

Opportunities and Challenges for Evaluating the Health Significance of Functional Foods and Their Components

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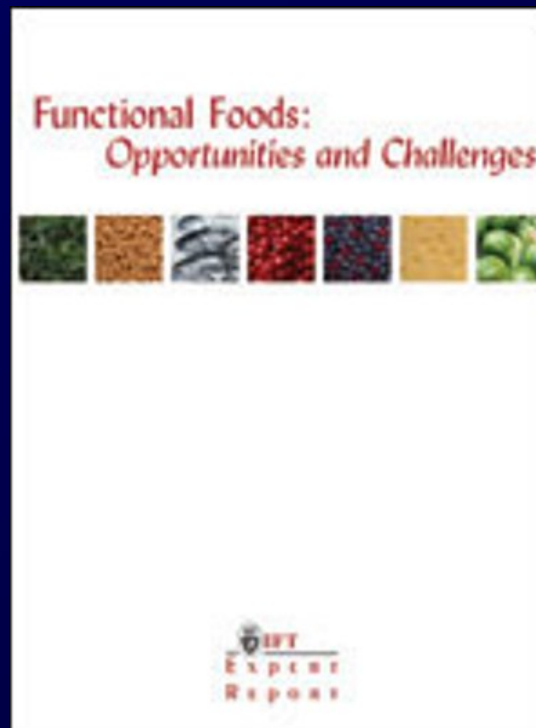
milnerj@mail.nih.gov

National Academy of Sciences Defines **Functional Foods:**

“encompass potentially healthful products,”
including “any modified food or food
ingredient that may provide a health benefit beyond the
traditional nutrients it contains.

Recent Report

Institute of Food
Technologists
IFT Experts Report
March 24, 2005



[http://members.ift
.org/IFT/Research
/IFTExpertReport
s/functionalfoods_
report.htm](http://members.ift.org/IFT/Research/IFTExpertReports/functionalfoods_report.htm)

**Hippocrates Proclaimed
almost 2500 years ago:**

**Let thy food be thy
medicine and thy medicine
be thy food**

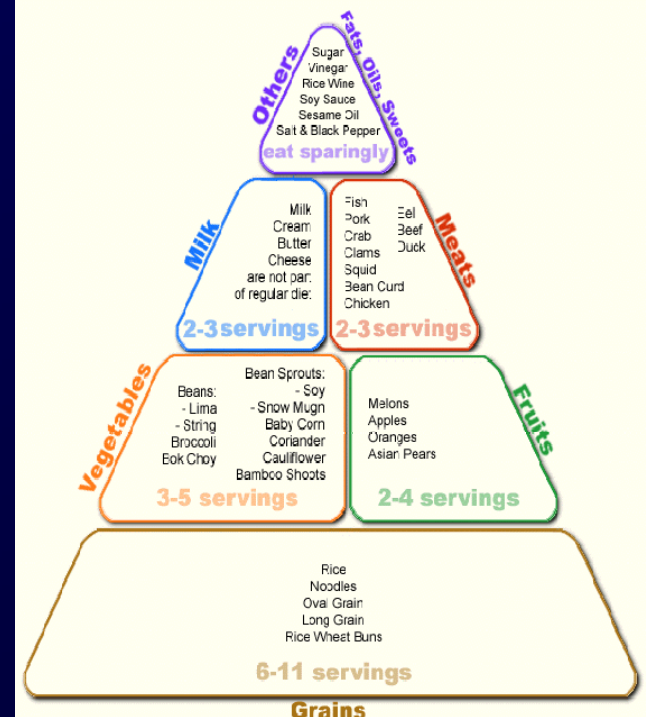
There Are Always A Few Skeptics!

Copyright 2002 by Randy Glasbergen. www.glasbergen.com



**“Snow White was poisoned by an apple,
Jack found a giant in his beanstalk, and look
what happened to Alice when she ate the mushroom!
And you wonder why I won’t eat fruit and vegetables!?”**

Public Health Approach



Suspect Functional Foods



■ Tomatoes

■ Spinach

■ Broccoli

■ Garlic

■ Nuts

■ Salmon

■ Oats

■ Blueberries

■ Green tea

■ Red wine



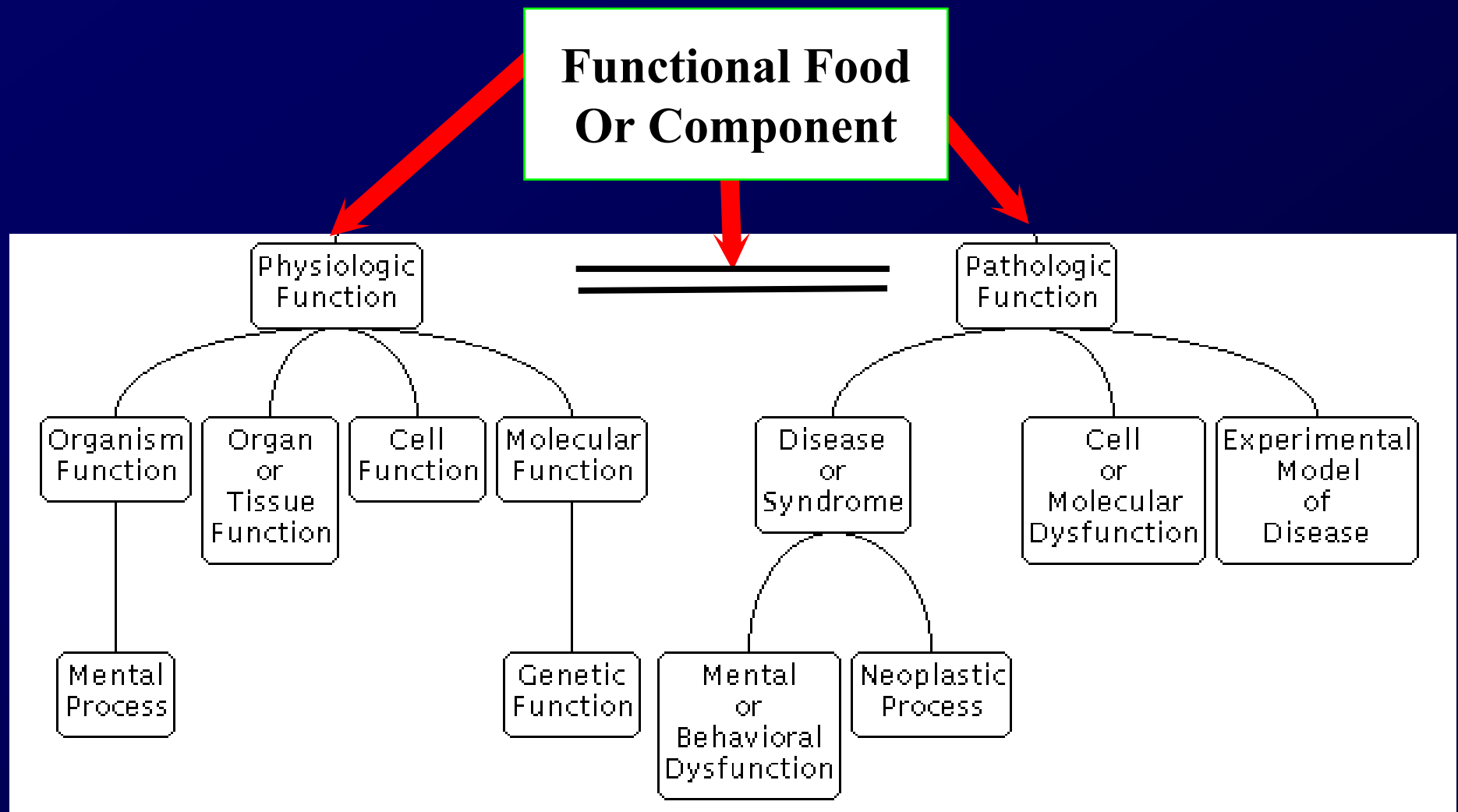
Time Magazine: January 21, 2002

Consumers are Bombarded With Many Confusing Messages

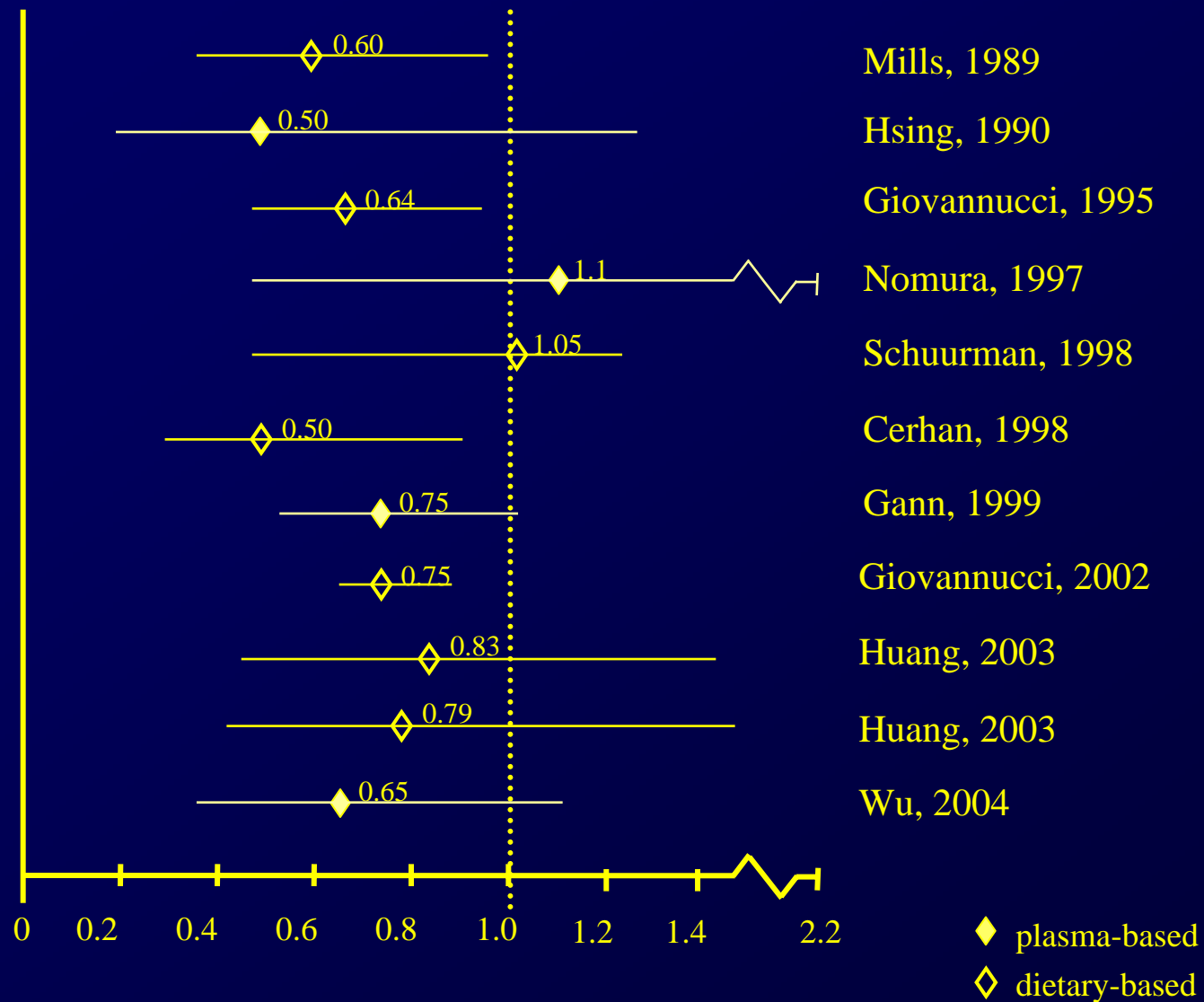


Messina (2006) Personal Communication

Question Remains About True Site of Action of Functional Foods and Health Benefits



RR (95% CI) High vs. Low Tomato Intake or Lycopene Prostate Cancer: Prospective Studies



Relative Risk of Breast Cancer: Influence of Soy

Asian

Lee '92 (total soy protein)
 p < 0.001 Premenopausal
 NS Postmenopausal
 Hirose '95 (bean curd, miso)
 Yuan '95 (tofu, soymilk)
 NS Premenopausal
 NS Postmenopausal
 NS p = 0.44–0.79 Shanghai, Tianjin

Wu '96 (tofu)
 p < 0.01 Premenopausal
 p < 0.05 Postmenopausal

Key '99 (soy)

Tofu

Miso

Zheng '99 (urinary isoflavonoids)

Dai '01 (soy)

NS All Breast Cancer

S Just ER⁺/PR⁺

Wu '02 (soy)

Yamamoto '03 (isoflavonoid consumption)

Premenopausal

Postmenopausal

Wu '04 (soy)

Ingram '97 (urinary isoflavones)

NS Diadzein

p = 0.009 Equol

Witte '97 (soy)

den Tonkelaar '01 (urinary phytoestrogens)

NS Postmenopausal

Horn-Ross '01 (phytoestrogen intake)

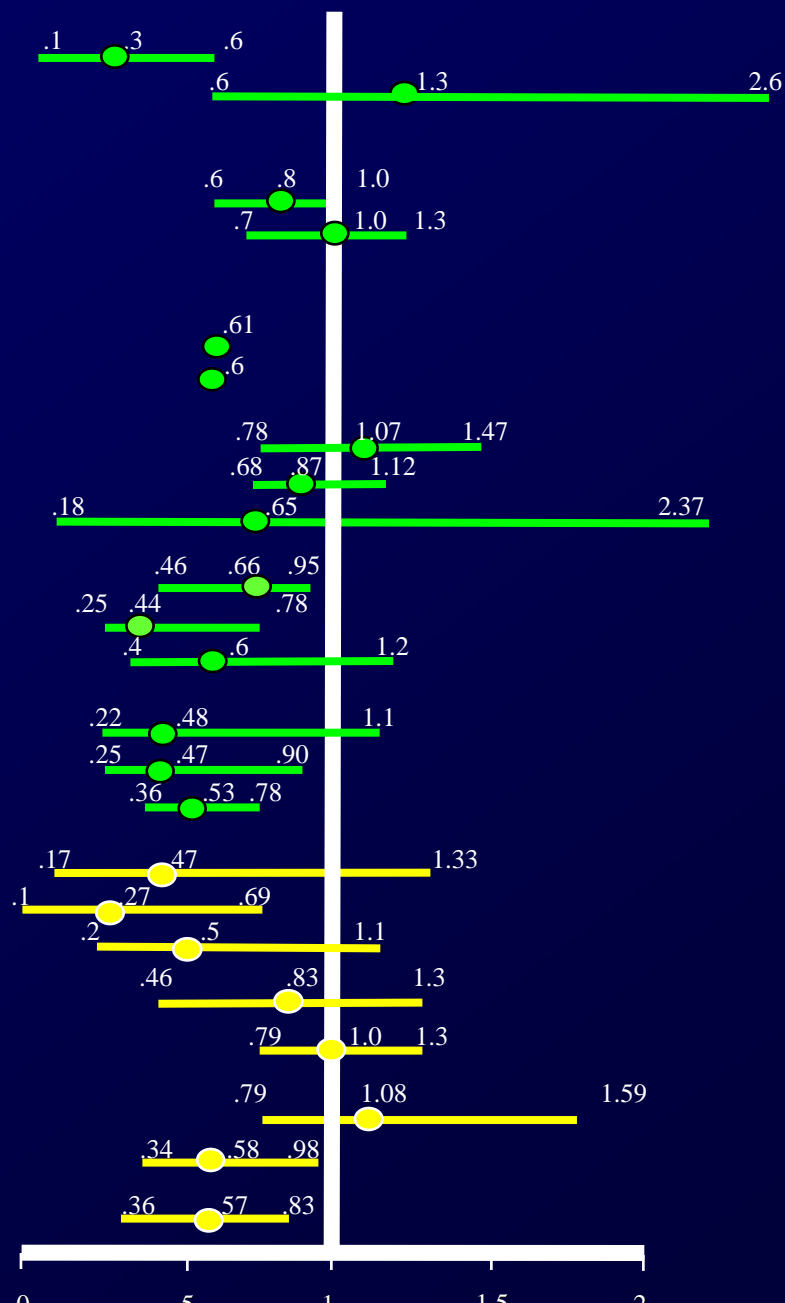
Keinan-Boker '02 (food content)

NS Isoflavones

S Lignans

Linseisen '04 (isoflavone intake)

daidzein and genistein



Western

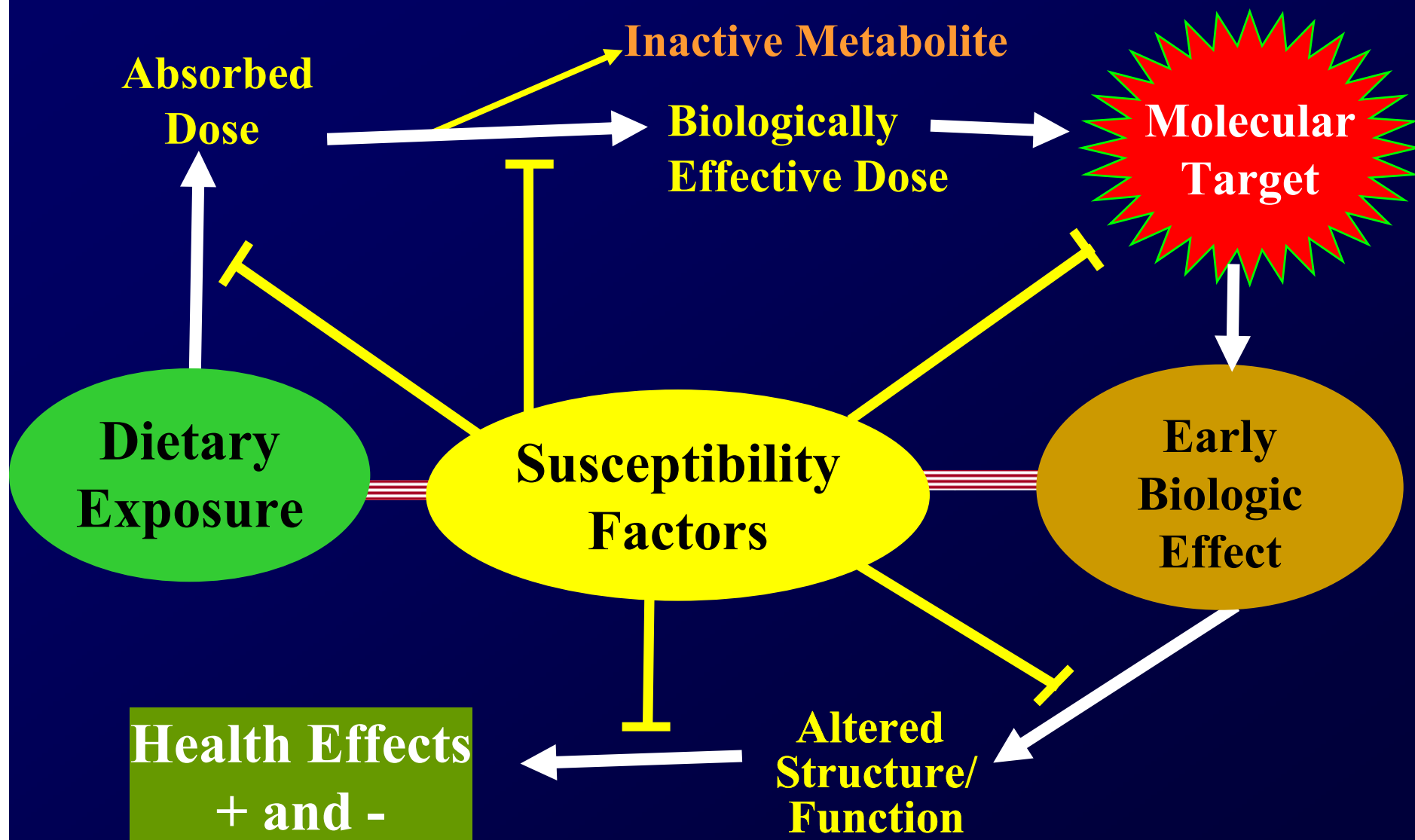
Various Approaches Used to Showcase Functional Foods

