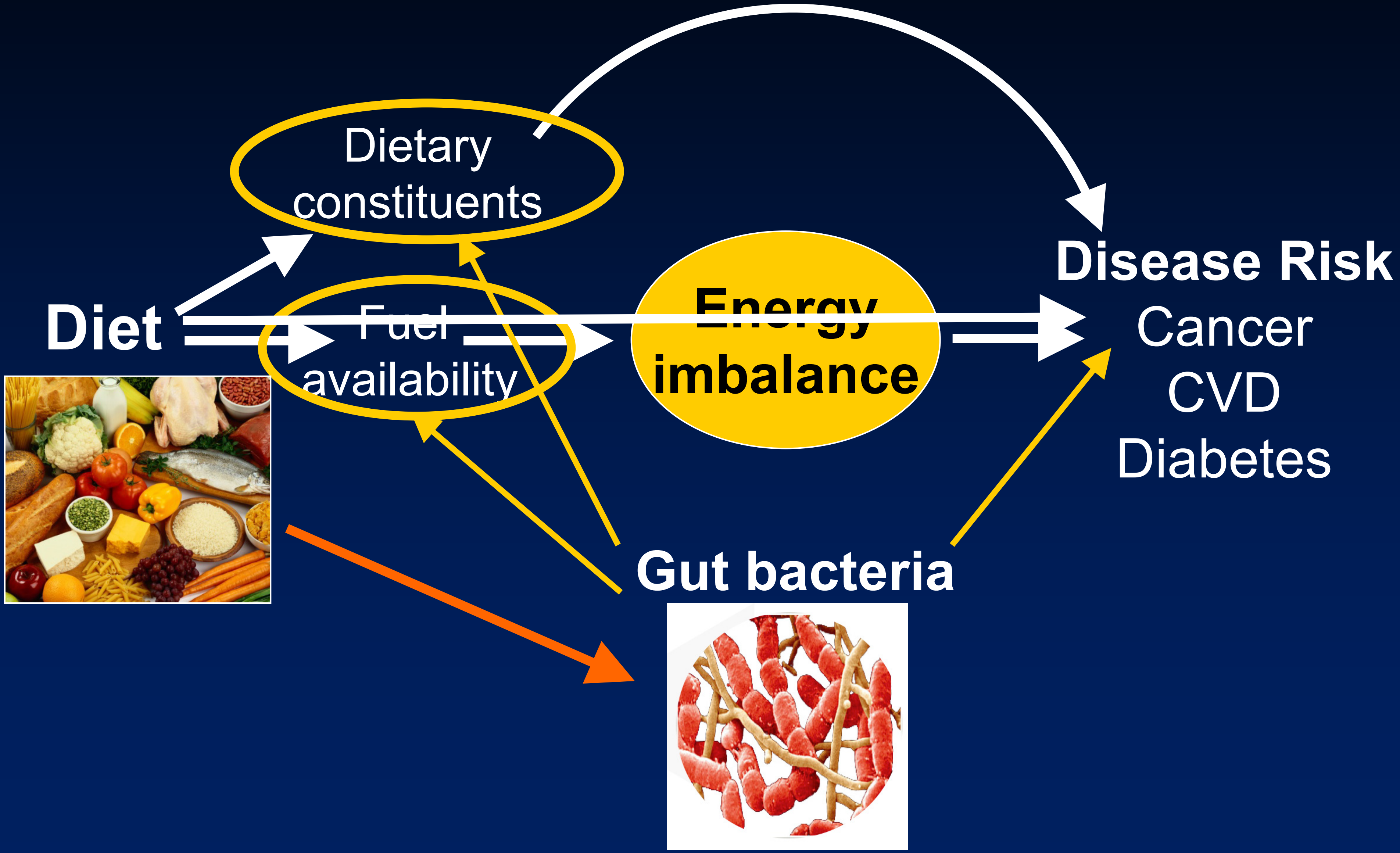
"Human Microbiome Science: Vision for the Future "
July 24-26, 2013, Bethesda, MD

Gut Microbial Metabolism of Food Constituents: Modulating Human Dietary Exposures

Johanna W. Lampe, PhD, RD
Meredith A.J. Hullar, PhD
Division of Public Health Sciences
Fred Hutchinson Cancer Research Center, Seattle WA

Relationship of Diet and the Gut Microbiome to Health and Disease



Diet

Dietary constituents

Fuel availability

Energy imbalance

Disease Risk
Cancer
CVD
Diabetes

Gut bacteria

Outline

- What are the gut microbes doing with our food?
- What is the effect of the gut microbiome on host dietary exposures?
- How might this influence disease risk?

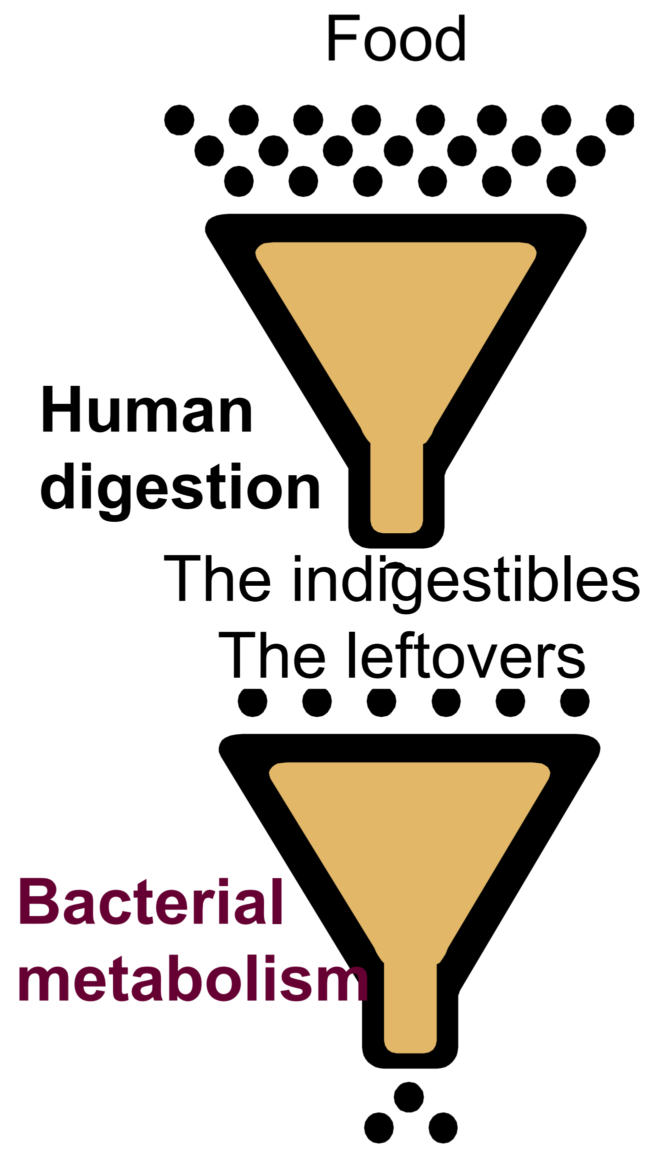
- Gaps, needs, and challenges

The human diet is complex.

