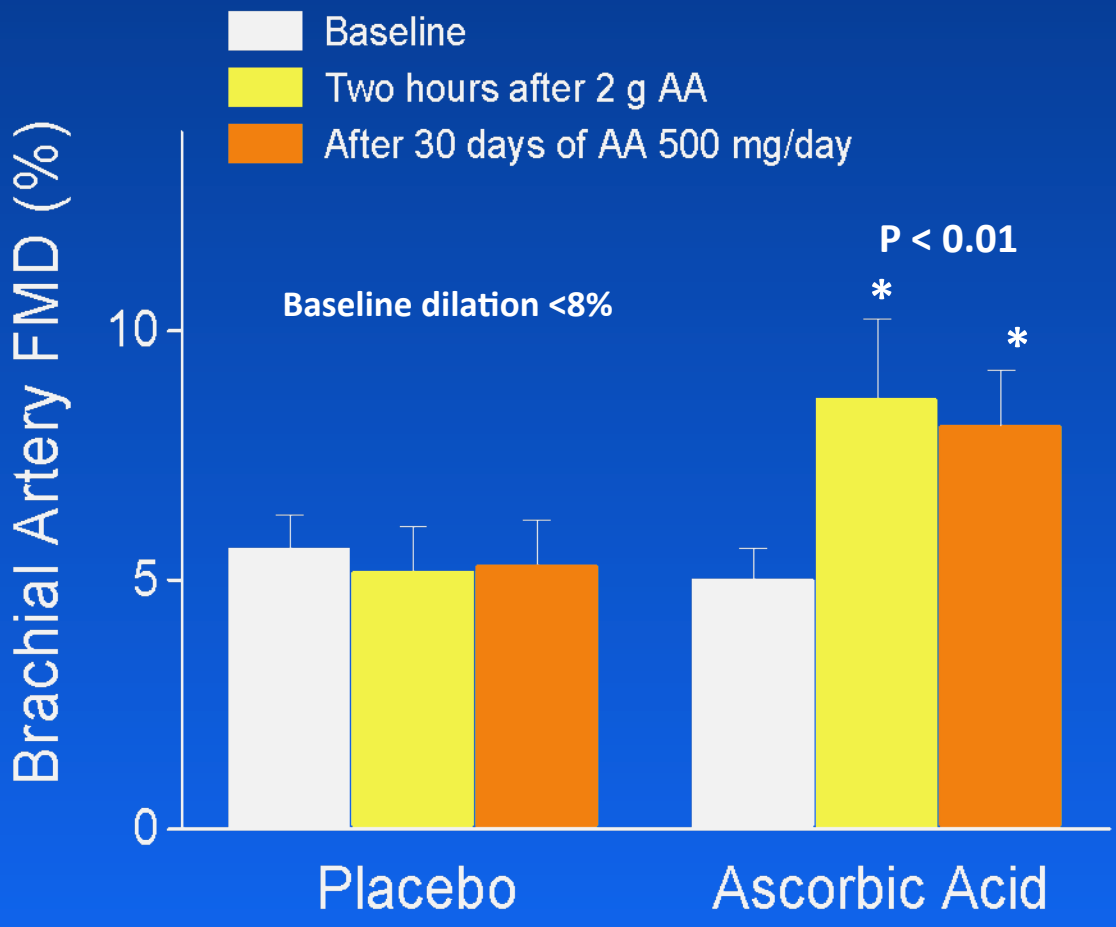
Role of inflammation and oxidative stress in atherosclerosis



Diaz, Frei *et al. New Engl. J. Med*. 1997;337:408-416

Vitamin C Treatment (Short- or Long-Term) Improves Vascular Function (EDNO-Dependent Vasodilation) in CAD Patients

- 48 patients with angiographically proven CAD
- Randomized, double-blind, placebo controlled study
- Visit 1: brachial ultrasound at baseline and 2 hours after 2 grams of ascorbic acid
- Visit 2: brachial ultrasound after 30 days of 500 mg/day of ascorbic acid

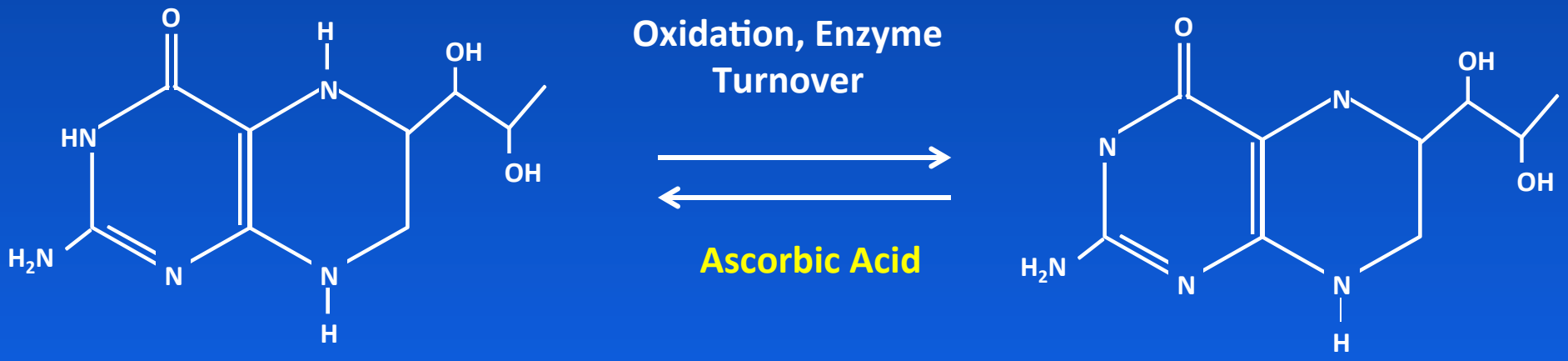


Levine *et al. Circulation* 1996;93:1107-1113
Gokce *et al. Circulation* 1999;99:3234-3240

Vitamin C (500 mg–12 g by IV infusion or orally) Improves Vascular Function (EDNO-Dependent Vasodilation):

- in CAD patients Levine et al. (1996) Circulation 93:1107-1113; Ito et al. (1998) Am J Cardiol 82:762-767; Gokce et al. (1999) Circulation 99:3234-3240
- in NIDDM and IDDM patients Ting et al. (1996) J Clin Invest 97:22-28; Timimi et al. (1998) J Am Coll Cardiol 31:552-557; Beckman et al. (2001) Circulation 103:1618-1623; Antoniades et al. (2004) Diabet Med 21:552-558
- in chronic smokers Heitzer et al. (1996) Circulation 94:6-9; Motoyama et al. (1997) Am J Physiol 273:H1644-H1650; Schindler et al. (2000) Cardiology 94:239-246
- in hypercholesterolemic subjects Ting et al. (1997) Circulation 95:2617-2622; Perticone et al. (2000) Atherosclerosis 152:511-518
- in hypertensive patients Solzbach et al. (1997) Circulation 96:1513-1519; Taddei et al. (1998) Circulation 97:2222-2229; Natali et al. (2000) ATVB 20:2401-2406
- following a single high-fat meal Plotnick et al. (1997) JAMA 26;278: 1682-1686
- in patients with chronic heart failure Hornig et al. (1998) Circulation 97:363-368; Ito et al. (1998) Am J Cardiol 82:762-767
- in patients with angina pectoris Kugiyama et al. (1998) J Am Coll Cardiol 32:103-109; Hamabe et al. (2001) Am J Cardiol 87:1154-1159
- in experimental hyperhomocyst(e)inemia Kanani et al. (1999) Circulation 100:1161-1168; Hanratty et al. (2001) BMC Cardiovasc Disord 1:1

Mechanism of Vitamin C Action



Tetrahydrobiopterin
(BH_4)

Quinoid dihydrobiopterin
(QBH_2)

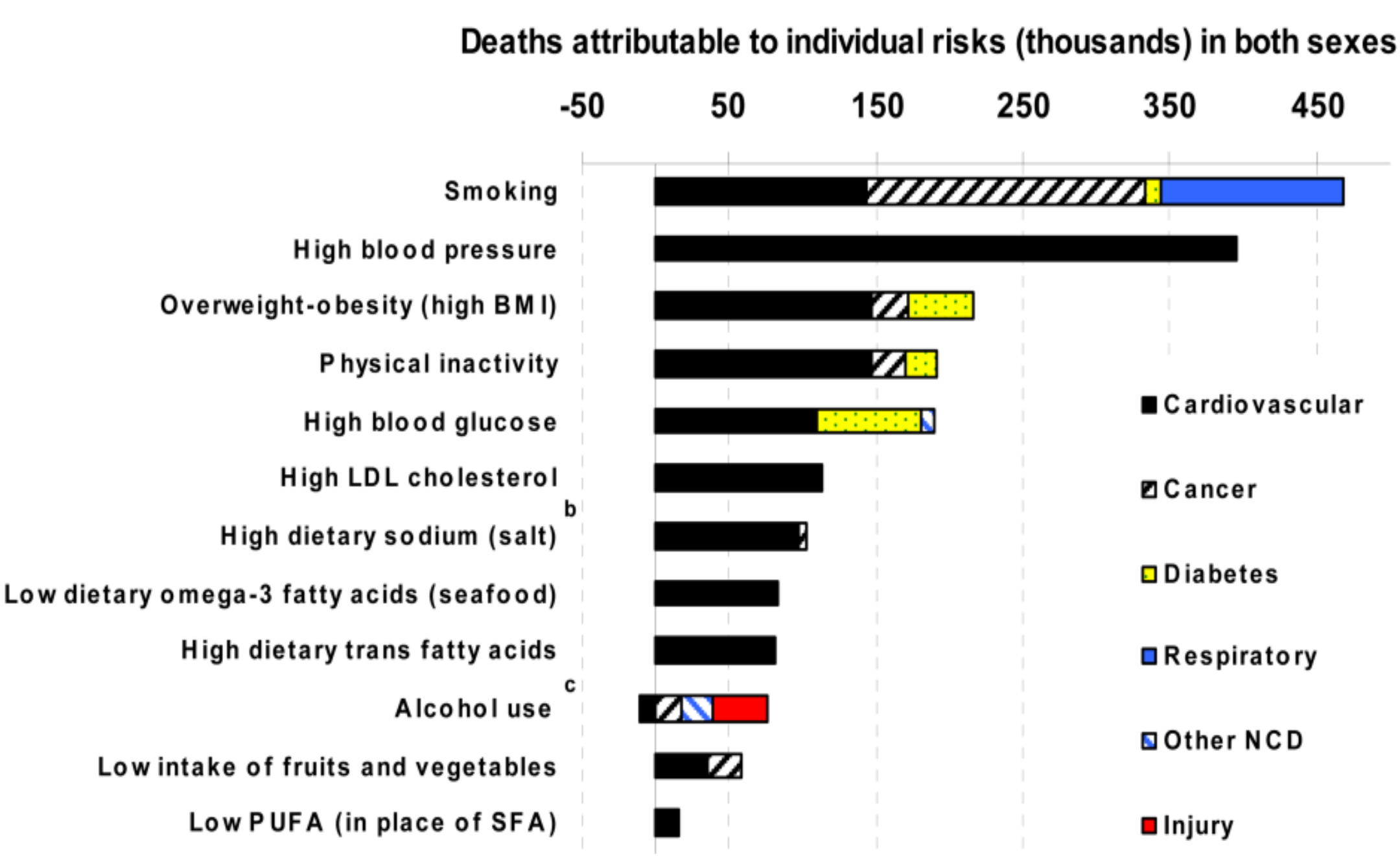
Plasma Vitamin C correlates with Unstable Coronary Syndrome (Angina Class) in CAD Patients (n = 149)

	Angina Class (1-4)	
Plasma Marker	R	*P*
α-Tocopherol (Vitamin E)	-.06	.68
LDL α-Tocopherol	-.18	.27
γ-Tocopherol	-.08	.56
β-Carotene	.07	.66
Lycopene	-.01	.95
Retinol	-.15	.36
Ascorbic Acid (Vitamin C)	-.43	.001
Uric Acid	.20	.12
Thiols	-.19	.15
Ceruloplasmin	.00	.97
Superoxide Dismutase (RBCs)	-.08	.64
Lag Phase (LDL Diene Conjugation)	-.13	.43
F_2-Isoprostanes	.02	.87

Vita *et al.* (1998) *J. Am. Coll. Cardiol.* 31, 980-986

Vitamin C and Blood Pressure
Phase II RCT Evidence

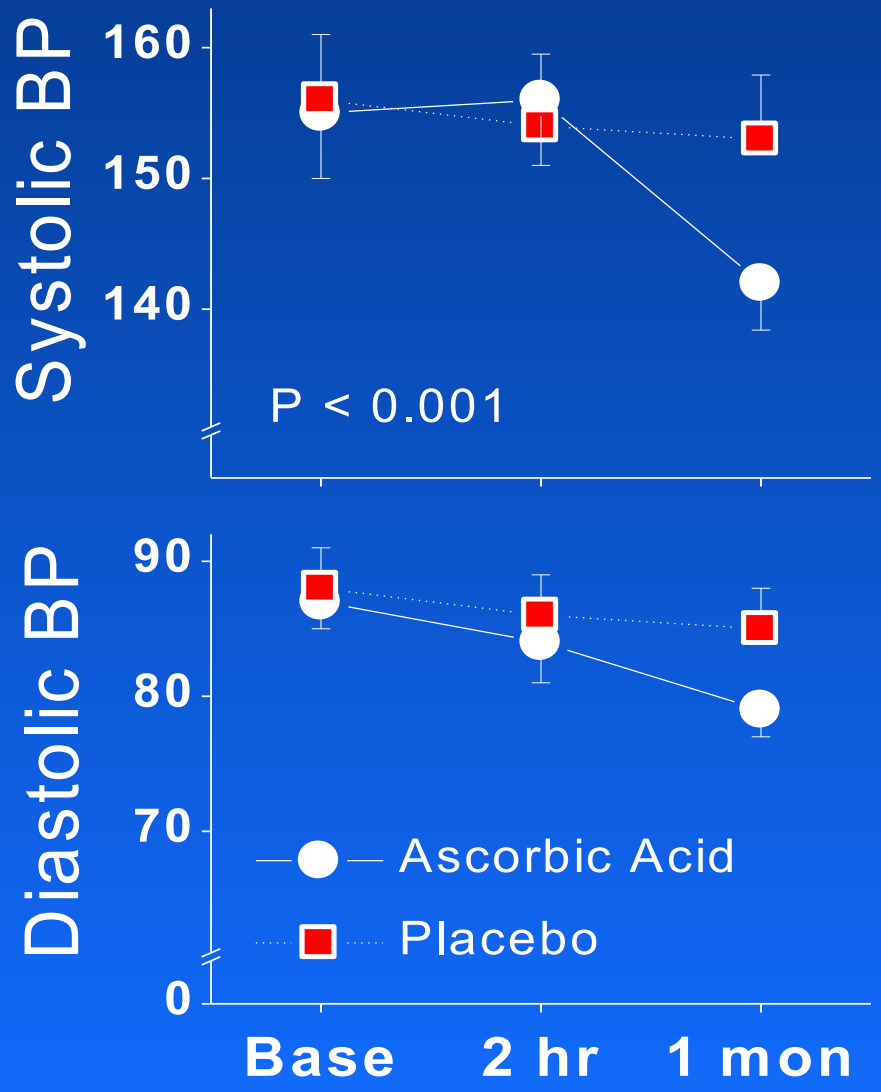
- Hypertension is a CV disease (ICD 10 Code 110-115: Hypertensive diseases)
- Second most common preventable cause of death in the U.S.



