Diet and the Human Gut Microbiome: Whose Diet is It Anyway?

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Relationship of Diet and the Gut Microbiome to Health and Disease

- Disease Risk
  - Cancer
  - CVD
  - Diabetes

- Dietary constituents
- Fuel availability
- Energy imbalance
- Gut bacteria
Outline

- What are the gut microbes doing with our food?
- How does this affect what our bodies are exposed to?
- How might this influence disease risk?
Microbes and Disease

- **Microbes as infectious agents**
  - Account for ~20% of cancers worldwide
  - Cervical, liver and gastric cancers
  - Direct effects

- **Microbes as modifiers of physiology**

- **Microbes as modifiers of exposures**
  - Metabolize dietary constituents, drugs, carcinogens
  - Affecting energetics and obesity
The human diet is complex.

- 1000s of compounds
- Variety of methods of food preparation
  - Structure and particle size
  - Bioavailability to host
WE ARE WHAT WE EAT: Mammals and Their Gut Microbes Cluster by Diet

Ley et al., Science, 2008
Gut Microbial Metabolism -- Designed to make the most of the situation

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

Food

Human digestion

The indigestibles

The leftovers

Bacterial metabolism
Distribution of Metabolic Pathways in the Gut Microbiome

Xenobiotic biodegradation
- phytochemicals
- pyrolysis products
- drugs

Fermentation of Carbohydrates: Production of Short Chain Fatty Acids

Acetate
Propionate
Butyrate + gases

Butyrate

- Serves as energy source for colon cells
- Increases satiety
- Reduces inflammation and oxidative stress
- Decreases tumorigenesis
- Improves gut barrier function

Microbial Metabolism of Proteins & Amino Acids

Proteins Peptides

hydrolysis

Aromatic Amino acids

α, β elimination

Aromatic

Other Amino acids

deamination

decarboxylation

Other Amino acids

deamination & fermentation

Sulfur Amino acids

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

- Modulates expression of pro- and anti-inflammatory genes
- Strengthens epithelial cell barrier properties
- Decreases pathogen colonization

Bansal T et al, *PNAS* 2010
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)

Impact on health:
- Toxic to colon cells 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 crossover study of 5 protein doses for 10 days each:
  - 0 – 600 g meat /d
- Measured fecal sulfide excretion

Microbial Conversion of Nitrate to Nitrite

Microbial Nitrate Reductase

\[ \text{Nitrate} \quad \text{Nitrite} \]
\[ \text{NO}_3^- \quad \text{NO}_2^- \]

\textit{N-nitroso compounds}
- nitrosamines
- nitrosamides
- nitrosoguanidine

\rightarrow

DNA adducts

DNA damage

Cancer Risk
Microbial metabolism important in production of trimethylamine oxide (TMAO).

- TMAO levels and choline and betaine increased after feeding phosphatidylcholine.
- Plasma TMAO decreased after antibiotics and reappeared after antibiotic withdrawal.

Tang et al. *NEJM*, 2013
Major Adverse Cardiovascular (CVD) Events Increase by Amounts of Plasma TMAO

- 4007 adults undergoing elective cardiac catheterization
- 3-y follow-up for major adverse CVD events.
- Increased plasma TMAO associated with increased risk of CVD.

Tang et al. *NEJM*, 2013
Dietary Bioactive Phytochemicals

Phenolics
- Phenolic acids
- Flavonoids
- Stilbenes
- Coumarins
- Tannins

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
Gut Microbial Activity and Impact on Phytochemical Exposure

- Glucosinolates in cruciferous vegetables
- Isoflavones in soy
- Isoxanthohumol in hops
- Lignans in whole grains and vegetables
Is broccoli really all it’s cracked up to be?