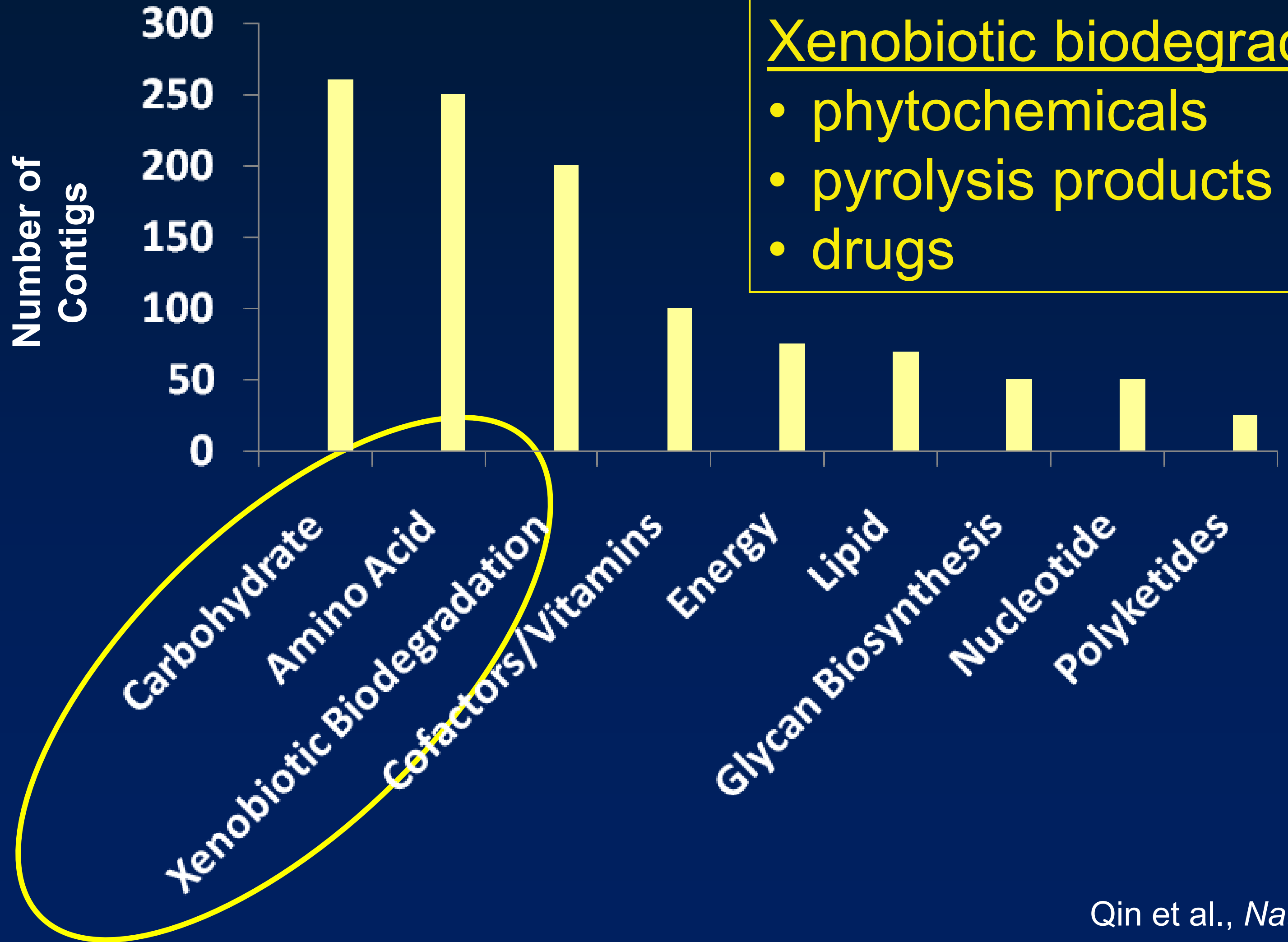
- 1000s of compounds
- Variety of methods of food preparation
  - Structure and particle size
  - Bioavailability to host

Gut Microbial Metabolism -- Designed to make the most of the situation

- Fermentation
- Reduction
  - -- nitrate, sulfate
- Esterification
- Aromatic fission
- Hydrolysis/deconjugation
  - -- glycosides
  - -- glucuronide conjugates



