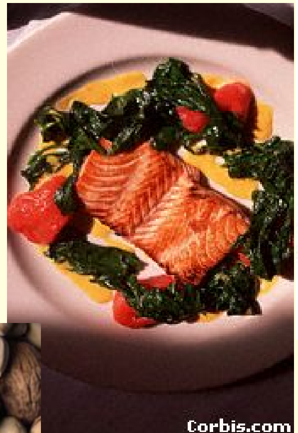





Bioactive Compounds and CVD

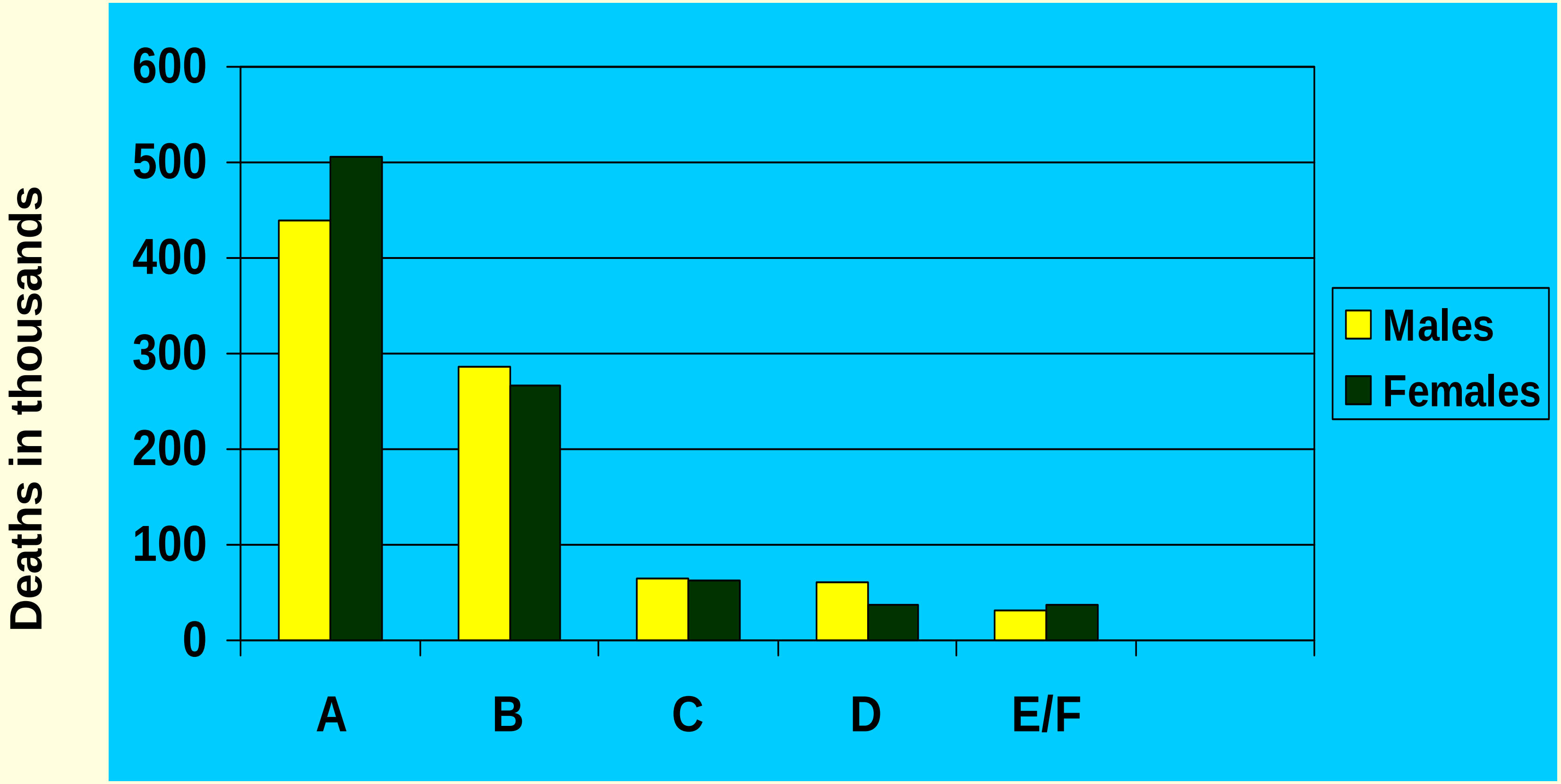
Knowledge Gained From Establishing Their Role in Risk Reduction and Mechanisms of Action

Penny Kris-Etherton, PhD, RD
Pennsylvania State University

Outline

- Evidence in support of lowering LDL cholesterol
- Phytochemicals
  - Major classes defined
  - Food sources
  - Effects on CVD risk factors
- Challenges in studying the clinical effects of phytochemicals
  - Biological diversity
  - External factors that modulate response
- Case Study: Plant-derived Omega-3 Fatty Acids
- Summary

Leading Causes of Death for All Males and Females in the U.S.



A. Total CVD
B. Cancer
C. Accidents
D. Chronic Lower Respiratory Diseases
E. (Males) Diabetes Mellitus
F. (Females) Influenza and Pneumonia

AHA Heart and Stroke Facts, 2003

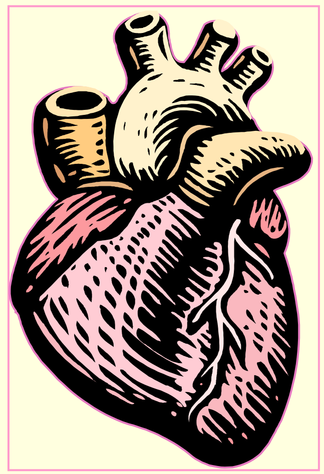
CVD

Heart Attack

Stroke

Angina

Arrythmias



Modifiable Risk Factors

- Total - C
  LDL - C
- HDL – C
- Hypertension
- Thrombogenic/hemostatic
- Diabetes
- Obesity
- Smoking
- Physical Inactivity

Non-modifiable Risk Factors

- Age
- Male >45, Female >55
- Family History of Premature CHD

Source: ATP III Report, 2001

Trends in Total Cholesterol Levels in the U.S.

NHANES I 1976-1980	NHANES II 1988-1994	NHANES III 1999-2000
213 mg/dL	205 mg/dL	203 mg/dL

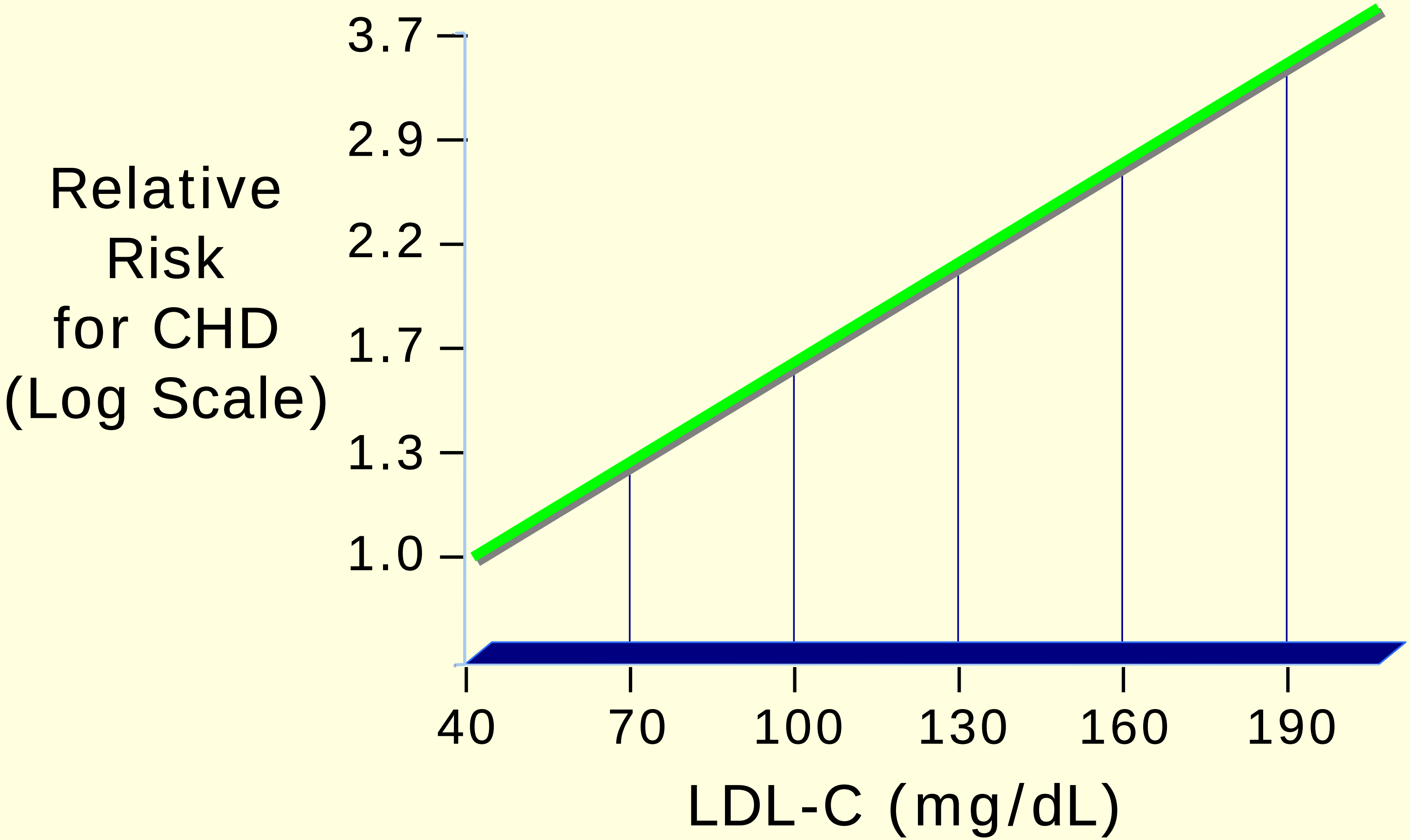
Adpated from Ford et al., Circulation 107:2185, 2003

Adult Intake of Total Fat, SFA and PUFA in US (midpoint of NHANES surveys)

	1972 (NHANES I)	1978 (NHANES II)	1990 (NHANES III)
Total Fat % Kcal	36.4	36.3	34.1
SFA	13.2	12.8	11.7
PUFA ALA** EPA+DHA**	4.3	5.7	7.1 0.6-0.7** 0.1**

Ernst et al, 1997, **Kris-Etherton et al, 2000

“The Lower, the Better”



Grundy SM et al. *Circulation* 2004;110:227–239.

