

# “Nutrigenomics and Personalized Medicine”

**John Milner**

**Nutritional Science Research Group**

**Division Cancer Prevention,**

**National Cancer Institute**

**[milnerj@mail.nih.gov](mailto:milnerj@mail.nih.gov)**

# “Nutrigenomics and Personalized Medicine”

## Learning Objectives

- Define a bioactive food component
- Understand intraindividual variations in response
- Define interaction between cancer and food components such as selenium
- Define how diet can shift transcriptomic expression
- Explain long term effects of soy intake as it relates to cancer



**Unprecedented opportunities exist for the expanded use of foods and components to achieve genetic potential, increase productivity and reduce the risk of disease, including cancer**

**Hippocrates Proclaimed  
almost 2500 years ago:**

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

# Public Health Approach



## Eating for Healthy Adult New Zealanders



# There Are Always A Few Skeptics!

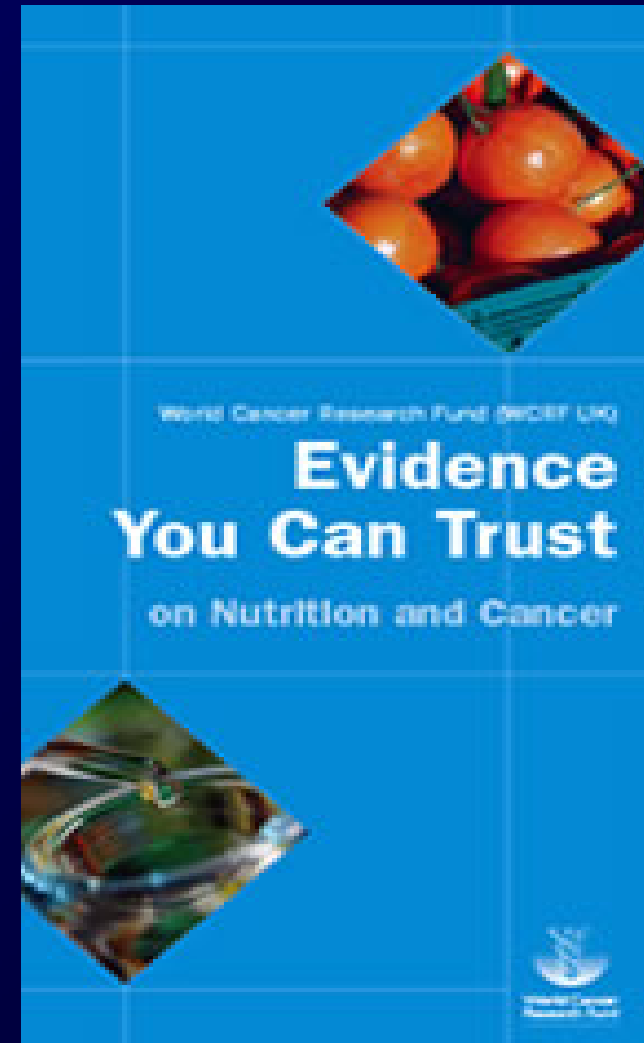
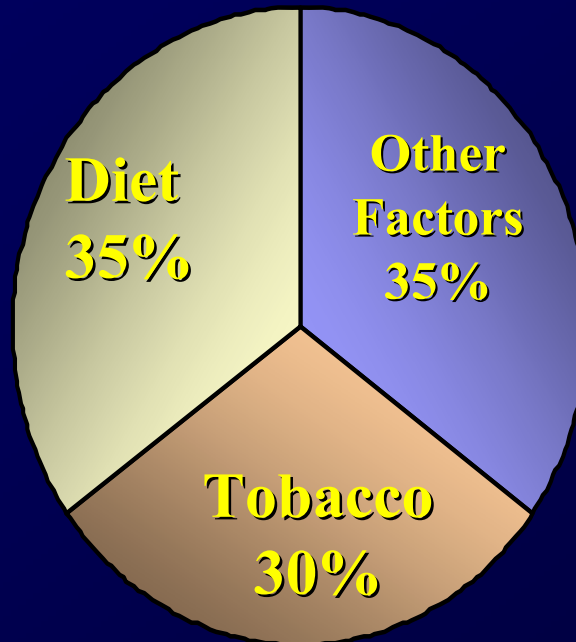
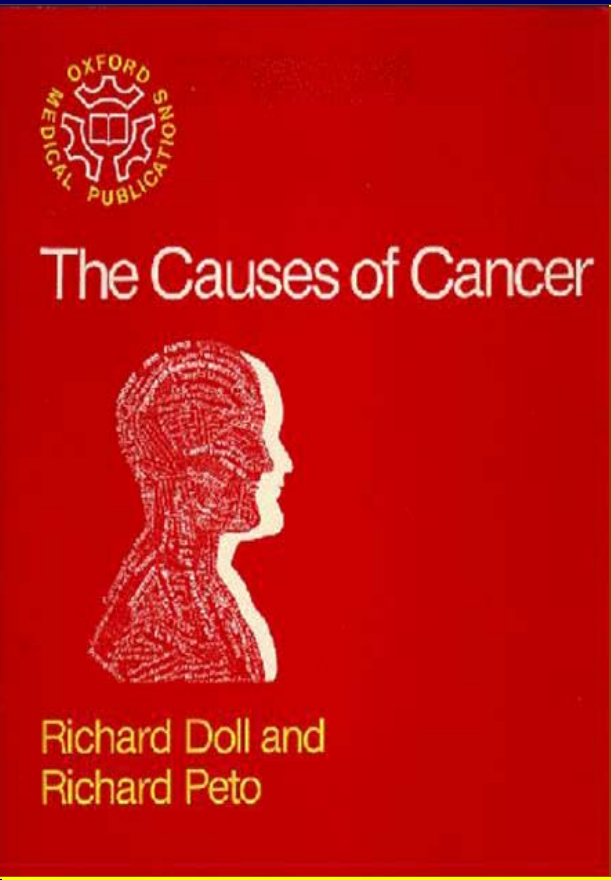
Copyright 2002 by Randy Glasbergen. [www.glasbergen.com](http://www.glasbergen.com)



**“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!”**

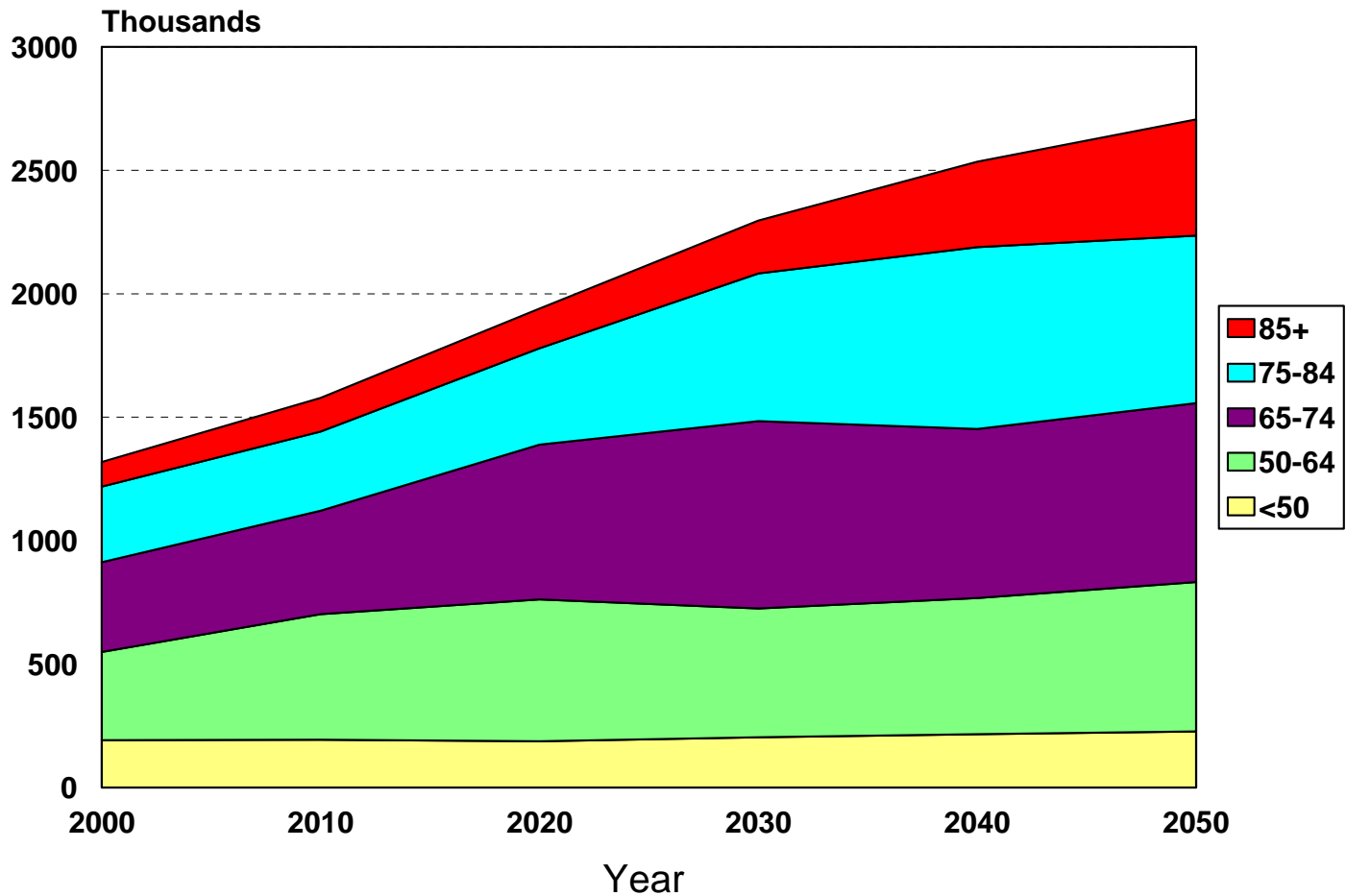
# The Causes of Cancer

– Richard Doll & Richard Peto, 1981



**In 2006 about 720K new cases in men and about 670K cases in women. Death attributed to cancer in 2006 will be about 290K and 270K, respectively**

## Projections of Cancer Cases between 2000 to 2050 by Age



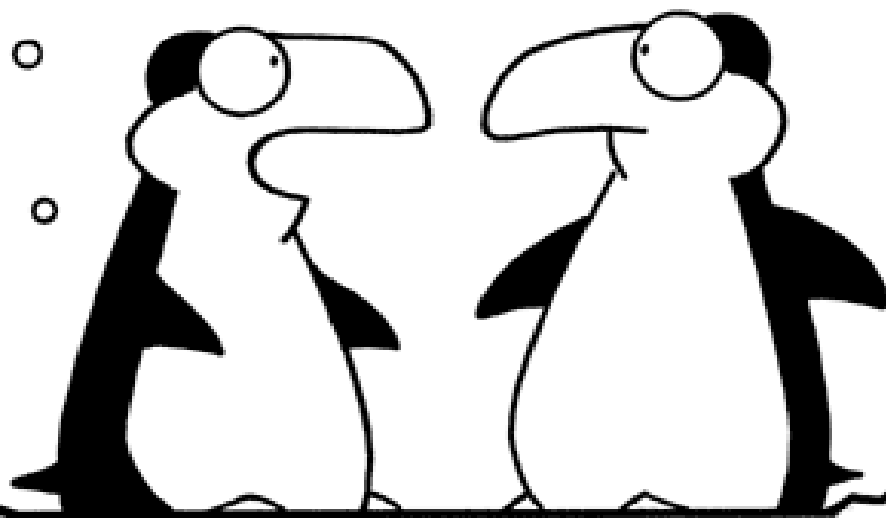
Number of  
 cancer  
 cases  
 Expected to  
 increase  
 due to  
 Growing  
 and aging  
 population





# While Diet Linked to Cancer Risk: Much Confusion Exist About What to Eat

Copyright 2003 by Randy Glasbergen.  
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**“Low fat diets don’t work. I eat fish every day  
and my butt still drags on the ground!”**

# 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)

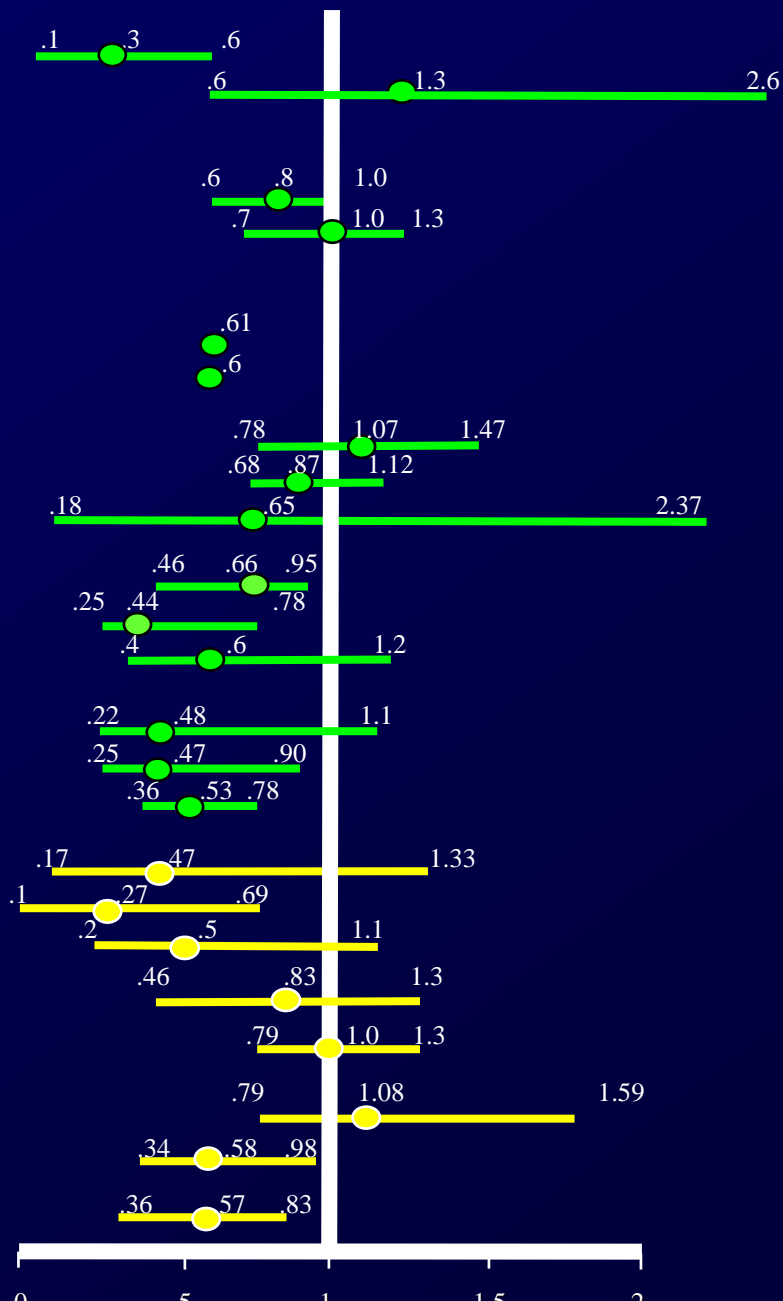
Keinan-Boker '02 (food content)

NS Isoflavones

S Lignans

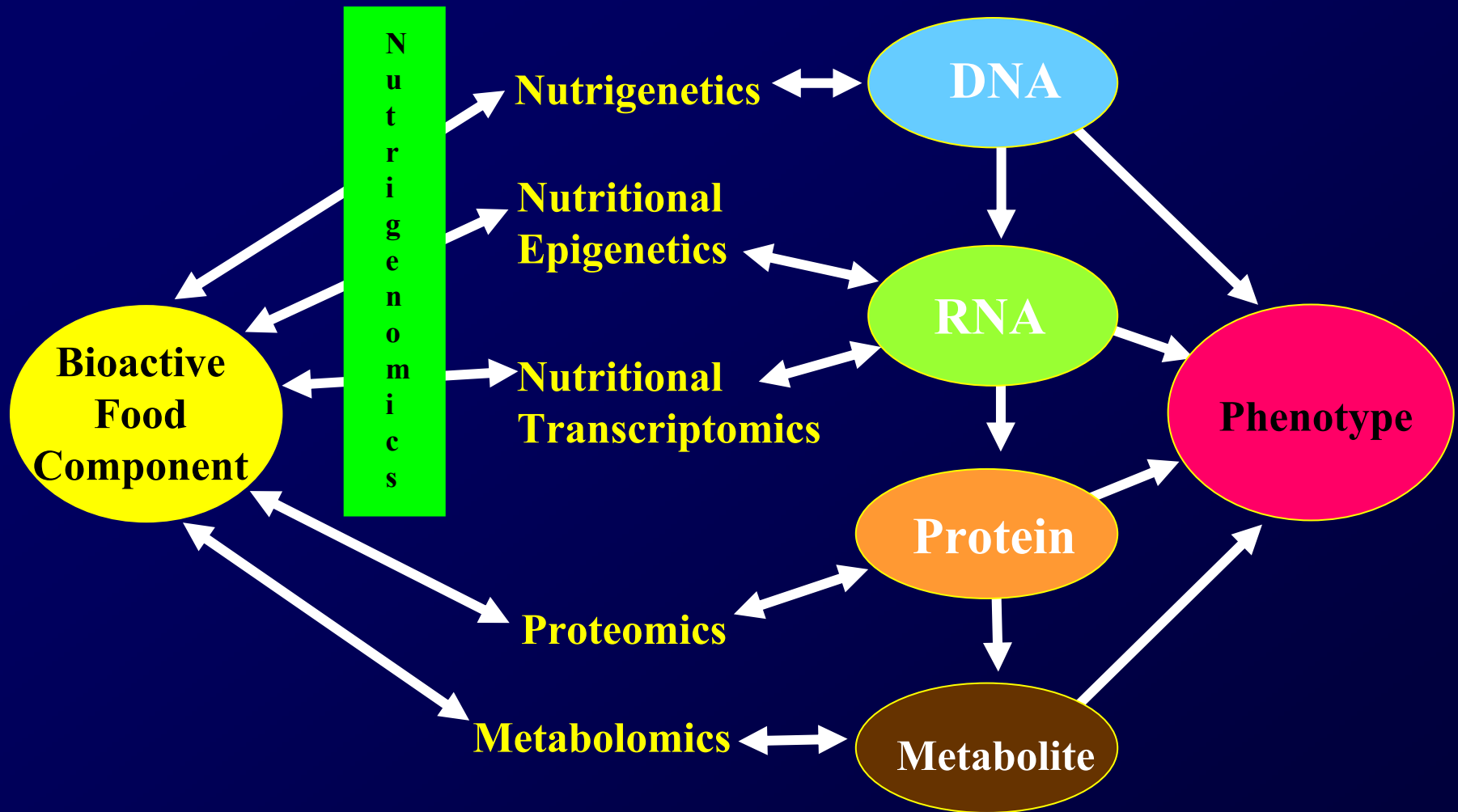
Linseisen '04 (isoflavone intake)

daidzein and genistein



## Western

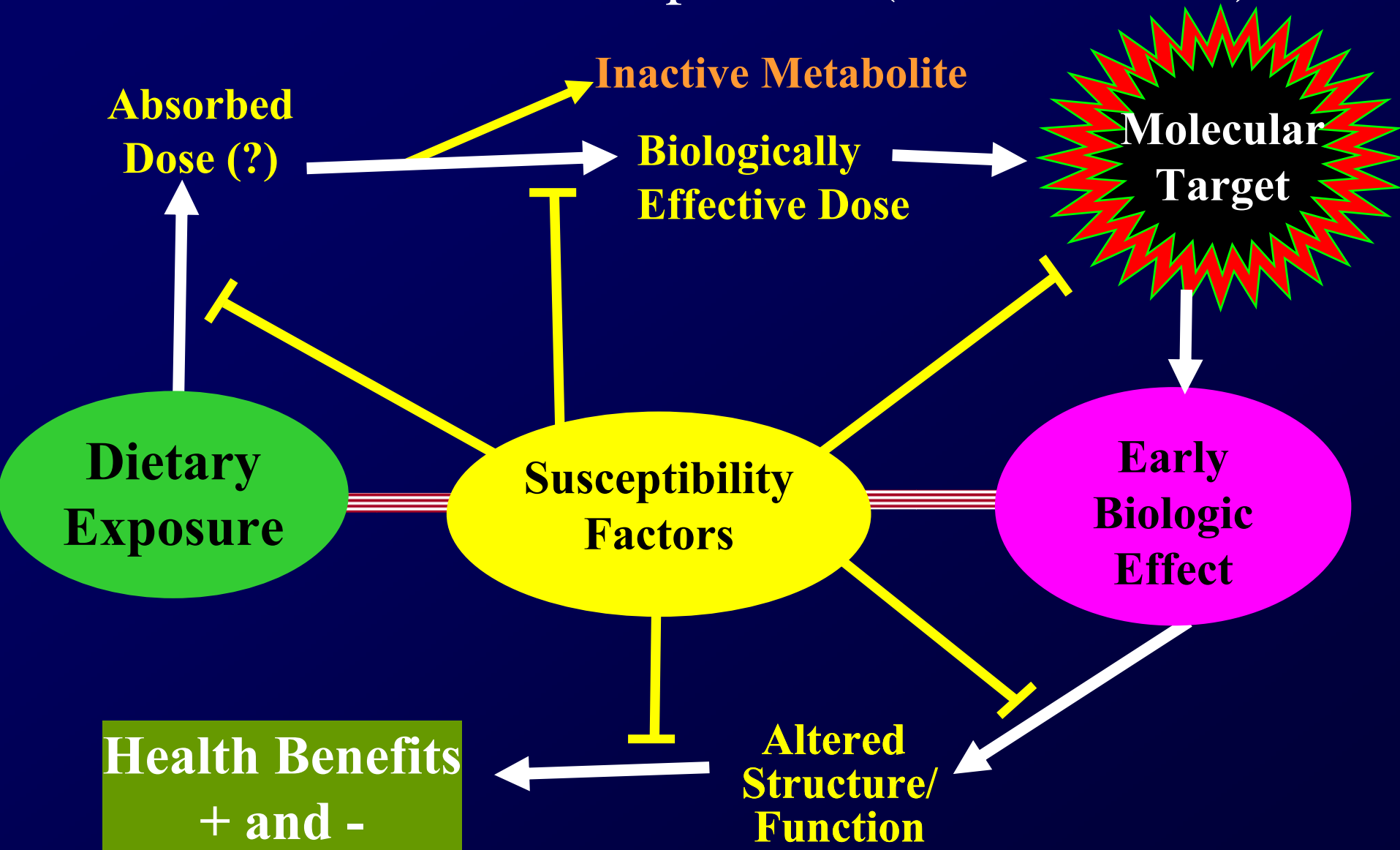
# The “Omics” May Explain Variation in Response



