THE RELATIONSHIP BETWEEN DIET, INTESTINAL MICROBIOTA, IMMUNITY AND HEALTH

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Outline

• What are intestinal microbiota?
• Functions of intestinal microbiota
• Role of intestinal microbiota on immunity
• Factors influencing the composition of intestinal microbiota
• Microbial imbalance and disease
• Manipulation of intestinal microbiota
• Conclusions
WHAT ARE INTESTINAL MICROBIOTA?
What are Intestinal Microbiota?

- **Microbiota:** community of micro-organisms present in one environment eg. the gut. Includes bacteria, archaea, fungi and viruses.
- **Microbiome:** all the DNA, or genomes, of all the micro-organisms present in one environment eg. human body. Estimated to contain 100-times more genes than human genome.

Information collected on the human microbiome in recent years is by data generated by:

- European Metagenomics of the Human Intestinal Tract (MetaHIT)
- NIH funded Human Microbiome Project (HMP)

- GIT is home to the largest community of bacteria, estimated to contain more than 100 trillion bacterial cells.
- Guts of healthy adults harbor more than 1000 species of bacteria.
- Almost 150 species per individual (divided into 200 strains).
Intestinal Microbiota

- Microbial content of GI tract changes along its length.
- Ranges from a low number and narrow diversity in stomach, to larger numbers of more diverse microbes in large intestine.
- Large intestine constitutes over 70% of all microbes found in body.
STOMACH: Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria and Fusobacteria: $10^1-10^3 \text{ ml}^{-1}$

SMALL INTESTINE: Firmicutes, Actinobacteria, Bacteroidetes: $10^3-10^7 \text{ g}^{-1}$

LARGE INTESTINE: Firmicutes, Bacteroidetes, Actinobacteria: $10^{11}-10^{12} \text{ g}^{-1}$
Intestinal Microbiota Composition

- Comprised of 6-7 difference bacteria phyla:
  1. Bacteroidetes (include *Bacteroides*, *Prevotella* genera)
  2. Firmicutes (include *Clostridium*, *Enterococcus*, *Lactobacillus*, *Ruminococcus* genera)
  3. Actinobacteria (include *Bifidobacterium*)
  4. Proteobacteria (include *Enterobacteriaceae*)
  5. Verrucomicrobiota
  6. Euryarchaeota

- *Bacteriodes* active in several functions including energy production and conversion; amino acid transport and metabolism; CHO metabolism. (15-30% phyla)
- *Firmicutes* active in CHO metabolism. (60-80% phyla)
Intestinal Microbiota Composition

• A study followed changes in the microbiome of 37 adults for up to 5 years.
• \(\sim 60–70\%\) of bacterial strains present remained unchanged throughout study.
• **Bacteroidetes + Actinobacteria populations were less susceptible to changes; Firmicutes + Proteobacteria were significantly less stable.**
• Taxa present in individuals remain fairly constant over time, but *relative abundance* subject to change.
• Evidence suggests that diet shapes relative abundance of dominant phyla, and populations of specific bacterial groups influenced by macronutrient composition.
Enterotypes

- Attempts to identify ‘core’ microbiota (shared between 95% of individuals tested).
- International cohort of 39 individuals.
- 3 clusters or ‘enterotypes’ based on metagenomics sequences (dominated by particular bacterial genus):
  1. Bacteroides  
     Associated with high protein and fat diets.
  2. Prevotella  
     Associated with CHO (plant fiber) intake.
  3. Ruminococcus
- Differ in their specific gene functions.
- Stable in terms of age, gender, ethnicity, BMI.
- Long term dietary habits found to be primary predictor of enterotype.
FUNCTIONS OF INTESTINAL MICROBIOTA
### Functions of Intestinal Microbiota

