The Impact of the Intestinal Microbiome: Exploring the Relationship of the Intestinal Micro Flora to Diet, Digestion and Disease

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Objectives

• The participant will be able to discuss the general makeup of the intestinal microbiome in health.
• The participant will be able to delineate potential mechanisms that cause an alteration in the stable microbiome, predisposing to disease.
• The participant will recognize potential pathways to minimize disruption of the microbiome.
• The participant will be able to determine how medical treatments and dietary interventions may affect the intestinal microbiome and the potential “brain-gut” impact.
Disclosures

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• Funding support:
  AAP, Newman’s Foundation, Nutricia, Autism Research Institute

• I do not intend to speak about specific products and will acknowledge any funded research.
The changing face of gut microbes

Your Inner Ecosystem
In your body, bacteria outnumber your own cells 10 to 1.
Who's in control?

FELLOW TRAVELLERS
First results from the Human Microbiome Project highlight the healthy variation in our microbial selves
PAGES 194, 207 & 213

MINDFULNESS
FEELING THE PRESSURE
The brain for emotionally neutral hydrogen
PAGE 47

CLIMATE CHANGE
GET USED TO UNCERTAINTY
Climate modeling
PAGE 157

TITAN'S ELUSIVE METHANE
Tropical lakes on Saturn's enigmatic moon
PAGE 257
Microbiomes

mi·cro·bi·ome
ˌmīkrōˈbīōm/
noun
plural noun: microbiomes
the microorganisms in a particular environment (including the body or a part of the body).
"we depend on a vast army of microbes to stay alive: a microbiome that protects us against germs, breaks down food to release energy, and produces vitamins"
the combined genetic material of the microorganisms in a particular environment.
More than microbiomes

• Archaea-single celled
• Fungome-fungal proteins implicated in pathogenesis
• Virome-the collection of viruses in and on the human body. Viruses in the human body infect both human cells as well as other microbes such as bacteria.
• Metabolome-the total number of metabolites present within an organism, cell, or tissue. These metabolites are products of digestion and also products of the microbiota
**THE HUMAN MICROBIOME**

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

- **25 SPECIES** in the stomach include:
  - Helicobacter pylori
  - Streptococcus thermophilus

- **500–1,000 SPECIES** in the intestines include:
  - Lactobacillus casei
  - Lactobacillus reuteri
  - Lactobacillus gasseri
  - Escherichia coli
  - Bacteroides fragilis
  - Bacteroides thetaiotaomicron
  - Lactobacillus rhamnosus
  - Clostridium difficile

- **60 SPECIES** in the urogenital tract include:
  - Ureaplasma parvum
  - Corynebacterium aurimucosum

- **600+ SPECIES** in the mouth, pharynx and respiratory system include:
  - Streptococcus viridans
  - Neisseria sicca
  - Candida albicans
  - Streptococcus salivarius

- **1,000 SPECIES** in the skin include:
  - Pityrosporum ovale
  - Staphylococcus epidermidis
  - Corynebacterium jeikeium
  - Trichosporon
  - Staphylococcus haemolyticus

**SOURCES:** NATIONAL INSTITUTES OF HEALTH, SCIENTIFIC AMERICAN; HUMAN MICROBIOME PROJECT

Dean Tweed • POSTMEDIA NEWS / IMAGE: Fotolia
Cycle of healthy microbiota transmission and host development

Interferences

**Antibiotics**
Taken by girl and boy, gravid woman, prepartum and postpartum, infant

**C-section**
Baby does not go through birth canal

**Washing of skin with antibacterials**
Intrapartum, baby (vernix), baby, girls, women

**Oral ingestion of antibacterials**
Children, women, men

MG Dominguez-Bello & MJ Blaser 2015;7::307fs39
MA Fischbach & JA Segre Cell 2016;164:1288-1300
Development of the gut microbiota