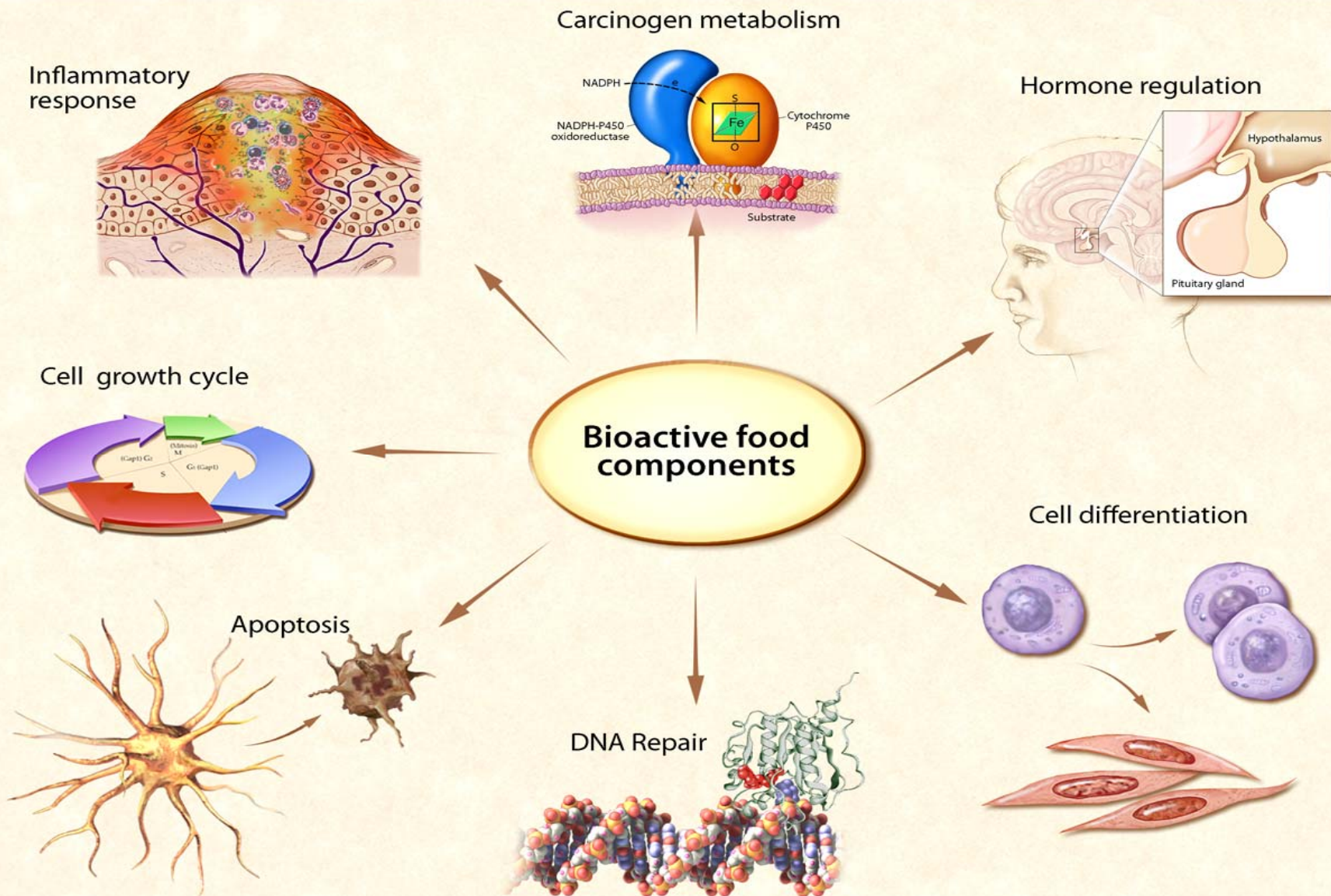
*“Nutritional Preemption”*

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

# Biological Determinants of the Response to Bioactive Food Components (3 Biomarkers)



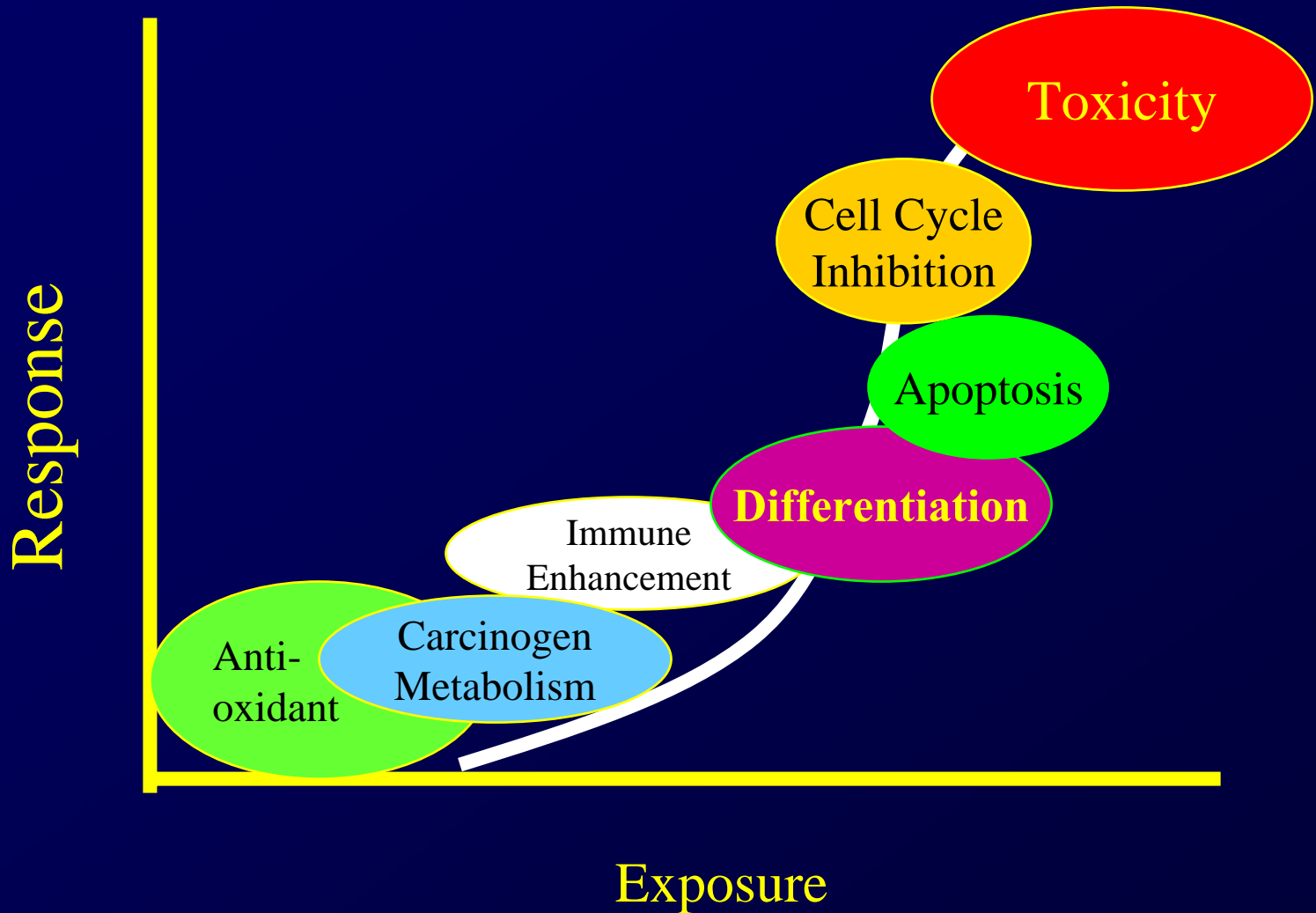
# Future Is To Focus on the Process Needing Modification



**Credentialing of nutrients and molecular targets is likely the future?**

Credentialing is defined as “omic” changes that bring about a phenotypic change

# Biomarkers of Quantity and Intended Use



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

# Suspect Functional Foods With Health Benefits

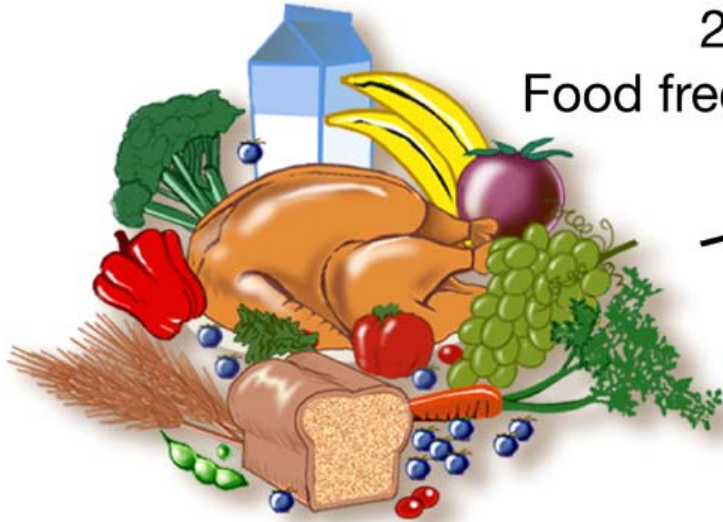


- Soy
- Tomatoes
- Spinach
- Broccoli
- Garlic
- Nuts
- Salmon
- Oats
- Blueberries
- Green tea
- Red wine

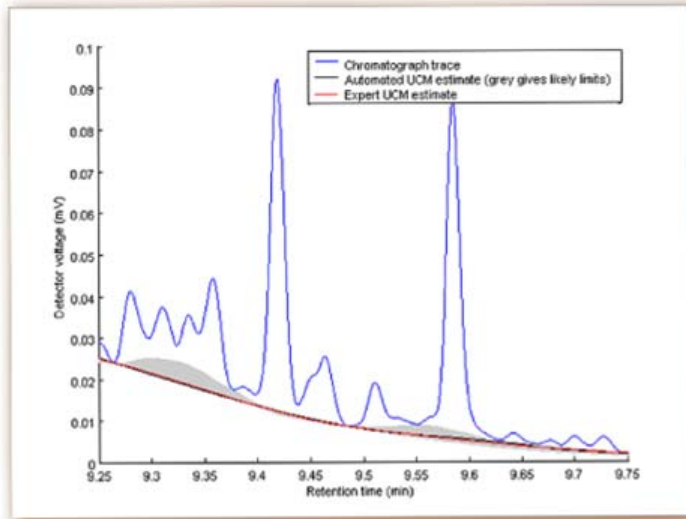
24 hour record

Food frequency questionnaire

Food diary



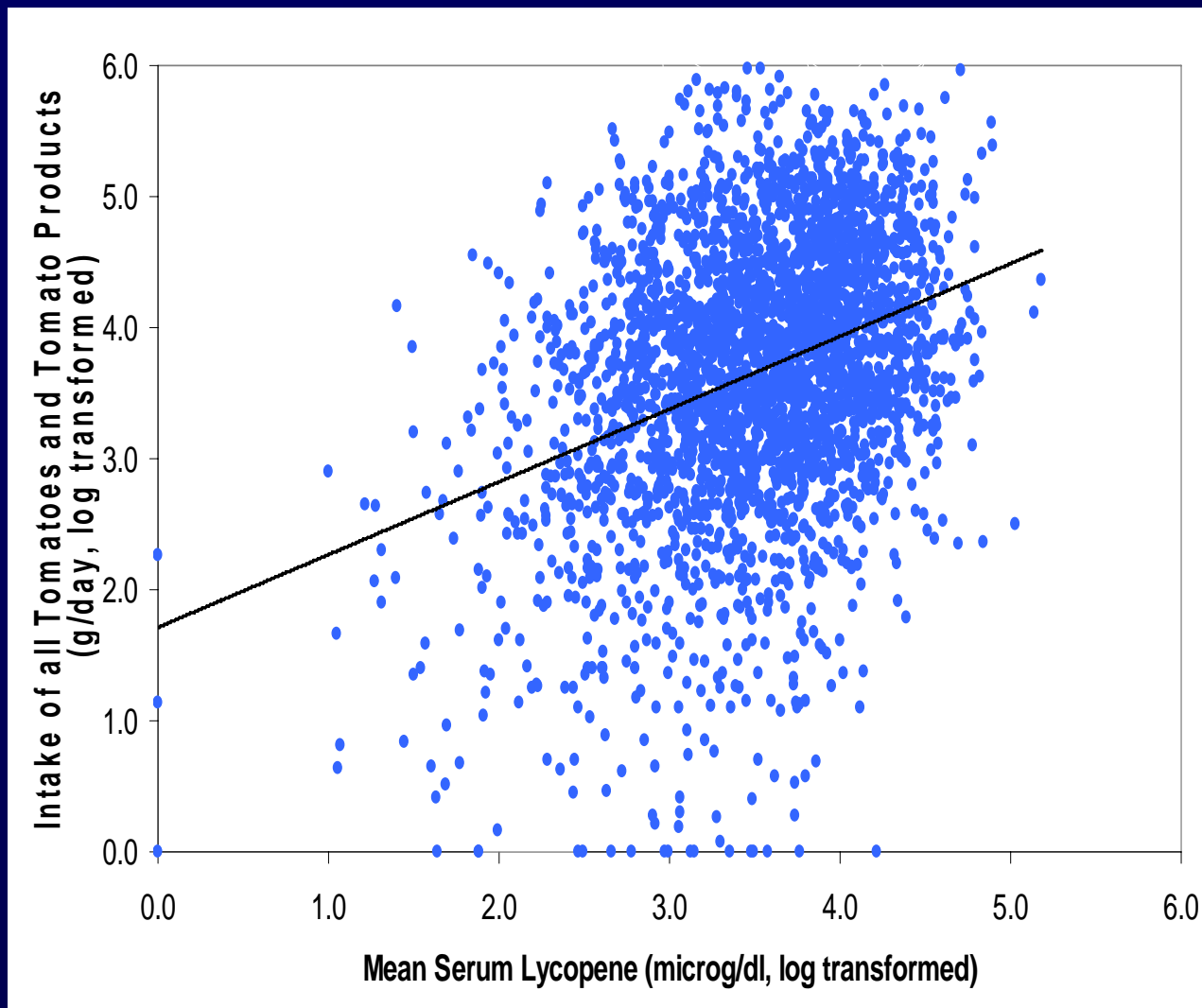
Physiological exposures



Absorption  
Metabolism  
Distribution  
Excretion



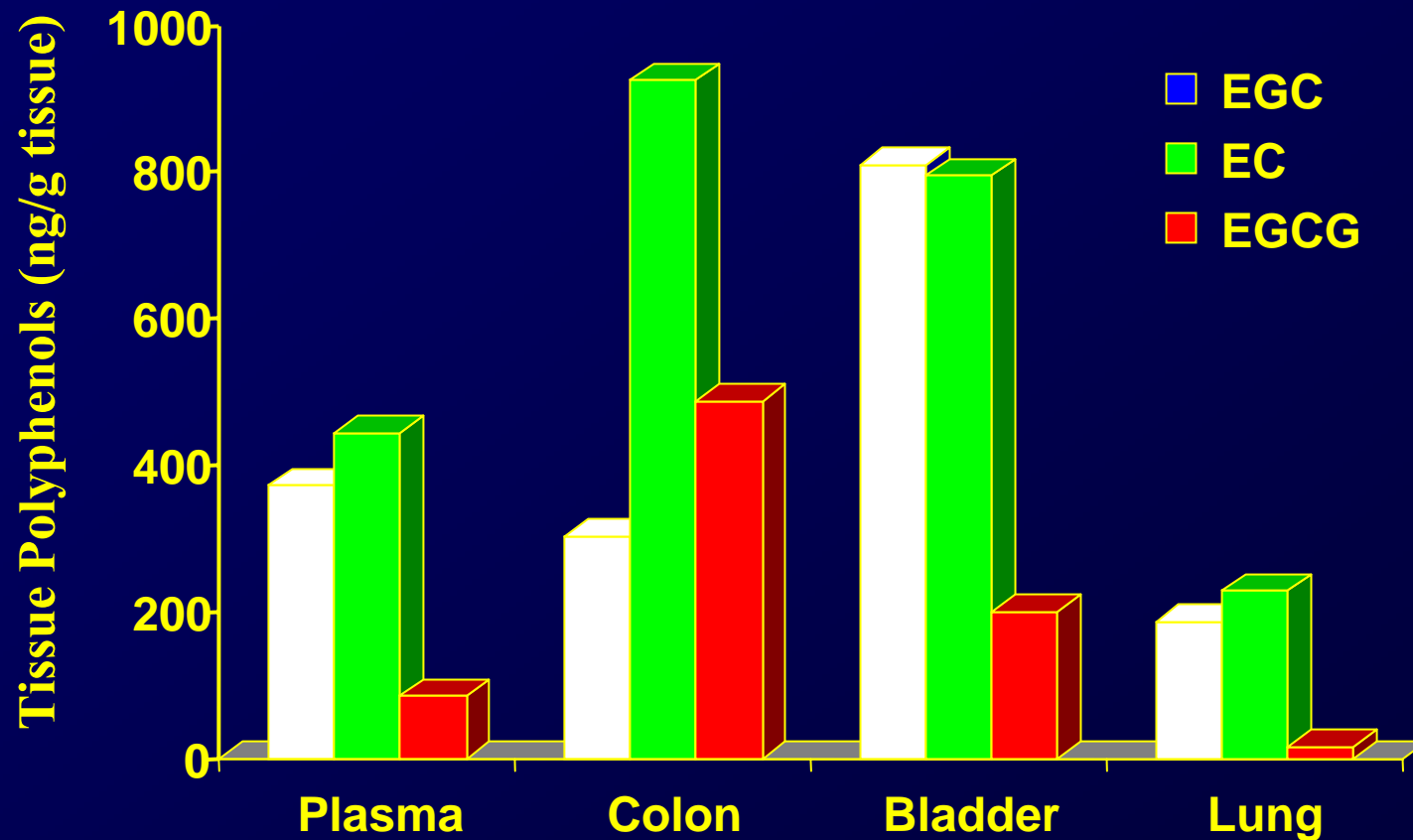
# Individual Consumption of All Tomatoes and Tomato Products and Serum Lycopene Levels (EPIC Cross-sectional Study in 3000 subjects)



Corr = 0.23

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

# Not All Tissues Equally Influenced by Bioactive Food Components



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

**Functional Food  
Or Component**

Physiologic  
Function

Organism  
Function

Organ  
or  
Tissue  
Function

Cell  
Function

Molecular  
Function

Mental  
Process

Genetic  
Function

Mental  
or  
Behavioral  
Dysfunction

Neoplastic  
Process

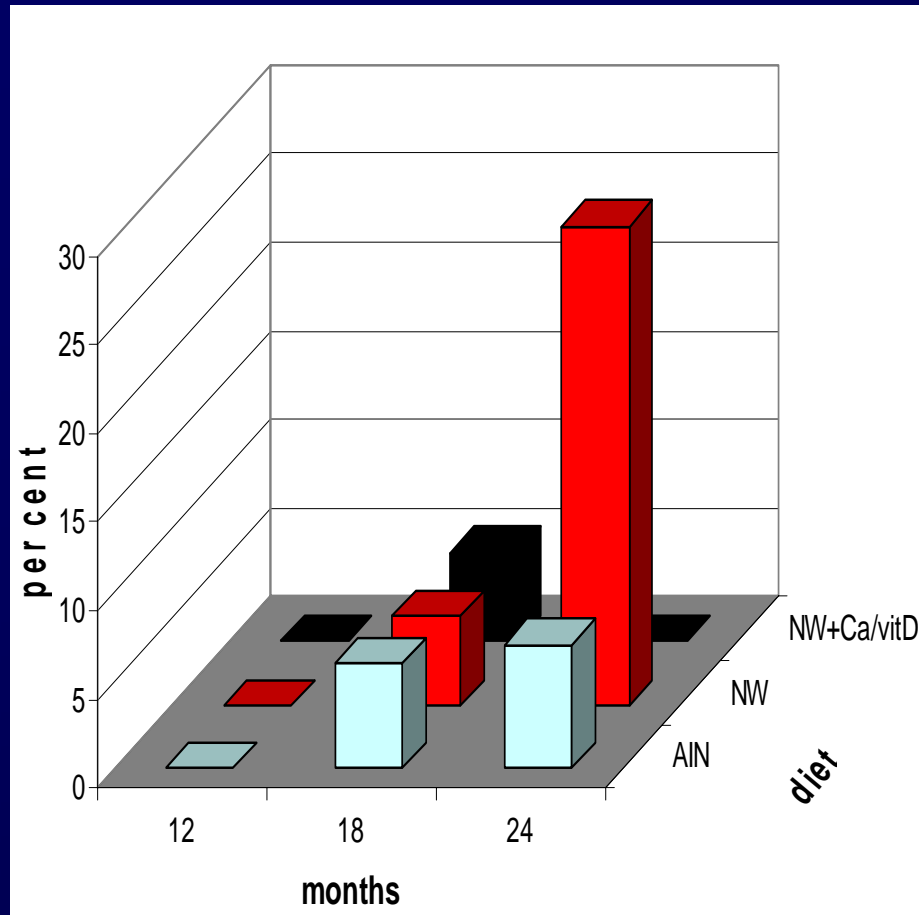
Pathologic  
Function

Disease  
or  
Syndrome

Cell  
or  
Molecular  
Dysfunction

Experimental  
Model  
of  
Disease

## Diet Influences Colon Tumor Incidence in C57Bl/6 Mice



***Approximately 25% of the mice develop a single tumor***

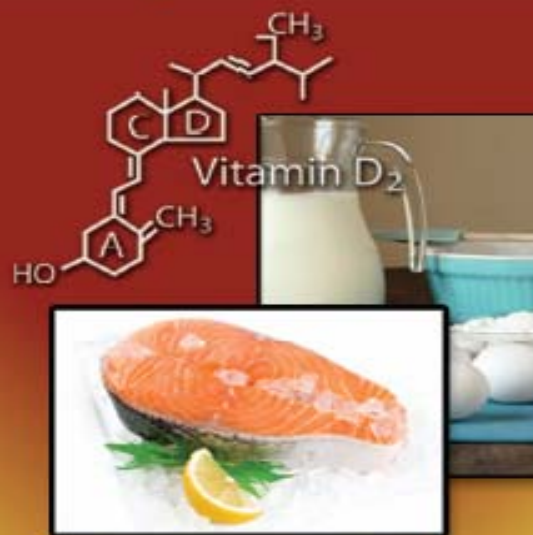
***.....this is a new mouse model of sporadic colon cancer,  
which represents the vast majority of human colon cancer***