Danaei et al., 2009; 6(4):e1000058

Vitamin C Lowers Blood Pressure in Patients with Essential Hypertension

- 39 patients with hypertension, age 49±13 years
- Randomized, double-blind, placebo controlled study
- Visit One: blood pressure measured at baseline and 2 hours after 2 grams of ascorbic acid
- Visit Two: blood pressure measured after 30 days of 500 mg/day of ascorbic acid



Duffy *et al. Lancet* 1999;354:2048-2049

Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials[1–3]

Stephen P Juraschek, Eliseo Guallar, Lawrence J Appel, and Edgar R Miller III

ABSTRACT

Background: In observational studies, increased vitamin C intake, vitamin C supplementation, and higher blood concentrations of vitamin C are associated with lower blood pressure (BP). However, evidence for blood pressure–lowering effects of vitamin C in clinical trials is inconsistent.

Objective: The objective was to conduct a systematic review and meta-analysis of clinical trials that examined the effects of vitamin C supplementation on BP.

Design: We searched Medline, EMBASE, and Central databases from 1966 to 2011. Prespecified inclusion criteria were as follows: *1*) use of a randomized controlled trial design; *2*) trial reported effects on systolic BP (SBP) or diastolic BP (DBP) or both; *3*) trial used oral vitamin C and concurrent control groups; and *4*) trial had a minimum duration of 2 wk. BP effects were pooled by random-effects models, with trials weighted by inverse variance.

Results: Twenty-nine trials met eligibility criteria for the primary analysis. The median dose was 500 mg/d, the median duration was 8 wk, and trial sizes ranged from 10 to 120 participants. The pooled changes in SBP and DBP were −3.84 mm Hg (95% CI: −5.29, −2.38 mm Hg; $P < 0.01$) and −1.48 mm Hg (95% CI: −2.86, −0.10 mm Hg; $P = 0.04$), respectively. In trials in hypertensive participants, corresponding reductions in SBP and DBP were −4.85 mm Hg ($P < 0.01$) and −1.67 mm Hg ($P = 0.17$). After the inclusion of 9 trials with imputed BP effects, BP effects were attenuated but remained significant.

Conclusions: In short-term trials, vitamin C supplementation reduced SBP and DBP. Long-term trials on the effects of vitamin C supplementation on BP and clinical events are needed. *Am J Clin Nutr* 2012;95:1079–88.

A large number of small randomized controlled trials have evaluated the effect of vitamin C supplementation on BP (11–48), but the results were inconsistent, possibly because of heterogeneous methods and the small sample size of individual trials (49, 50). Our objective was to conduct a systematic review and meta-analysis of randomized controlled trials to determine the effects of vitamin C supplementation on BP in adults.

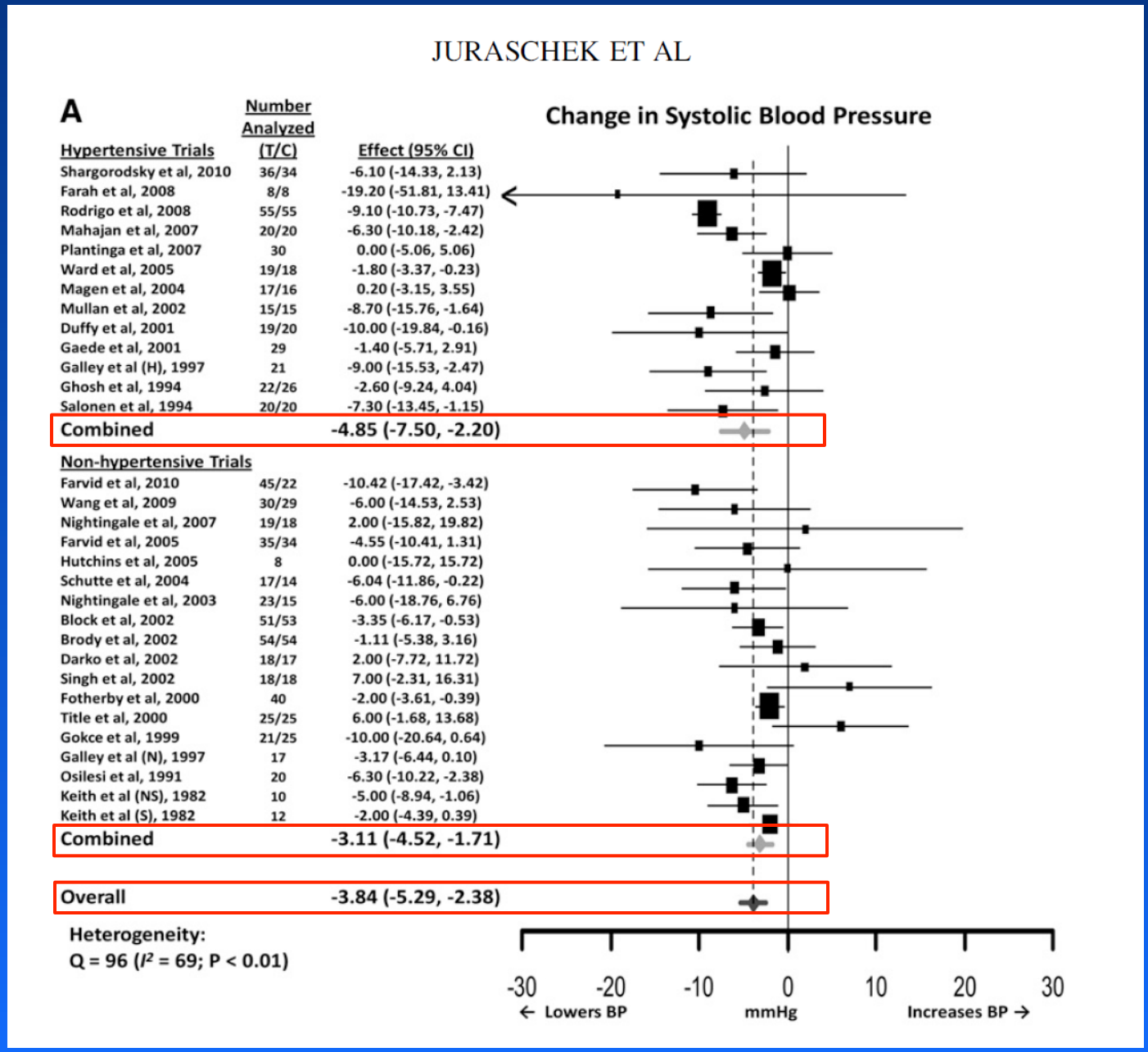
METHODS

Search strategy and eligibility criteria

We performed a search of Medline, EMBASE, and the Cochrane Central Register of Controlled Trials (Central) databases from January 1966 through December 2010 using the following terms: *blood pressure*, *hypertension*, *hypertensive*, *hypotension*, *hypotensive*, *endothelial dysfunction*, *endothelial function*, *ascorbic acid*, *antioxidant(s)*, *vitamin(s)*, *randomized controlled trials*, and *clinical trials*. The search was confined to human studies without language restrictions. *See* the online supplemental material under "Supplemental data" in the online issue for details. We reviewed bibliographies of original research and previous reviews to complement the search.

Prespecified inclusion criteria were as follows: *1*) use of a randomized controlled trial design, *2*) trial reported effects on systolic BP (SBP) or diastolic BP (DBP) or both, *3*) trial used oral vitamin C supplementation and concurrent control groups, and *4*) trial had a minimum duration of 2 wk. Exclusion criteria were as follows: *1*) trials that enrolled pregnant women, children, or patients with end-stage renal disease; 2) trials in which

Effects of Vitamin C Supplementation on Blood Pressure: Meta-Analysis of 29 RCTs (median 500 mg/d, 8 wks)



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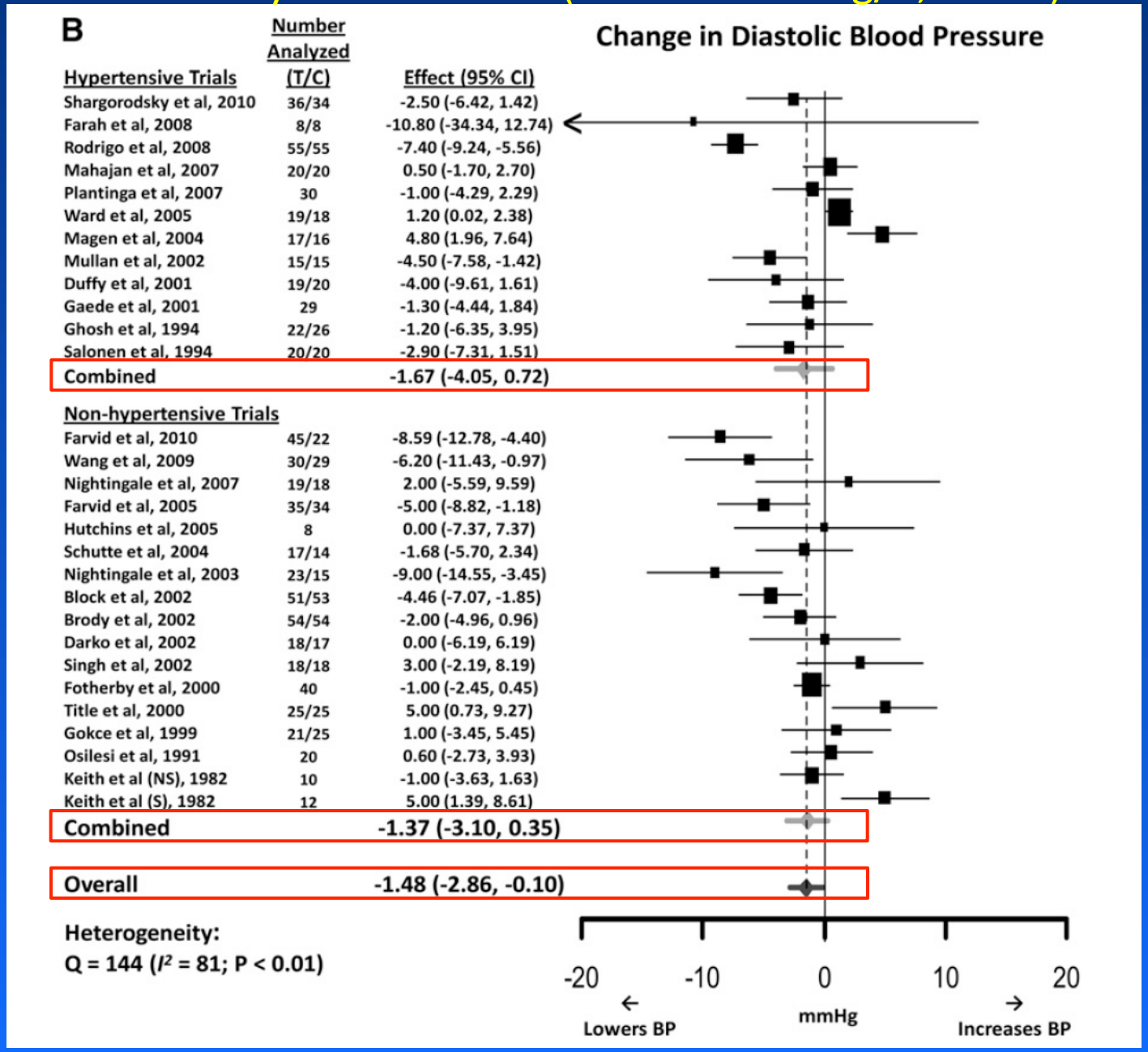
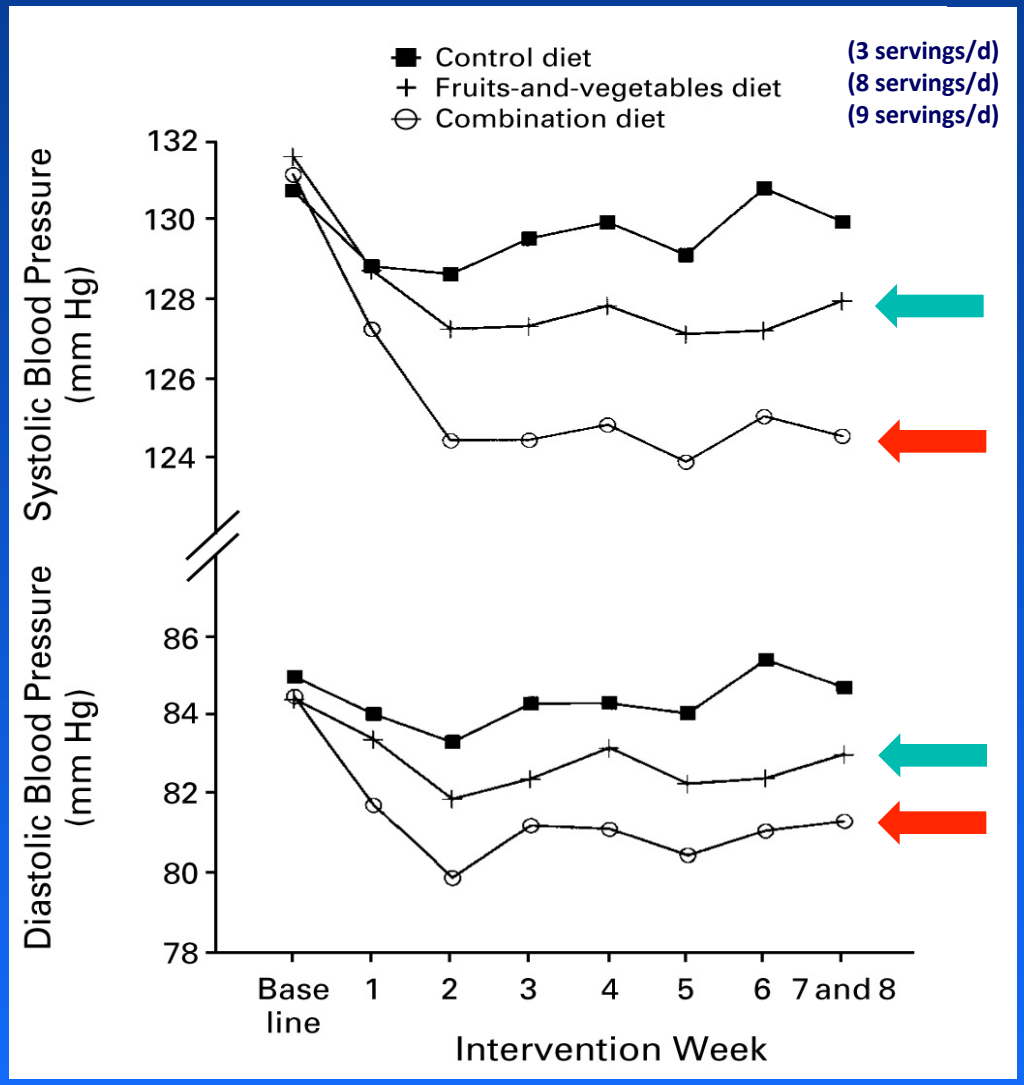


Fruit & Vegetable Intake and Blood Pressure



After 8 weeks, the SBP and DBP of those on the fruit and vegetable diet were significantly lower than those on the typical American diet.

