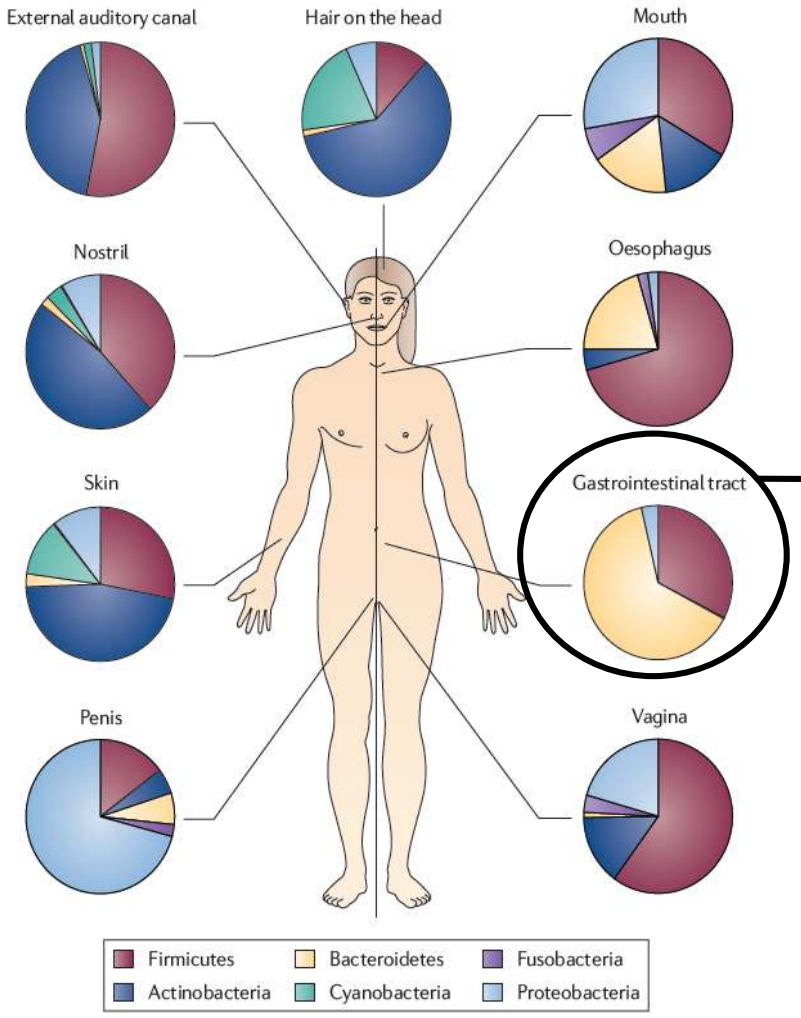
PennCHOP
MICROBIOME PROGRAM

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

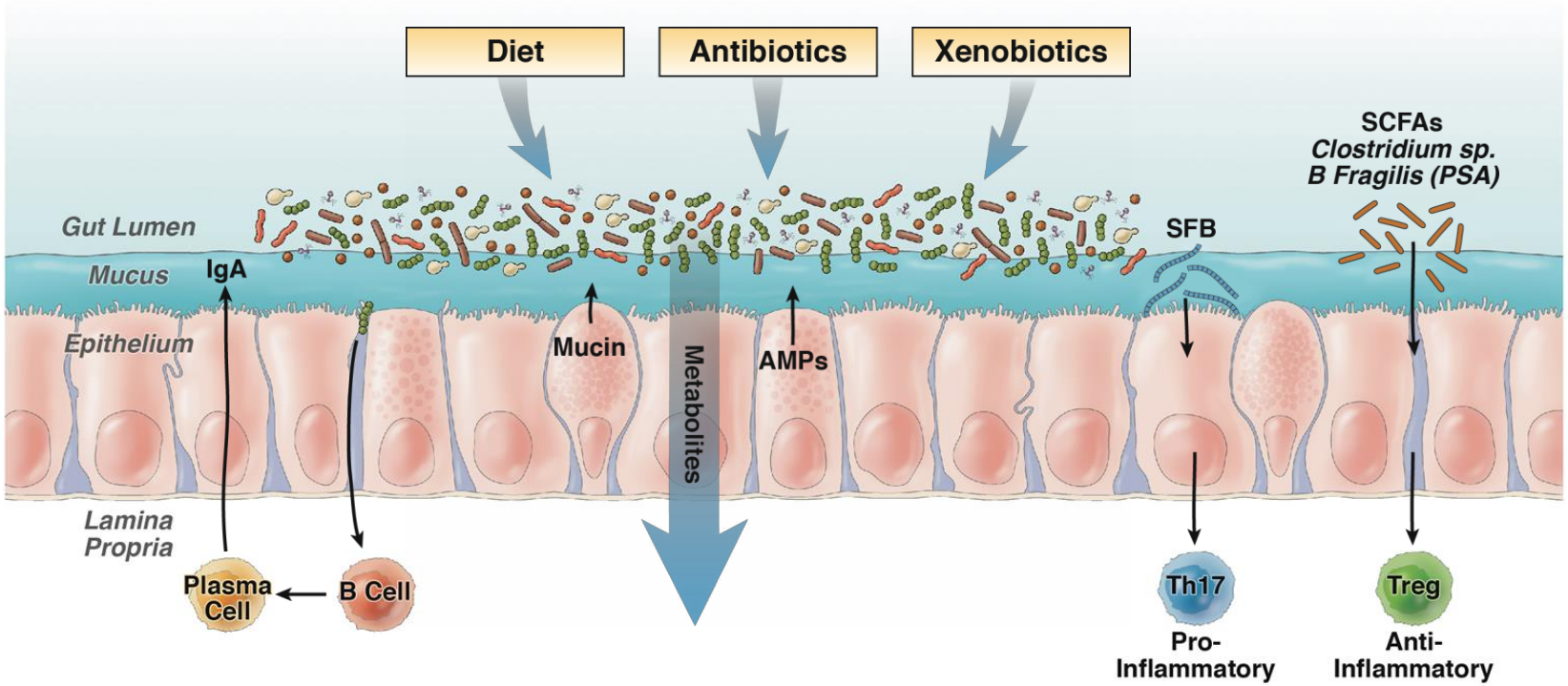


- **Comprised of Bacteria, Viruses, others (Archaea, Eukaryotes)**
- **Distinctive microbiomes at each body site (gut, lung, skin, mucosa etc.)**

<u>The Gut Microbiota</u>

- **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**

Nat. Rev. Micro. 2011;9:279-290



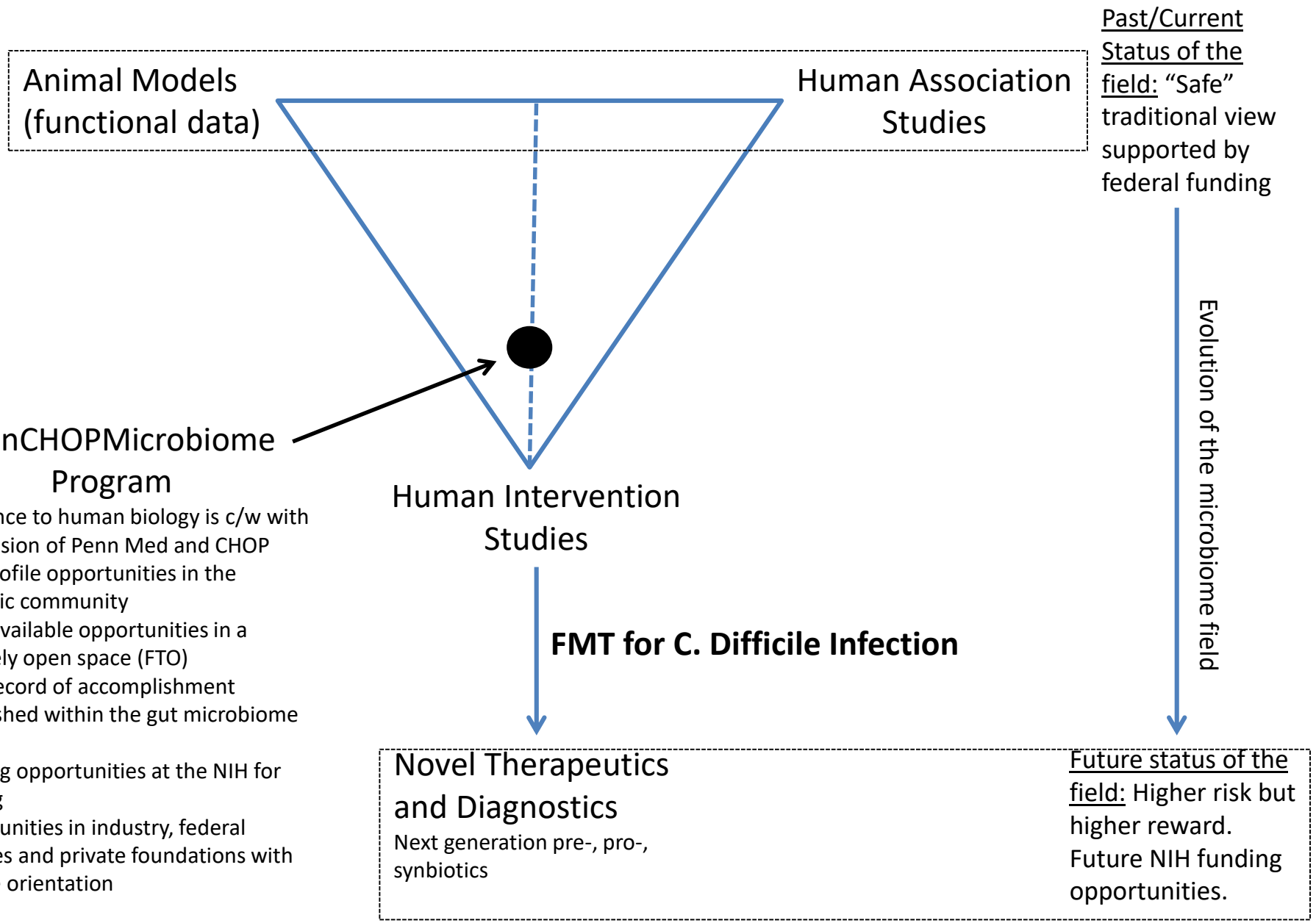
Diabetes: Type 1 DM (MyD88-dependent in NOD Mice); Type 2 DM (TLR4 and TLR5 KOs)