***Yang et al. J Nutr. 2005;135:2710-4.***

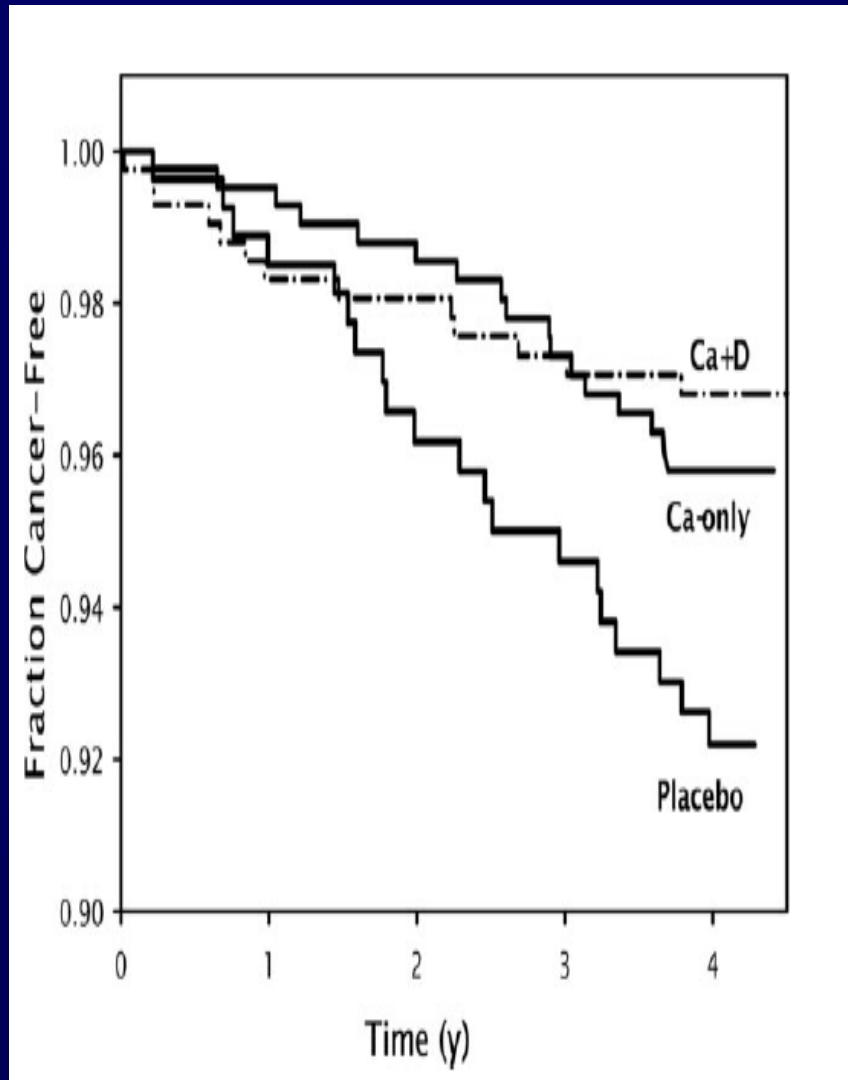
Lister Hill Auditorium, NIH Campus | Bethesda, MD

# Vitamin D and Cancer: Current Dilemmas/Future Needs

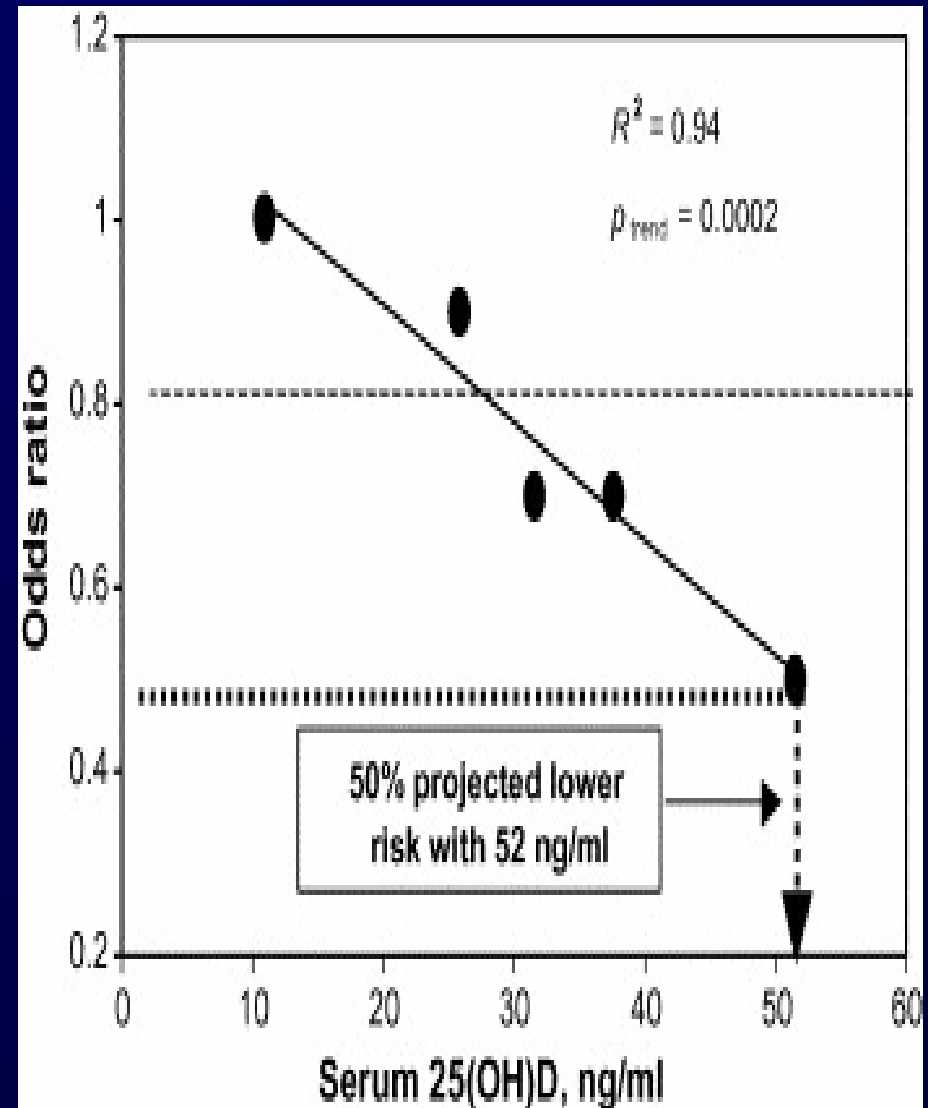
May 7-8, 2007



# Vitamin D, Calcium and Cancer



**Lappe et al. Am J Clin Nutr  
2007;85:1586–91**



**Garland et al. J Steroid Biochem  
Mol Biol. 2007;103:708-11**

# The Genetic Revolution Is Providing New Insights into a Number of Health Issues Including the Role of Diet in Cancer Prevention

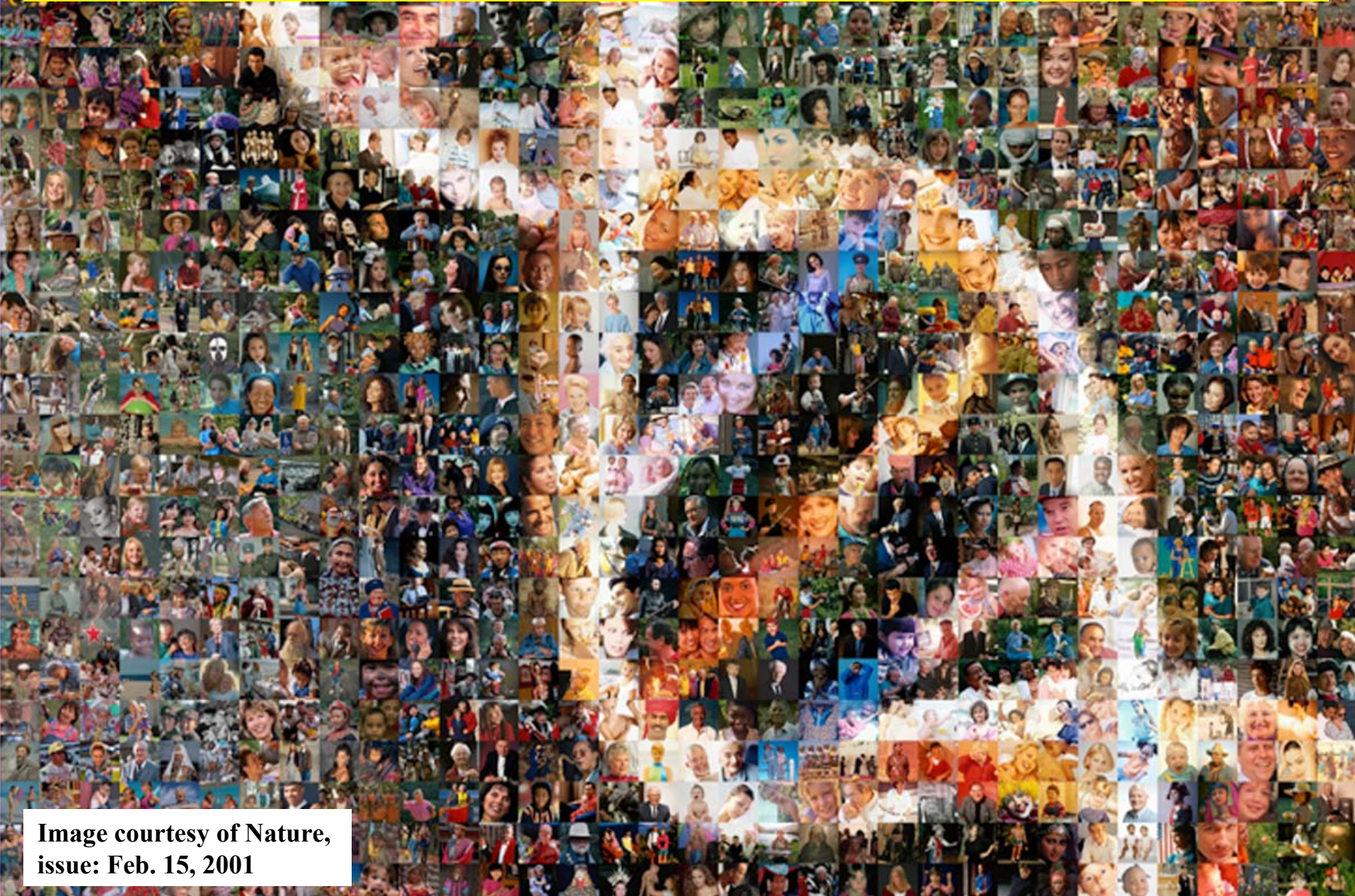
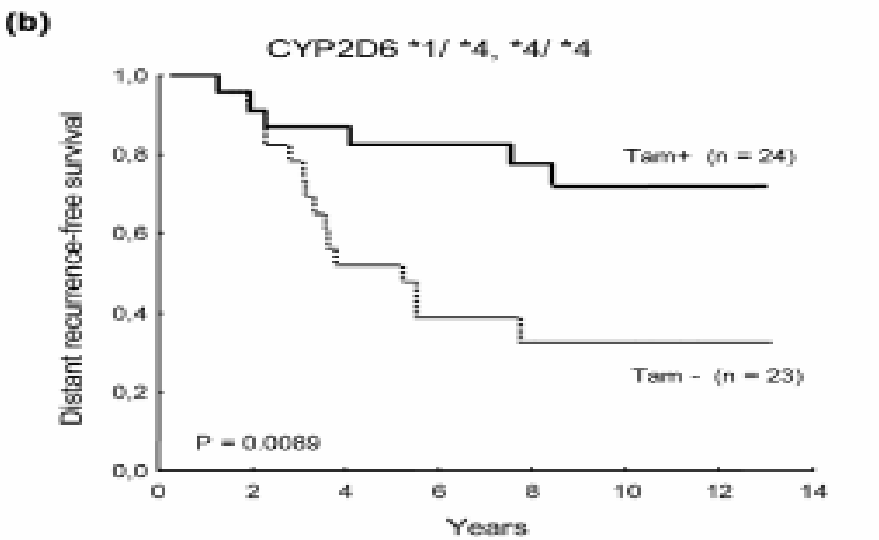
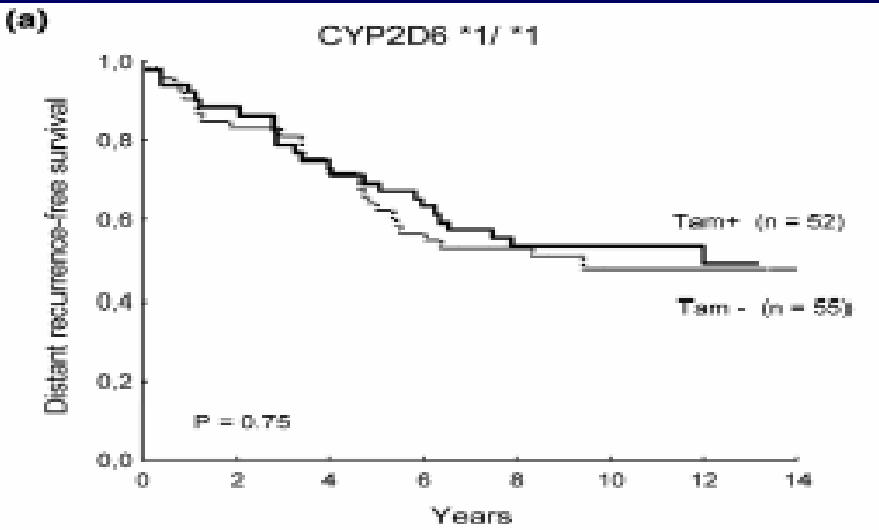


Image courtesy of Nature,  
issue: Feb. 15, 2001

# Genotype of Metabolic Enzymes Modifies the Benefit of Tamoxifen

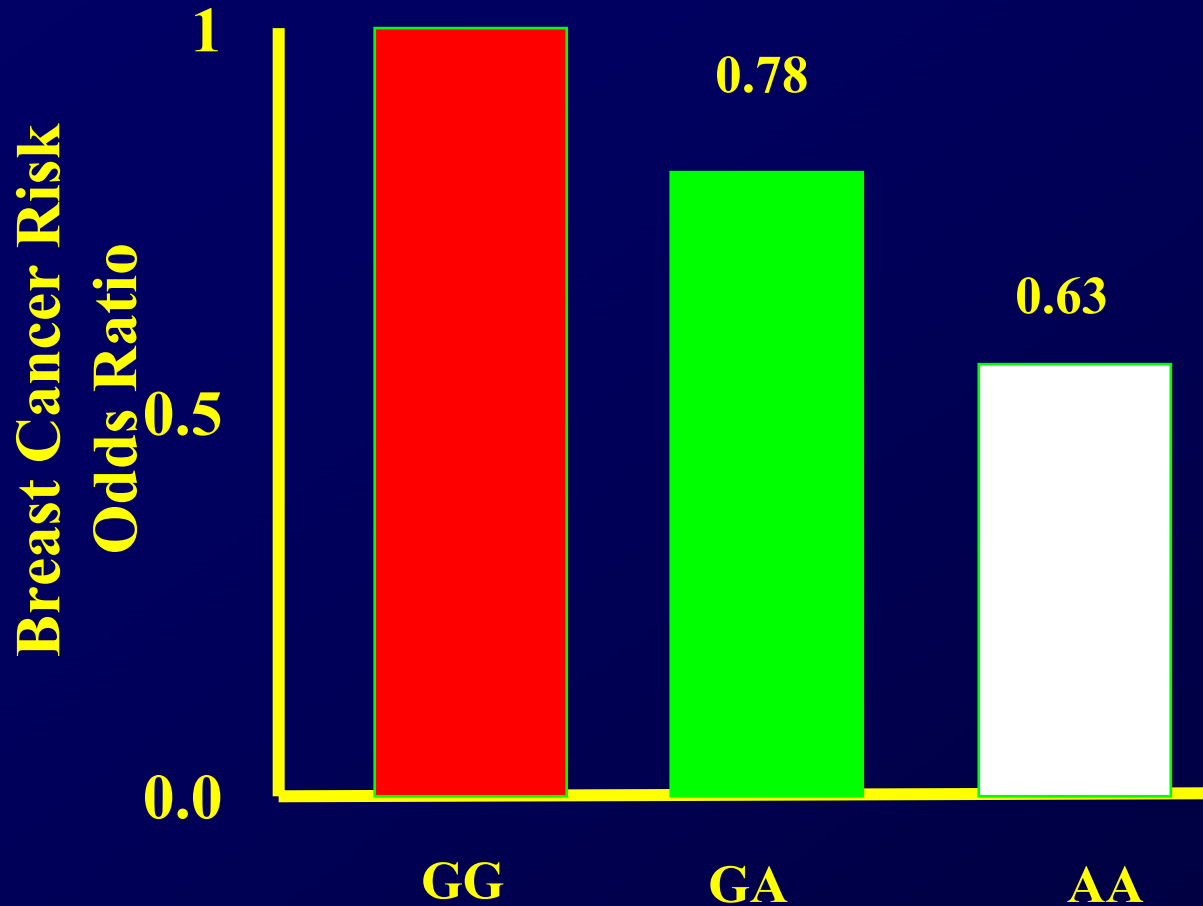


- Cytochrome P4502D6 is involved in the metabolism of tamoxifen
- Among individuals homozygous for CYP2D6\*1 genotype, there was no benefit of tamoxifen
- Patients containing at least one CYP2D6\*4 allele had better survival (RR=0.28) when randomized to tamoxifen



# All Individuals Will Not Respond Identically!

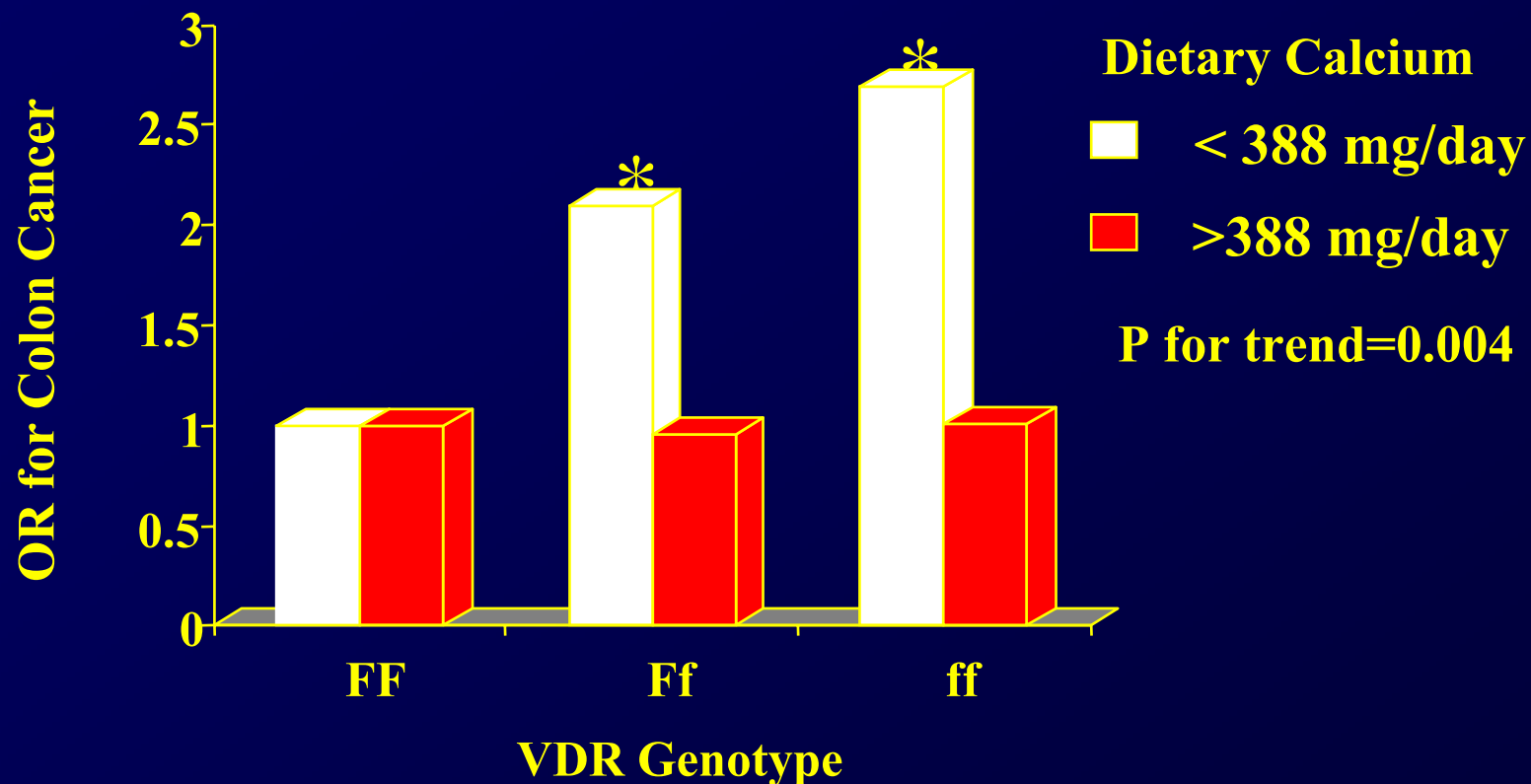
## Myeloperoxidase Polymorphism (G463A) and Higher Fruit/Vegetable Intake