SBP and DBP of those on the combination (DASH) diet were even lower.

Appel et al. *N Engl J Med* 1997;336:1117-1124

Epidemiologic Studies of Vitamin C and Incidence of CHD or Stroke

Prospective Cohort Studies based on Plasma or Serum Levels of Vitamin C

Prospective Cohort Studies: Highest Plasma or Serum Vitamin C Levels are associated with Lowest CVD Risk (CHD or Stroke)

Reference	Study population	Mean vitamin C level associated with health effect	Disease outcome
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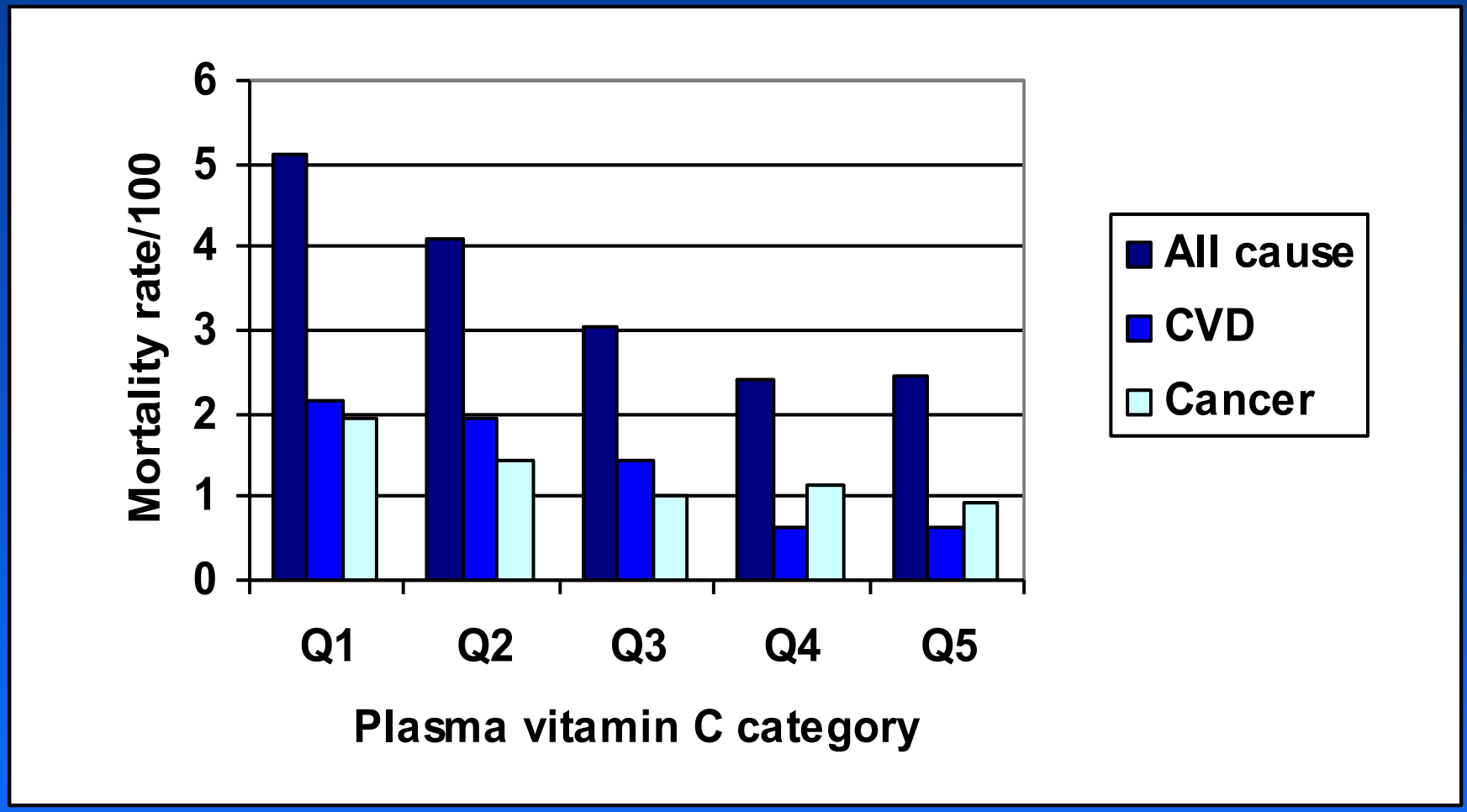
Kurl et al. Stroke 2002;33:1568-73	2,419 middle aged men	>65.0 µmol/L	Stroke

Frei, Birlouez-Aragon, and Lykkesfeldt. *Crit Rev Food Sci Nutr* **2012;52(9):815-29**

Mortality from all causes, cardiovascular diseases, and cancer in >19,000 men and women 45-79 years by category of plasma vitamin C

European Prospective Investigation into Cancer (EPIC) Norfolk 1993-2000



Khaw et al. *Lancet* 2001;357:657-663

Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study

Kay-Tee Khaw, Sheila Bingham, Ailsa Welch, Robert Luben, Nicholas Wareham, Suzy Oakes, Nicholas Day

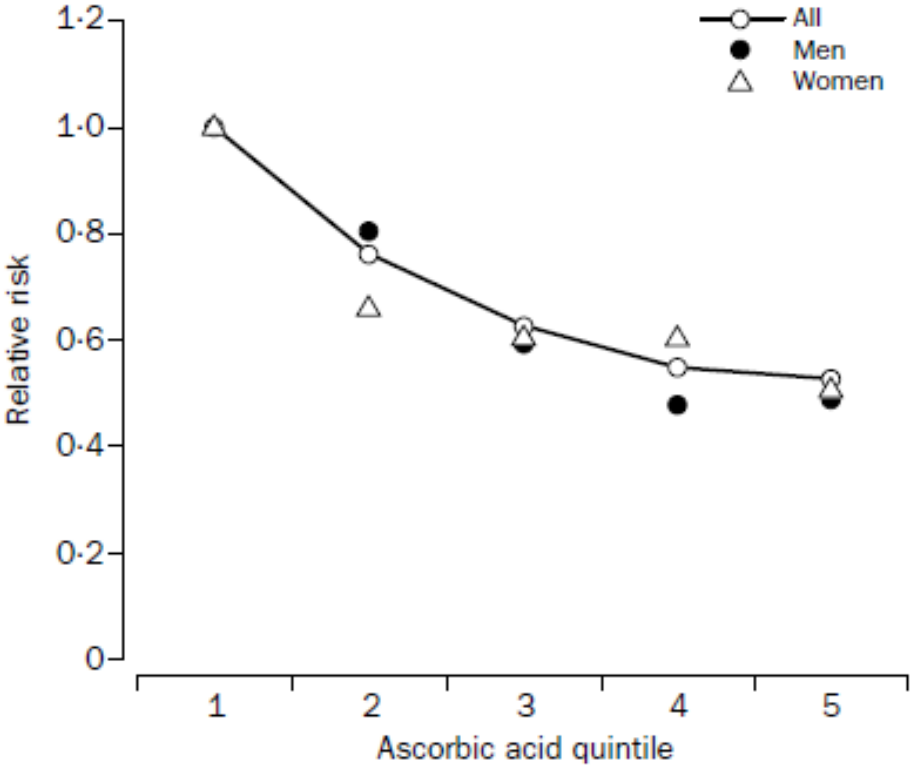


Figure 1: **Relative risk of total mortality by quintile of plasma ascorbic acid**

Age-adjusted and sex adjusted Cox regression model for relative risk, including a quadratic term for ascorbic-acid.

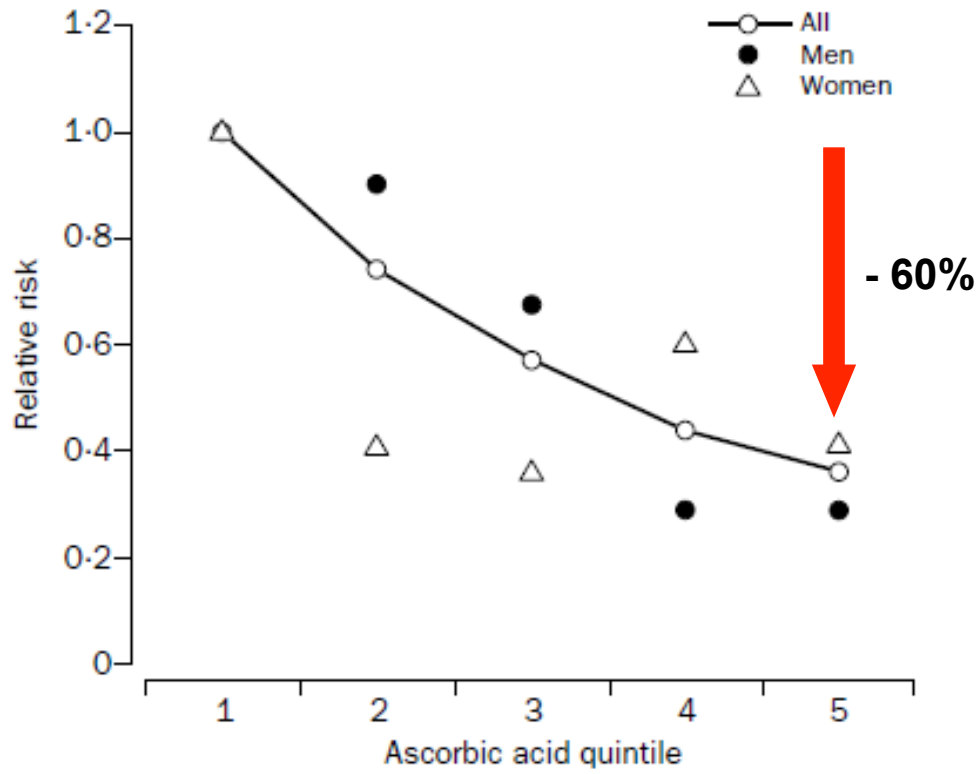


Figure 2: **Relative risk for cardiovascular disease mortality by quintile of plasma ascorbic acid**

Age-adjusted and sex-adjusted Cox regression model for relative risk, including a quadratic term for ascorbic acid.

Plasma vitamin C concentrations predict risk of incident stroke over 10 y in 20 649 participants of the European Prospective Investigation into Cancer–Norfolk prospective population study[1–3]

Phyo K Myint, Robert N Luben, Ailsa A Welch, Sheila A Bingham, Nicholas J Wareham, and Kay-Tee Khaw

Am J Clin Nutr 2008;87:64–9.

TABLE 2
Relative risks (and 95% CIs) for risk of stroke by quartile of plasma vitamin C concentration at baseline in the European Prospective Investigation into Cancer (EPIC)–Norfolk population (1993–1977 to 2005)[1]

	No. of events	Plasma vitamin C quartile				*P*
		1 (< 41 μmol/L)	2 (41–53 μmol/L)	3 (54–65 μmol/L)	4 (≥ 66 μmol/L)	
Model A	448	1.00	0.76 (0.61, 0.96)	0.57 (0.43, 0.75)	0.49 (0.37, 0.64)	<0.0001
Model B	448	1.00	0.80 (0.63, 1.01)	0.60 (0.45, 0.79)	0.51 (0.39, 0.68)	<0.0001
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Model F	381	1.00	0.91 (0.70, 1.17)	0.67 (0.50, 0.91)	0.61 (0.45, 0.84)	0.006
Model G	428	1.00	0.80 (0.63, 1.02)	0.62 (0.46, 0.82)	0.58 (0.43, 0.78)	0.001
Model H	448	1.00	0.83 (0.65, 1.05)	0.67 (0.47, 0.83)	0.57 (0.42, 0.76)	<0.0001
Model I	427	1.00	0.91 (0.72, 1.16)	0.69 (0.52, 0.92)	0.60 (0.44, 0.81)	0.003

[1] A Cox-proportional hazards model was used. Model A was adjusted for age and sex. Model B was adjusted for age, sex, and smoking status. Model C was adjusted for age, sex, smoking status, BMI, systolic blood pressure (by 10-mm Hg increase), cholesterol, physical activity, and prevalent myocardial infarction and diabetes. Model D was adjusted as in model C and for social class and alcohol consumption. Model E was adjusted as in model D and for any supplement use. Model F was adjusted as in model E after exclusion of prevalent myocardial infarction and cancer. Model G was adjusted as in model E after exclusion of vitamin C supplement users. Model H was adjusted as in model E and for fruit and vegetable consumption. Model I was adjusted as in model E after exclusion of strokes occurring within 2 y of follow-up.

