“YOU ARE WHAT YOUR BUGS EAT!”

Diet, the Gut Microbiota and its Metabolome in Human Health and Disease

Gary D. Wu, M.D.

Ferdinand G. Weisbrod Professor in Gastroenterology
Perelman School of Medicine
University of Pennsylvania

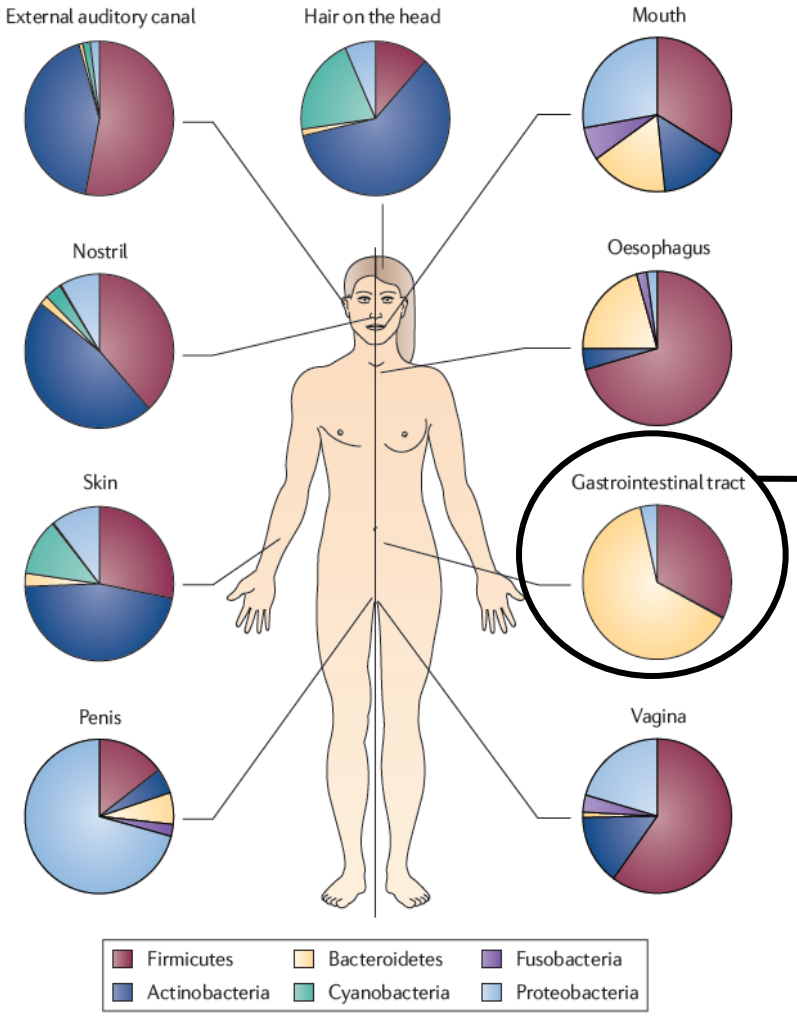
Agenda

The Intestinal Microbiome, Early Life Events, and Association with Disease (Asthma)

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

Current and Future Status of the Microbiome Field: Fecal Microbiota Transplantation (FMT) and Beyond

The Human Microbiome

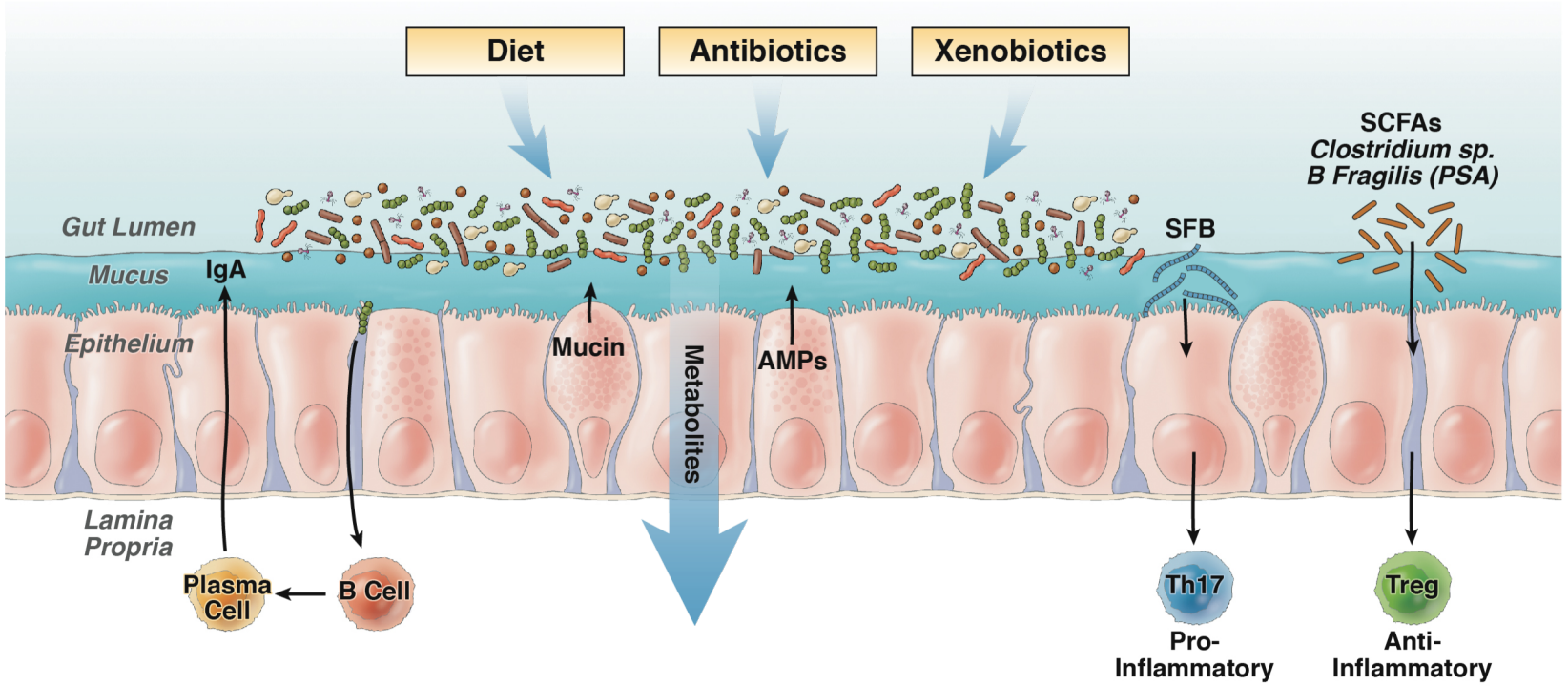


- **Comprised of Bacteria, Viruses, others (Archaea, Eukaryotes)**
- **Distinctive microbiomes at each body site (gut, lung, skin, mucosa etc.)**

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**

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



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

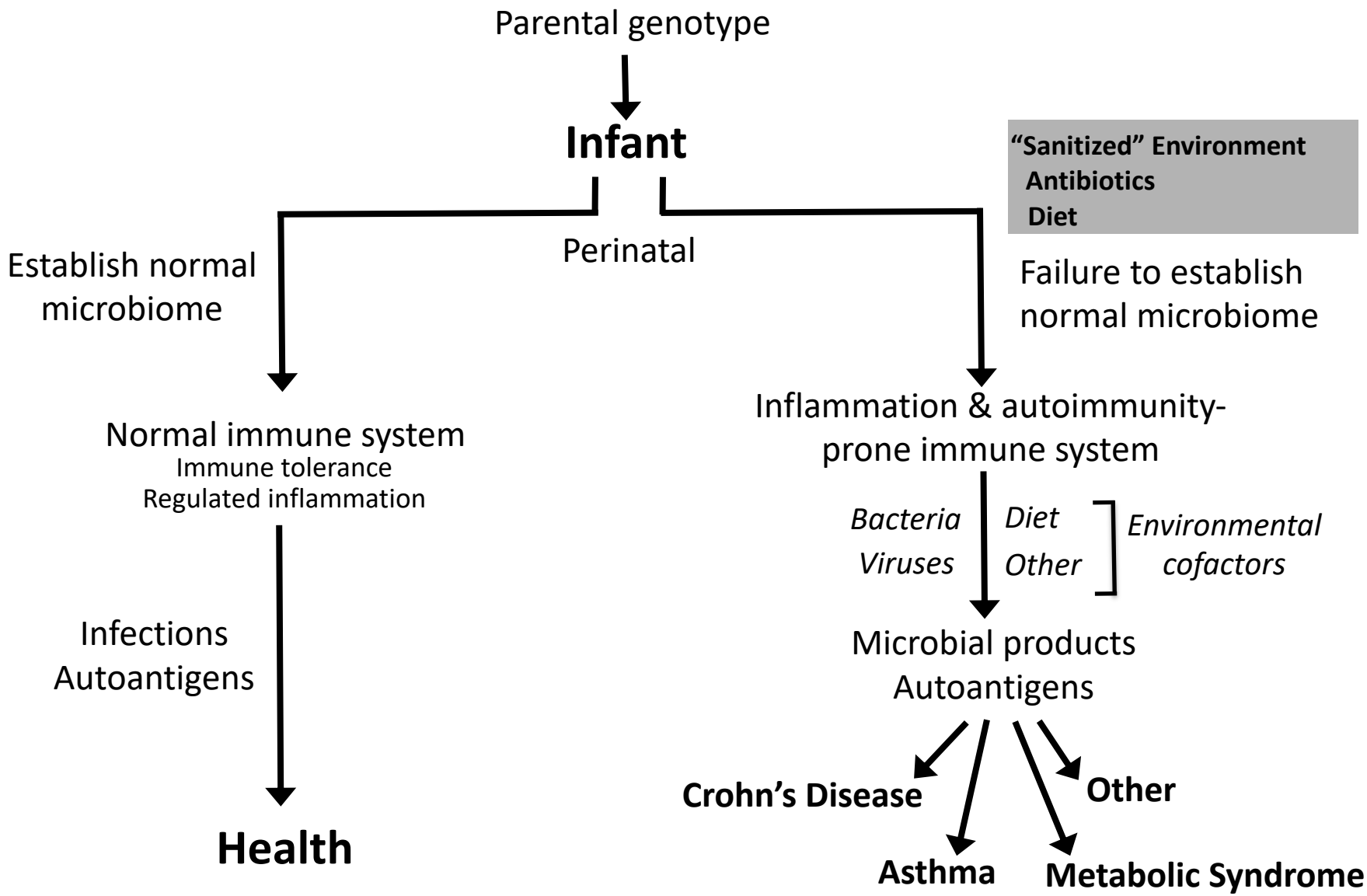
Atherosclerosis: Oral, gut and plaque microbiota; Microbial metabolism of choline to TMA