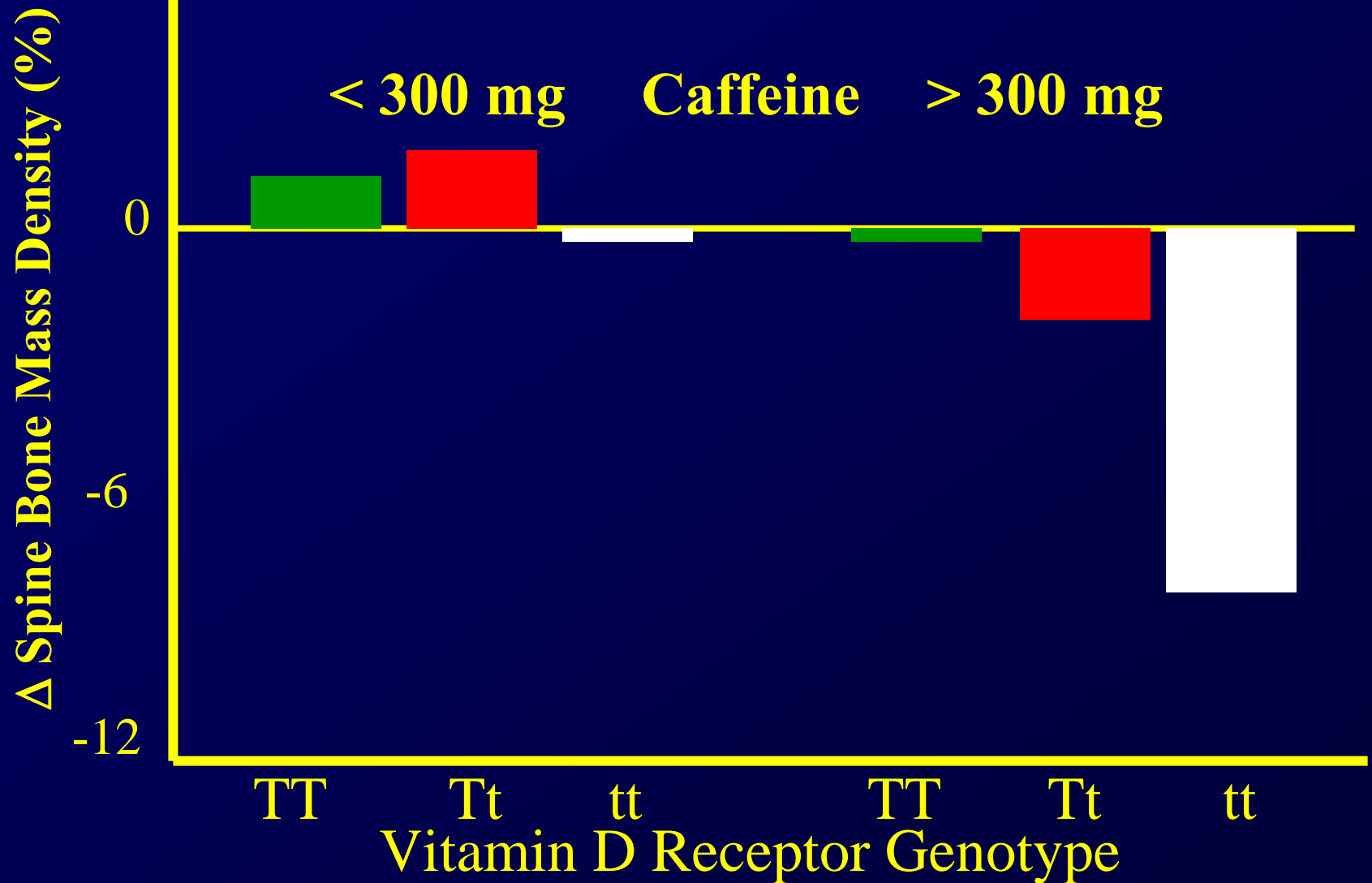
Risk not related to polymorphism when intakes was low. Low and high consumption based on median values of control group: fruit and vegetable, 29 svg/wk

Premenopausal- greatest response to increased fruits and vegetables

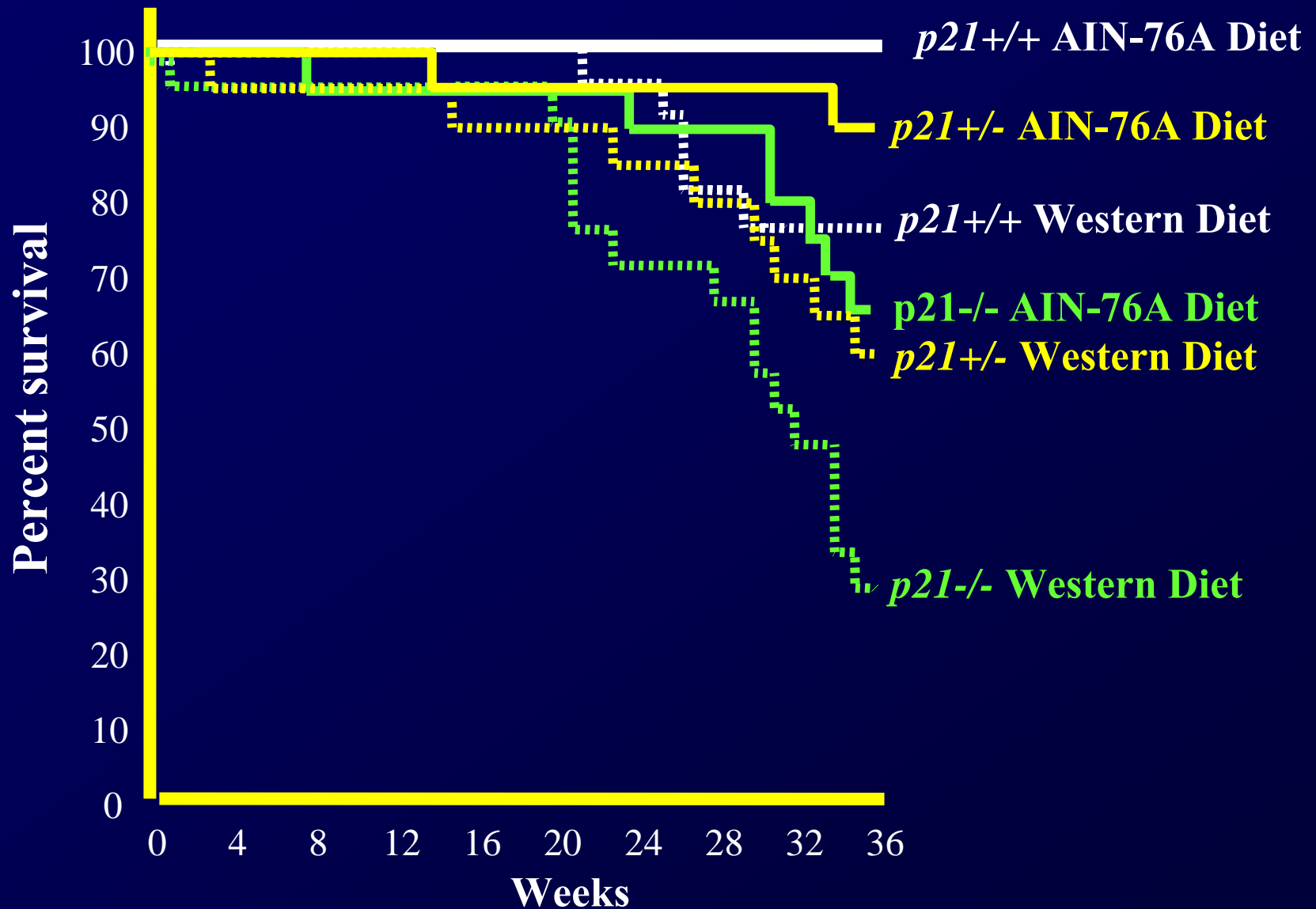
# Genetic Information May Assist in Identifying Those Who Must Assure Adequate Intakes



# Genetic Information May Help Identify Those At Risk and to Formulate Appropriate Interventions

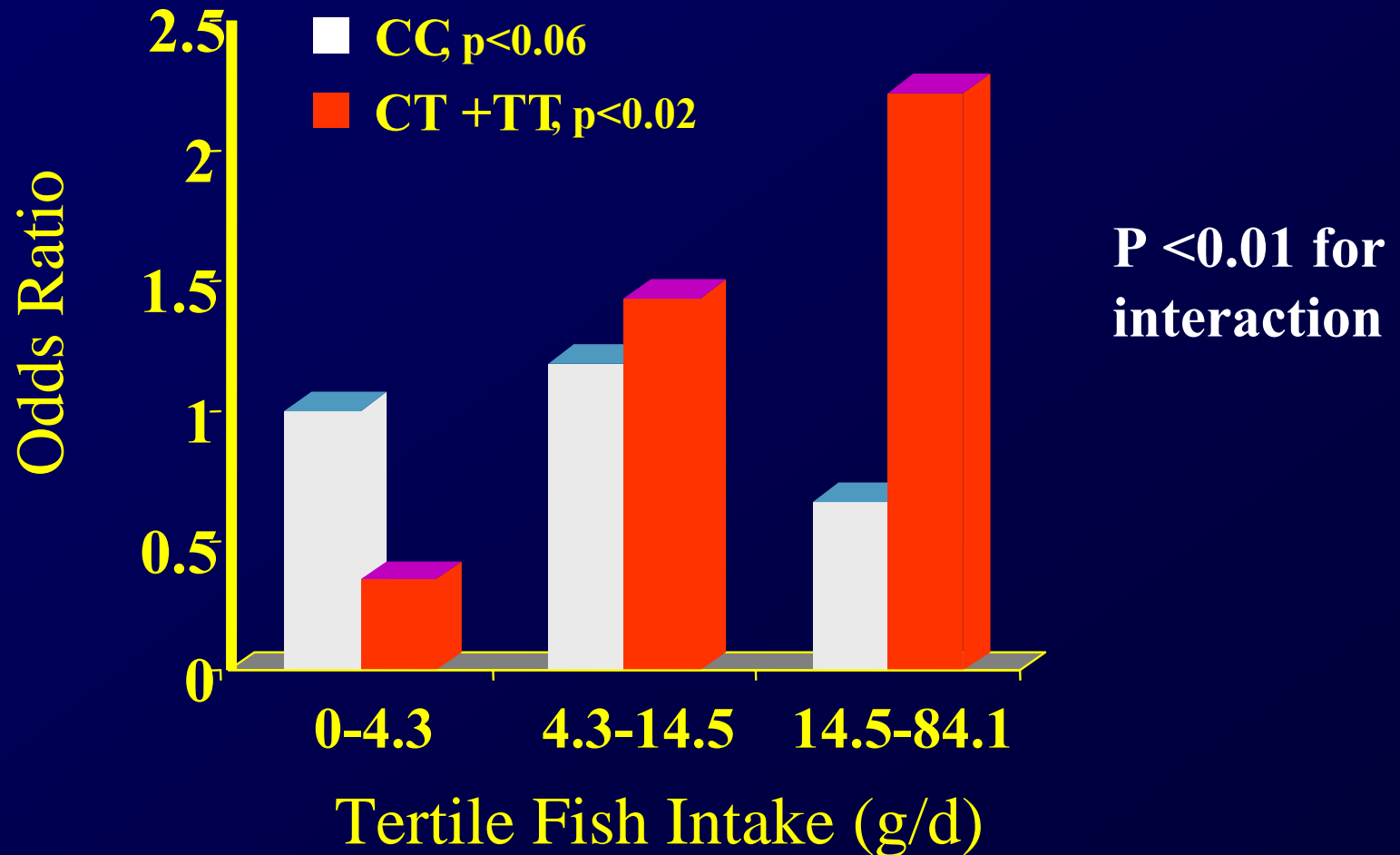


# Not Knowing Genomics Can Cause Misinterpretation

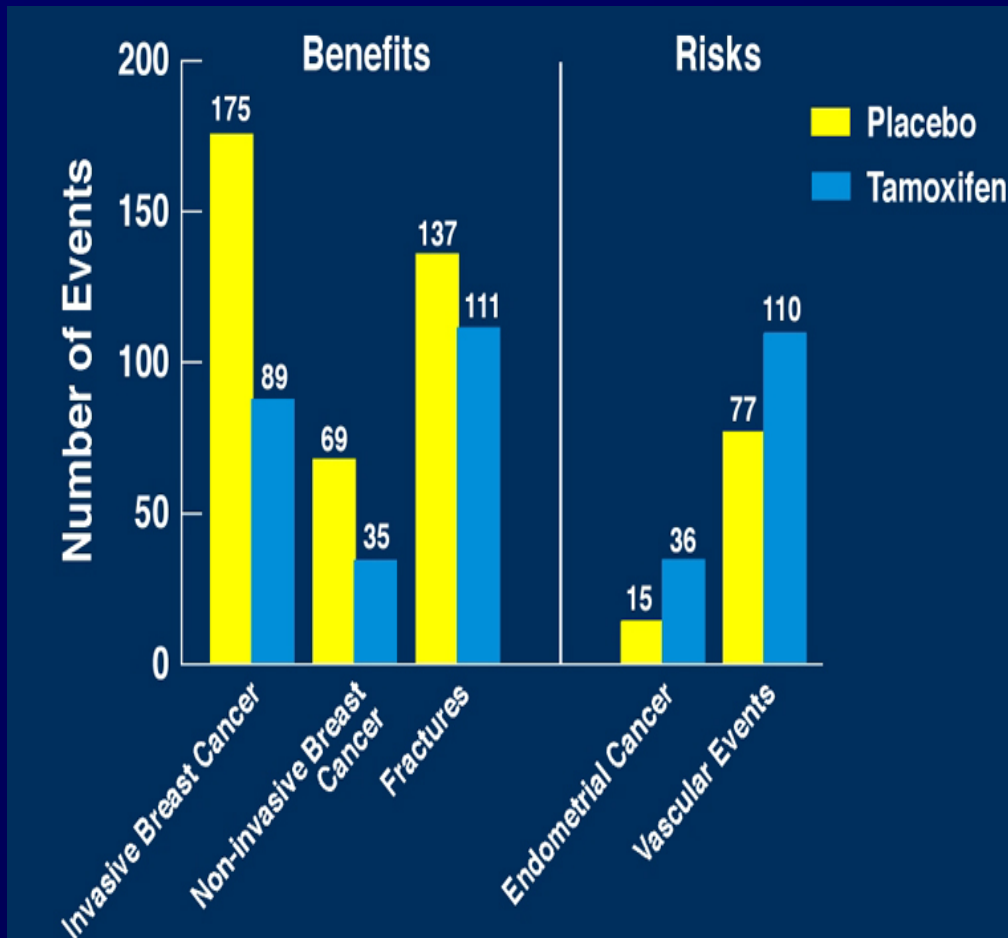


# PPAR $\Delta$ Genotype, Fish Consumption and Colon Cancer

(384 cases and 403 polyp-free controls 789C $\rightarrow$ T )



# Breast Cancer Prevention Trial (BCPT) Events Among Participants



**Is It Logical  
To Assume  
That Foods  
Or their  
Components  
Result in No  
Risk??**

# **More Folate May Not Be Beneficial to Some**

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

**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.**

**Folic Acid Supplement Intervention May Also Increase Colon Cancer Risk**

**Cole et al. JAMA. 2007;297(21):2351-9.**

**Concerns about Several Other Food Components**

**603 association of polymorphisms and disease**

**166 studies with at least 3 population**

**Only 6 reproducible (>75 % studies)**

**Hirschhorn et al. 2002 Genet Med 4:45-61.**



# Genetic Testing Promises vs Reality!

- **Commercial Nutrition-Gene Test**
  - Genelex Sciona 19 genes including MTHFR \$395
  - Gene Care CVD nutritional genetic test (South Africa) MTHFR (Hcyst), apoA1 (HDL) +9 others \$400
- **Exceeding complex area since about 30, 000 Genes, 8-10 Million SNPs**

**\*\*\*Opportunities available for SBIR and STTR for Kits (Screens) for Predicting Risk**

# Diets Are Complex Since Contain More than 25,000 Bioactive Components

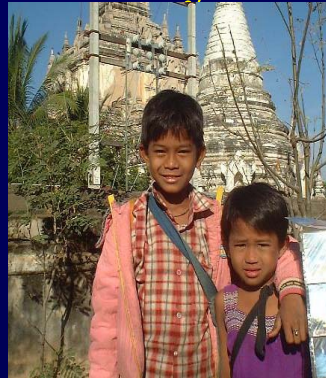


- **Essential Nutrients-** Ca, Zn, Se, Folate, Vitamins D, C, E
- **Non-Essential**
  - Phytochemicals-** Carotenoids, Flavonoids (Anthocyanin), Isothiocyanates, Allyl Sulfur,
  - Zoochemicals** - Conjugated linoleic acid, n-3 fatty acids
  - Fungochemicals** - Several compounds in mushrooms
  - Bacteriochemical**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

**Complexity of diet is Illustrated by the Vast Number of Foods and Components which can modify the cancer process**

# 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

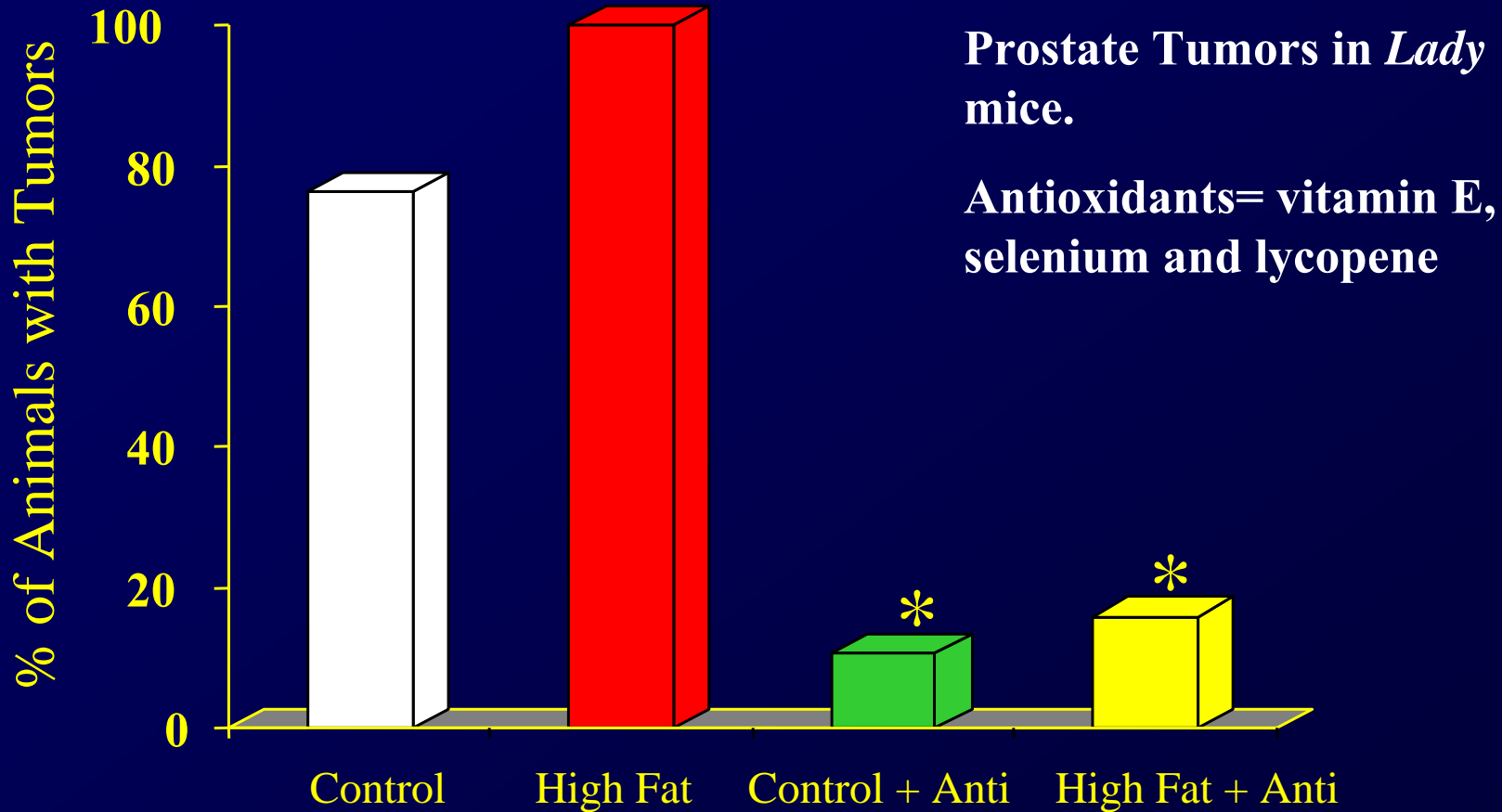
**Niculescu et al (2006) J Nutr. Biochem**