<table>
<thead>
<tr>
<th>Generate vitamins eg. K, B12, thiamine, folate, biotin. Enhance absorption of minerals and vitamins. <em>(Bifidobacterium)</em></th>
<th>Mediate synthesis of bile acids <em>(Lactobacillus, Bifidobacterium, Bacteroides)</em></th>
<th>Produce many chemical of a hormonal nature eg. HPA hormones; neurotransmitters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcompete and eliminate enteropathic bacteria eg. <em>E. coli</em></td>
<td>Help maintain appropriate intestinal pH</td>
<td>Exert anti-diabetic effects; lower cholesterol levels; <em>(Lactobacillus and Bifidobacterium)</em></td>
</tr>
<tr>
<td>Exert anti-inflammatory effects at local and systemic levels</td>
<td>Produce short chain fatty acids (SCFA) from indigestible fibers</td>
<td>Improve lactose intolerance</td>
</tr>
</tbody>
</table>

Microbes play key roles in metabolic, nutritional, physiological and immunological processes in the human body.
Short Chain Fatty Acids (SCFA)

- SCFA’s are metabolites generated by the fermentation of fiber by colonic microbiota.
- Main SCFA: **acetate; butyrate; propionate**.

<table>
<thead>
<tr>
<th>Butyrate:</th>
<th>Propionate:</th>
<th>Acetate:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Principal energy source for colonic epithelial cells</td>
<td>• Regulate intestinal physiology and immune function.</td>
<td>• Acts as substrate for lipogenesis and gluconeogenesis.</td>
</tr>
<tr>
<td>• Modulates intestinal inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Promotes genomic stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Regulates colonocyte differentiation and apoptosis.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ROLE OF INTESTINAL MICROBIOTA ON IMMUNITY
Intestinal Microbiota and Immune System

- Microbiota is important in inducing regulatory mechanisms to keep mucosal and systemic immunity in balance, so that humans are tolerant of harmless bacteria, yet still able to react to pathogens.

- Cells of the gut and immune system continuously interact to distinguish between non-pathogenic commensal microflora, harmless food particles, and pathogenic microorganisms.

- Intestinal microbiota play a crucial role in the development of local and systemic immunity, as well as maintaining colonic homeostasis.

- Dysbiosis, imbalances in gut microbiota, could lead to altered immune functions and increased risk of disease.
Role of Specific Bacteria in Immunity

Monocolonization with *B. fragilis*

Promotes $T_{reg}$ cells and induces anti-inflammatory cytokine IL-10 production, which may protect from chemically induced colitis, and help maintain intestinal homeostasis.

Monocolonization with *Clostridia*

Promotes IL-10 producing $T_{reg}$ cells.

Monocolonization with *Clostridium coccoides*

Major producers of butyrate, protect against inflammatory damage, help promote intestinal epithelial barrier integrity.
## Tests on Germ Free Mice

<table>
<thead>
<tr>
<th>T Cells</th>
<th>Angiogenin-4</th>
</tr>
</thead>
</table>
| • In germ-free (GF) mice, there is a deficiency of T cells (CD3+) which can result in increased bacterial translocation.  
• Adding *Bacteroides fragilis* has been shown to increase T cells.  
• Thus, important reciprocal regulation between microbiota and T cell development. | • Expression of angiogenin-4 (a potent antimicrobial peptide) is diminished in GF mice.  
• Inoculation with *Bacteroides thetaiotaomicron* can induce its expression. |
Regulation of Microbiota: Innate and Adaptive Immunity

**Innate: Toll-like receptors**
- Critical mediators of innate immune recognition of pathogens and commensals.
- Play a critical role in maintaining intestinal homeostasis.
- Regulate microbiota composition.
- Mice lacking TLR5 had altered intestinal microbiota.

**Adaptive: T cells**
- T cell deficient mice exhibit microbiota alterations.
- $T_{\text{reg}}$ and Th17 cells provide help to B cells in production of IgA in intestine.
- IgA can affect composition of microbiota and support host-microbiota homeostasis by:
  1. Restricting growth or inflammatory effects of commensals.
  2. Enforcing maintenance of a diverse and ‘healthy’ microbiota composition.
Crucial relationship between intestinal microbiota and immunity. Microbiota promotes development of host immune responses, and the host immune response has the capacity to prevent excess bacterial translocation and inflammation.
FACTORS INFLUENCING THE COMPOSITION OF INTESTINAL MICROBIOTA
Factors influencing gut microbiota

Genetics
Stress
Pregnancy
Type of Birth
Nutrition
AGE
Age
Exercise
Antibiotic

Cerda et al, 2016
Age – Birth

- Human microbiota is established at birth.
- Intestinal microbiota composition plays an important role in immune system development. Childhood allergies??
- The mode of delivery strongly affects the composition of microbiota.