Plasma Vitamin C Levels Are Decreased and Correlated With Brain Damage in Patients With Intracranial Hemorrhage or Head Trauma

Maria Cristina Polidori, MD; Patrizia Mecocci, MD, PhD; Balz Frei, PhD

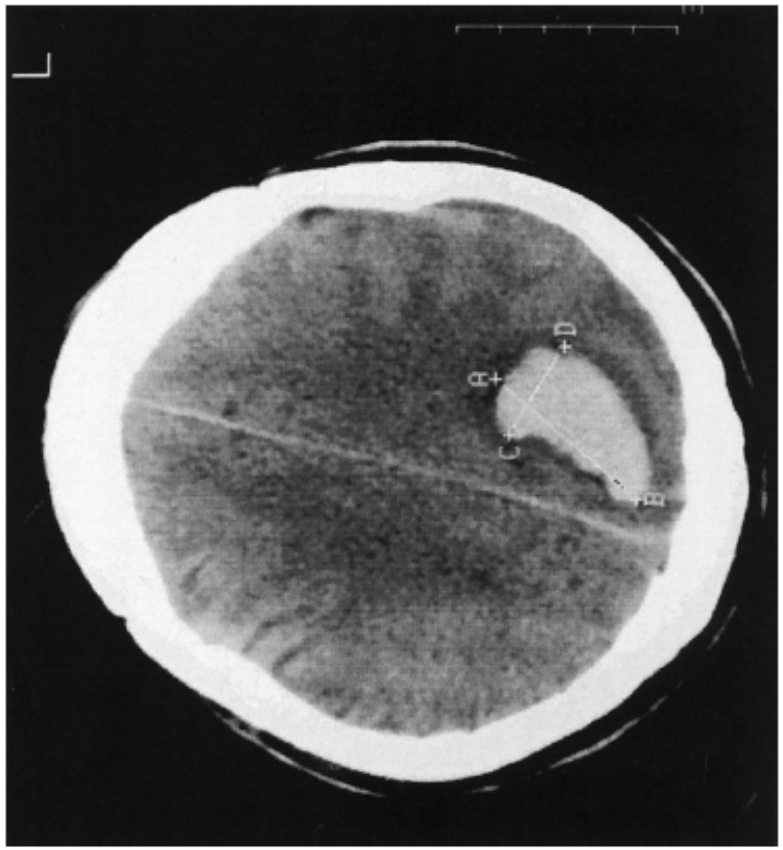


Figure 1. CT scan of patient showing ICH (occipital). Major diameter of lesion, 3.9 cm (group B).

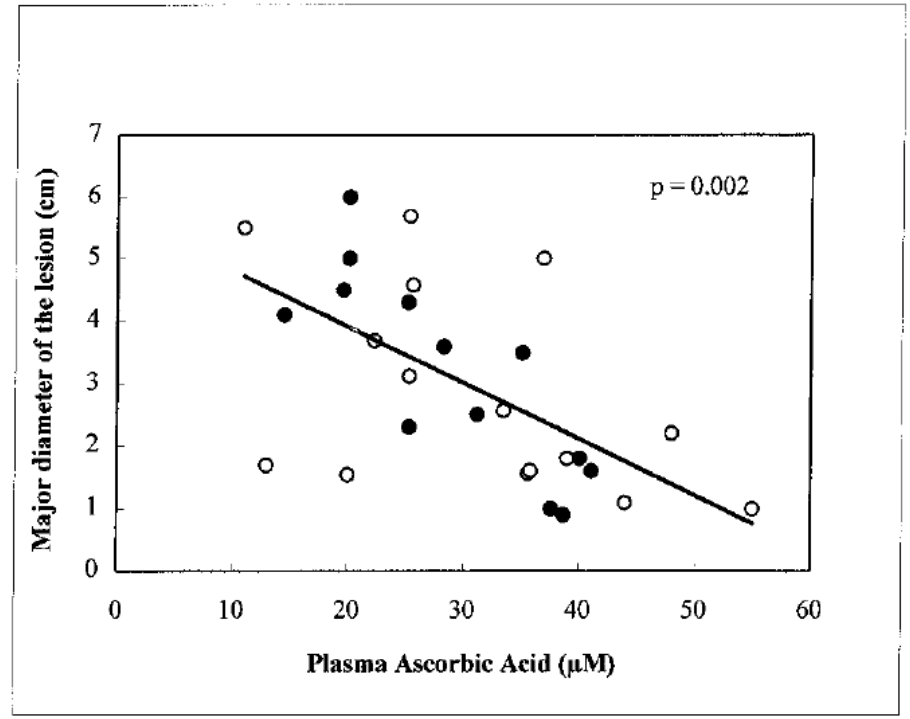
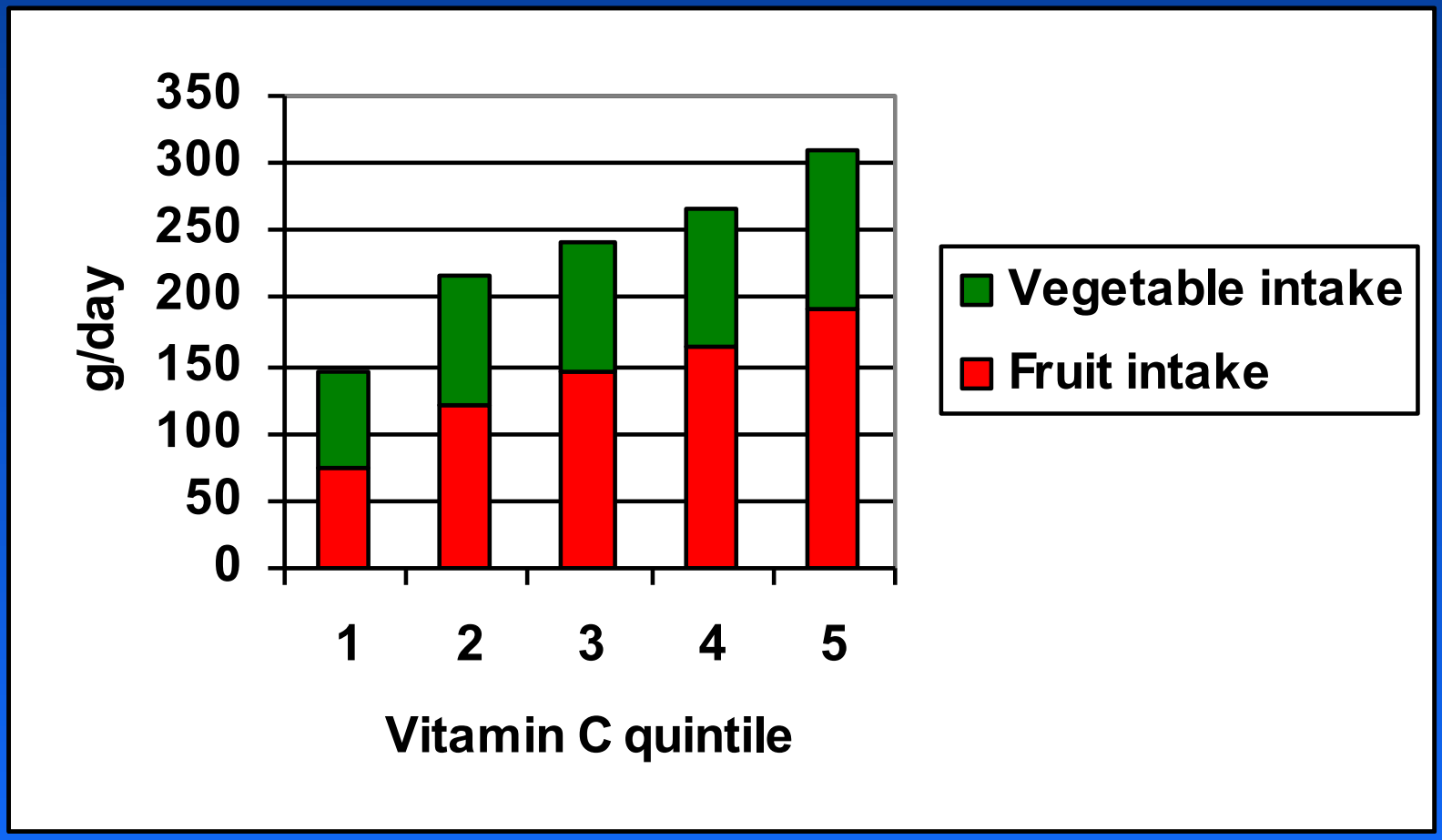


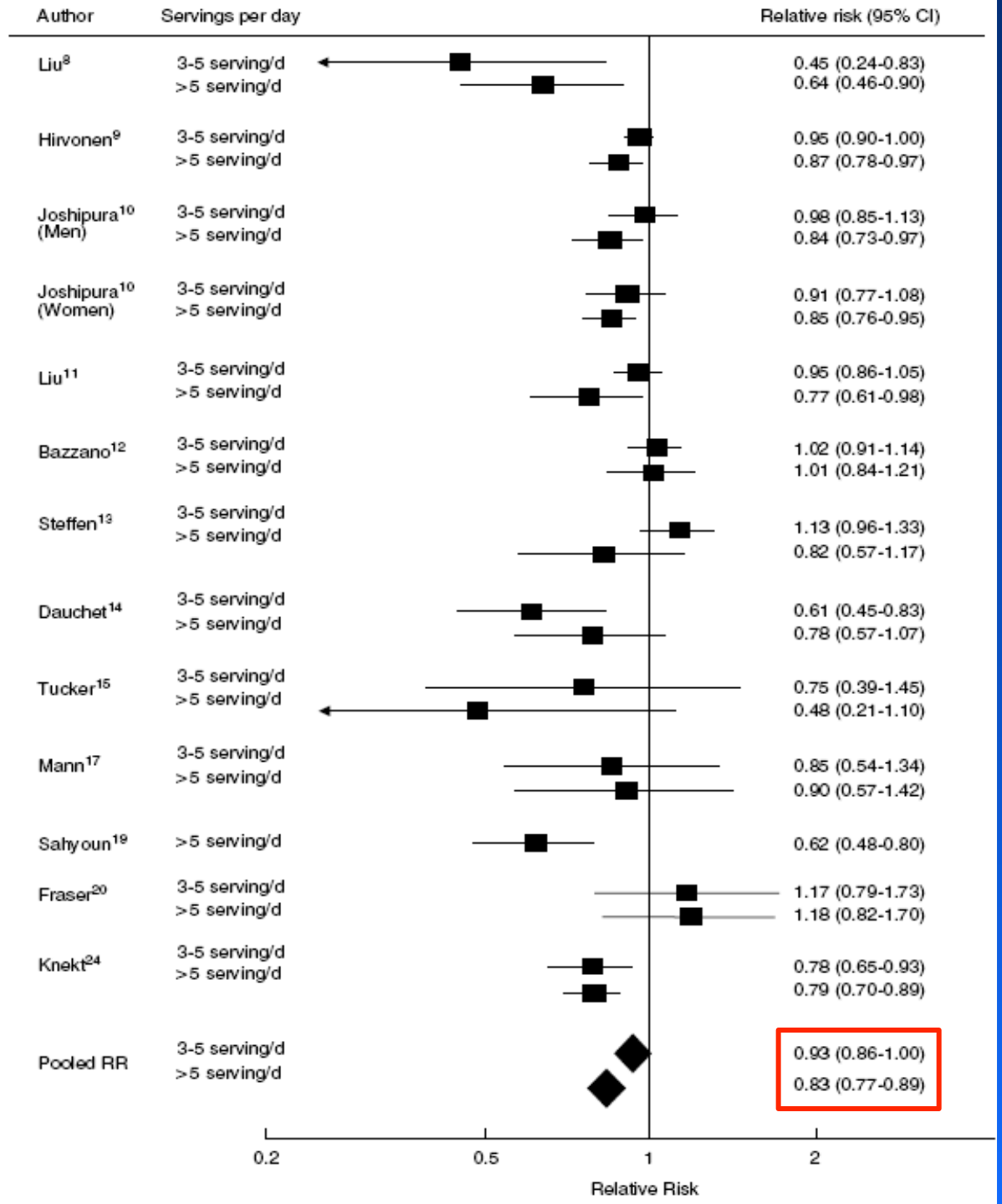
Figure 3. Plasma levels (μmol/L) of AA in all patients are significantly inversely correlated with major diameter of hemorrhage (closed circles; $\rho = -0.47$, $P = 0.002$) and contusion (open circles; $\rho = -0.54$, $P = 0.002$).

Stroke 2001;32:898-902

Fruit and vegetable intake by quintile of plasma vitamin C in men (EPIC Norfolk 1993-1997)



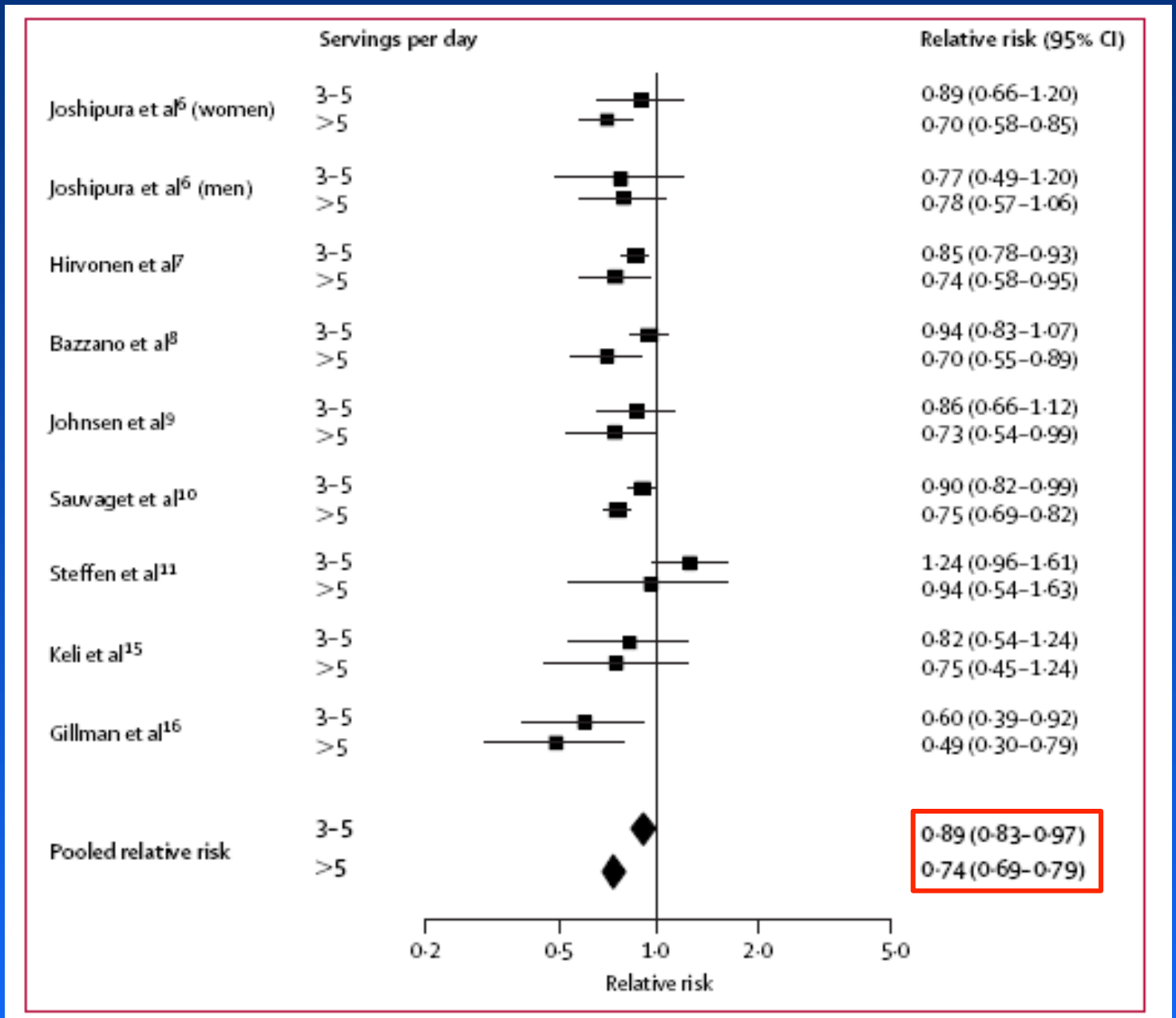
Khaw et al. *Lancet* 2001



Fruit & Vegetable Intake and Coronary Heart Disease: 17% Reduction

(Vitamin C/EPIC: 60%)

