“YOU ARE WHAT YOUR BUGS EAT!”

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

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<th>Agenda</th>
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<td>The Intestinal Microbiome, Early Life Events, and Association with Disease (Asthma)</td>
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<td>Diet and the Gut Microbiome and its Metabolome in Health and Disease</td>
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<td>Current and Future Status of the Microbiome Field: Fecal Microbiota Transplantation (FMT) and Beyond</td>
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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
Host Gene-Microbial Interactions in the Pathogenesis of Immune-Mediated Diseases in “Modern Society”

Parental genotype → Infant

Establish normal microbiome → Normal immune system (Immune tolerance, Regulated inflammation)

Perinatal → Inflammation & autoimmunity-prone immune system

“Sanitized” Environment
Antibiotics
Diet

Failure to establish normal microbiome → Infections, Autoantigens

Health

Bacteria
Viruses
Diet
Other

Environmental cofactors

Microbial products
Autoantigens

Crohn’s Disease
Asthma
Metabolic Syndrome

Other

Adapted from Virgin et al. Cell 2011;147:44
The Early Human Gut Microbiota

PNAS 2011;108:4578
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., Mauricio 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 Hollreich, 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 hyperreactivity and eosinophilia.

- Analogies to peanut allergies.

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<tr>
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<th>Conventionally Housed</th>
<th>Germ-free</th>
<th>Adult Microbial Colonization</th>
<th>Perinatal Microbial Colonization</th>
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<tr>
<td>Colonic and Lung iNKT Cells</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
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<tr>
<td>Oxazolone Colitis and Asthma</td>
<td>+</td>
<td>++++</td>
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Olszak et al. Science 2012;336:489
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
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
Complex plant polysaccharides

Gut microbiota

SCFA

Colonic epithelium

- MCT1
- Main energy source
- Less oxidative DNA damage
- Regulation of proliferation
- Maintenance of barrier function
- Tumor suppression
- Cytokine production

Receptors and mechanisms

- GPR43
- MCT1
- Histone deacetylase inhibition
- GPR109A
- GPR41

Immune system

- Enhanced ROS burst
- More phagocytosis
- Induction of apoptosis
- Modulation of recruitment
- Cytokine production

Nature Immunology 12, 5–9 (2011)
Dietary Fiber and the Intestinal Mucus Barrier

Gnotobiotic mice with characterized human gut microbiota

Dietary fiber deprivation

Infection with enteric pathogen

Colon

Fiber-rich diet

Mature mucus layer: intact barrier function

Microbiota eroded mucus layer: barrier dysfunction

Mucus layer
Fiber-degrading microbiota
Mucus-degrading microbiota
Mucosal pathogen
Bacterial dietary-fiber degradation
Bacterial host-secreted mucus degradation

Dietary Effects on Human Gut Microbiome and its Association with Disease

**ARTICLE**

Richness of human gut microbiome correlates with metabolic markers

**LETTER**

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
Cell Metabolism

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

Graphical Abstract

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

Authors

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

Correspondence

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PNAS 2010;107:14691–14696
Personalized Nutrition by Prediction of Glycemic Responses

David Zeedi, Tal Korem, Niv Zmora, David Israeli, Daphna Rothschild, Adina Weinberger, Orly Ben-Yacov, Dar Lador, Talli Avnit-Sagi, Maya Lotan-Pompan, Jotham Suez, Jemal Ali Mahdi, Elad Matot, Gal Malka, Noa Kosower, Michal Rein, Gili Zilberman-Schapira, Lenka Dohnalova, Meirav Pevsner-Fischer, Ron Bikovsky, Zamir Halpern, Eran Elinav, and Eran Segal

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

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

Bacteria that have the CutC gene

Choline

\[ \text{Choline} \rightarrow \text{TMA-lyase (CutC Gene)} \rightarrow \text{Trimethyl Amine (TMA)} \]

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.

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

Fig. S3. Environmental and phylogenetic distribution of choline utilization. (A) Sources of sequenced bacterial isolates that possess a complete cut gene cluster. (B) Phylogenetic distribution of putative choline-degrading human gastrointestinal tract isolates.

Fig. S4. SDS-PAGE of cell lysates from heterologous expressions of CutC and CutD in E. coli using a 4–15% (wt/vol) polyacrylamide Tris-HCl gel. Lane 1, 10–250 kDa protein ladder (New England Biolabs); lane 2, wild-type CutC + CutD; lane 3, CutC C498A mutant + CutD; lane 4, CutC G821A mutant + CutD; and lane 5, empty pET-29b vector control. The calculated molecular weight of CutC (Dde_3282) is 96 kDa.
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. Hazen1,3,*

Abstract

Introduction

Trimethylamine (TMA) N-oxide (TMAO), a gut-microbial metabolite, is formed from cytosine and vitamin B7 derivatives that are abundant in foods such as meat, egg yolks, and high-fat dairy products—serve as dietary precursors for TMAO generation in mice and humans, a metabolite that accelerates cardio-metabolic diseases. Trimethylamine (TMA) is a structural analog of choline, 3,3-dimethyl-1-butanol that is found in the gut microbiota where it is evolved by TMA lyases, and to both inhibit TMA production and formation of TMAO specifically and non-lethally microbial pathway(s) involved in TMA production has not yet been established that humans with genetic defects in FMO3 suffer from atherosclerosis. Toward that end, recent studies confirm that both choline-diet-enhanced atherosclerosis and incident major adverse cardiac events in multiple independent studies suggest that targeting gut microbial pathways (Brady et al., 2013; Koeth et al., 2013; Steenbergen et al., 2015; Wang et al., 2011, 2015, Cell, 2015; Lever et al., 2014; Mente et al., 2015; Tang et al., 2013, 2014; Trøseid et al., 2015; Wang et al., 2013). In brief, TMAO-lowering interventions are, thus, of considerable interest for their potential therapeutic benefit for atherosclerosis susceptibility and TMAO formation are transmissible traits (Bennett et al., 2013; Koeth et al., 2013; Shih et al., 2015; Wang et al., 2015). However, while numerous beneficial effects with FMO3 inhibitors in general may serve as a potential therapeutic approach for the treatment of cardiometabolic diseases.

