Overview/State of the Science on Microbiota, Diet & Dietary Patterns

Gary D. Wu, M.D.
Ferdinand G. Weisbrod Professor of Medicine
Division of Gastroenterology
Perelman School of Medicine
University of Pennsylvania
The Human Microbiome

- Comprised of Bacteria, Viruses, others (Archaea, Eukaryotes)
- Distinctive microbiomes at each body site (gut, lung, skin, mucosa etc.)

**The Gut Microbiota**
- Human gut is home to ~ 100 trillion bacterial cells
- Density of $10^{11}$ to $10^{12}$ per gram in the colon
- Large numbers of species present, many uncultured

**Diabetes:** Type 1 DM (MyD88-dependent in NOD Mice); Type 2 DM (TLR4 and TLR5 KOs)

**Atherosclerosis:** Oral, gut and plaque microbiota; Microbial metabolism of choline to TMA

**Asthma:** Sanitized environment

**Colon Cancer:** Enterotoxigenic *Bacteroides fragilis* and *Fusobacterium*

**Inflammatory Bowel Disease:** Dysbiosis
PennCHOPMicrobiome Program

- Relevance to human biology is c/w with the mission of Penn Med and CHOP
- High profile opportunities in the scientific community
- Many available opportunities in a relatively open space (FTO)
- Track record of accomplishment established within the gut microbiome group
- Growing opportunities at the NIH for funding
- Opportunities in industry, federal agencies and private foundations with disease orientation

- Past/Current Status of the field: “Safe” traditional view supported by federal funding
- Future status of the field: Higher risk but higher reward. Future NIH funding opportunities.

Human Association Studies

FMT for C. Difficile Infection

Evolution of the microbiome field

Novel Therapeutics and Diagnostics

Next generation pre-, pro-, synbiotics
Agenda

Diet and the Gut Microbiome: Of Mice and Men

Dietary Fiber, the Gut Microbiota, and the Intestinal Mucosal Barrier

Diet and the Gut Microbiome and its Metabolome in Health and Disease
Challenges in characterizing the effects of diet on the human gut microbiome

- Humans are poorly adherent to standardized dietary regimes
- Current tools to characterize dietary composition and intake are imperfect
- The reciprocal nature of dietary composition to maintain isocaloric consumption make it difficult to determine the factor responsible for an observed outcome
- Diet can have profound impacts on host biology independent of the gut microbiota
- Both intensive controlled feeding experiments and large outpatient cohort studies are expensive and challenging to complete

The utility of animal models in studying the interaction between diet and the gut microbiome

- Tight control of defined diets over long periods of time
- Multiple biological replicates feasible
- Germ-free animals can be used to examine the effect of diet independent of the gut microbiome
- Defined microbial consortia or complete human gut microbiota studies can be performed in gnotobiotic animals
- Cause-and-effect relationships involving diet and the gut microbiota can be determined

But, do
Extreme and Consistent Effect of the Diet on the Murine Gut Microbiota vs. Small Effects in Humans

Controls Feeding Experiment: CAFÉ

Wu et al. Science 2011;334:105-8

- 10 Healthy volunteers
- Randomized to high fat vs. low fat diet
- 10 day inpatient stay with same meals each day
- Daily stool sample collection

Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production

Gary D Wu,1 Charlene Compehe,2 Eric Z. Chen,3 Sarah A Smith,4 Rachana D Shah,4 Kyle Bittinger,5 Christel Chehoud,5 Lindsey G Albenberg,6 Lisa Nessel,7 Erin Gilroy,8 Julie Star,1 Aalim M Wijie,9 Harry J Flint,9 David C Metz,1 Michael J Bennett,9 Honghe Li,9 Frederic D Bushman,9 James D Lewis9