He et al. *J Hum Hyperten* 2007;21:717-728



Fruit & Vegetable Intake and Stroke: 26% Reduction

(Vitamin C/EPIC: 39-51%)

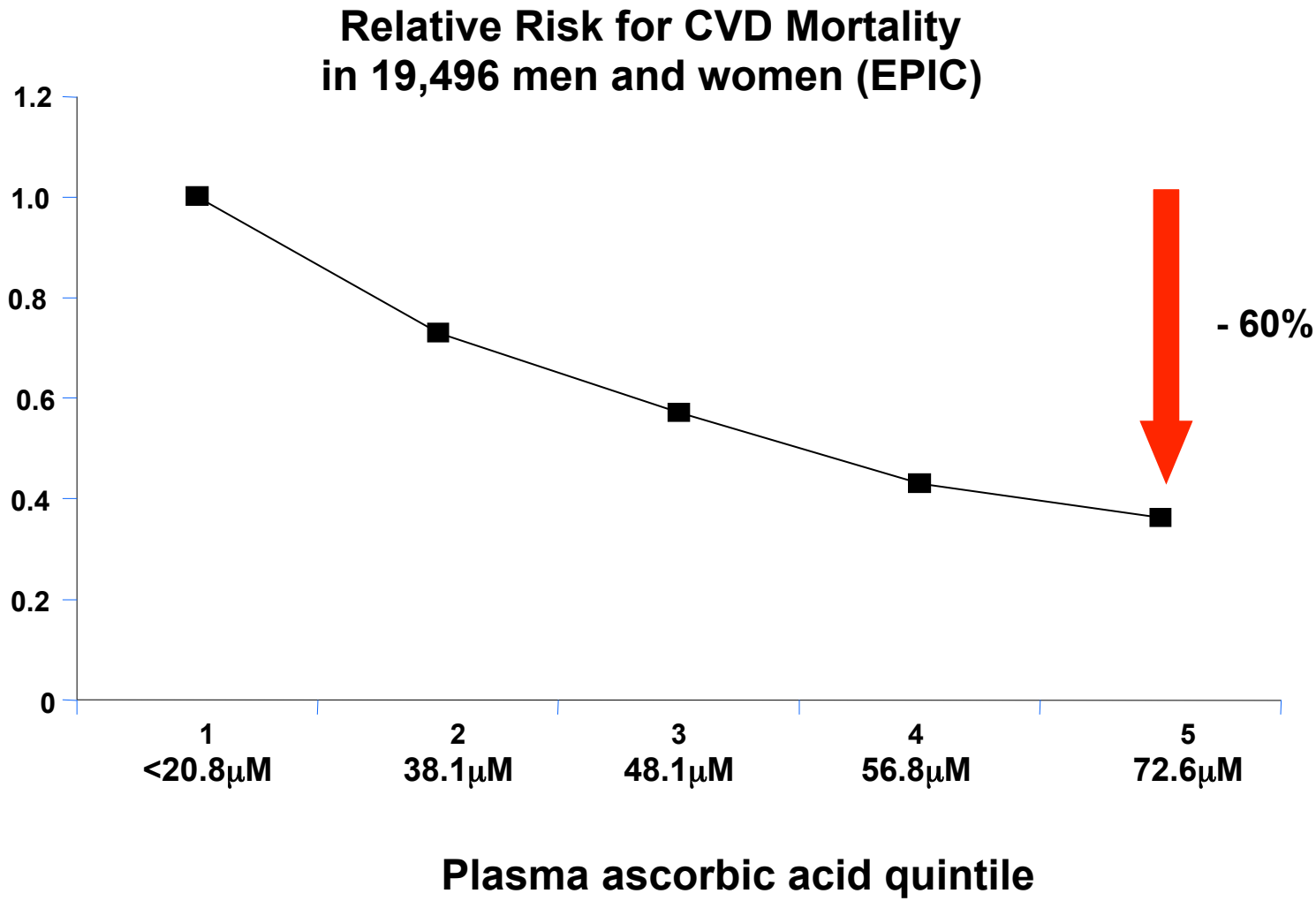
He et al. Lancet 2006;367:320-326

Vitamin C Supplement Use and Coronary Heart Disease Risk in the Nurses' Health Study

	RR	95% CI
Supplement Use	0.72*	(0.61-0.86)
Dose of Vitamin C Supplements		
1-400 mg/day	0.82	(0.60-1.12)
401-749 mg/day	0.69*	(0.55-0.87)
750 mg/day or more	0.71*	(0.55-0.92)
Duration of Vitamin C Supplements		
<2 years	1.12	(0.45-2.76)
2-4 years	0.77	(0.63-1.12)
5-9 years	0.84	(0.63-1.12)
10 or more years	0.70*	(0.52-0.94)

Osganian et al., *JACC* 2003

What amount of vitamin C do humans have to consume to achieve optimum plasma and tissue levels and reap the full health benefits of vitamin C?



Khaw et al., Lancet 2001;134:657

Prospective Cohort Studies: Highest Plasma or Serum Vitamin C Levels (64-85 μM) are associated with Lowest CVD Risk (CHD or Stroke)

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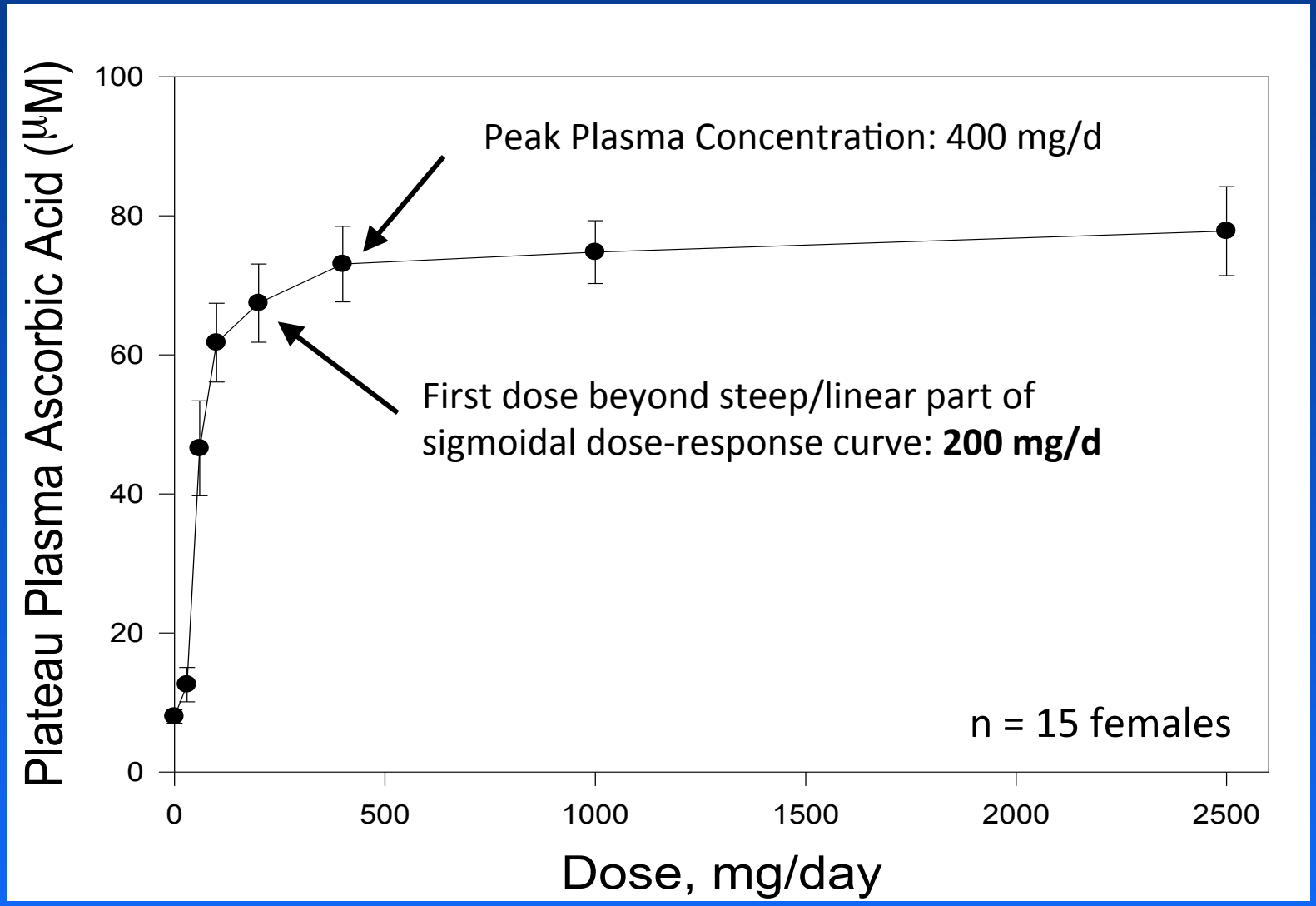
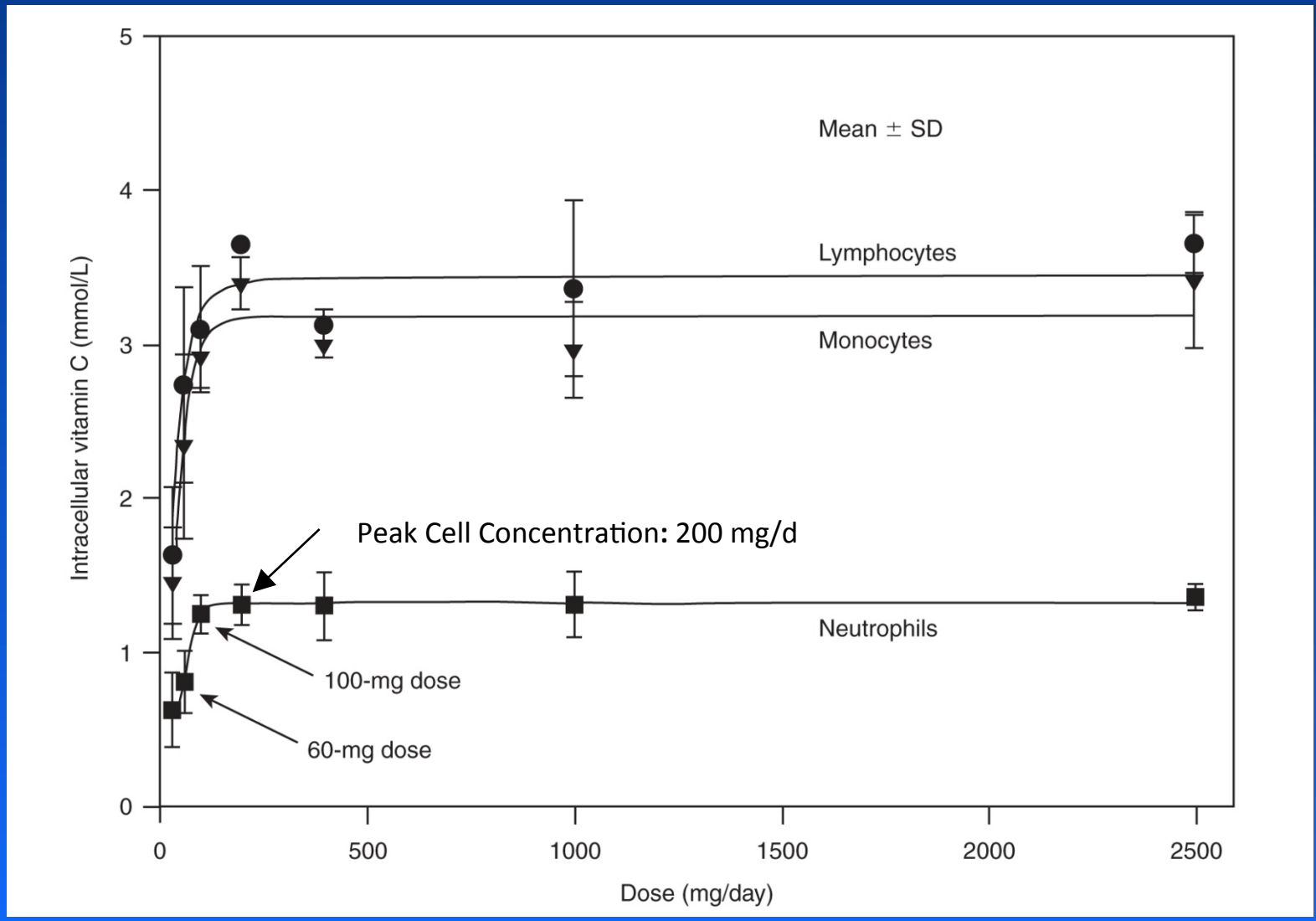
Frei, Birlouez-Aragon, and Lykkesfeldt. *Crit Rev Food Sci Nutr* **2012;52(9):815-29**