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



- 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

Agenda

Diet and the Gut Microbiome: Of Mice and Men

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

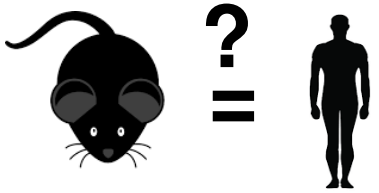
Diet and the Gut Microbiome and its Metabolome in Health and Disease

Challenges in characterizing the effects of diet on the human gut microbiome

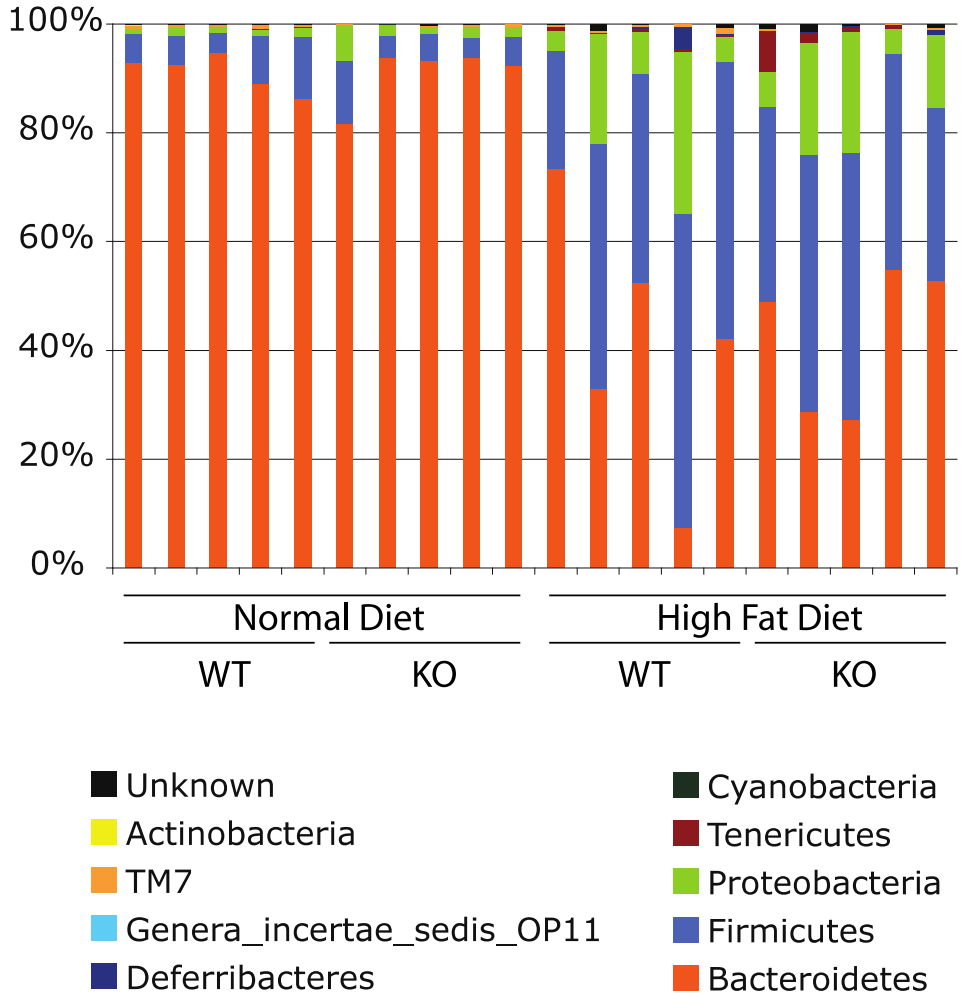
- Humans are poorly adherent to standardized dietary regimes
- Current tools to characterize dietary composition and intake are imperfect
- The reciprocal nature of dietary composition to maintain isocaloric consumption make it difficult to determine the factor responsible for an observed outcome
- Diet 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

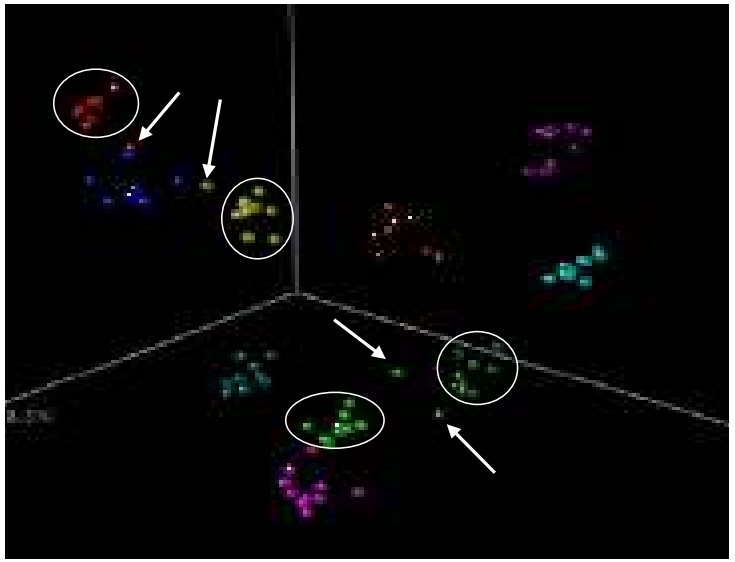


Hidebrandt MA et al. Gastroenterology 2009

Controlled 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 Compher,[2] Eric Z Chen,[3] Sarah A Smith,[1] Rachana D Shah,[4] Kyle Bittinger,[5] Christel Chehoud,[5] Lindsey G Albenberg,[6] Lisa Nessel,[3] Erin Gilroy,[3] Julie Star,[1] Aalim M Weljie,[7] Harry J Flint,[8] David C Metz,[1] Michael J Bennett,[9] Hongzhe Li,[3] Frederic D Bushman,[5] James D Lewis[1,3]

Gut 2016

Agenda

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Diet and the Gut Microbiome and its Metabolome in Health and Disease

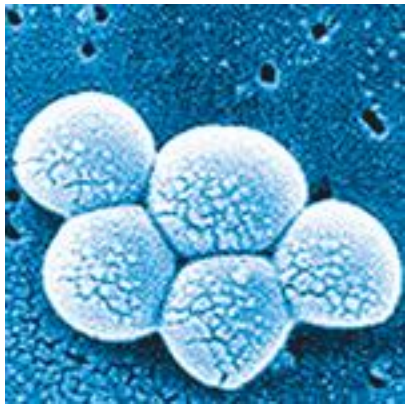
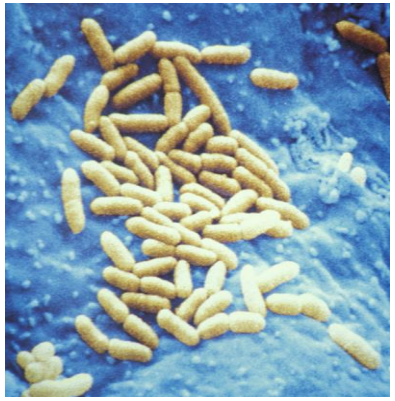
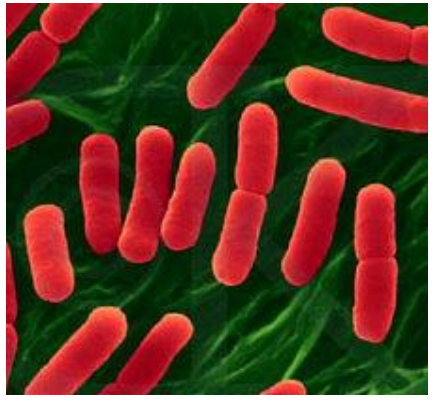
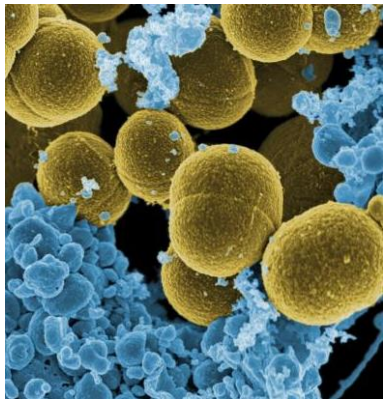
Host-Microbial Mutualism the Gut

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 carhohydrate esterases (CE)
- High proportion of these are encoded in microbial genomes: Carbohydrate-active enzymes (CAZymes)