Hidebrandt MA et al. Gastroenterology 2009

Gut 2016
Agenda

Diet and the Gut Microbiome: Of Mice and Men

Dietary Fiber, the Gut Microbiota, and the Intestinal Mucosal Barrier

Diet and the Gut Microbiome and its Metabolome in Health and Disease
Host-Microbial Mutualism the Gut

**Host benefits to bacteria**
- Provides a unique niche
- Intestinal mucus provides a source of nutrition

**Bacteria benefits the host**
- Fermentation of indigestible carbohydrates and the production of SCFAs
- Biotransformation of conjugated bile acids
- Urease activity participates in nitrogen balance
- Synthesis of certain vitamins
- Metabolize drugs
- Education of the mucosal immune system
How Do Bacteria Digest Complex Carbohydrates for Fermentation?

- 130 families of glycoside hydrolases (GH), 22 polysaccharide lysases (PL), and 16 carbohydrate esterases (CE)
- High proportion of these are encoded in microbial genomes: Carbohydrate-active enzymes (CAZymes)

**Figure 5. Bacteroides thetaiotaomicron sux system.** (A) shows the order of genes in the sux cluster that is responsible for starch utilization in this species. (B) shows the inferred organization of gene products on or near the bacterial cell surface (OM outer membrane, CM cytoplasmic membrane). Starch molecules are shown as sugar chains, at various stages of hydrolysis.
Dietary Fiber and the Intestinal Mucus Barrier

A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility

Mahesh S. Desai, Anna M. Seekatz, Nicole M. Koropatkin, Nobuhiko Kamada, Christina A. Hickey, Mathis Wolter, Nicholas A. Pudlo, Sho Kitamoto, Nicolas Terrapon, Arnaud Muller, Vincent B. Young, Bernard Henrissat, Paul Wilmes...

Volume 167, Issue 5, 2016, 1339–1353
Diet and the Gut Microbiome: Of Mice and Men

Dietary Fiber, the Gut Microbiota, and the Intestinal Mucosal Barrier

Diet and the Gut Microbiome and its Metabolome in Health and Disease
Dietary Effects on Human Gut Microbiome and its Association with Disease

• Individuals with marked obesity, insulin resistance, dyslipidemia, and inflammatory phenotype have low bacterial richness

• Increased consumption of an agrarian diet, rich in fruits and vegetables with higher fiber, is associated with increased bacterial gene richness

• Energy-restricted diets increase bacterial gene richness

Decrease gut microbiome “richness” (decreased number of various bacteria and their genes) is associated with both disease states and the consumption of a Westernized diet

Wu et al. Science 2011;334:105-8
Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella

Authors
Petia Kovatcheva-Datchary, Anne Nilsson, Rozita Akrami, ..., Eric Martens, Inger Björck, Fredrik Bäckhed

Correspondence
fredrik.backhed@wlab.gu.se

In Brief
Diet affects the gut microbiota composition, though large inter-individual variations exist. Kovatcheva-Datchary et al. reveal that subjects with improved glucose metabolism after barley kernel supplementation have increased Prevotella in their gut microbiota. Prevotella plays a direct role in the beneficial response, supporting the importance of personalized approaches to improve metabolism.

Highlights
- Prevotella/Bacteroides is associated with a beneficial response to barley kernels
- Prevotella-enriched microbial interactions are higher in barley kernel responders
- Prevotella protects against Bacteroides-induced glucose intolerance
- Prevotella promotes increased hepatic glycogen storage in mice
Personalizing Responses to Diet Using the Gut Microbiome

Personalized Nutrition by Prediction of Glycemic Responses

David Zezevi,1,2,3 Tal Korem,1,2,3 Niv Zmora,3,4,5,6 David Israeli,6,8 Daphna Rothschild,1,2,3 Adina Weinberger,1,2,6 Orly Ben-Yacov,1,2 Dar Lador,1,2 Tal Avnit-Sagi,1,2 Maya Lotan-Pomp,1,2 Jotham Suez,2 Jemal Ali Mahdi,2 Elad Matot,7 Gali Malka,4 Noa Kosover,3 Michal Rein,7 Gil Ziberman-Schapira,7 Lenka Dohnalová,8 Meirav Pevaser-Fischer,3 Rony Bikovsky,6,8 Zamir Haipen,3,8 Eran Elinav,3,5,7 and Eran Segal1,2,3