**demonstrated that isoflavone treatment in subjects who have the capacity to produce equol differentially affects gene expression as compared with nonproducers.**

**Expression of a large number of genes was altered by isoflavone treatment, including induction of genes associated with cyclic adenosine 3',5'-monophosphate (cAMP) signaling and cell differentiation and decreased expression of genes associated with cyclin-dependent kinase activity and cell division.**

**In general, isoflavones had a stronger effect on some putative estrogen-responsive genes in equol producers than in nonproducers.**

# Transgenic and Knockout Models Key to Identifying Sites of Action of Food Components



# Response Also Evident in Humans

## But Tissue Specific Response

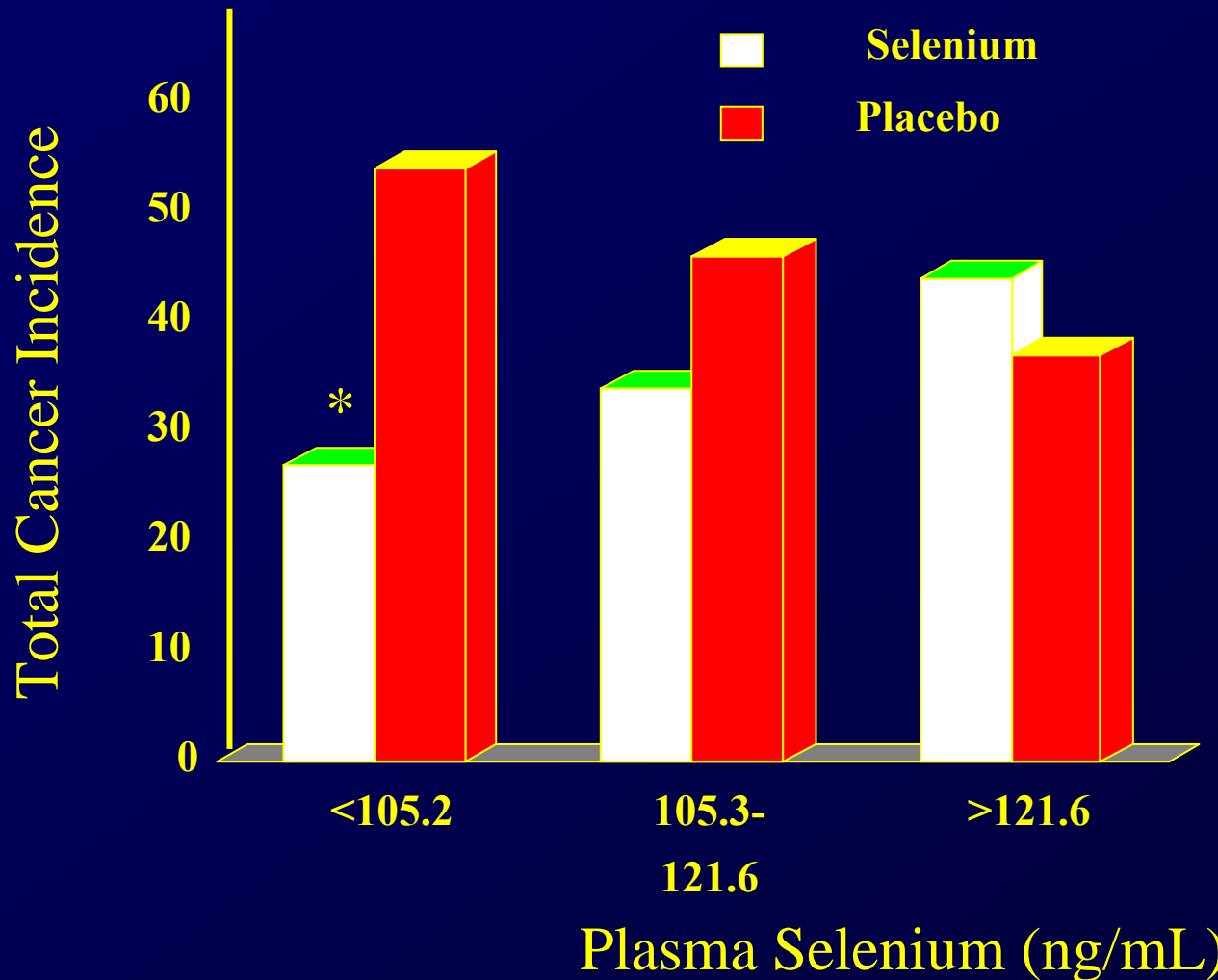
Design Phase III trial with 1,312 skin cancer subjects

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

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

**Clark et al. JAMA 276: 1957, 1996.**

# Also Clear That All People Do Not Respond Identically



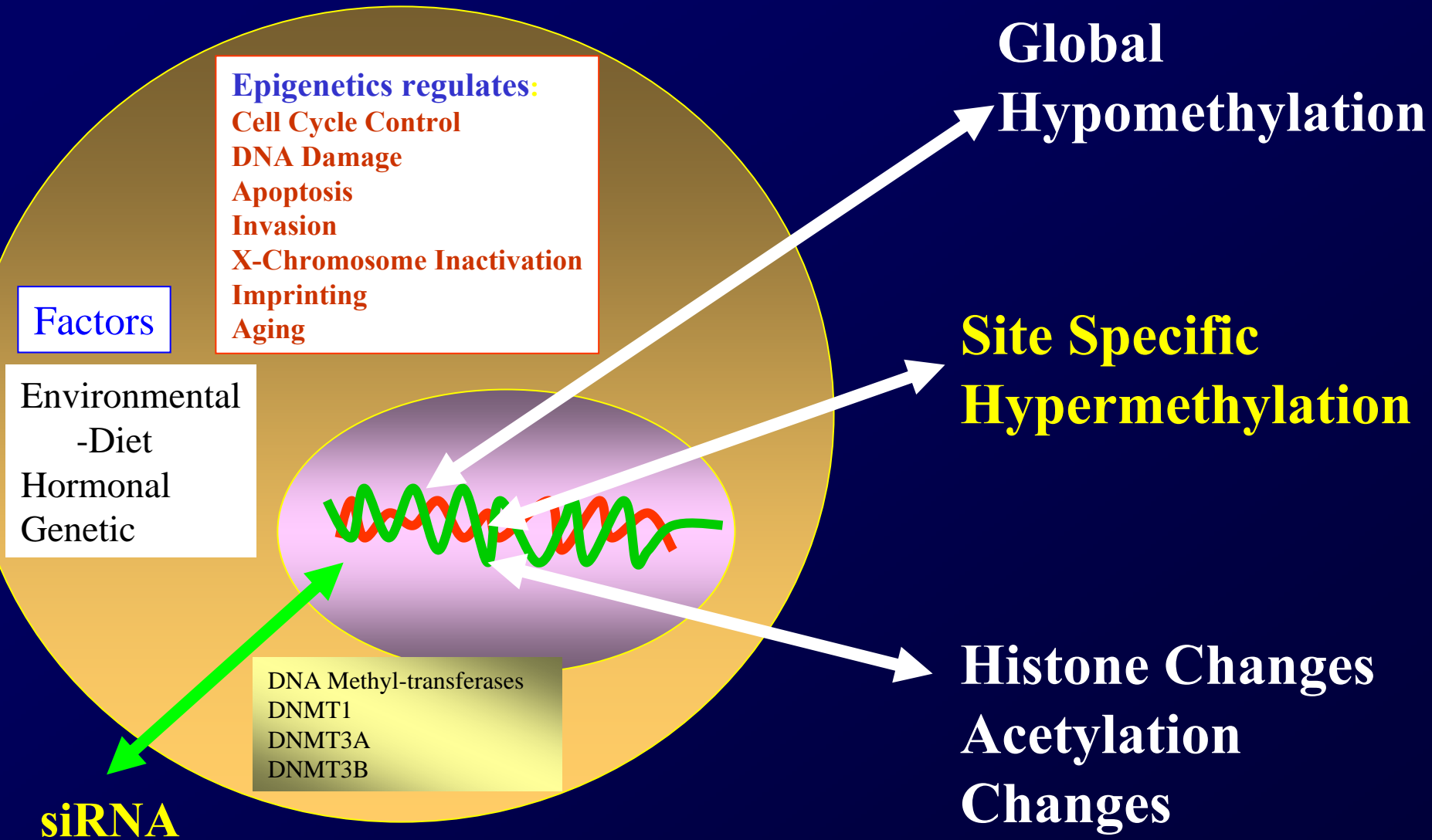


MY  
GENOME  
MADE ME  
DO IT!

FEYERS



# Epigenetic Regulation of Cancer



Verma and Srivastava (2002) Lancet Oncology 3:755-63.

PA-06-414 Diet, Epigenetic Events, and Cancer Prevention.

**LTR Hypomethylated**



**Yellow Mouse**

**High risk cancer, diabetes, obesity & reduced lifespan**

**When to Intervene??**

**Maternal Supplements with zinc, methionine betaine, choline, folate, B<sub>12</sub>**



**Or Genistein**

**LTR Hypermethylated**



**Agouti Mouse**

**Lower risk of cancer, diabetes, obesity and prolonged life**

# Oxidation and Chromatin Structure

**Oxidation of either a single guanine to 8-oxoguanine or of a single 5mC to 5-hydroxymethylcytosine**

**significantly inhibits binding of the methyl-CpG binding proteins to the oligonucleotide duplex, reducing the binding affinity by at least an order of magnitude.**

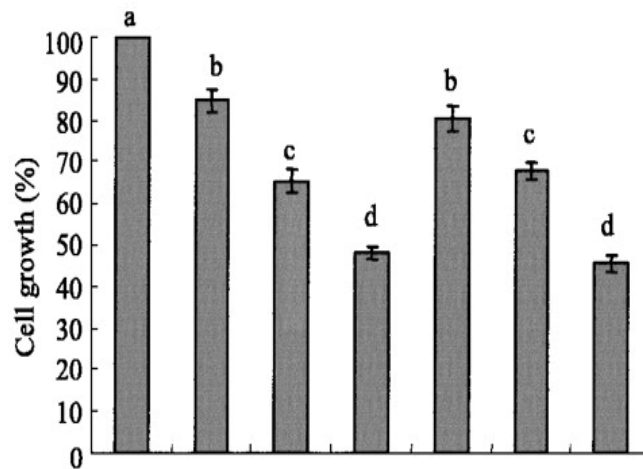
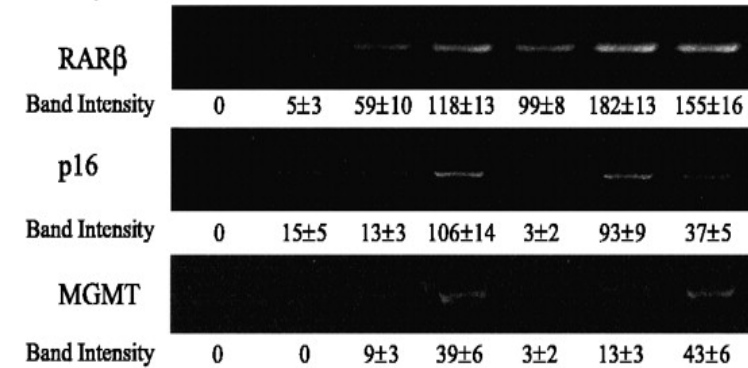
**Oxidative damage to DNA could therefore result in heritable, epigenetic changes in chromatin organization.**

**Valinluck et al. Nucleic Acids Res. (2004) 32:4100.**

# Reactivation of Genes by Dietary Components

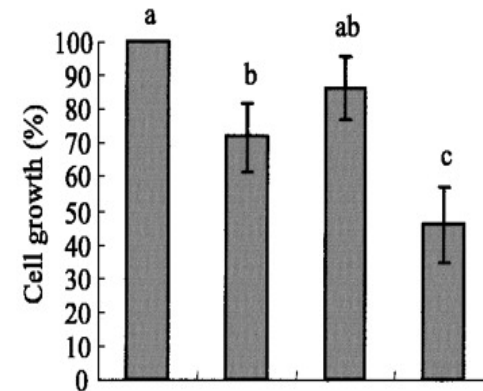
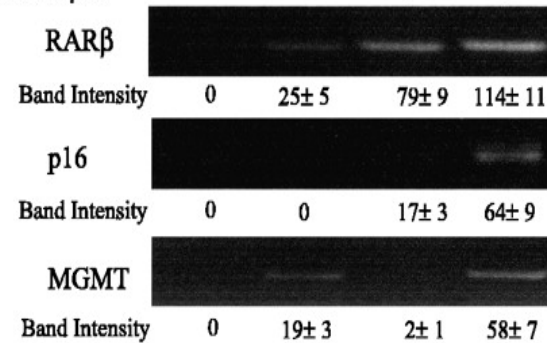
**A**

Genistein 5 $\mu$ M	-	+	-	+	-	+	-
TSA 0.5 $\mu$ M	-	-	+	+	-	-	+
DAC 2 $\mu$ M	-	-	-	-	+	+	+



**B**

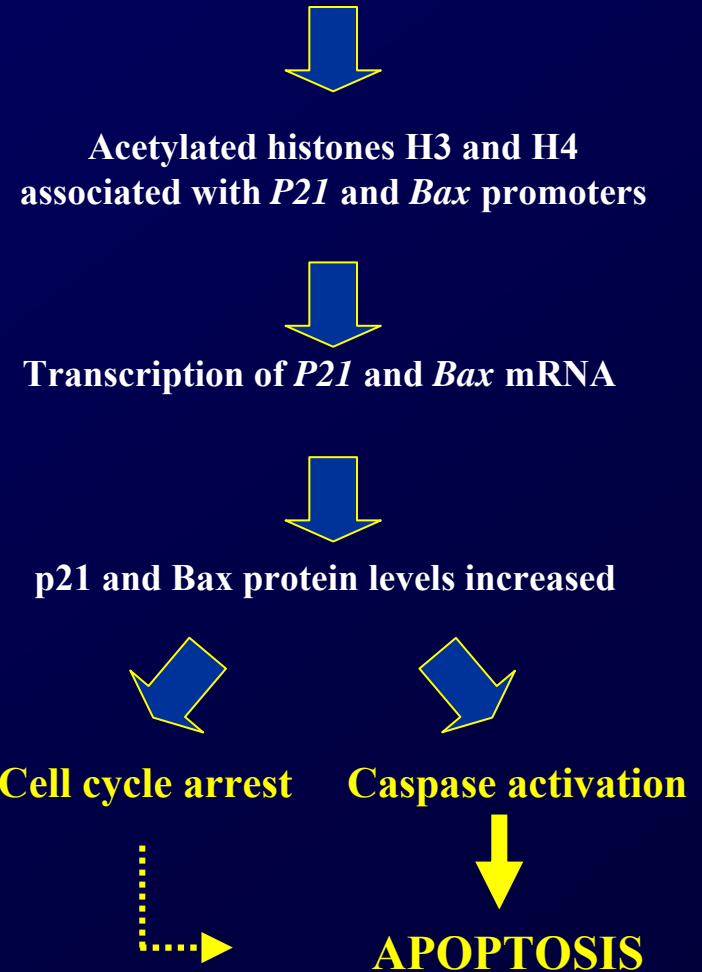
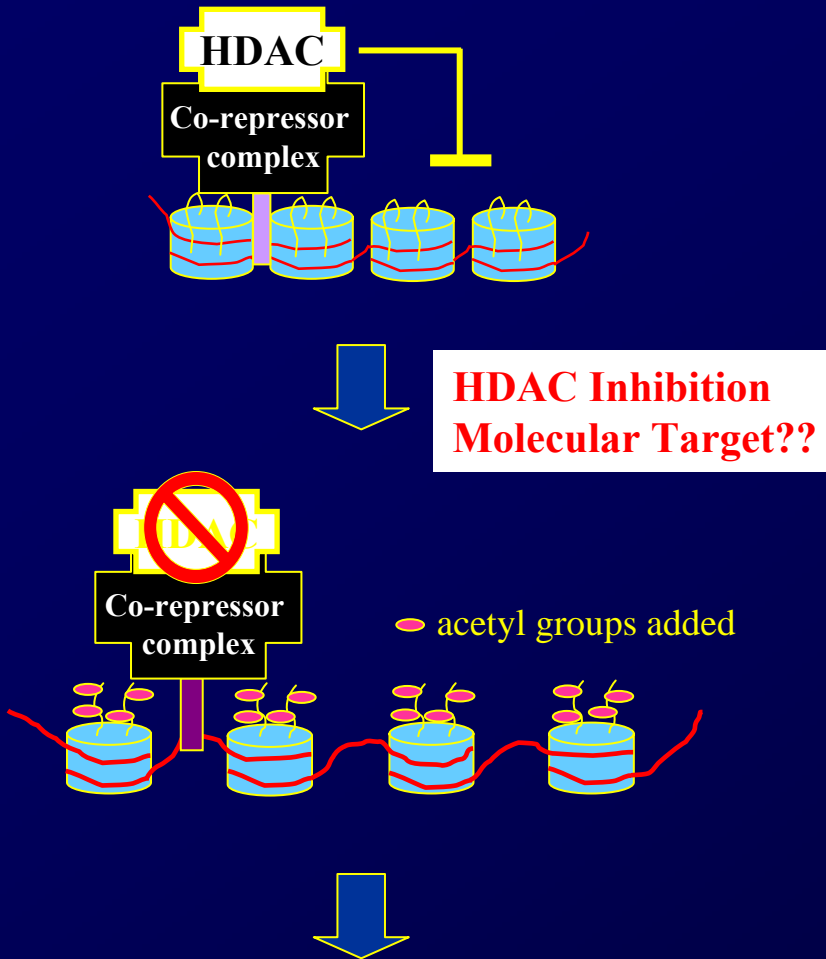
Genistein 5 $\mu$ M	-	+	-	+
SFN 15 $\mu$ M	-	-	+	+



Trichostatin =TSA 5-aza-dCyd= DAC

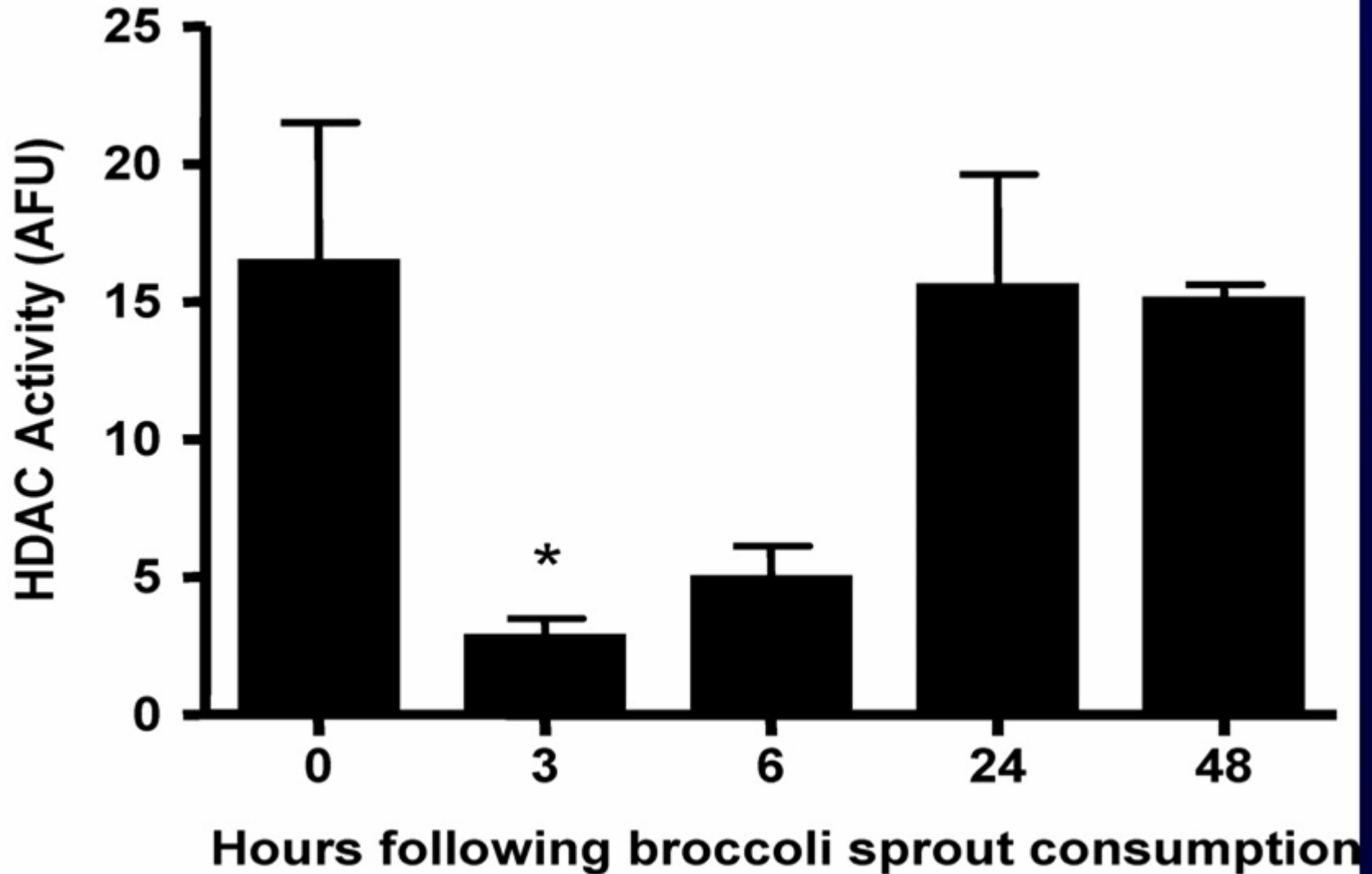
**Fang et al. Clin Cancer Res. (2005) 11:7033-41.**

