PROBIOTICS

• Live microorganisms that confer a health benefit on the host when administered in adequate amounts
• Effect is strain-specific
• Dosage is strain-specific
• Common probiotic families: *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, *Streptococcus*, *Enterococcus*

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Subspecies</th>
<th>Strain designation</th>
<th>International strain depository designation</th>
<th>Strain nickname</th>
<th>Product name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td><em>rhamnosus</em></td>
<td>None</td>
<td>GG</td>
<td>ATTC 53103</td>
<td>LGG</td>
<td>Culturelle</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td><em>animalis</em></td>
<td><em>lactis</em></td>
<td>DN-173 010</td>
<td>CNCM I-2494</td>
<td>Bifidus regularis</td>
<td>Activia yogurt</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td><em>longum</em></td>
<td><em>longum</em></td>
<td>35624</td>
<td>NCIMB 41003</td>
<td>Bifantis</td>
<td>Align</td>
</tr>
</tbody>
</table>

ATCC, American Type Culture Collection; CNCM, National Collection of Microorganisms Cultures; NCIMB, National Collection of Industrial and Marine Bacteria.
PROBIOTIC MECHANISMS

<table>
<thead>
<tr>
<th><strong>Probiotics</strong></th>
<th><strong>Immunologic benefits</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Activate local macrophages to increase antigen presentation to B lymphocytes and increase secretory immunoglobulin A (IgA) production both locally and systemically</td>
</tr>
<tr>
<td></td>
<td>• Modulate cytokine profiles</td>
</tr>
<tr>
<td></td>
<td>• Induce tolerance to food antigens</td>
</tr>
<tr>
<td></td>
<td><strong>Nonimmunologic benefits</strong></td>
</tr>
<tr>
<td></td>
<td>• Digest food and compete for nutrients with pathogens</td>
</tr>
<tr>
<td></td>
<td>• Alter local pH to create an unfavorable local environment for pathogens</td>
</tr>
<tr>
<td></td>
<td>• Produce bacteriocins to inhibit pathogens</td>
</tr>
<tr>
<td></td>
<td>• Scavenge superoxide radicals</td>
</tr>
<tr>
<td></td>
<td>• Stimulate epithelial mucus production</td>
</tr>
<tr>
<td></td>
<td>• Enhance intestinal barrier function</td>
</tr>
<tr>
<td></td>
<td>• Compete for adhesion with pathogens</td>
</tr>
<tr>
<td></td>
<td>• Modify pathogen-derived toxins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Prebiotics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Metabolic effects: production of short-chain fatty acids, absorption of ions (Ca, Fe, Mg)</td>
</tr>
<tr>
<td>• Enhancing host immunity (IgA production, cytokine modulation, etc.)</td>
</tr>
</tbody>
</table>

World Gastroenterology Organisation Probiotics and Prebiotics, 2017
APPLICATIONS

• Diarrhea
  • Treatment and prevention of acute diarrhea
  • Prevention of antibiotic-associated diarrhea, *C. difficile* diarrhea, radiation-induced diarrhea
• *H. pylori* eradication
  • In conjunction with antibiotics
• Hepatic encephalitis

• Inflammatory Bowel Disease (IBD)
  • Pouchitis
  • Ulcerative Colitis
  • Crohn’s Disease
• Irritable Bowel Syndrome (IBS)
• Lactose malabsorption
• Necrotizing Enterocolitis
• Nonalcoholic Fatty Liver Disease

*Not to be used in critically ill or immunocompromised individuals*
PROBIOTIC BENEFITS

- Weight: Mixed results offering no effect or weight loss
- Improve cholesterol levels (↓VLDL & LDL, ↑HDL)
- Decrease fat storage (visceral and subcutaneous)
- Decrease serum leptin
- Increase satiety
- Decrease insulin resistance
- Improve glucose tolerance
- Decrease fasting glycaemia
- Enhance SCFA production
- Increase GLP-1 release (via increase in SCFA)
- Decrease intestinal permeability
- Decrease inflammatory response
PROBIOTIC STUDIES

• **Mice**
  - Probiotic VSL#3
    - ↑ GLP-1 concentration
    - ↓ food intake
    - ↓ weight gain
    - Improved glucose tolerance
    - ↑ Bacteroidetes and Bifidobacteria
    - ↓ Firmicutes