Plasma ascorbate concentrations as a function of vitamin C dose



Levine *et al.* (2001) *PNAS* 98:9842-6

Intracellular ascorbate concentrations as a function of vitamin C dose



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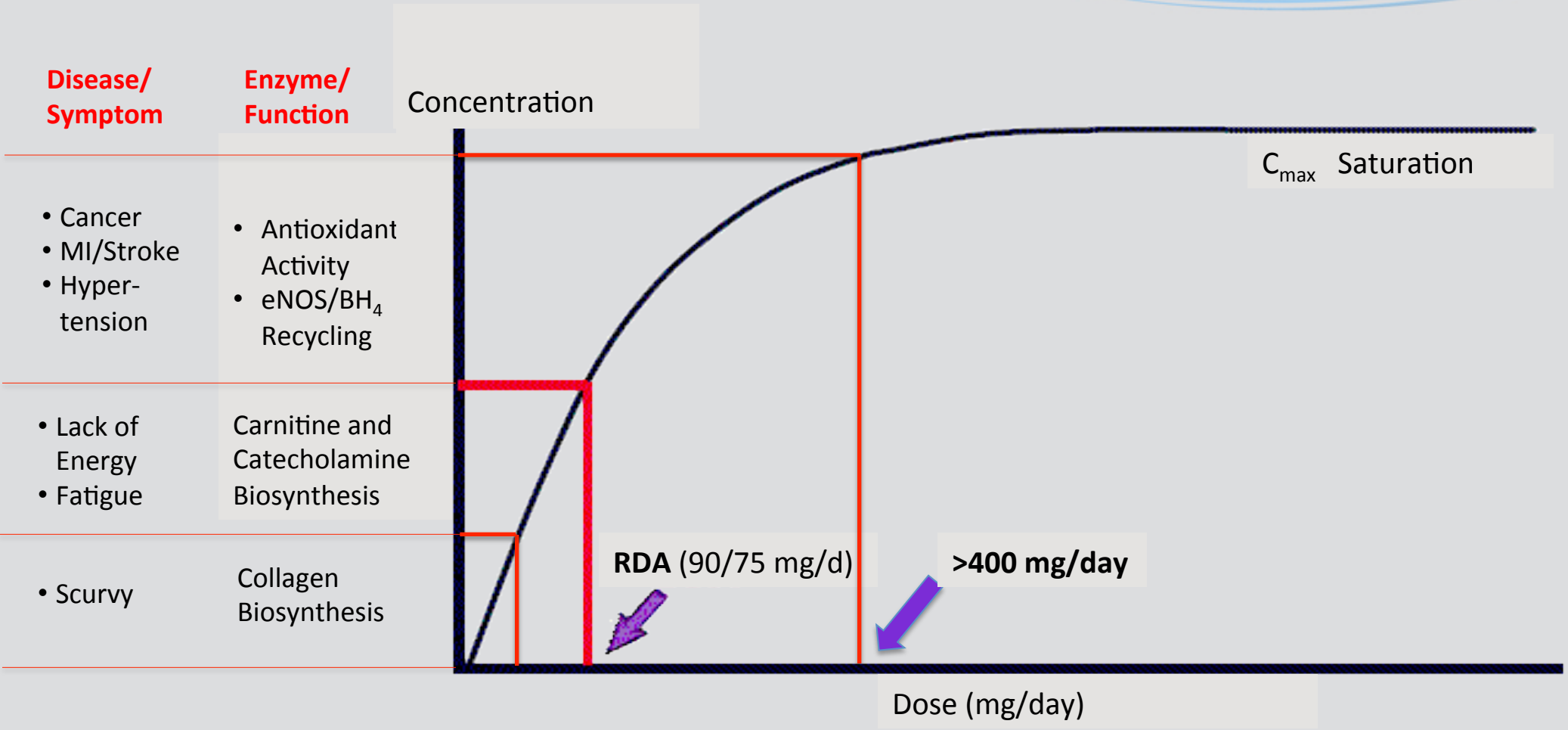
Osganian et al., *JACC* 2003

Plasma Ascorbate Concentration: Vitamin C Dose vs. Function

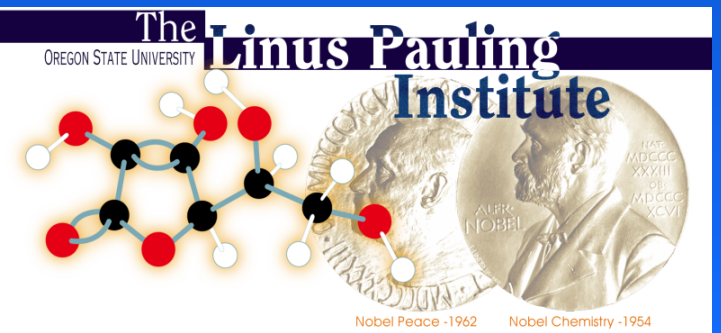


Acknowledgements

- Frei lab, esp. Cristina Polidori, Anitra Carr, and Alexander Michels
- Bruce N. Ames (University of California, Berkeley)
- John F. Keaney and Joseph Vita (Boston University School of Medicine)
- Jason Morrow (deceased) (Vanderbilt University)
- Mark Levine (NIDDK, NIH)

Funding provided by:

US National Institutes of Health (NHLBI, NCCAM, NIEHS) and American Heart Association



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Home of the Linus Pauling Institute at Oregon State University, Corvallis, USA

Vitamin C and CVD (CHD and Stroke)

Prospective Cohort Studies (Observational)

- Based on dietary intake assessment of vitamin C

or

- Based on plasma (serum) levels of vitamin C
  - More accurate measure of nutrient exposure: not affected by recall bias, and taking into account loss of vitamin C during food storage and preparation as well as genetic polymorphisms in vitamin C transport

How do genetic factors influence plasma vitamin C levels?

A new twist on an old vitamin: human polymorphisms in the gene encoding the sodium-dependent vitamin C transporter 1[1,2]

Alexander J Michels, Tory M Hagen, and Balz Frei

Am J Clin Nutr 2010;92:271–2. Printed in USA. © 2010 American Society for Nutrition 271

Vitamin C levels in blood are influenced by polymorphisms in glutathione S-transferases

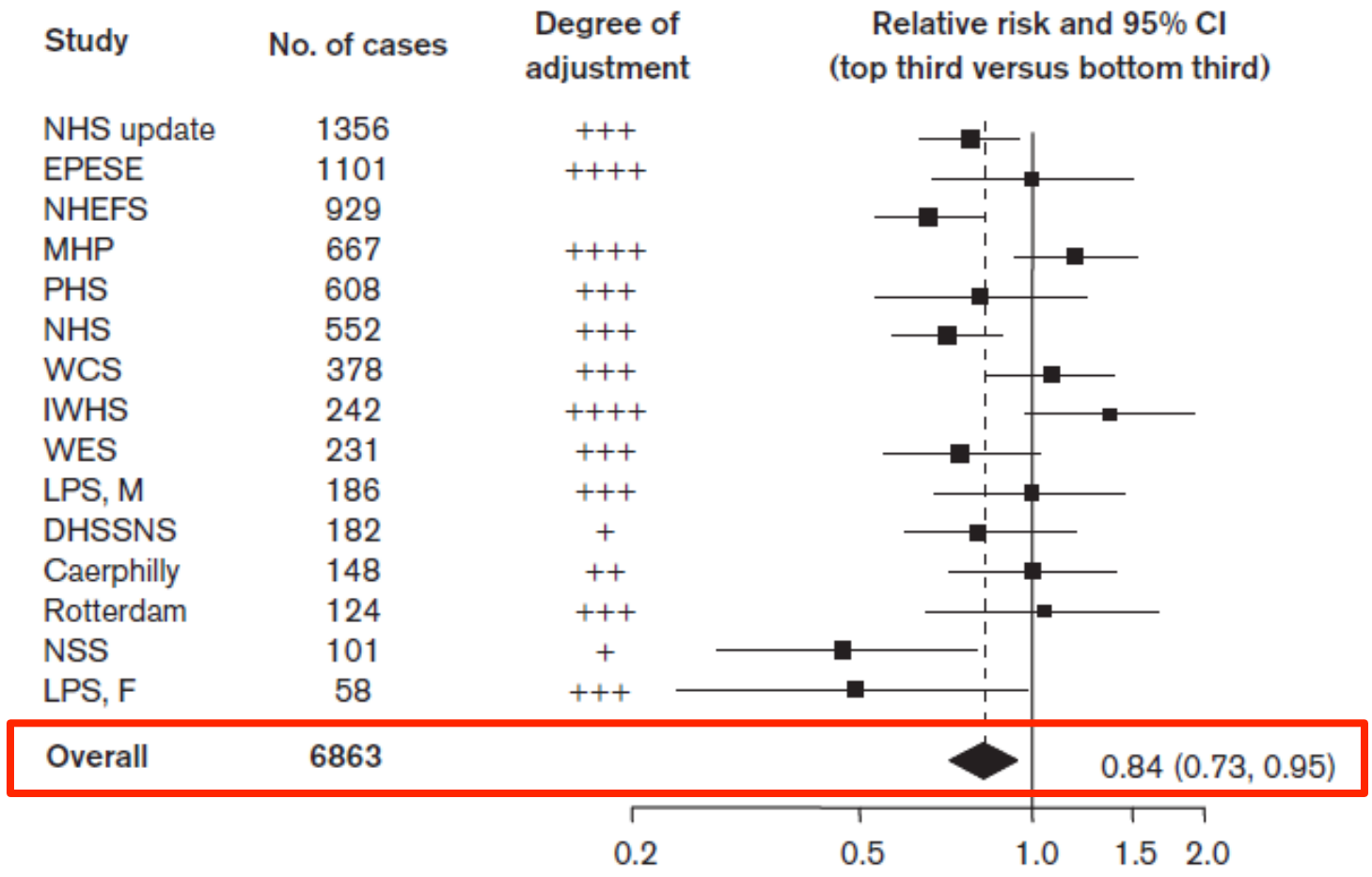
Alexandra Horska · Csilla Mislanova · Stefano Bonassi · Marcello Ceppi · Katarina Volkovova · Maria Dusinska

Eur J Nutr
DOI 10.1007/s00394-010-0147-2

Vitamin C Deficiency and Scurvy Are Not Only a Dietary Problem but Are Codetermined by the Haptoglobin Polymorphism

Clinical Chemistry 53, No. 8, 2007 1397

Meta-analysis of 15 prospective cohort studies of vitamin C intake: 16% lower CHD risk



Ye Z, Song H. *Eur J Cardiovasc Prev Rehabil*. 2008;15:26-34.

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Loria et al. Am J Clin Nutr 2000;72:139-45	7,071 men and women	≥73.8 µmol/L in men ≥85.2 µmol/L in women	Cancer, all-cause mortality
Jenab et al. Carcinogenesis 2006; 27:2250-7	215 cases and 416 controls	>82.0 µmol/L in cases >75.0 µmol/L in controls	Gastric cancer

Frei, Birlouez-Aragon, and Lykkesfeldt. *Crit Rev Food Sci Nutr* **2012;52(9):815-29.**

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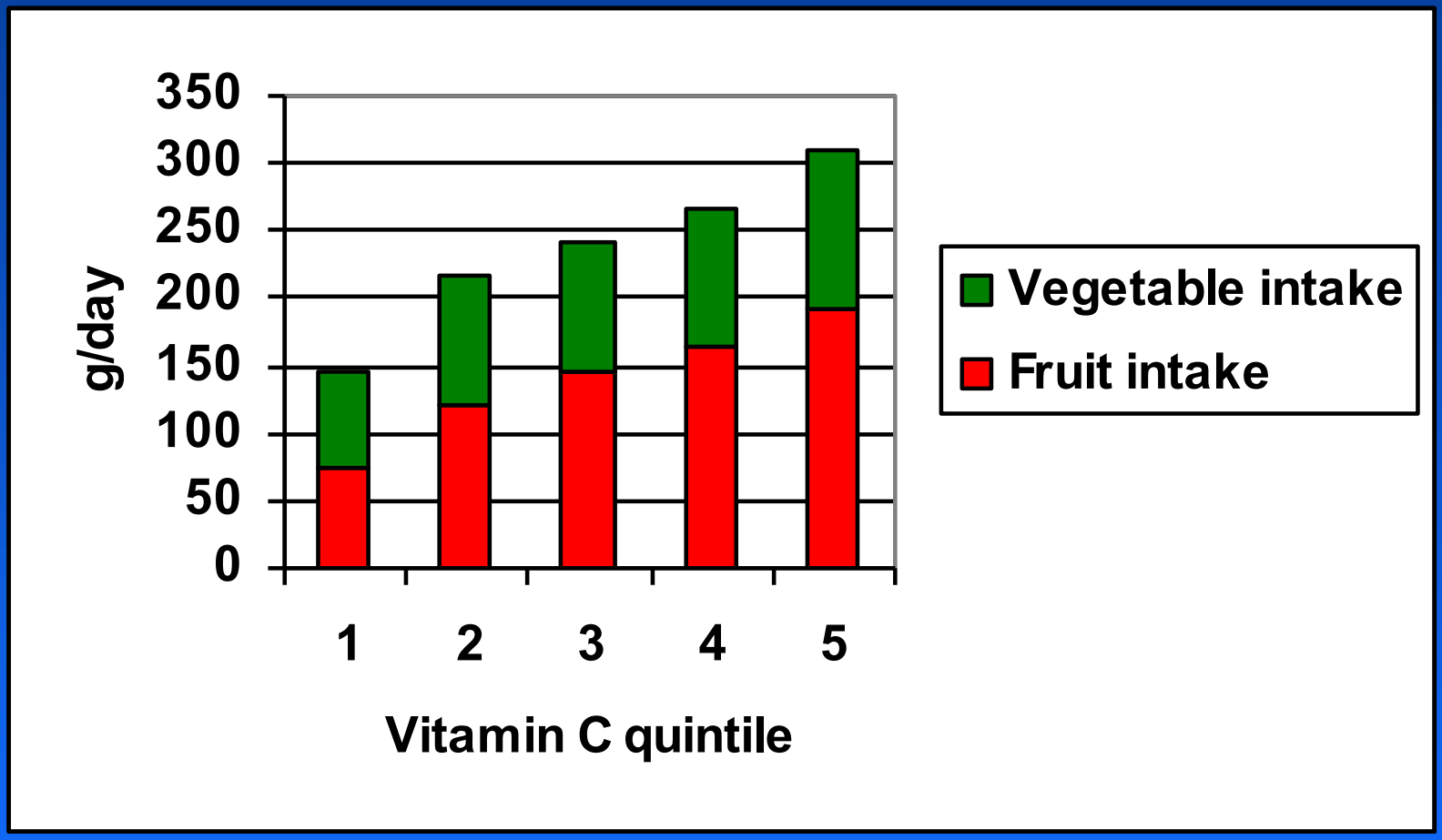
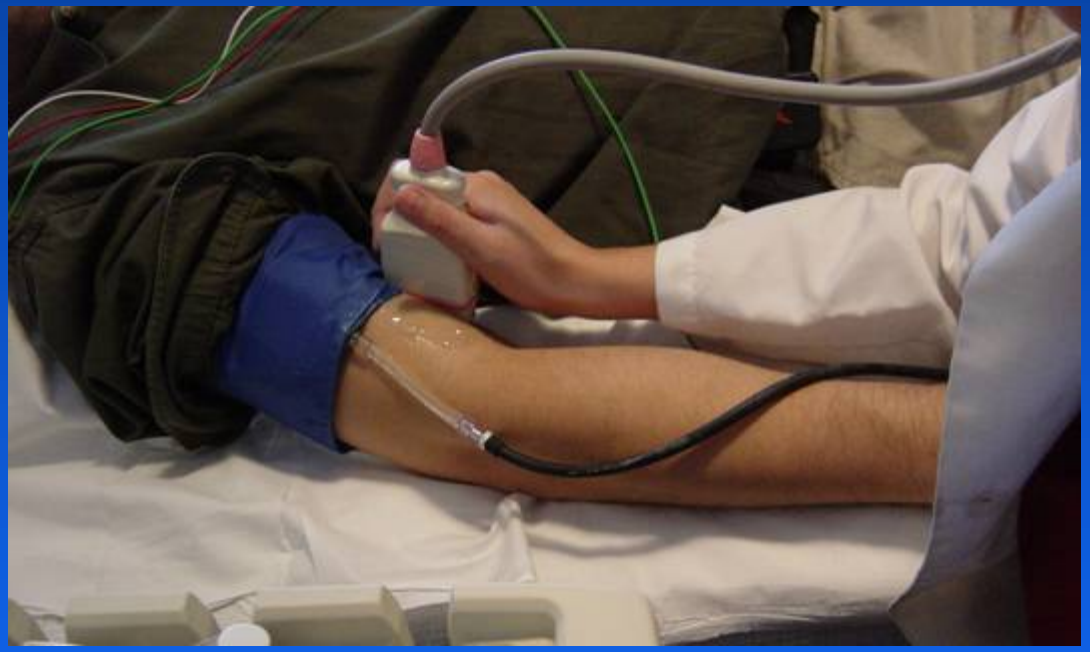
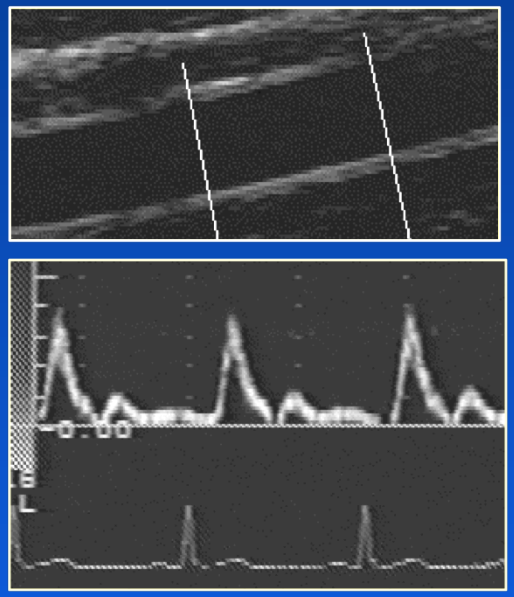
Fruit and vegetable intake by quintile of plasma vitamin C in men (EPIC Norfolk 1993-1997)