Features of Therapeutic Lifestyle Changes

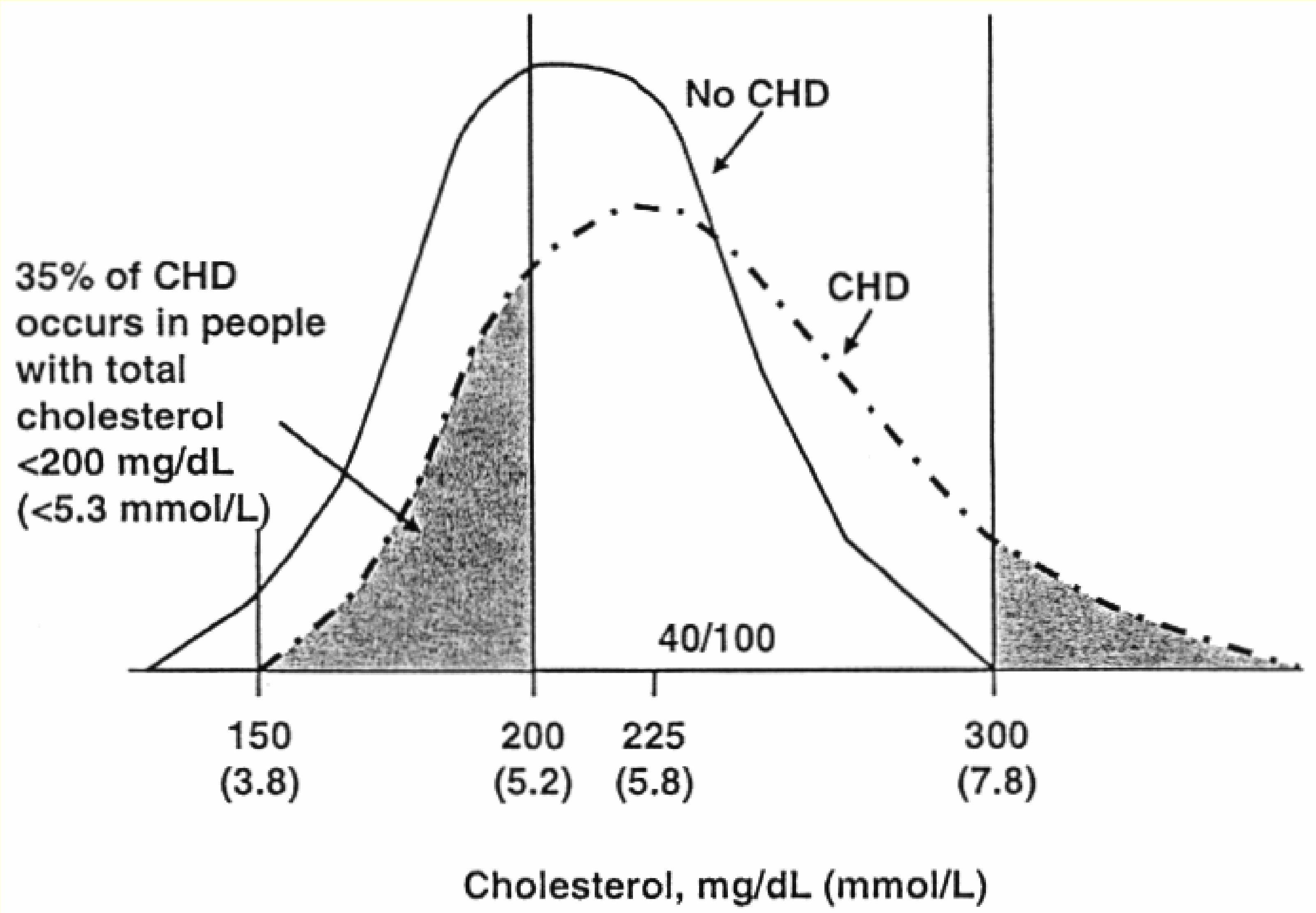
LDL-C Raising Nutrients	
Saturated (and *Trans*) Fats	**< 7% Kcal**
Dietary Cholesterol	**<200 mg/d**
Therapeutic Options for LDL-C Lowering	
Plant stanols/sterols	**2 g/d**
Increased viscous(soluble) fiber	**10-25 g/d**
Total Calories	
Maintain desirable body weight/ prevent weight gain	
Physical Activity	
Exercise to expend at least 200 kcal/d	

LDL-C Reduction Achievable by Diet

Dietary Component	Dietary Change	Approximate LDL Reduction
Major Interventions		
Saturated Fat , *trans* fat	<7% of calories, minimum	8-10%
Dietary cholesterol	<200 mg/d	3-5%
Weight Reduction	lose 10 lbs	5-8%
Other LDL-Lowering Options		
Viscous fiber	5-10 g/d	3-5%
Plant Sterol/Stanol Esters	2 g/d	6-15%
Cumulative Estimate		20-30%

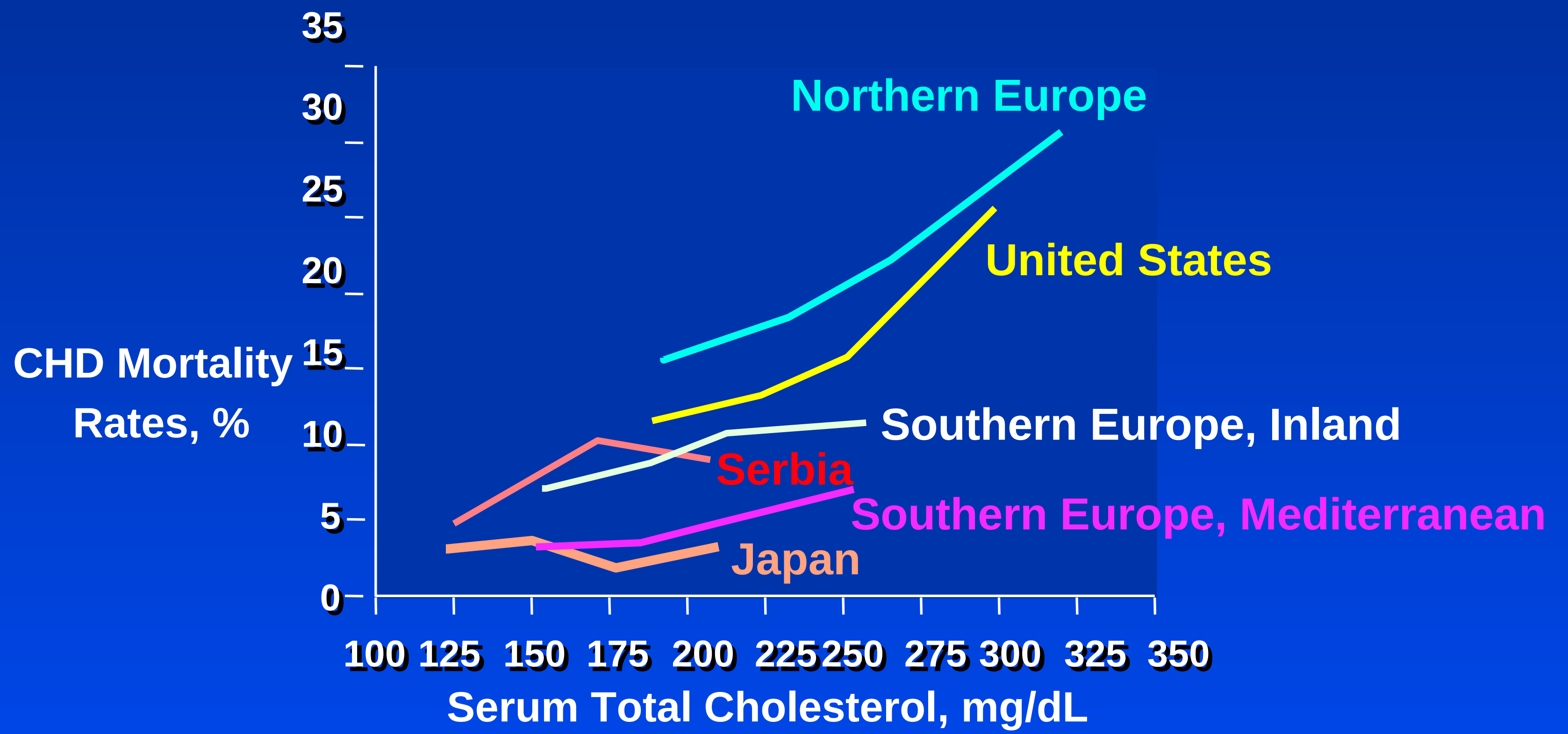
Adapted from Jenkins et al., 2000

Distribution of Plasma Total Cholesterol Levels in Individuals with and without Coronary Heart Disease



Libby, Am J Med, 2004

Serum Cholesterol and 25-Year CHD Mortality[1]: Seven Countries Study



1

adjusted for age, smoking and systolic BP

Source: Verschuren et al. JAMA, 1995

CVD

Heart Attack

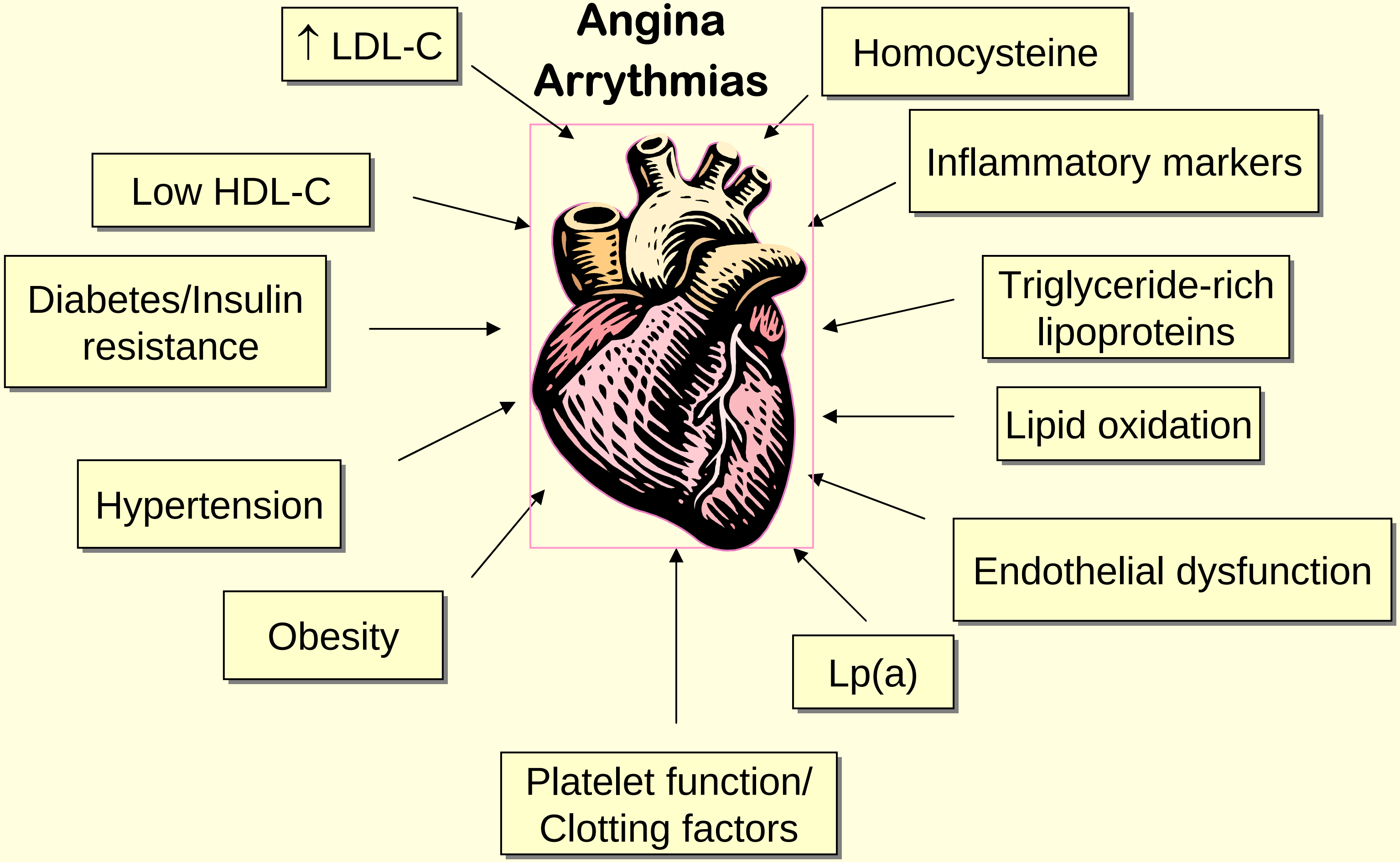
Stroke

Angina

Arrythmias

<u>Established</u> Risk Factors

<u>Emerging</u> Risk Factors



Source: Mustad, V. Current Atheroscler Reports, 2000, 2:461-66.