• Fetal intestine: “sterile”  Or maybe not!
• Initial colonization determined by:
  – Delivery mode (caesarian section vs. vaginal)
  – Diet (breast feeding vs. formula feedings)
  – Hygiene (exposure to pathogens)
  – Medication (antibiotics)
• Temporal changes over the first years of life

P LaRosa et al. PNAS 2014;111:12522-7 (premature babies)
Gut diversity in 15 vaginally delivered and 9 caesarian section infants

Changing gut microbiota diversity
(330 Danish infants, 9-36 mos old)

Low gut microbiota diversity in during the first month of life precedes asthma at school age

Asthma 7y

Shannon Diversity index

1 week 1 month 12 months

Asthma 7@ yr (n=8)

No asthma 7y (n=39)

Reduced gut diversity (FLVR) at 100 days predicts childhood asthma
Athletes had a higher diversity of gut micro-organisms, representing 22 distinct phyla, which in turn positively correlated with protein consumption.

Clarke SF et al. Gut 2014;63:1913-1920
Low diversity (less modular) gut microbiota in IBD and in obesity

124 Danish & Spanish adults
42 BMI >30
25 IBD

S Greenblum et al., PNAS 2012;109:594-599
Core microbiome in obese and lean twins: Missouri Adolescent Female Twin Study


with restoration after gastric bypass: H Zhang et al. PNAS 2009;106:2365-70
Twin Studies in UC-Microbiome

- Patients with UC had different gene expression profiles and lower levels of biodiversity than their healthy twins, as well as unusual aerobic bacteria.

- Patients with UC had lower percentages of potentially protective bacterial species than their healthy twins (lactose fermenters).

Twin Study Indicates Loss of Interaction Between Microbiota and Mucosa of Patients With Ulcerative Colitis
PATRICIA LEPAGE, et al, GASTROENTEROLOGY 2011;141:227–236
Abnormal Microbiome in Autism

• A growing number of studies point to altered microbiome in populations of children with autism.
• A consistent pathogen has not been reported
• Patterns of microbe alterations are being described and the effects vetted, high Clostridial species, diminished Bacteriodetes

Potential Etiologic Factors of Microbiome Disruption in Autism, Clinical Therapeutics, Buie, T
Volume 37 Number 5, 2015
Duodenal Microbiome in Autism

CORRELATION BETWEEN INTESTINAL DISACCHARIDASES AND MICROBIOME IN CHILDREN WITH AUTISM
Rafail I. Kushak, Timothy M. Buie et al

• In autism group, 17 out of 21 subjects and in controls 18 out of 19 subjects were lactase deficient.
• In samples from autism subjects, the relative abundance of genus Bacteroides, Faecalibacterium, and Clostridium showed a statistically significant positive correlation with lactase activity.
• The duodenal microbiome in neurotypical children was different than in children with autism.

Conclusion: There are differences at the genus and species level in the duodenal microbiota in children with autism that could be influenced by maldigestion of lactose or nutritional differences in food consumption.

Paper accepted JPGN 10/2016
Limitations of available studies

• Only within the last 2-3 years is it possible to evaluate the microbiome to species rather than phyla (families of organisms) using current technology

• Even now, proposals looking only at species without metabolic or proteomic studies are finding difficulty getting funded
Microbiota in immunity & inflammation

Y Belkaid & TW Hand, Cell 2014;157:121-141
Non-caloric artificial sweeteners alter the gut microbiome in mice and man
Saccharin, sucralose, aspartame; effects reduced by antibiotics; effects transferrable to germ-free mice

Contrasting opinion (based on doses in animals)
B Magnuson. Calorie Control Council
Atlanta, GA, March 13, 2015

Dietary emulsifiers impact the mouse gut microbiota promoting metabolic s., colitis
B Chassaing et al. Nature 2015;519:92-96
Distribution of microbes in the gut

- Present in all parts of the intestinal tract
- Increase from esophagus to colon
  - acid production
  - bile
  - motility
  - ileocecal valve

- Surface-lumen axis: more anaerobes in the outer mucus
- FISH: bacteria are not in direct contact with the mucosa
  - at least, in healthy subjects (vs. Crohn’s disease)