- **Conventional product marketed with new information**
 - Oats soluble fiber claim
- **Conventional product with added or enhanced ingredient**
 - Spread w/ added plant sterols
- **“New” product to deliver functional ingredient**
 - Beverages– functional teas, water
- **Removing negative functionality**
 - Oils with heart-healthy profile

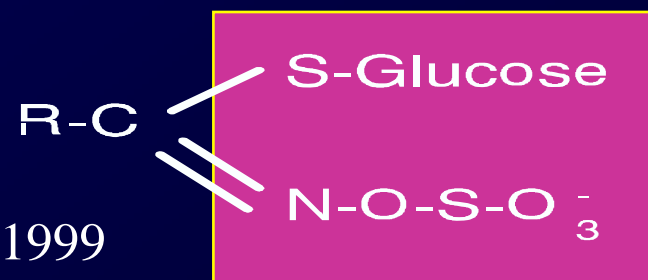
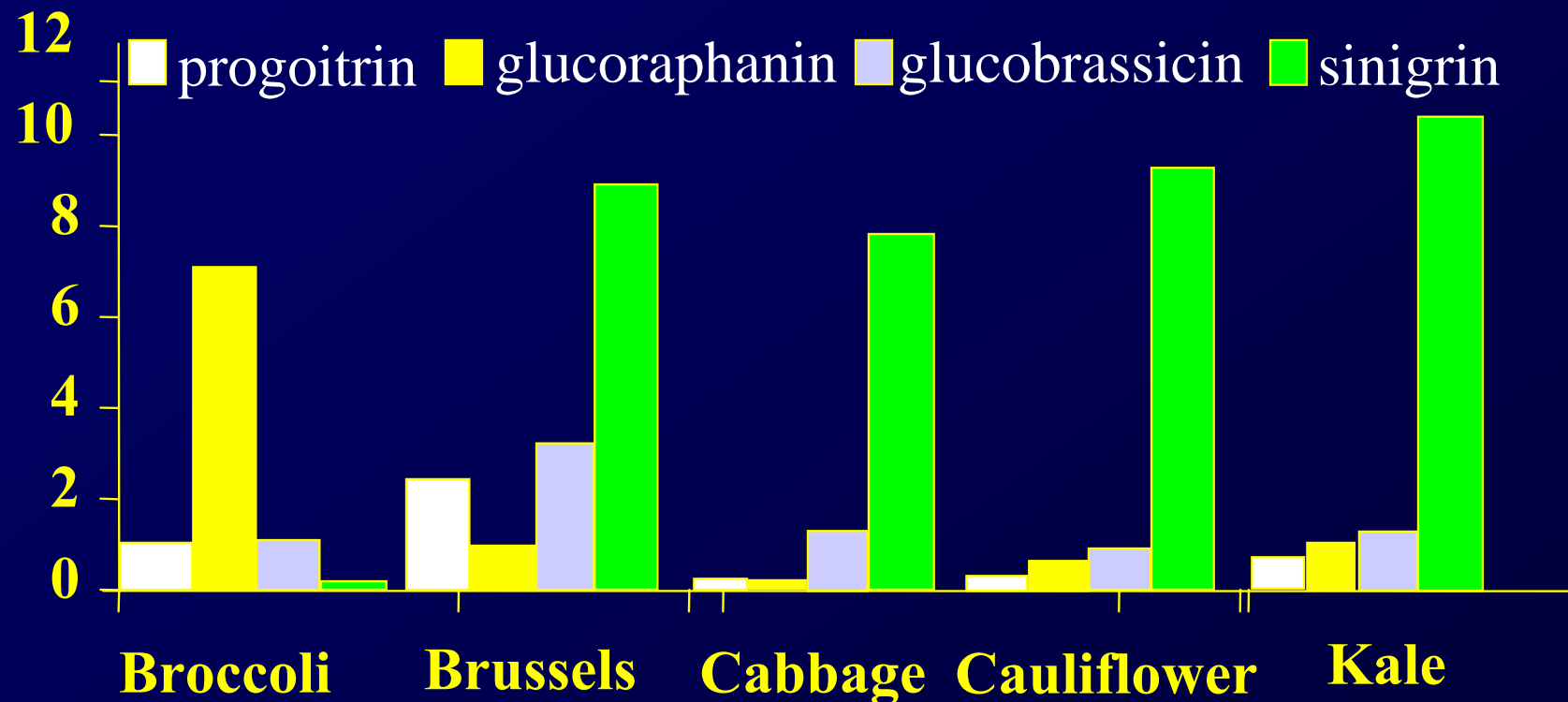
Functional Foods Have Economic Importance

- **Estimates of functional foods market vary from \$20 and \$120 billion, depends on what is included**
 - Compare to about \$6 billion organics and about \$500 billion total food
- **Clear impact in some markets**
 - Calcium-enhanced OJ is 1/5 of OJ market
 - Soy beverages rapid growth
 - Functional breads and grains 10% of market

Three Types Biomarkers Needed To Truly Assess Benefits

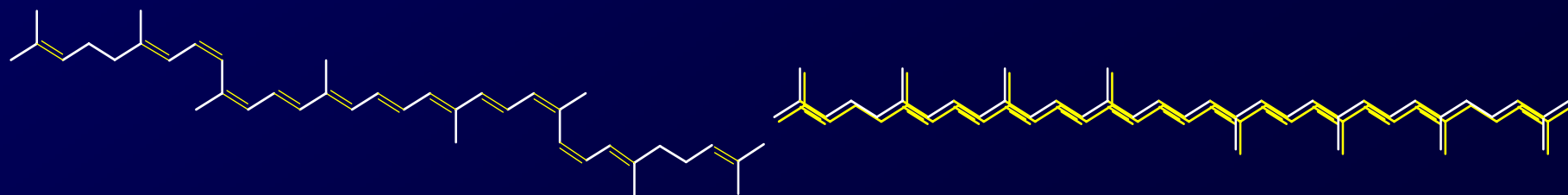


Glucosinolate Variation Among Cruciferous vegetables ($\mu\text{mol/g Dry Weight}$)



Kushad et al, J Agric Food Chem. 47(4):1541, 1999

Tomato Varieties



Foods May Influence Multiple Processes Because of Multiple Components

Phytochemical: Fresh/Canned: Tomato Sauce:



Lycopene	3.0	15.9
Phytoene	1.9	3.0
Phytofluene	0.8	1.3
β-carotene	1.5	3.2
Cyclolycopene	0.1(juice)	
Quercetin (free)	0.1-0.2	3.8-9.5
Quercetin (conj)	1.2-21.5	10.9-61.4
Naringenin	0.8-4.5	2.5
Chlorogenic acid	0.9-4.2	0.7
Ascorbate	5.0-14.0	
Tomatine	0.5	

* (mg/100 g)

Domestic cooking of tomatoes
significantly increases naringenin
and chlorogenic acid bioavailability
as indicated by plasma levels

Bugianesi et al. Eur J Nutr. 2004

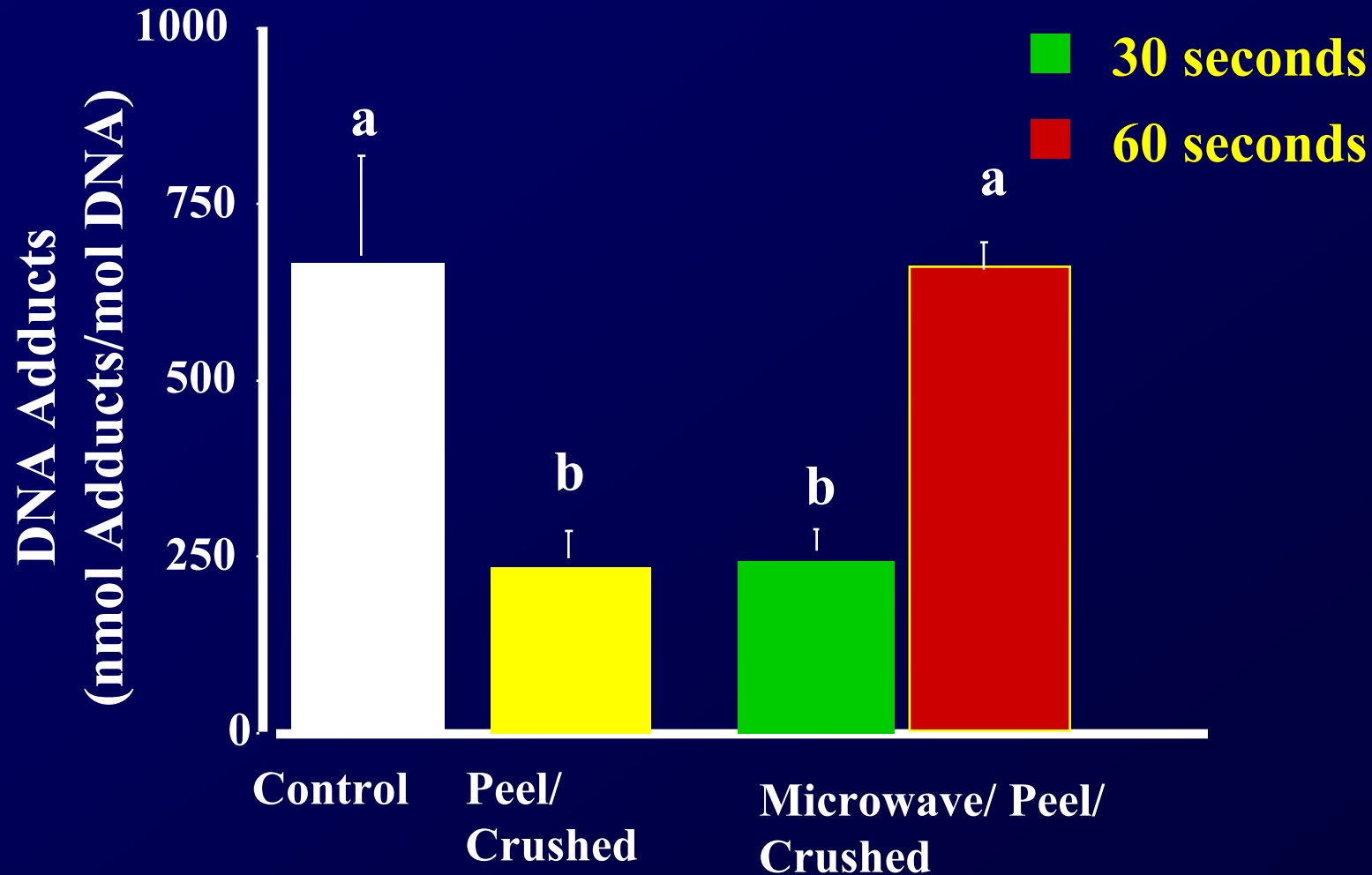
Dec;43(6):360-6

Cooking tomatoes with olive oil/
sausage increases bioavailability of
lycopene

Fielding et al. Asia Pac J Clin Nutr.

2005;14(2):131-6.

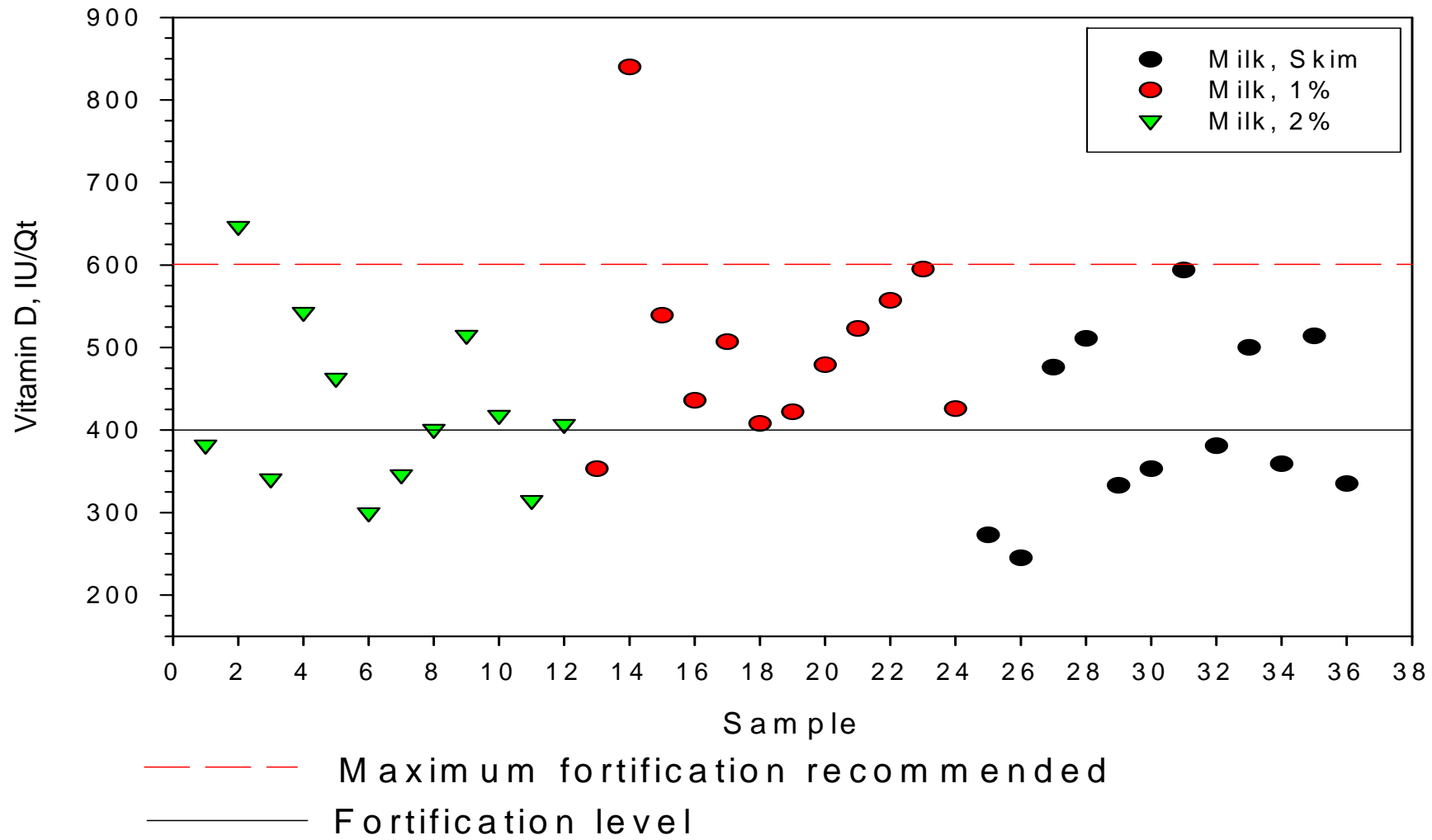
Heating Garlic Can Reduce the Biological Consequences