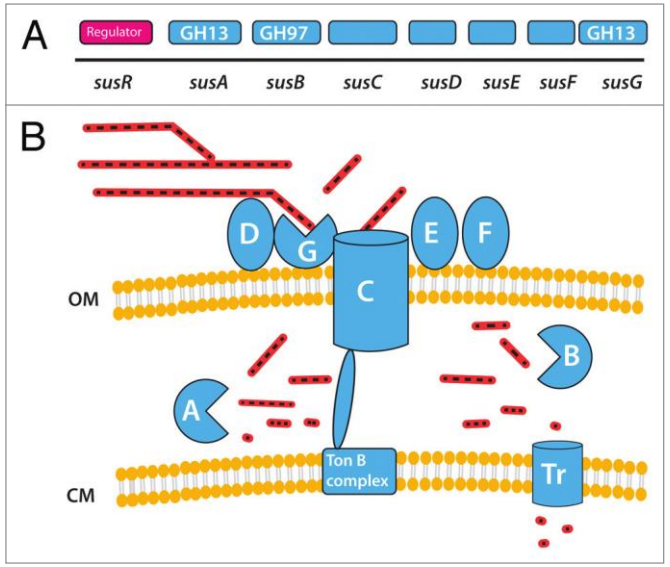
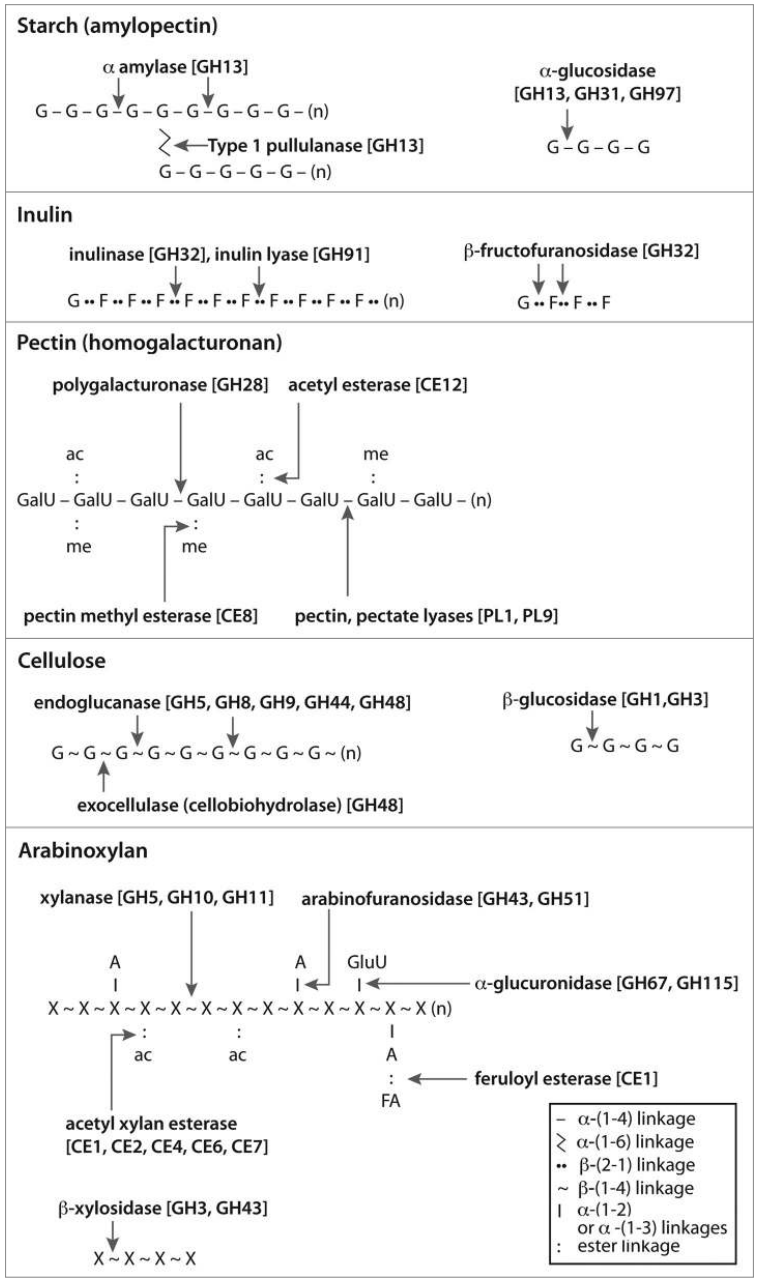
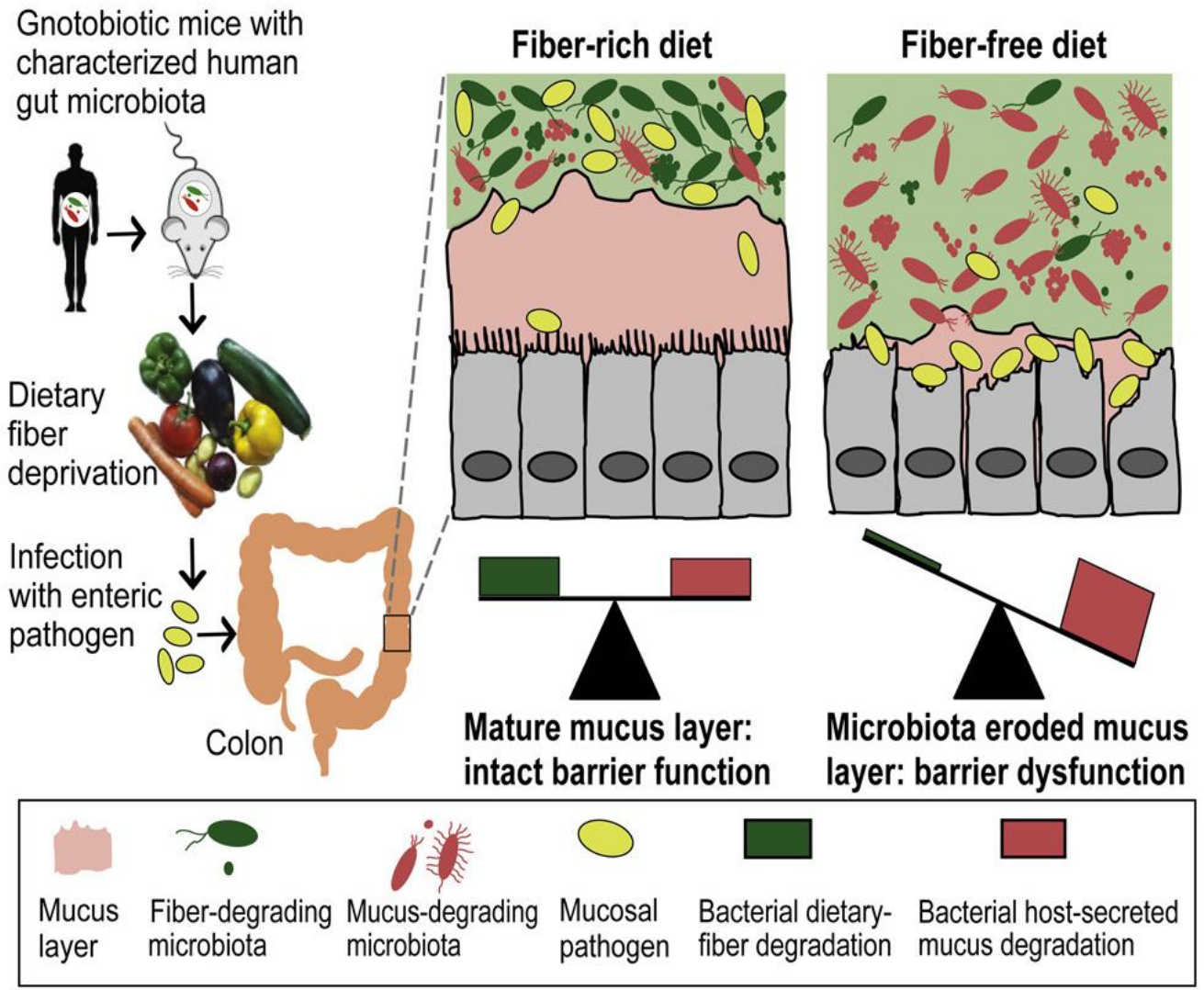


Figure 5. *Bacteroides thetaiotaomicron sus* system. (A) shows the order of genes in the *sus* cluster that is responsible for starch utilization in this species. (B) shows the inferred organization of gene products on or near the bacterial cell surface (OM outer membrane, CM cytoplasmic membrane). Starch 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

Agenda

Diet and the Gut Microbiome: Of Mice and Men

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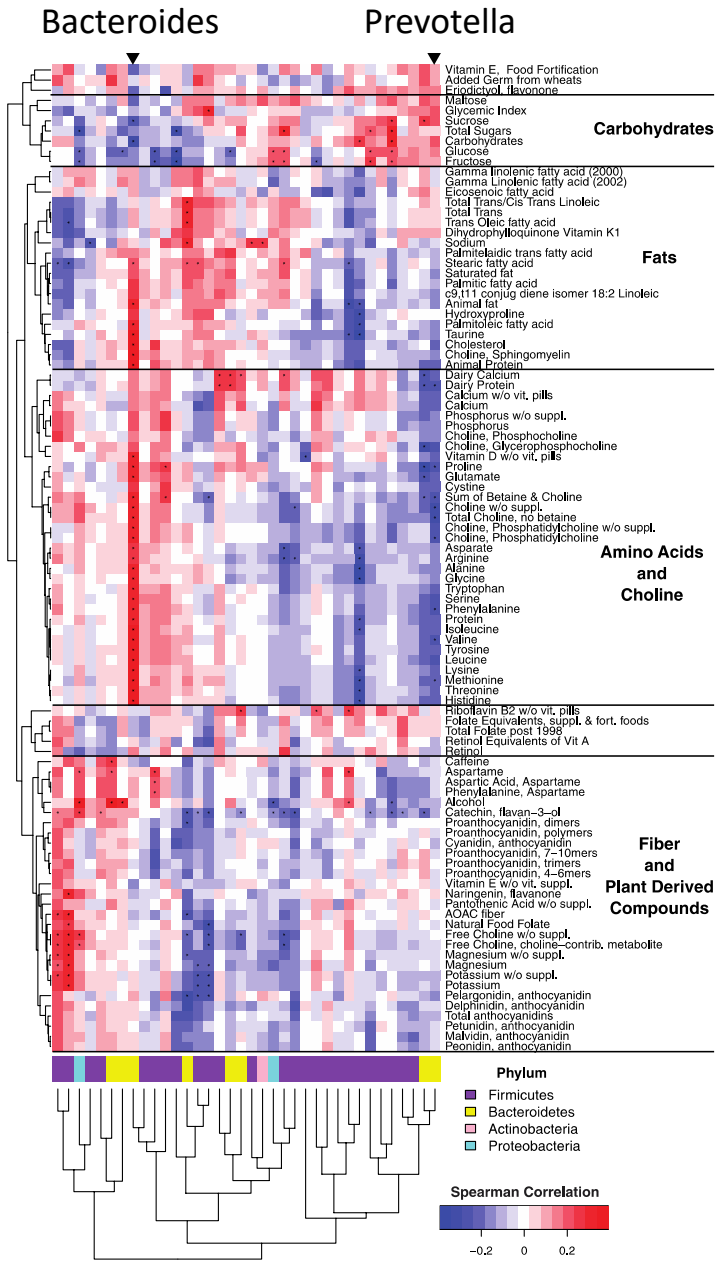
Diet and the Gut Microbiome and its Metabolome in Health and Disease