Asthma: Sanitized environment

Colon Cancer: Enterotoxigenic *Bacteroides fragilis and Fusobacterium*

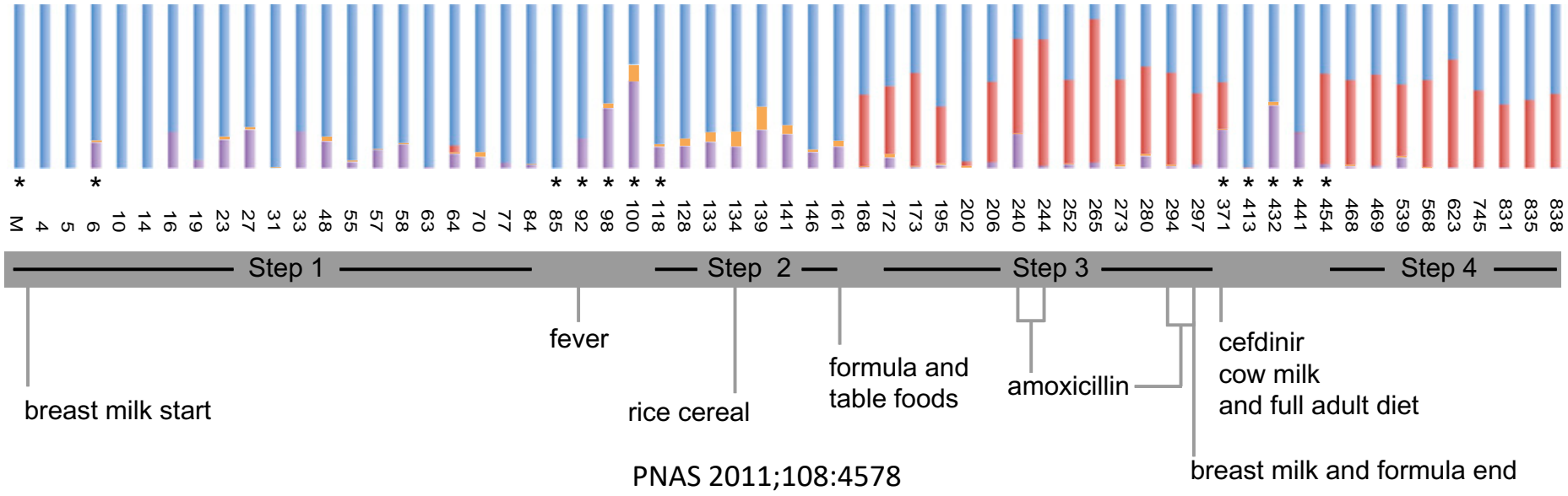
Inflammatory Bowel Disease: Dysbiosis

Host Gene-Microbial Interactions in the Pathogenesis of Immune-Mediated Diseases in "Modern Society"



Adapted from Virgin et al. Cell 2011;147:44

The Early Human Gut Microbiota



PNAS 2011;108:4578

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 4, 2016 VOL. 375 NO. 5

Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children

Michelle M. Stein, B.S., Cara L. Hrusch, Ph.D., Justyna Gozdz, B.A., Catherine Igartua, B.S., Vadim Pivniouk, Ph.D., Sean E. Murray, B.S., Julie G. Ledford, Ph.D., Mauricius Marques dos Santos, B.S., Rebecca L. Anderson, M.S., Nervana Metwali, Ph.D., Julia W. Neilson, Ph.D., Raina M. Maier, Ph.D., Jack A. Gilbert, Ph.D., Mark Holbreich, M.D., Peter S. Thorne, Ph.D., Fernando D. Martinez, M.D., Erika von Mutius, M.D., Donata Vercelli, M.D., Carole Ober, Ph.D., and Anne I. Sperling, Ph.D.

- Asthma was 4 and 6 times lower in the Amish relative to Hutterites.
- Differences in microbial composition were also observed in dust samples from Amish and Hutterite homes.
- Profound differences functions immune cells were also found between the two groups of children.
- In a mouse model of experimental allergic asthma, dust extracts from Amish but not Hutterite homes significantly inhibited airway hyperractivity and eosinophilia.

➢ Analogies to peanut allergies.

	Conventionally Housed	Germ-free	Adult Microbial Colonization	Perinatal Microbial Colonization
Colonic and Lung iNKT Cells	+	++++	++++	+
Oxazolone Colitis and Asthma	+	++++	++++	+

Olszak et al. Science 2012;336:489

Agenda

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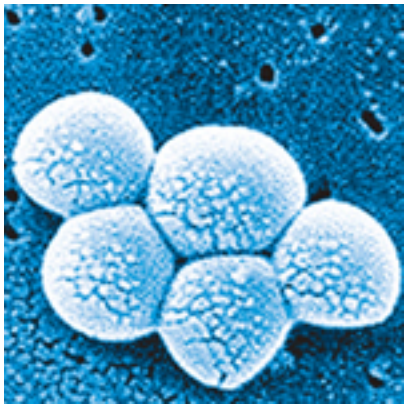
Host-Microbial Mutualism the Gut

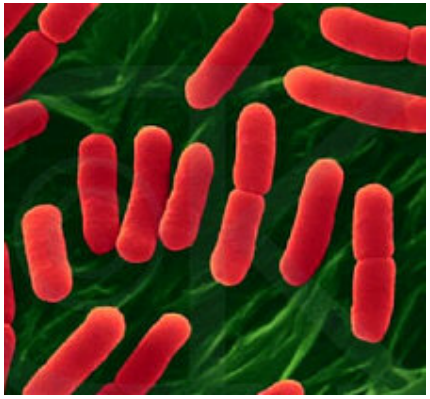
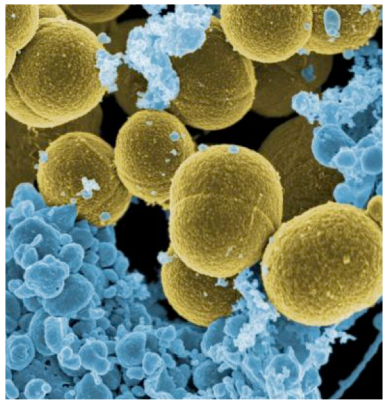
Host benefits to bacteria

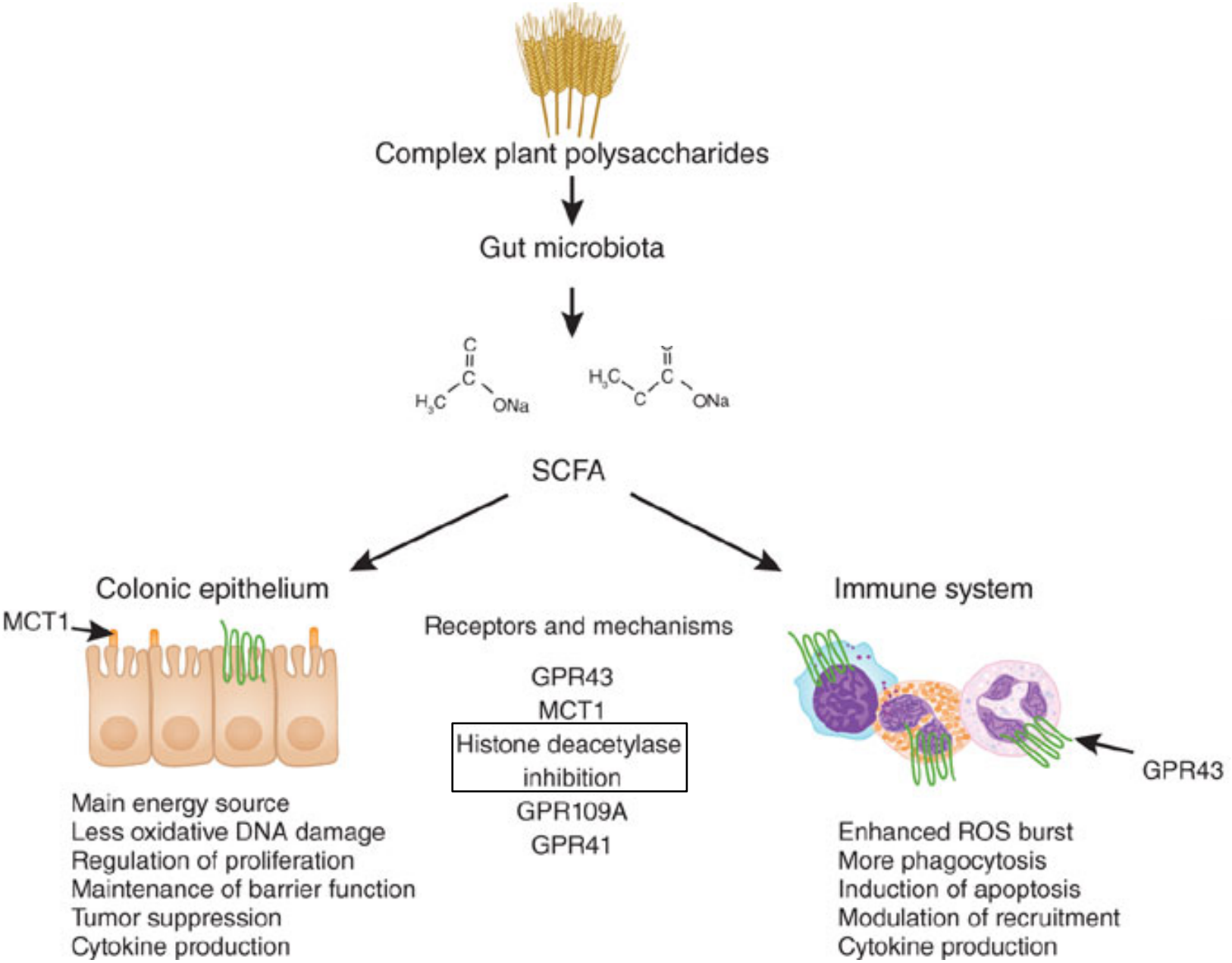
- •Provides a unique niche
- → •Intestinal mucus provides a source of nutrition