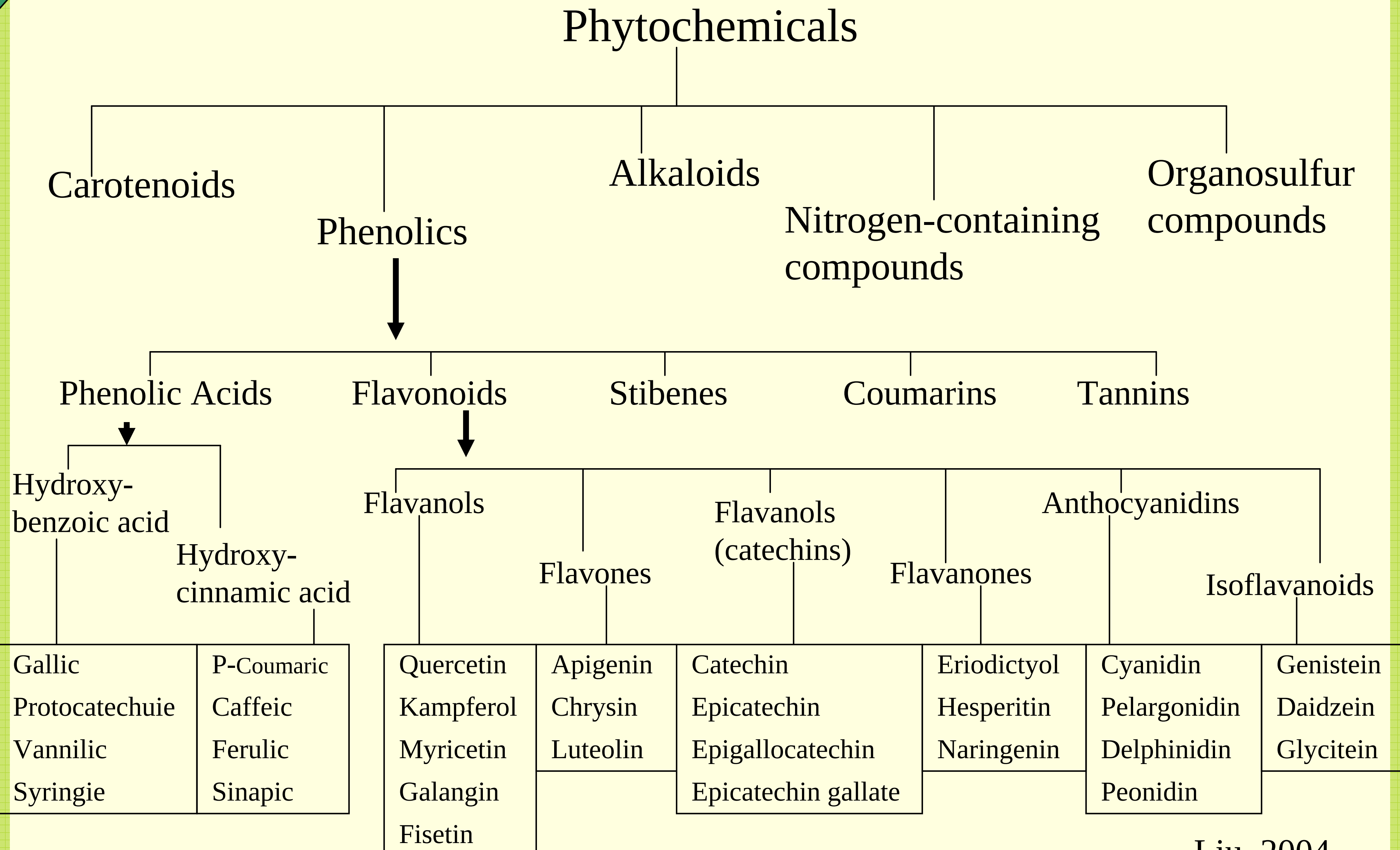
Classification of Dietary Phytochemicals

Phytochemicals

- Carotenoids
  - α-carotene
  - β-carotene
  - β-cryptoxanthin
  - Lutein
  - Zeaxanthin
  - Astaxanthin
  - Lycopene
- Phenolics
  - See next slide
- Alkaloids
- Nitrogen-containing compounds
- Organosulfur compounds
  - Isothiocyanates
  - Indoles
  - Allylic sulfur compounds

Liu, 2004

Classification of Dietary Phytochemicals



Liu, 2004

Food Sources of Bioactive Compounds – Effects on CVD Risk Factors

Bioactive Compound	Examples	Food Sources	Effect on CVD Risk Factor	Studies
Phenolics → Flavonoids → Flavonols	**Quercetin, Kaempferol, Myricetin, Galangin, Fisctin**	Onions, apples, tea, berries, olives, red wine, cocoa	**↓ TC, LDL-C oxid. ↓ platelet aggreg. ↑ HDL-C ↑ Antioxidant effect**	**Tzeng et al., 1991 Chung et al., 1993 Peterson et al., 1998 Bravo et al., 1998 McAnlis et al., 1999**
Flavanols	**Catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate**	Green/black tea, cocoa, plums, apples, berries, pecans	**↓ LDL oxid. ↓ platelet aggreg. ↓ BP ↑ insulin sensitivity ↑ FMD**	**Grassi et al., 2005 Kondo et al., 1996 Waterhouse et al., 1996 Kondo et al., 1999 Rein et al., 2000 Duffy et al., 2001**

Food Sources of Bioactive Compounds – Effects on CVD Risk Factors

Bioactive Compound	Examples	Food Sources	Effect on CVD Risk Factor	Studies
Organosulfur compounds	Isothiocyanates Indoles Allylic sulfur compounds	Garlic, leeks, onions, cruciferous vegetables like broccoli and cauliflower	↓ TC, LDL-C ↓ LDL-C Oxid. ↓ TG ↓ BP ↓ thrombosis ↓ platelet aggregation ↑ Antioxidant effect	**Warshafsky et al., 1993** **Matsuura, 2001** **Jain et al., 1993** **Silagy & Neil, 1994** **Borek, 2001** **Steiner & Li, 2001**

Food Sources of Bioactive Compounds – Effects on CVD Risk Factors

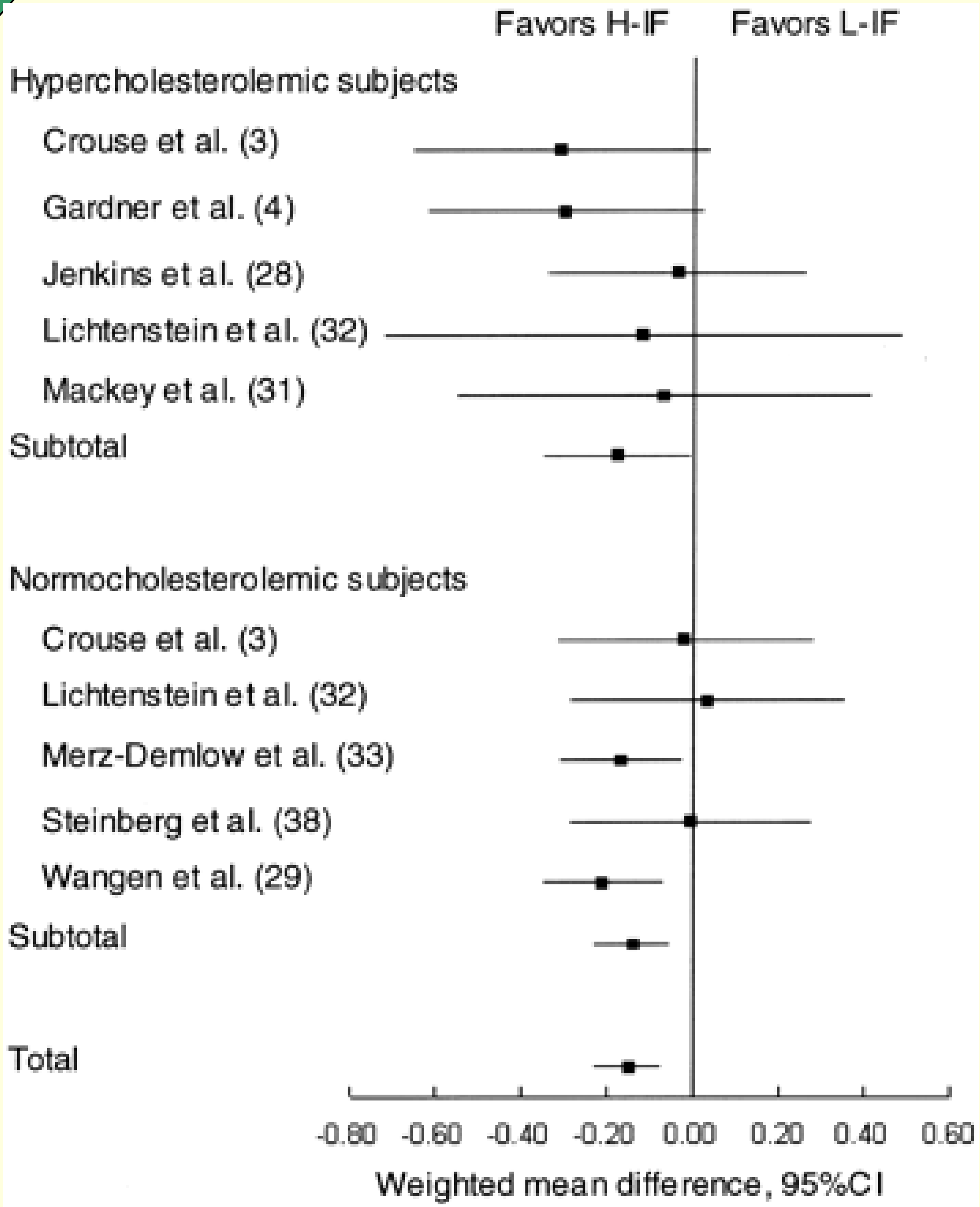
Bioactive Compound	Examples	Food Sources	Effect on CVD Risk Factor	Studies
Carotenoids	α-Carotene, β-Carotene, Lutein Zeaxanthin, β-Cryptoxanthin Lycopene	carrots, sweet potatoes, winter squash, pumpkin, papaya, mango, watermelons, apricots	↓ LDL-C ↓ LDL-C Oxid. ↑ Antioxidant effect	**Agarwal et al., 1998** **Lowe et al., 1996** **Fuhrman et al., 1997**

Food Sources of Bioactive Compounds – Effects on CVD Risk Factors

Bioactive Compound	Examples	Food Sources	Effect on CVD Risk Factor	Studies
Phytoestrogens ↓ Lignans		Whole grains, nuts, Flaxseed oil	May ↓ total and LDL-C ↓ postprandial glucose absorption ↓ markers of inflammation ↑ arterial compliance ↑ improved insulin sensitivity ↓ LDL oxidation	**Nestel et al., 1999**

Food Sources of Bioactive Compounds – Effects on CVD Risk Factors

Bioactive Compound	Examples	Food Sources	Effect on CVD Risk Factor	Studies
Phytoestrogens ↓ Isoflavones	Genistein Daidzein Glycitein	Clover, peas, soybeans Soy protein with genistein Isolated soy protein (ISP) with isoflavones	**↑ FMD** **↑ post-occlusion peak flow velocity** **↑ HDL-C** **↓ LDL-C in hypercholesrolemic subjects** **↓ thrombosis** **↓ LDL-C oxidation**	**Anderson et al., 1995** **Squadrito et al., 2002** **Steinberg et al., 2003** **Anthony et al., 1996** **Crouse et al., 1999** **Jenkins et al., 2002** **Tikkanen et al., 1998**