**Cruciferous Vegetables and Cancer**

- Cruciferous vegetable intake shows most consistent association with lower risk of certain cancers:
  - lung, colorectal, breast, prostate, pancreatic cancer

- Isothiocyanates and indoles:
  - Are chemopreventive in animal models
  - Decrease inflammation and oxidative stress
  - Induce cell differentiation and apoptosis
  - Improve carcinogen metabolizing capacity
Isothiocyanates from Glucosinolates in Cruciferous Vegetables

\[ \text{S-D-Glucose} \quad \overset{R-C}{\text{N-O-SO}_3^-} \quad \text{Glucosinolate} \]

\[ \text{Glucose} \quad \overset{\text{Thioglucoosidase (Myrosinase)}}{\text{SH}} \quad \overset{R-C}{\text{N-O-SO}_3^-} \]

\[ \text{HSO}_4^- \quad \overset{R-N=C=S}{\text{Isothiocyanate}} \]
Excretion of Total Isothiocyanates from Broccoli Sprouts

% of dose

0 20 40 60 80 100

Chewed Unchewed

Uncooked

Shapiro et al, Cancer Epidemiol Biomarkers Prev, 2001
Excretion of Total Isothiocyanates from Broccoli Sprouts

% of dose

0 20 40 60 80 100

Chewed Unchewed Cooked Myrosinase-pretreated

Uncooked

Shapiro et al, Cancer Epidemiol Biomarkers Prev, 2001
Inverse association between urinary ITC excretion and aflatoxin-DNA adducts: Wide 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

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

Daidzein → Dihydrodaidzein → Cis/Trans-isoflavan-4-ol → Equol

80-90% of individuals produce O-Desmethylangolensin

20-60% of individuals produce Equol
Soy Interventions
Equol-Producing Capacity Associated with:

- Greater lengthening of menstrual cycle follicular phase.
  Cassidy et al., *Am J Clin Nutr*, 1994

- Lower blood concentrations of certain estrogens, androgens, and cortisol, and higher SHBG and mid-luteal phase progesterone
  Duncan et al., *Cancer Epi Biomark Prev*, 2000

- Improved bone mineral density in post-menopausal women.

- Differential gene expression in peripheral lymphocytes of equol producers and non-producers.
Equol-Producing Capacity: Observational Studies

- Positively associated with better estrogen metabolite ratios in premenopausal and postmenopausal women.
  

- Breast density 39% lower in equol producers.
  
  Frankenfeld et al, *Cancer Epidemiol Biomarkers Prev*, 2004

- Significant interaction between soy intake and equol-producer status in predicting breast density in postmenopausal women.
  
  Fuhrman et al., *Cancer Epidemiol Biomarkers Prev*, 2008
Relationship of Diet and the Gut Microbiome to Obesity and Chronic Disease

- Energy imbalance
- Disease Risk: Cancer, CVD, Diabetes
- Gut bacteria

Diet → Fuel availability → Energy imbalance → Disease Risk
Gut Microbiota and Host Body Fat

- Conventionally raised and colonized adult mice, vs germ-free, mice have~ 40% more total body fat.

Bäckhed et al. PNAS 2004;101:15718-23
Intestinal Microbiota Transfer From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome

- Significant change in fecal microbiota composition after infusion from lean controls.
- 2.5-fold increase in butyrate-producing bacteria in stool and intestinal biopsies.

Vrieze et al., Gastroenterol, 2012
Mechanisms Linking Microbiota to Obesity:

How could bacteria contribute to obesity?

- Stimulating dietary fat absorption in the small intestine.
- Providing increased energy to host as short-chain fatty acids.
- Causing systemic inflammation via lipopolysaccharide (LPS), an endotoxin from Gram-negative bacteria.

Semova et al., *Cell Host Microbe*, 2012
Summary

- Gut microbes metabolize a variety of dietary components.
- Diet as consumed is not necessarily that experienced by the host.
- Certain gut microbial communities may predispose the host to greater body fat gain.
- Gut microbiota mediate host metabolism and obesity through a variety of mechanisms that still remain to be elucidated fully.