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

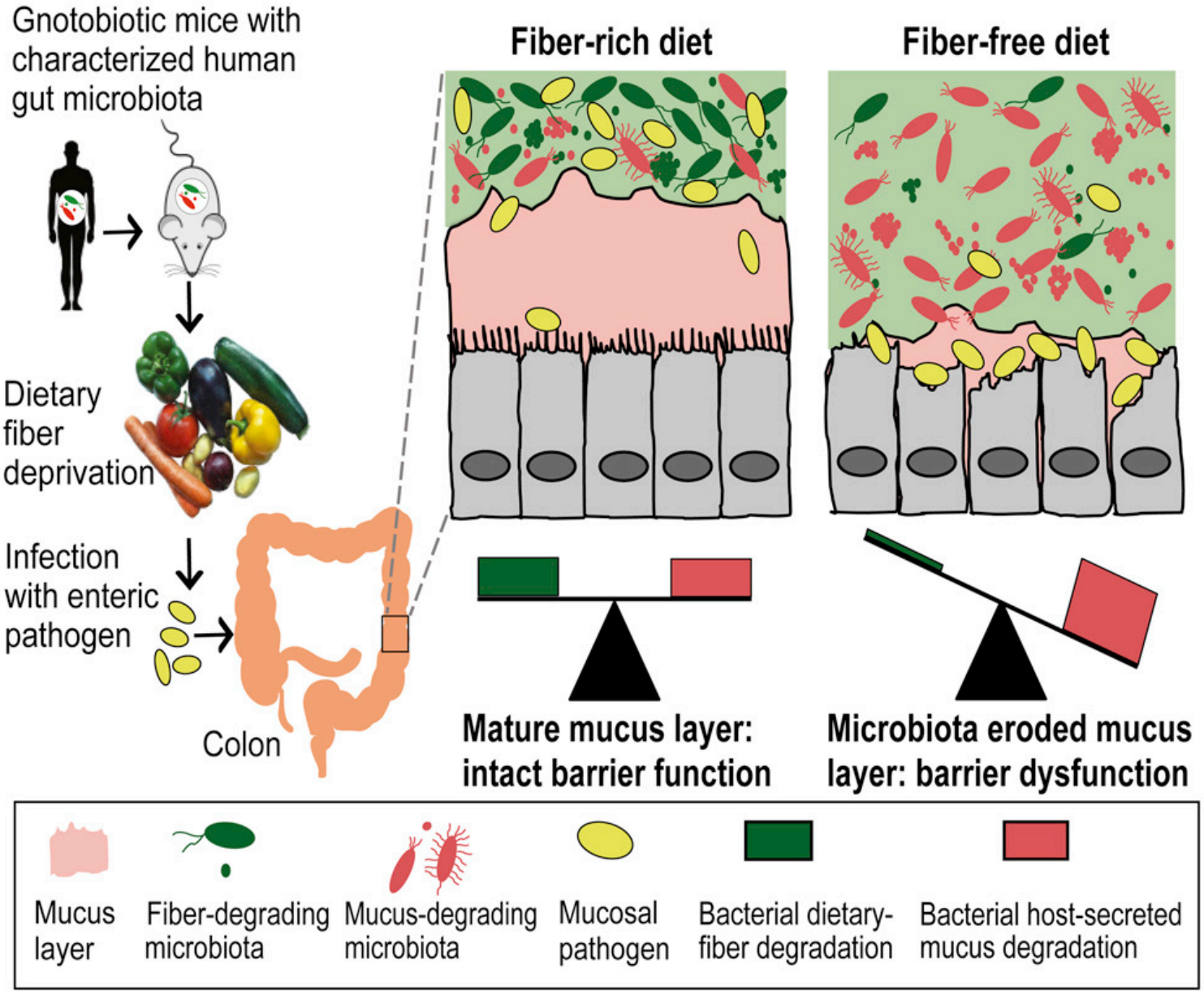




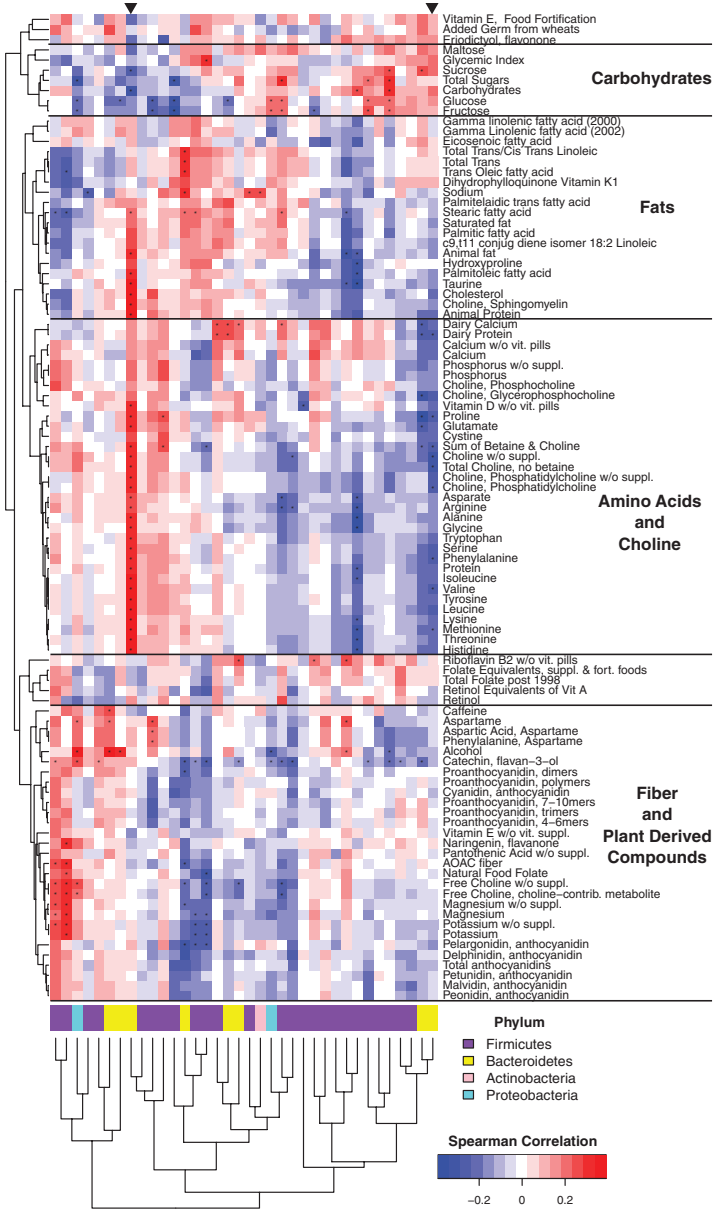
Nature Immunology 12, 5–9 (2011)

Dietary Fiber and the Intestinal Mucus Barrier



Desai, MS et al. Cell;167:2016

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

Emmanuelle Le Chatelier[1]*, Trine Nielsen[2]*, Junjie Qin[3]*, Edi Prifti[1]*, Falk Hildebrand[4,5], Gwen Falony[4,5], Mathieu Almeida[1], Manimozhiyan Arumugam[2,3,6], Jean-Michel Batto[1], Sean Kennedy[1], Pierre Leonard[1], Junhua Li[3,7], Kristoffer Burgdorf[2], Niels Grarup[2], Torben Jørgensen[8,9,10], Ivan Brandslund[11,12], Henrik Bjørn Nielsen[2], Agnieszka S. Junckerr[13], Marcelo Bertalan[13], Florence Levenez[1], Nicolas Pons[1], Simon Rasmussen[2,3], Shinichi Sunagawa[6], Julien Tap[1,6], Sebastian Tims[14], Erwin G. Zoetendal[14], Søren Brunak[15], Karine Clément[2,5,16,17], Joël Doré[1,18], Michiel Kleerebezem[14], Karsten Kristiansen[19], Pierre Renault[18], Thomas Sicheritz-Ponten[15], Willem M. de Vos[14,20], Jean-Daniel Zucker[13,16,21], Jeroen Raes[4,5], Torben Hansen[8,22], MetaHIT consortium†, Peer Bork[6], Jun Wang[3,19,23,24,25], S. Dusko Ehrlich[1] & Oluf Pedersen[2,26,27,28]

LETTER doi:10.1038/nature12480

Dietary intervention impact on gut microbial gene richness

Aurélie Cotillard[1,2]*, Sean P. Kennedy[3]*, Ling Chun Kong[1,2,4]*, Edi Prifti[1,2,3]*, Nicolas Pons[3]*, Emmanuelle Le Chatelier[3], Mathieu Almeida[3], Benoit Quinquis[3], Florence Levenez[3,5], Nathalie Galleron[3], Sophie Gougis[4], Salwa Rizkalla[1,2,4], Jean-Michel Batto[3,5], Pierre Renault[3], ANR MicroObes consortium†, Joel Doré[3,5], Jean-Daniel Zucker[1,2,6], Karine Clément[1,2,4] & Stanislav Dusko Ehrlich[3]

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

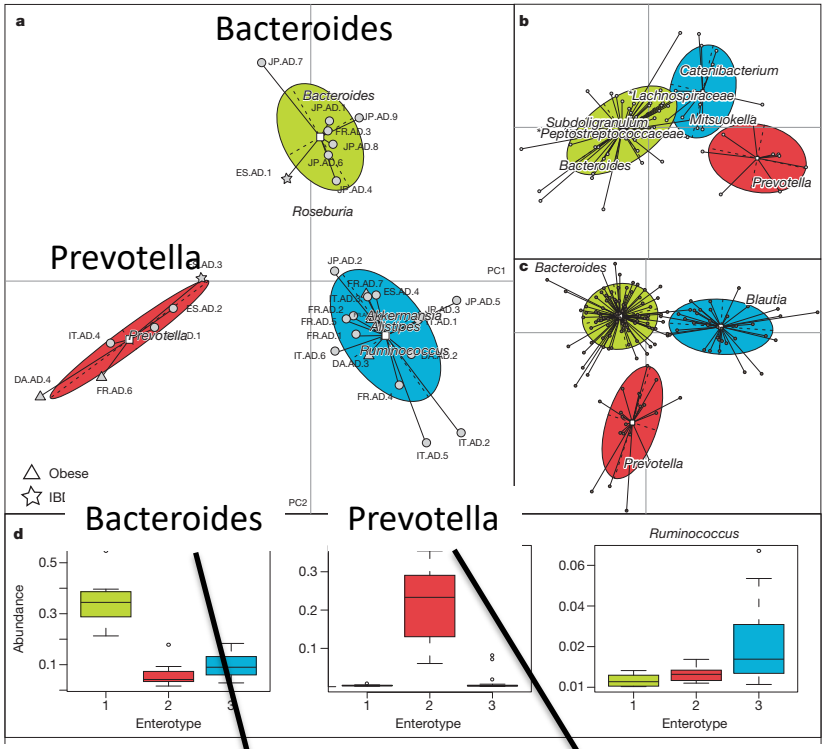
- Individuals with marked obesity, insulin resistance, dyslipidemia, and inflammatory phenotype have low bacterial richness
- Increased consumption of an agrarian diet, rich in fruits and vegetables with higher fiber, is associated with increased bacterial gene richness
- Energy-restricted diets increase bacterial gene richness