The gut microbiota and host health: a new clinical frontier.
JR Marchesi et al. Gut 2016;65;330-339
Reduced bacterial diversity (dysbiosis): an emerging theme across diseases

- Microbiota affected by:
  - Infections
  - Antibiotics
  - Xenobiotics
- Diabetes mellitus
- Obesity
- Cancers: gastric, colonic
- Inflammatory bowel diseases
- Necrotizing enterocolitis
- Irritable bowel syndrome, colic

Scientific American
June 2012
Microbial composition of IBS vs. controls

Adults: M Rajlic-Stojanovic et al. Gastroenterology 2011;141:1792-1801
Children: D Saulnier et al. Gastroenterology 2011;141:1782-1791
Control of Brain Development, Function, and Behavior by the Microbiome

Pathways linking the microbiome and central nervous system

- Intestinal lumen
- Intestinal barrier

Vagus nerve

Vagal stimulation

Circulatory system

- Neurotransmitters
- Hormones
- Metabolites
- Immune signaling

Immune system

- MAMPs
- Metabolites

TR Sampson & SK Mazmanian Cell Host Microbe 2015:17:564-576
Can we fix anything?

• The microbiome is relatively established early in childhood at 2-3 years of age (setting point).
• Subsequent to this, there are perturbations like antibiotic exposures but following these, over weeks to months, the microbiome migrates back to previous baseline (whether healthy or unhealthy at the time).
• Establishing a healthy microbiome by this setting point should be our goal.
Nature’s first Functional Food

WHAT’S IN HUMAN MILK

Human milk oligosaccharides (HMOs) are food for friendly bacteria like *Bifidobacterium infantis*. Shorter chain HMOs in particular are almost entirely consumed by this microbe.

A Petherik. Nature 2010;468:S5-S7
T Gura. Science 2014;345:747-749
Human milk oligosaccharides

- Large component of breast milk (5-10 g/L)
- Complex mixture of galacto-oligosaccharides
- Bifidogenic properties
- Concentration affects microbiome composition
- Normally, not present in infant formulas

T Gura. Science 2014;345:747-749
Benefits of prebiotics for infants

Systematic review of 11 RCT’s (n=1,459)

- Results in softer, more frequent stools
- Increases #’s of Bifidobacter & Lactobacilli
- Reduces stool pH
- No effect on weight accretion
- Clinical impact not assessed as an outcome
  - might protect against harmful bacteria
  - may reduce infection rates

Criteria for prebiotic classification

1. **Resistance** to gastric acid, enzyme hydrolysis and intestinal absorption
2. **Fermentation** by intestinal microflora
3. **Selective stimulation of growth** and/or activity of intestinal bacteria that contribute to health

AM Bronawell et al. J Nutr 2012;142:962-974

*lacS* utilization by lactic acid-producing bacteria

JM Andersen et al. PNAS 2011;108:17785-17790
Gut epithelial barrier integrity impacted by diet

Fiber

• 269 incident cases of CD (incidence, 8/100,000 person-years)
• 338 cases of UC (incidence, 10/100,000 person-years).

• High fiber intake in the highest quintile was associated with a 40% reduction in risk of CD

• This apparent reduction appeared to be greatest for fiber derived from fruits

• Fiber from cereals, whole grains, or legumes did not modify risk

• Neither total intake of dietary fiber nor intake of fiber from specific sources appeared to be significantly associated with risk of UC.

A Prospective Study of Long-term Intake of Dietary Fiber and Risk of Crohn's Disease and Ulcerative Colitis
Mechanisms of action of probiotics:

Colonization resistance:
- Competitive exclusion

Barrier function: tight junctions
- Reduce macromolecular permeability, bacterial translocation

Metabolic effects:
- Bacteriocins
- Decrease pH
- Quorum sensing

Innate & adaptive immunity:
- IgA, IgG, IgM, Tregs
- Mucins, TFF

Modulation of signal transduction:
- NF-κB
- IFNγ
- MAPK
- TC
- DC
- PC

P Sherman et al. Nutr Clin Pract 2009;24:10-14
Subject global assessment IBS symptoms after 4 wks:

$p = 0.0118$

PJ Whorwell et al. Am J Gastroenterol 2006;101:1581-90
Probiotics in experimental colitis: timing matters!