Song and Milner J Nutr 1999;129:657-61

NFNAP Vitamin D in Milk

12 Locations Nationwide, 2001

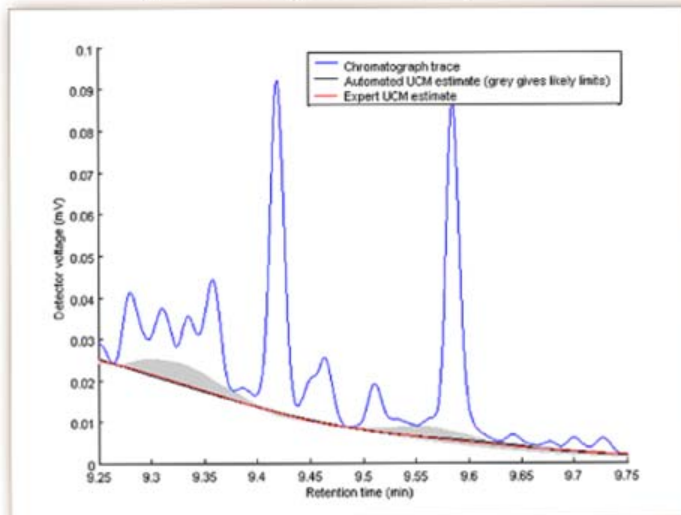




24 hour record
Food frequency questionnaire
Food diary



Physiological exposures

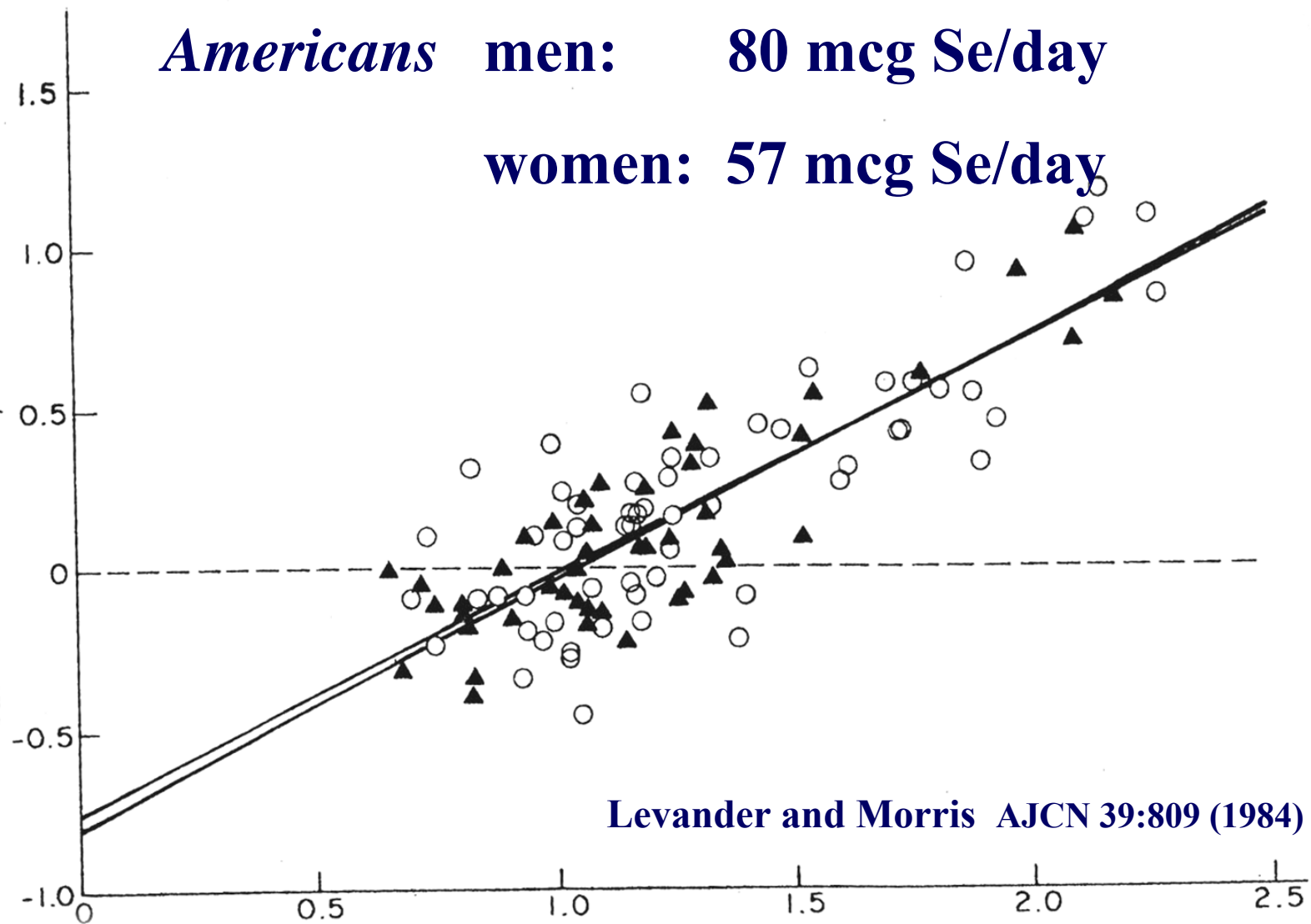


Absorption
Metabolism
Distribution
Excretion

Selenium Balance ($\mu\text{g/kg/d}$)

Americans men: 80 mcg Se/day

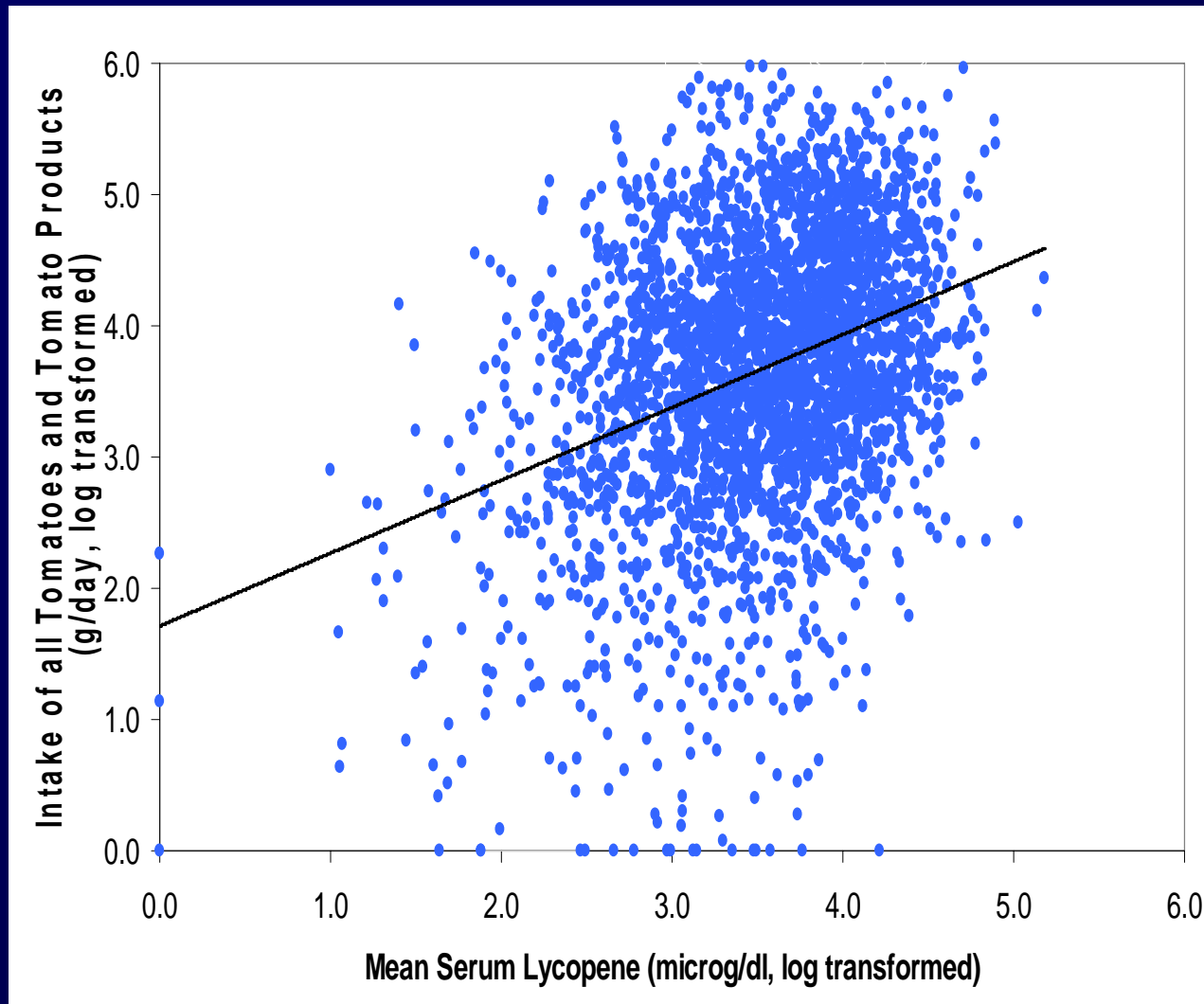
women: 57 mcg Se/day



Levander and Morris AJCN 39:809 (1984)

Selenium Intake ($\mu\text{g/kg/d}$)

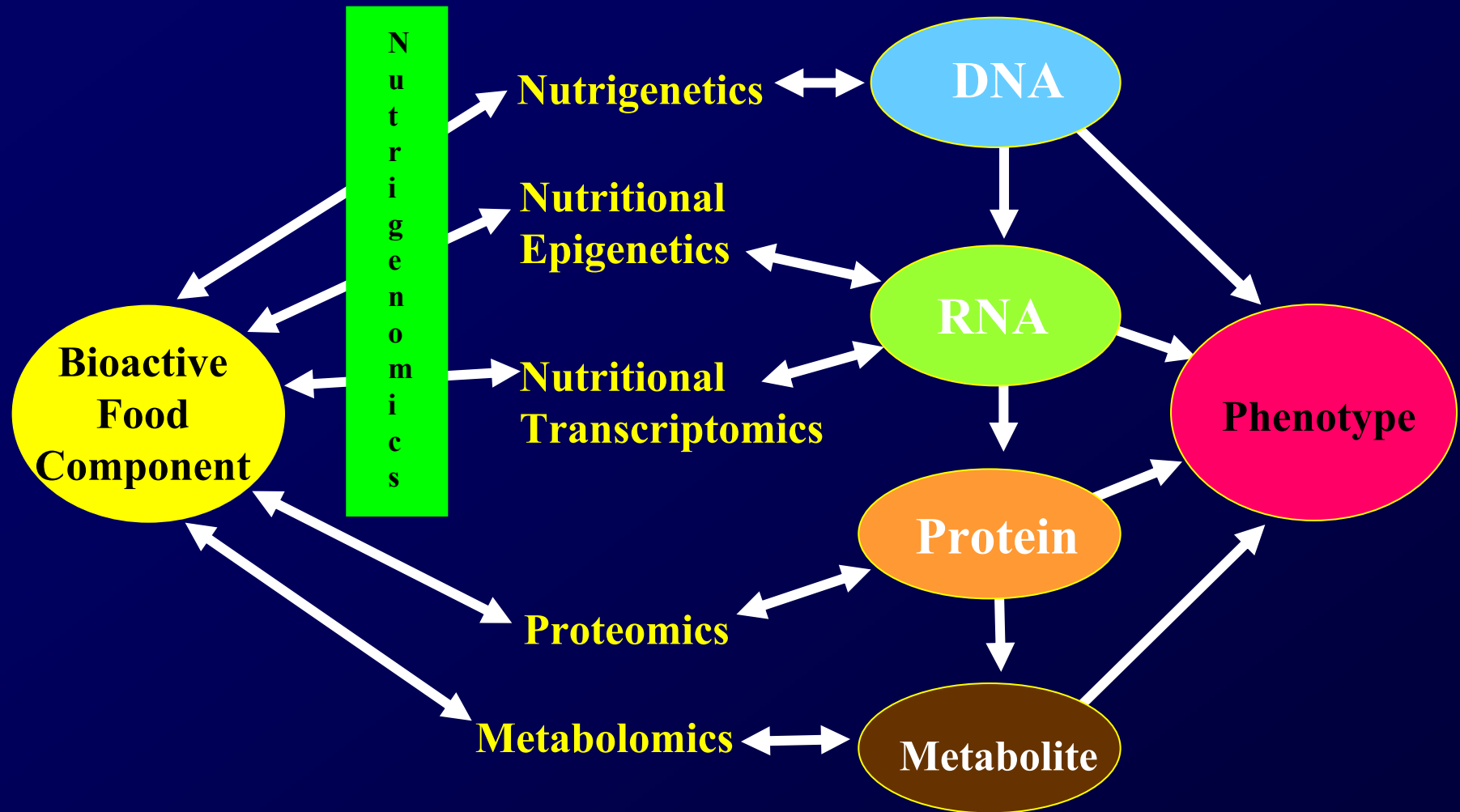
Individual Consumption of All Tomatoes and Tomato Products and Serum Lycopene Levels (EPIC Cross-sectional Study in 3000 subjects)



Corr = 0.23

Jenab et al. J. Nutr. 135:2032, 2005

The “Omics” May Explain Variation in Response



“Nutritional Preemption”

Concept that bioactive food components can be introduced at points of initiation & progression for pathway leading to an unhealthy or lethal phenotype

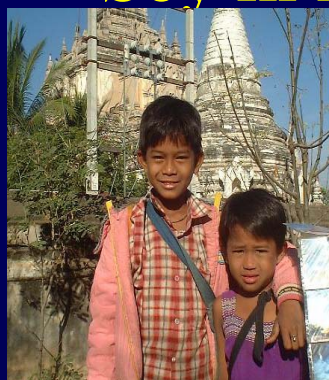
Many Bioactive Food Components May Influence Health



- **Essential Nutrients-** Ca, Zn, Se, Folate, C, E
- **Non-Essential**
 - Phytochemicals-**
Carotenoids, Flavonoids, Indoles, Isothiocyanates, Allyl Sulfur
 - Zoochemicals** - Conjugated linoleic acid, n-3 fatty acids
 - Fungochemicals** - Several compounds in mushrooms
 - Bacteriochemical** - Those formed from food fermentations and those resulting from intestinal flora

Human Genome Establishes Microbial Populations?

Bacterial Formed Equol May Account for Part of the Anticancer Properties from Soy in Asian-Americans



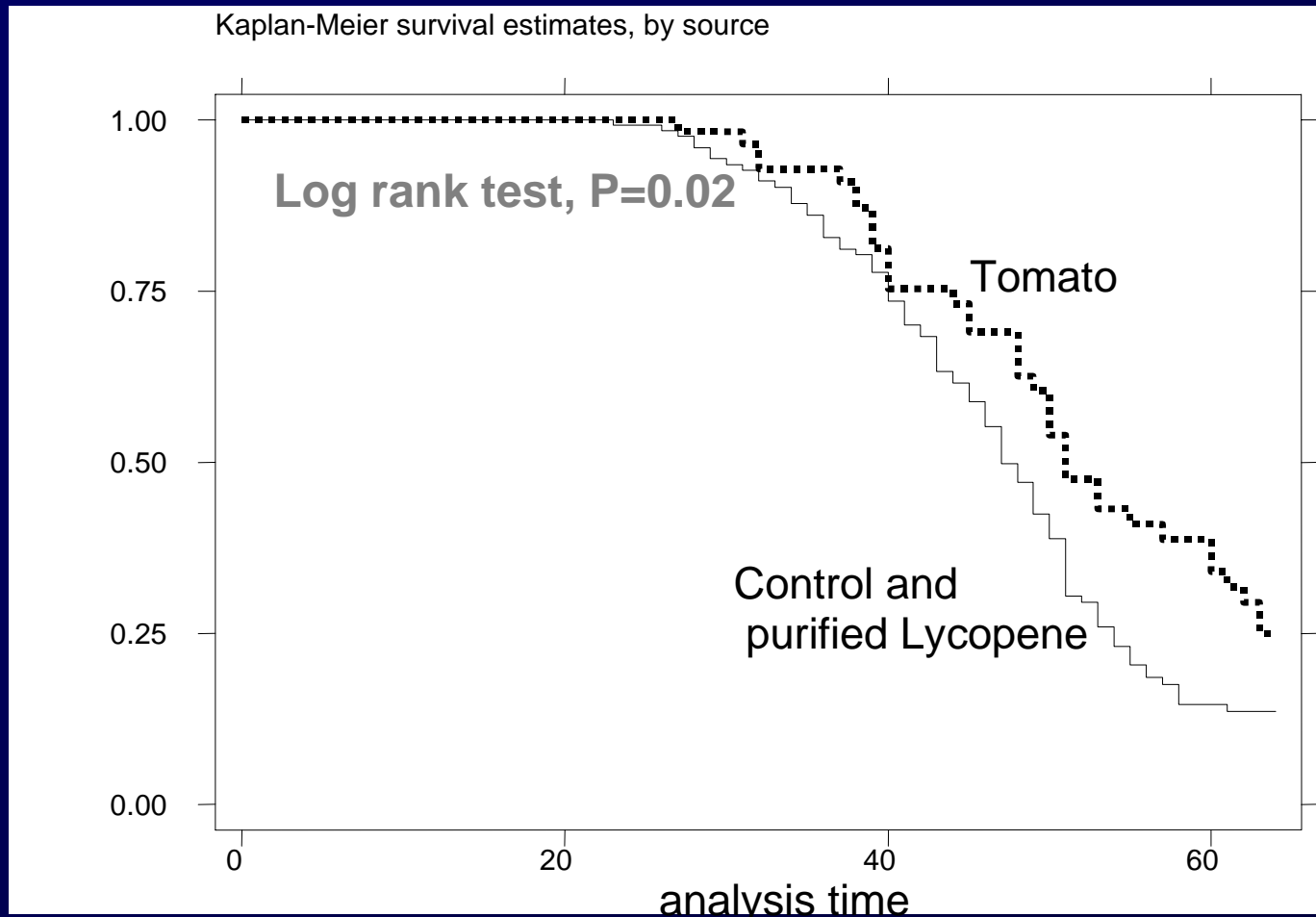
In addition to genistein microorganisms in subpopulations form equol which possesses anticancer properties (**J Nutr. 2006 Apr;136(4):946-52. Arch Microbiol. 2005;183:45–55**). The mechanism by which equol may offer protection remains unresolved but gene expression differences are evident in equol producers

(Niculescu et al (2006) J Nutr. Biochem



Some Individual Foods Offer Advantages Over Their Isolated Constituents

Tomato Powder but not Lycopene Inhibits Prostate Cancer



Boileau et al. JNCI 95:1578-1586, 2003.

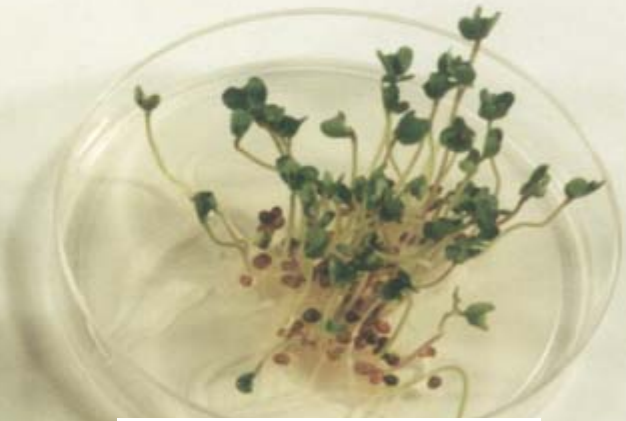
Choices But the Same Biological Response?