• **Humans**
  - Probiotic VSL#3 in obese children with NASH
    - ↓ liver fat
    - ↑ fasting GLP-1
  - Probiotic *Lactobacillus* LG2055 in obese adults
    - ↓ abdominal obesity
    - ↓ weight

Parekh et al., 2014, Frontiers in Endocrinology 5
PROBIOTIC FOOD PRODUCTS

- Food products: Yogurt, frozen fermented dairy, freeze-dried yogurt, ice cream, cheeses, whey drink, whey cheese, soymilk, fruit & juices
- Food products suggested to need $10^7$ CFU per g or mL of food
- Probiotic must remain viable through GI tract with a minimum of $10^6$ CFU/g to offer benefit
- Many commercial yogurts do not deliver required level of probiotic bacteria
- Companies must use processing techniques compatible with bacteria survival
ACTIVE CULTURE FOOD SOURCES

- Yogurt
- Kefir
- Fermented vegetables
- Kimchi
- Pickles
- Sauerkraut
- Miso
- Tempeh
- Kombucha
- Buttermilk
- Fermented cheese (feta, blue, cheddar, goat)
HOW TO EVALUATE A PROBIOTIC SUPPLEMENT

- Marketing and trade names are not regulated
- Structure and function claims do not require FDA approval
- Items can be labeled as probiotics without substantiation
- No general dose: Effective dose is species specific, ranging 100 million – 10+ billion CFU/dose
- Evaluate live cultures at expiration date, NOT time of manufacture
**WHAT TO LOOK FOR**

**WHO RECOMMENDATIONS FOR PROBIOTIC LABELS**

<table>
<thead>
<tr>
<th>Expiration Date</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony forming units (CFUs): minimum number viable microorganisms at expiration date</td>
<td><em>Genus species</em> (strain) For all microbes contained in the product</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact us at:</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="http://productwebsite.com">http://productwebsite.com</a></td>
</tr>
<tr>
<td>Company Name</td>
</tr>
<tr>
<td>Address</td>
</tr>
<tr>
<td>Phone number</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Health Benefit (eg, improves GI transit time)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Suggested serving size or dose</th>
</tr>
</thead>
</table>

| Proper storage conditions (Climate changes, exposure to moisture and oxygen may be important to keep probiotics alive) |
PROBIOTIC SUPPLEMENTS

• Resources
  • WGO: World Gastroenterology Organisation Global Guidelines Probiotics and Prebiotics (February 2017)
    • Includes evidence-based indications for probiotics, bacteria strains, probiotic companies with descriptions and websites
  • ISSAP: International Scientific Association for Probiotics and Prebiotics
    • Has infographic handouts and consumer guides for probiotics, prebiotics and fermented foods
  • Clinical Guide to Probiotic Products (website or app)
    • Probiotic supplement or food product, dose, evidence
    • App: Probiotic Guide *US version
MEDICATIONS

Antibiotics
Metformin
Proton Pump Inhibitors (PPIs)
• Taxonomic differences seen in those with T2D than in those without diabetes
• Dysbiosis associated with T2D not only a result of dysglycemia
• Fewer butyrate producing taxa in T2D
• Functional analysis showed an increase in butyrate and propionate production in pts treated with Metformin
  • SCFAs may increase intestinal gluconeogenesis, which may decrease hepatic gluconeogenesis and decrease appetite and weight (mice)
• Metformin may reverse changes in gut microbiota caused by T2D to levels similar to non-DM
• Metformin increases Akkermansia muciniphila
  • Associated with metabolic improvements
• Mice on a High Fat Diet had more Firmicutes and fewer Bacteroidetes than the Normal Diet group
  • Metformin increased Bacteroidetes
PROTON PUMP INHIBITORS

- Proton pump inhibitors (PPIs) usually taken recurrently and long term
- Decrease microbiota richness and diversity
- Gut dysbiosis increases risk of enteric infections
- PPIs increase risk of enteric infections
- 65% increased incidence of *C. difficile* infection with PPIs
- Altered pH in stomach (acid suppression) may allow for survival of more species from food and oral mucus
  - Acidity is a defense against pathogens
ANTIBIOTICS