Highlight:
- High interpersonal variability in post-meal glucose observed in an 800-person cohort
- Using personal and microbiome features enables accurate glucose response prediction
- Prediction is accurate and superior to common practice in an independent cohort
- Short-term personalized dietary interventions successfully lower post-meal glucose

Quantifying Diet-Induced Metabolic Changes of the Human Gut Microbiome

Sohan Singha, Tapan Sethi, Fatma Karakousis-Sch Pry, Ami Menashe, Partih Seo, Dalia Pujol-Guix, Tomasz Wrona, CatherineUsage, brake Natalia, I Pola, Oliver, Linda Heymsley, Arne Frühauf, Madhuri Jain, Stephanie, and Jaya Thubrikar
Diet, the Gut Microbiome, and its Metabolome

Holmes et al. *Cell Met.* 2012;16:559

How are plasma metabolites in humans influenced by the gut microbiome via diet?
Effect of Diet on Metabolite Production by the Gut Microbiota and its Impact on Disease

Human microbiome sample

Extract DNA

- 16S rRNA gene sequencing
- 18S rRNA gene sequencing
- Total DNA sequencing (shotgun metagenomics)

1. Compare sequences to ribosomal databases
2. Compare sequences to reference genomes
3. Compare sequences to genomic databases

Gene content

- Bacteria and Archaea
- Fungus/Yeast
- Viruses

Identify relative frequencies and pathways

What organisms are present and what is their relative abundance?

What are the functions of the community?

Extract small molecules

- Mass spectroscopy (metabolomics)

Compare to small molecule reference libraries

Metabolite characterization
Food And Resulting Microbial Metabolites (FARMM)

**Objective:** Determine the relation between dietary composition, gut microbiome composition, and the metabolic products that ultimately are present in the gut lumen and the plasma of humans.
Study Diet: Western omnivore (n=10), Modulen IBD (n=10), Vegan (n=10)

Antibiotics

- Shotgun metagenomic sequencing
- Fecal and plasma metabolomics
- Immune profiling of LPMC

**Diet Induced Changes in Metabolites and Microbiome (Aim 1)**

**Differences Identify Metabolites Produced or Consumed by Microbes (Aim 2)**

**Correlate Increase/Decrease of Metabolites with Recurrence of Microbes and Diet to Identify Key Drivers (Aims 2 and 3)**

**Manuscript in preparation**
Composition of the Gut Microbiota in FARMM

Intervention
Pre-Intervention
Post-Intervention

study_group
- Modulen
- Vegan
- Western

study_day
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15

Manuscript in preparation
Study Diet: Western omnivore (n=10), Modulen IBD (n=10), Vegan (n=10)

<table>
<thead>
<tr>
<th>Vanco / Neo</th>
<th>PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>*</td>
</tr>
<tr>
<td>9</td>
<td>*</td>
</tr>
<tr>
<td>12</td>
<td>*</td>
</tr>
<tr>
<td>15</td>
<td>*</td>
</tr>
</tbody>
</table>

Diet Induced Changes in Metabolites and Microbiome (Aim 1)

Differences Identify Metabolites Produced or Consumed by Microbes (Aim 2)

Correlate Increase/Decrease of Metabolites with Recurrence of Microbes and Diet to Identify Key Drivers (Aims 2 and 3)

Paired t-tests to determine categories of variation

- p.value < 0.05/4 & log \( x_t/x_{t-1} \) > 0
- p.value ≥ 0.05/4
- p.value < 0.05/4 & log \( x_t/x_{t-1} \) < 0