**MARKET STAGE
BROCCOLI**



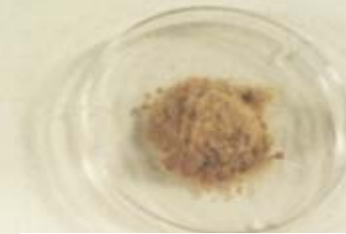
150 grams

3-DAY SPROUTS



3 grams

**FREEZE-DRIED
SPROUT EXTRACT**



150 mg

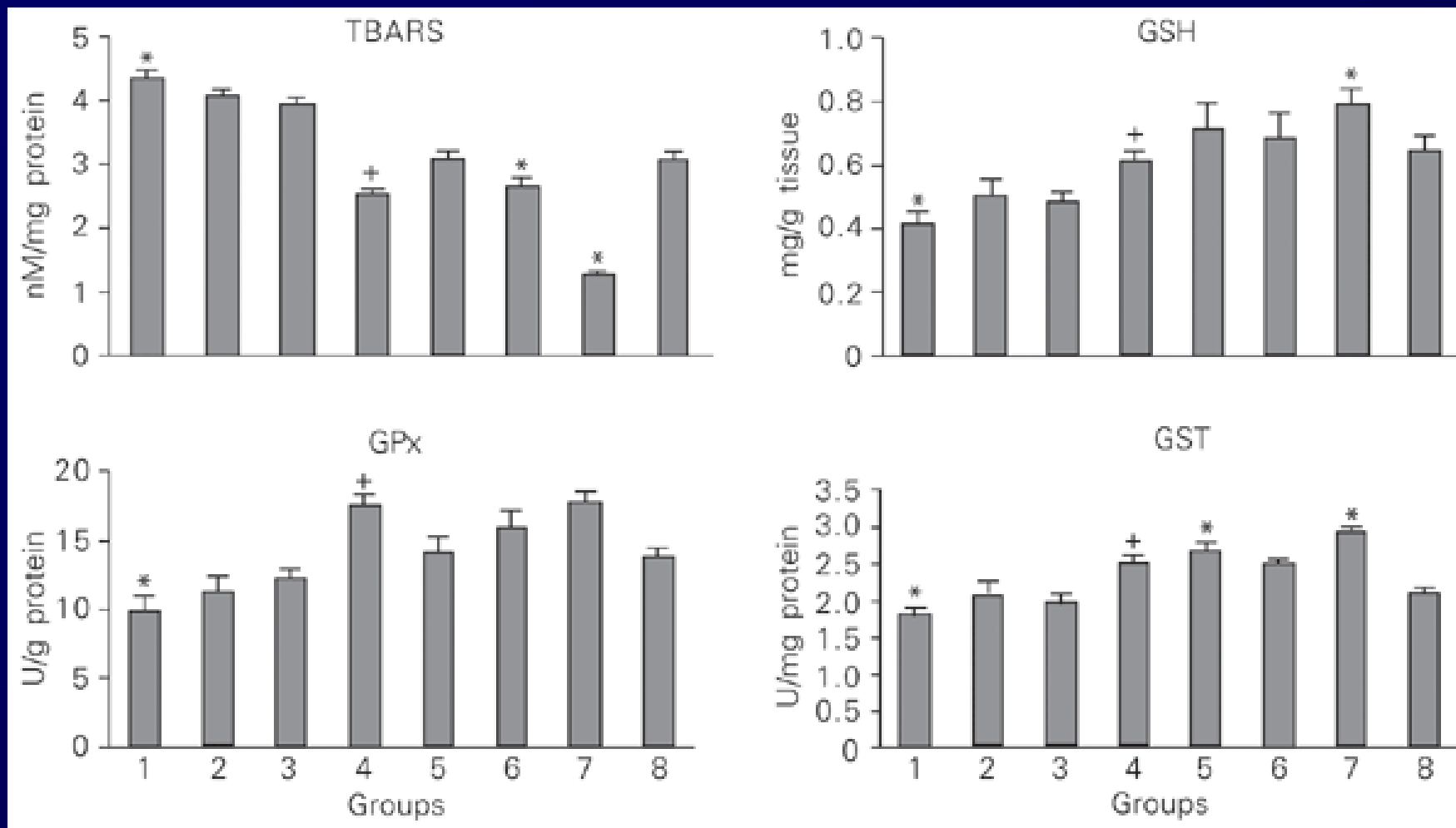
All preparations contain the same quantity
of detoxification enzyme inducer activity

Components are “complex mixtures” - act synergistically



“Caution: This tomato soup combined with our chicken noodle soup can form a lethal nerve gas.”

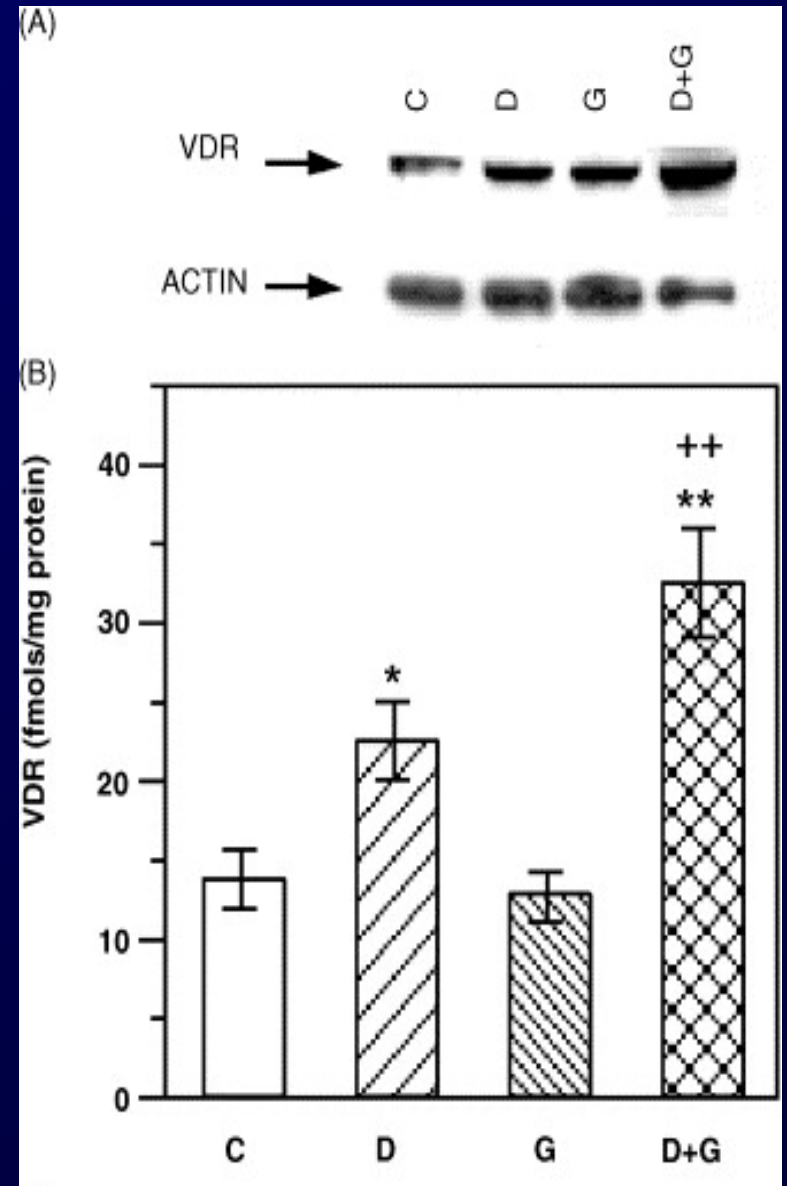
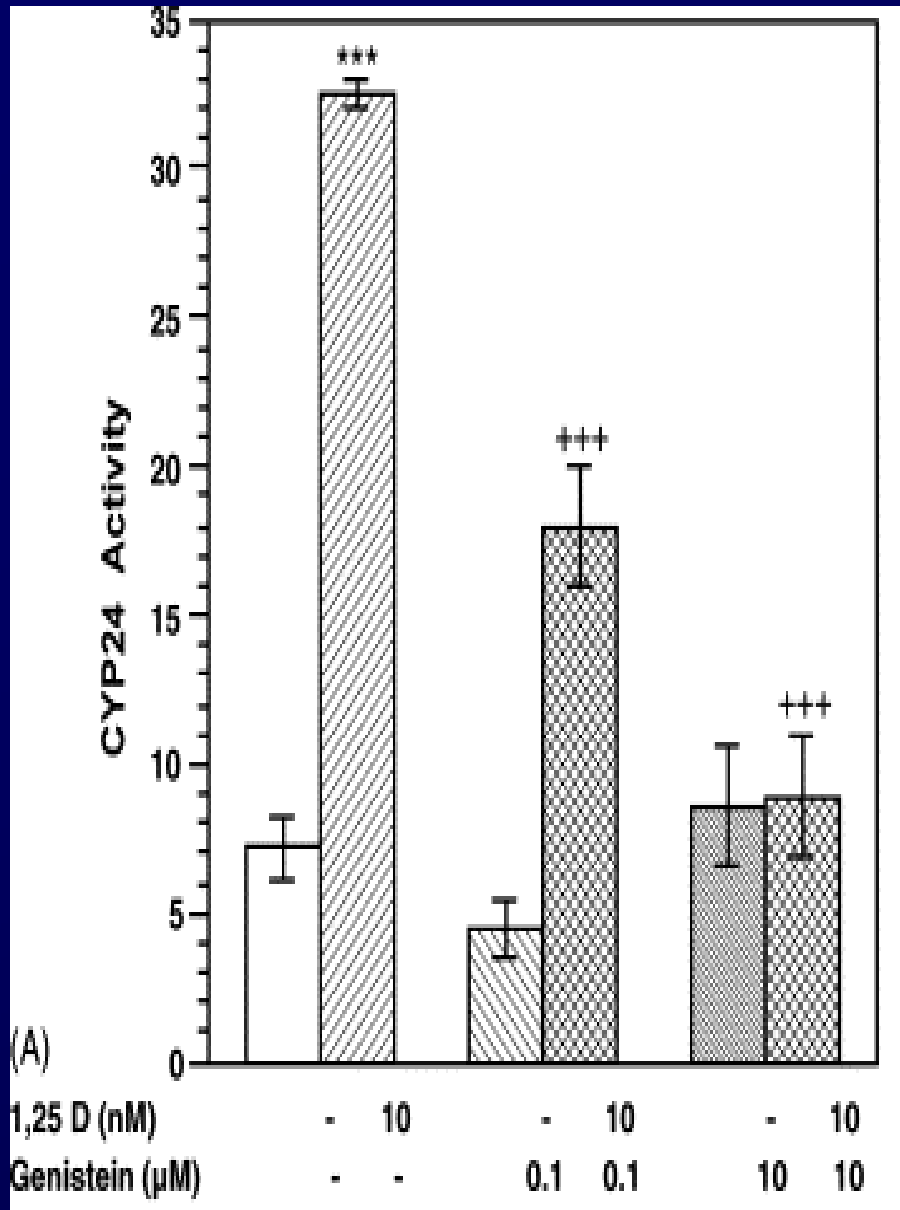
Garlic and Tomato Interactions



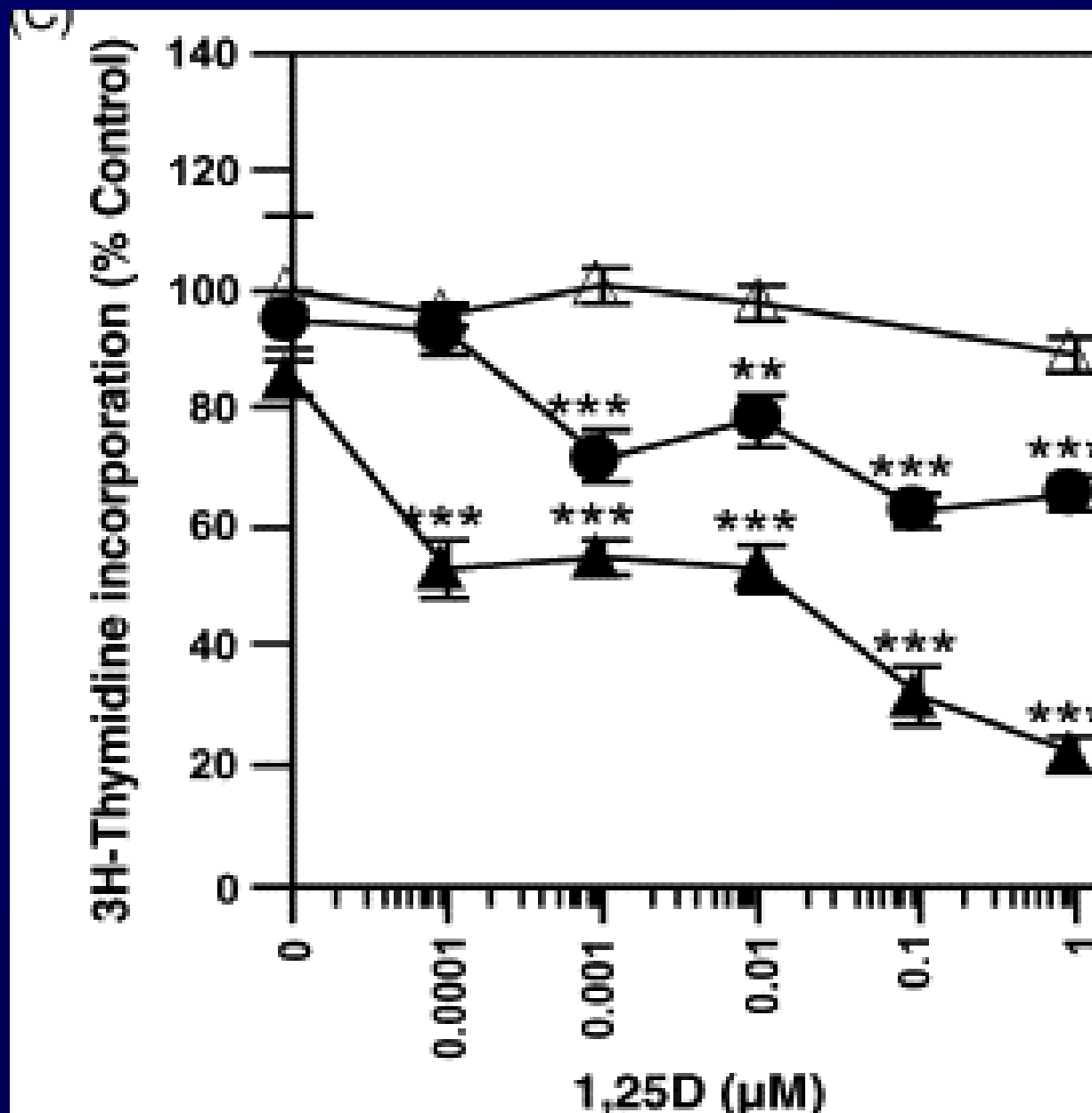
1 = DMBA; 2 = DMBA + tomato; 3 = DMBA + garlic; 4 = DMBA + tomato + garlic; 5 = tomato; 6 = garlic; 7 = tomato + garlic; 8 = control.

Bhuvaneswari et al. Braz J Med Biol Res, (2004) 37:1029 .

Vitamin D and Genistein Interact



Swami et al. (2005) Mol Cell Endocrinol. 241(1-2):49-61.



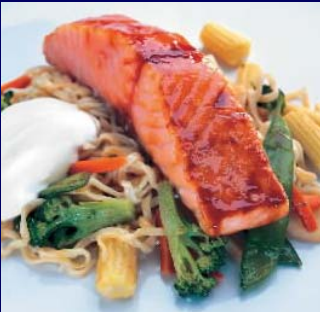
0 Genistein

10 μM Genistein

100 μM Genistein

Swami et al. (2005) Mol Cell Endocrinol. 241(1-2):49-61.

Polymeals May Offer Special Attributes



<u>Dietary Component</u>	<u>% Reduction</u>
--------------------------	--------------------

- | | |
|--|-----|
| ➤ Wine (150 ml/day) | 32% |
| ➤ Fish (114 g 4x/week) | 14% |
| ➤ Dark Chocolate (100 g/day) | 21% |
| ➤ Fruits and Vegetables
(400 g/day) | 21% |
| ➤ Garlic (2.7 g/day) | 25% |
| ➤ Almonds (68 g/day) | 12% |