Dietary Effects on Human Gut Microbiome and its Association with Disease



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

ARTICLE

doi:10.1038/nature12506

Richness of human gut microbiome correlates with metabolic markers

LETTER

doi:10.1038/nature12480

Dietary intervention impact on gut microbial gene richness

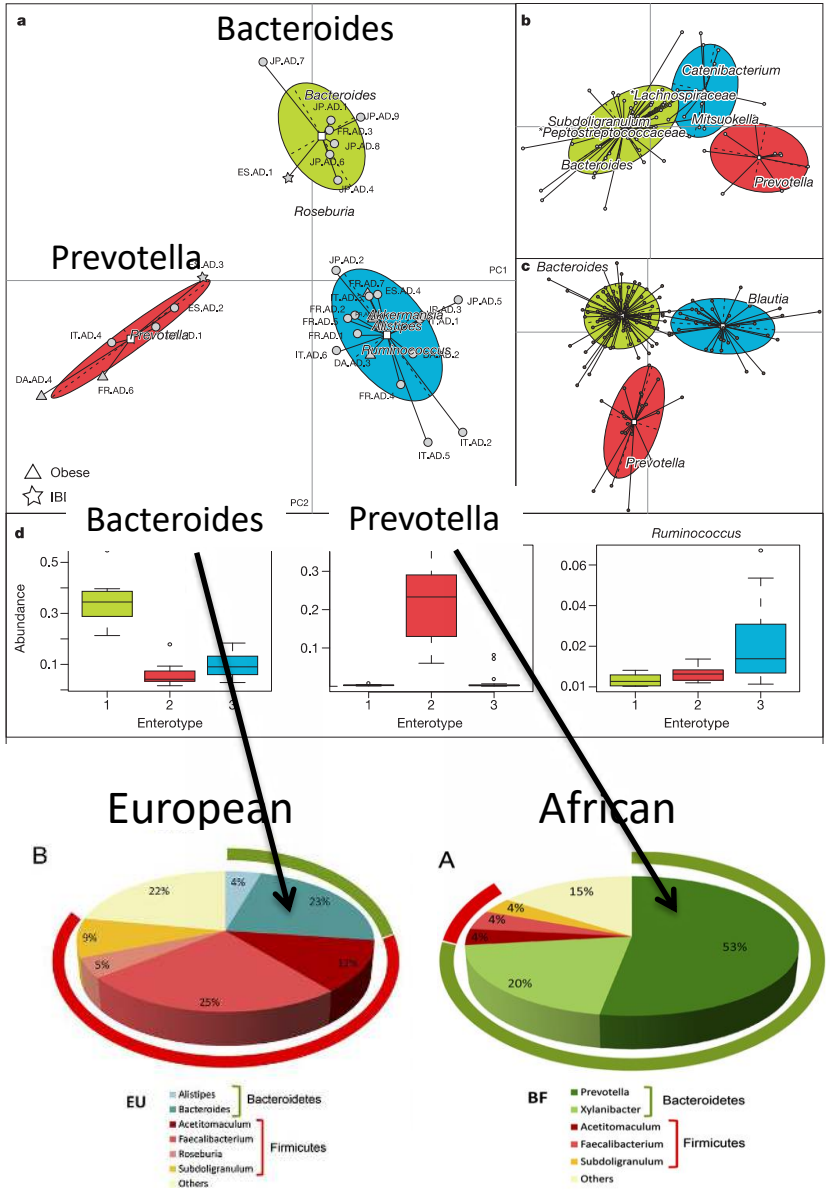
Decrease gut microbiome "richness" (decreased number of various bacteria and their genes) is associated with both disease states and the consumption of a Westernized diet

- 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

ARTICLE
doi:10.1038/nature09944

Enterotypes of the human gut microbiome

Manimozhiyan Arumugam[1]*, Jeroen Raes[1,2]*, Eric Pelletier[3,4,5], Denis Le Paslier[3,4,5], Takuji Yamada[1], Daniel R. Mende[1], Gabriel R. Fernandes[1,6], Julien Tap[1,7], Thomas Bruls[3,4,5], Jean-Michel Batto[7], Marcelo Bertalan[8], Natalia Borruel[9], Francesc Casellas[9], Leyden Fernandez[10], Laurent Gautier[8], Torben Hansen[11,12], Masahira Hattori[13], Tetsuya Hayashi[14], Michiel Kleerebezem[15], Ken Kurokawa[16], Marion Leclerc[7], Florence Levenez[7], Chaysavanh Manichanh[9], H. Bjørn Nielsen[8], Trine Nielsen[11], Nicolas Pons[7], Julie Poulain[3], Junjie Qin[17], Thomas Sicheritz-Ponten[8,18], Sebastian Tims[15], David Torrents[10,19], Edgardo Ugarte[3], Erwin G. Zoetendal[15], Jun Wang[17,20], Francisco Guarner[9], Oluf Pedersen[11,21,22,23], Willem M. de Vos[15,24], Søren Brunak[8], Joel Doré[7], MetaHIT Consortium†, Jean Weissenbach[3,4,5], S. Dusko Ehrlich[7] & Peer Bork[1,25]

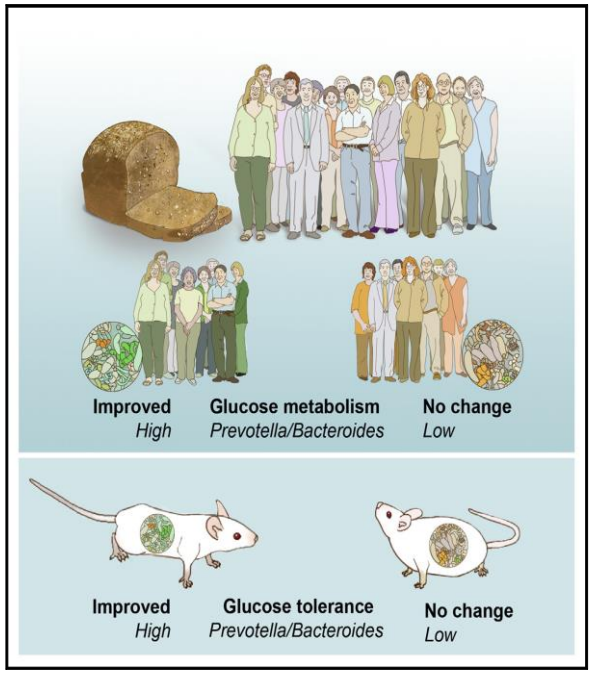


PNAS 2010;107:14691–14696

Cell Metabolism
Clinical and Translational Report

Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*

Graphical Abstract



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.

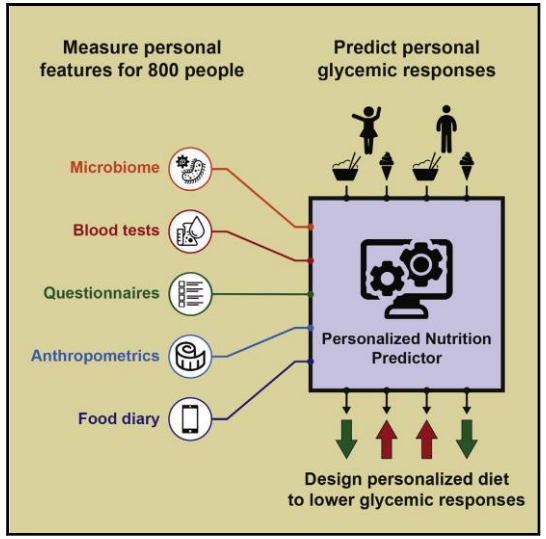
Highlights

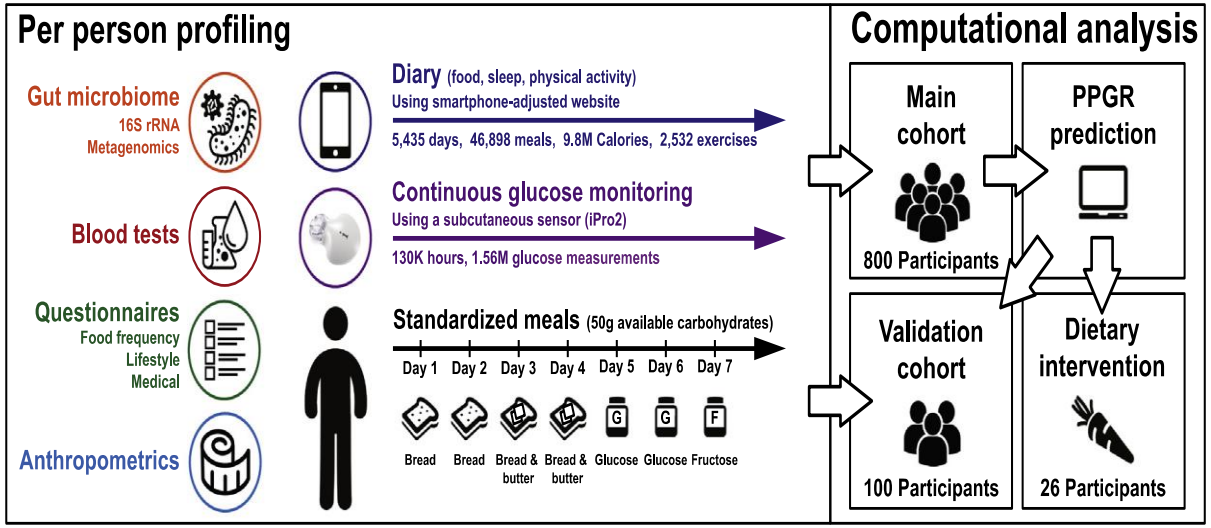
- *Prevotella*/*Bacteroides* is associated with a beneficial response to barley kernels
- *Prevotella*-enriched microbial interactions are higher in barley kernel responders
- *Prevotella* protects against *Bacteroides*-induced glucose intolerance
- *Prevotella* promotes increased hepatic glycogen storage in mice