Methods

In this study, we investigated the impact of targeted inhibition of the gut microbial TMA pathway with DMB, a 3,3-dimethyl-1-butanol to inhibit TMAO production, on diet-induced atherosclerosis. A randomized, dietary oligonucleotide-based approach, results in the reduction of both circulating TMAO levels and diet-enhanced atherosclerosis susceptibility and transfers to both inhibit TMA production and formation of TMAO specifically and non-lethally microbial pathway(s) involved in TMA production has not yet been established that humans with genetic defects in FMO3 suffer from atherosclerosis.

Results

We report that DMB, a 3,3-dimethyl-1-butanol attenuates choline diet-enhanced endogenous macrophage foam cell formation and atherosclerotic lesion inhibition in mice fed a high-choline or L-carnitine diet. DMB from physiologic polymicrobial cultures (e.g., intestinal flora) inhibits microbial TMA lyase activity, interfering with the formation of TMA and TMAO, both enhances atherogenesis in animal models and is associated with cardiovascular risks in clinical studies. Here, we establish that human gut microbiota-dependent metabolite, both enhances atherogenesis in animal models and is associated with cardiovascular risks in clinical studies.

Discussion

We establish a new non-lethal, microbial pathway involved in TMA production and a non-lethal microbial pathway(s) involved in TMA production has not yet been established that humans with genetic defects in FMO3 suffer from atherosclerosis. Toward that end, recent studies confirm that both choline-diet-enhanced atherosclerosis and incident major adverse cardiac events in multiple independent studies suggest that targeting gut microbial pathways (Brady et al., 2013; Koeth et al., 2013; Steenbergen et al., 2015; Wang et al., 2011, 2015, Cell, 2015; Lever et al., 2014; Mente et al., 2015; Tang et al., 2013, 2014; Trøseid et al., 2015; Wang et al., 2013). In brief, TMAO-lowering interventions are, thus, of considerable interest for their potential therapeutic benefit for atherosclerosis susceptibility.

Correspondence

Jennifer A. Buffa, 1*, Joseph A. DiDonato, 1, Aldons J. Lusis, 2, and Stanley L. Hazen 1,3,*

Acknowledgments

We thank the other members of the Hazen Research Team and the Core Facilities at the Cleveland Clinic for their support and assistance.

Conflict of Interest

Conflict of interest statements are available for this article at http://dx.doi.org/10.1016/j.cell.2015.11.055.

References

Additional references are available in the Supplementary Information.

http://dx.doi.org/10.1016/j.cell.2015.11.055

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http://dx.doi.org/10.1016/j.cell.2015.11.055

December 17, 2015

Wang et al., 2013, Cell, 2015; Lever et al., 2014; Mente et al., 2015; Tang et al., 2013, 2014; Trøseid et al., 2015; Wang et al., 2011, 2015, Cell, 2015; Lever et al., 2014; Mente et al., 2015; Tang et al., 2013, 2014; Trøseid et al., 2015; Wang et al., 2013.
- Antibiotics
- Neurotransmitters
- Immune Modulators
- Siderophores
- Nuclear Hormone Receptor Agonists
- GPCR Agonists
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
Evolution of the Microbiome Field

Animal Models (functional data) -> Human Association Studies

Past/Current Status of the field: “Safe” traditional view

Future status of the field: Higher risk but higher reward.

Evolution of the microbiome field

Human Intervention Studies

FMT for C. Difficile Infection

Clinical Practice

Regulation

IP & Business

Science and Technology

Novel Therapeutics and Diagnostics
Next generation pre-, pro-, synbiotics

[Graphical representation of the microbiome field evolution]
**Clostridium difficile infection (CDI)**

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

**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
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.

**FDA regulation of FMT by requiring a Investigator New Drug application (IND):**

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

<table>
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<tr>
<th>Evolution of Scientific and Product Development</th>
<th>FMT</th>
<th>Processed Fecal Products</th>
<th>Defined Microbial Consortia</th>
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<tr>
<td>CDI</td>
<td>Yes</td>
<td>Likely Yes</td>
<td>Likely Yes</td>
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<td>Other diseases</td>
<td>?</td>
<td>?</td>
<td>Focus of technology development</td>
</tr>
<tr>
<td>Safety</td>
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</tr>
</tbody>
</table>

Initiation

Safey

Other diseases

Focus of technology development

Safety

Sustainability

References:
Petrof EO. Microbiome. 2013 Jan 9;1(1):3
The human small intestinal and colonic microbiota in vitro: Community structure and function


* Authors contributed equally to this work
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 \textit{in vivo}
  - Difficult to study
    - Static composition (Single time point)
    - Dynamic response to stimuli
- Develop a small intestine \textit{in vitro} model
  - Glass cultivars/bioreactors
  - Mimic physiological conditions
    - Temperature, pH control, Agitation
    - Inflow \rightarrow outflow

**Small Intestine**

- Ileostomy Sample
- Colon
- Fecal Sample

- Allow communities to develop

**Sample harvested**

- Metagenomics
- Shotgun sequencing
- Functional Analysis

1. \textit{Similarity of community to inoculum}
2. \textit{Compare functionality to in vivo reports}
3. \textit{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

Microbiota
- Bacteria (Enterotype, CAG Richness/Diversity)
- Viruses
- Archaea
- Fungi

Host

Metabolome