Distribution of Metabolic Pathways in the Gut Microbiome

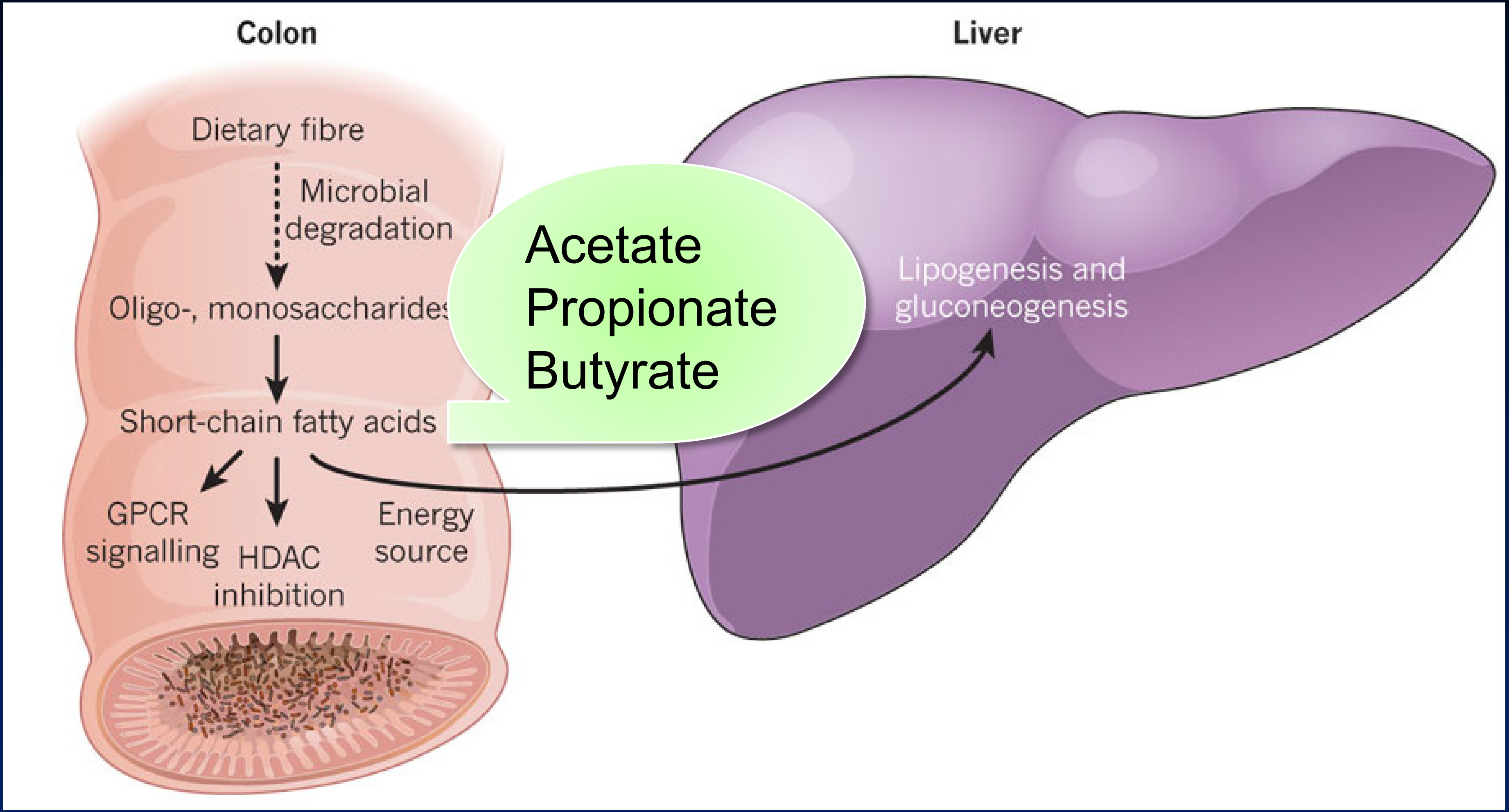


Xenobiotic biodegradation

- phytochemicals
- pyrolysis products
- drugs

Qin et al., *Nature*, 2010, 464:59

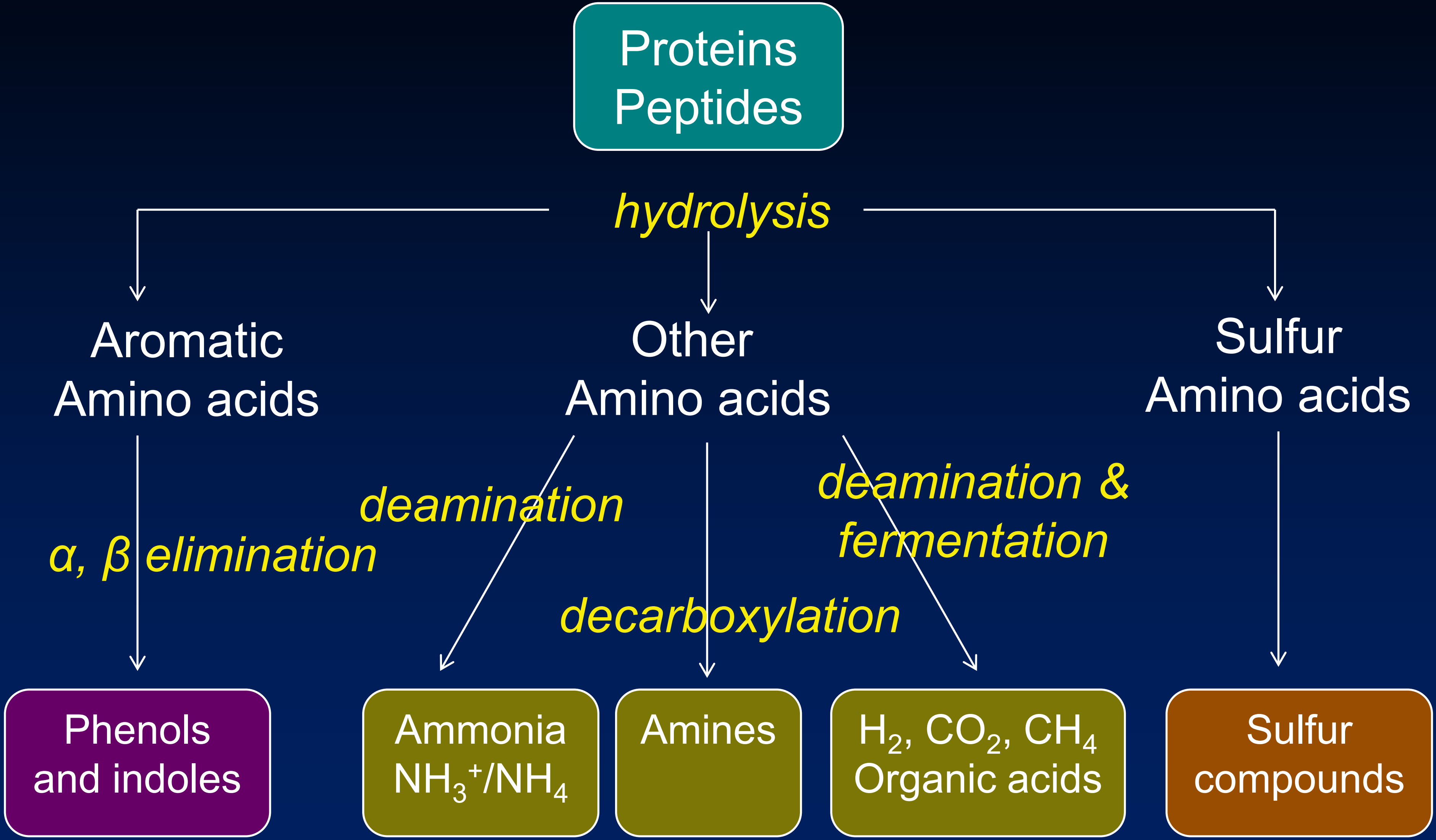
Fermentation of Carbohydrates



Acetate
Propionate
Butyrate

Tremaroli & Bäckhed, *Nature*, 2012

Microbial Metabolism of Proteins & Amino Acids



Proteins
Peptides

hydrolysis

Aromatic
Amino acids

Other
Amino acids

Sulfur
Amino acids

α, β elimination

deamination

decarboxylation

deamination &
fermentation

Phenols
and indoles

Ammonia
NH_3^+/NH_4

Amines

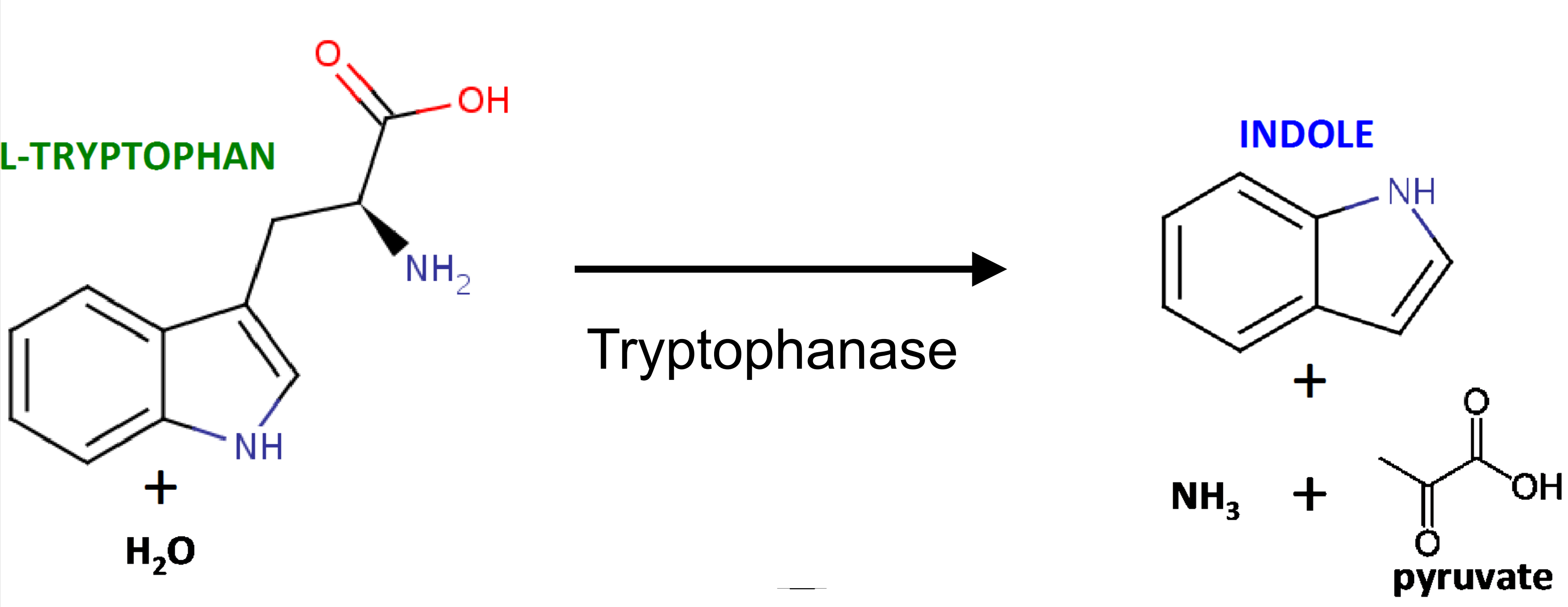
H_2, CO_2, CH_4
Organic acids

Sulfur
compounds

Adapted from Nyangale et al. *J Proteome Res*, 2012

Aromatic Amino Acid Metabolism:

Conversion of *L*-Tryptophan to Indole



- Concentration in human and rodent lumen – 0.1 to 4 mM
- Modulates expression of pro- and anti-inflammatory genes
- Strengthens epithelial cell barrier properties
- Decreases pathogen colonization

Bansal T et al. *PNAS* 2010
Slide courtesy of R Alaniz, Texas A&M

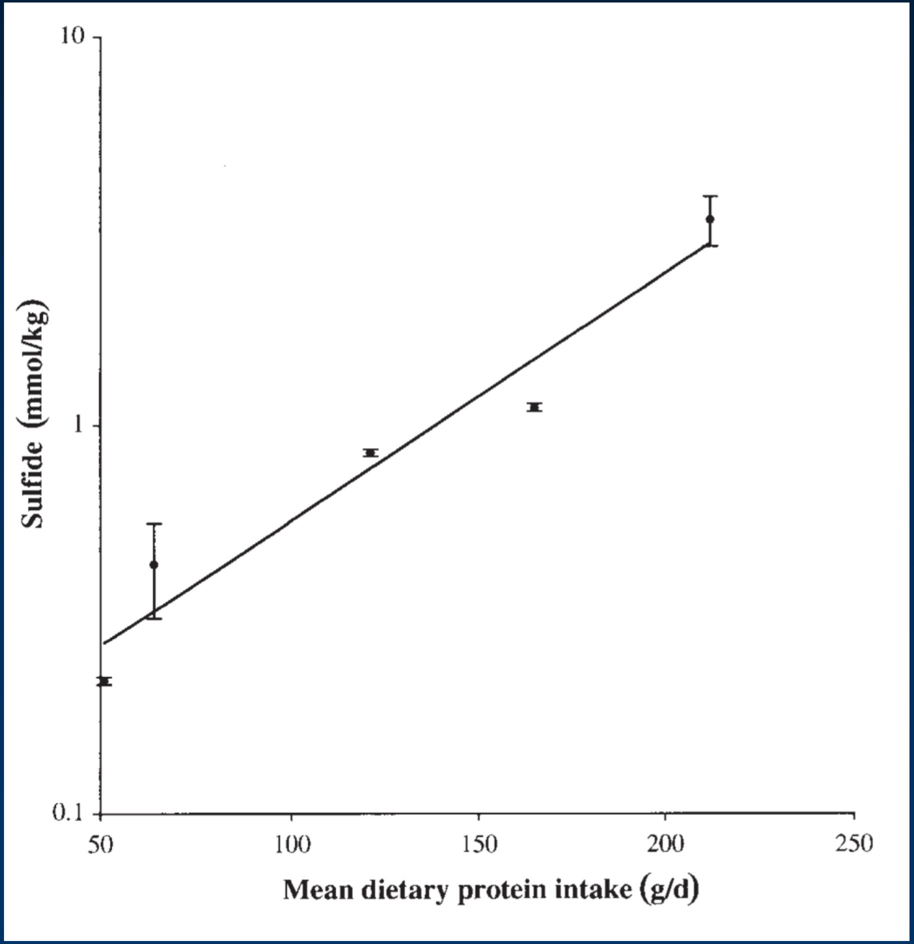
Sulfur Amino Acid Metabolism:
Generation of Hydrogen Sulfide (H_2S)

Produced by gut bacteria:

- Fermentation of sulfur-containing amino acids (methionine, cysteine, cystine, and taurine)
- Action of sulfate-reducing bacteria on inorganic sulfur (sulfate and sulfites)

- Toxic to colonocytes both in vitro and in vivo
- Contributes to inflammation (UC and colon cancer)

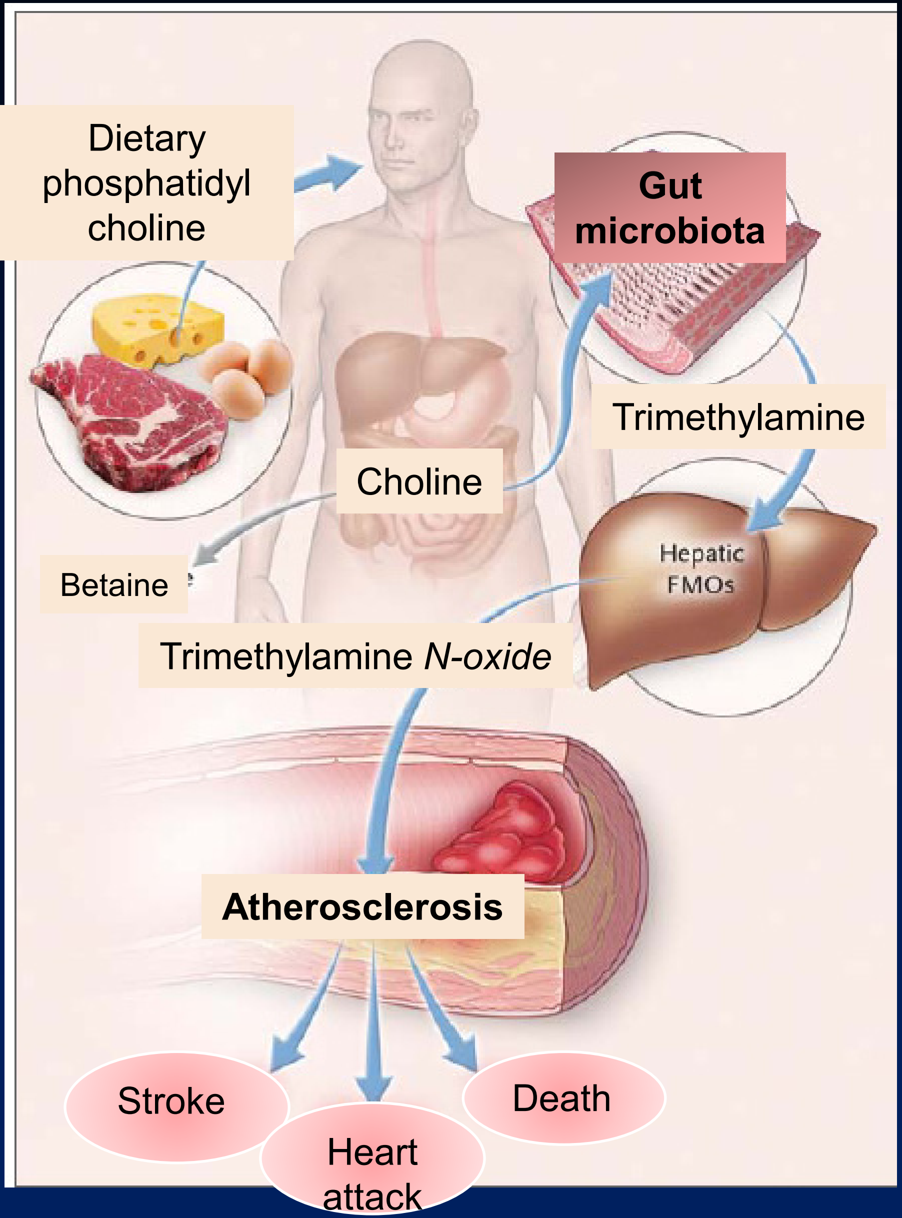
Fecal sulfide concentrations increase with increased protein intake in a controlled feeding study



- 5 male volunteers
- Randomized cross-over study of 5 protein doses for 10 days each:
- 0 – 600 g meat /d
- Measured fecal sulfide excretion