Personalizing Reponses to Diet Using the Gut Microbiome

Personalized Nutrition by Prediction of Glycemic Responses

David Zeevi,[1,2,8] Tal Korem,[1,2,8] Niv Zmora,[3,4,5,8] David Israeli,[6,8] Daphna Rothschild,[1,2] Adina Weinberger,[1,2] Orly Ben-Yacov,[1,2] Dar Lador,[1,2] Tali Avnit-Sagi,[1,2] Maya Lotan-Pompan,[1,2] Jotham Suez,[3] Jemal Ali Mahdi,[3] Elad Matot,[1,2] Gal Malka,[1,2] Noa Kosower,[1,2] Michal Rein,[1,2] Gili Zilberman-Schapira,[3] Lenka Dohnalová,[3] Meirav Pevsner-Fischer,[3] Rony Bikovsky,[1,2] Zamir Halpern,[5,7] Eran Elinav,[3,9,*] and Eran Segal[1,2,9,*]





Highlights

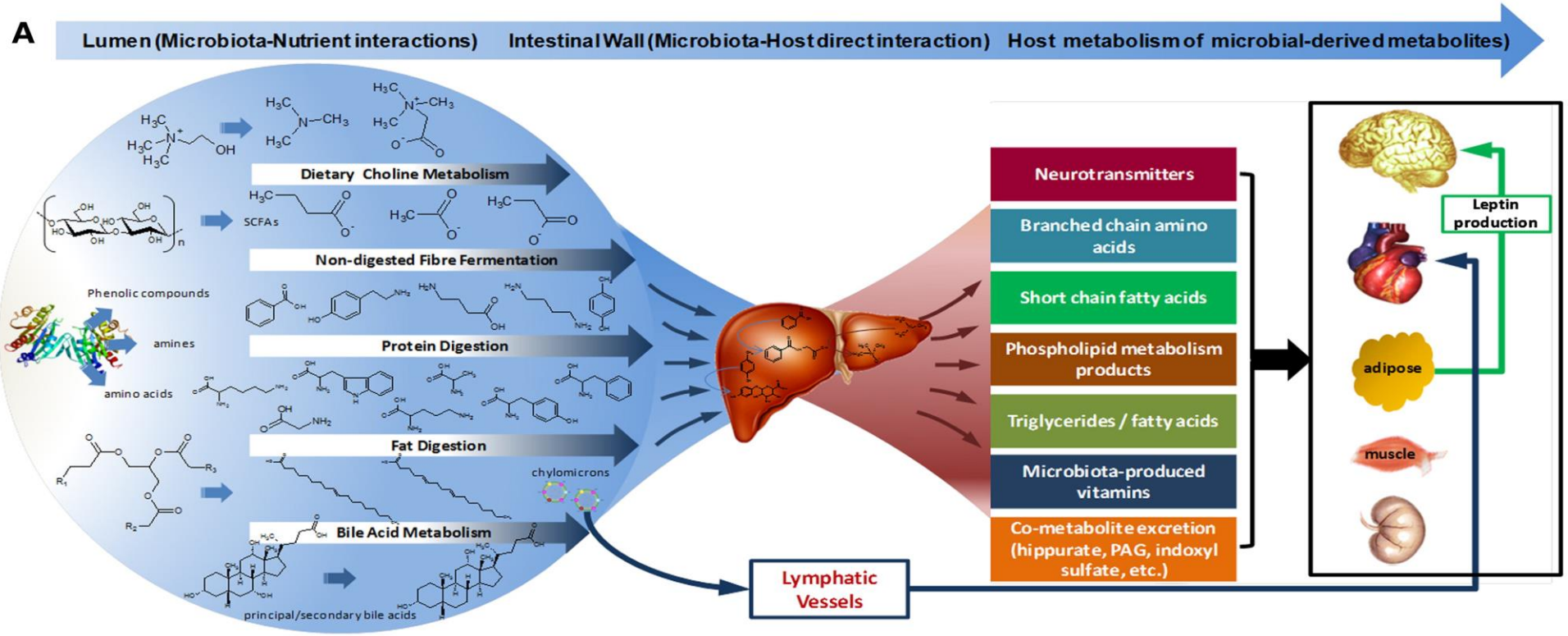
- High interpersonal variability in post-meal glucose observed in an 800-person cohort
- Using personal and microbiome features enables accurate glucose response prediction
- Prediction is accurate and superior to common practice in an independent cohort
- Short-term personalized dietary interventions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

CellPress
Cell Metabolism
Resource

Quantifying Diet-Induced Metabolic Changes of the Human Gut Microbiome

Saeed Shoaie,[1] Pouyan Ghaffari,[1] Petia Kovatcheva-Datchary,[2] Adil Mardinoglu,[1] Partho Sen,[1] Estelle Pujos-Guillot,[3] Tomas de Wouters,[4] Catherine Juste,[4] Salwa Rizkalla,[5,6] Julien Chilloux,[7] Lesley Hoyles,[7] Jeremy K. Nicholson,[7] MICRO-Obes Consortium, Joel Dore,[4] Marc E. Dumas,[7] Karine Clement,[5,6,8] Fredrik Bäckhed,[2,9] and Jens Nielsen[1,*]

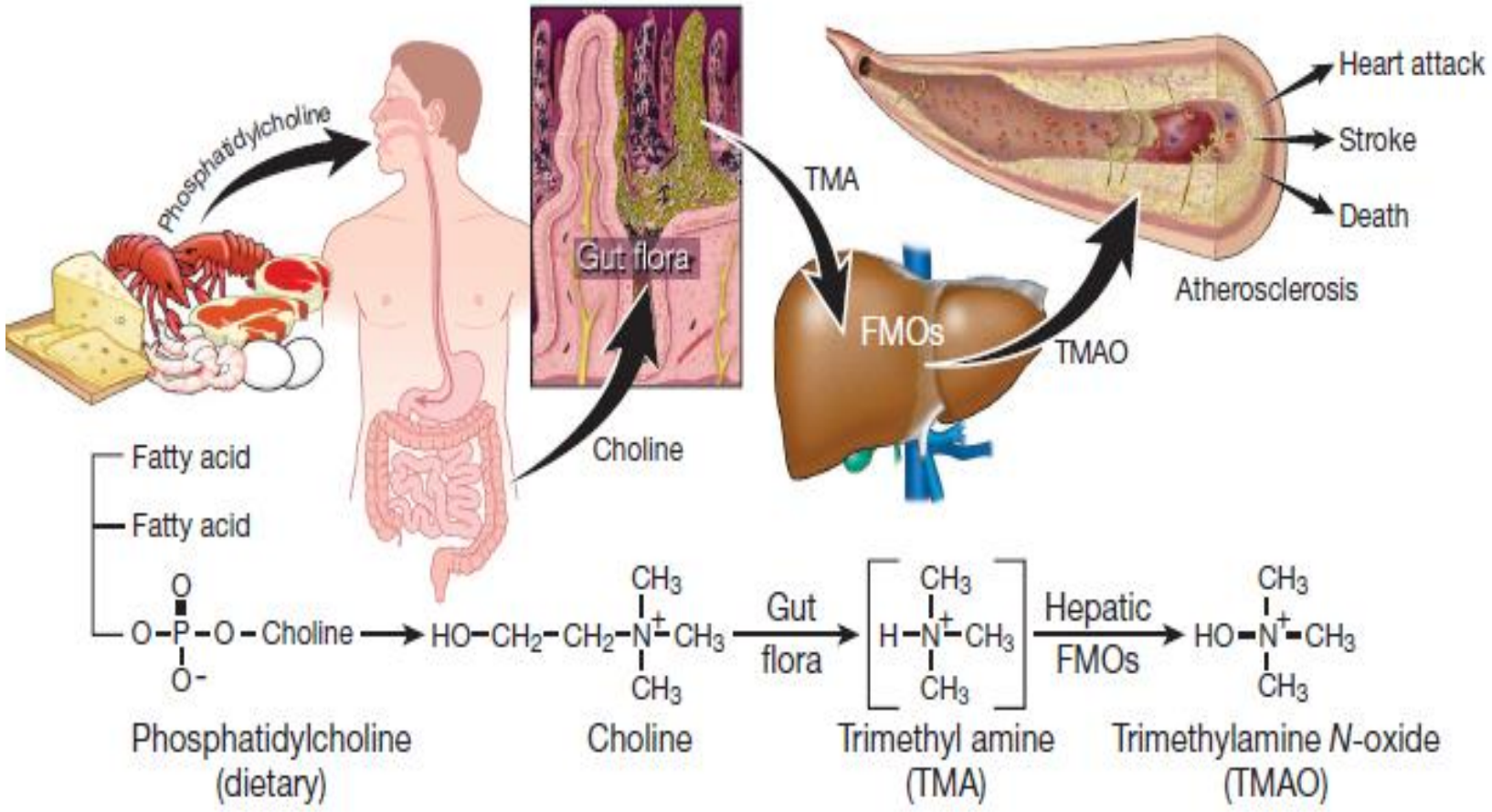
Diet, the Gut Microbiome, and its Metabolome