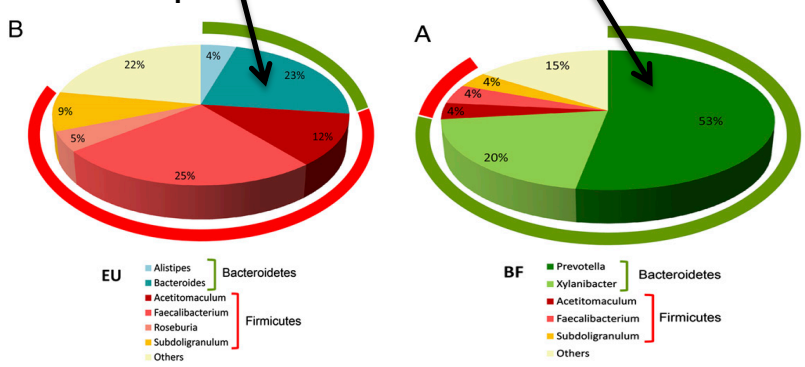
ARTICLE

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[9], 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]





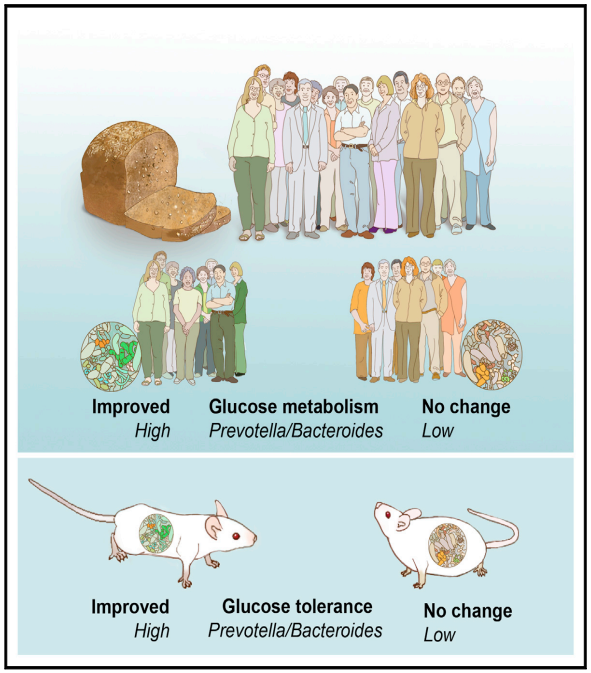
PNAS 2010;107:14691–14696

Clinical and Translational Report

Cell Metabolism

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

Graphical Abstract



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

Authors

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

Correspondence

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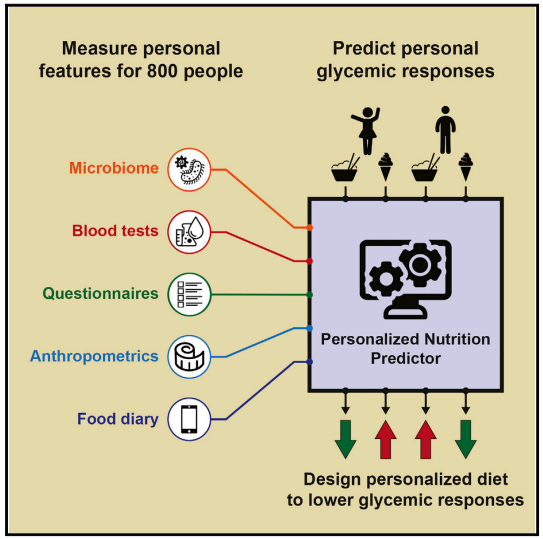
In Brief

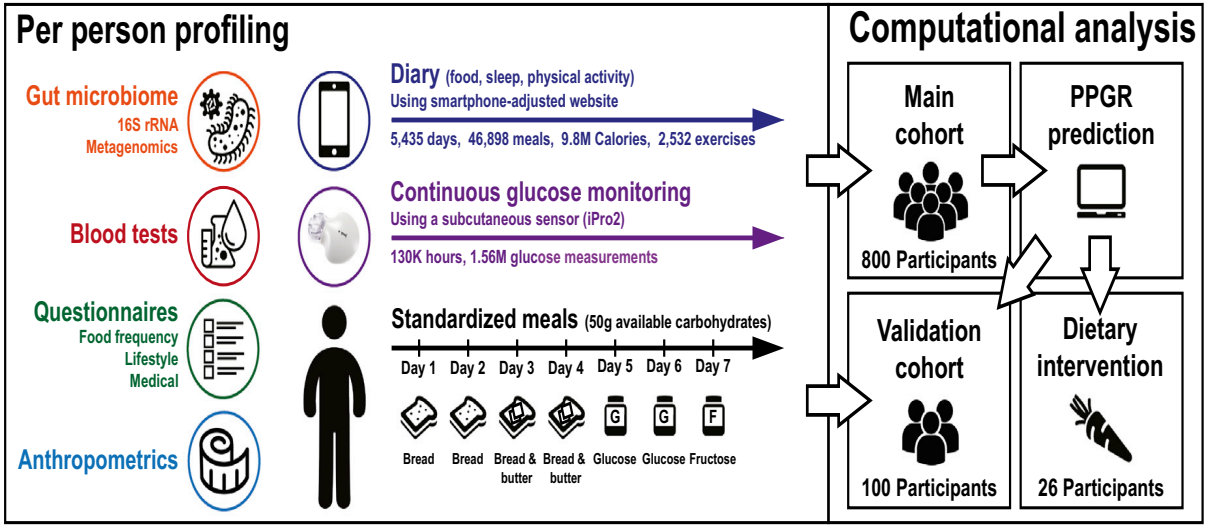
Diet affects the gut microbiota composition, though large inter-individual variations exist. Kovatcheva-Datchary et al. reveal that subjects with improved glucose metabolism after barley kernel supplementation have increased *Prevotella* in their gut microbiota. *Prevotella* plays a direct role in the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

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,*]