Magee et al. *Am J Clin Nutr,* 2000

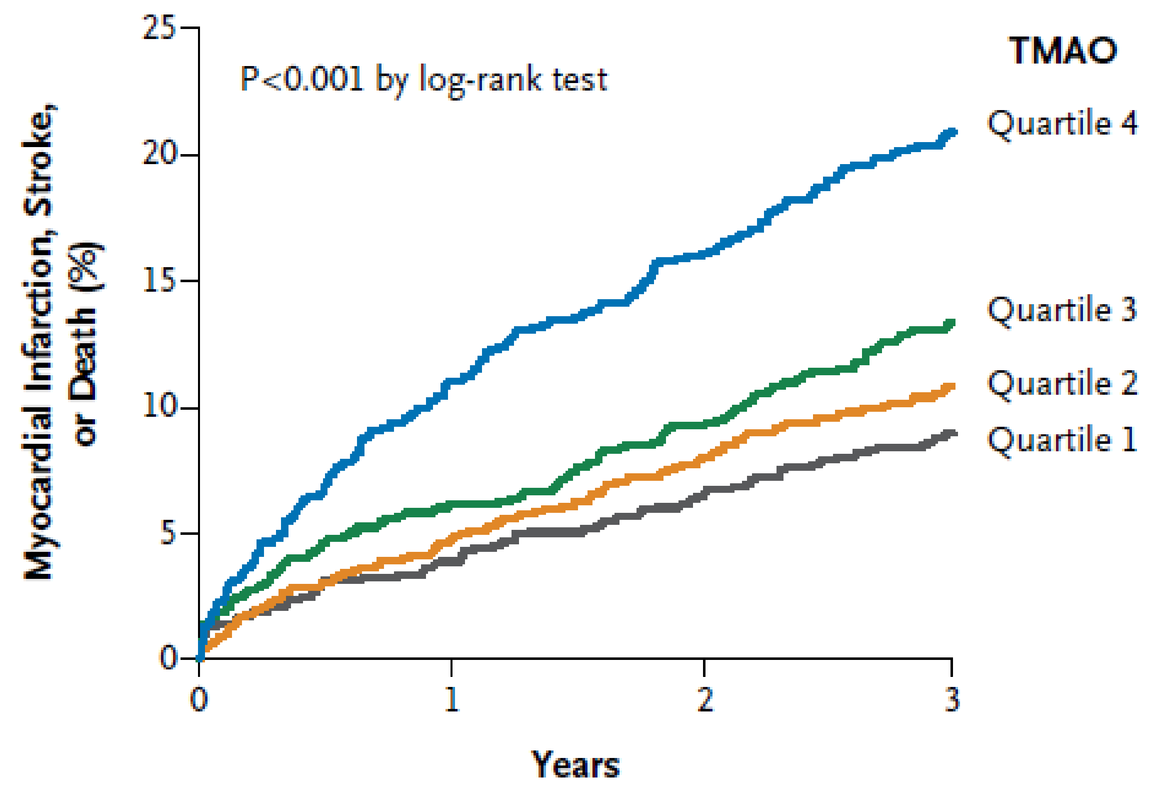
Conversion of Choline to Trimethylamine



- Microbial metabolism important in production of TMAO.
- Levels of TMAO and choline and betaine increased after a phosphatidylcholine challenge (2 eggs and [d9]-phosphatidylcholine).
- Plasma TMAO suppressed after antibiotics and reappeared after antibiotic withdrawal.

Tang et al. *NEJM*, 2013

Major Adverse Cardiovascular Events Increase by Quartile of Plasma TMAO



- 4007 adults undergoing elective diagnostic cardiac catheterization
- 3-y F/U for major adverse CVD events.
- Increased plasma TMAO associated with increased risk of CVD event.

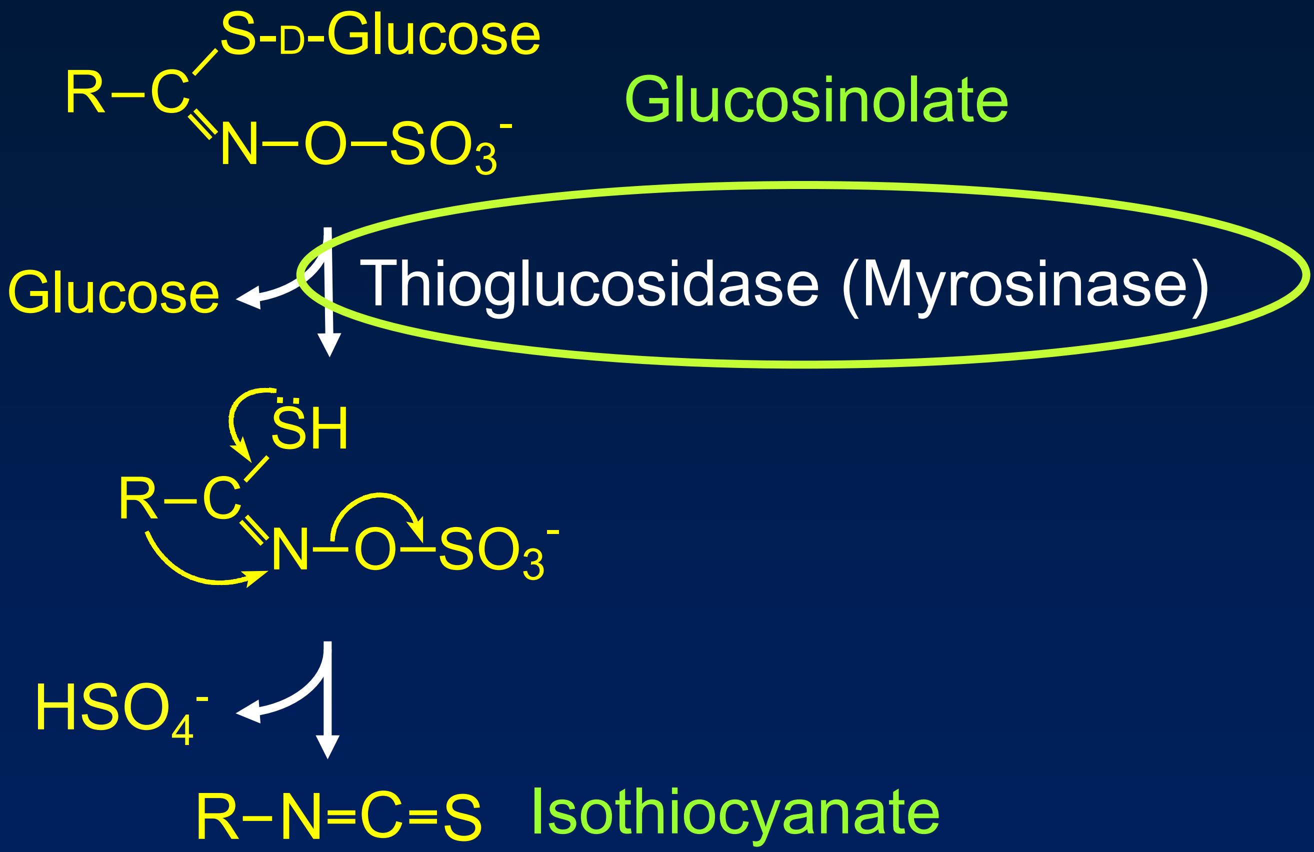
Tang et al. *NEJM,* 2013

Dietary Bioactive Phytochemicals

- Phenolics
  - Phenolic acids
  - Stilbenes
  - Curcuminoids
  - Chalcones
  - Lignans
  - Flavonoids
  - Isoflavones
- Terpenoids
  - Phenolic terpenes
  - Carotenoids
  - Saponins
  - Phytosterols
- Organosulfurs
  - Thiosulfinates
- N-containing compounds
  - Glucosinolates
  - Indoles