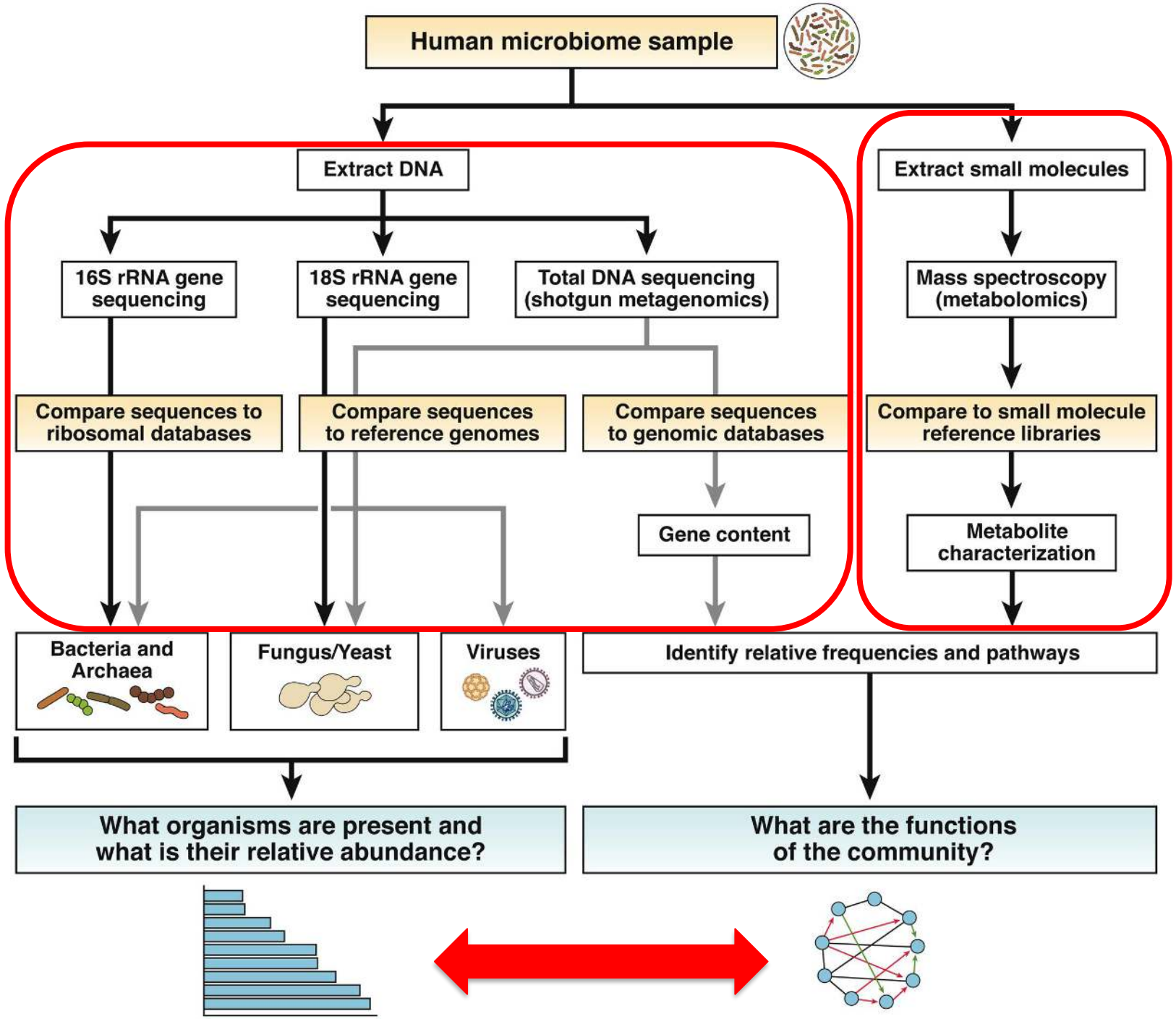
Holmes et al. *Cell Met.* 2012;16:559

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



Wang et al. *Nature*. 2011;472:57-63


Human microbiome sample
Extract DNA
16S rRNA gene sequencing
18S rRNA gene sequencing
Total DNA sequencing (shotgun metagenomics)
Compare sequences to ribosomal databases
Compare sequences to reference genomes
Compare sequences to genomic databases
Gene content
Extract small molecules
Mass spectroscopy (metabolomics)
Compare to small molecule reference libraries
Metabolite characterization
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?

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)

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

Antibiotics

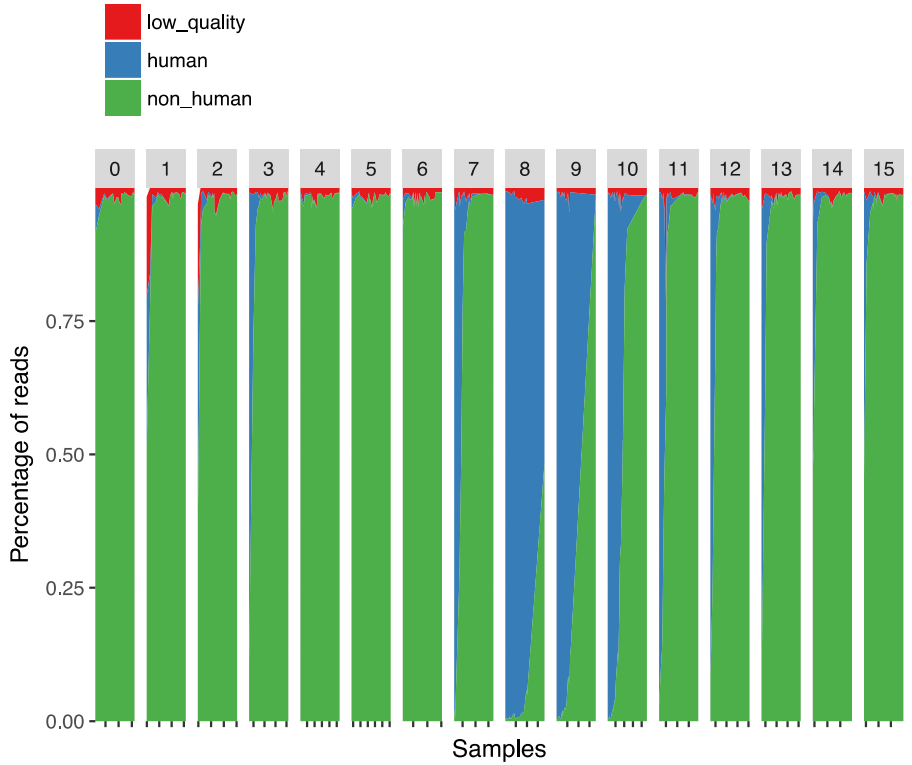
PEG

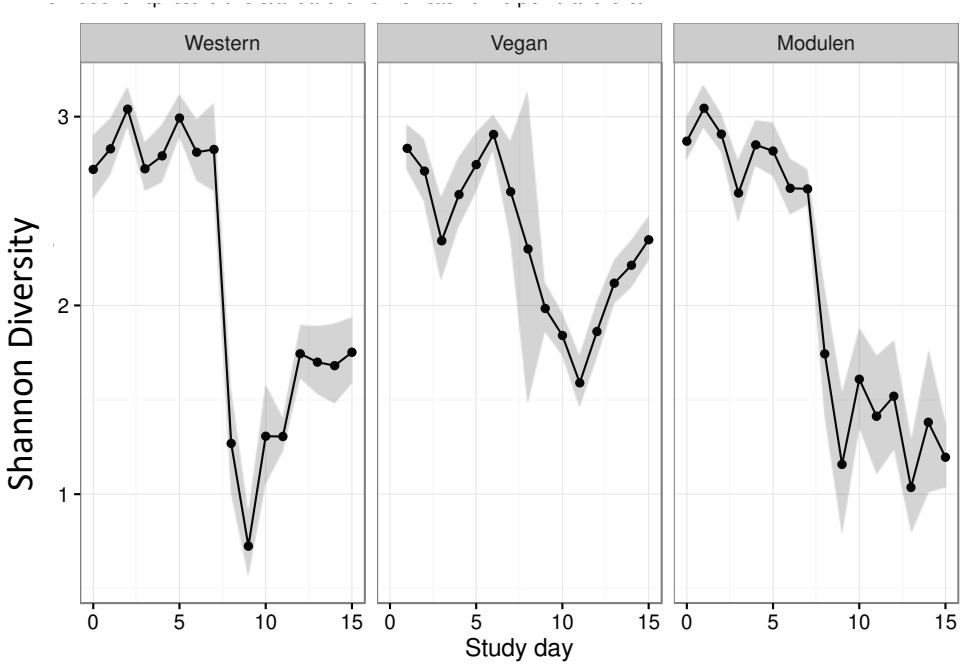
* * * * *

Diet Induced Changes in Metabolites and Microbiome (Aim 1)