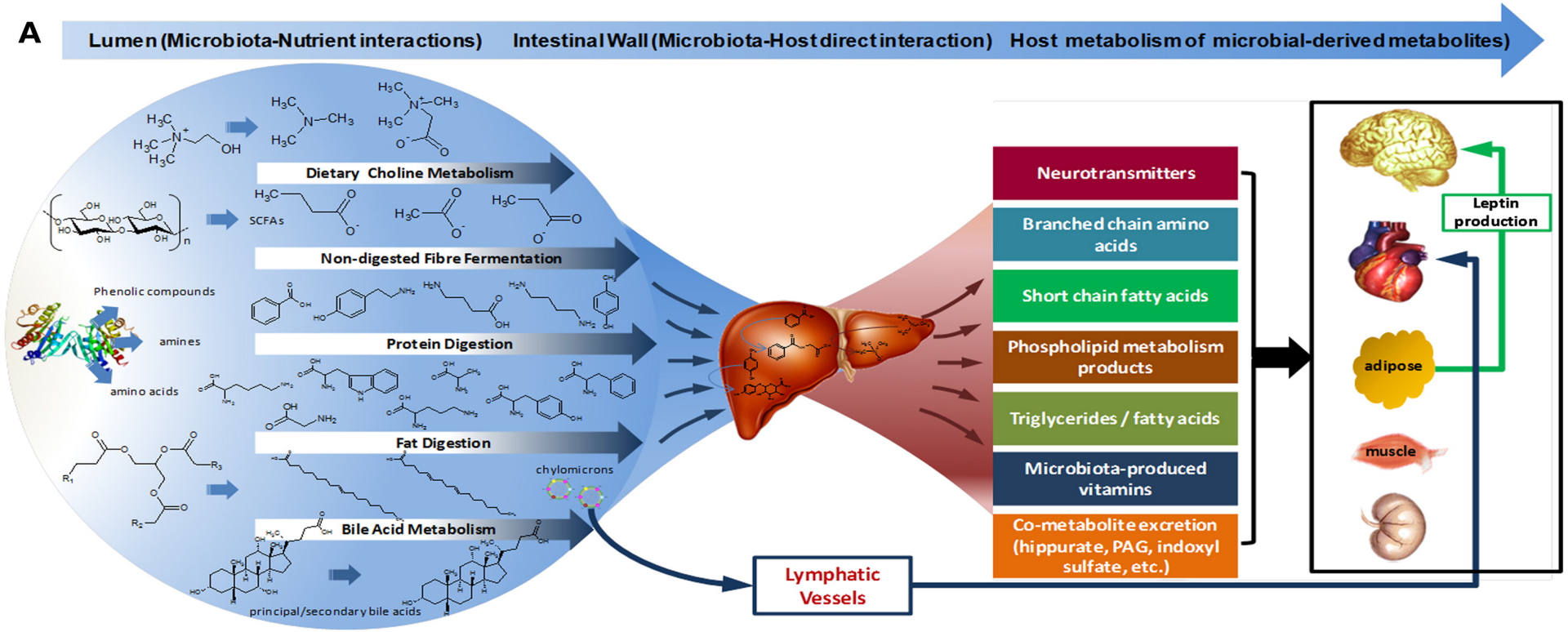




Highlights

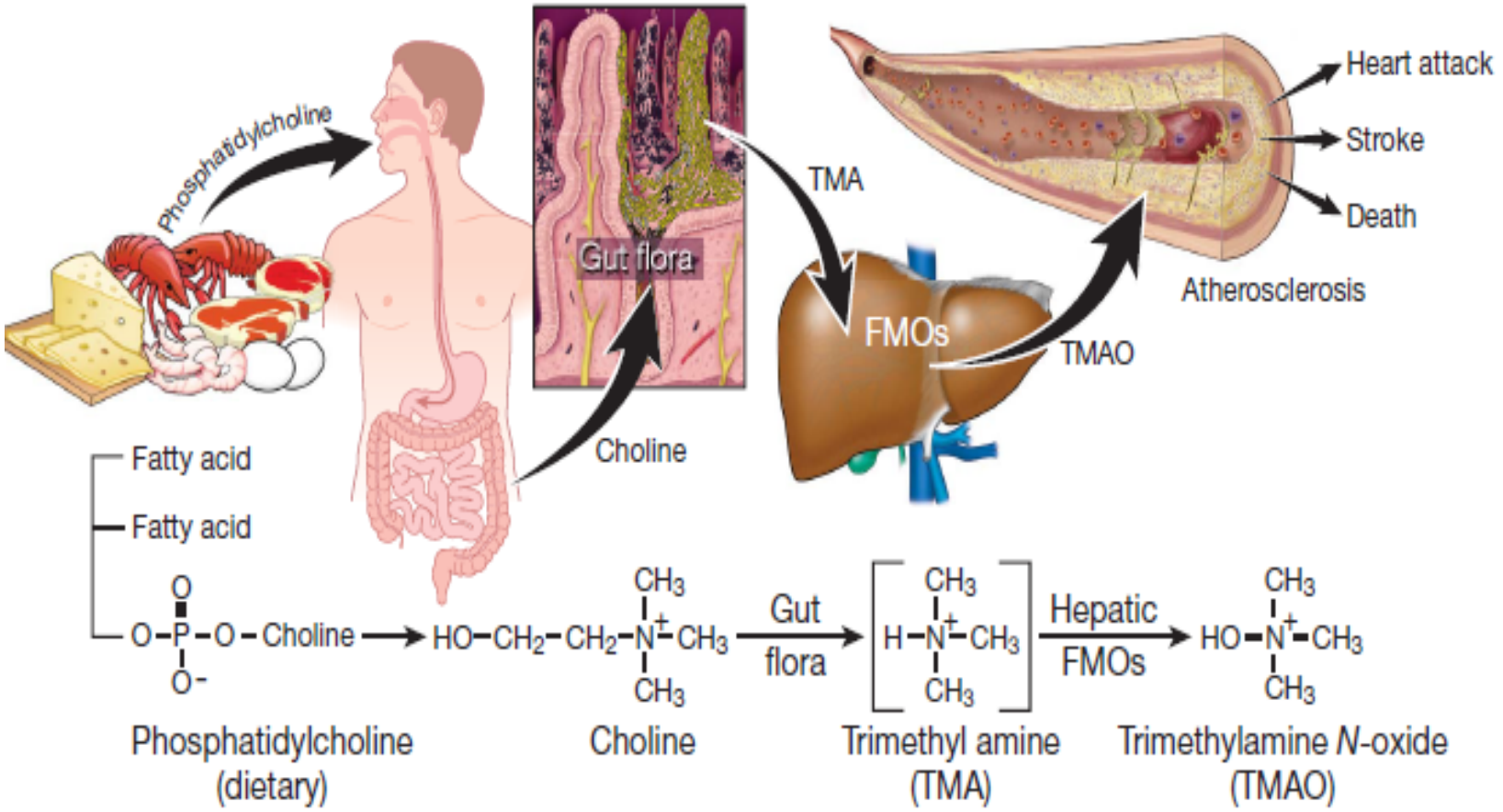
- High interpersonal variability in post-meal glucose observed in an 800-person cohort
- Using personal and microbiome features enables accurate glucose response prediction
- Prediction is accurate and superior to common practice in an independent cohort
- Short-term personalized dietary interventions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

Diet, the Gut Microbiome, and its Metabolome



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

Effect of Diet on Metabolite Production by the Gut Microbiota and its Impact on Disease



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

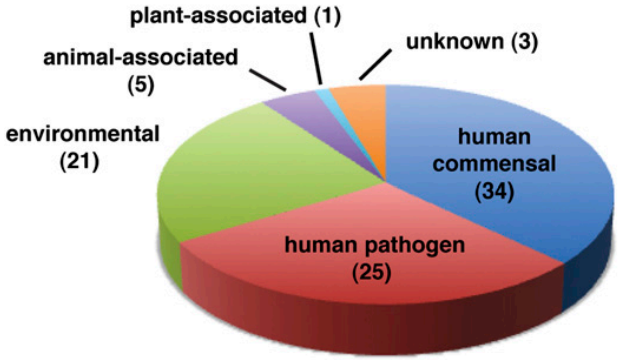
The CutC Bacterial Gene Converts Choline into TMA: Implications for Human Health

Choline

Choline
TMA-lyase
(CutC Gene)

Trimethyl Amine (TMA)

Bacteria that have the CutC gene



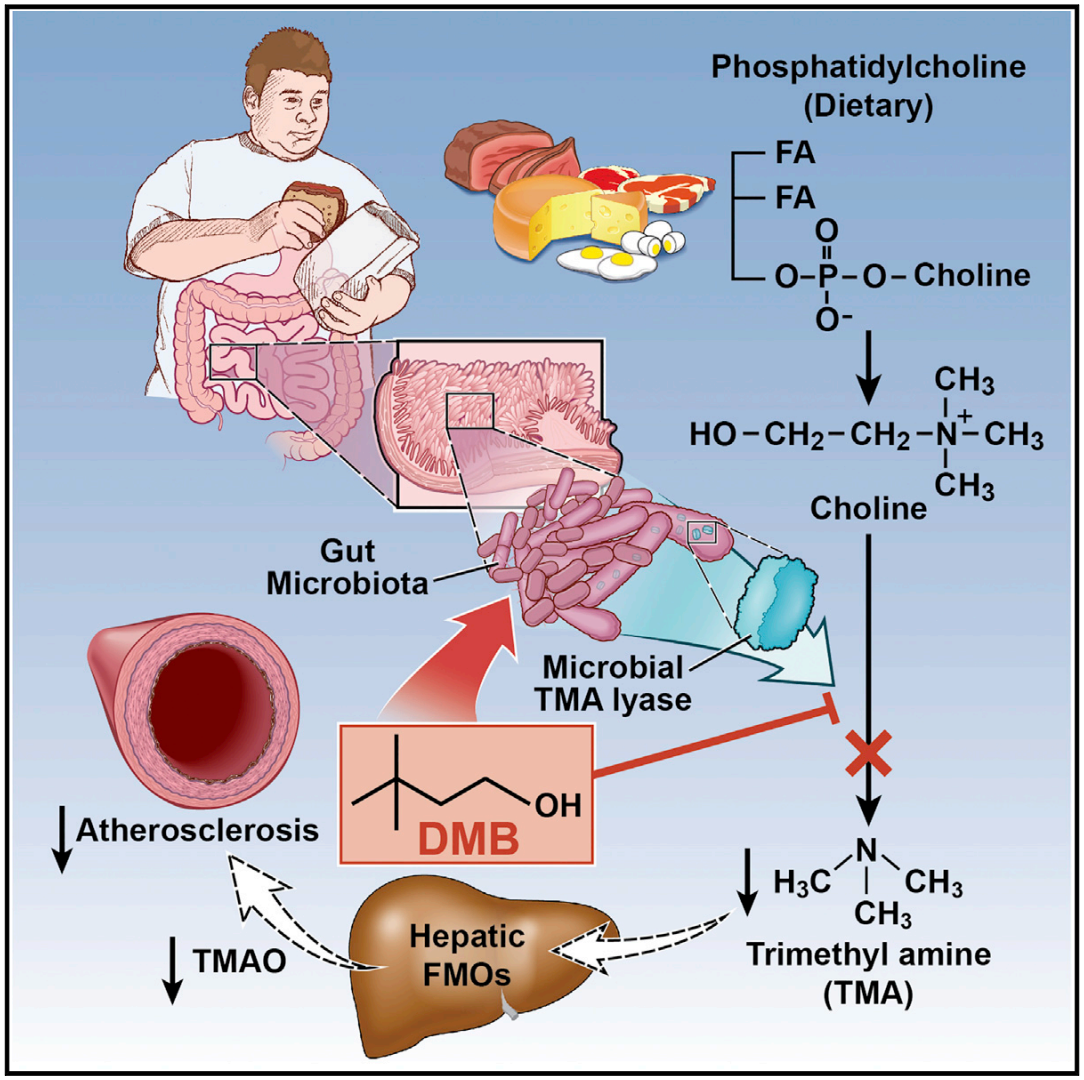
Craciun S , and Balskus E P
PNAS 2012;109:21307-21312

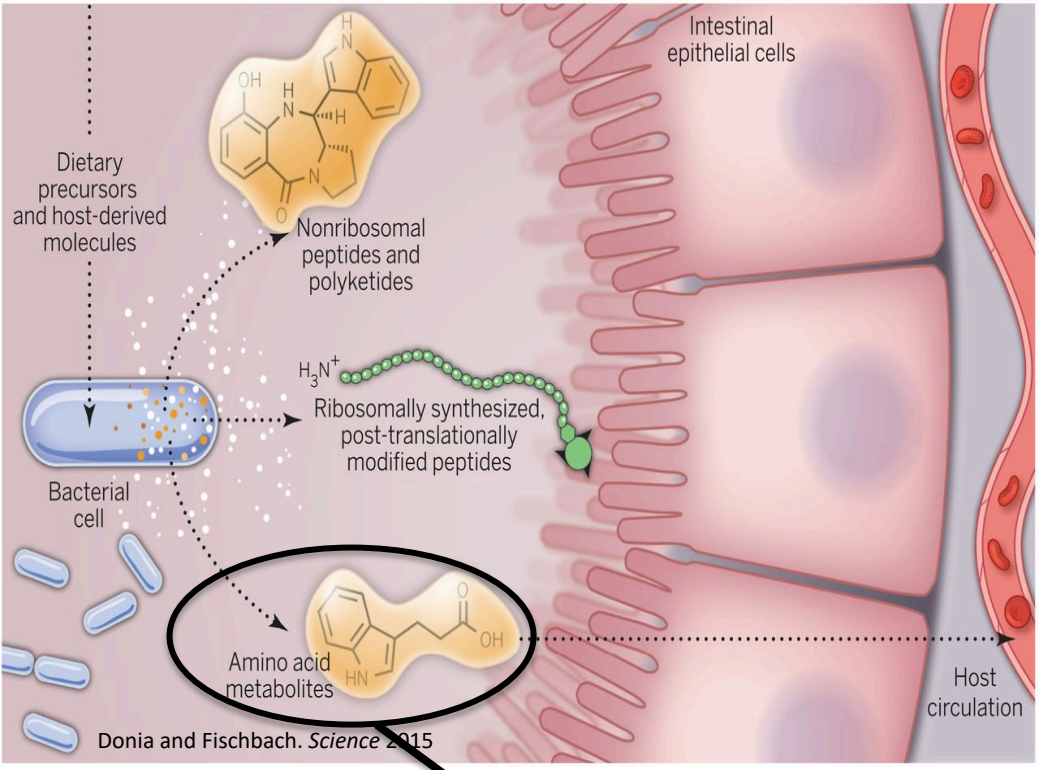
Developing Innovative Strategies to Prevent and Treat Human Disease

- Quantify the risk for heart disease by characterizing the abundance of bacteria in the gut that have a CutC gene.
- Develop “medical foods” to reduce the production of TMA by bacteria from the diet.
- Reduce CutC expressing bacteria in the gut or develop drugs to inhibit CutC activity in bacteria.

Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis

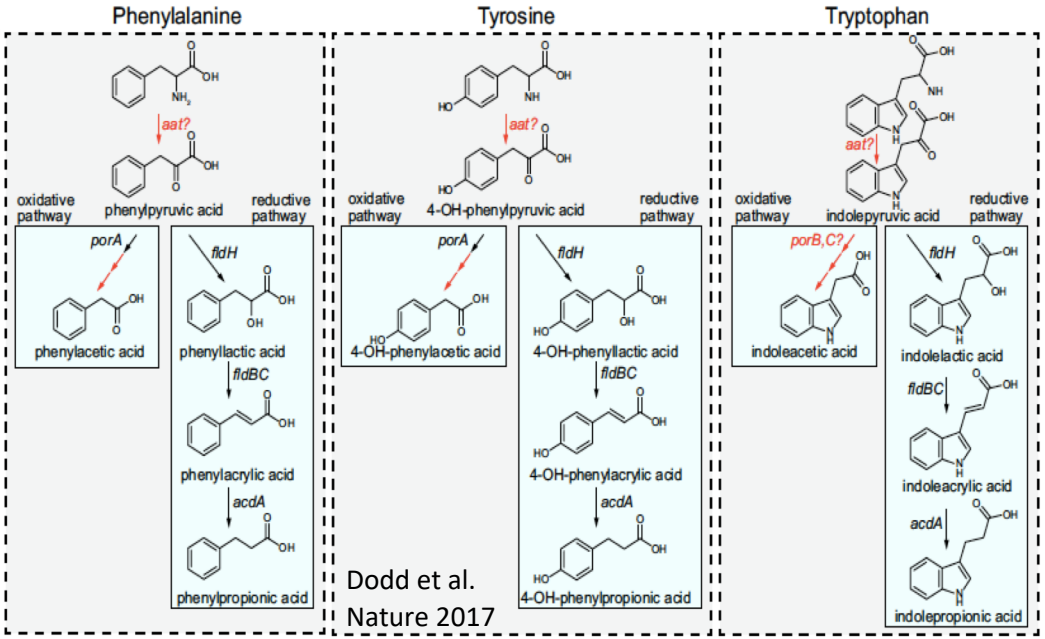
Zeneng Wang,[1,*] Adam B. Roberts,[1] Jennifer A. Buffa,[1] Bruce S. Levison,[1] Weifei Zhu,[1] Elin Org,[2] Xiaodong Gu,[1] Ying Huang,[1] Maryam Zamanian-Daryoush,[1] Miranda K. Culley,[1] Anthony J. DiDonato,[1] Xiaoming Fu,[1] Jennie E. Hazen,[1] Daniel Krajcik,[1] Joseph A. DiDonato,[1] Aldons J. Lusis,[2] and Stanley L. Hazen[1,3,*]





Donia and Fischbach. *Science* 2015

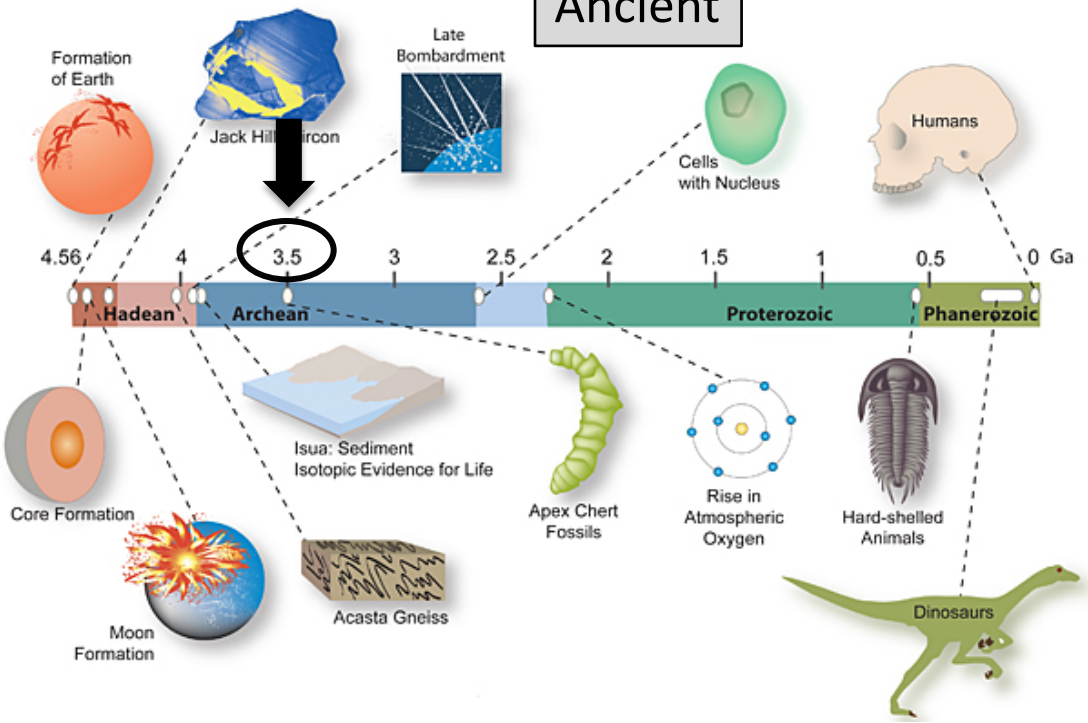
- Antibiotics
- Neurotransmitters
- Immune Modulators
- Siderophores
- Nuclear Hormone Receptor Agonists
- GPCR Agonists



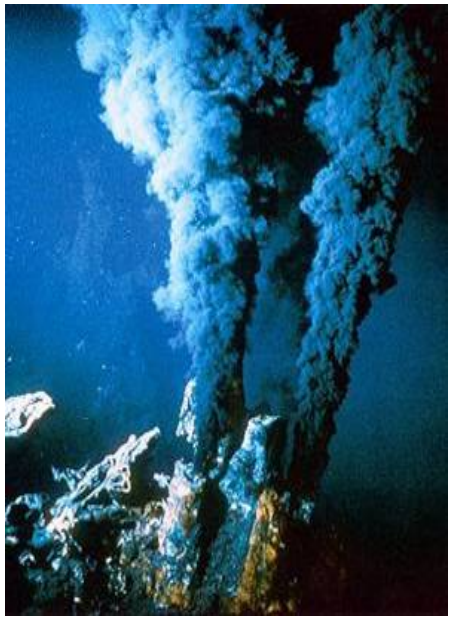
Dodd et al. Nature 2017

Methanobrevibacter smithii is the Archaeon in the gut that produces methane. Arachaea are:

Ancient

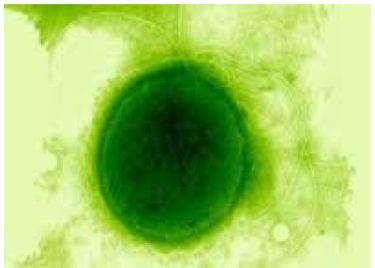


Extremophiles

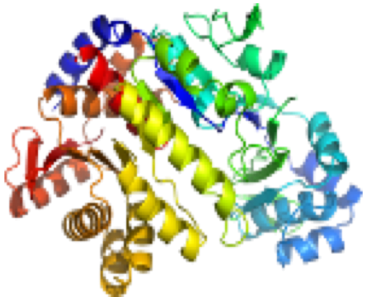


Very helpful in science!