Adapted from Scalbert et al, J. Agric. Food Chem. 2011, 59, 4331–48

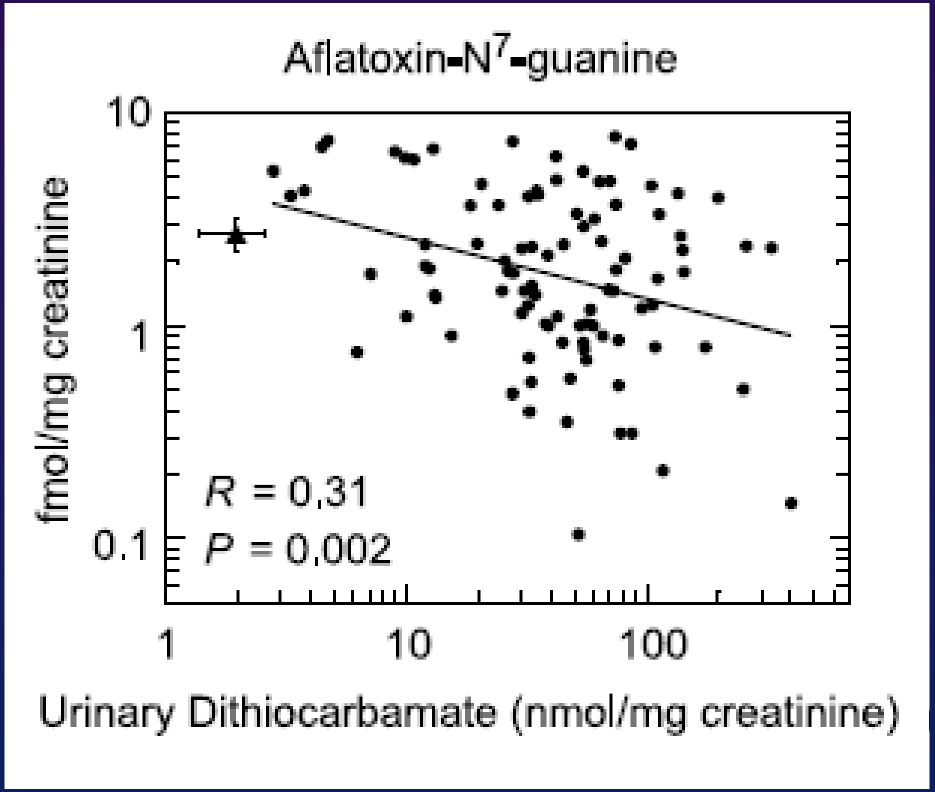
Isothiocyanates from Glucosinolates in Cruciferous Vegetables



Yuesheng Zhang, Roswell Park Cancer Institute, Buffalo, NY

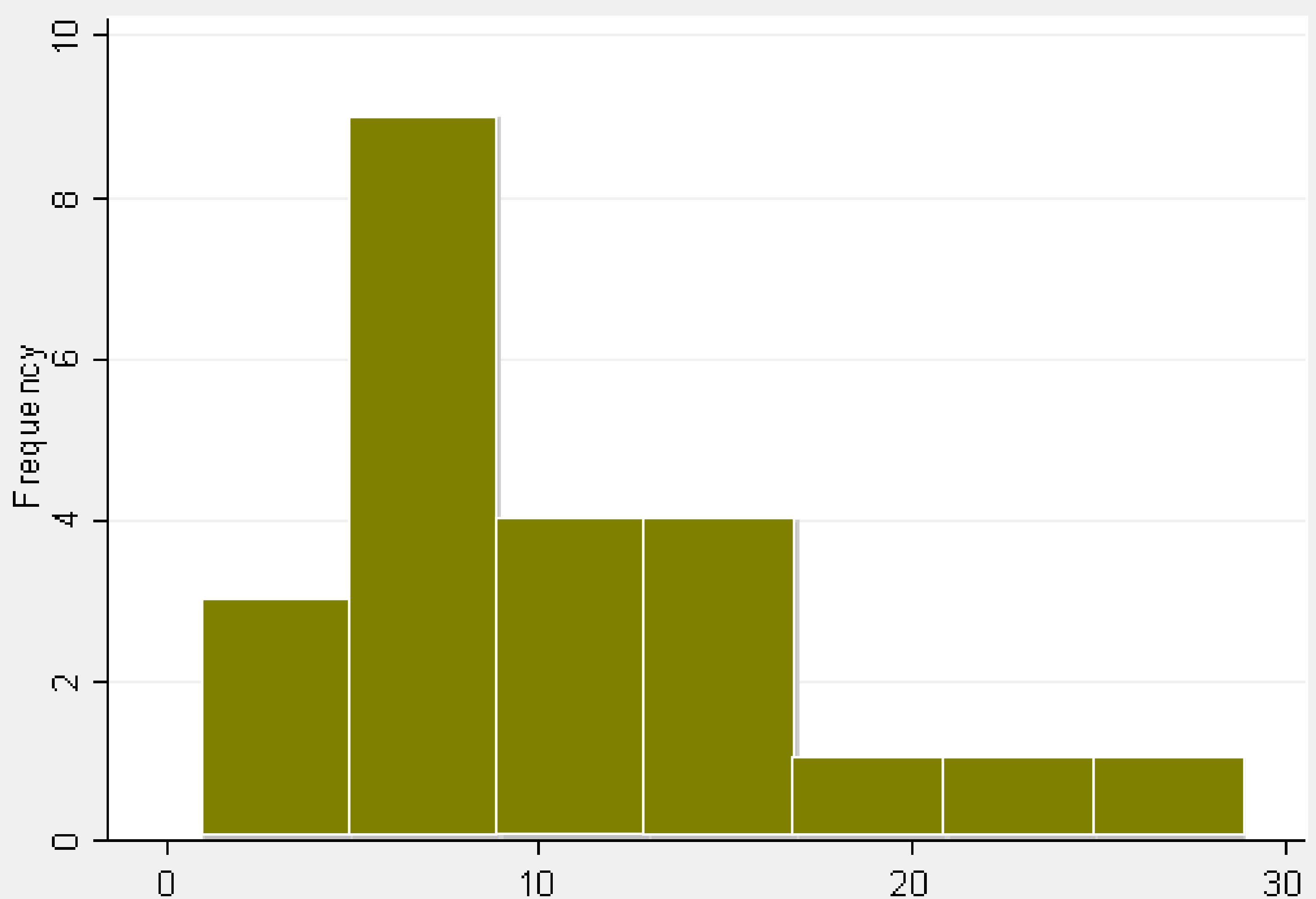
Inverse association between urinary ITC excretion and aflatoxin-DNA adducts – Interindividual variation in ITC bioavailability

- N=200, Qidong, China
- Randomized, parallel arm, 2-week trial
- 400 umol glucoraphanin/d vs. placebo
- Urinary ITC recovery 1-45% of dose