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

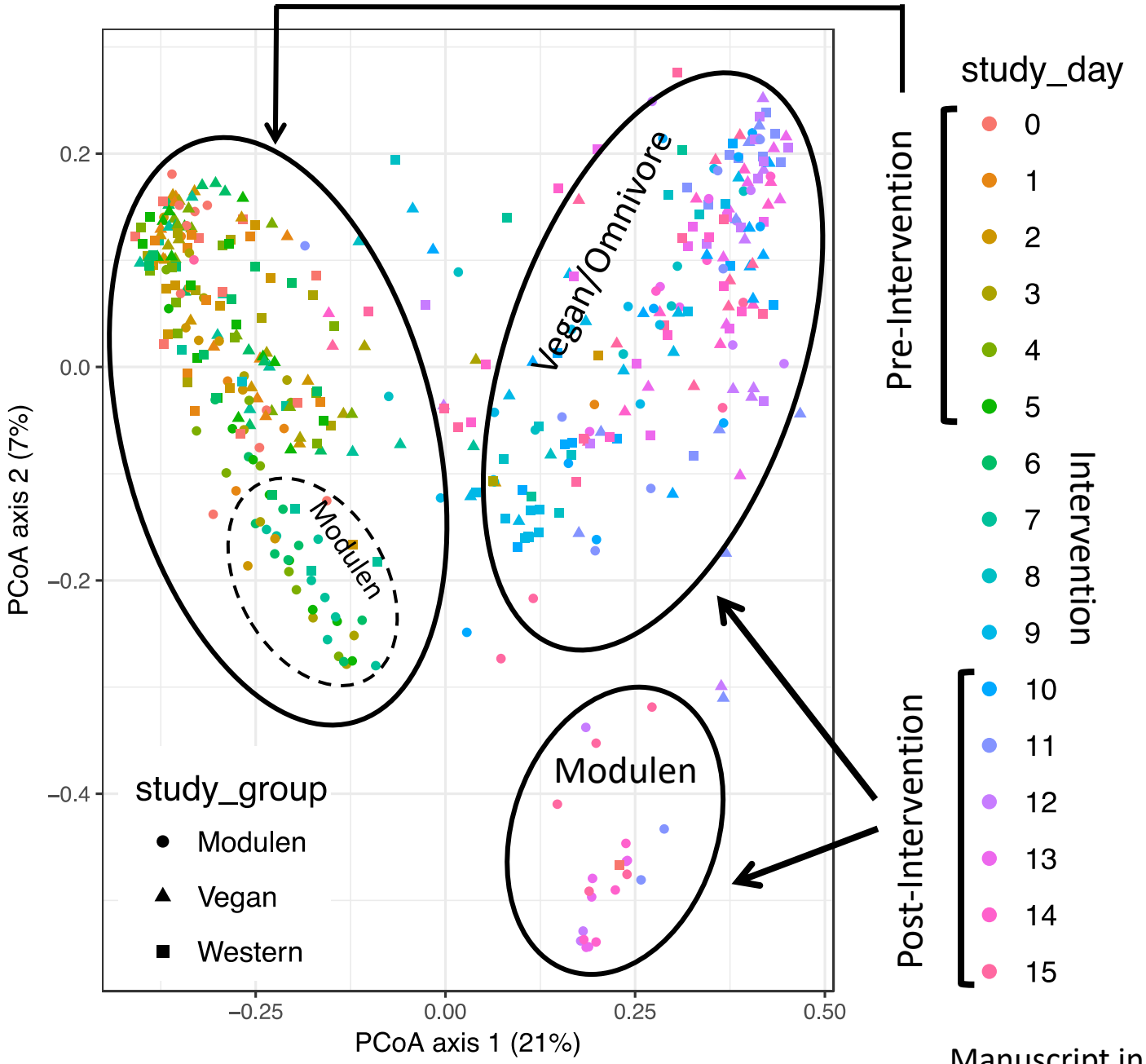
Correlate Increase/ Decrease of Metabolites with Recurrence of Microbes and Diet to Identify Key Drivers (Aims 2 and 3)



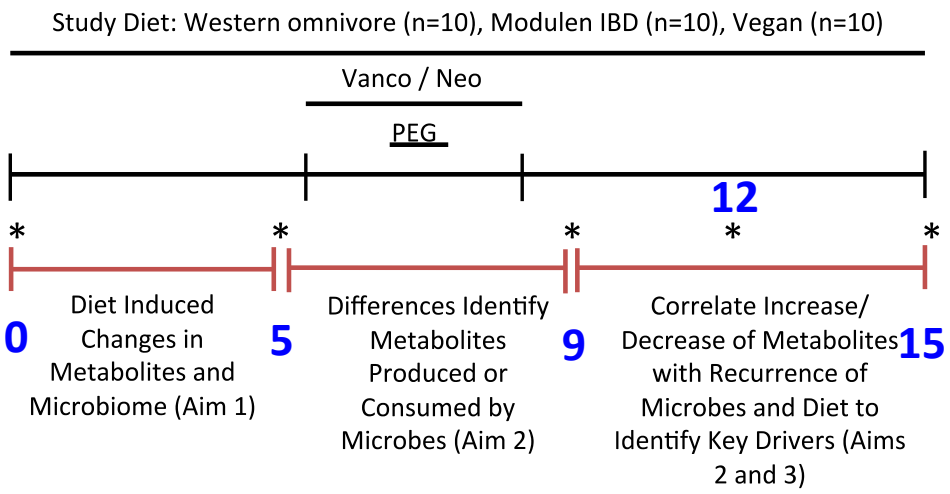


Manuscript in preparation

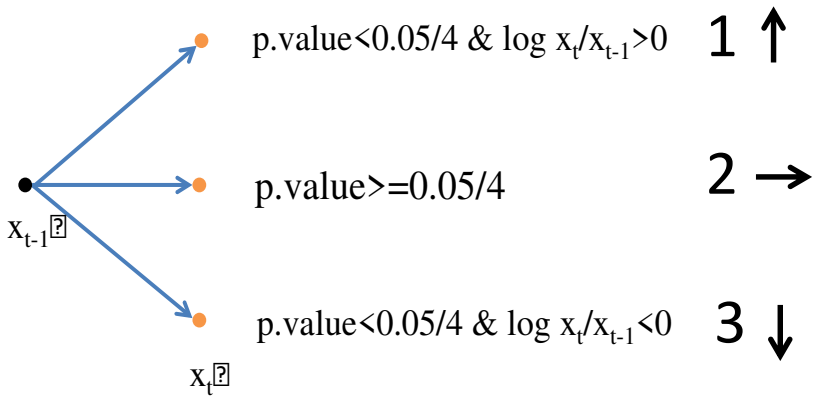
Composition of the Gut Microbiota in FARMM