Thermus aquaticus



PCR



1993 NOBEL PRIZE IN CHEMISTRY
AWARDED TO
KARY MULLIS

Agenda

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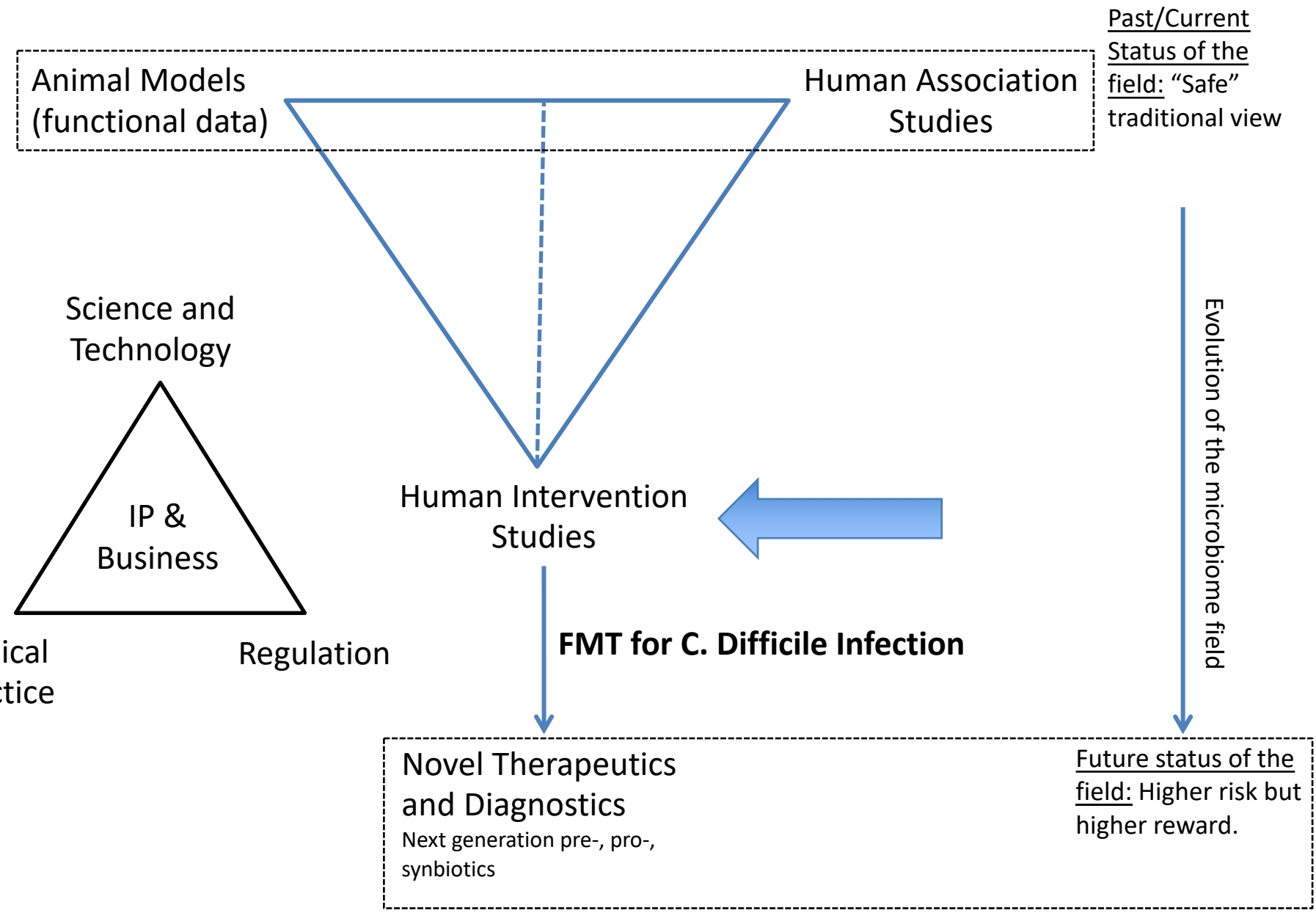
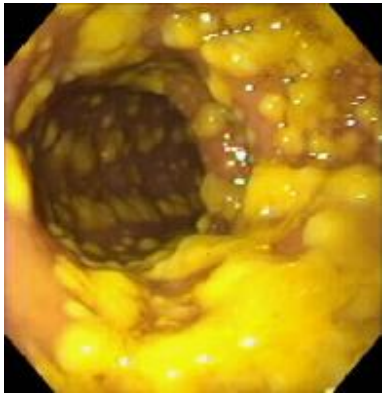
Evolution of the Microbiome Field

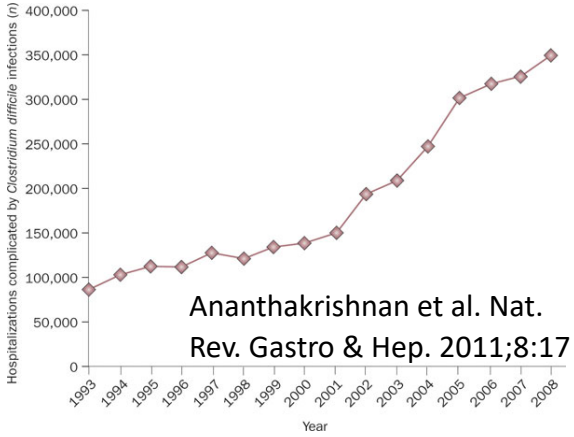


Clostridium difficile infection (CDI)

- Overgrowth of a toxin producing bacterium
- Caused by a disruption of the normal gut microbiota through the use of antibiotics



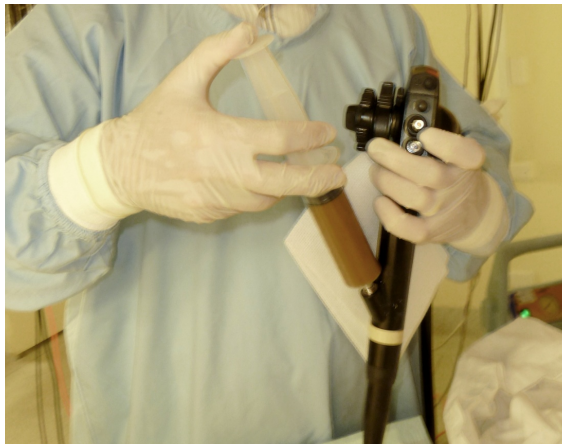
Medscape C. Diff Colitis



Ananthakrishnan et al. Nat. Rev. Gastro & Hep. 2011;8:17

Fecal Microbiota Transplantation (FMT): A success story for the Treatment of Refractory CDI

- Prescreening of donors to prevent transmission of currently known pathogens
- Homogenization, filtration, and administration usually through a colonoscope



Success rate of around 90% when fecal microbiota transplantation (FMT) is used to treat CDI

FMT: Clinical trials

- ***C difficile* infection (38)**
- **Crohn's (5)**
- **Ulcerative Colitis (15)**
- Pouchitis (3)
- IBD (9)
- IBS (6)
- Constipation (4)
- NAFLD/NASH (3)
- PSC
- Intestinal pseudo-obstruction
- Autologous FMT (preventative)

- Obesity/metabolic syndrome (5)
- HIV
- DM-II (2)
- Pancreatitis (2)
- Hepatitis B
- MRSA enterocolitis
- Drug-resistant organisms (4)
- Hepatic encephalopathy (2)
- Post-stem cell transplant (2)

Clinicaltrials.gov 06/02/2016

You Shouldn't Do it Just Because You Can—Caution about FMT and the Need for Regulation

Although the short-term infectious risks of FMT seem to be definable and quantifiable, we should remember the entire generation of patients infected with HCV by blood transfusion before this pathogen was identified.

The field should move cautiously because the long-term consequences of FMT in humans are unknown.

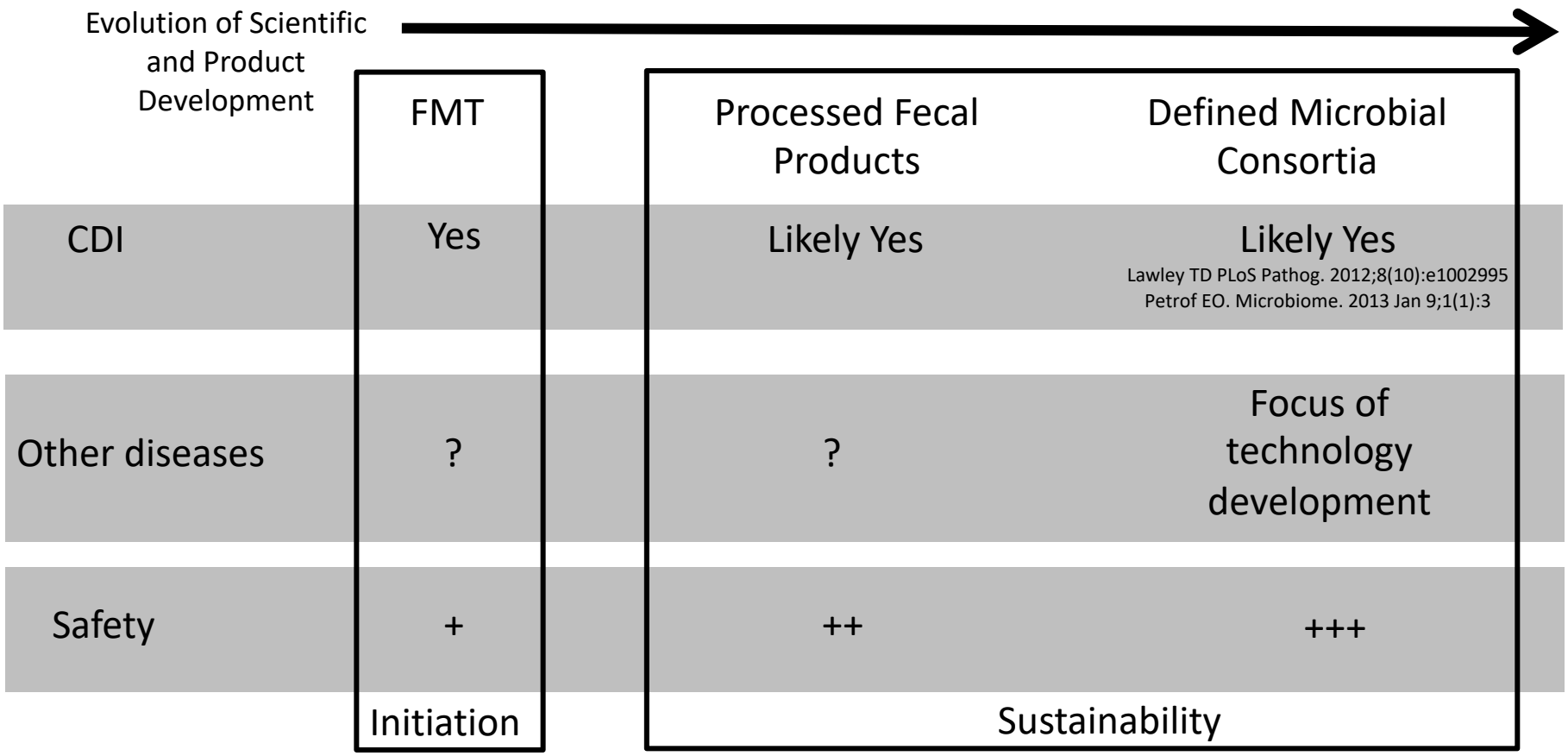
- The gut microbiome contains a highly complex and dense community of microbes that include bacteria, fungi and viruses, many of which have not been fully characterized.
- It is a dynamic and living consortium that can change over time in ways that scientists cannot currently fully predict.
- Animal model data suggests that the gut microbiome may play a role in the pathogenesis of several human diseases.

<u>FDA regulation of FMT by requiring a Investigator New Drug application (IND):</u>

4/25/13: FDA Center for Biologics Evaluation and Research (CBER): Publically announces the need for an IND.

6/17/13: "Discretionary Oversight" announced by CEBR.