76% decreased risk of CVD



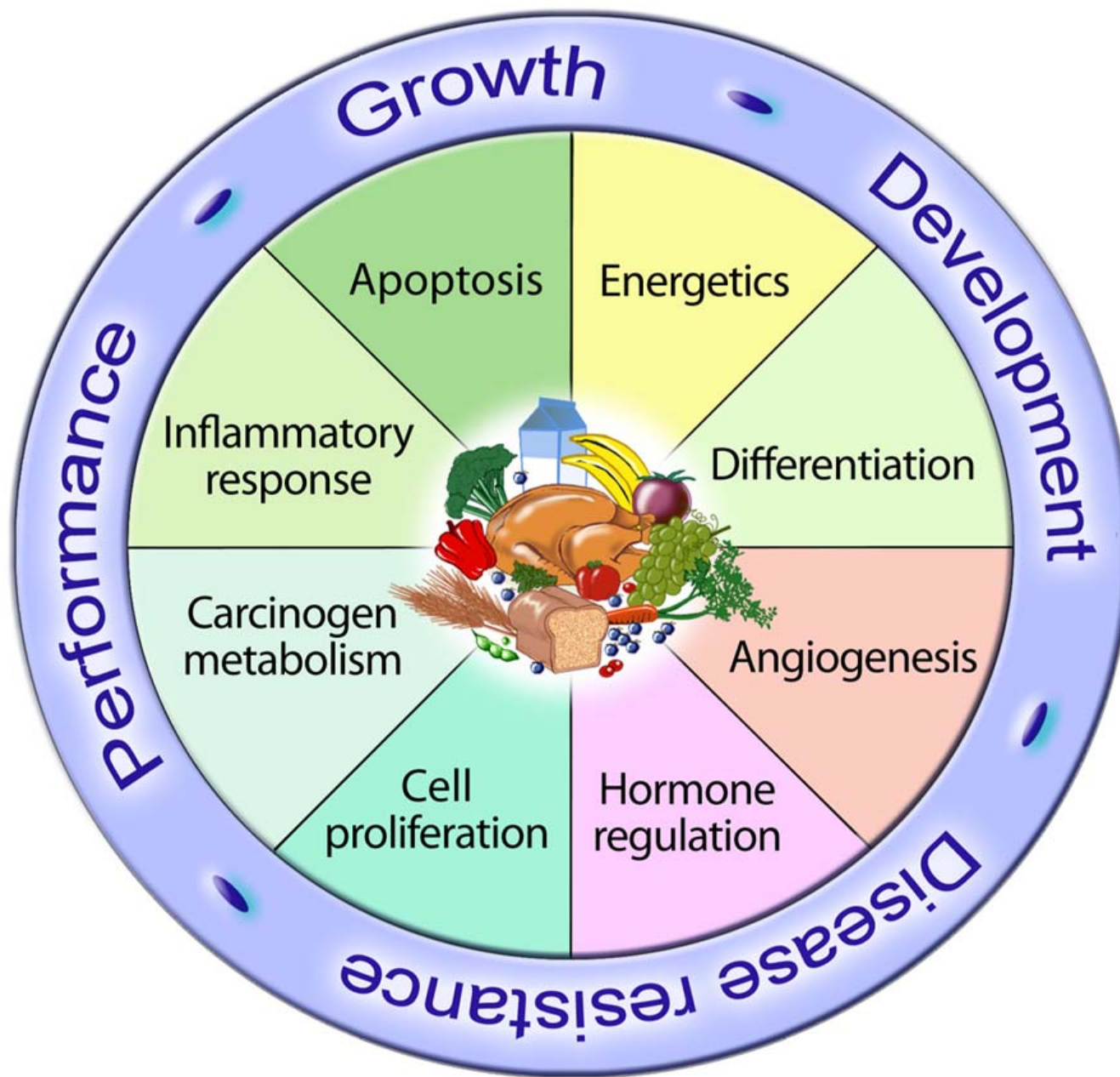
Not All Tissues Equally Responsive

Phase III trial with 1,312 skin cancer subjects

Se (200 µg) as Brewers Yeast vs. Placebo, follow-up 6.4 yrs

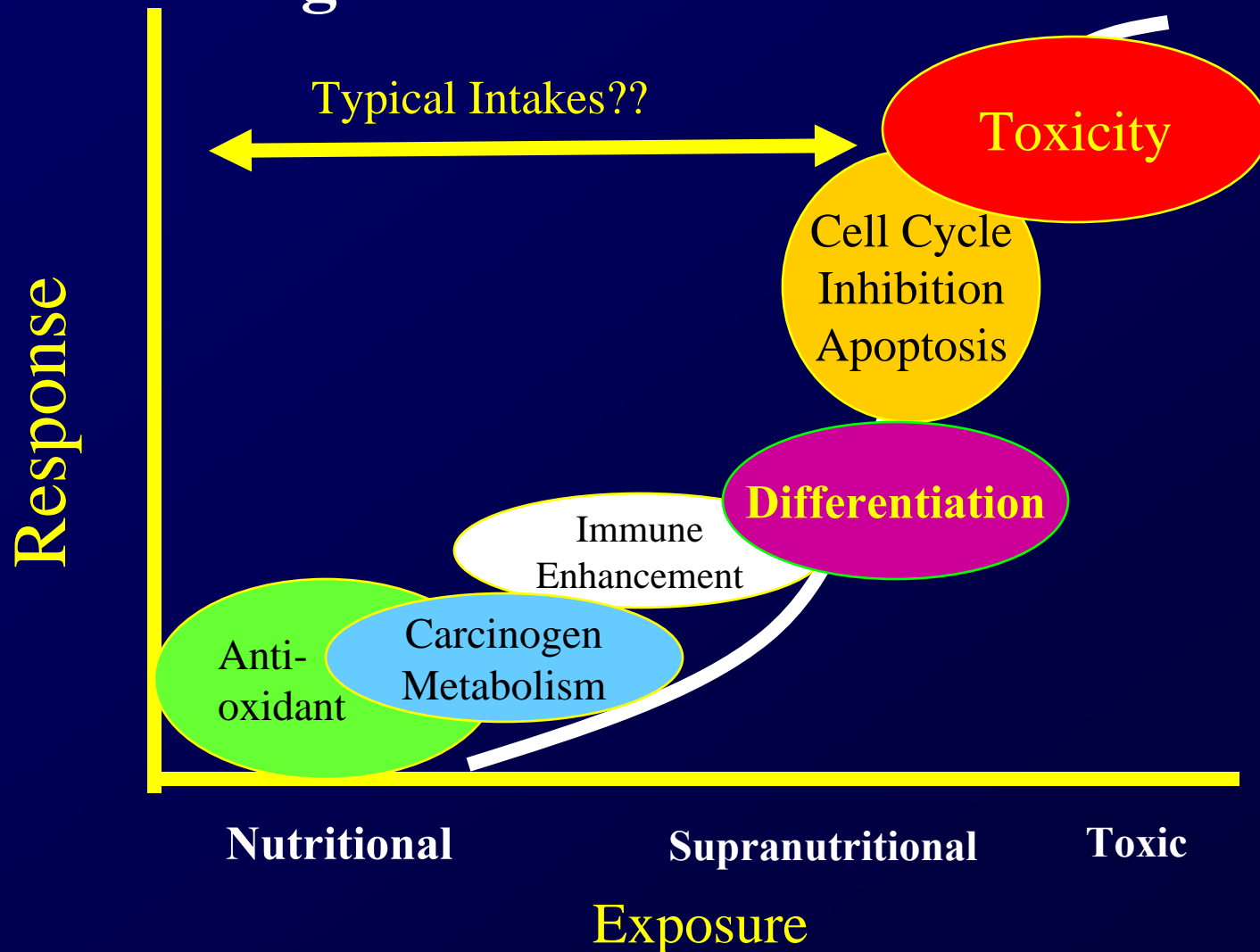
	Selenium	Placebo	RR	97% CI
Skin Cancer				
Squamous cell	218	190	1.14	0.93-1.39
Basal cell	377	350	1.10	0.95-1.28
Cancer (- Skin)				
Lung	17	31	0.54	0.30-0.98
Prostate	13	35	0.37	0.18-0.71
Colorectal	8	19	0.42	0.18-0.95
All Sites (- Skin)	77	119	0.63	0.47-0.85

Clark et al. JAMA 276: 1957, 1996.



**Response
Depends on
Intended
Use and
Biological
Process
Being
Modified**

Another Questions Must Be “What is the Amount Needed to Bring About a Desired Outcome?”



Modified from Combs and Gray, Parmacol. Ther. 79: 179-192, 1998.

Breast Cancer Prevention

Phase 1 Study of Indole-3-Carbinol

Eligible Women

N=17

Indole-3-Carbinol

200 mg BID x 2 wks

Indole-3-Carbinol

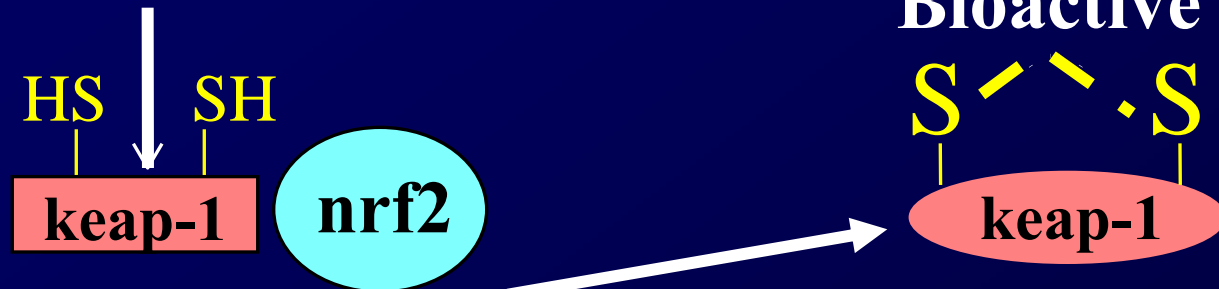
400 mg BID x 2 wks

Results: **4x ↑ in CYP1A2 @ 4 wks (16/17) with 800mg**
50% ↑ in urinary 2-OH-estrone/16 α -OH-estrone ratio
with 400 mg, adding more did not change

Reed et al., Cancer Epidemiol Biomarkers Prev. 2005 14:1953-60.

Do Combinations Reduce The Quantity Needed For A Response?

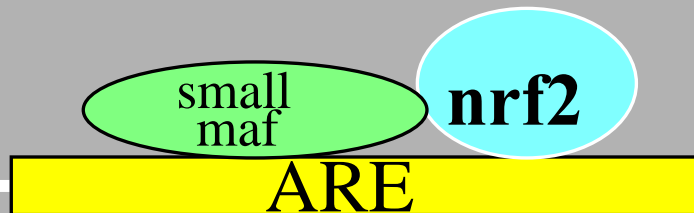
Organosulfur compounds (garlic)
Selenium (fish)
Isothiocyanates (broccoli)



Cytoplasm



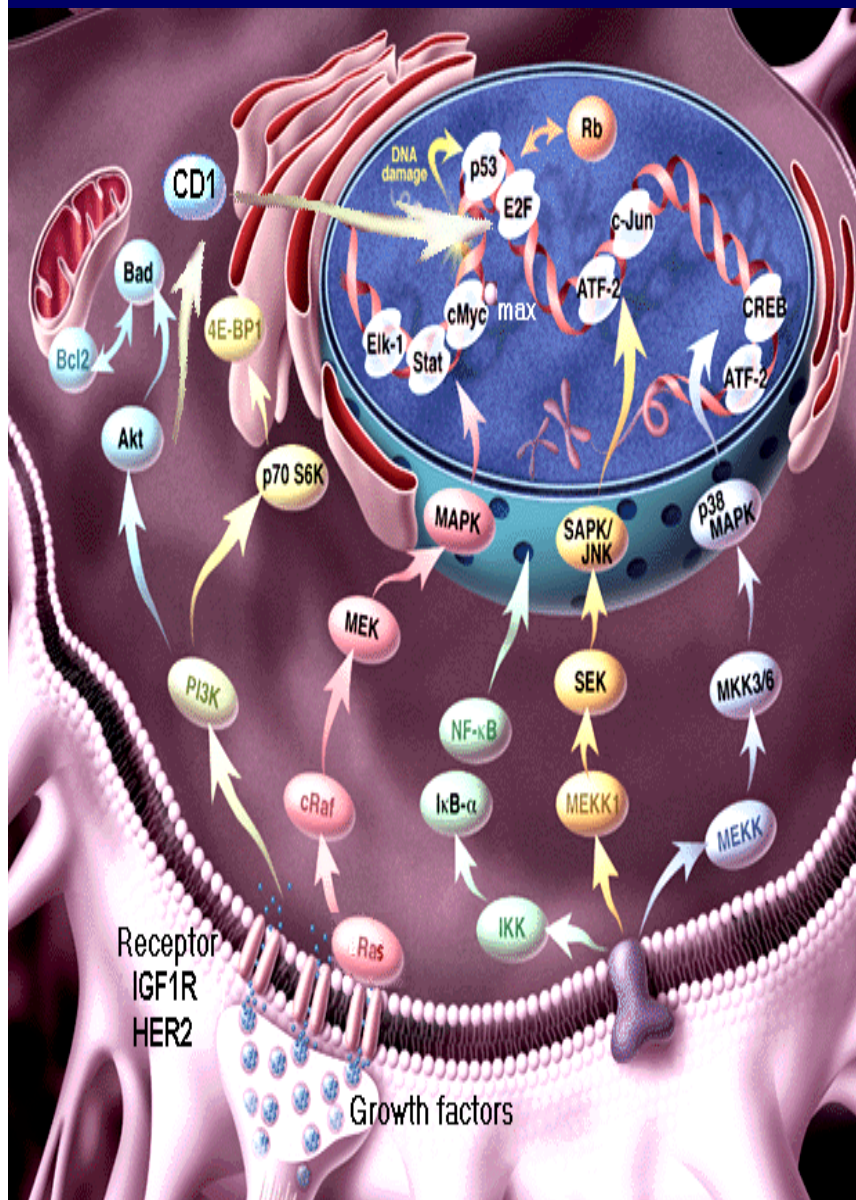
Nucleus



“antioxidant responsive element”

Increased GST, QR

Drugs-Foods May Have Same Target



Recent News Release suggests:

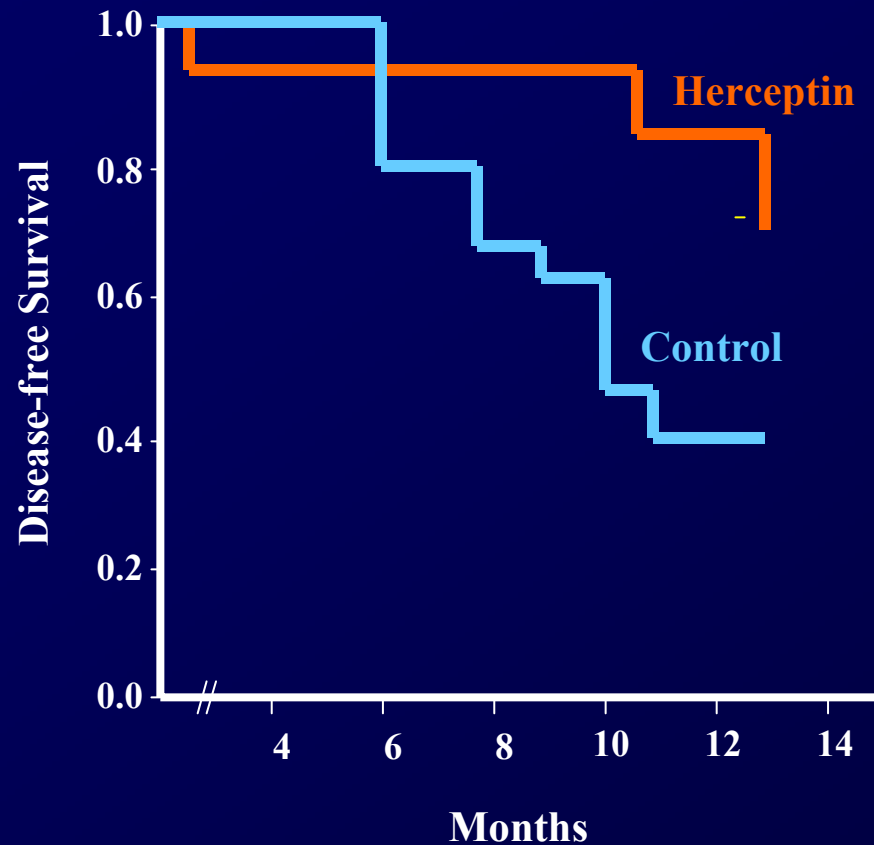
Herceptin is a Novel Pioneering Drug for Personalized Medicine Approach Based on Pharmacogenomics to block Her2-neu expression.

Evidence Has Existed for Some Time:

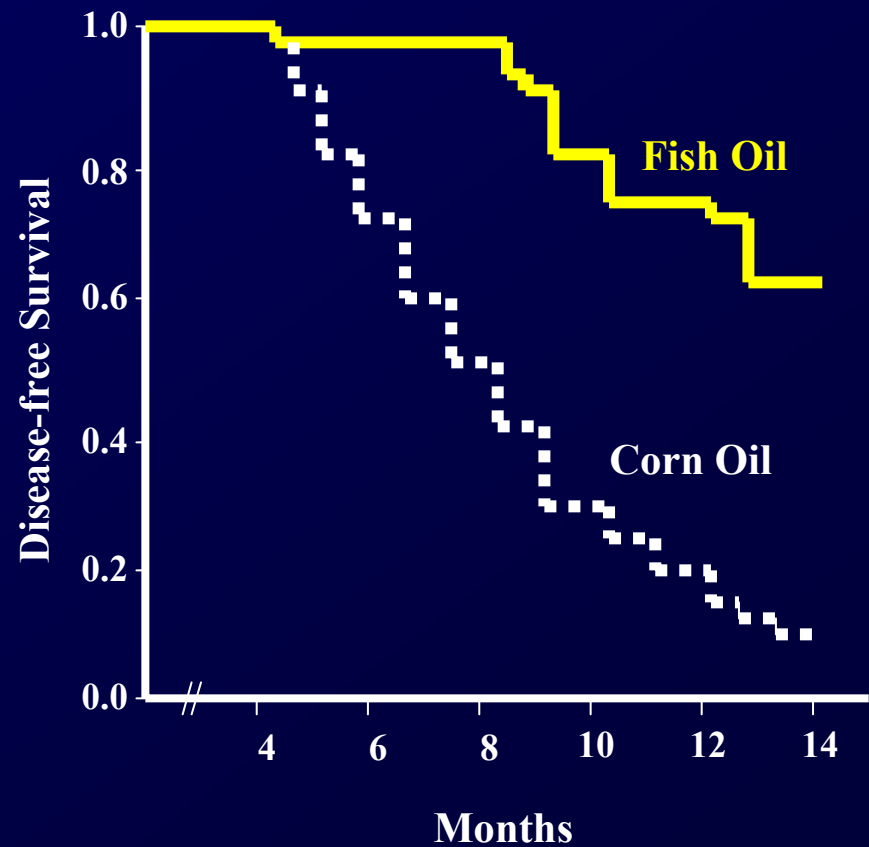
EGCG from Green Tea, Oleic Acid from Olive Oil, and n-3 fatty acids from Fish Oil and Apigenin from parsley, thyme, and peppermint since they also can significantly influence HER2neu expression!

So why are we not being more proactive in showcasing dietary effects?

Herceptin and Dietary fish oil increased the latency time to mammary gland tumor development in the HER-2 transgenic mice

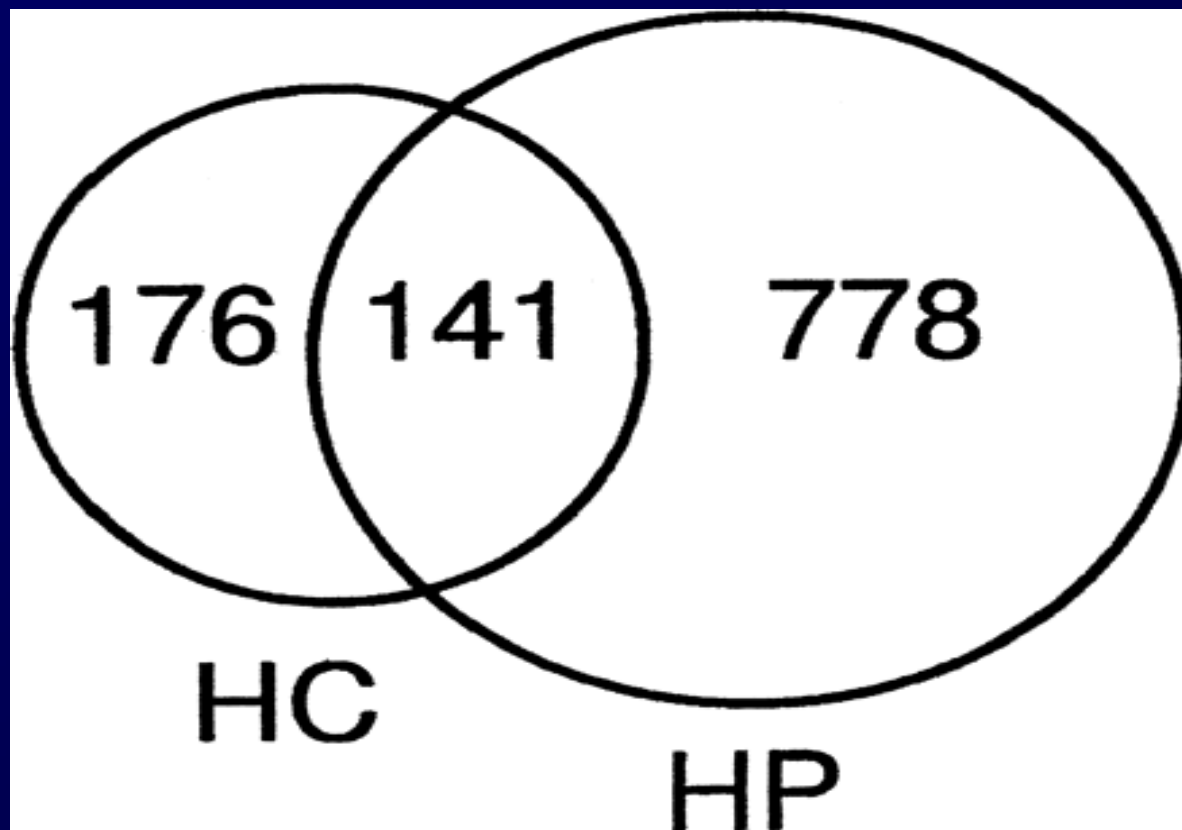


Finkle D et al Clin Cancer Res 10: 2499-511, 2004



Yee LD et al J Nutr 135: 983-8, 2005

Challenge to Foods May Help Identify Those Who Benefit Most to Specific Foods/Components



van Erk et al. (2006) Am J Clin Nutr;84:1233-41

Food/Food Components
(n-3/Butyrate/ Herbs/Spices,)