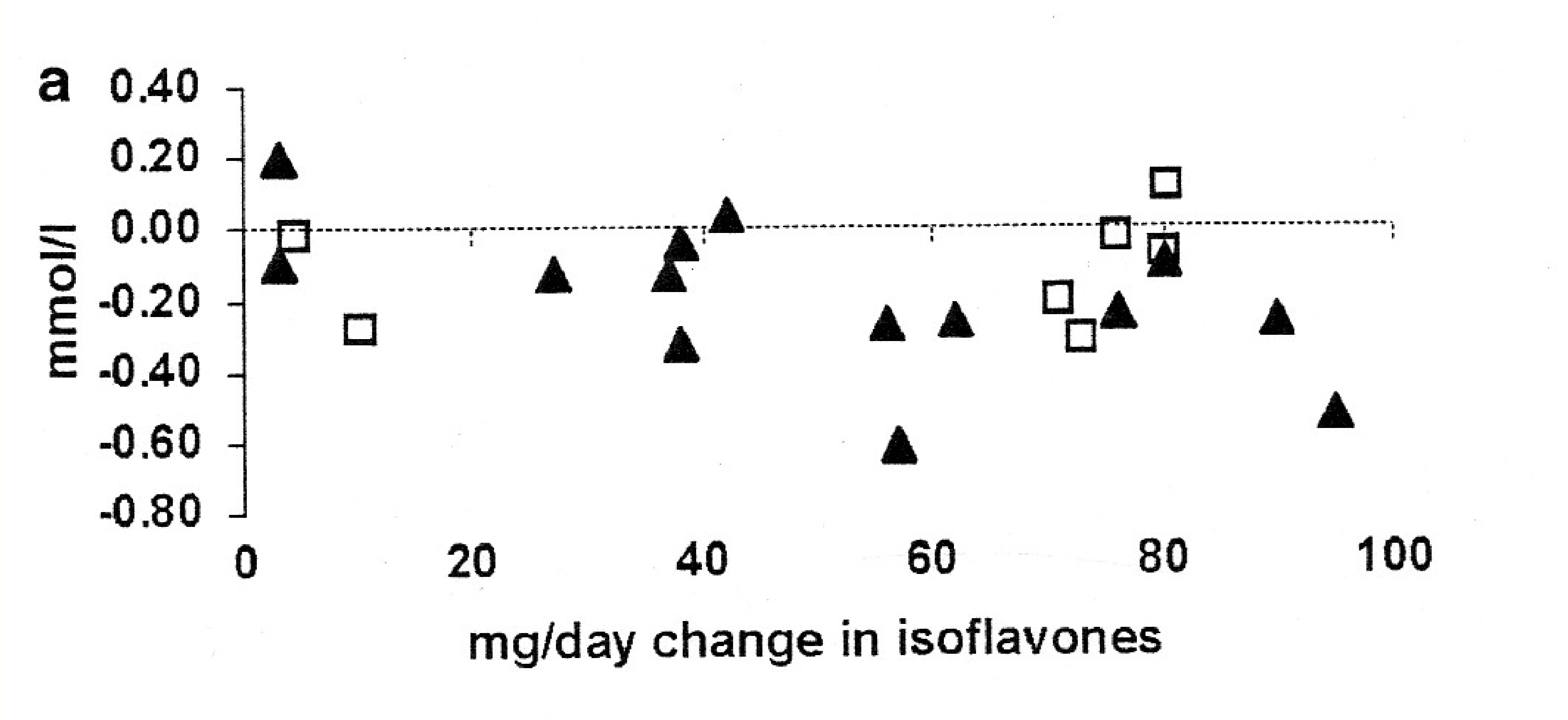


Meta-Analysis: Significant Effect of High Isoflavone Intake on LDL-C

Zhuo et al., 2004

No Significant Effect on Plasma LDL-C as a Function of Soy Isoflavone Levels after Dietary Interventions (meta-analysis includes studies with both stringent and general criteria selection)

LDL



Weggemans et al., 2003

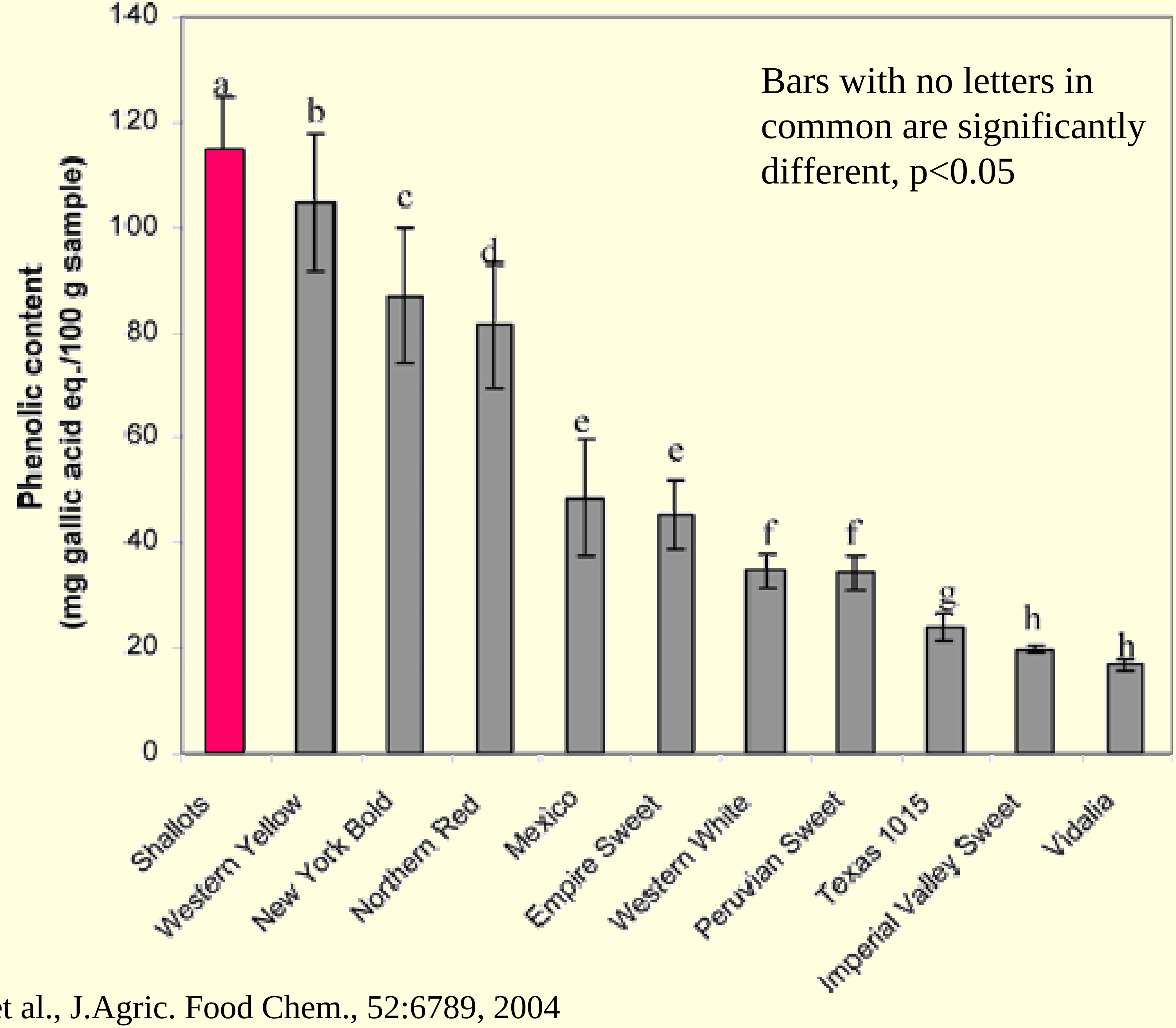
Variability of Phytochemical Content of Foods

- Plant variety*
- Ripeness at the time of harvest
- Environmental factors
- Processing
- Storage
- Method of culinary preparation*

* To be discussed

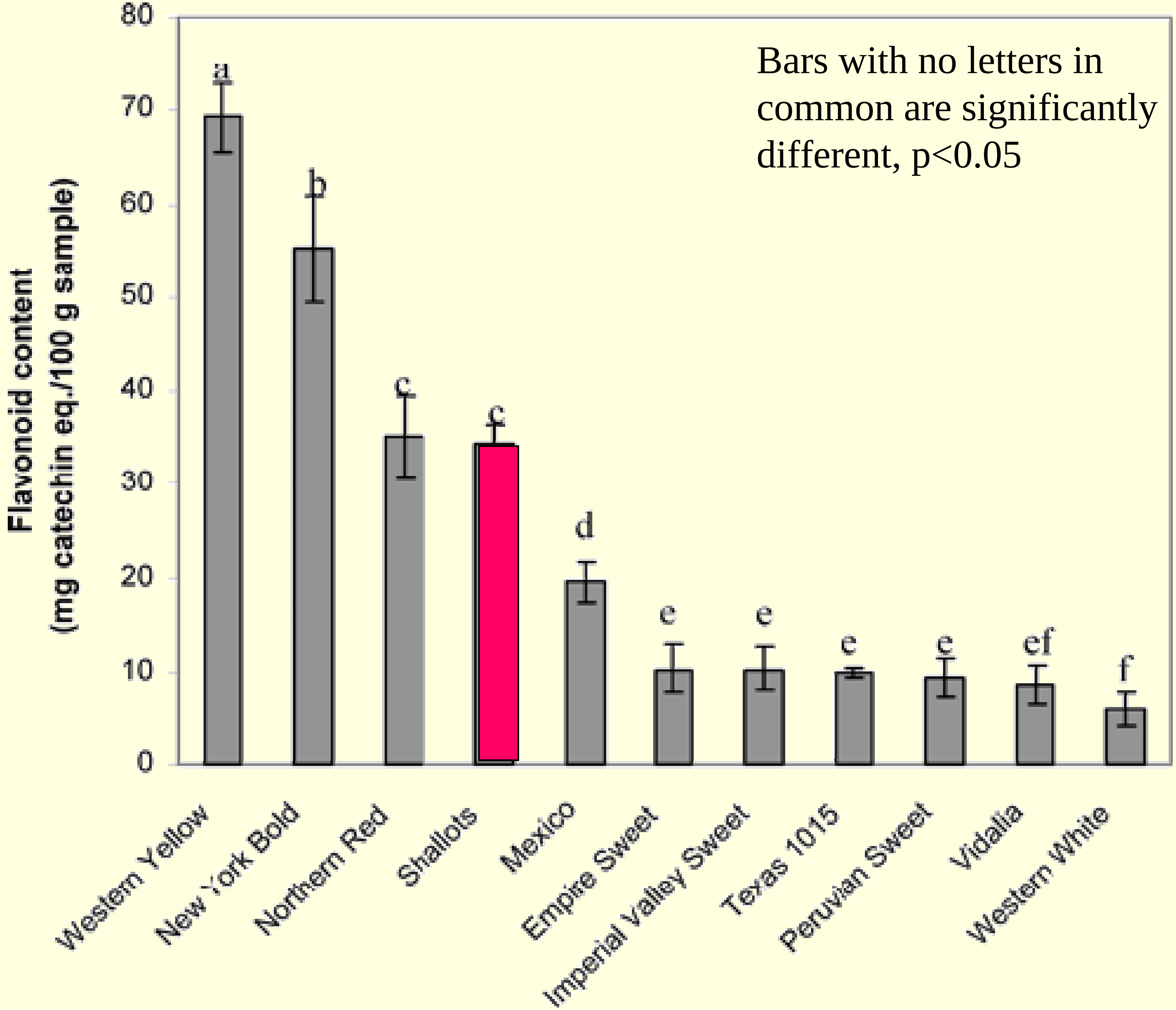
Manach et al., Am J Clin Nutr 79: 727, 2004

