Microbes, Man and Medicine

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Microbes appeared 3.5 billion years ago

Microbial Colonization

• Humans came much later.
• Dominance by microbes is still absolute as colonization by bacteria rapidly takes place within 24h of birth.
  – Colonization by maternal gut and vaginal bacteria is promoted by vaginal delivery.
  – Bacteria moves caudally over a period of several days.
  – Microbial flora is established by 3rd to 4th week of age.
  – >1000 species (>80% not culturable).
• Colonization continues from Mom’s milk

Bacterial DNA in Mom’s Milk (M) and Infant Feces (F) Match

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Bacteria in breast milk - *Staph, Strep, Lacto, Bifido*  
Cesarean-delivered Infant Could Still be Colonized by Mom’s Gut Bacteria via Breast Milk

- Six women took *Lactobacillus rhamnosus strain GG* (ConAgra, Omaha, NE) as a probiotic until delivery.
- Four of 6 infants were delivered vaginally and 2 by c-section.
- All infants were breast fed after birth.
- None of the infants were fed probiotics.
- *Lactobacillus GG* DNA were searched for using molecular assay in infant feces.
- *Lactobacillus GG* were present in all 4 vaginally delivered infants and in 1 out of 2 c-section delivered infant.

Maternal dendritic cells penetrate mucosa to “grab” gut bacteria and colonize mammary gland.

https://images.nature.com/full/nature-assets/ni/journal/v8/n11/images/ni1526-F2.jpg
Human Milk Oligosaccharides (HMOS) Shape Our Gut Microbiome

HMOS or fructooligosaccharides (FOS) promote growth of Bifidobacteria

Fermentation byproducts from HMOS (organic acids) suppress growth of pathogens

An abnormal microbiome may result if mother is not able to deliver required oligosaccharides.

Yu ZT et al. Glycobiology 2013;23(2):169-177
Gut Bacteria Must Resist Removal

1-3 billion cells per hour are shed by small intestine. 100-300 million cells per hour are shed by large intestine (Xu J, Gordon JI. PNAS 2003;100:10452-10259)

Entrenched “resident” bacteria are able to establish themselves by embedding in biofilms (Sonnenburg JL, Angenent LT, Gordon JI. Nature Immunology 2004;5:569-573)

Biofilms are dense communities of bacteria that attach themselves to surfaces by encrusting in a polysaccharide matrix that resist shear forces. Free-living bacteria = “planktonic” phenotype.

High Density of Microbes as a Biofilm is Found in Colon and Stool

Macfarlane S and Macfarlane GT. Appl Environ Micro 2006;72:6204-6211
Microbiome = the community of microorganisms that shares our body space

Pop: 100 Trillion

Microbes in and on our body outnumber human cells 10:1
We have 23,000 genes; our microbiome has 8,000,000 genes
Microbes Shape Host Structure
Normal Intestinal Capillary Formation Depends on *B. thetaiotamicron*
Microbes Shape Nutrition and Metabolism
Gut Bacteria Are Crucial to Nutrition & Metabolism

• Extract nutrients from indigestibles such as cellulose via bacterial fermentation.
• Bacterial fermentation is crucial for herbivores and important to omnivores/vegetarians like humans and rodents.
• Volatile fatty acids (acetic, propionic and butyric acids) and gases (hydrogen, methane, carbon dioxide and hydrogen sulfide) are produced and absorbed by diffusion.
• Infusing acetate/placebo into the distal colon of 12 obese men increased fasting fat oxidation $1.78+/-0.28$ vs. $-0.78+/-0.89$ g fat $2\ h^{-1}$ (p=0.015) (Canfora EE, et al. Sci Rep. 2017; 7: 2360. Published online 2017 May 24. doi: 10.1038/s41598-017-02546-x)
Microbiome and Obesity
Colonization by donor-derived gut microbes (CONV-D) increases fat deposition.

Gut Microbes Promotes Fat Storage by Suppressing Intestinal Expression of FIAF, a LPL Inhibitor (lower FIAF, higher LPL, more fat stored).