Inflammatory Stimuli
(chemicals, ROS, bacteria,
viruses)

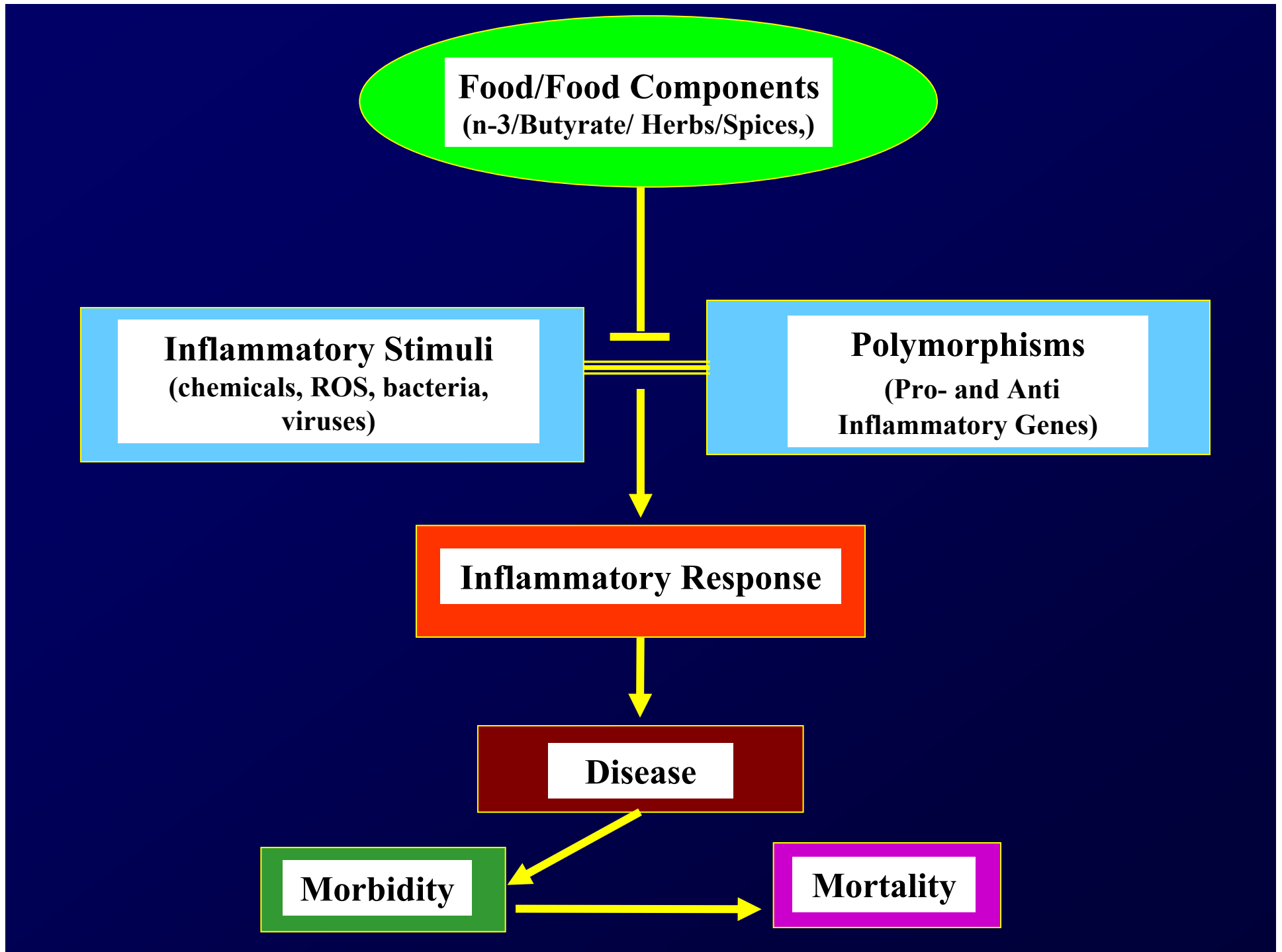
Polymorphisms
(Pro- and Anti
Inflammatory Genes)

Inflammatory Response

Disease

Morbidity

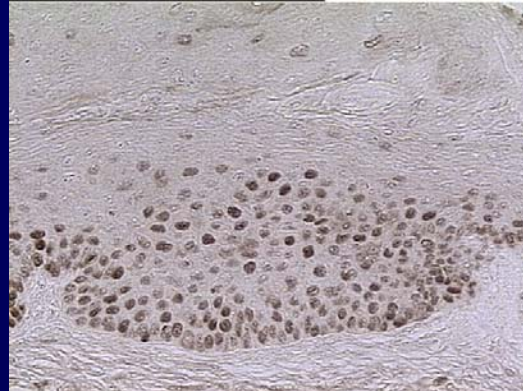
Mortality



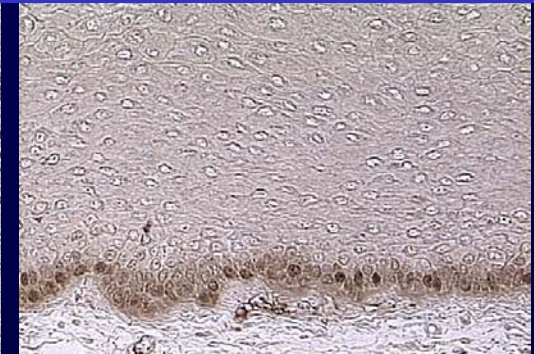
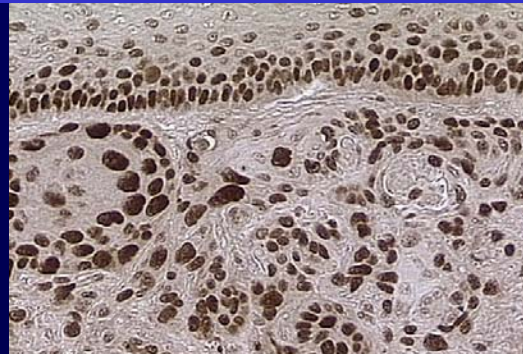
K14-HPV16
Transgenic
Mouse

Background
Mouse

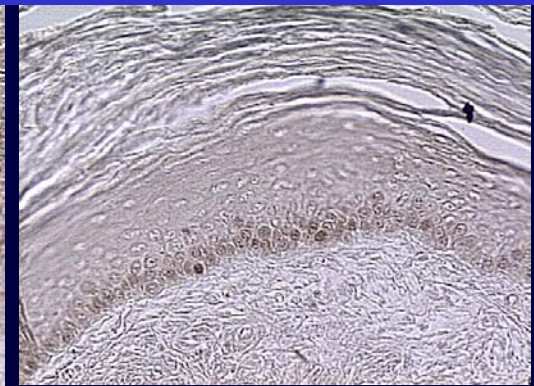
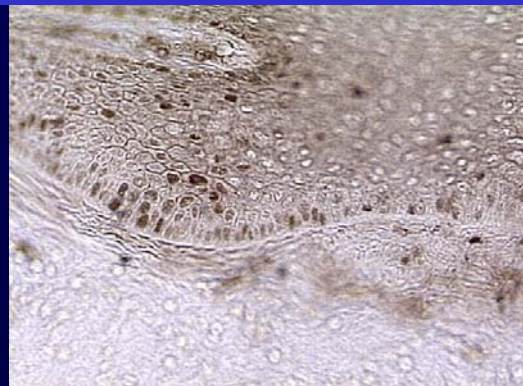
No Estrogen
Normal Diet



Estrogen
Normal Diet



Estrogen
Diet + I3C



Proliferation Assay: PCNA by
immunohistochemistry

Auborn et al, Personal Communication

**Elevated thiobarbituric acid reactive substances,
conjugated dienes and lowered activities of
superoxide dismutase, catalase, glutathione
peroxidase, glutathione-S-transferase and reduced
glutathione in the liver, heart, kidney, intestine and
aorta observed**

in rats fed the high fat diet

**was minimized or eliminated by supplementation
with**

**black pepper or the active principle of black pepper,
piperine.**

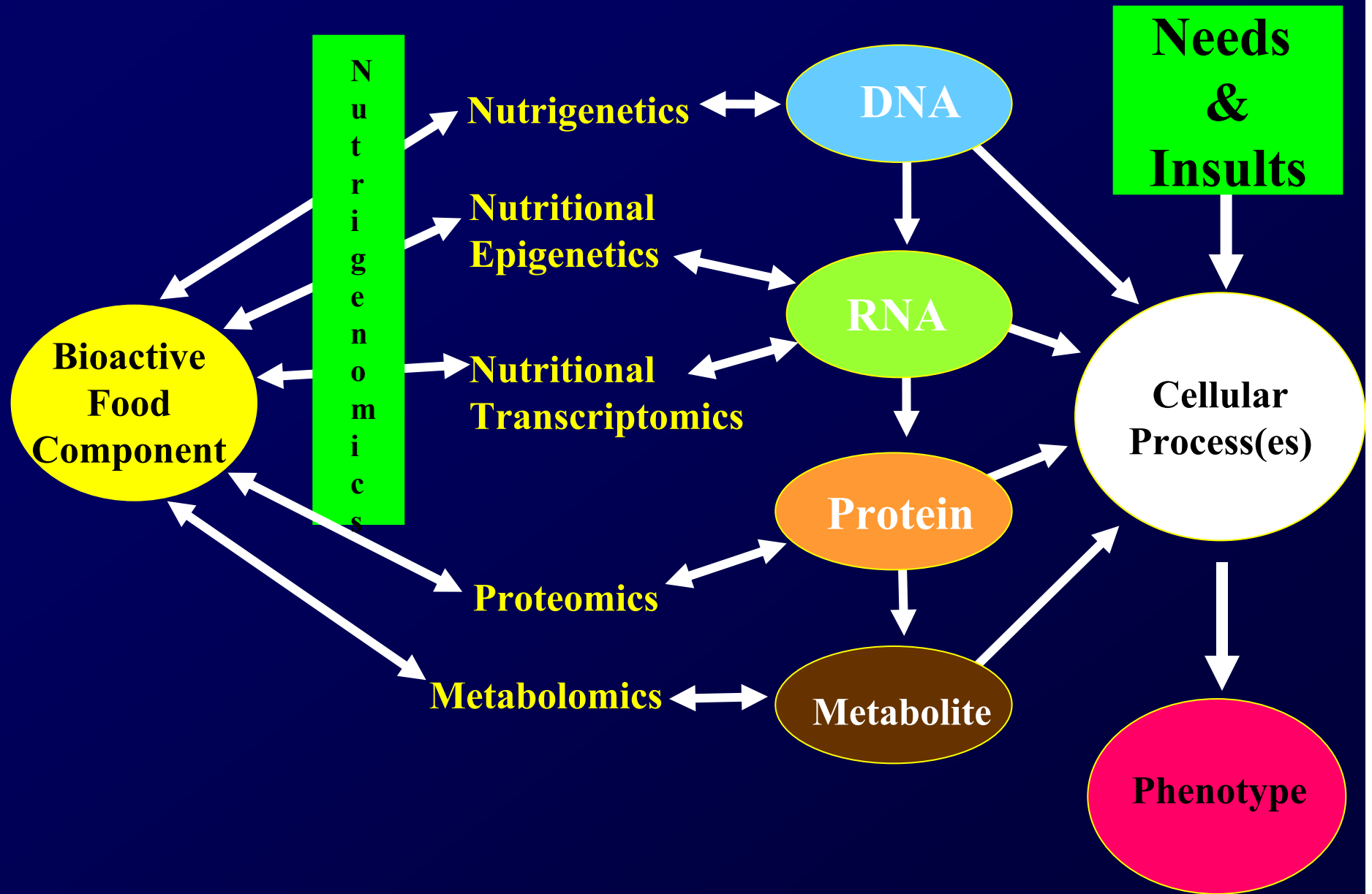
Vijayakumar et al. (2004) Redox Rep.9(2):105-10.

Tea and Human Oral Precancerous Lesions

- A double-blind intervention trial of 59 patients with oral mucosa leukoplakia
- Twenty-nine patients received tea administered orally and topically; 30 patients received placebo treatment
- After 6 months, the size of oral lesion decreased in 38% of the treated group and in 10% of the placebo group; the lesion increased in 3.4% of the treated group and in 6.7% of the placebo group
- The incidence of micronucleated exfoliated oral mucosa cells in the treated group (0.54%) was lower than the control group (1.13%)

Li et al. Proc. Soc. Expr. Biol. Med. 220: 218-224, 1999.

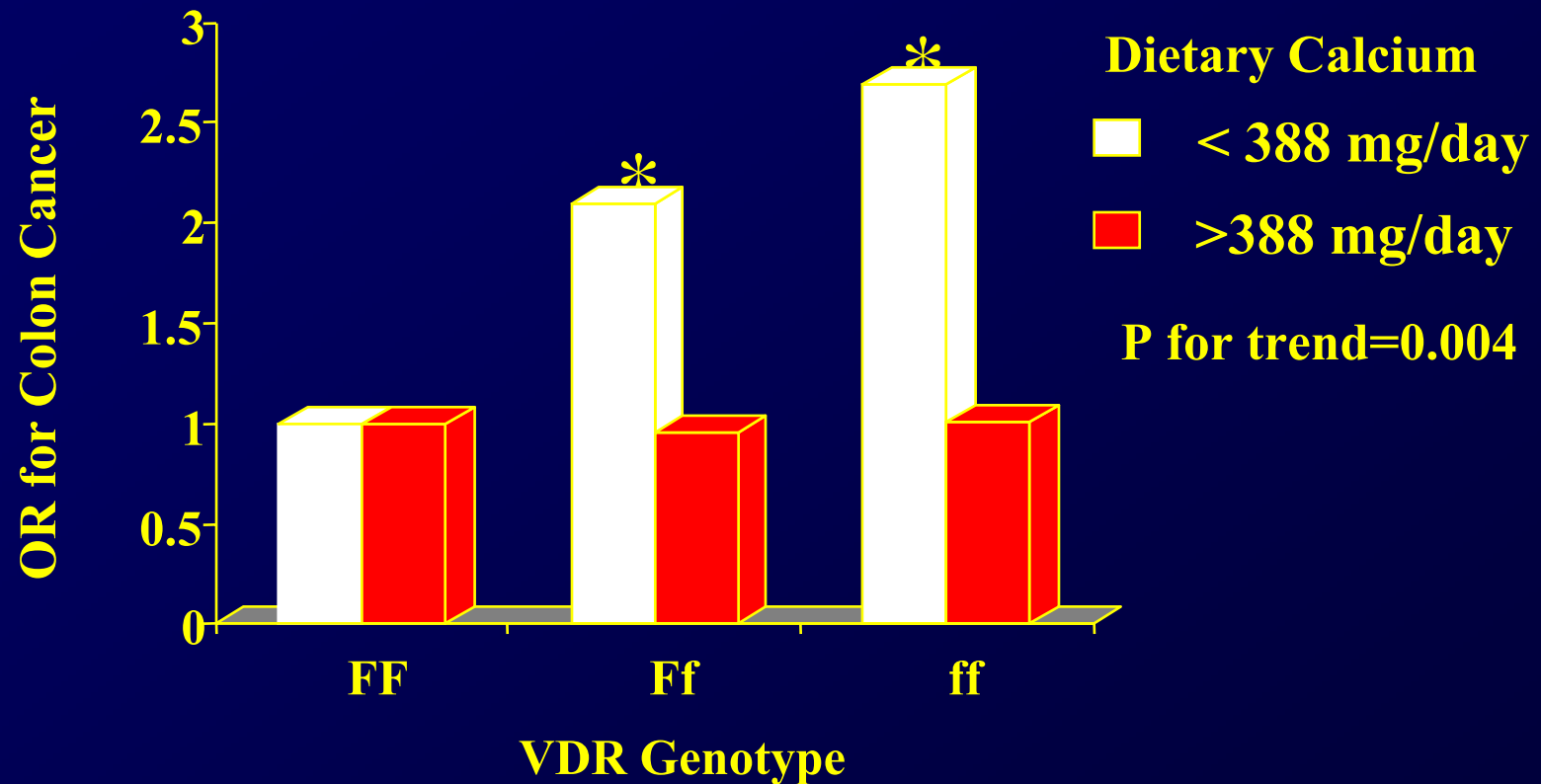
Green tea's anti-cancer effects 'highly unlikely', says FDA





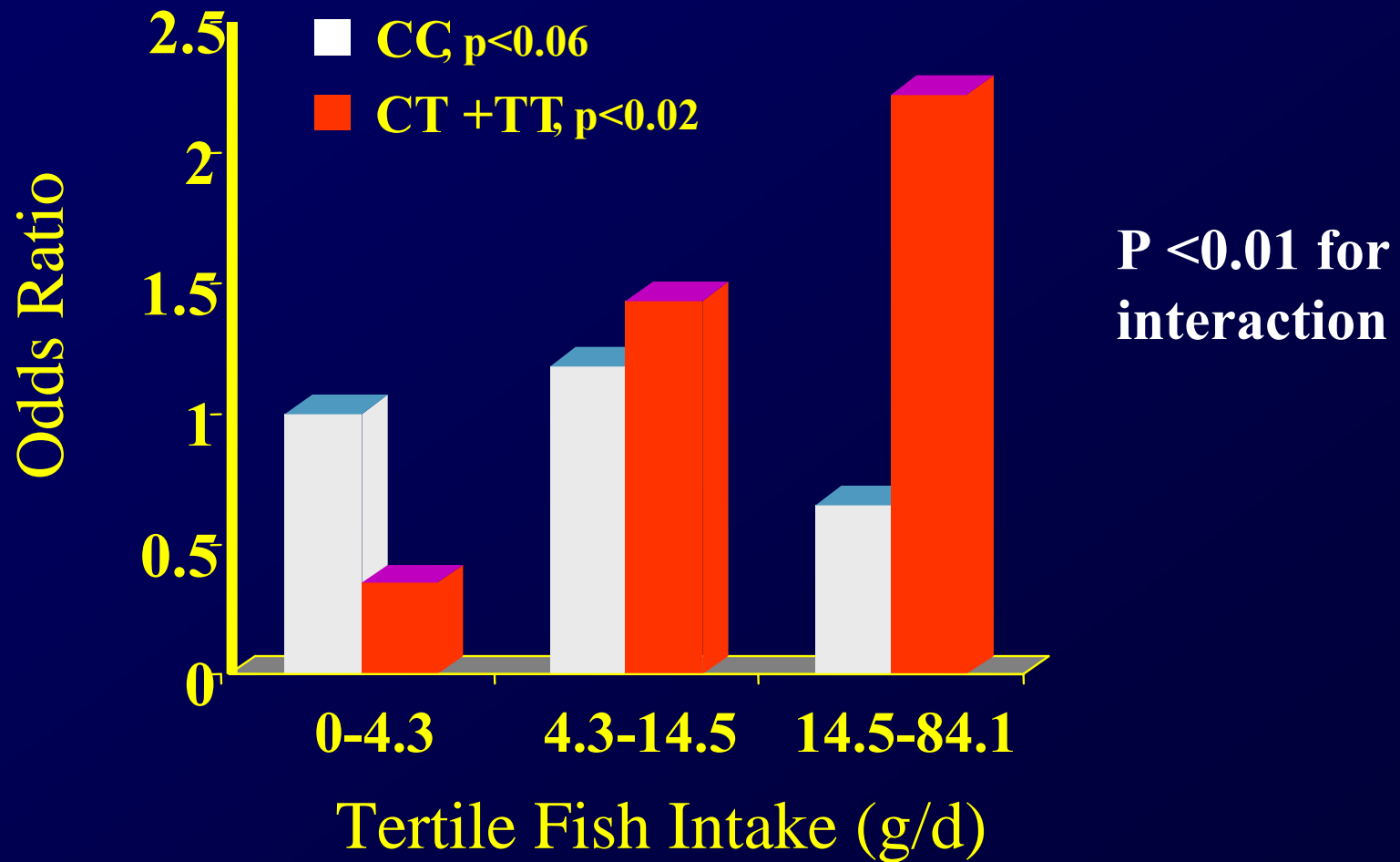
**One Size Does Not Fit All! Omic Information Will Help Identify
Responders to Foods and Components**