Variable Phenolic Content of 10 Onion Varieties



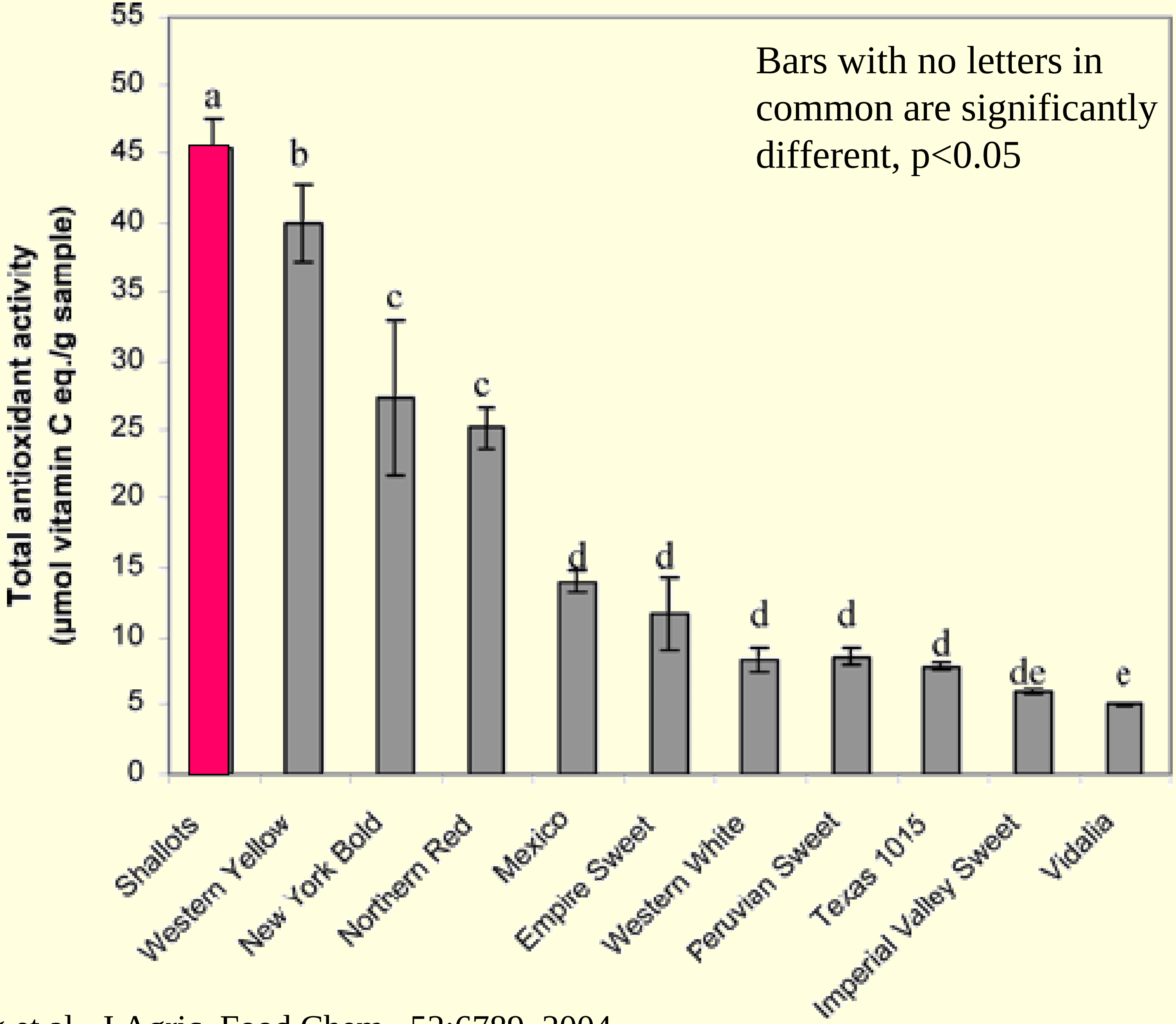
Yang et al., J.Agric. Food Chem., 52:6789, 2004

Variable Flavonoid Content of 10 Onion Varieties



Yang et al., J.Agric. Food Chem., 52:6789, 2004

Variable Antioxidant Content of 10 Onion Varieties



Yang et al., J.Agric. Food Chem., 52:6789, 2004

Variable Carotenoid Content of 11 Wheat Varieties

Lutein

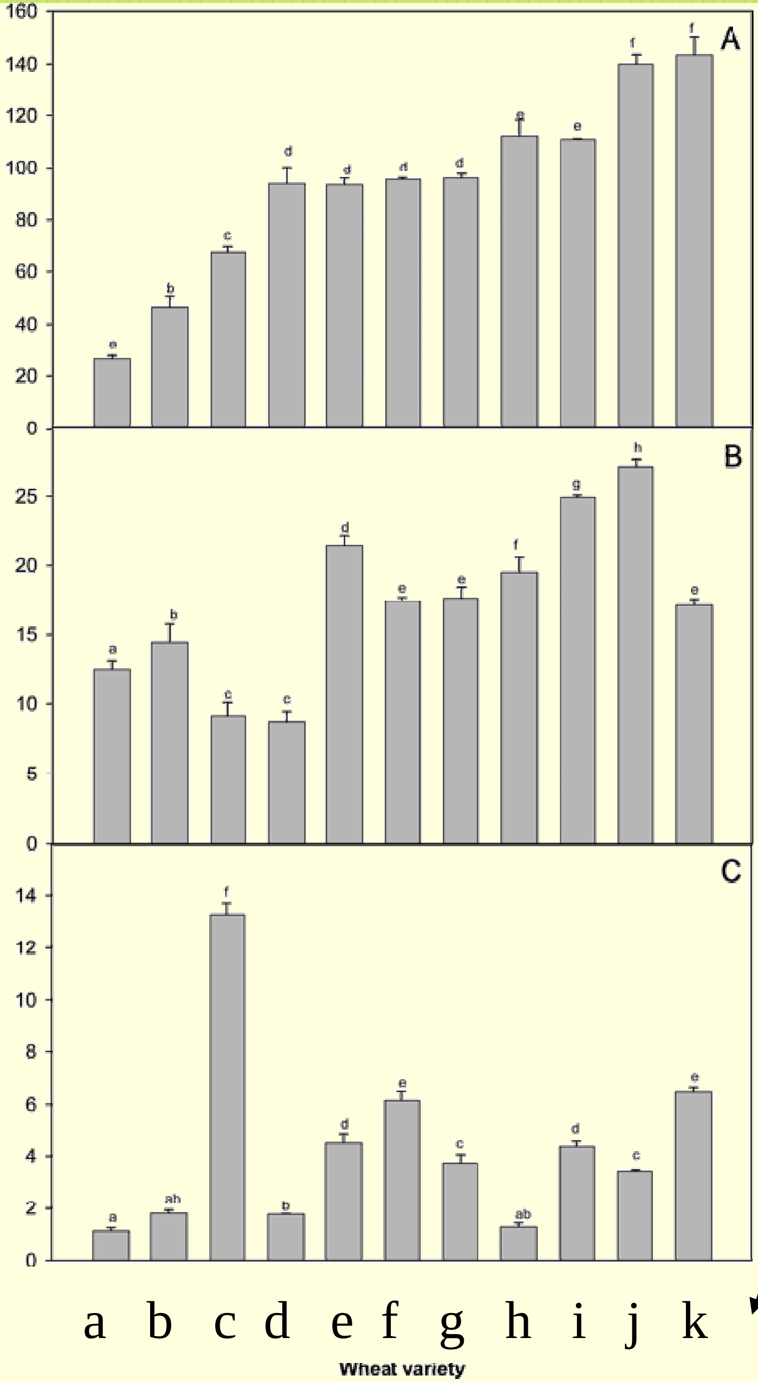
(µg/100 g grain

Zeaxanthin

(µg/100 g grain

β-cryptoxanthin

(µg/100 g grain



a-W7985
b-Jenneh Khetifa
c-Stoa
d-Cham-1
e- Clark's cream
f- NY6432-18
g- Opata
h- Caledonia
i- Sinton
j- Superior
k- Roane

Adom et al., J Agric Food Chem, 51:7825, 2003

Various Cooking Methods Affect the Flavonoid Content in Onion

- Microwave cooking without water better retains flavonoids and ascorbic acid
- Frying does not affect flavonoid intake
- The boiling of onion leads to about 30% loss of quercetin glycosides, which transfers to the boiling water

Ioku et al., J Nutr Sci Vitaminol., 47:78, 2001

Garlic Products on the Market

Not all garlic preparations may lower cholesterol levels

Type of Product	Main Components
Garlic Oil	Only 1% oil-soluble sulfur compounds in 99% vegetable oil
	No water-soluble fraction
	No allicin
Garlic Oil Macerate	Oil-soluble sulfur compounds and alliin
	No allicin
Garlic powder	Alliin and a small amount of oil-soluble sulfur compounds
	No allicin
Aged garlic extract	Mainly water soluble compounds
	Small amount of oil-soluble sulfur compounds

Amagase et al., J Nutr, 131: 955S, 2001

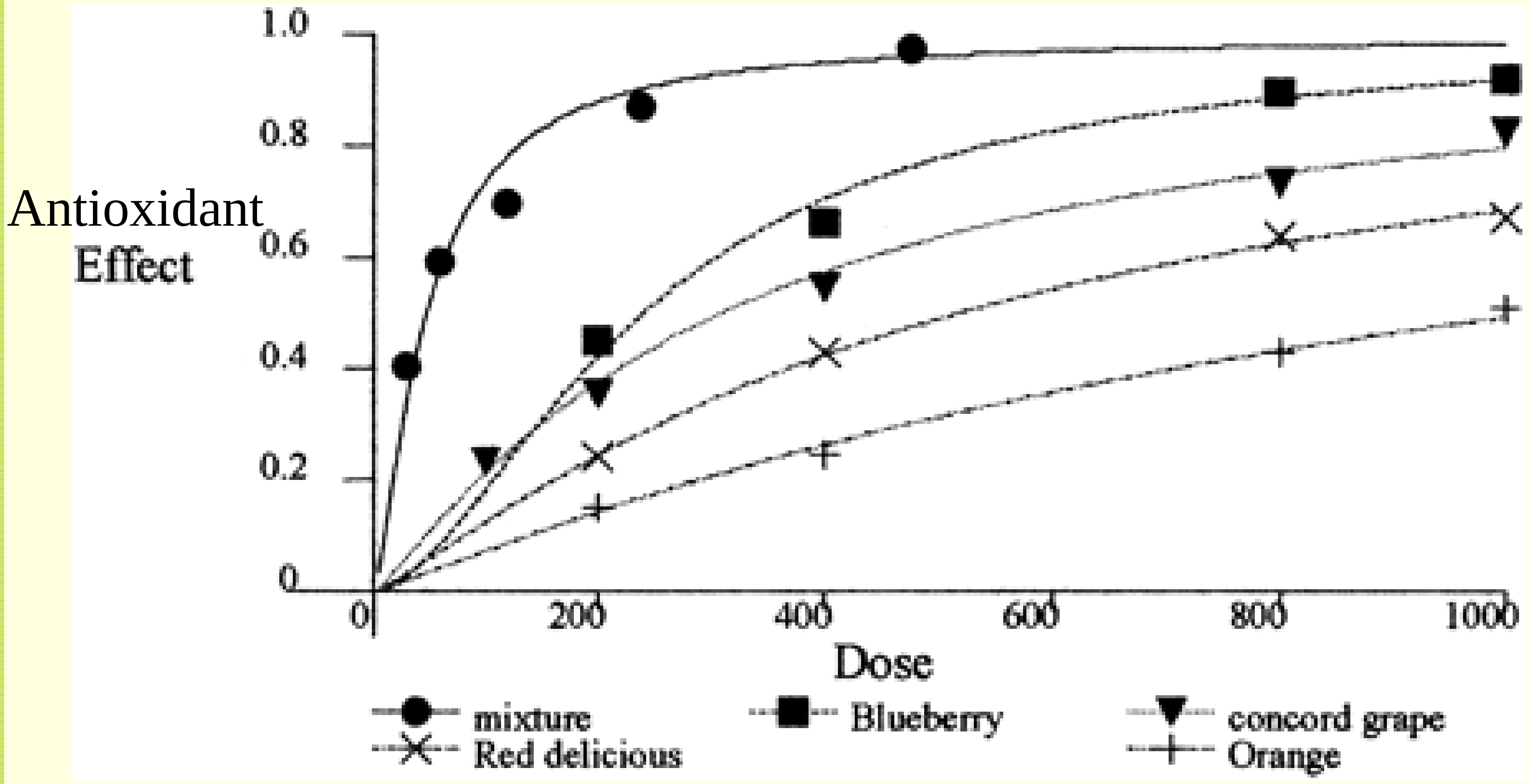
Bioavailability of Phytochemicals: Factors to Consider