Backhead F. et al. PNAS 2004;101:15718
Fat Mass Increases in Germ-Free Mice Receiving Stool from Obese Human Donor

Ridaura VK et al. Science 2013; 341;1079-1089
Antibiotic Effects Vary in Experiments Depending on Recipient Microbiome:

Microbiome Normal – Bad Effect
Microbiome Abnormal – Good Effect
Microbiome and Metabolic Syndrome
To test the role of microbes in metabolic syndrome, a diet-induced mouse model of obesity characterized by glucose intolerance and fatty liver was treated with antibiotics (nor + amp) vs. placebo (control).
Glycemic Profile Improved with Antibiotics

Membrez M et al. FASEB J 2008; 22((7): 2416-2426

B

Blood glucose during OGTT

Control
Nor+Amp

mg/dL

0 100 200 300 400 500

0 20 40 60 80 100 120 140

Time (min)
Liver Fat Load Decreased with Antibiotics

Membrez M et al. FASEB J 2008; 22((7): 2416-2426
Antibiotics Corrected High Fat Diet -induced Leaky Gut

- CT = Normal Diet
- CT-Ab = Normal Diet and Antibiotics (amp+neo)
- HF = High Fat Diet
- HF-Ab = High Fat Diet and Antibiotics

Cani PD et al. Diabetes 2008; 57. 1470-1481
Antibiotics Corrected High Fat Diet-induced Endotoxemia

- CT = Normal Diet
- CT-Ab = Normal Diet and Antibiotics
- HF = High Fat Diet
- HF-Ab = High Fat Diet and Antibiotics

Cani PD et al. Diabetes 2008; 57. 1470-1481
High-fat feeding → Change Gut flora → Increased permeability → Increased LPS absorption → Increased endotoxemia → Inflammation → Metabolic disorders
Low Dose Antibiotic Exposure and Weight Gain
Antibiotics is a Growth-Promoting Feed Supplement

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>Daily Gain</th>
<th>Feed/lb Gain</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>22.9 lb</td>
<td>179.5 lb</td>
<td>1.40 lb</td>
<td>3.38 lb</td>
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<tr>
<td>Chlortetracycline 10g per ton</td>
<td>22.9 lb</td>
<td>192.5 lb</td>
<td>1.51 lb</td>
<td>3.26 lb</td>
</tr>
<tr>
<td>Chlortetracycline 50g per ton</td>
<td>22.8 lb</td>
<td>192.5 lb</td>
<td>1.52 lb</td>
<td>3.21 lb</td>
</tr>
</tbody>
</table>

Mechanism of action: Antibiotics reduce population of *Lactobacillus* species that produces bile salt hydrolases (BSH); BSH converts micelle-forming primary bile salts to secondary bile salts; less BSH means more primary bile salts and more efficient fat assimilation.

Luecke RW, et al. 1956
(https://www.animalsciencepublications.org/publications/jas/pdfs/15/.../JAN015003076...)
Disrupting the Microbiome Early in Life may Increase Obesity Risk
Low Dose Penicillin Given to Weaning Pups Leads to Increased Fat Stores

• A cohort of 979 children were measured repeatedly at 7 time points during the first 10 years of life.
• Children exposed to 1 course of antibiotics in the first 6 months had increased weight and height.
• Exposure in the second year of life was associated with both increased weight and height when children were exposed to 2 or more antibiotics.
• Association was strongest with beta-lactam agents.
• Causality with obesity requires longer studies.

Mbakwa CA et al. Pediatr. 2016 Sep;176:105-113
Prenatal Exposure to Antibiotics may Increase Obesity and Disease Risk
Prenatal Exposure to Antibiotics Leads to Higher BMI

436 mother-child pair followed to age 7; Ab exposure is associated with 84% greater risk of obesity. Mueller NT, et al. *Int J Obes (Lond)*. 2015 April ; 39(4): 665–670
Mouse Offsprings Were More Likely to Develop Diabetes When Their Diabetic Mothers Were Treated with Antibiotics During Pregnancy.