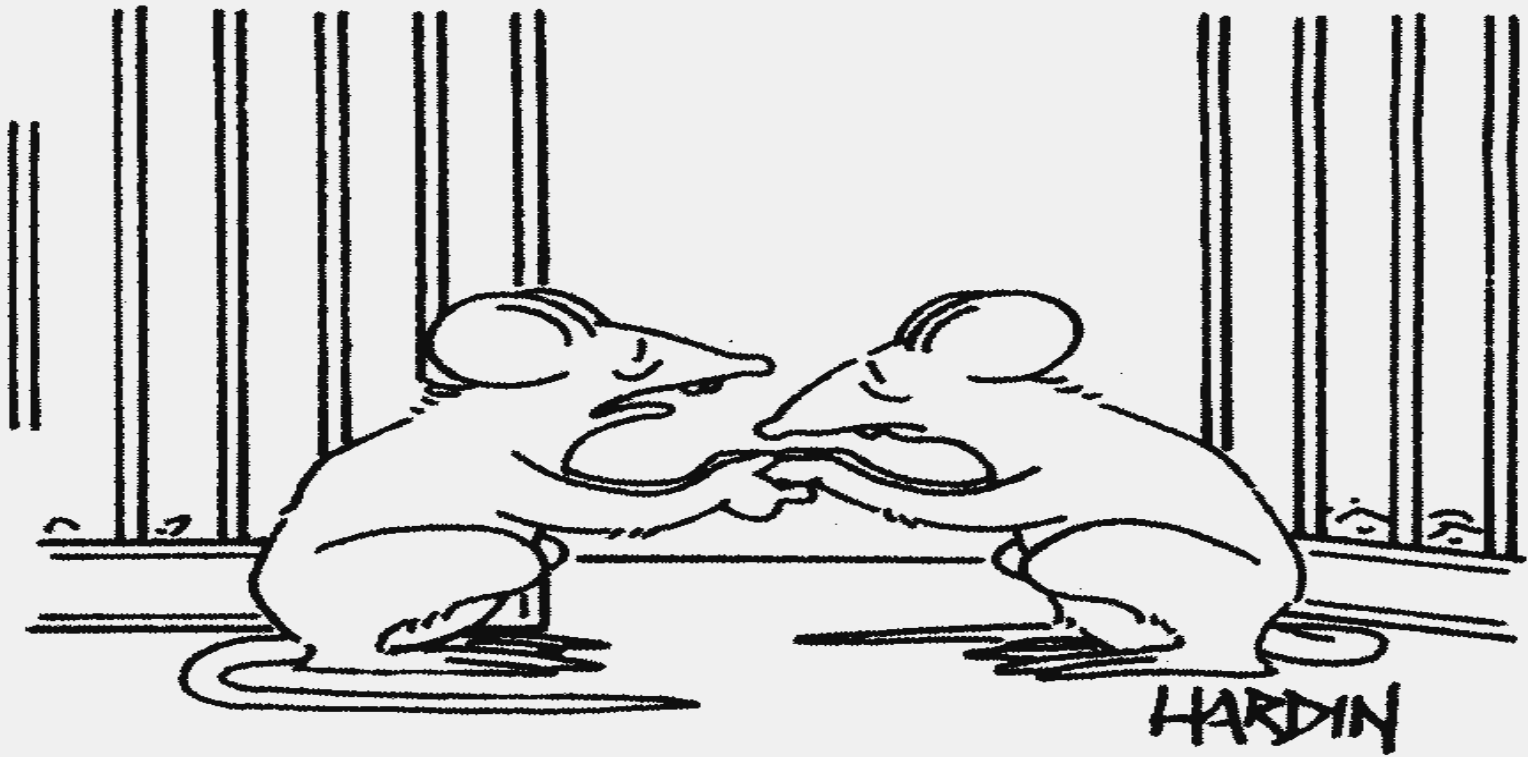
# Histones Can Be Regulated by Isothiocyanates, Allyl Sulfur, Genistein and Butyrate



Influence of diet on other histone modification process remains unknown

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

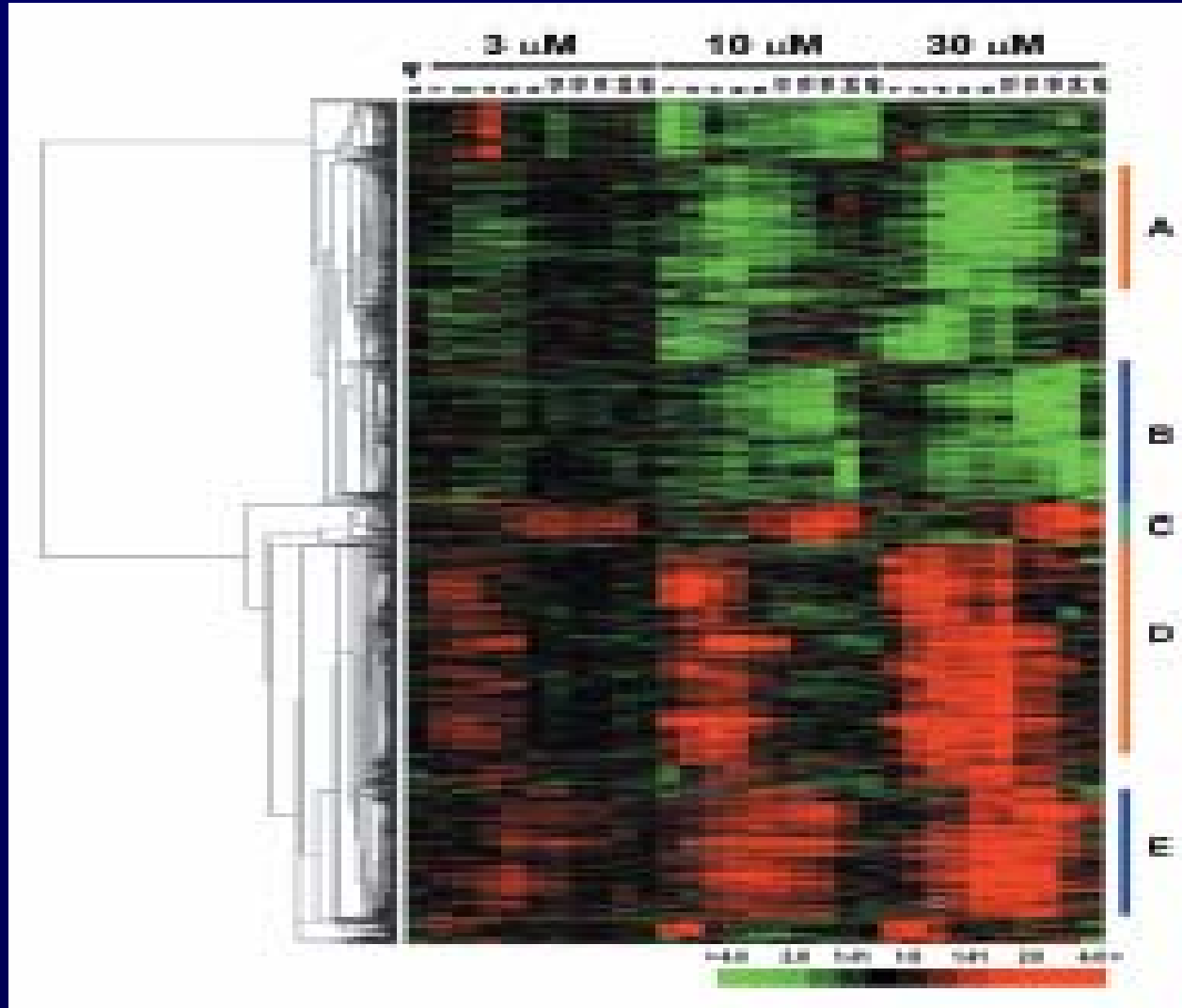




"It would never work, Nichole; I'm in the experimental group and you're in the control."

**Dietary Intervention Can Cause Shifts in Transcriptomic Expression**

# Transcriptomic Markers Are Providing Clues About Molecular Targets for Specific Food Components



Selenium in prostate cancer cells:

**Androgen Signaling**

**Proliferation/Cell cycle**

**Detoxification**

**Immune/stress**

**Apoptosis**

**Transcription**

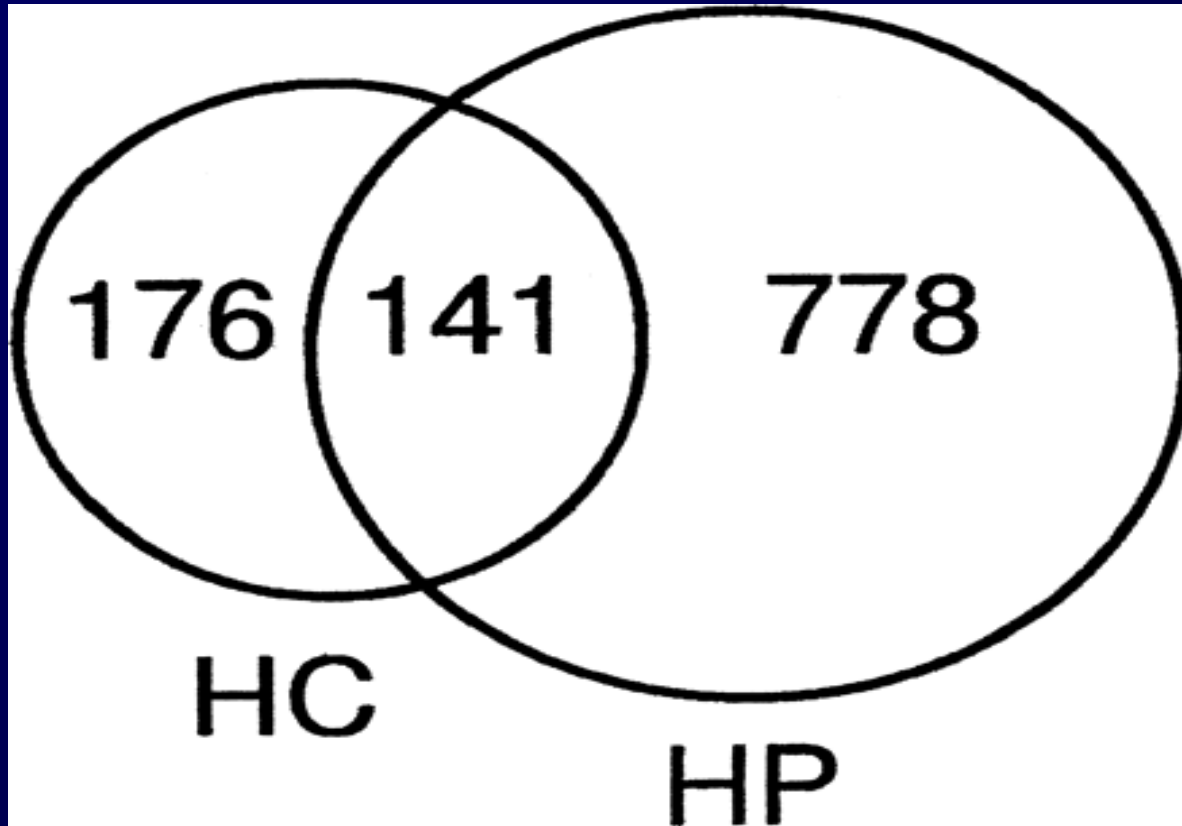
**Signal Transduction**

**Cell Shape**

Zhao et al., (2004) *Molec. Biol. Cell* 15: 506-519.



**Differentially expressed genes in human blood leukocytes after consumption of the high-protein (HP) breakfast, the high-carbohydrate (HC)**



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

# Combinations May Influence The Quantity Needed For A Response?

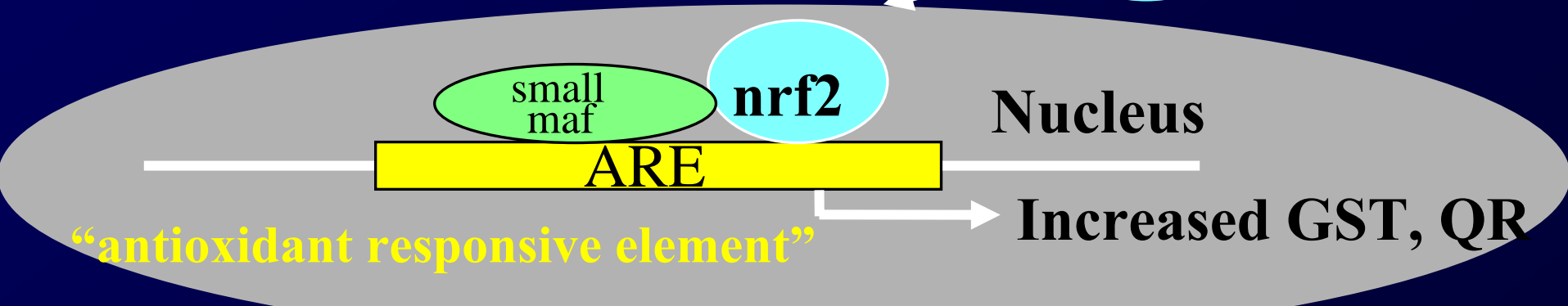
Garlic, fish, broccoli



Active Intermediate (radical??)



Cytoplasm

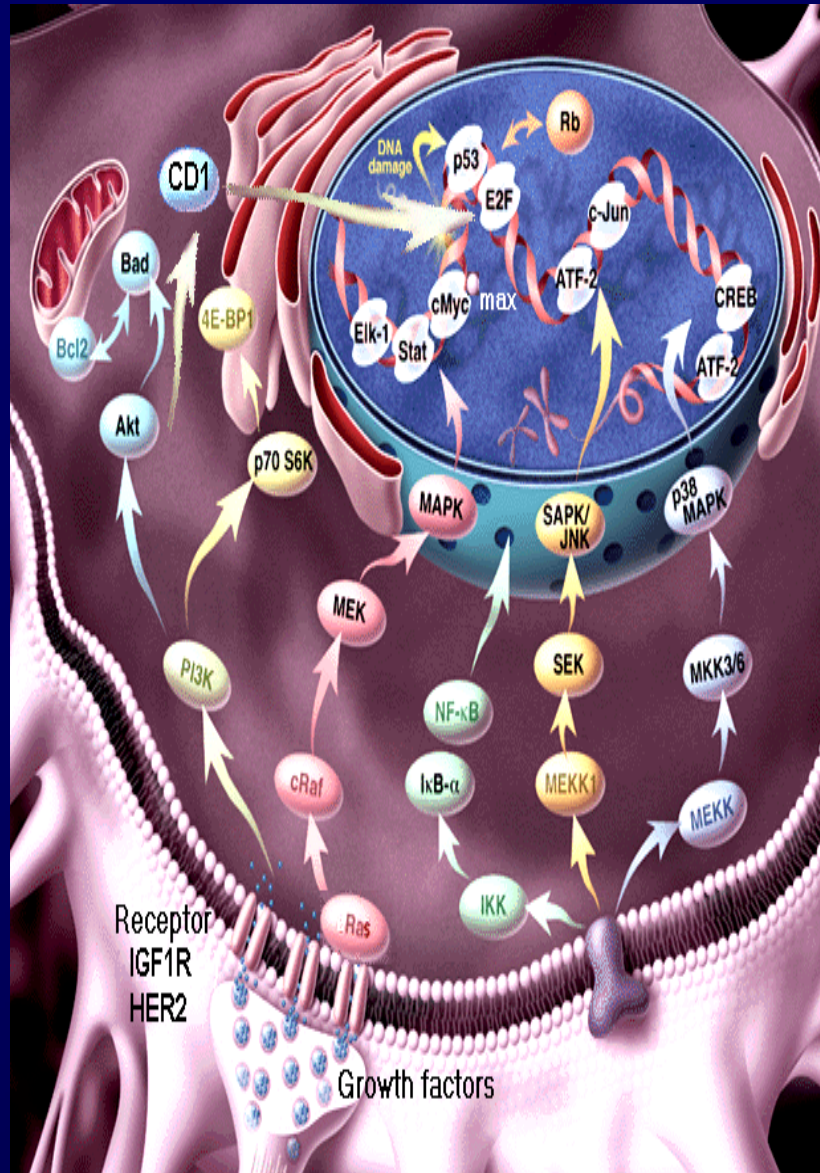


“antioxidant responsive element”

Nucleus

Increased GST, QR

# A Molecular Approach to Medicine



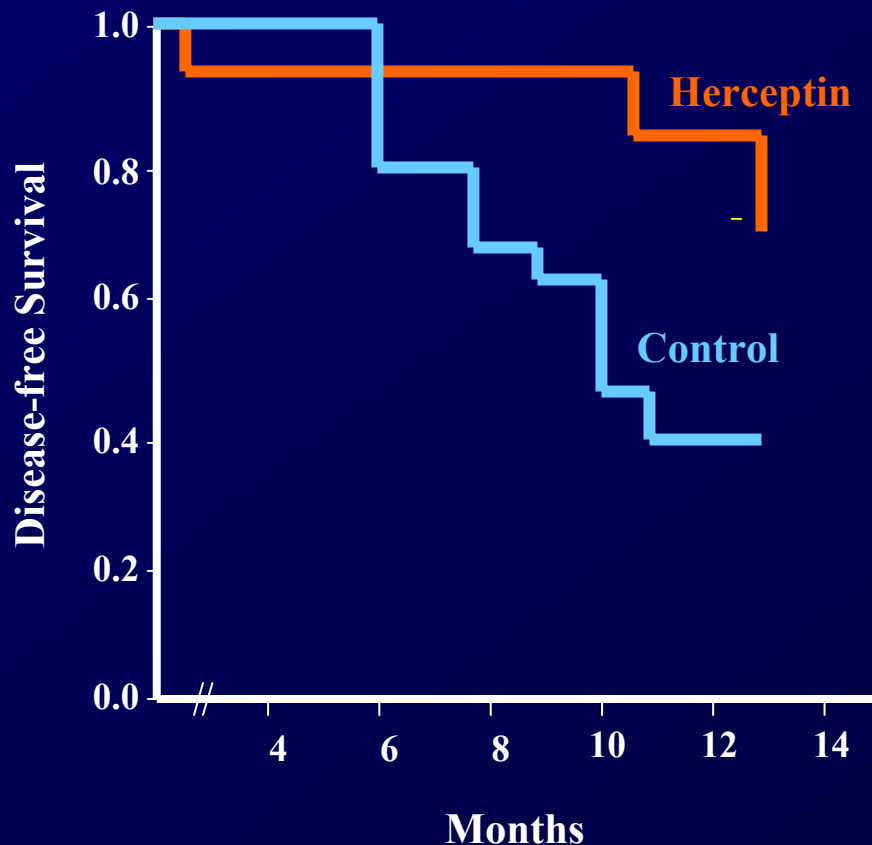
**Recent News Release suggests:**  
Herceptin is a Novel Pioneering Drug for Personalized Medicine Approach Based on Pharmacogenomics to block Her2-neu expression.

**Evidence Has Existed for Years that:**

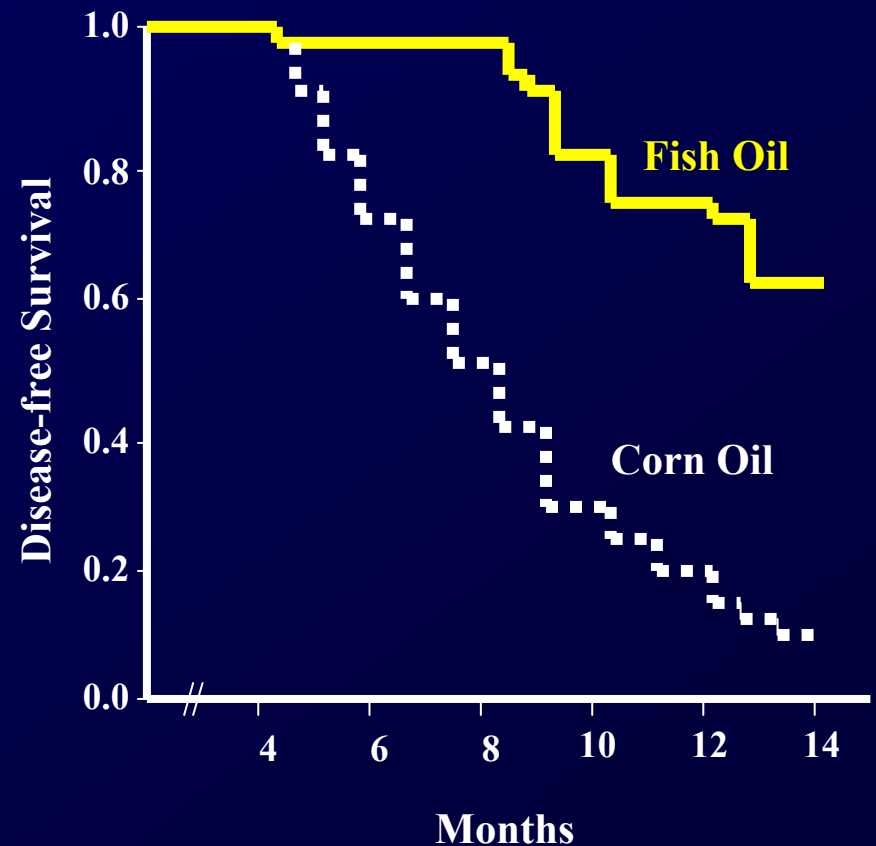
EGCG from Green Tea, Oleic Acid from Olive Oil, and n-3 fatty acids from Fish Oil and Apigenin from parsley, thyme, and peppermint can significantly influence HER2neu expression!