- Intestinal absorption
  - Influence of chemical structure
  - Food matrix*
  - Excretion back into the intestinal lumen
  - Role of microflora – catabolism of bioactive compounds and production of active metabolites
- Transport, metabolism and elimination
  - Circulating metabolites*
  - Cellular uptake
  - Intracellular metabolism
  - Target tissue accumulation
- Physiological factors*

* To be discussed

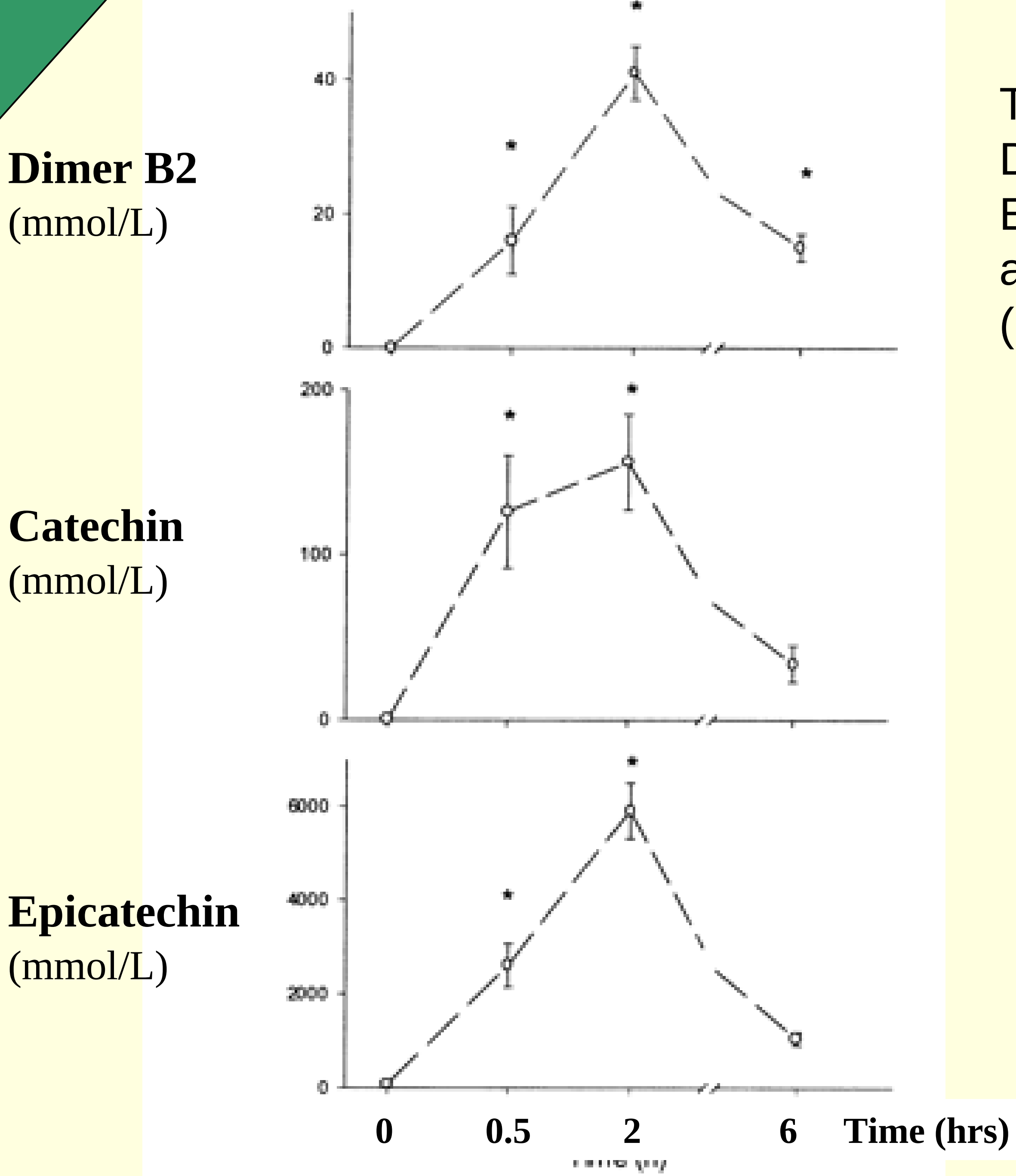
Manach et al., Am J Clin Nutr 79: 727, 2004

Greater Dose-response Antioxidant Effect of Fruit Mixture vs. Individual Fruits



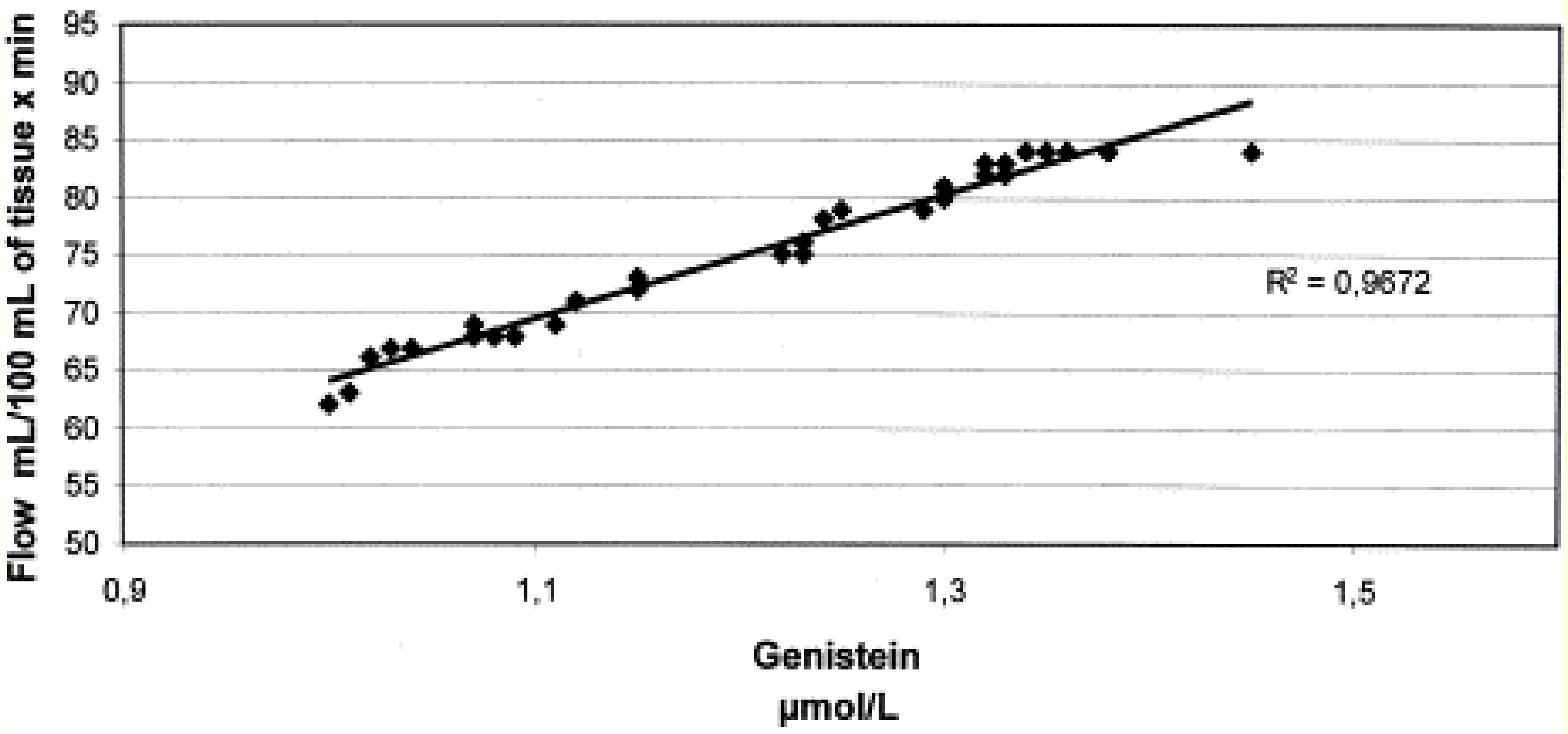
Liu, 2004

Time course for Plasma Dimer B2, Catechin and Epicatechin Concentrations after Consumption of Cocoa (dose =0.375 g/kg BW, n=5)



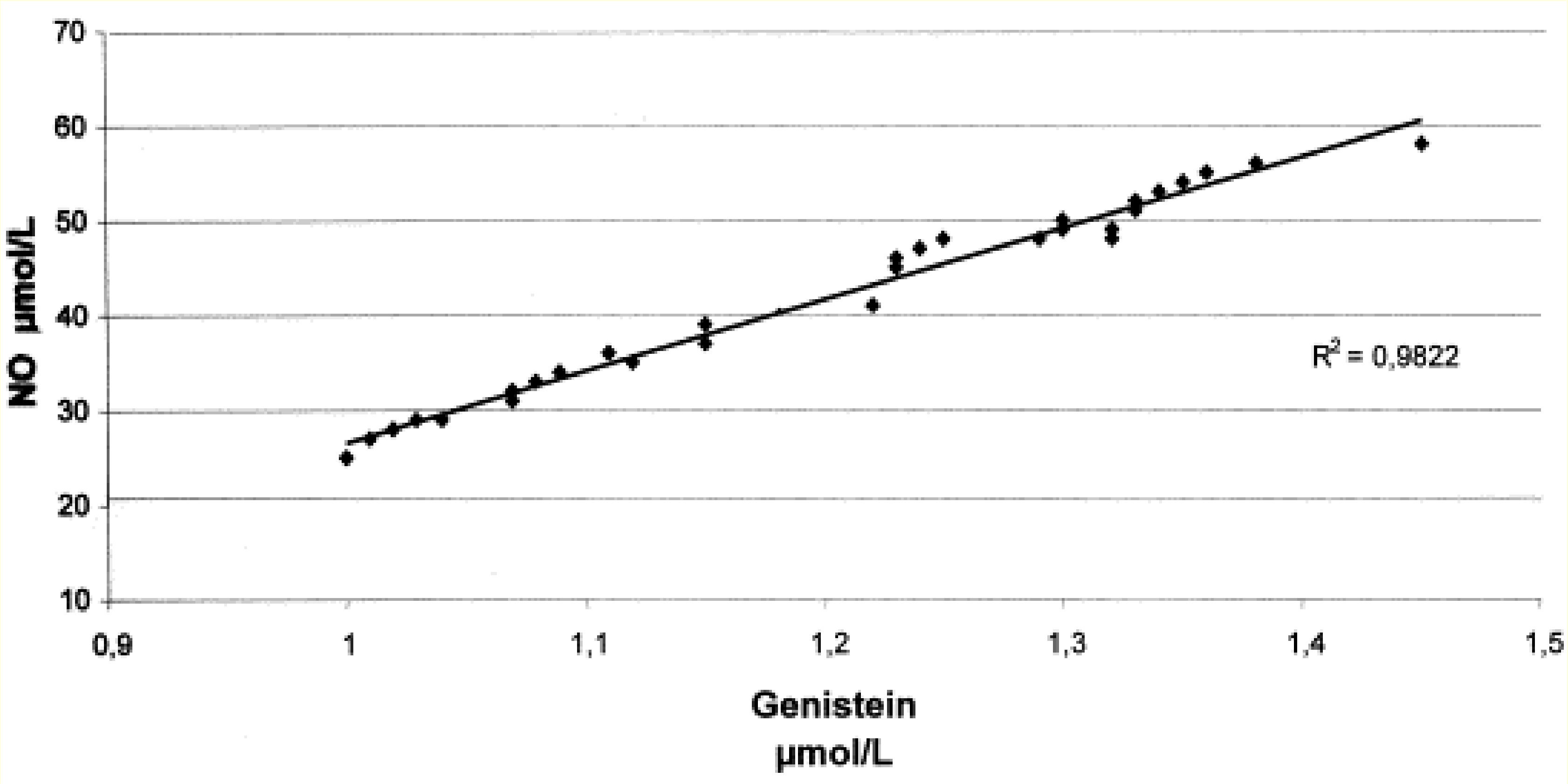
Holt et al., Am J Clin Nutr: 76, 798, 2002