Kensler et al, *Cancer Epidemiol Biomarkers Prev*, 14:2605, 2005

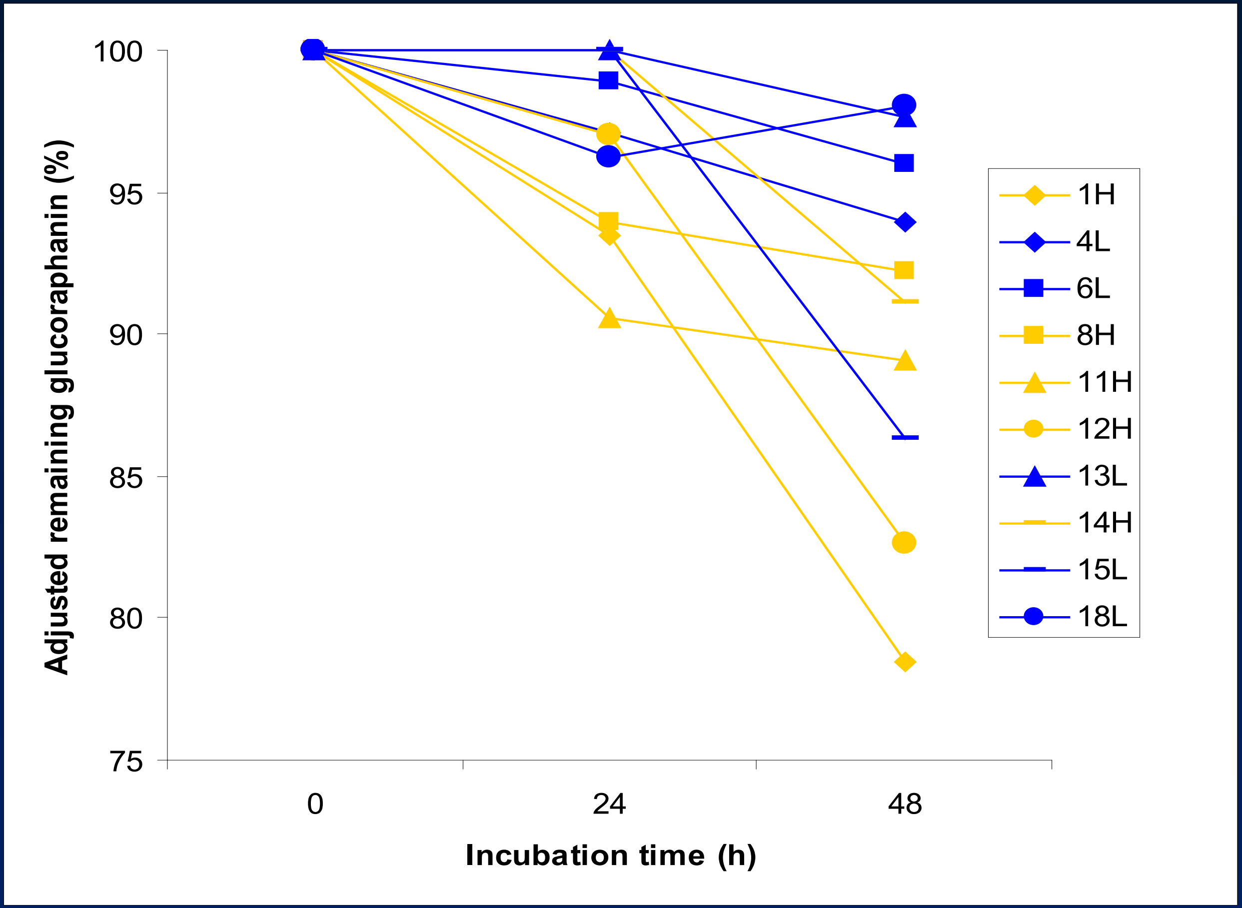
Isothiocyanate Recovery in Urine Ranged from 1 to 28% with 200 g Cooked Broccoli



% ITC excreted in urine after 200 g broccoli

Li *et al., Br J Nutr*, 2011

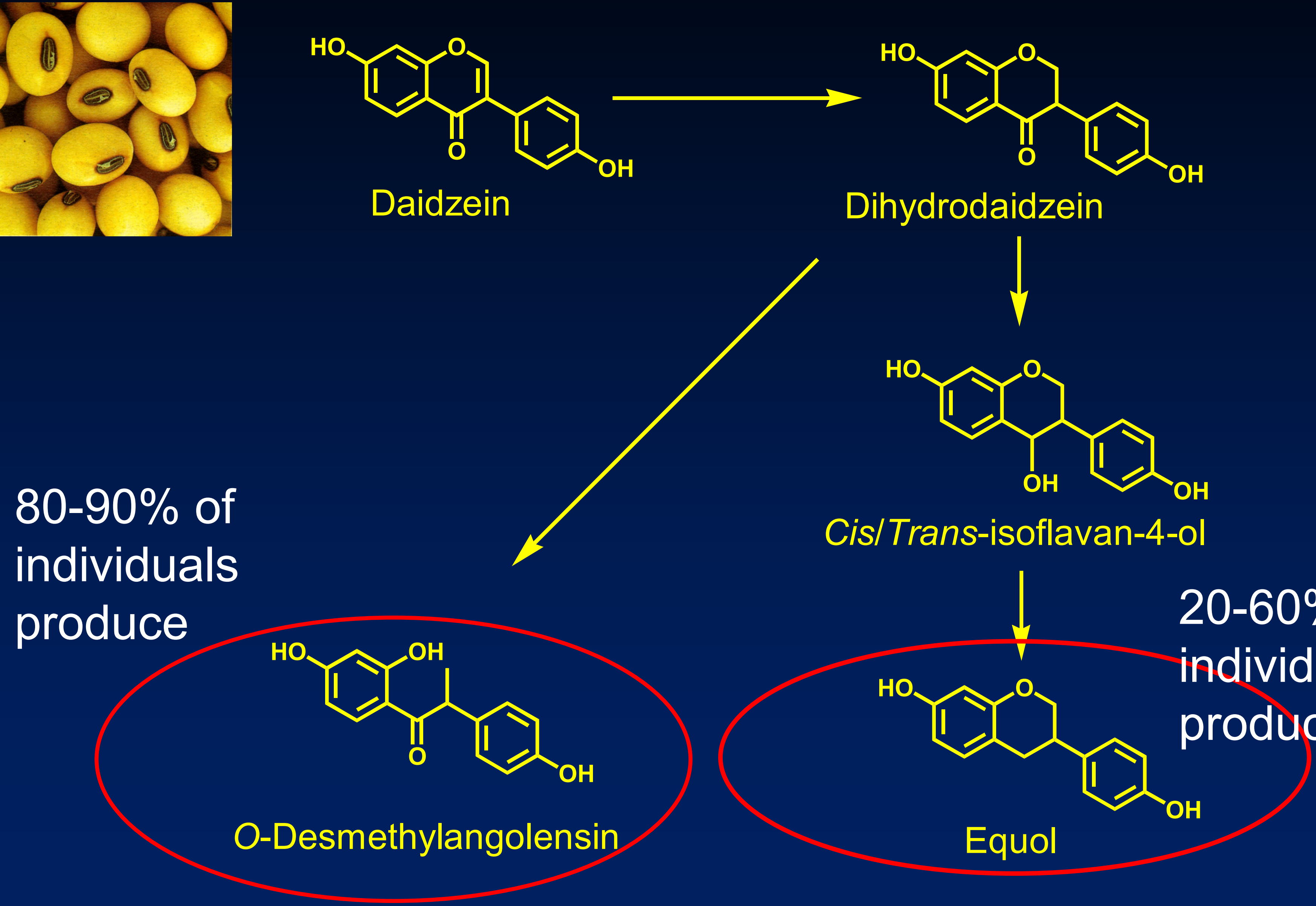
Fecal Bacterial Degradation of Glucosinolates In Vitro Differs by ITC-Excreter Status



- Low- and high-ITC excreters identified with standardized broccoli meal
- Fecal bacteria incubated with glucoraphanin for 48 h