Khaw et al. *Lancet* 2001

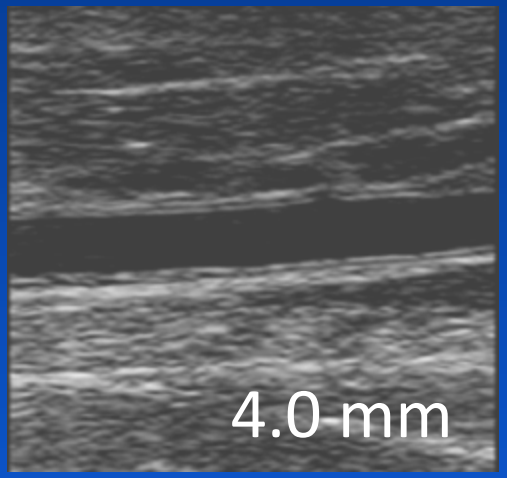
Non-Invasive Measurement of NO-Mediated Vasodilation

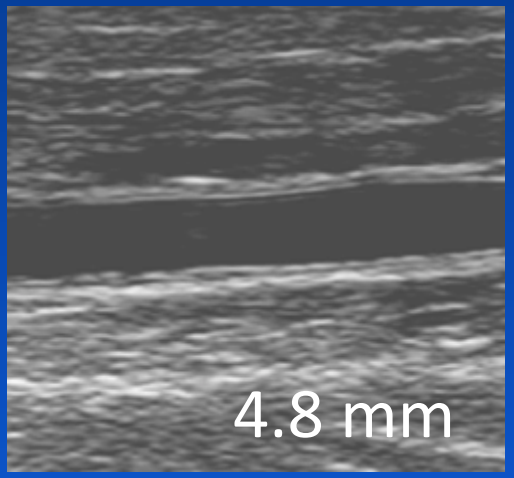




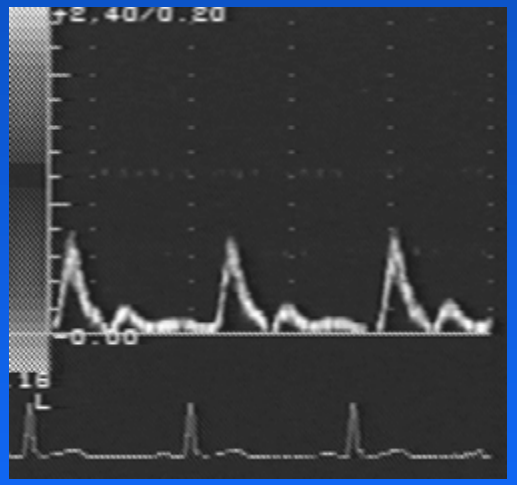
Real-time display of flow velocity and vessel diameter

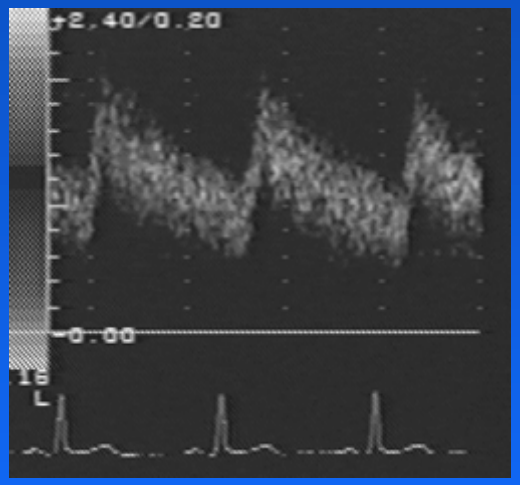
Ultrasound Evaluation of Brachial Artery Endothelial Function





20% Vasodilation





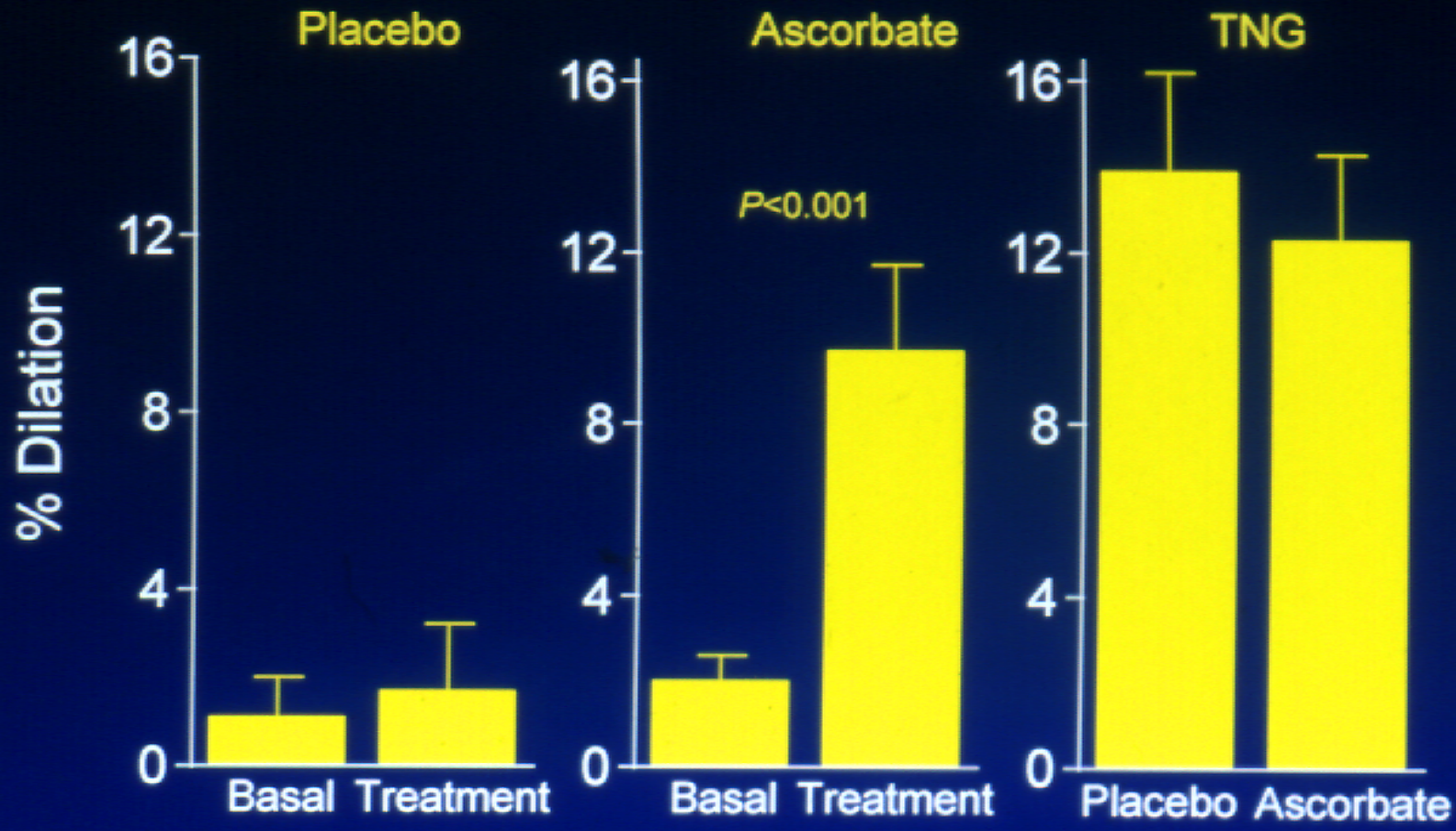
Increased blood flow

Baseline

Hyperemia

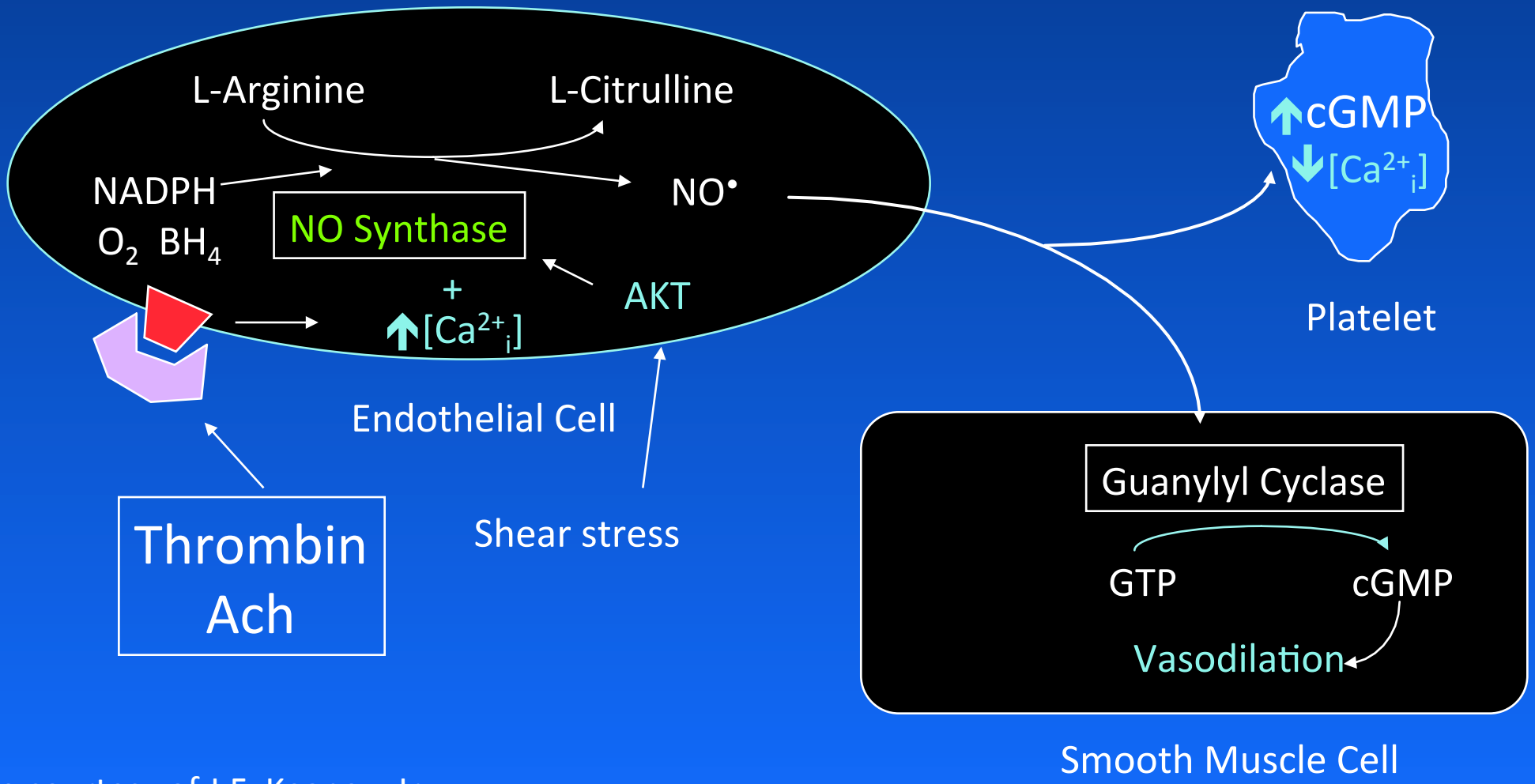
Ascorbate Improves Brachial Vascular Function in CAD Patients

Patients with angiographically documented CAD and <5% dilator response



Levine, Frei, et al. *Circulation* 1996;93:1107-1113

Synthesis and Action of EDNO



Slide courtesy of J.F. Keaney, Jr.