• Human microbiota is overexposed to antibiotics (ABX)
  • Medical use and use in farm animals and crops
• Broad-spectrum antibiotics affect 30% of bacteria in gut
• Disrupt the ecology of the microbiome: Decrease some beneficial bacterial and allow for growth of pathogens
• Rapid and significant alteration = decreased richness, diversity and evenness (dysbiosis)
• Dysbiosis may persist for months to years
• Some degree of resilience, but even after populations return, they may not fully recover
ANTIBIOTICS

- Impact of antibiotics depends on the functions performed by specific bacteria
- Affect gene expression, protein activity and metabolism of gut microbiota
- Alter effectiveness of immune response, decreasing the ability to fight infections
- May thin the mucin layer in the epithelium and increase immune stimulation leading to inflammation
- Microbiota functionality mimic states seen in disease conditions
- Decreased diversity and altered composition seen in infants treated with antibiotics AND in infants not treated with antibiotics, but whose mothers received antibiotics prior to delivery
ANTIBIOTICS THROUGH THE LIFE CYCLE

- **Infants**
  - Majority of women are given antibiotics during pregnancy and delivery
  - 11 types of broad-spectrum ABX cross the placenta
  - Gut microbiota of preterm infants differs from full term infants
  - Preterm infants are often given broad-spectrum ABX further altering the microbiota diversity

- **Children**
  - Malnutrition alters the gut microbiota; ABX treatment can allow for nutritional recovery in children with severe acute malnutrition & decrease mortality
  - ABX increase weight in children (both malnourished and not)
  - Shift the ecology toward *Firmicutes*
  - Increased risk T2D with repeat use of some ABX

- **Adults**
  - Antibiotic-associated diarrhea
  - Infection: *C. difficile*-associated diarrhea
ANTIBIOTIC-RELATED DYSBIOSIS

• Increased susceptibility to disease
  • Intestinal infection
  • Overgrowth of pathogens
  • Antibiotic-associated diarrhea

• Altered immune system homeostasis
  • Increase atopic (allergenic) disease, inflammatory disease, and autoimmune disease associated with dysbiosis
  • Associated with antibiotic use in early life & in children with maternal antibiotic use during pregnancy
  • Antibiotic use associated with necrotizing enterocolitis (infants) and Crohn’s disease

• Deregulated metabolism
  • Increase obesity (humans and animals)
END OF THE ANTIBIOTIC-ERA

• 2015: 50,000 deaths from antibiotic-resistant pathogens in US and Europe
• Excess exposure leads to bacterial resistance
• Gut microbiota is a “reservoir of antibiotic resistance”
• Antibiotic resistant microorganisms are increasing while new antibiotic discovery is decreasing
• Antibiotic resistance genes (ARGs)
  • Found ARGs for 50 our to 68 antibiotic classes
  • ARGs highest for antibiotics that have been around longer and in those used in animals
  • Children have ARGs that are vertically inherited from the maternal microbiome
• Need more narrow-spectrum, pathogen-specific ABX
COMBATING ANTIBIOTICS

• Probiotics
  • Restore microbiota
  • Used to treat C. difficile, antibiotic-associated diarrhea and infectious diarrhea
  • Commonly Lactobacillus or Bifidobacterium species
  • May help IBD and Crohn’s disease

• Fecal Microbiota Transplants
  • Use healthy fecal microbes
  • Treat C. difficile infection by suppressing C. difficile blooms

• Phage Therapy
  • Phages are predatory toward bacteria
  • Target specific bacteria (decrease off-target effects)
  • Self-replicating
SLEEP
SLEEP

• Chronic sleep loss is associated with weight gain, obesity and type 2 diabetes
• One night of sleep deprivation leads to impaired insulin sensitivity
• Circadian Rhythm (CD) = 24hr biological clock (sleep/wake cycle for plants, animals, fungi and bacteria)
• CD corresponds with gene expression; over ⅓ genes depend on the circadian clock.
• Gut microbiota fluctuate in composition and gene expression in a circadian-dependent manner
• Artificial light, irregular sleep schedules, shift work and travel-related jet lag may alter CD
SLEEP DISTURBANCES TO MICROBIOME (HUMAN)

• Sleep deprivation
  • Took fecal samples & did OGTT (normal wt men):
    • After 2 days normal rest
    • After 2 days of sleep deprivation (4hr, 15min)
  • Sleep deprivation subjects had increased Firmicutes:Bacteroidetes ratio (associated with obesity)
  • No effect on SCFA
  • Fasting and postprandial insulin sensitivity decreased