Li *et al.*, *Br J Nutr*, 2011

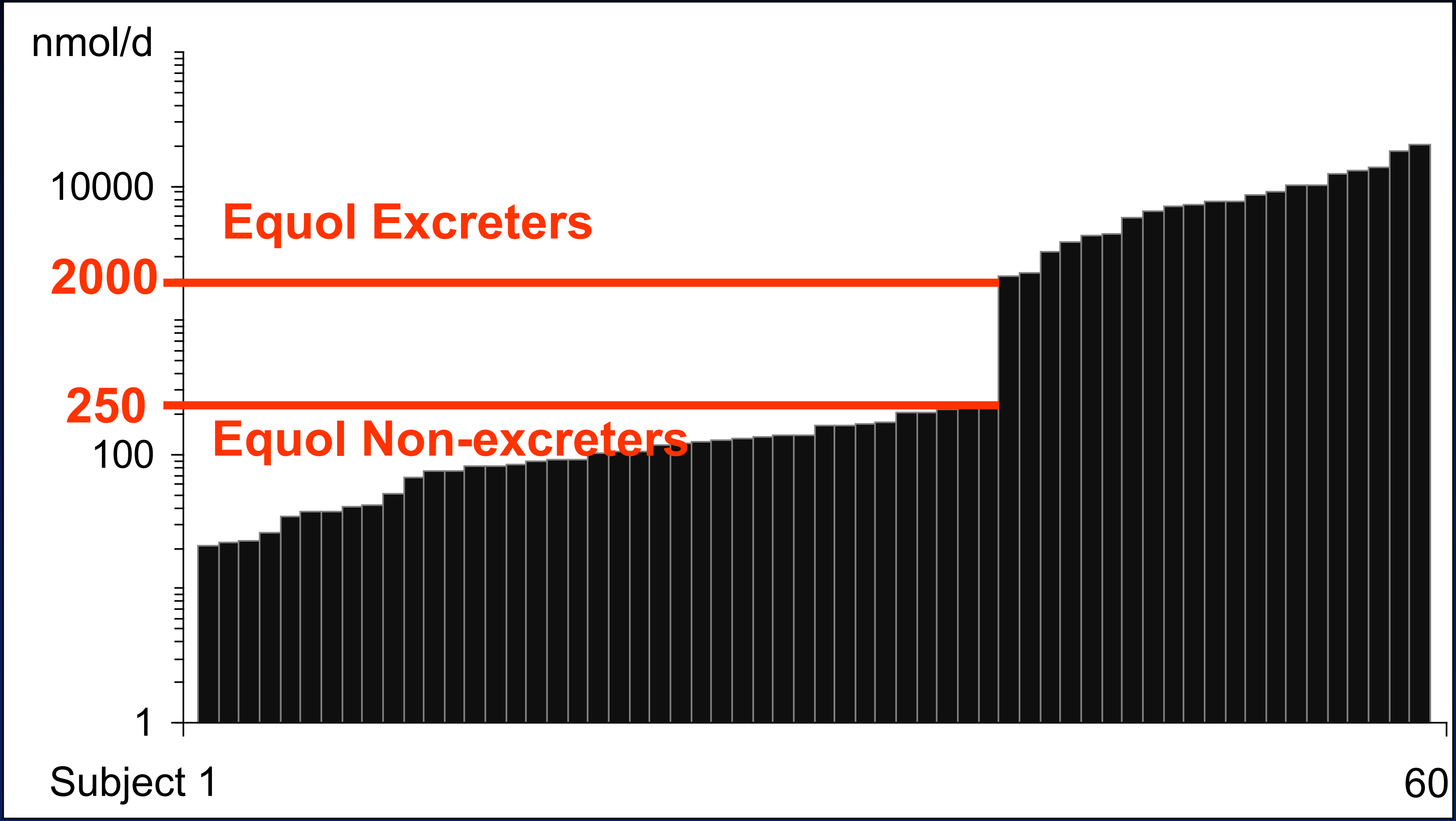
Microbial Production of Equol and ODMA



80-90% of individuals produce

20-60% of individuals produce

Urinary Equol Excretion with Soy Challenge



Lampe et al., *PSEBM* 217:335-339, 1998

Soy Interventions
Equol-Producing Capacity Associated with:

- Greater lengthening of menstrual cycle follicular phase.

  Cassidy et al., *Am J Clin Nutr* 60:333, 1994.

- Lower estrone, estrone-sulfate, testosterone, DHEA, DHEA-sulfate, androstenedione, and cortisol, and higher SHBG and mid-luteal phase progesterone

  Duncan et al., *Cancer Epi Biomark Prev* 9:581, 2000.

- Improved bone mineral density in post-menopausal women.

  Lydeking-Olsen et al, *Eur J Nutr* 43: 246, 2004.

- Differential gene expression in peripheral lymphocytes of equol producers and non-producers.

  Niculescu et al, *J Nutr Biochem* 18:380, 2007.

Equol-Producing Capacity and Health: Observational Studies

- Positively associated with 2-OH/16αOHE1 ratios in premenopausal and postmenopausal women.

Atkinson et al, *J Steroid Biochem Mol Biol* 86:71, 2003
Frankenfeld et al, *J Steroid Biochem Mol Biol* 88:399, 2004

- Mammographic density 39% lower in equol producers.

Frankenfeld et al,*Cancer Epidemiol Biomarkers Prev* 13:1156, 2004

- Plasma equol concentrations inversely associated with prostate cancer risk in Japanese men.

Akaza et al., *Jpn J Clin Oncol* 32:296, 2002

- Significant interaction between soy intake and equol-producer status in predicting breast density in postmenopausal women.

Fuhrman et al., *Cancer Epidemiol Biomarkers Prev* 17:33, 2008

What Human Gut Microbes Produce S-(-)Equol?

Daidzin ► Daidzein ► Dihydrodaidzein ► Equol

Daidzein ► Equol

- *Adlercreutzia equolfaciens*
- *Bacteroides ovatus*
- *Bifidobacterium*
- *Eggerthella sp YY7918*
- *Enterococcus faecium*
- *Finegoldia magna*
- *Lactobacillus mucosae*
- *Lactococcus garvieae*
- *Ruminococcus productus*
- *Slackia sp HE 8*
- *Streptococcus intermedius*
- *Veillonella sp*

Daidzin ►Dihydrodaidzein

- *Clostridium-like bacterium*

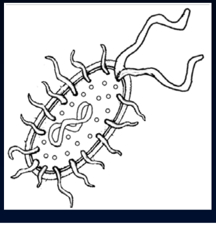
Dihydrodaidzein ► Equol

- *Eggerthella sp Julong 732*

Summerized in Setchell and Clerici, *J Nutr,* 2010.

Microbial Metabolism of Dietary Components

Summary





- Gut microbial metabolism modifies a variety of dietary components.
- Differences in gut microbial community capacity to handle substrates is detectable as metabolic phenotypes.
- Diet as consumed is not necessarily that experienced by the host.
- The gut microbiome needs to be considered in context of host diet to understand its impact on metabolism and disease risk.

Gaps, Needs and Challenges: More Specific to Nutrition

- **Challenge**: Testing causality of gut microbiome’s contribution to health and disease in humans.
- **Need:**
  - Prospective cohorts with repeated measures of exposure (i.e., diet, etc) and samples for gut microbiome characterization.
  - Well-controlled dietary interventions to understand inter-individual variation in bacterial metabolic phenotypes in the context of diet.
  - Accurate model systems of human dietary metabolism and associated microbiota.

Gaps, Needs and Challenges: Broader Considerations

- To facilitate transdisciplinary research to allow for integrated breadth and depth of knowledge.
- Methods of assessing composite functionality of the gut microbiome and integration of the structure and function of microbial systems.
- Computational methods to integrate high-dimensional microbiome and metabolome data.

J Lampe Lab
Meredith Hullar
Lisa Levy
Fei Li
Sandi Navarro
Wendy Thomas
Elizabeth Traylor
Seth Yoder

Texas A&M University
Robert Chapkin
Ivan Ivanov

FHCRC and UW collaborators
Mario Kratz
Marian Neuhouser
Tim Randolph
Ali Shojae

University of Bristol
Charlotte Atkinson

University of Helsinki
Kristiina Wähälä

Supported by: US National Cancer Institute
FHCRC

FRED
HUTCHINSON
CANCER
RESEARCH
CENTER