Genetic Information May Assist in Identifying Those Who Must Assure Adequate Intakes

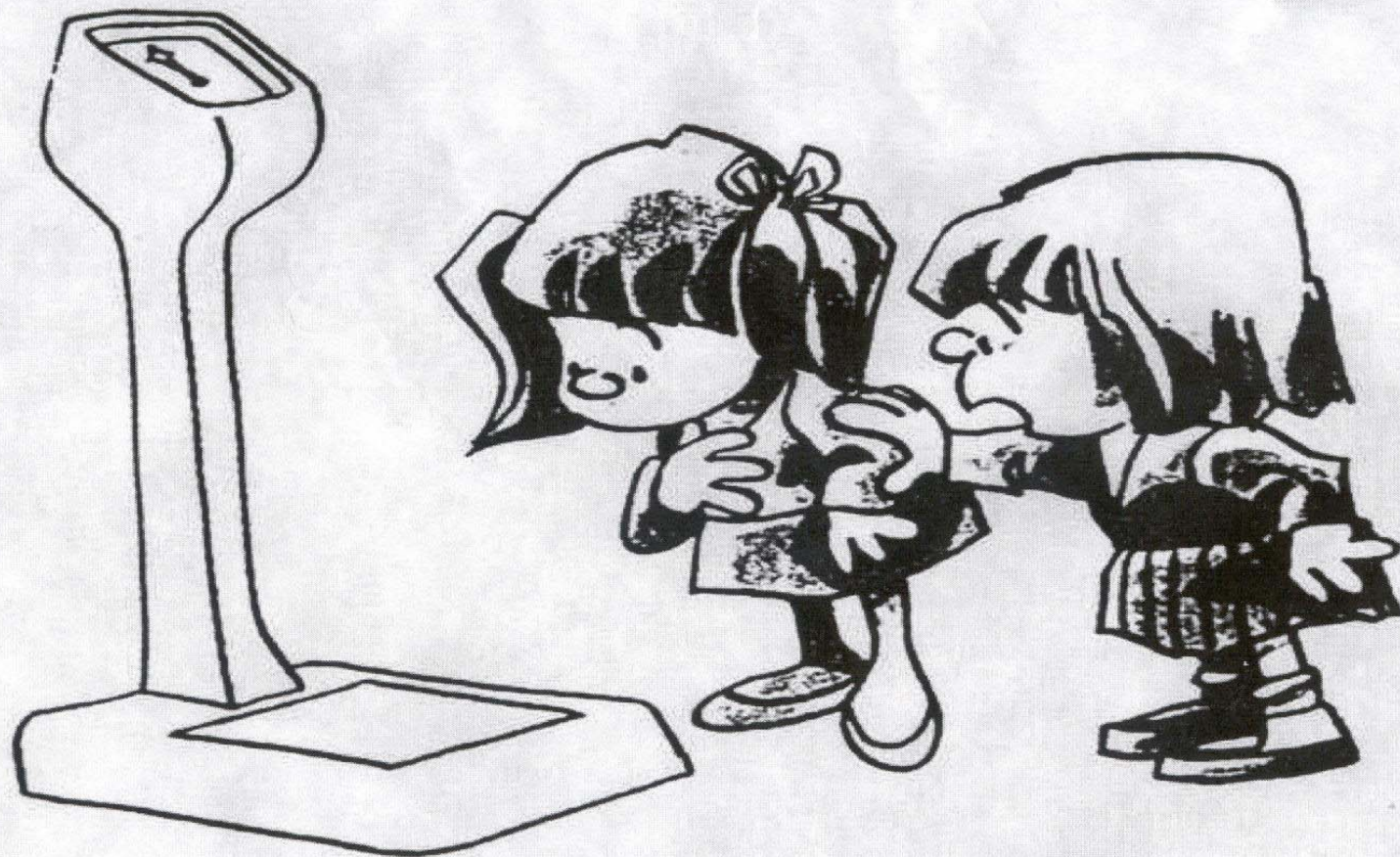


Wong et al. Carcinogenesis, 24: 1091-1095, 2003

PPAR γ Genotype, Fish Consumption and Colon Cancer (384 cases and 403 polyp-free controls 789C-->T)



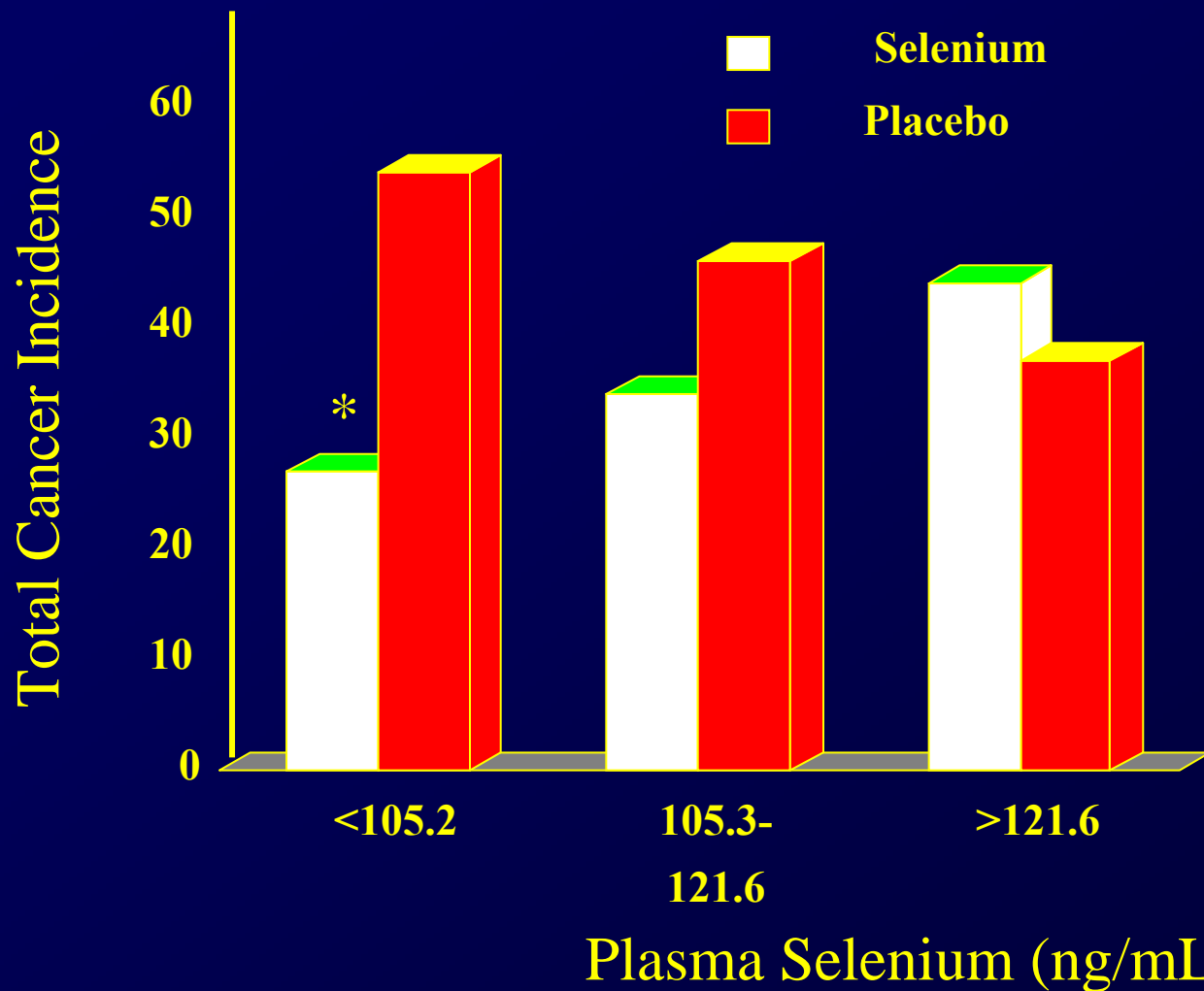
Siezen et al., Carcinogenesis 26:229-457, 2005



"Don't step on it... it makes you cry."

**More is Not Always Better. Energy Intakes and Expenditures Are
Linked to Health! Possible Harm Not Limited to Calories!**

Total Cancer Incidence By Treatment Group and Baseline Plasma Selenium



Duffield-Lillico et al., (2002) Cancer, Epidem. Biomarkers & Prev., 11: 630

More Folate May Not Be Beneficial to Some

Our results do not support the hypothesis that high folate intake reduces breast cancer risk; instead, they suggest that a high intake, generally attributable to supplemental folic acid, may increase the risk in postmenopausal women.

Stolzenberg-Solomon (2006) Am J Clin Nutr. 83:895-904.

When to Is the Best Time
to
Intervene
and
For How Long???

LTR Hypomethylated



Yellow Mouse

High risk cancer, diabetes, obesity & reduced lifespan

When to Intervene??

**Maternal Supplements
with
zinc, methionine
betaine, choline,
folate, B₁₂**



**Or
Genistein**

LTR Hypermethylated



Agouti Mouse

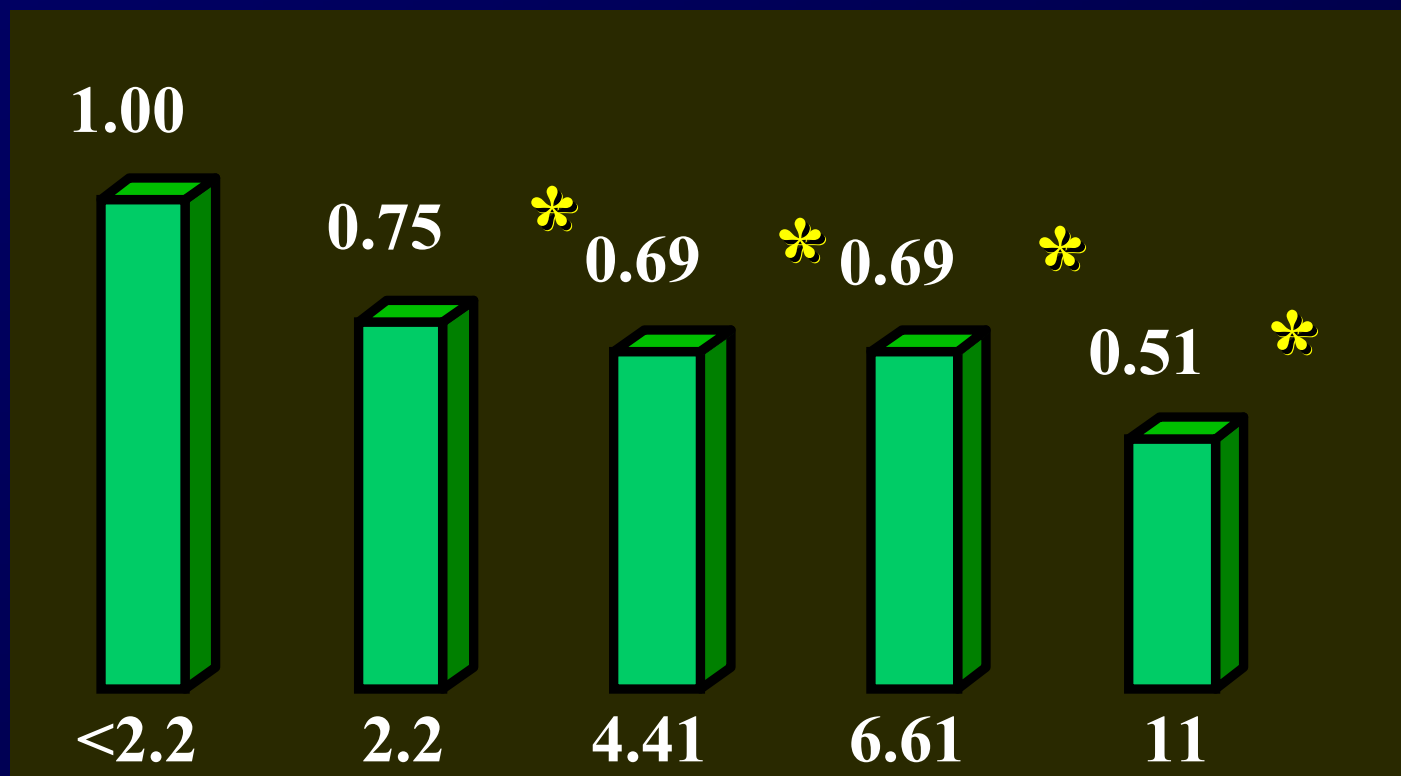
Lower risk of cancer, diabetes, obesity and prolonged life

Cooney et al. J Nutr 132:2393S (2002); Dolinoy et al. Envir. Health Perspect 114: 567 (2006)

Teenage (13-15 y) Soy Intake & Adult Breast Cancer Risk

(Shanghai: 1459 cases, 1556 cont)

Adjusted
Odds ratio



Soy protein intake (g/d)

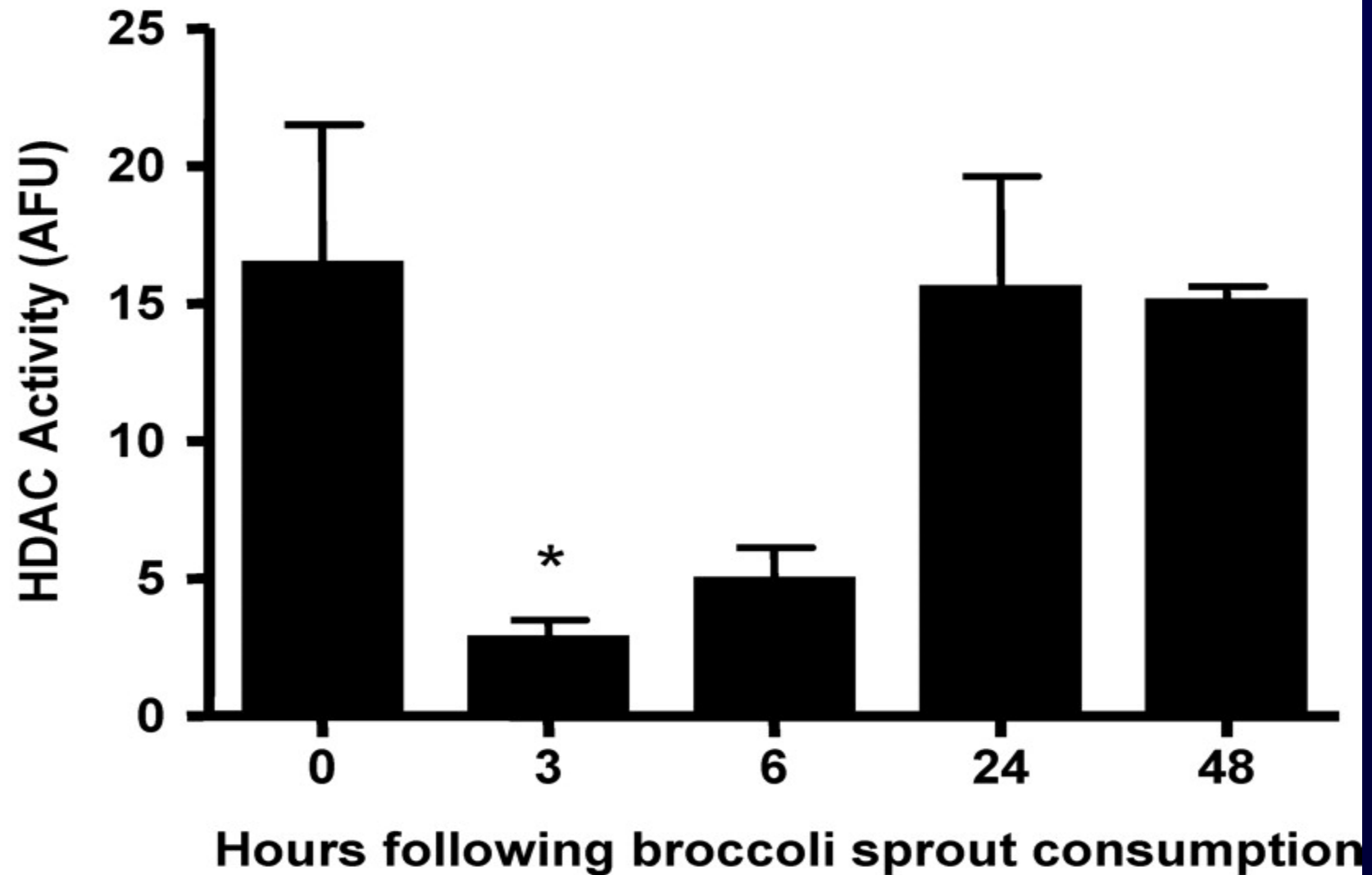
CEBP 10:
481, 2001

Results: pre/post, x age 47.

Other legumes not protective.

Shu et al., Cancer Epid Biomarkers Prev 10:483-488, 2001.