**Vaginal Delivery**
- 1st encounter with microorganisms occurs in birth canal
- Colonization initiated by vaginal and intestinal microbiota, as well as the environment.

**Cesarean Delivery**
- Environment plays a major role in colonizing bacteria.
- During early weeks of life, infants will have lower and less diverse bacterial count.
- Less *bifidobacteria* and *bacteroides*.
- Take months to develop normal population microbiota.
Age – Infancy

- Factors influencing infant microbiota are:
  - Type of infant feeding
  - Infant hospitalization
  - Antibiotic use

- A complex and more stable community (similar to adult microbiota) established at 2-3 years of age.

<table>
<thead>
<tr>
<th>0-9 Months (Newborn)</th>
<th>9-18 Months (Infant-Pre-Toddler)</th>
<th>18-36 Months (Toddler)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breast-Fed Characteristics (BF)</strong></td>
<td><strong>Introduction of Weaning &amp; Solid Food</strong></td>
<td><strong>Diet-Influenced Microbiome Profile</strong></td>
</tr>
<tr>
<td>• Low Species Diversity</td>
<td>• Increased Species Diversity</td>
<td>• Stable Gut Microbiome Formation</td>
</tr>
<tr>
<td>• Bacterial Composition Flux</td>
<td>• Bacterial Composition Flux Persists</td>
<td>• Increased Species Diversity</td>
</tr>
<tr>
<td>• Major Phyla: Actinobacteria &amp; Firmicutes</td>
<td>• Increasing Butyrate Producing Bacteria</td>
<td>• Breast-Feeding History Ceases To Impact Gut Microbiome Profile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Increasing Butyrate Producing Bacteria Abundance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Major Phyla: Bacteroidetes &amp; Firmicutes</td>
</tr>
<tr>
<td><strong>Formula-Fed Characteristics (FF)</strong></td>
<td></td>
<td>• Dietary Intake Strongly Influences Abundances (Prevotella vs Firmicutes)</td>
</tr>
<tr>
<td>• Low Species Diversity</td>
<td></td>
<td>• Major Phyla: Bacteroidetes &amp; Firmicutes</td>
</tr>
<tr>
<td>• Bacterial Composition Flux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Major Phyla: Actinobacteria &amp; Bacteroidetes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Voreades et al, 2014
Age - Adulthood

- Composition of intestinal microbiota remains relatively stable.

- This stability imparts resilience to disturbance, ensuring continued gut function.

- In a disease context, however, such stability and resilience could be detrimental if the gut community is pathogenic.
Age - Elderly

- Aging is associated with reduced number and species diversity of microbiota.

- Considerable variation in reported microbial composition of elderly.

- Microbiota affected by various physiological changes that elderly experience, as well as modifications in lifestyle, nutritional behavior, infection rates, inflammatory disease, medication.

- Individual differences continue to occur.
Diet

• Diet continues to be the most important determinant in shaping the composition, diversity and richness of intestinal microbiota.

• Diets rich in fruits, vegetables, fibers associated with higher richness and diversity.
Diet

- ‘Western Diets’ had higher intakes of sugar, starch, fat, animal protein.
- ‘Rural’ plant rich diet-high fiber and non-animal protein (Burkina Faso).
- European children had higher levels of Enterobacteriaceae.
- Gut microbiota co-evolved with plant rich diet, maximizing energy extraction from fiber and protecting from inflammation and non-infectious GI disease.