Positive Correlation between Brachial Artery Flow Levels and Genistein Levels



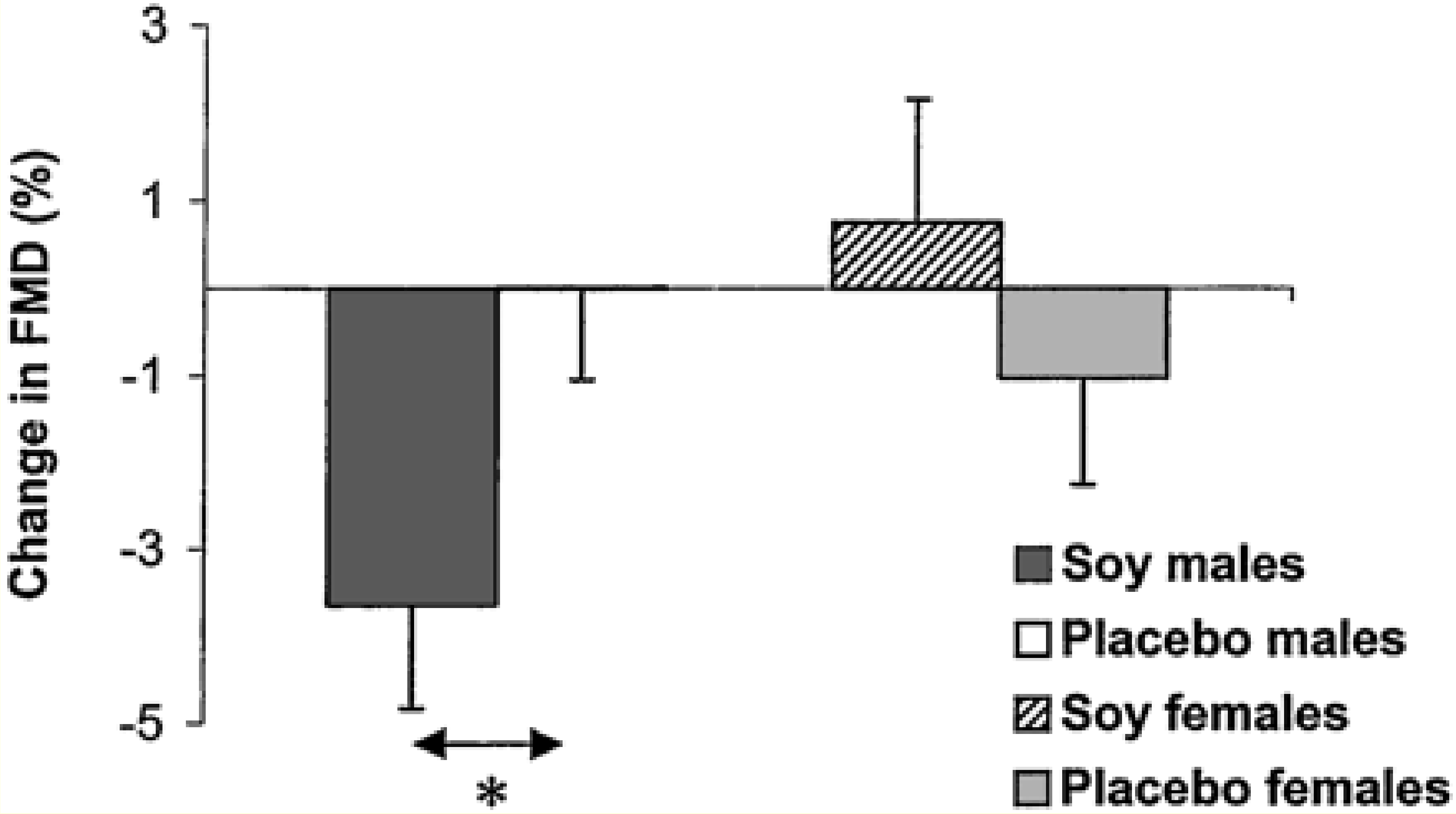
Squadrito et al., 2002

Positive Correlation between Plasma NO Levels and Genistein Levels



Squadrito et al., 2002

Gender Differences in Response to Soy Intake on Flow Mediated Dilation



Teede et al., J Clin Endocrinol Metab, 86: 3053, 2001

Gene Polymorphisms Affect Diet Responses

- Polymorphisms at Apo A1/C3/A4 gene cluster and the Apo E gene explain inter-individual variability in lipid/lipoprotein responses to diet.
  - Ordovas and Schaefer, Br J Nutr 83 (Suppl 1): s-127, 2000
- CYP7A1 A-278C Polymorphism affects plasma lipid response to dietary cholesterol
  - Hofman et al., J Nutr 134: 2200, 2004
- PPARα Leu – 162 Val polymorphism contributes variability in lipid/lipoprotein response to dietary P:S ratio
  - Paradis et al. Am J Clin Nutr 81: 523, 2005

LDL-Cholesterol Lowering is Less in Overweight vs. Normal Weight Men on a Hi-SFA Diet Compared to NCEP I Diet

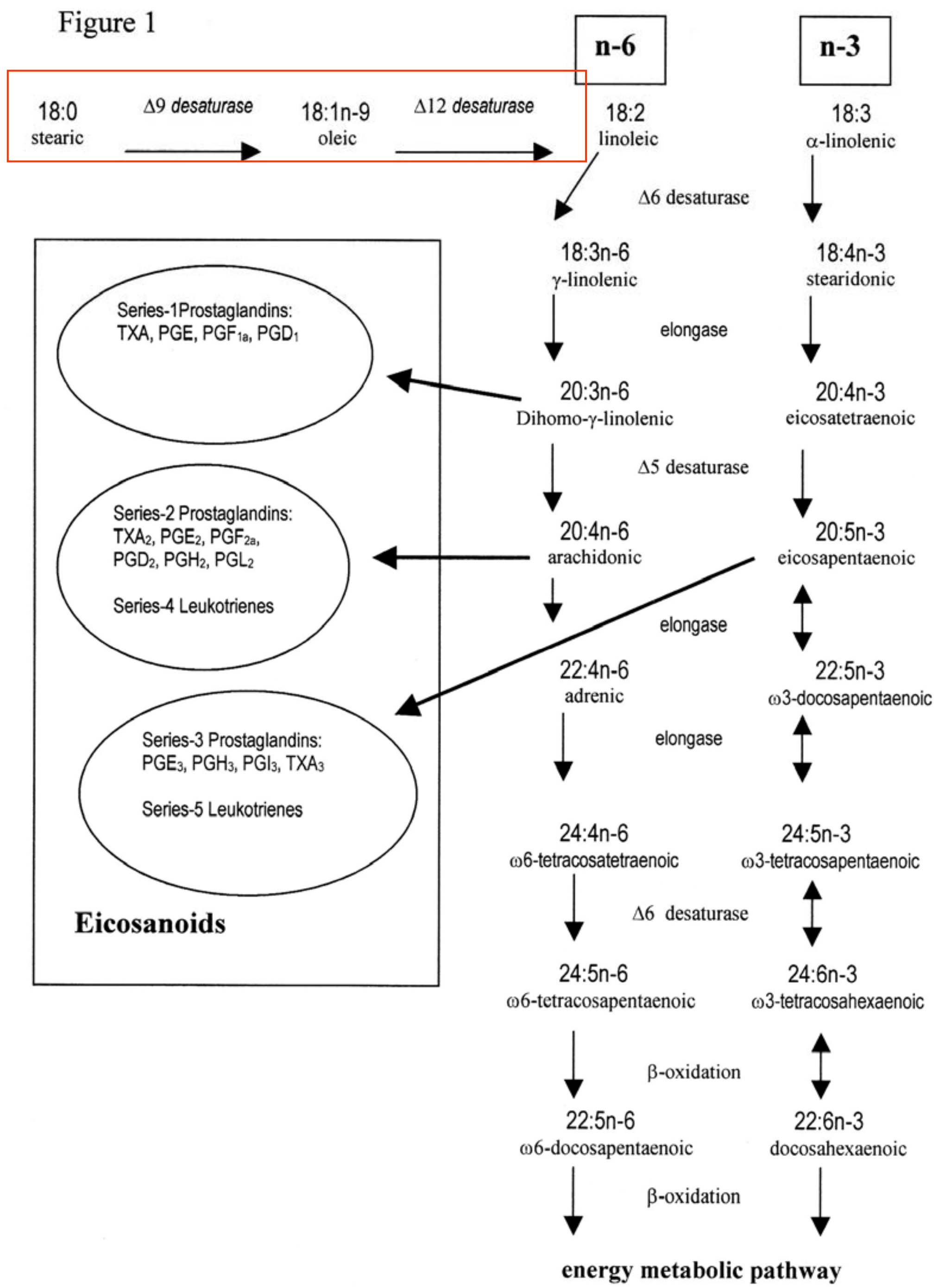
	Cholesterol mmol/L (%)	LDL-C mmol/L (%)	HDL-C mmol/L (%)	TG mmol/L (%)
BMI< 25 kg/m2 n=26	-0.67 (-16)	-0.55 (-21)	-0.1 (-8)	-0.04 (-5)
BMI > 25 kg/m2 n=15	-0.30 (-7)	-0.24 (-9)	-0.05 (-5)	-0.07 (-5)

Values are means and %;
Change is significantly different, $p<0.05$

Jansen et al., J Nutr, 128:1144, 1998

Eicosanoids from n-6 and n-3 PUFA

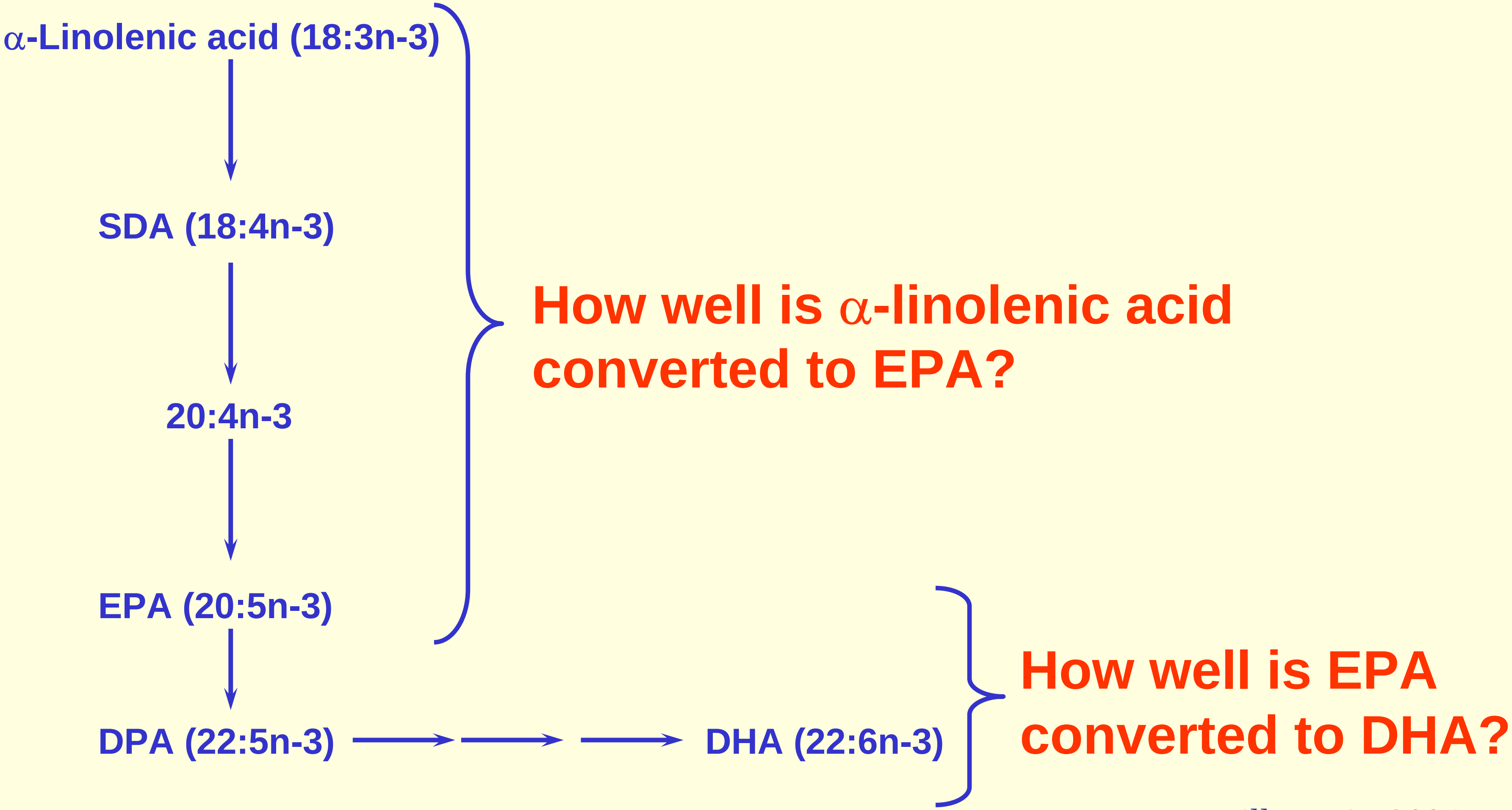
Not in mammals



Leonard et al., 2004

Balk et al., 2004

Is α-linolenic acid a good precursor for EPA and DHA in humans?