Tormo-Badia N et al. doi: 10.1111/sji.12205
Microbiome and Cardiovascular Disease
Red Meat Consumption is Associated with Cardiovascular Disease

**Red Meats have High Concentration of Carnitine**

Carnitine is from lysine and methionine

### Selected food sources of carnitine

<table>
<thead>
<tr>
<th>Food</th>
<th>mg</th>
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<tbody>
<tr>
<td>Beef steak, cooked, 4 ounces</td>
<td>56–162</td>
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<tr>
<td>Ground beef, cooked, 4 ounces</td>
<td>87–99</td>
</tr>
<tr>
<td>Milk, whole, 1 cup</td>
<td>8</td>
</tr>
<tr>
<td>Codfish, cooked, 4 ounces</td>
<td>4–7</td>
</tr>
<tr>
<td>Chicken breast, cooked, 4 ounces</td>
<td>3–5</td>
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<tr>
<td>Ice cream, ½ cup</td>
<td>3</td>
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<tr>
<td>Cheese, cheddar, 2 ounces</td>
<td>2</td>
</tr>
<tr>
<td>Whole–wheat bread, 2 slices</td>
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<tr>
<td>Asparagus, cooked, ½ cup</td>
<td>0.1</td>
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</table>

Trimethylamine N-oxide (TMAO) is a Product of Carnitine

Omnivores but not Vegans Convert Carnitine to TMAO

Formation of TMAO from L-carnitine is nil in vegans but high in Omnivores. Correspondingly, baseline urinary TMAO is low in vegan when compared to omnivores.

Is the different response between vegans and omnivores explained by their microbiome?
Subjects give L-carnitine, urinary TMAO is measured at Visit 1. After antibiotics, TMAO measured again at visit 2 then again after 3-week wait for repopulation of gut microbes.

Antibiotics Decrease Carnitine – Induced Atherosclerosis

CAD Risk Increases with Increasing Plasma [Carnitine]

Closed Circle = Carnitine Before
Open Circle = Carnitine After

N=2595

Red meat consumption is linked to CVD. Carnitine and choline are abundant in red meat and are metabolized by gut bacteria to trimethylamine (TMA) which is further metabolized by a liver enzyme FMO to trimethylamine-N-oxide (TMAO) which is proatherogenic and associated with CVD.

Resveratrol in Red Wine Reduces Cardiovascular Risk

• Light (1-7/week) and moderate (8-21) wine drinkers had a 26% and 36% reduction respectively in cardiovascular mortality (Gronbaeck M et al. Ann Intern Med 2000; 133:411-419)

• An antioxidant in red wine called resveratrol (RSV) is considered the beneficial substance. However, in spite of many proposed mechanisms, it was not known how resveratrol exerts this effect (Vidavalur R, et al. Exp Clin Cardiol 2006;11(3):217-225)
Resveratrol Alters Gut Microbiome to Reduce Conversion of Choline to TMAO

Volume 7 Issue 2 e02210-15
Resveratrol or Antibiotics Protects Against Atherosclerotic Plaque Formation

Chen ML, et al. mBio. March/April 2016 Volume 7 Issue 2 e02210-15

ApoE⁻/⁻ mice
Microbes and Behavior
Introduction of Bacteria into Gut Induces Anxiety-like Behavior with Less Exploration

% Positive Change (less symptoms) in Perceived Stress Scale (PSs) and Hospital Anxiety and Depression Scale (HADs) are Greater with Probiotics

L. Helveticus and B. longum or placebo x 30 days in 25 healthy volunteers, scores measured baseline vs. follow-up

Swapping Microbiome as Therapy
Antibiotic Results in Simplified Community and a Loss of Colonization Resistance

Fecal Transplantation Resolves Relapsing C. difficile Infection in Mice

Clindamycin-induced C. difficile infection relapses x 2 after vancomycin treatments but was suppressed by single fecal transplantation from healthy donor by oral gavage with lasting benefit.

FMT by Colonoscopy or NG Tube
Improved Diarrhea due to *C. difficile*

Twenty patients with refractory or recurrent *C. difficile* diarrhea were treated with FMT delivered by either colonoscopy or NG tube (Youngster I, et al. Clinical Infectious Diseases 2014;58(11):1515-22)


Shakeri, H.; Hadaegh, H.; Abedi, F.; Tajabadi-Ebrahim, M.; Mazrooi, N.; Ghandi, Y.; Asemi, Z. Consumption of symbiotic bread decreases triacylglycerol and VLDL levels while increasing HDL levels in serum from patients with type-2 diabetes. Lipids 2014, 49, 695–701. [CrossRef] [PubMed]