De Filippo et al, 2010
### Diet

<table>
<thead>
<tr>
<th>Community dwelling elderly</th>
<th>Long-term residential care elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Diet more diverse; low/moderate fat; high fiber.</td>
<td>• Diet less diverse; moderate/high fat; low/moderate fiber.</td>
</tr>
<tr>
<td>• Had more diverse microbiota.</td>
<td>• Had less diverse microbiota;</td>
</tr>
<tr>
<td>• The gut microbiota grouped more closely with healthy young adults, indicating that age itself may not be the driving factor of microbial change.</td>
<td>• Likely to be more frail, have inflammation and high clinical markers.</td>
</tr>
</tbody>
</table>

(Claesson et al, 2012)
Diet - Carbohydrate

- CHO are the principle carbon and energy source for colonic microbes.

- Complex CHO travel to the distal gut for fermentation by the dense community of microbes.

- The amount of fiber that can be metabolized depends on several factors, including microbial composition.

- Many of the health benefits of fiber result from its fermentation by colonic microbiota and metabolites produced eg. SCFA.
Diet - Protein

• Serves as major source of nitrogen for colonic microbial growth, needed for production of SCFA.

• Fermentation of amino acids may produce greater diversity of gases and metabolites, increases nitrogenous substrate for microbiota which increases eg. ammonia, phenols, certain amines and hydrogen sulfide.

• These may lead to leaky gut, inflammation, DNA damage and cancer progression as shown in animal studies and in vitro.

• Microbial metabolism of L-carnitine (abundant in red meat) may generate TMAO which may increase risk of atherosclerosis.
Diet - Fat

• High fat diets associated with colonic microbial changes at phylum and genus levels.

• High fat diets increase circulating levels of LPS, a potent inflammatory agent linked to development of common metabolic diseases.

• Secondary bile acids are produced by 7 α-dihydroxylation of primary bile acids by colonic microbiota. Can contribute to microbial imbalances. Potential carcinogens and may increase risk of Colorectal cancer and GI diseases.
Diet - Probiotics

- WHO Definition: “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host.”
- Mechanism of action is multifactorial and may be strain specific.
- Most studied and common probiotic preparations: *lactobacilli* and *bifidobacteria*.
- Live cells in probiotics inevitably lose viability through processing and possible due to hostile environment of stomach.

Power et al, 2014
Diet - Prebiotics

• Definition: non-digestible food ingredients (oligosaccharides or short polysaccharides), which have been shown to selectively stimulate growth of beneficial bacterial populations in the large intestine.

• Most studied: inulin, oligofructose, galactofructose, galacto-oligosaccharides, xylo-oligosaccharides.

• Upon fermentation, they lead to an increase in abundance of *bifidobacteria* or some butyrate producers.
Diet – Polyphenols

• Diverse class of plant secondary metabolites, associated with color, taste and defense actions of foods eg. berries, tea, cocoas.

• Gut microbiota play a vital role in transforming these into absorbable biologically active species; act on nearly 95% of dietary polyphenols reaching colon.

• Some studies showed that polyphenol extracts eg. cocoa derived flavanols can modulate microbiota by increasing abundance of bididobacteria and lactobacilli.
Diet – Vegetarianism

Long-term consumption of agrarian plant-based diets has been associated with:

- Greater taxonomic and bacterial gene diversity
- Higher levels of SCFA production
- Lowered intestinal pH
- Reduce pathogenic bacteria
Plant Based Vs. Animal Based Diets

- Significant change occurred a single day after animal-based diet reached distal gut microbiota.

- Subjects’ gut microbiota reverted to original structure 2 days after animal-based diet ended.