**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

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

**Inflammatory Stimuli**  
(chemicals, ROS, bacteria,  
viruses)

**Polymorphisms**  
(Pro- and Anti  
Inflammatory Genes)

**Inflammatory Response**

**Disease**

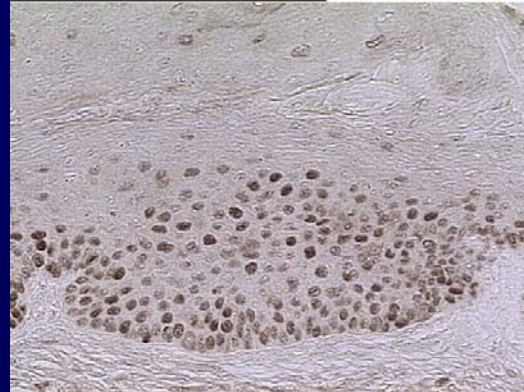
**Morbidity**

**Mortality**

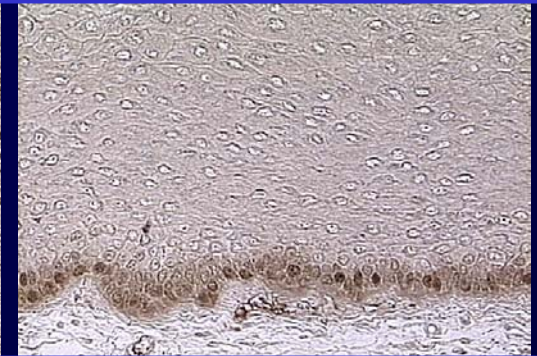
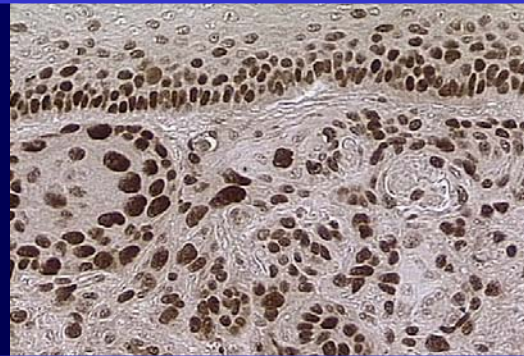
**K14-HPV16  
Transgenic  
Mouse**

**Background  
Mouse**

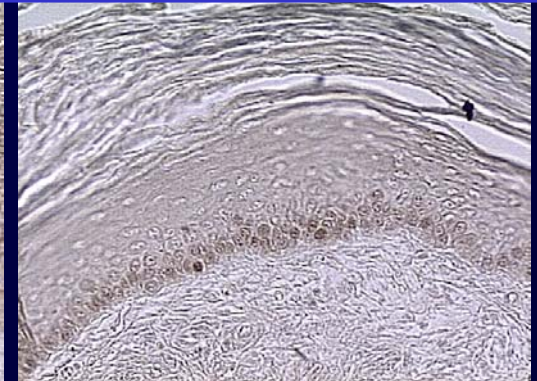
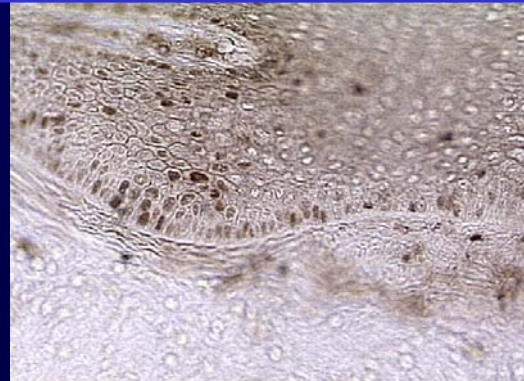
**No Estrogen  
Normal Diet**



**Estrogen  
Normal Diet**



**Estrogen  
Diet + I3C**



**Proliferation Assay: PCNA by  
immunohistochemistry**

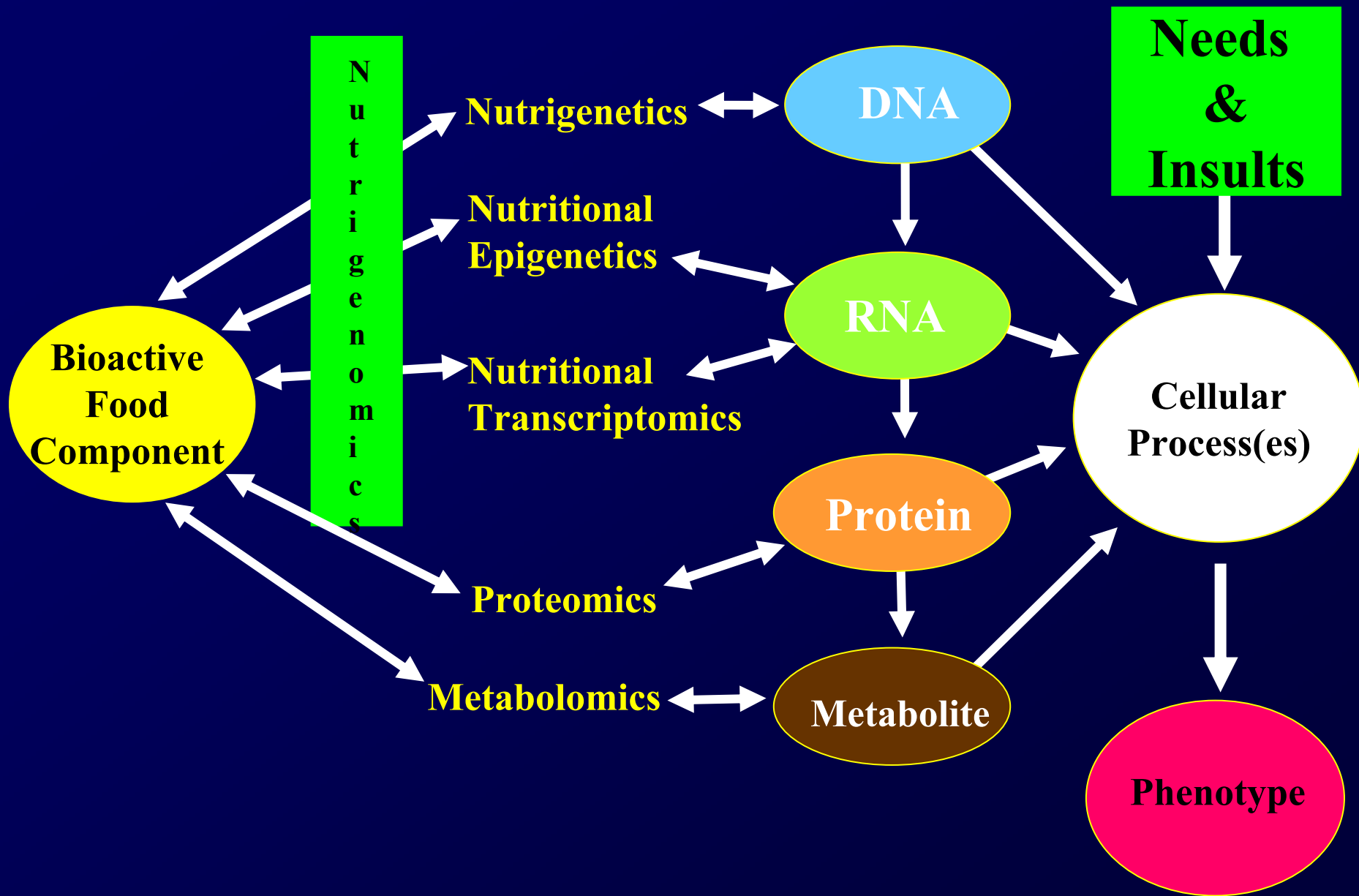
**Elevated thiobarbituric acid reactive substances, conjugated dienes and lowered activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione in the liver, heart, kidney, intestine and aorta observed**

**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.**





# Proteomics and Metabolomic Technologies and the Response to Bioactive Food Components



**Mitchell et al. (2005) Proteomics 5: 2238**  
**Identified 2 peaks that reflect with 76% accuracy broccoli intake**

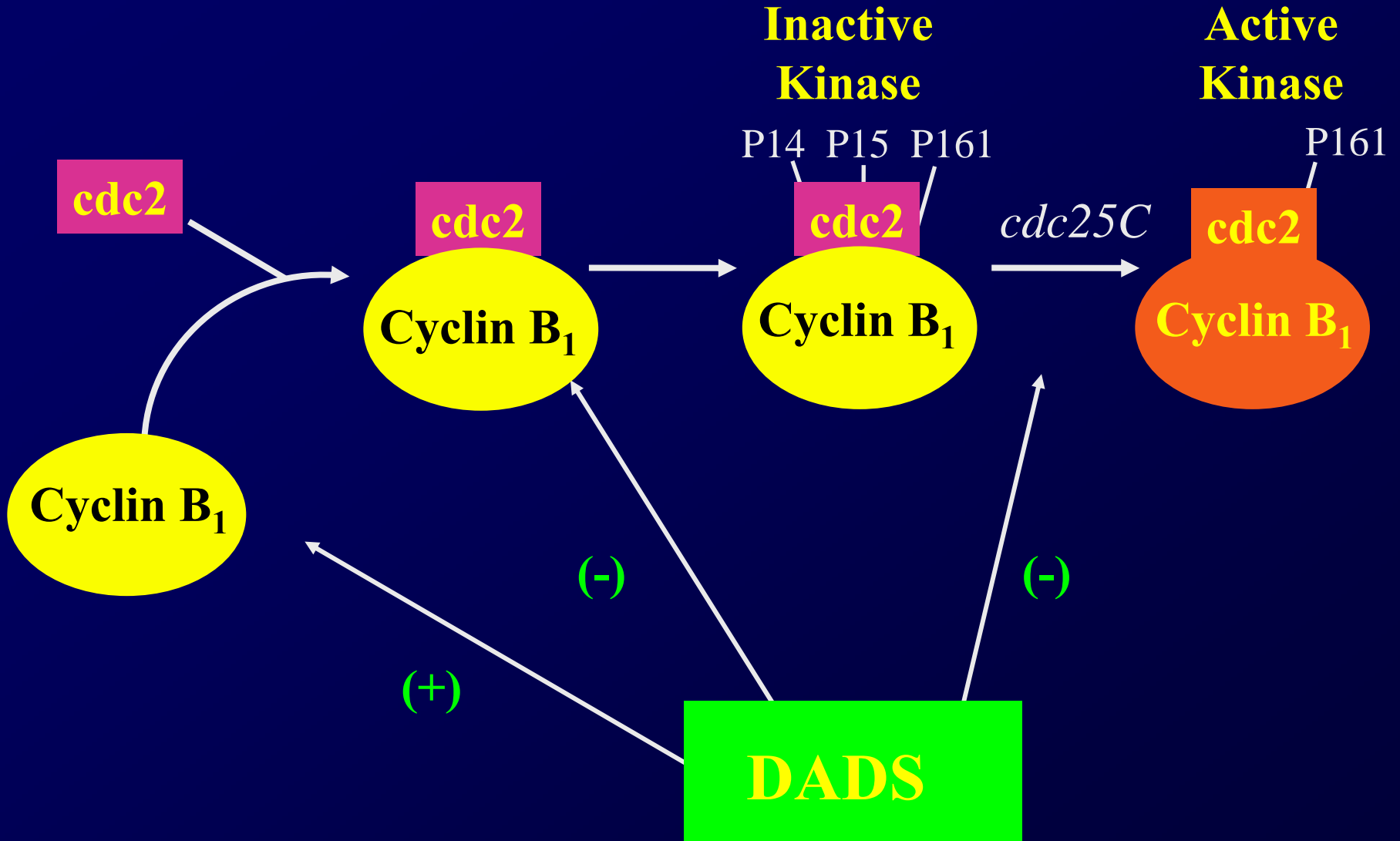
**Wenzel et al. (2004) Proteomics 4:2160**  
**Various proteins modified by quercetin**

**Yang et al (2004) Obesity Res 12:1179**  
**Exercise and quantitative proteomics**

**Linke et al (2006) J Chrom 1123:160.**  
**Vitamin A dependent proteins.**

**Mayr et al. (2005) Arterioscler Thromb Vasc Biol. 25:2135.** **Metabolomics demonstrate gene interactions with inflammation, oxidative stress, and energy metabolism.**

# Primary Effect of Allyl Sulfur is to Block Cell Division by Changing Phosphorylation



# We Must Be Careful About Messages to Public



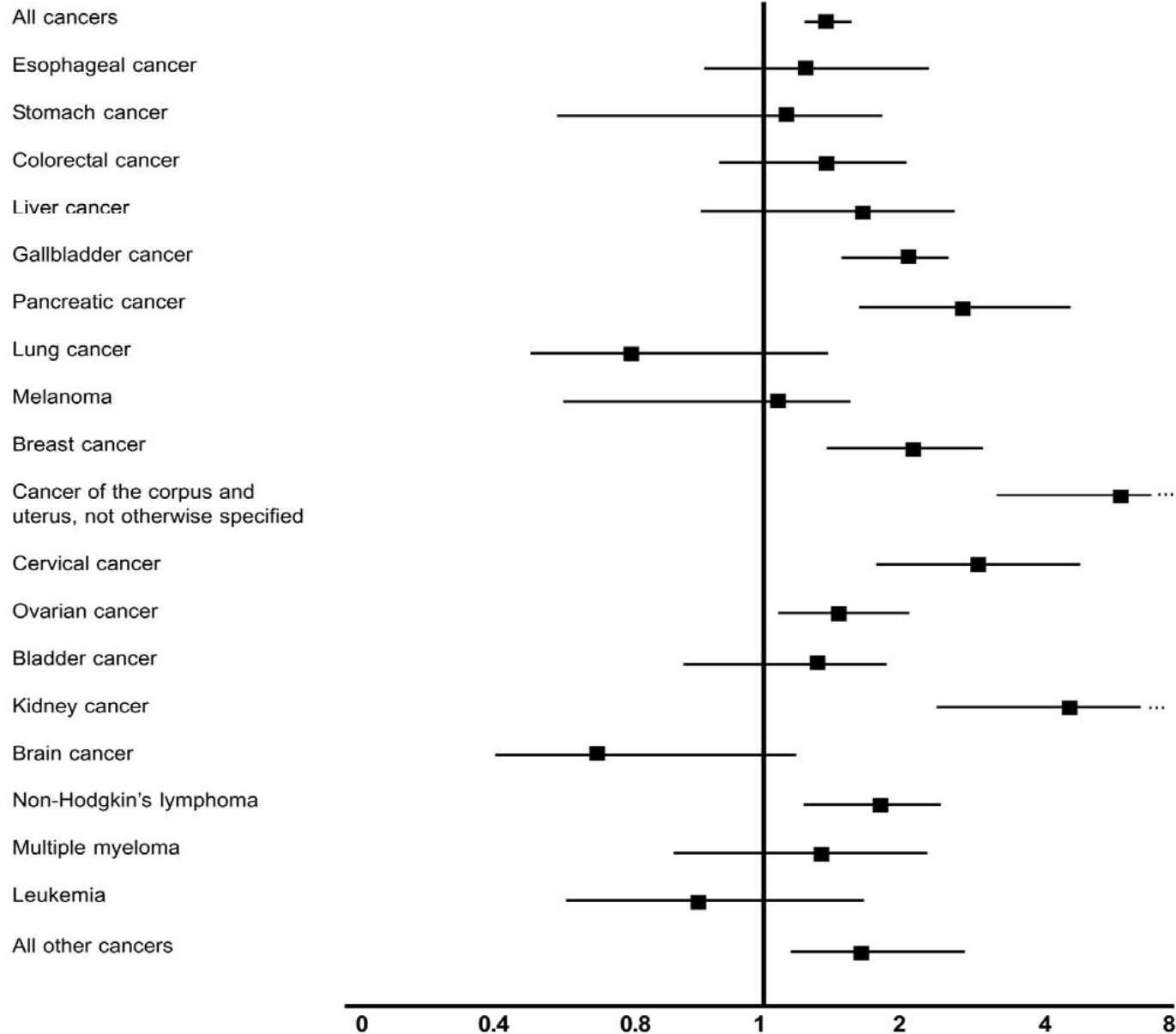
**One Size Does Not Fit All! Genomic Background Will Help Identify Responders to Foods and Components**



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

# Body Mass index and Cancer Mortality Women

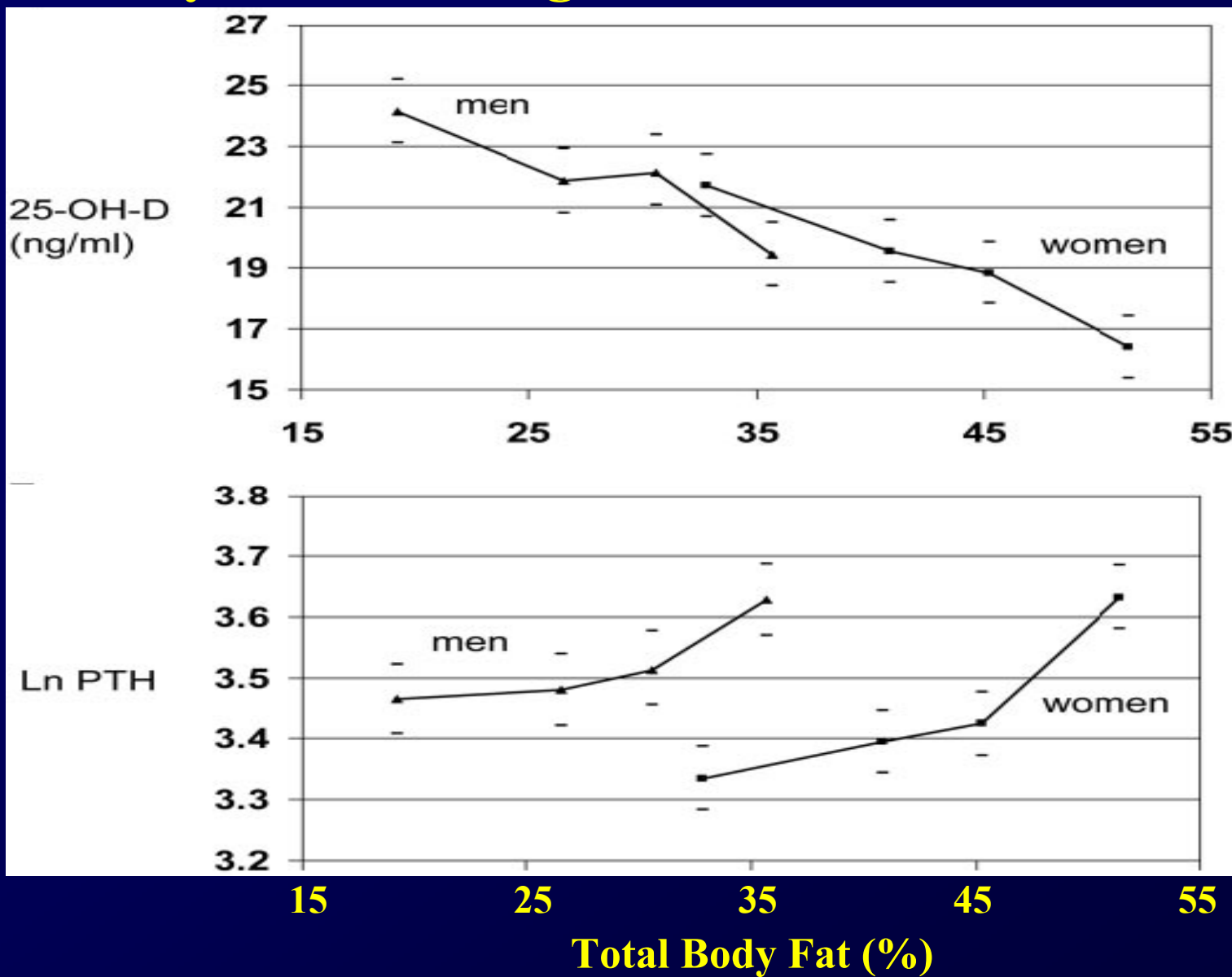
## Type of Cancer



20% of cancer deaths in women

Source: Calle et al NEJM 2003; 348: 1625-38

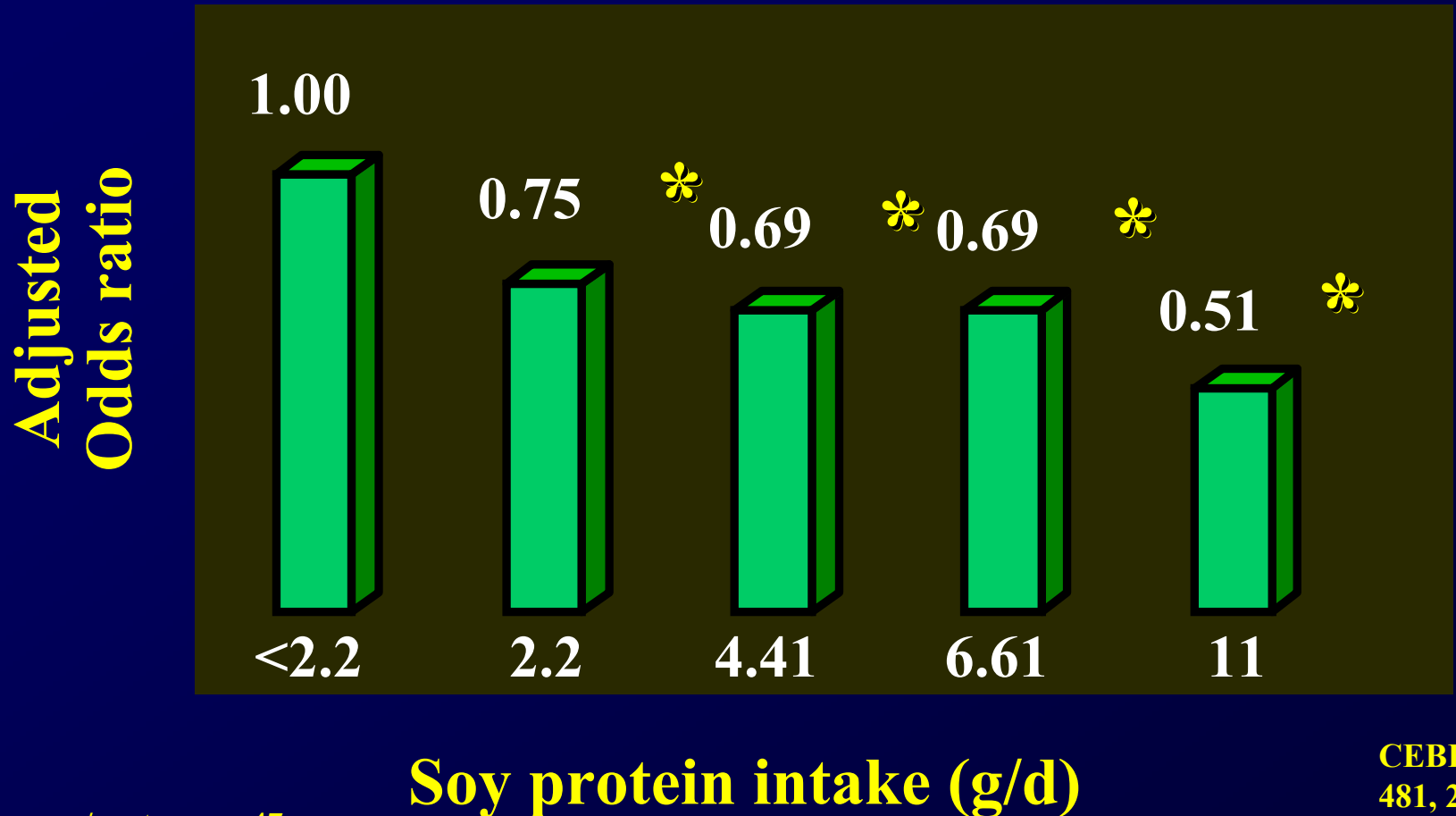
# Could Obesity Be Reflecting Some Other Metabolic Effect??