Examples of category 2312
- PGE2 (Western)
- Hydrocinnamate (Modulen)

Manuscript in preparation
Patterns of Fecal Metabolites Over Time in FARMM

Log10(Sum)

Manuscript in preparation
The Bidirectionality of Gut Microbiome Investigation

- Defined environmental conditions
- Defined genetics
- Monotonous diet

High signal-to-noise ratio

Proof-of-concept cause-and-effect relationships in a modest sized cohort

- Free living in a highly variable environment
- Genetic diversity
- Variable diet

Low signal-to-noise ratio

BUT

Small effect sizes over large populations can be highly impactful: Clean water, vaccinations, healthy diet

Embracing the complexity of human biology through the use of high dimensional analytic technologies together with advanced computational and biostatistical platforms
A Comparison of Diet Between Omnivores and Vegans

A Comparison of the Plasma Metabolome Between Omnivores and Vegans

The Urinary Metabolome

Predicting an Omnivore vs. Vegan Diet by Random Forest

Produced by the microbiota
The Convergence of Systems and Reductionist Approaches in Complex Trait Analysis

Evan G. Williams¹ and Johan Auwerx¹,⁎

¹Laboratory of Integrative and Systems Physiology, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
⁎Correspondence: admin.auwerx@epfl.ch
http://dx.doi.org/10.1016/j.cell.2015.06.024

Research into the genetic and environmental factors behind complex trait variation has traditionally been segregated into distinct scientific camps. The reductionist approach aims to decrypt phenotypic variability bit by bit, founded on the underlying hypothesis that genome-to-phenome relations are largely constructed from the additive effects of their molecular players. In contrast, the systems approach aims to examine large-scale interactions of many components simultaneously, on the premise that interactions in gene networks can be both linear and non-linear. Both approaches are complementary, and they are becoming increasingly intertwined due to developments in gene editing tools, omics technologies, and population resources. Together, these strategies are beginning to drive the next era in complex trait research, paving the way to improve agriculture and toward more personalized medicine.
“Penn Intestinal Microbiome Project Group”

*Co-Principal Investigators

Patient/subject recruitment and phenotyping, dietary assessment, sample collection and processing

Robert Baldassano, MD (CHOP)

*James D. Lewis, MD (Penn)

*Gary D. Wu, MD (Penn)

Charlene Compher, PhD, RD (Penn)
Andrew Gewirtz, PhD (GSU)

Microbiology

Mark Goulian, PhD (Penn)
Jay Zhu, PhD (Penn)

Biological Oxymetry

Sergei Vinogradov, PhD (Penn)

Jun Chen, Sam Minot, Serena Dollive, Eric Chen, Christian Hoffmann, Ying-Yu Chen, Jennifer Hwang, Erin Gilroy, Kernika Gupta, Lisa Nessel, Lindsey Albenberg, Judith Kelsen, Colleen Judge, Christel Chehoud, David Shen, Rohini Sinha, David Metz, Tatiana Esipova, Susan Parrott, Elliot Friedman, Josie Ni, Sarah Smith, Lillian Chau, Erika Panfen, Andrew Lin, Sarah Smith, Jack Jiang, Yun Li

DNA sequencing, data analysis, and mathematical modeling

*Frederic D. Bushman, PhD (Penn)
Hongzhe Li, PhD (Penn)
Kyle Bittinger, PhD (CHOP)
Costas Maranas, PhD (PSU)

Metabolomics

Michael Bennett, PhD (CHOP)
Marc Yudkoff, MD (CHOP)
Clary Clish, PhD, Ramnik Xavier, MD, and Jonathan Braun, MD-PhD
Andrew Patterson, PhD (PSU)

Proteomics

Benjamin Garcia, PhD (Penn)

The Joint Penn-CHOP Center for Digestive, Liver, and Pancreatic Medicine

Center for Molecular Studies in Digestive and Liver Diseases (P30 DK050306)