- Animal-based diet had greater impact on gut microbiota than plant-based diet.
Long-Term Gut Microbiome Bacterial Dominance Shift

Bacteroides Pole

Prevotella Pole

Plant polysaccharide
Meat/Fat
Plant polysaccharide
Meat/Fat
Plant polysaccharide
Meat/Fat
Plant polysaccharide
Meat/Fat
<table>
<thead>
<tr>
<th>Diet</th>
<th>Bacteria Altered</th>
<th>Effect on Bacteria</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat</td>
<td><strong>Bifidobacteria</strong> spp.</td>
<td>Decreased (absent)</td>
<td>[45]</td>
</tr>
<tr>
<td>High-fat and high-sugar</td>
<td><em>Clostridium innocuum</em>, <em>Catenibacterium mitsuokai</em> and <em>Enterococcus</em> spp.</td>
<td>Increased</td>
<td>[18]</td>
</tr>
<tr>
<td>Carbohydrate-reduced</td>
<td><strong>Bacteroides</strong> spp.</td>
<td>Decreased</td>
<td>[18]</td>
</tr>
<tr>
<td>Calorie-restricted</td>
<td><em>Clostridium coccooides</em>, <em>Lactobacillus</em> spp. and <em>Bifidobacteria</em> spp.</td>
<td>Decreased (growth prevented)</td>
<td>[48]</td>
</tr>
<tr>
<td>Complex carbohydrates</td>
<td><strong>Mycobacterium avium</strong> subspecies <em>paratuberculosis</em> and <em>Enterobacteriaceae</em></td>
<td>Decreased</td>
<td>[49]</td>
</tr>
<tr>
<td>Refined sugars</td>
<td><em>B. longum</em> subspecies <em>longum</em>, <em>B. breve</em> and <em>B. thetaiotaomicron</em></td>
<td>Increased</td>
<td>[53]</td>
</tr>
<tr>
<td>Vegetarian</td>
<td><strong>C. difficile</strong> and <strong>C. perfringens</strong></td>
<td>Increased</td>
<td>[54, 55]</td>
</tr>
<tr>
<td>High <em>n</em>-6 PUFA from safflower oil</td>
<td><strong>Bacteroidetes</strong></td>
<td>Decreased</td>
<td>[59, 60]</td>
</tr>
<tr>
<td></td>
<td>Firmicutes, <em>Actinobacteria</em> and <em>Proteobacteria</em></td>
<td>Increased</td>
<td>[59, 60]</td>
</tr>
<tr>
<td></td>
<td><em>δ-Proteobacteria</em></td>
<td>Increased</td>
<td>[61]</td>
</tr>
<tr>
<td>Animal milk fat</td>
<td><em>δ-Proteobacteria</em></td>
<td>Increased</td>
<td>[62]</td>
</tr>
</tbody>
</table>
Antibiotics

• Disturbs the composition and diversity of microbiota; affects metabolic activity of gut microbiota.

• Antibiotic treatment in elderly patients has been shown to:
  ➢ Increase intestinal abundance of proteolytic bacteria
  ➢ Potentially eliminate certain bacterial communities
  ➢ Associated with the overgrowth of Clostridium difficile

• Microbiota may be resilient and resemble pre-treatment in days or weeks. Other data showed that alterations in microbiota composition persist for a long time, and may not return to pre-treatment levels.
Selected features affecting the establishment and maintenance of microbiota and factors influencing composition of microbiota.

- **Staphylococcus**
- **Corynebacterium**
- **Propionibacterium** spp.
- ↓ Bacterial numbers

**C-section**

**Vaginal delivery**

- **Lactobacillus**
- **Prevotella**
- **Atopobium**
- **Sneathia** spp.

**Birth**

**Infancy (0–2 years)**

- **Unstable**

**Adulthood**

- **Stable**

**Old age**

- **Unstable**

**Diet**

- **Antibiotics**
- **Illness**

**Weaning**

- **Common core** genera
  - **Bacteroides**
  - **Clostridium**
  - **Ruminococcus**
  - **Eubacterium**
  - **Parabacteroides**
  - **Coprococcus**
  - **Dorea**
  - **Alistipes**
  - **Collinsella**
  - **Lachnospira**
  - **Roseburia**
  - **Faecalibacterium**