Effect of 68 g of BroccoSprouts on Histone Deacetylase Activity in Humans

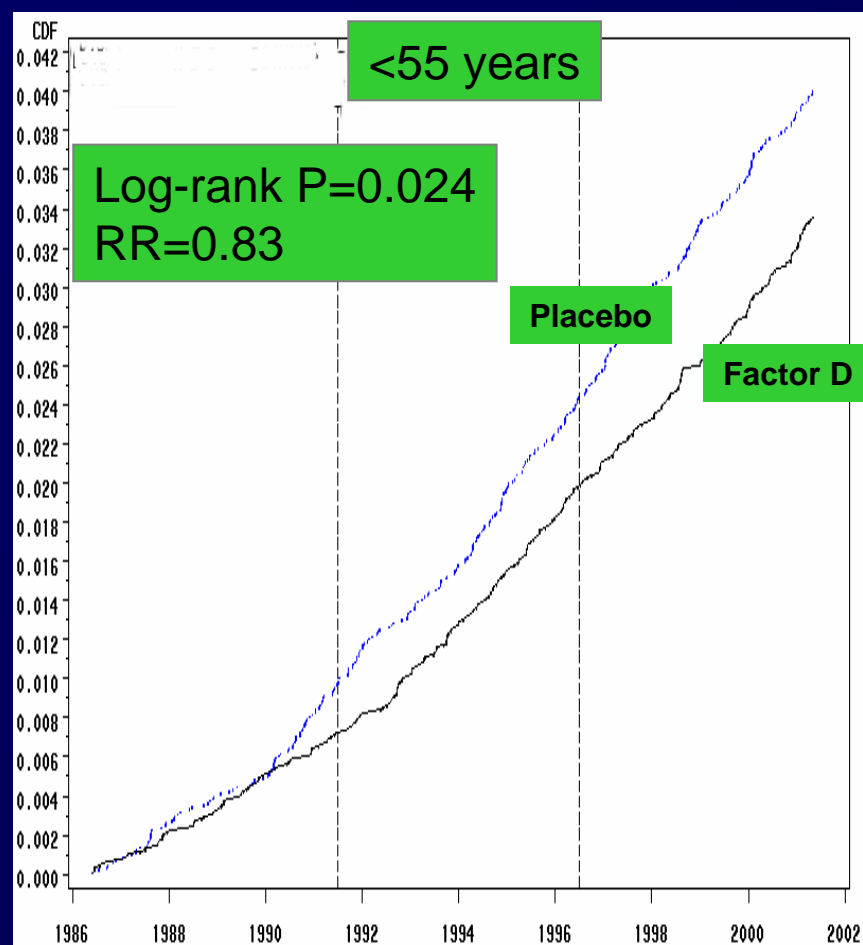


Myzak et al. (2007) Exp Biol Med 232:227-34.

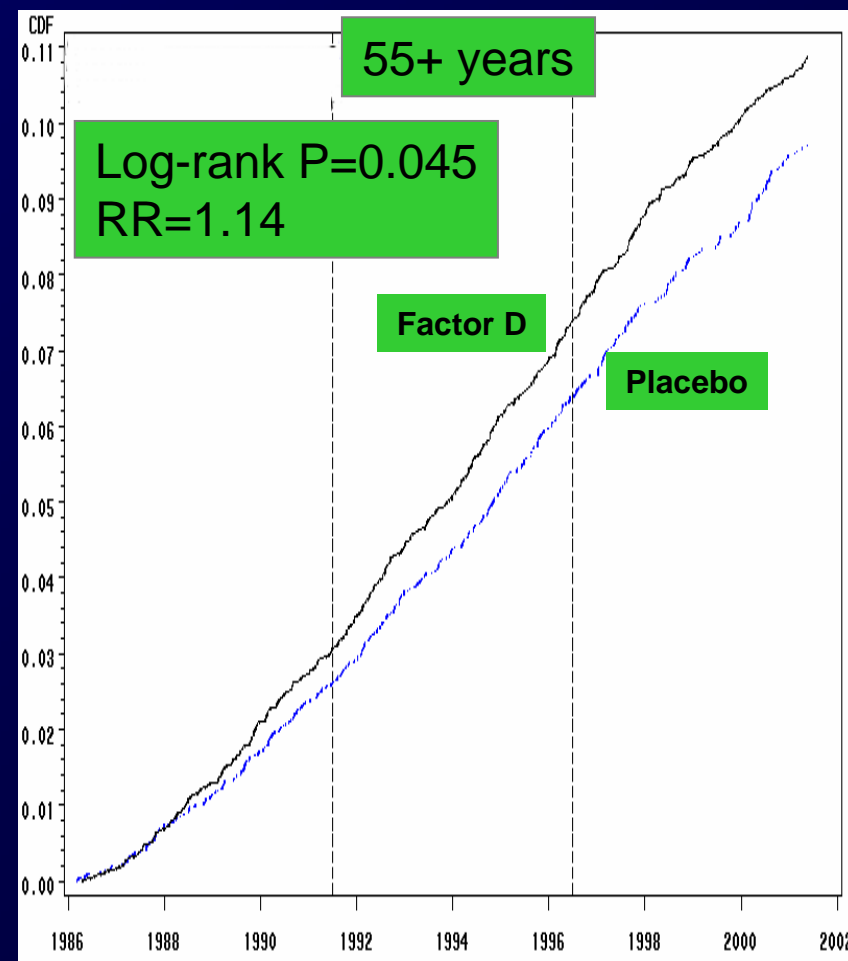
Linxian Nutrition Intervention Trial

Esophageal cancer mortality by factor D (N=1515)

Factor D= Selenium, β -carotene, vitamin E



Esophageal Cancer Death Time (Year)

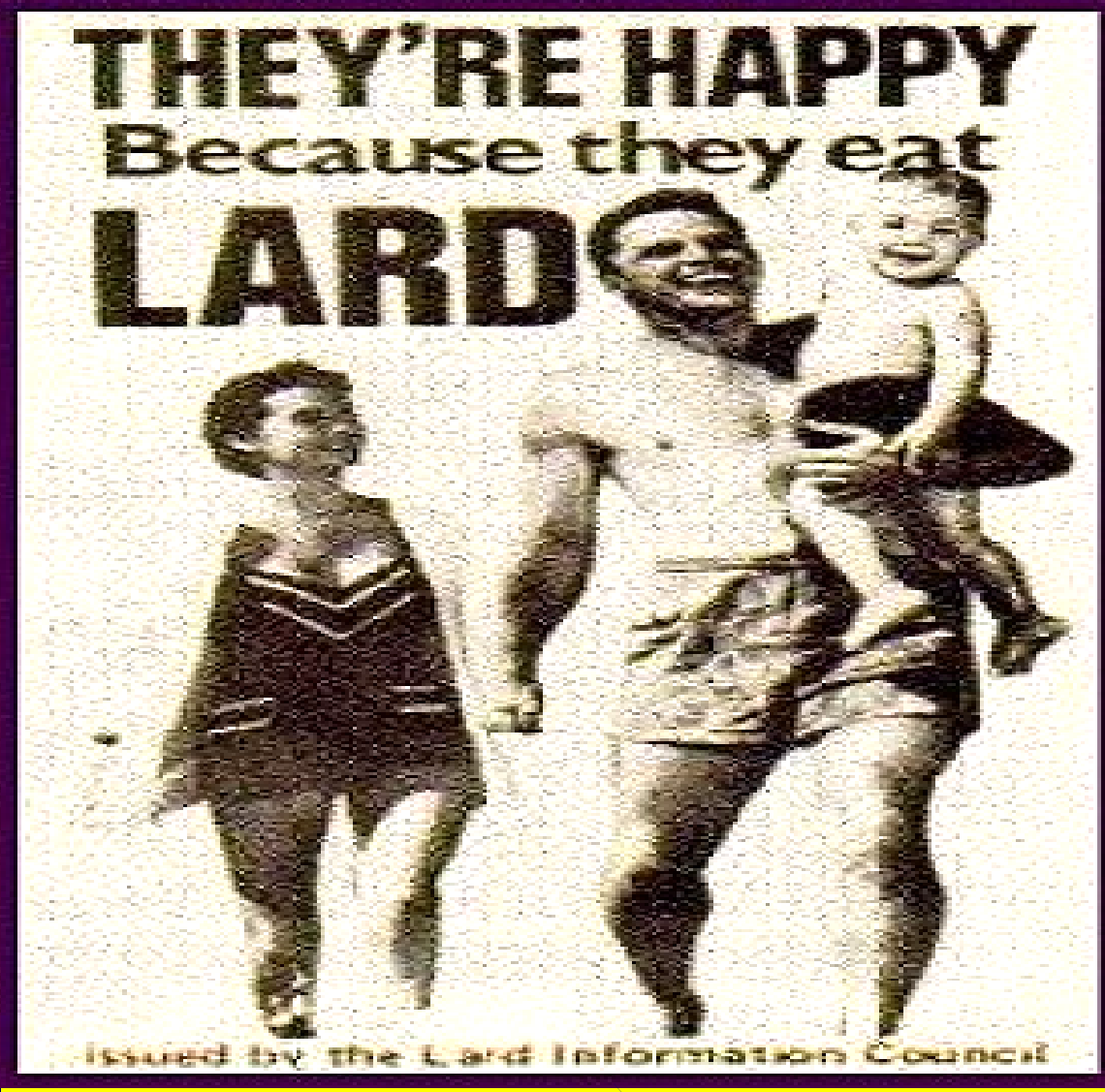


Esophageal Cancer Death Time (Year)

Taylor, P. et al., Gastroenterology 2005 (abstract)

**When I knew all of life's answers,
they changed all the questions!**

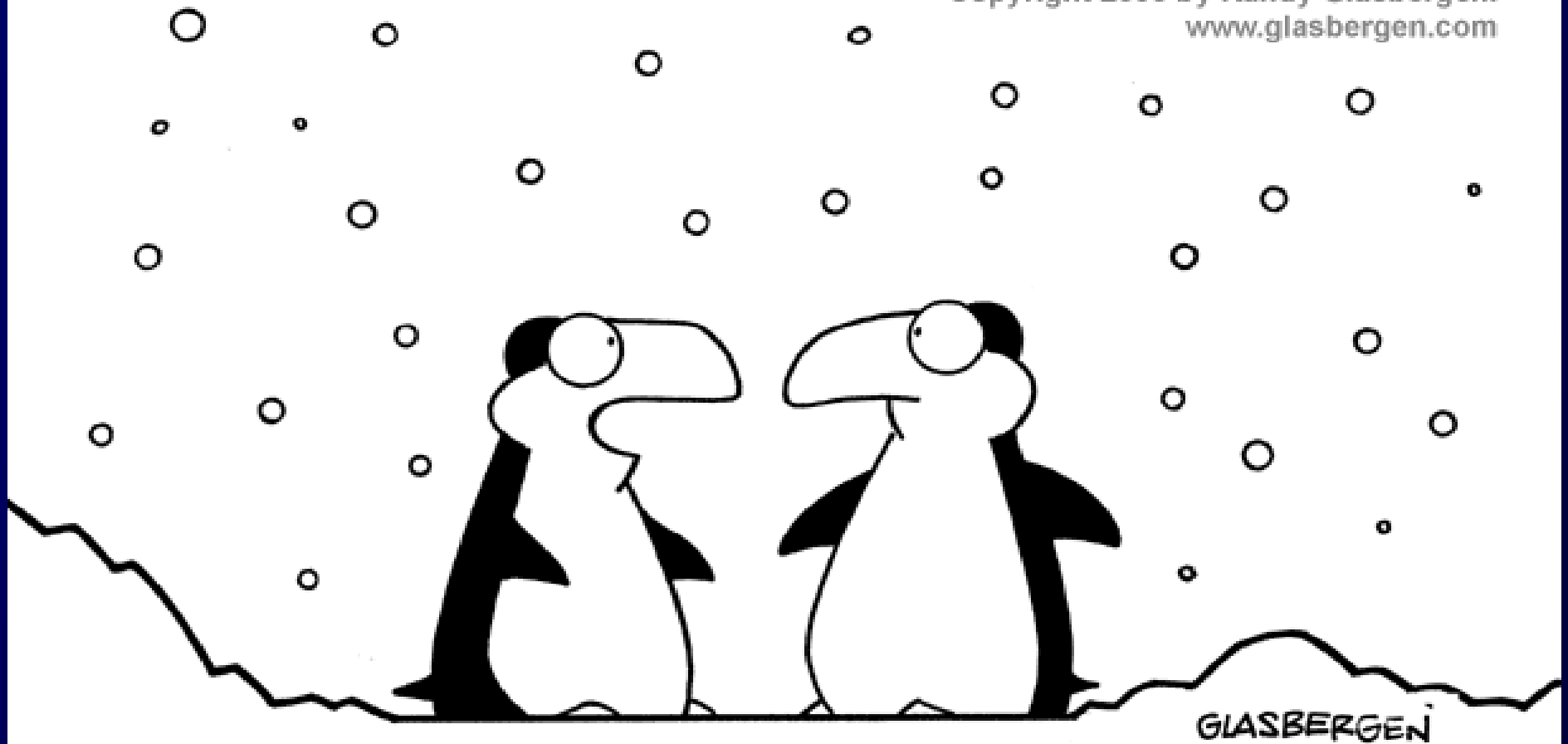




1940's Advertisement United Kingdom. While we have come a long way, we still can not identify who will and will not benefit from dietary intervention strategies

Much Confusion Exist About What Consumers Should and Should Not Do About Eating Behaviors

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www.glasbergen.com



**“Low fat diets don’t work. I eat fish every day
and my butt still drags on the ground!”**

FDA

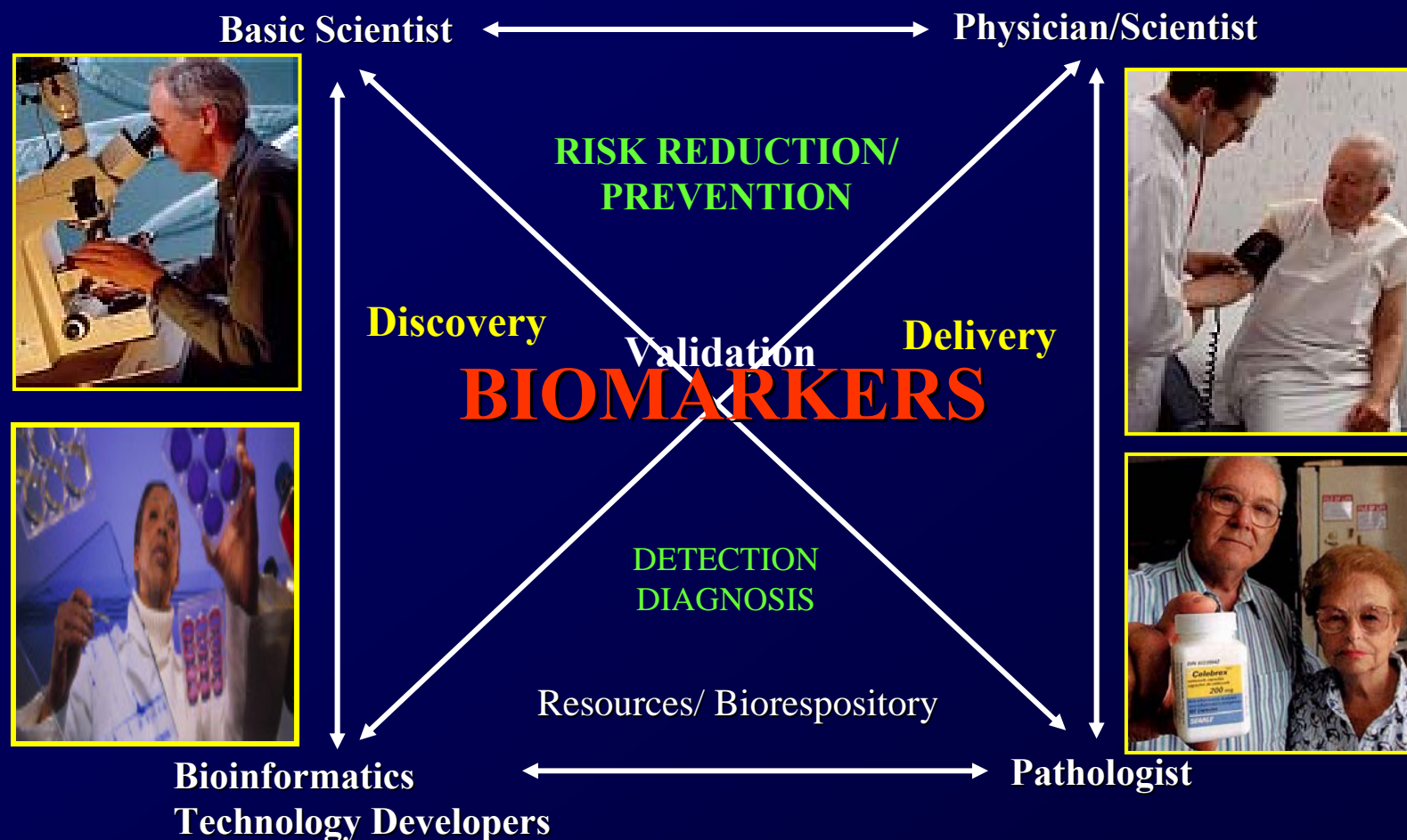
2002P-0122:

Request Rulemaking on Functional Foods & Establish Advisory Committee

Some Issues

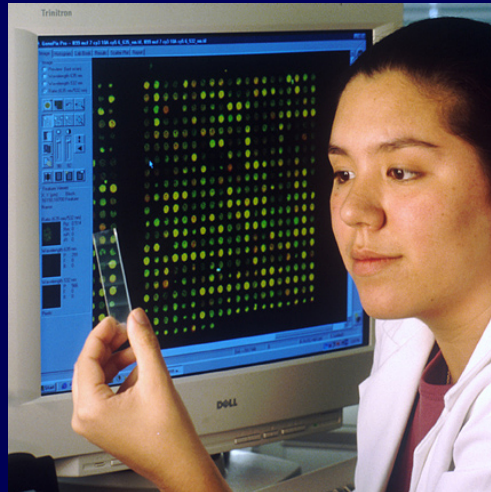
- 1. Novel Ingredients-validated response for food required?**
- 2. Safety- reasonable certainty of no harm?**
- 3. Prior Notification about marketing?**
- 4. IFT proposed independent experts to evaluate, not necessarily viewed as authoritative body?**
- 5. Is blocking a process consistent with normal regulation associated with taste, aroma or nutritive value characteristics.**
- 6. Etc.**

Systems Biology Approach Needed for Functional Foods and Biomarkers Related to Health

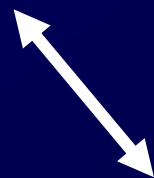


**Could Defining a Nutritional Phenotype Be
Key to Defining Functional Foods?**

The Future Paradigm: *Transform Medicine from Curative to Preemptive*



Predictive ↔ Personalized ↔ Preemptive



Participatory