**Timing of Dietary Change  
Also  
Important!!**

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

(Shanghai: 1459 cases, 1556 cont)



CEBP 10:  
481, 2001

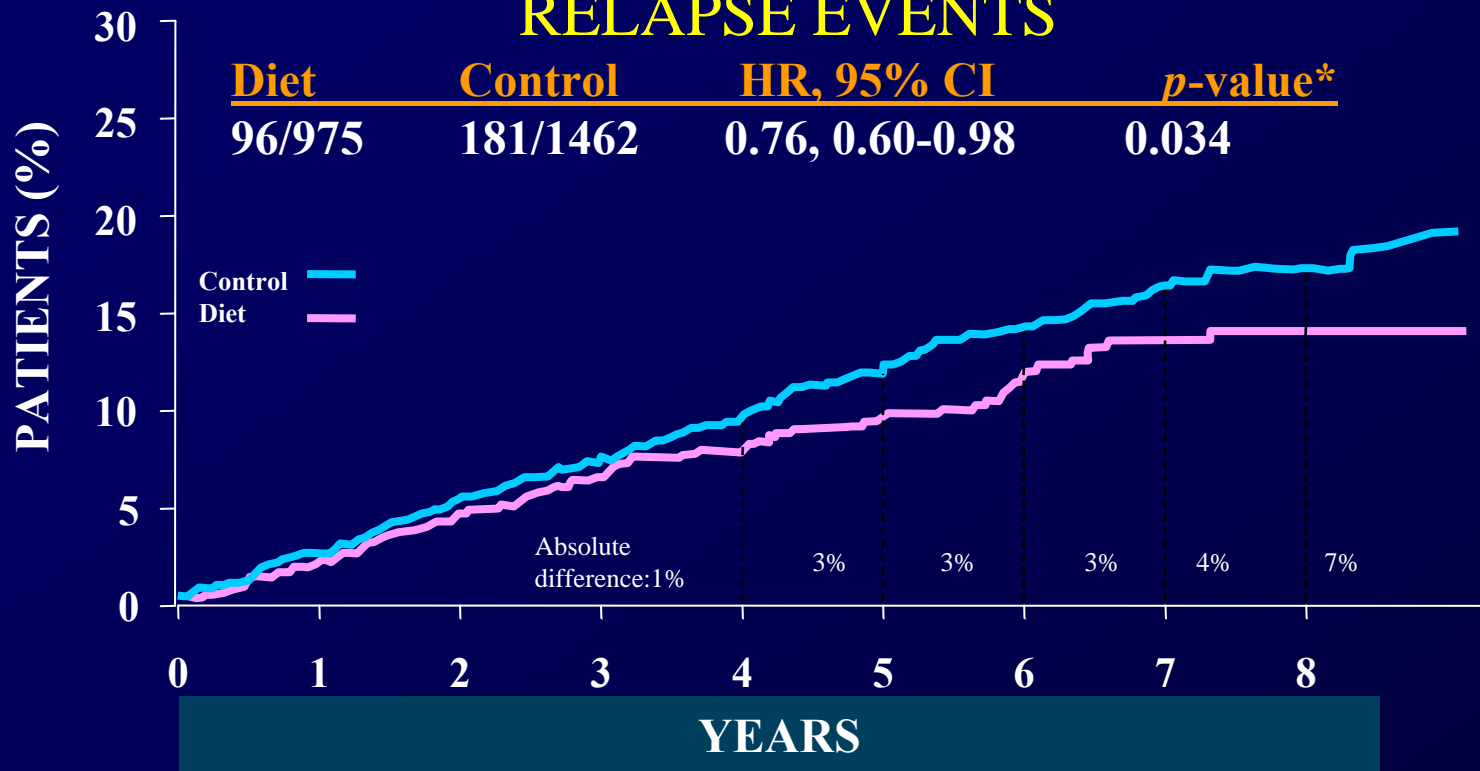
Results: pre/post, x age 47.  
Other legumes not protective.

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

# May Take Time to Observe A Response!

## Women's Intervention Nutrition Study (WINS)

### RELAPSE EVENTS



Diet	975	949	907	807	647	490	342	201	96
Control	1462	1416	1352	1197	965	756	529	326	151

\* From adjusted Cox proportional hazards model including: stratification factors, ER status, tumor size, and surgery (mastectomy/lumpectomy), p value = 0.067 by unadjusted log rank test

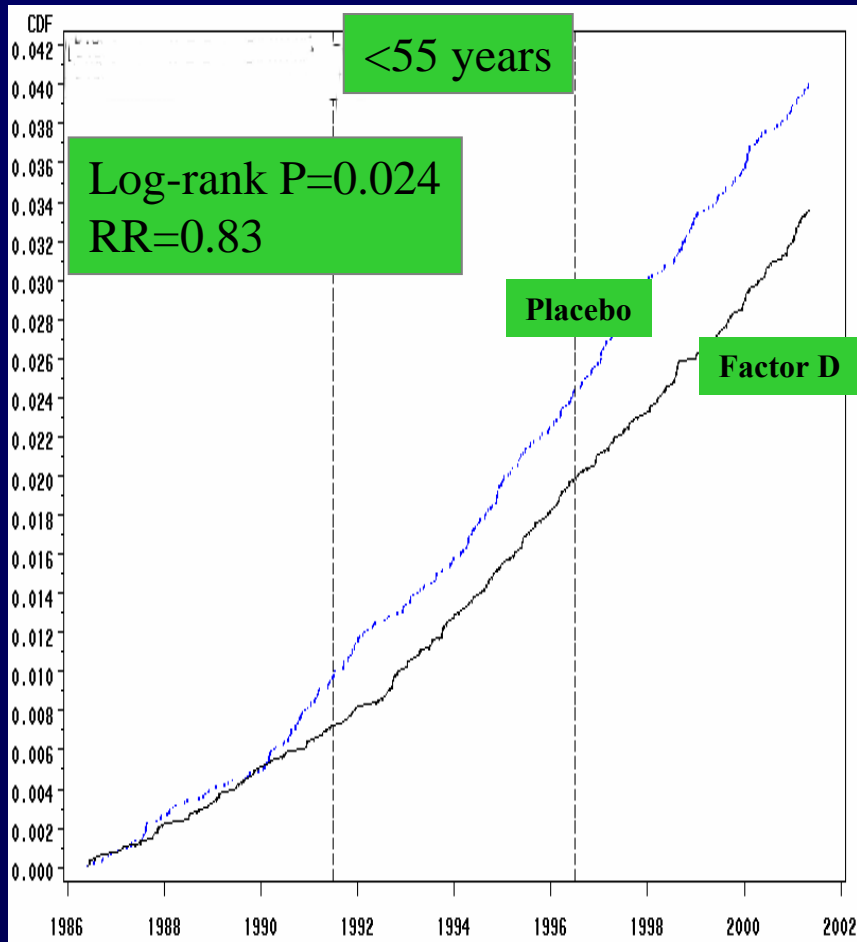
**Chlebowski et al (2006) J Natl Cancer Inst. 98:1767-76.**



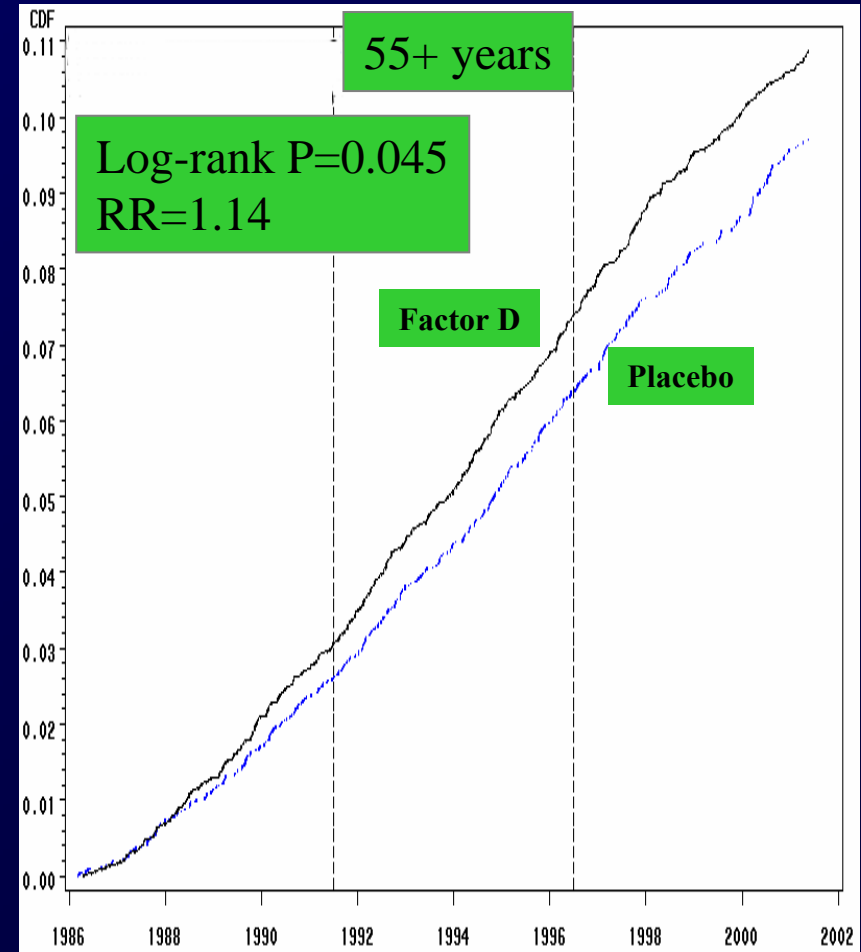
# Linxian Nutrition Intervention Trial

## Esophageal cancer mortality by factor D (N=1515)

**Factor D= Selenium,  $\beta$ -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!**

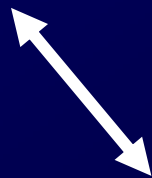


# The Future

*Transform Medicine from Curative to Preemptive*

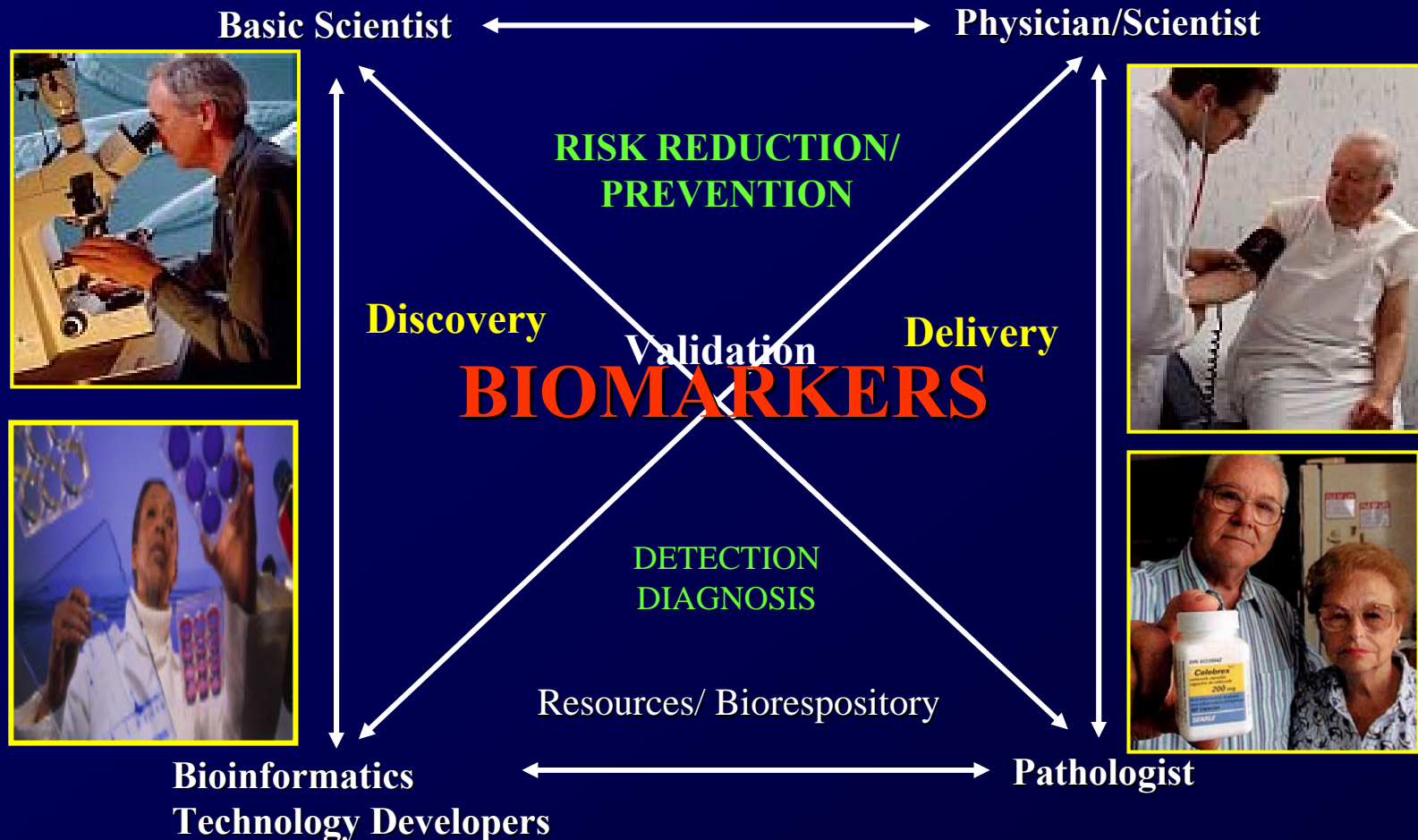


Predictive ↔ Personalized ↔ Preemptive



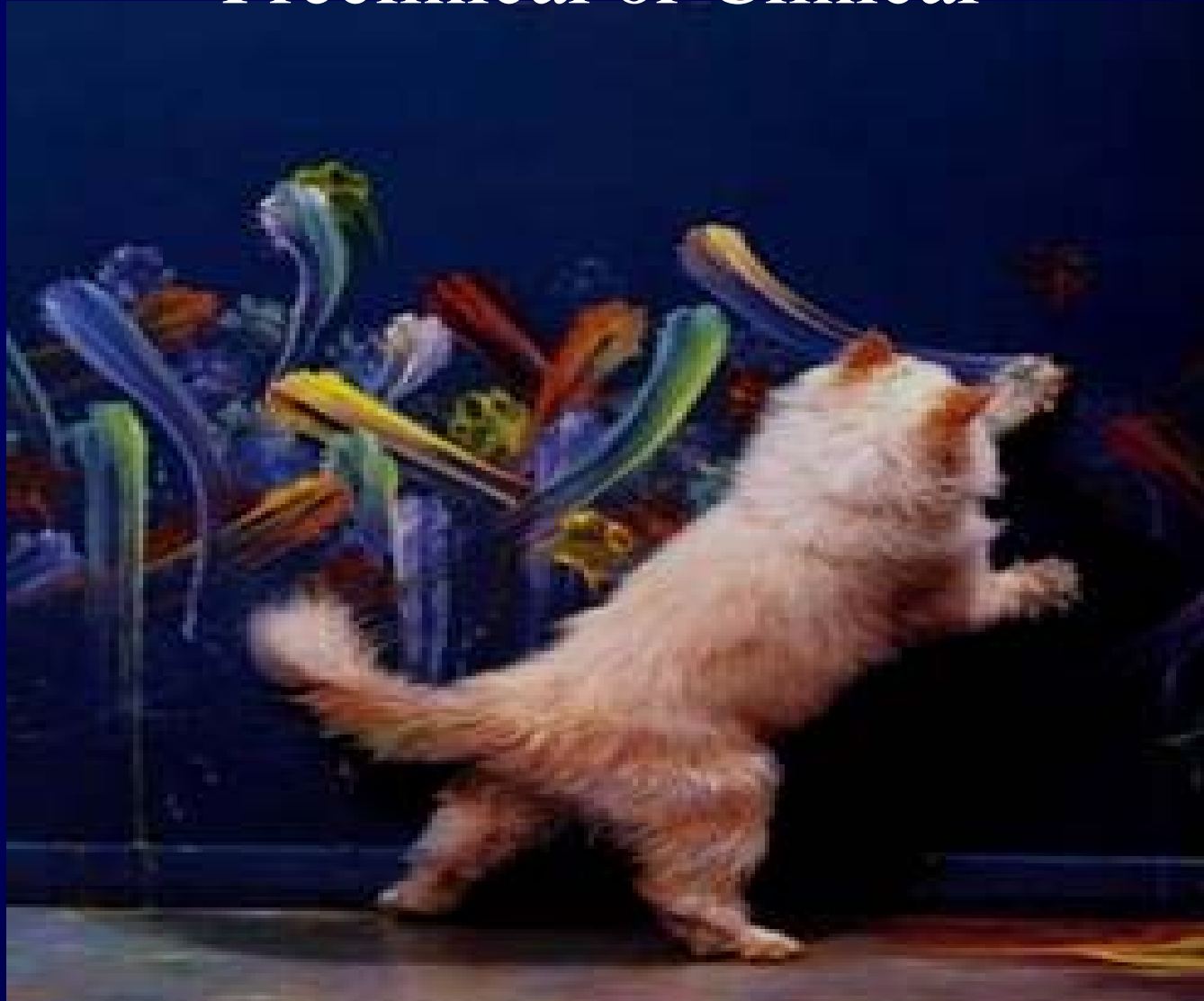
*Participatory*

# SYSYEMS BIOLOGY APPROACH TO BIOMARKERS RESEARCH



**Could Defining a Nutritional Phenotype Be the Future for Reducing Risk and Enhancing Therapy ?**

# Be Creative in Whatever You Do Preclinical or Clinical



# Develop Partnership: Build on Strengths



**Unprecedented Analytical Issues Surround**

**Defining:**

**Which Dietary Components Are Most  
Important**

**In What Amounts and When!!**

**Who will Respond Favorable  
and**

**Who will be Placed at Risk**

<http://www3.cancer.gov/prevention/nutrition/>



# Review

- **Why is there variation in response to specific food components?**
  1. **Different genetic metabolism**
  2. **Different absorption profiles**
  3. **Different excretion time profiles**
  4. **All of the above**
  
- **Which of the following terms applies to the food components effect on DNA?**
  1. **Nutrigenetics**
  2. **Nutritional Epigenetics**
  3. **Nutritional Transcriptomics.**
  
- **Of the 3 Vitamin D receptor genotypes (TT, Tt & tt) which is the most effected by increased caffeine intake?**
  1. **TT**
  2. **Tt**
  3. **tt**
  
- **What dietary changes mimic the effects of Herceptin in HER2 neu expression?**
  1. **EGCG from green tea**
  2. **N-3 fatty acids from Fish Oil**
  3. **Apigenin from parsley, thyme and peppermint**
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