**Formula-fed**

- **More complex**
- ↑ **B. fragilis**
- ↑ **E. coli**
- ↑ **C. difficile**

**Breast-fed**

- **Bifidobacterium**
- **Ruminococcus**

**Prebiotic/probiotic supplementation**

- **Bifidobacterium**
- **Lactobacillus**

**Enterotypes?**

- **Bacteroides**
- **Prevotella**
- **Ruminococcus**

**↑ Fusobacterium**

**↓ Facultative anaerobes**

**Proteolytic activity**

**↑ Bacteroides**

**↓ Bifidobacterium**

**Amylolytic activity**

**↓ SCFA**

Power et al, 2014
MICROBIAL IMBALANCE AND DISEASE
Intestinal Microbiota and Disease

• Link between dysbiosis and disease pathogenesis still unclear.
• Does dysbiosis cause disease, or does disease lead to changes in intestinal microbiota?
• Diseases certainly have an effect as they may include changes to dietary habits; bowel function; medications.
• Dysbiosis is linked to:
  ➢ Obesity
  ➢ Cardiovascular disease
  ➢ Colorectal cancer
  ➢ Diabetes type 2
  ➢ Rheumatoid arthritis
  ➢ Inflammatory bowel disease
  ➢ Celiac disease
A

Germ-free animals are protected from high-fat diet-induced obesity

High-fat / High-sugar
Western diet

Obesity

Altered
Microbiota
Composition

B

Germ-free animals adopt phenotype of microbiota donor

Donor weight
Normal
Obese
Underweight

Microbiota
Transfer

Adoption of
phenotype
Intestinal Microbiota and Obesity

• Several mechanisms have been proposed to account for these observations:

1. Fermentation of indigestible CHO by GIT microbiota leads to increased intestinal absorption of monosaccharides and SCFA followed by increased hepatic lipogenesis.

2. High fat diets trigger increased transfer of lipopolysaccharide (LPS) from intestinal lumen to blood causing low-grade inflammation. High fat also reduce intestinal barrier integrity.

3. GIT microbiota suppress the formation of angiopoietin-like protein 4, an inhibitor of lipoprotein lipase. This can lead to increased TAG storage in adipose tissue.

4. Abundance of Firmicutes and decrease in Bacteroidetes in obese mice and humans. Firmicutes may have larger capacity to harvest energy from diet; can lead to higher fat mass.
Intestinal Microbiota and Cardiovascular Disease

• Link between microbiota and CVD: microbial metabolism of dietary phosphatidylcholine into proatherosclerotic metabolite-N-oxide (TMAO).

• Plasma TMAO levels associated with increased risk of CVD event in patients with CVD risk factors.

Brown et al, 2014
MANIPULATION OF INTESTINAL MICROBIOTA
Probiotics and Prebiotics

• An in vitro study showed promise that the elderly gut microbiota can be modulated with species of *Bifidobacterium* and *Lactobacillus* along with two prebiotics.

• Probiotic/prebiotic combinations added to the culture increased the *Bifidobacterium* and *Lactobacillus* count in the vessel representing the distal colon, and decreased the Bacteroides count.

• Another study providing inulin supplementation to an elderly cohort increased *Bifidobacterium* levels.

• Thus, beneficial bacteria in the form of probiotics and the indigestible fibers of prebiotics have potential to help restore stability, increase diversity and beneficially alter the immune system in the aging gut.
Dietary Sources

• Fermented foods: kefir; yogurt; miso; soy sauce; sauerkraut

• Inulin is naturally present in bananas, berries, onions, garlic, leeks, asparagus, artichokes, honey

• Raw, unpasteurized aged cheese (sheep and goats milk)

• Plant based diet (beans, whole-grains; cruciferous vegetables)
Conclusions

• Intestinal microbiota provide a powerful route to influence health.

• Play crucial role in host metabolism and immune system development.

• Microbes residing in our GIT comprise a dynamic community that changes throughout lifespan.

• Still unclear if dysbiosis contribute to disease pathogenesis and symptoms, or if it is a consequence of disease.

• With better understanding of mechanisms and contributions of microbiota to disease (and dietary influences), therapeutics could potentially be developed to modulate microbiota to prevent/treat disease.
References