Probiotics are not all alike

Table 4. Evidence-based adult indications for probiotics in gastroenterology clinical practice.

<table>
<thead>
<tr>
<th>Indication and probiotic strain</th>
<th>Recommended dosage</th>
<th>Evidence level</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of acute infectious diarrhea</td>
<td>Saccharomyces boulardii</td>
<td>$10^9$ CFU per capsule of 250 mg, 2-6 capsules per day</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus rhamnosus GG or L paracasei B 21060</td>
<td>$10^9$ CFU twice-daily</td>
<td>2b</td>
</tr>
<tr>
<td>Prevention of antibiotic-associated diarrhea</td>
<td>S boulardii</td>
<td>1 g or $4\times10^9$ CFU per day</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td>L rhamnosus GG</td>
<td>$10^{10}-10^{11}$ CFU twice-daily</td>
<td>1b</td>
</tr>
<tr>
<td>Prevention of C difficile-associated diarrhea</td>
<td>S boulardii</td>
<td>2-3$\times10^9$ CFU for 28 days, followed up for another 4 weeks</td>
<td>1b</td>
</tr>
<tr>
<td>Coadjuvant therapy for H pylori eradication</td>
<td>L rhamnosus GG</td>
<td>$6\times10^9$ CFU twice daily</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td>S boulardii</td>
<td>500 mg-1 g or $2-4\times10^9$ CFU per day</td>
<td>1b</td>
</tr>
</tbody>
</table>

Recommended dose and evidence level were obtained according to World Gastroenterology Organisation Global Guidelines for Probiotics and Prebiotics published in October 2011. These guidelines are available at http://www.worldgastroenterology.org/probiotics-prebiotics.html. CFU: colony-forming units.
Probiotics DO NOT Set Up Residence

No long-term persistence of AH1206: “non-persisters”

Long-term persistence of AH1206: “persisters”

30% of those supplemented established long term engraftment
Maldonado-Gomez et al., 2016, Cell Host & Microbe 20, 515–526 October 12, 2016
Probiotics reduce symptoms of functional abdominal pain in childhood

S Guandalini et al. JPGN 2010;51:24-30 (VSL#3)
A Gawronska et al. APT 2007;25:177-184 (LGG)
Reviews of *L. reuteri* DSM 17938 in colic

T Harb et al. JPGN 2016;62:668-686
Gut-Brain Connections
L. reuteri MM4-1A impacts brain health

Maternal high-fat diet alters offspring gut microbiome, social behavior, PVN oxytocin levels, and VTA plasticity

Maternal Regular Diet (MRD) Offspring
- Normal
- Social
- Normal

Maternal High-Fat Diet (MHFD) Offspring
- Dysbiosis
- Impaired
- Impaired

Precision microbial reconstitution in MHFD offspring restores social behavior, oxytocin levels, and VTA plasticity

MHFD Offspring + Lactobacillus reuteri
- Treated
- Social
- Restored

SA Buffington et al. Cell 2016;165:1762-1775
Probiotics increase neurotransmitters in male mice (*L. rhamnosus*, JB-1 for 4 wks)

- Glutamate+glutamine
- N-acetyl aspartate + N-acetyl glutamate
- GABA

Magnetic resonance spectroscopy

Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,1,2,* Sara W. McBride,1 Sophia Hsien,1 Gil Sharon,1 Embriette R. Hyde,3 Tyler McCue,3 Julian A. Codelli,2 Janet Chow,1 Sarah E. Reisman,2 Joseph F. Petrosino,3 Paul H. Patterson,1,4,* and Sarkis K. Mazmanian1,4,*