Benedict et al., 2016, Molecular Metabolism 5
SLEEP DISTURBANCES TO MICROBIOME (MICE)

• Obstructive Sleep Apnea (OSA)
  • Microbiota Changes
    • Intermittent hypoxia was mimicked in mice over a 6 week period; Measured partial pressure of oxygen in feces & did taxonomic analysis of fecal microbiota
    • Intermittent hypoxia lead to increased abundance of Firmicutes and decreased Bacteroidetes
    • Increased Prevotella and Desulfovibrio (mucus-degrading microbes) may lead to alterations of intestinal permeability
  • Blood Pressure
    • Mimicked OSA conditions in rats fed either a normal chow diet or high-fat diet (HFD)
    • Mice with OSA on HFD had increased BP after 7 and 14 days
    • Mice with OSA on normal diet had no increase in BP
    • HFD and OSA decreased butyrate producing species and increased lactate producing species
    • FMT of bacteria from mice with OSA on a HFD resulted in HTN to the recipient

Moreno-Indias et al., 2015, European Respiratory Journal 45(4)
Durgan et al., 2016, Hypertension
Fecal Microbiota Transplants
Fecal Microbiota Transplants (FMT)

- Very effective in eliminating *C. difficile* infection
  - Success rate of ~90%
- Route: Enema, colonoscopy, nasogastric or nasoduodenal tube or freeze dried capsules
- Freeze-dried fecal matter in acid-resistant capsules designed to open in the small intestine
- Samples are filtered, diluted & screened
  - Donors may have harmful bacteria, viruses & parasites
- Don’t DIY
FMT: WEIGHT & METABOLIC DYSFUNCTION

• Mice
  - FMT from mice with glucose intolerance into germ-free mice induced glucose intolerance
  - Bacteria transplanted from obese mice or obese humans into lean mice triggered weight gain

• Human
  - FMT from lean donors into subjects with metabolic syndrome increased insulin sensitivity (peripheral & hepatic), increased bacterial diversity & increased butyrate-producing bacteria
  - Incidental weight gain caused in recipients receiving FMT for C. difficile from obese donors

• New trial from Massachusetts General Hospital (ends June 2018) to evaluate effect of FMT in humans on insulin resistance, weight and body composition

Vrieze et al., 2012, Gastroenterology 143(4)
EXERCISE

• Possible Mechanisms
  • Alteration of bile acids
  • ↑ SCFA
  • ↓ Serum LPS (via signaling pathway)
  • Anti-inflammatory effect
  • Weight loss

• Rats
  • ↑ bacterial diversity in rats that exercised
  • ↑ Lactobacillus
  • ↑ % Bacteroidetes, ↓ Firmicutes
  • Bacteroidetes:Firmicutes ratio inversely correlated with exercise

• Humans (1 study)
  • Rugby players vs. healthy controls had greater bacterial diversity
  • Unable to distinguish if related to exercise or diet

Cerdá et al., 2016, Frontiers in Physiology 7
GASTRIC BYPASS

- Roux-en-Y gastric bypass (RYGB)
- Profiled gut microbiota before, 3 months after & 6 months after RYGB (2 studies)
  - Richness increased after RYBG
  - ↑ Bacteroides/Prevotella & E. coli at 3 months
  - ↓ Lactobacillus & Bifidobacterium
  - ↓ Firmicutes
  - ↓ Firmicutes:Bacteroidetes ratio
    - No relationship found in other studies
  - Half of changes to microbiota were independent of calorie intake
  - Increased association between white adipose tissue (WAT) genes and corresponding bacteria after RYGB

Kong et al., 2013, American Journal of Clinical Nutrition 98
Furet et al., 2010, Diabetes 59
TAKEAWAYS

• Research is still new; we need more human research to be conducted into ways to safely alter the gut microbiota and treat dysbiosis

• Diet: Need long-term changes and consistency

• Eat plant-based foods to increase fiber

• Consume probiotic- and prebiotic-rich foods

• Avoid a Western Diet (high-fat, high in refined grains & processed foods)

• Avoid unnecessary antibiotics

• Get enough sleep

• Exercise
THANK YOU