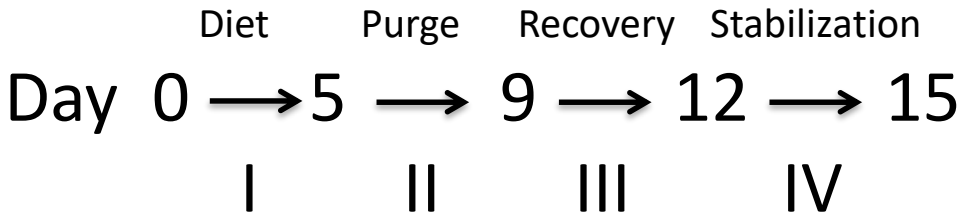


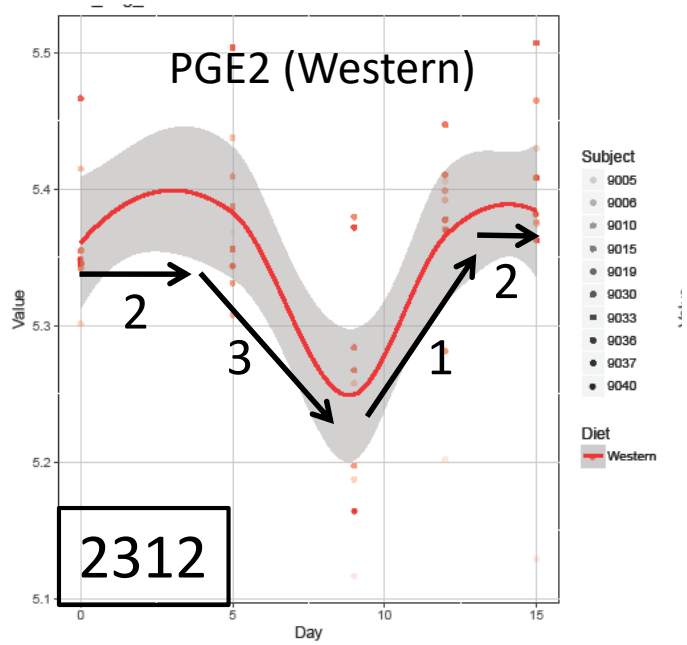
Manuscript in preparation

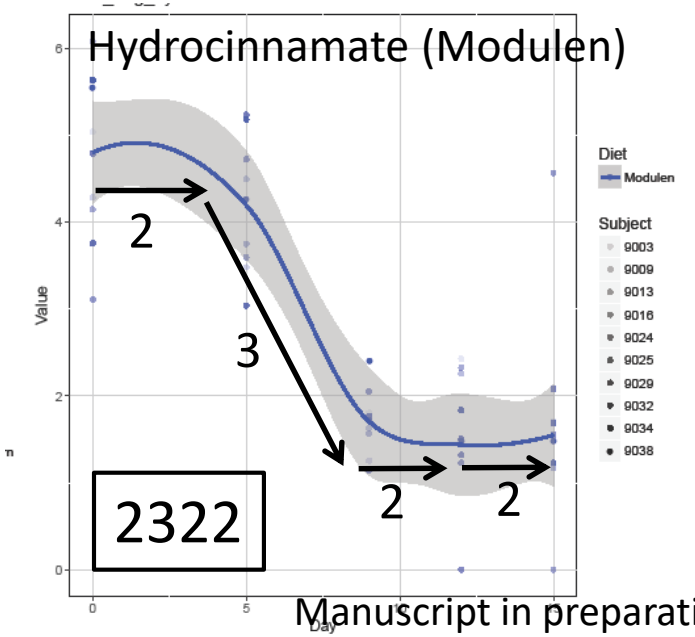


Paired t-test to determine categories of variation



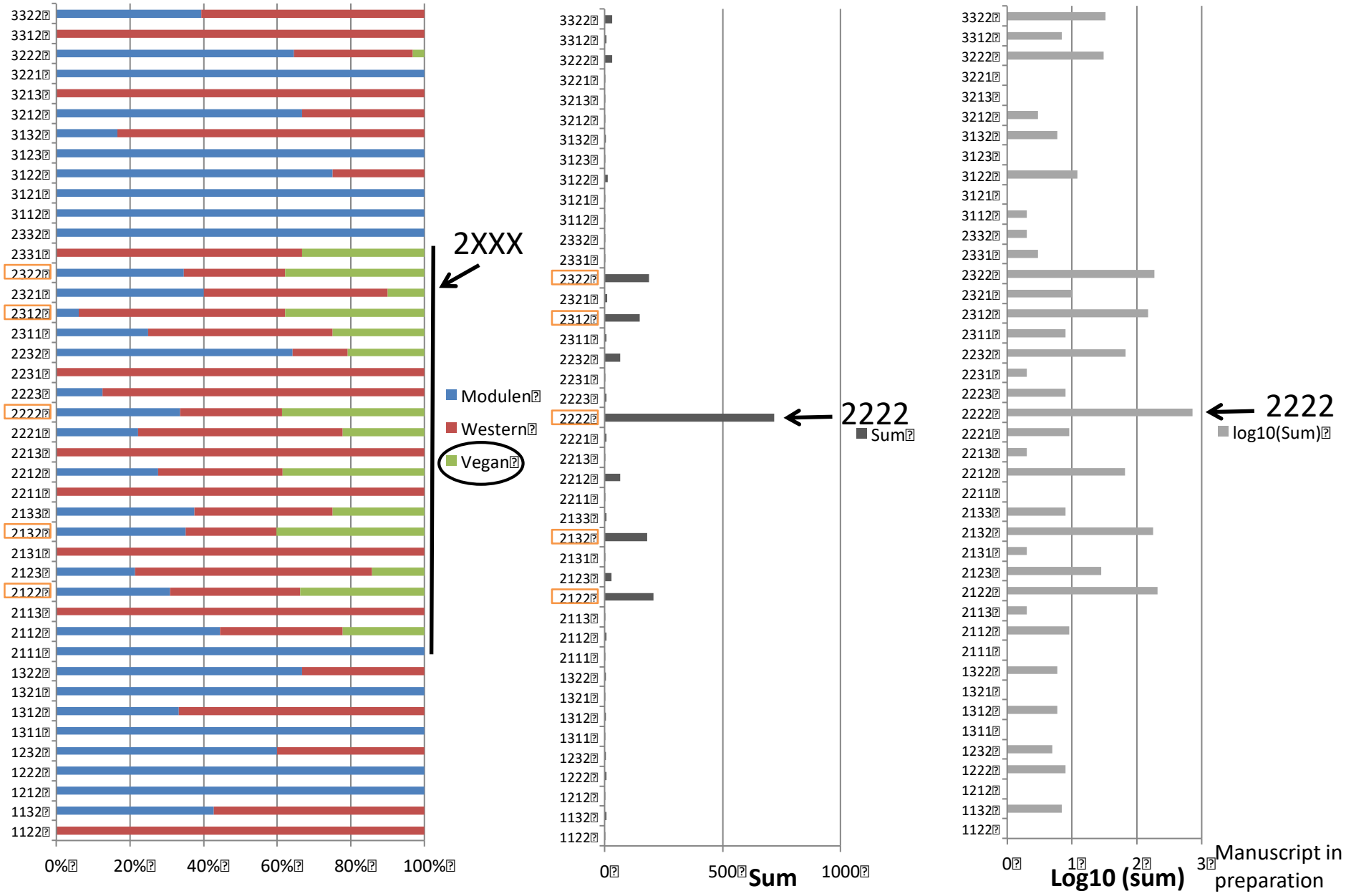




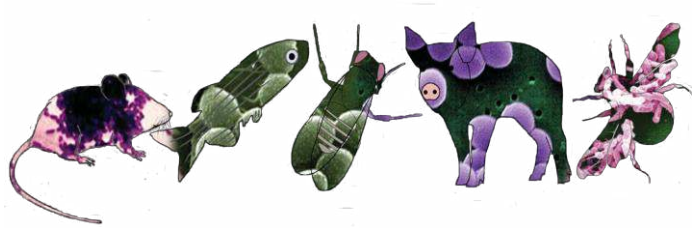


Manuscript in preparation

Patterns of Fecal Metabolites Over Time in FARMM



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

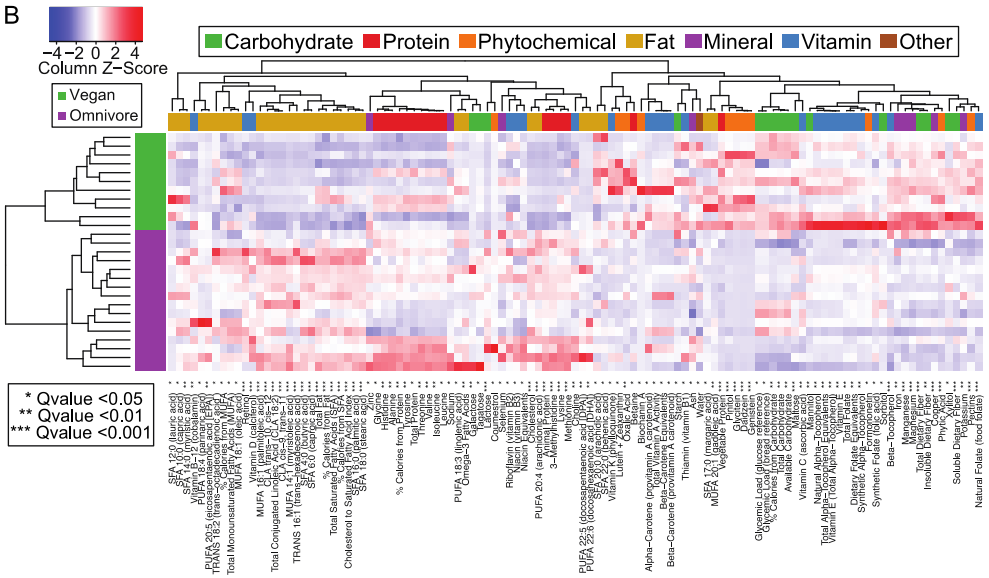
BUT

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

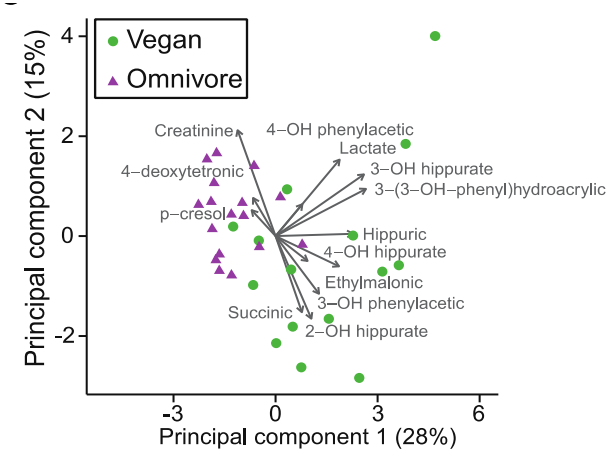
Embracing the complexity of human biology through the use of high dimensional analytic technologies together with advanced computational and biostatistical platforms

The Plasma and Urinary Metabolome as a Biomarker of Diet

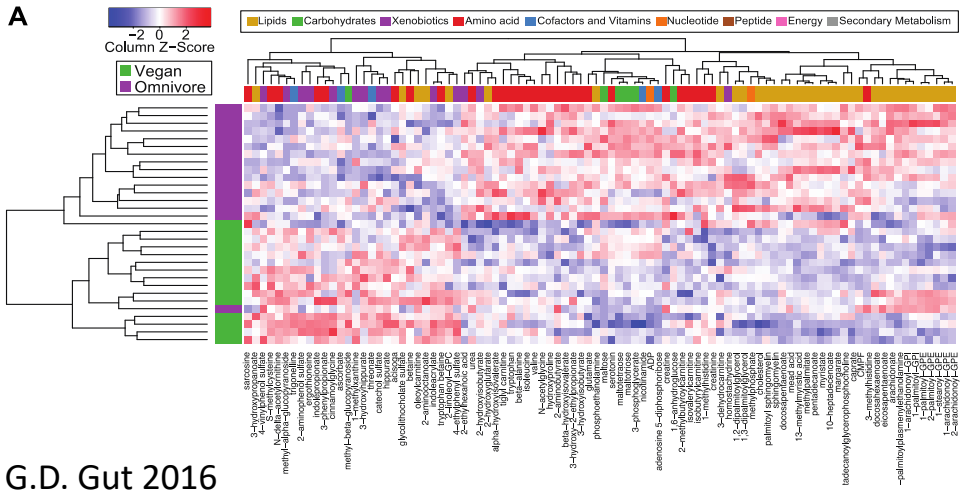
A Comparison of Diet Between Omnivores and Vegans



The Urinary Metabolome



A Comparison of the Plasma Metabolome Between Omnivores and Vegans



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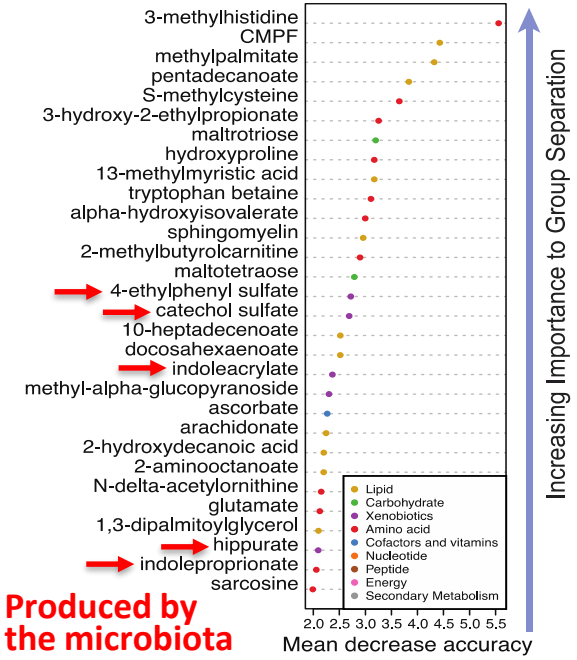
Predicting an Omnivore vs. Vegan Diet by Random Forest



→ = **Produced by the microbiota**

Leading Edge
Perspective

Cell

The Convergence of Systems and Reductionist Approaches in Complex Trait Analysis

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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.

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PennCHOP MICROBIOME PROGRAM

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

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