Cell 2013
Gut microbiome and the brain in humans

Altered fecal microbiota (reduced *Faecalibacterium* spp.) in active major depressive disorder (n=29), versus inactive MDD (n=17) & healthy controls (n=30)

Reduced cognitive reactivity (rumination and aggressive thoughts) in healthy adults receiving probiotics
L Steenbergen et al. Brain, Behavior, and Immunity 2015;48:258-264

Autism and autism-spectrum disorders?
F Mangiola et al. World J Gastroenterol 2016;7:361-368
[based on mouse studies in: *Cell* 2013;155:1451-63]
Probiotics Affect Brain Function

Consumption of fermented milk product with probiotic modulates brain activity.

- Yogurt culture (Bifidobacterium Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus, and Lactococcus lactis)

Probiotics modulate brain activity in women

FMPP denotes fermented milk product with 4 probiotic strains
Reduced activity in response to emotional faces attention task (n=12)
K Tillisch et al (E Mayer). Gastroenterology 2013;144:1394-1401
Anti-obesity effects of probiotics:

CLA production

↑ FIAF*

↑ Brown tissue thermogenesis

↓ Leptin

Altered activity in appetite centers

↓ Absorption of lipids

CLA: linoleic acid
FIAF: fasting-induced adipose factor

T Arora et al., Nutrition 2013;29:591-596
Insulin sensitivity by fecal transplant from lean donors

@ 6 wks
9 Dutch men

A Vrieze et al. Gastroenterology 2012;143:913-916
Prevention

• Evaluate C-Section Delivery, can we turn the pattern of elective, convenience delivery?
• Judicious antibiotic exposures to mother and baby
• ? Probiotics supplemented for “health”
• Focus families back onto breast feeding for as long as possible
• Fiber as food is back, fruit may be best
Antibiotics, birth mode and diet

• **Breast Milk** for 3 months or longer supported higher diversity of the microbiome for the first 2 years of life compared to formula fed infants.

• **C-Section Delivery** brought lower diversity compared with vaginal delivery for up to 2 years. (In another study performed by this group, diversity improved if infants born by C-section were exposed to maternal vaginal secretions.)

• **Antibiotics** lowered diversity for up to 2 years. The worst impact on the microbiota was antibiotic exposure from 6-12 months of age.

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Antibiotics, birth mode, and diet shape microbiome maturation during early life

- Nicholas A. Bokulich

Antibiotic effects

- **Monthly** microbiome, metabolomic and genomic testing on 39 infants for 3 years.
- Supports vaginal vs. C-sections biome differences (C-section often lacking protective Bacteroides species)
- Antibiotic exposure diminished diversity and promoted antibiotic resistance genes

Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability
Moran Yassour et al
*Science Translational Medicine* 15 Jun 2016: Vol. 8, Issue 343, pp. 343ra81
Diet

Particular diet models:

• Moderate/low Carbohydrate
• Protein maximized, ? Fat (at least no high fat)
• Fruit based fiber
• Yogurt/Fermented sources offering probiotics
• Unique restrictions need vetting
C-Section Rates Nationally

• The overall rate of caesarean section births in the U.S. was 32.7 percent in 2013
• The WHO supports that approximately 10 percent to 15 percent of C-section deliveries are medically necessary
• It is important to evaluate why high rates are present, risk, monitoring concerns, medicolegal concerns
C-Section Rates CA

• In California data from 244 hospitals regarding C-sections performed on first-time moms:
  • 26.5 percent in 2014 down from 27.4 percent in 2013
  • The federal government has set a goal of reducing C-sections in low-risk pregnancies to 23.9 percent by 2020.
  • The top performing 10 percent of hospitals had C-section rates of 19 percent or lower. Meanwhile,
  • The worst performing 10 percent had rates of 33 percent or higher. The number of births at a hospital doesn't seem to be a factor, since there is a mix of facilities with high and low volumes throughout the rankings.
Thanks

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