The Progression of Science, Reduction to Practice, and Development of New Gut Microbiota-Based Products



The human small intestinal and colonic microbiota *in vitro*: Community structure and function

Jenni Firrman*, Elliot S. Friedman*, William C. Strange, Jung-Jin Lee, Kyle Bittinger, LinShu Liu, Gary D. Wu

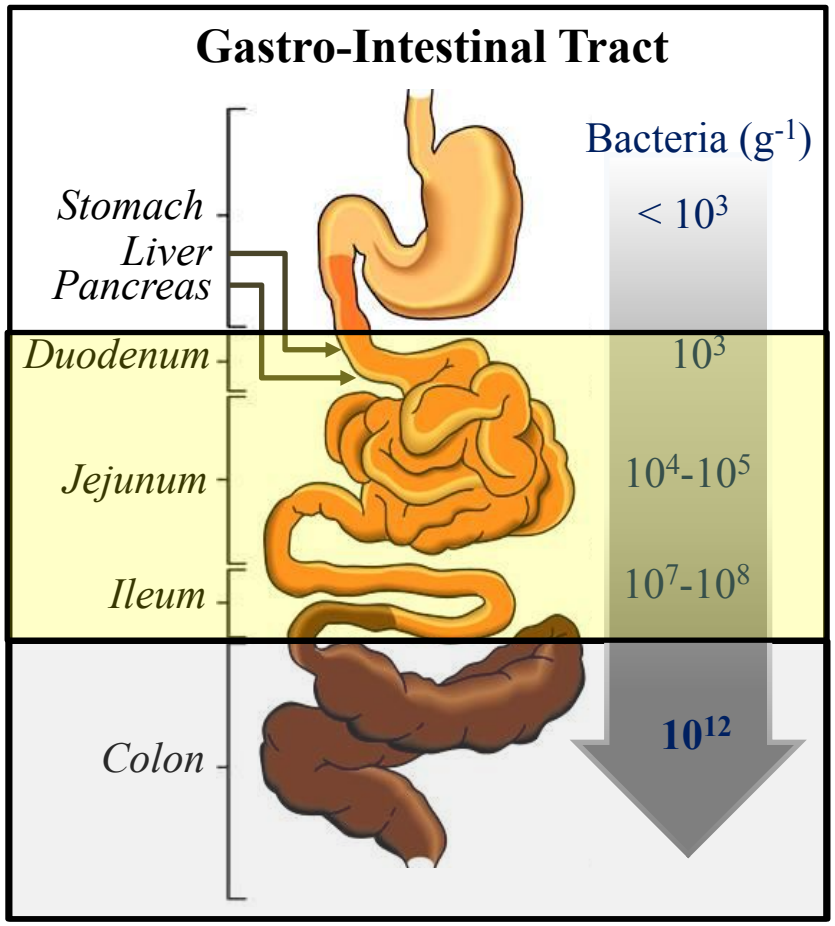
* Authors contributed equally to this work

USDA

Perelman School of Medicine
University of Pennsylvania

The Children's Hospital of Philadelphia®

The human gut microbiota

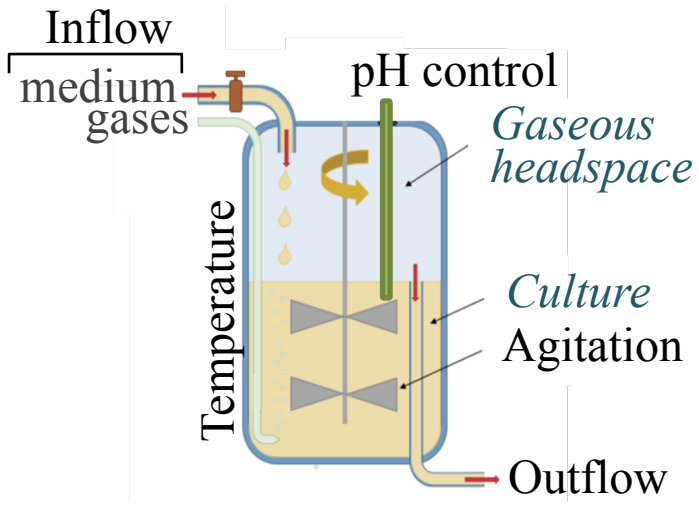


- Complex community of micro-organisms
- Present in the Gastro-Intestinal Tract (GIT)
  - Density changes longitudinally
  - Maximal concentration in the colon

Large amount of information on the colon microbiota; only limited information on the small intestine microbiota

- Small intestine contains a complex microbial community
  - Distinct from the colon microbiota
    - Less diverse with lower biomass
    - Due to functional and anatomical differences

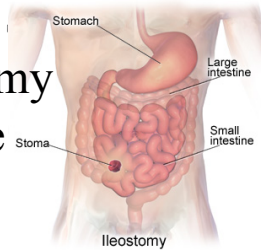
Experimental design



- Limited access to the small intestine *in vivo*
  - Difficult to study
    - Static composition (Single time point)
    - Dynamic response to stimuli
- Develop a small intestine *in vitro* model
  - Glass cultivars/bioreactors
  - Mimic physiological conditions
    - Temperature, pH control, Agitation
    - Inflow → outflow

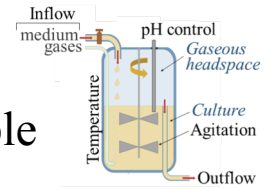
Small Intestine

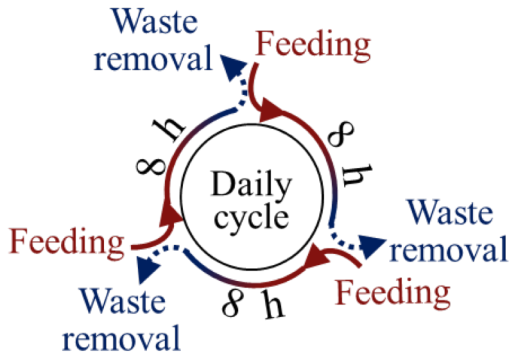
Ileostomy Sample



Colon

Fecal Sample





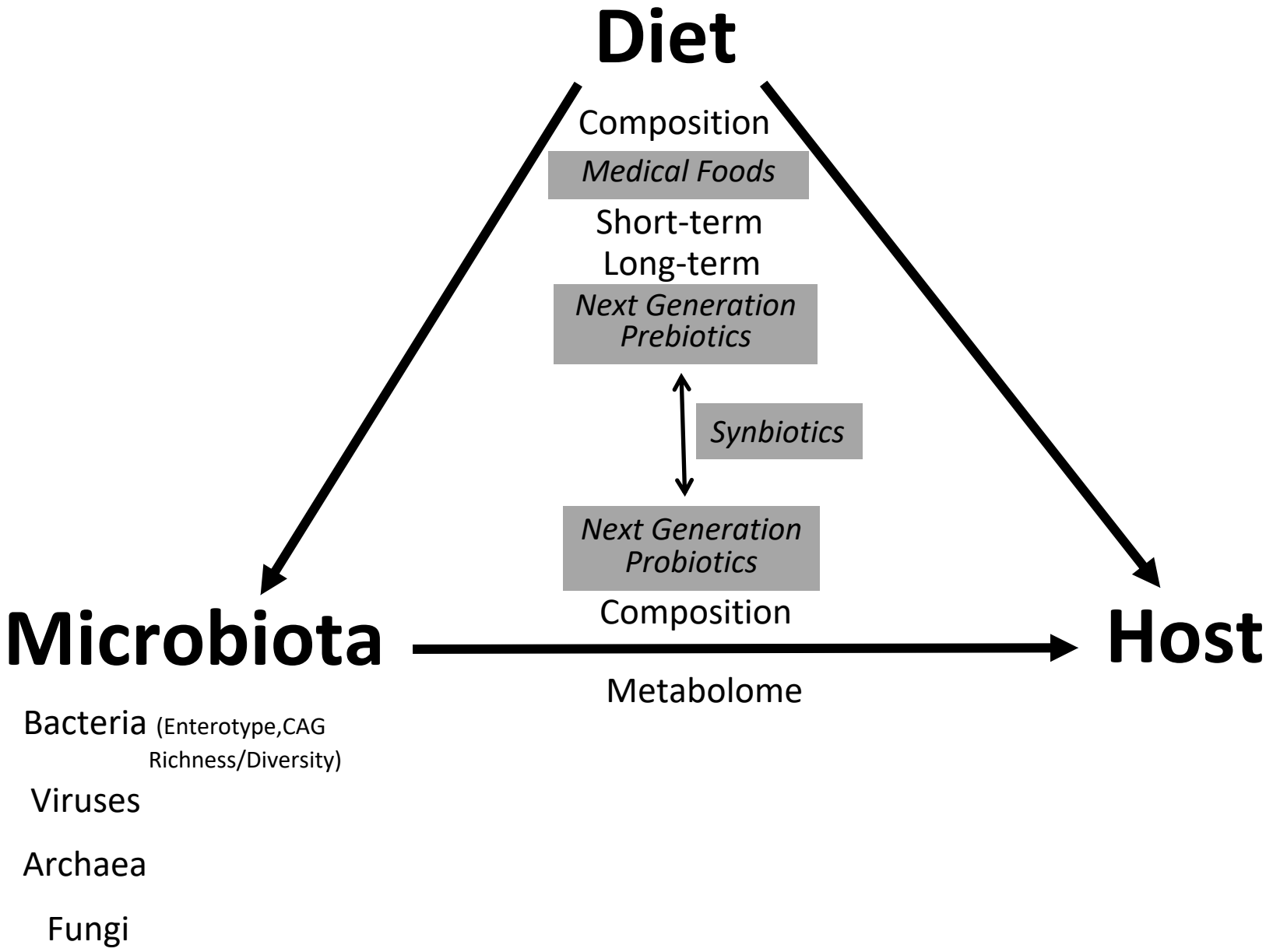
2 weeks

Allow communities to develop

Sample harvested

- Metagenomics
  - Shotgun sequencing
- Functional Analysis

1. *Similarity of community to inoculum*
2. *Compare functionality to in vivo reports*
3. *Gain a deeper understanding on the differences between these communities*


Diet
Composition
Medical Foods
Short-term
Long-term
Next Generation Prebiotics
Synbiotics
Next Generation Probiotics
Composition
Microbiota
Host
Metabolome
Bacteria (Enterotype,CAG
Richness/Diversity)
Viruses
Archaea
Fungi