Superoxide Inactivates Nitric Oxide

$$O_2\cdot^- + NO\cdot \longrightarrow ONOO^-$$

- Nitric oxide combines readily with superoxide
- Rate Constant = $1.9 \times 10^{10}\ M^{-1} \cdot s^{-1}$

Beckman et al. *PNAS 1990*

Kissner et al. *Chem. Res. Toxicol. 1997*

Superoxide Inactivates Nitric Oxide

Vitamin C

$$O_2\cdot^- + NO\cdot \not\longrightarrow ONOO^-$$

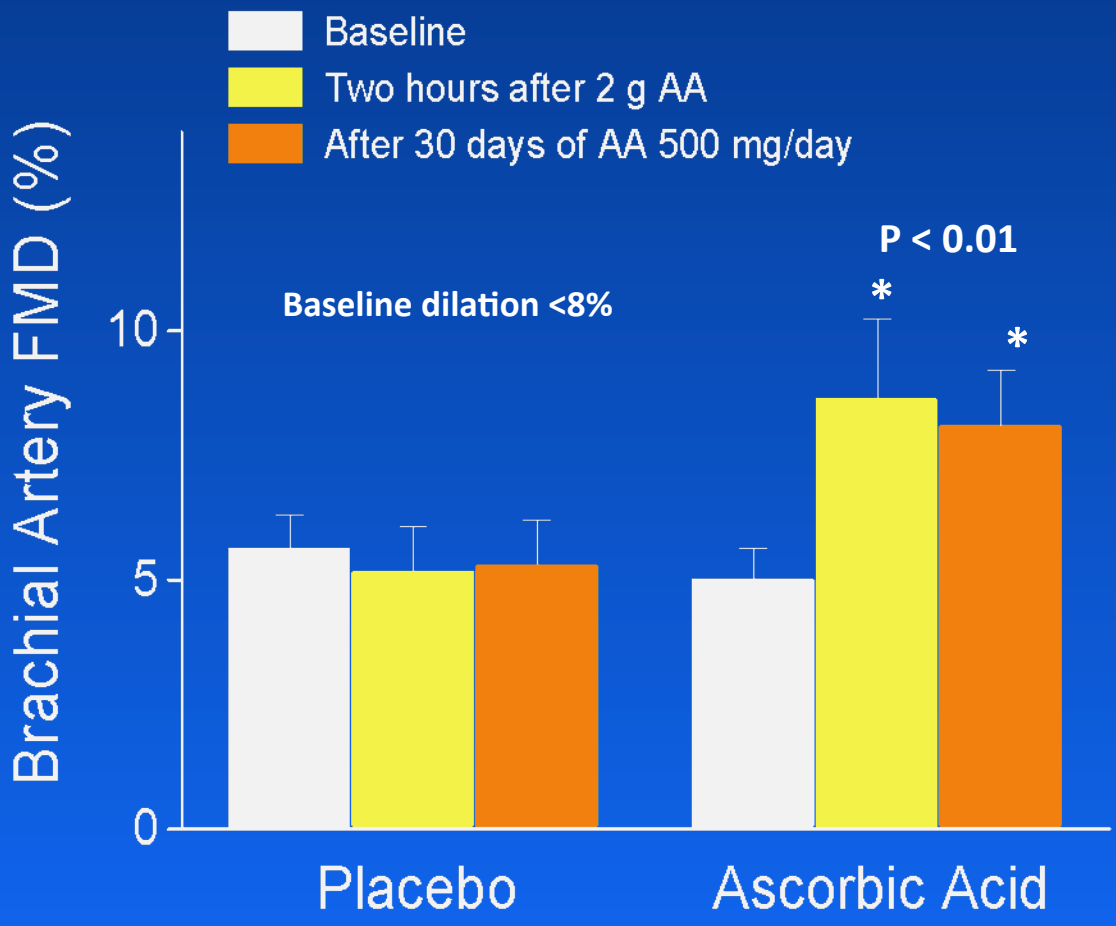
- Nitric oxide combines readily with superoxide
- Rate Constant = $1.9 \times 10^{10}\ M^{-1} \cdot s^{-1}$

Beckman et al. *PNAS 1990*

Kissner et al. *Chem. Res. Toxicol. 1997*

Vitamin C Treatment (Short- or Long-Term) Improves Brachial Artery EDNO Action in CAD Patients

- 48 patients with angiographically proven CAD
- Randomized, double-blind, placebo controlled study
- Visit 1: brachial ultrasound at baseline and 2 hours after 2 grams of ascorbic acid
- Visit 2: brachial ultrasound after 30 days of 500 mg/day of ascorbic acid



Levine *et al. Circulation* 1996;93:1107-1113
Gokce *et al. Circulation* 1999;99:3234-3240

Vitamin C (500 mg–12 g by IV infusion or orally) Improves Vascular Function (EDNO-Dependent Vasodilation):

- in CAD patients Levine et al. (1996) Circulation 93:1107-1113; Ito et al. (1998) Am J Cardiol 82:762-767; Gokce et al. (1999) Circulation 99:3234-3240
- in NIDDM and IDDM patients Ting et al. (1996) J Clin Invest 97:22-28; Timimi et al. (1998) J Am Coll Cardiol 31:552-557; Beckman et al. (2001) Circulation 103:1618-1623; Antoniades et al. (2004) Diabet Med 21:552-558
- in chronic smokers Heitzer et al. (1996) Circulation 94:6-9; Motoyama et al. (1997) Am J Physiol 273:H1644-H1650; Schindler et al. (2000) Cardiology 94:239-246
- in hypercholesterolemic subjects Ting et al. (1997) Circulation 95:2617-2622; Perticone et al. (2000) Atherosclerosis 152:511-518
- in hypertensive patients Solzbach et al. (1997) Circulation 96:1513-1519; Taddei et al. (1998) Circulation 97:2222-2229; Natali et al. (2000) ATVB 20:2401-2406
- following a single high-fat meal Plotnick et al. (1997) JAMA 26;278: 1682-1686
- in patients with chronic heart failure Hornig et al. (1998) Circulation 97:363-368; Ito et al. (1998) Am J Cardiol 82:762-767
- in patients with angina pectoris Kugiyama et al. (1998) J Am Coll Cardiol 32:103-109; Hamabe et al. (2001) Am J Cardiol 87:1154-1159
- in experimental hyperhomocyst(e)inemia Kanani et al. (1999) Circulation 100:1161-1168; Hanratty et al. (2001) BMC Cardiovasc Disord 1:1

Working Model

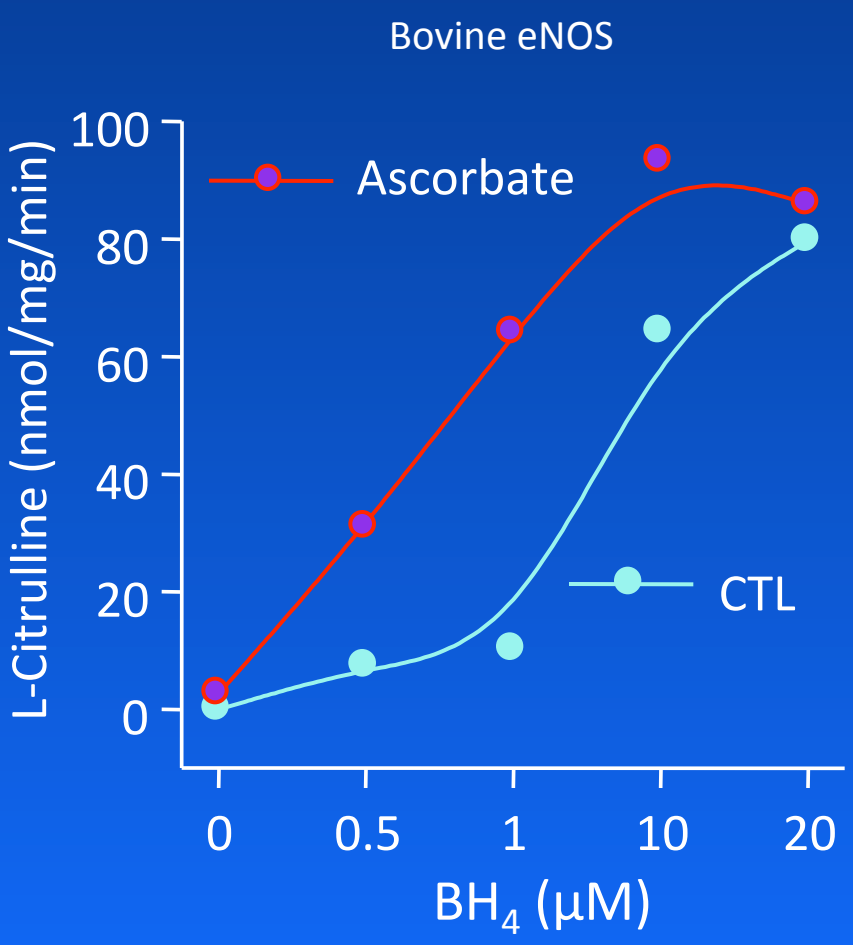
Vitamin C

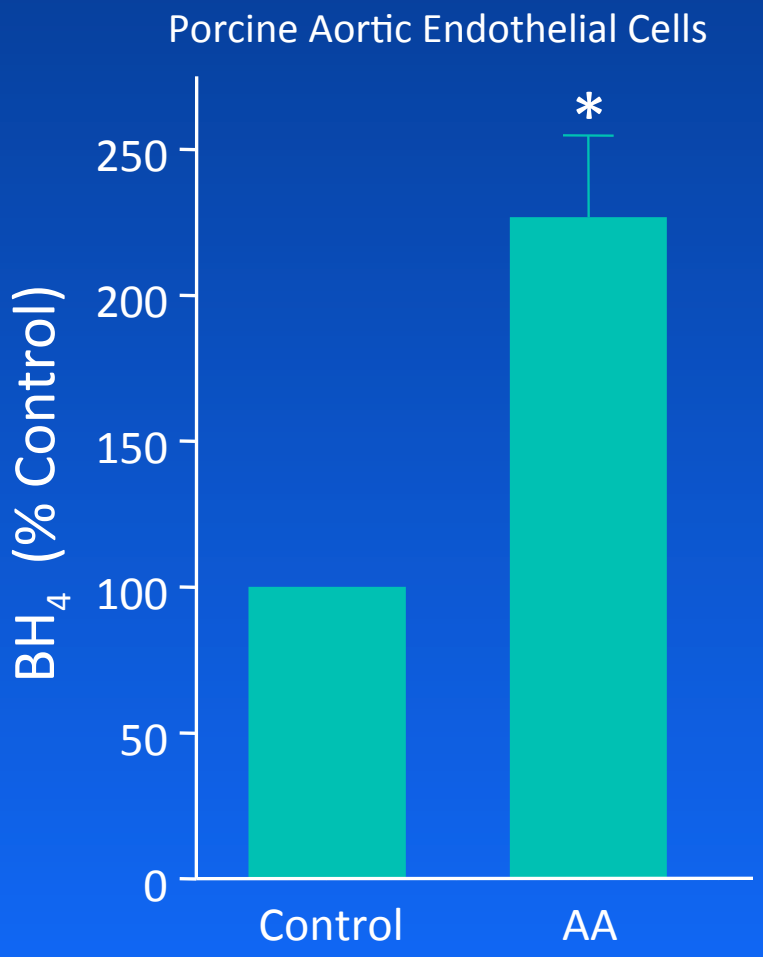
$O_2\cdot^- + NO\cdot \;\not\rightarrow\; ONOO^-$

$NO\cdot$ ($k_2 = 1.9 \times 10^{10}\ M^{-1} \cdot s^{-1}$)
(~ 0.1 μM)

Ascorbic Acid
($k_2 = 2 \times 10^5\ M^{-1} \cdot s^{-1}$)
(~ 0.1 μM x 10^5 = 10 mM)

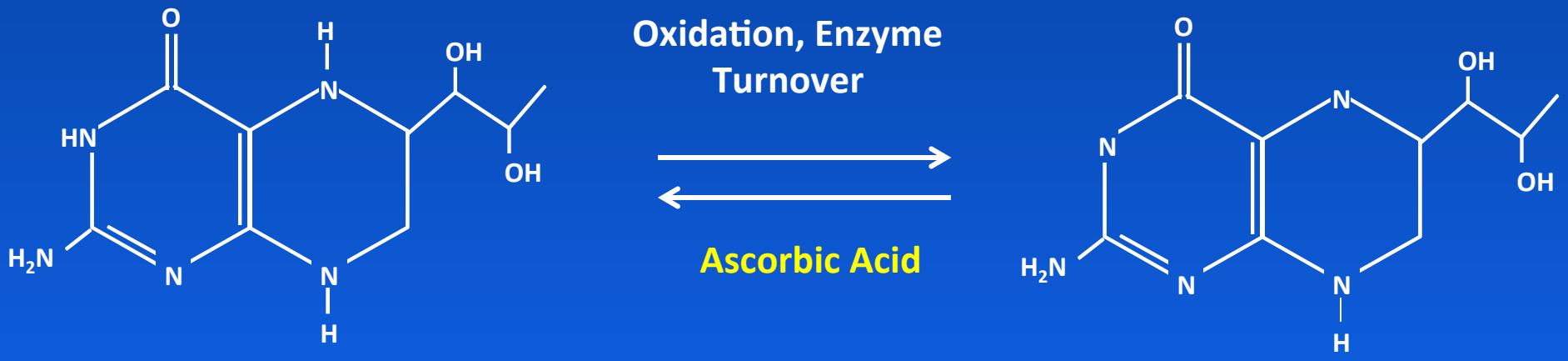
Ascorbic Acid enhances BH_4-Dependent eNOS Activity and Intracellular BH_4 Levels





Huang et al., J Biol Chem. 2000:275:17399

Mechanism of Vitamin C Action



Tetrahydrobiopterin
(BH_4)

Quinoid dihydrobiopterin
(QBH_2)