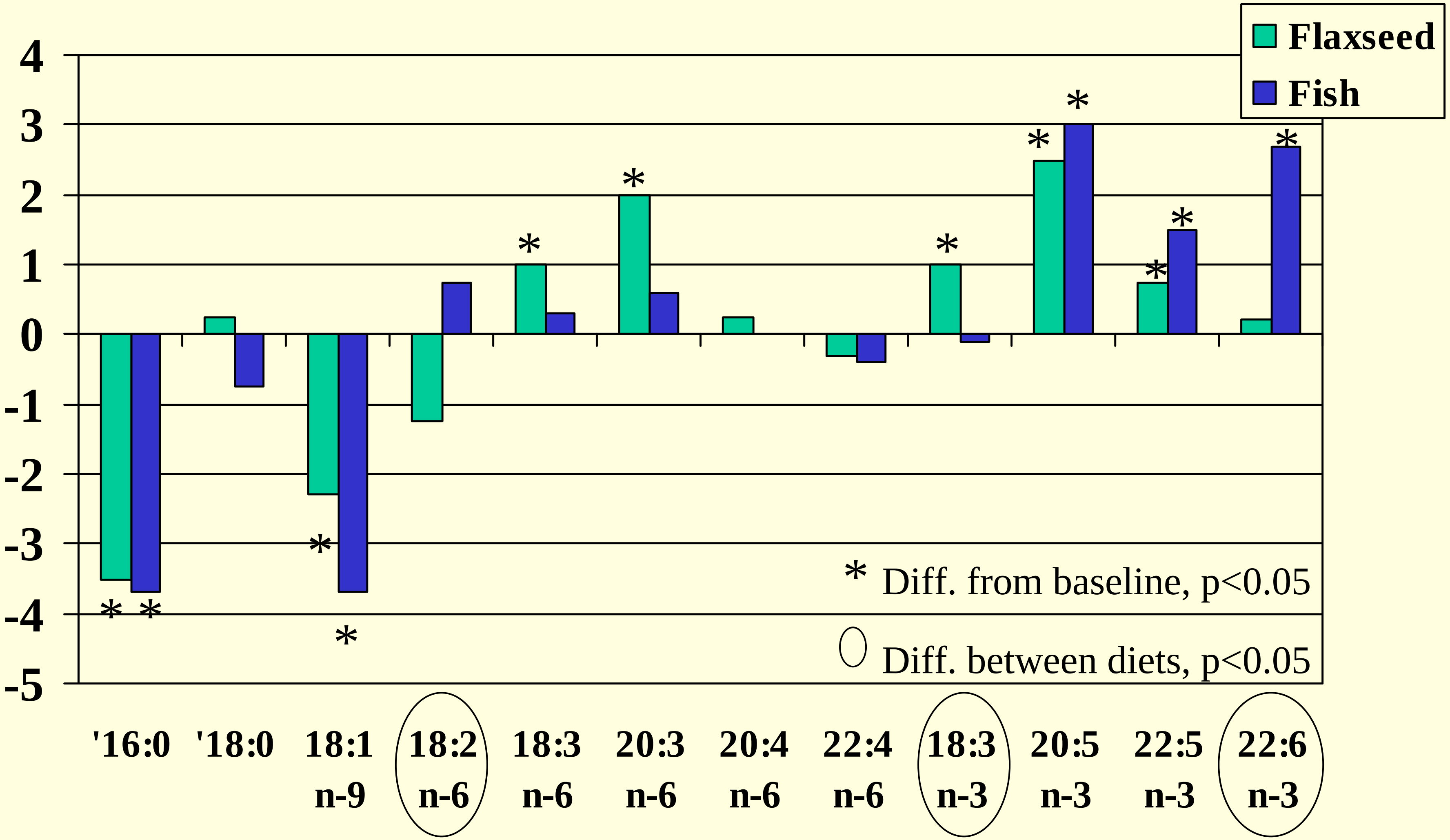


From Bill Harris, 2004

Approaches Used to Study Efficiency of ALA Conversion to Long-Chain n-3 PUFA

- Fatty acid composition of biological samples, primarily plasma and circulating cells.
- Stable isotope studies to trace ALA metabolism

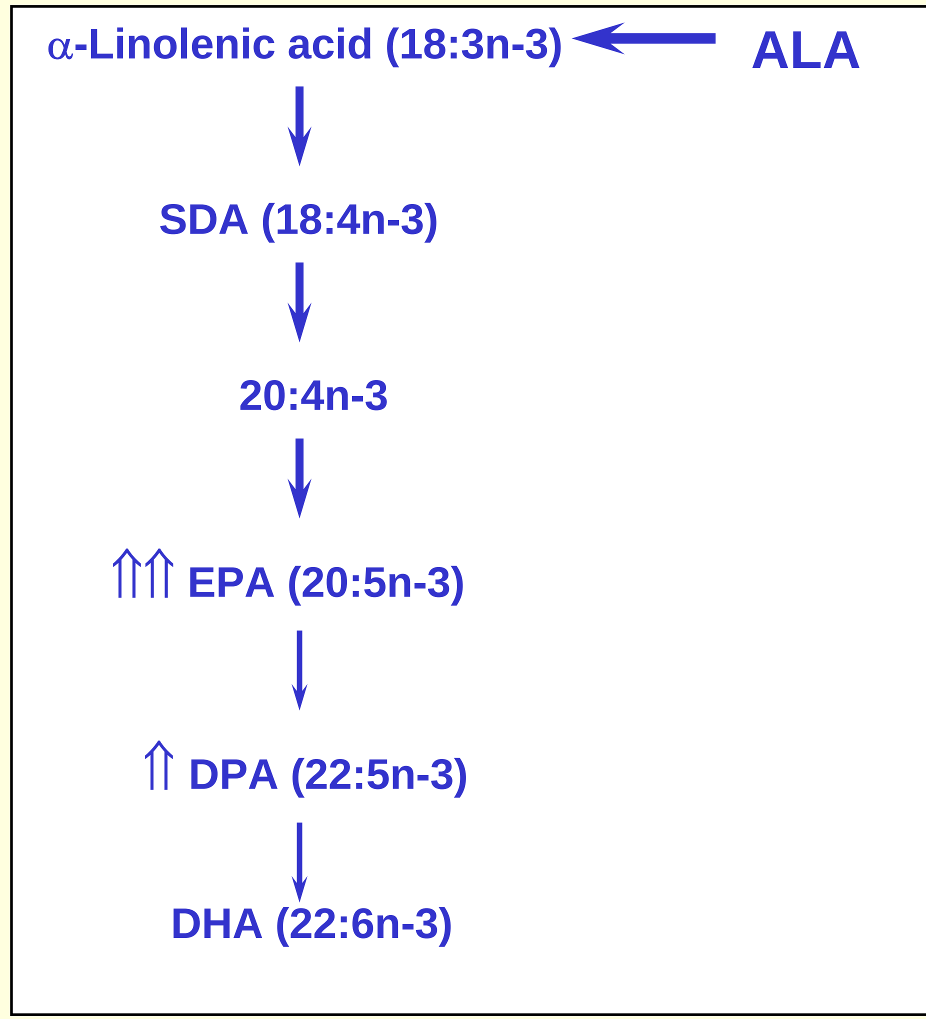
Erythrocyte Phospholipid Fatty Acid Profiles after 12 Week Supplementation of Flaxseed Oil Compared to Fish Oil



Summary : α-Linolenic acid

- Dietary ALA can increase plasma, platelet, red cell and white cell EPA and DPA status (ALA dose important)

- Dietary ALA does NOT increase plasma, platelet, red cell or white cell DHA status



From Bill Harris, 2004

Tracer Converstion to Long-Chain PUFA

Diet	DGLA	AA	EPA	DPA	DHA
Flax Oil (n = 6)	0.20	0.12	0.29	0.05	< 0.01
Sun Oil (n = 5)	0.29	0.26	0.19	0.02	<0.01
Both Diets (n = 11)	0.23	0.18	0.26	0.04	<0.01
P	<0.05	<0.05	NS	NS	NS

Overall conversion is maximum plasma ^{13}C content as a % of dose of either ^{13}C-LA or ^{13}C-ALA.

Hussein et al., J. Lipid Res. 46:269, 2005.

Summary: Stable Isotope Studies

- More than 75% of dietary ALA is oxidized
- Conversion of ALA to long-chain n-3 PUFA is limited

Influence of Species and Diet on PUFA Variability in Salmon (3 oz. cooked)

Fish	Kcal	Fat (g)	Total PUFA (g)	18:2 n-6 linoleic	18:3 n-3 ALA	20:5 n-3 EPA	22:5 n-3 DPA	22:6 n-3 DHA
Atlantic-wild	**155**	**6.91**	**2.78**	**0.19**	**0.32**	**0.35**	**0.31**	**1.22**
Atlantic-farmed	**175**	**10.5**	**3.76**	**0.58**	**0.10**	**0.59**	**--------**	**1.24**
Coho-wild	**118**	**3.65**	**1.08**	**0.05**	**0.05**	**0.34**	**--------**	**0.56**
Coho-farmed	**151**	**7.0**	**1.67**	**0.32**	**0.07**	**0.35**	**--------**	**0.74**

USDA Nutrient Database

EPA vs. DHA : What are the Relative Potencies?

- **Differential Effects on Lipoproteins**

  Leigh-Firbank et al., 2002
  - DHA: ↑ LDL-C
  - EPA: ↓ plasma TG

  Mori et al., 2000
  - 4 g/d EPA: ↓HDL3 (7%)
  - 4 g/d DHA: ↑ HDL2 (29%)
  - DHA: ↑ LDL-C (8%), ↑LDL size
- **Similar Effects on TGs**

  Woodman et al., 2002, Grimsgaard et al., 1997
  - 4 g/d EPA or DHA: ↓ plasma TG (19%)
  - 3.8 g/d EPA or 3.6 g/d DHA: ↓plasma TG (26 & 21%)
- **EPA: primarily responsible for ↓TG**

  Rambjor et al., 1996

EPA vs. DHA : What are the Relative Potencies?

- EPA, but not DHA, decreases mean platelet volume (early step in platelet aggregation)
  - Park et al., 2002
- DHA, but not EPA, lowers ambulatory blood pressure and heart rate in humans
  - Mori et al., 1999

Summary

- There are numerous bioactive compounds that have the potential to decrease CVD risk
- There's much to be learned and the physiology of bioactive compounds is complex
- The focus of current and future work should lead to population – based dietary recommendations