Obesity and the Microbiome

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Objectives

1. Summarize the origin and scope of the microbiome

2. Illustrate the complex interaction among the nervous system, digestive system, endocrine system, immune system and microbiota that initiates in the gut

3. Evaluate mechanisms by which our microbiome can affect obesity and where potential treatments might lie in the future
The human microbiome project says the human body has 100 trillion microscopic life forms living in it.

You call this living?
Definition and Origin: Gut Microbiota

Bacteria, viruses, fungi, archaea, phages and protozoa residing in the human intestines

Originates at Birth – though somewhat determine before

Large variation through first 3 years of life
  Vaginal Birth vs. C-Section
  Breast Fed vs. Bottle Fed
  When solids are introduced
  Regional Diet/Lifestyle
  Early antibiotic exposure
  Twin studies suggest only limited amount of microbiome is inherited

After age 3 microbial “signature” will not change much, though ratios, metabolites and function altered by a host of factors
Definition and Origin: Gut Microbiome

Over 100 Trillion microbes in the gut
Metagenomic sequencing revealed gastrointestinal microbiota contains 150 fold more genes than human genome

Lumped together 2-6 lbs  (about size of brain 2.6 lbs)

Current estimates suggest:

500,000 distinct metabolites produced by
7 million distinct genes (compared to 20,000 discovered in human genome)
Each metabolite can have up to 40,000 variants (depending on its relationship to other metabolites)
40% of metabolites circulating in humans actually produced by gut microbes

Bacterial Phyla

• 5 main Phyla found in digestive system
  - Actinobacteria, Bacteroides, Firmicutes, Proteobacteria, Verrucomicrobia

• 2 dominant – firmicutes and bacteroides account for 90%

• Changes across digestive tract – acidity/oxygenation
  - Proximal – firmicutes, lactobacilli, proteobacteria
  - Distal – bacteroides, firmicutes, akkermansia municiphilia

• More diversity – healthier, less autoimmune disease and obesity
  - Less diversity found in patients with DM2 and obesity

What are the functions of gut microbes?
Functions of Gut Bacteria

- Modulation of bone-mass density
- Promotion of fat storage
- Protection against epithelial injury
- Promotion of angiogenesis
- Resistance to pathogens
- Development and training of the immune system
- Breaking down food compounds
- Biosynthesis of vitamins and amino acids
- Modification of the nervous system
- Metabolism of therapeutics

Functions of Gut Bacteria: Digestion

Symbiotic relationship

Human enzymes cannot digest most fibers and prebiotics
<20 glycosidases identified in human genome involved in polysaccharide digestion
Pancreatic and salivary amylase - glycosidic linkages in starch polysaccharides
Intestinal brush border disaccharidases sucrase and lactase - sucrose, lactose

Carbohydrates that escape digestion by human enzymes = substrates for bacterial fermentation

Bacteria provide host with ~ 10% of its energy via fermentation of undigested dietary material (fiber, resistant starch) producing short-chain fatty acids
Functions of Gut Bacteria: Digestion

Digestion and absorption mainly occur in the stomach and proximal small intestine.

~66–95% proteins, 85% carbohydrates, and ~95% of fats are absorbed before entering the large intestine.

Soluble fibers, short-chain fructooligosaccharides + pectin, are metabolized by bacteria in ileum and ascending colon.

Highest density of gastrointestinal microorganisms found in the cecum + colon.

Less soluble fibers, cellulose, fermented in the distal colon.

Gut microbiota is predominantly involved in the fermentation of indigestible carbohydrates into short-chain fatty acids (SCFA).
Functions of Gut Bacteria: Short-Chain Fatty Acid Production

>95% of SCFA content are acetate, butyrate, and propionate

90-99% of SCFAs are absorbed in the gut or used by the microbiota

Important energy and signaling molecules

<table>
<thead>
<tr>
<th>SCFAs</th>
<th>Pathways/Reactions</th>
<th>Producers</th>
<th>References</th>
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<tbody>
<tr>
<td>Acetate</td>
<td>from pyruvate via acetyl-CoA</td>
<td>most of the enteric bacteria, e.g., Akkermansia muciniphila, Bacteroides spp., Bifidobacterium spp., Prevotella spp., Ruminococcus spp.</td>
<td>Louis et al., 2014; Rey et al., 2010</td>
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<td></td>
<td>Wood-Ljungdahl pathway</td>
<td>Blautia hydrogenotrophica, Clostridium spp., Streptococcus spp.</td>
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<tr>
<td></td>
<td>succinate pathway</td>
<td>Bacteroides spp., Phascolarctobacterium succinatutens, Dialister spp., Veillonella spp.</td>
<td>Louis et al., 2014; Scott et al., 2006</td>
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<td></td>
<td>acrylate pathway</td>
<td>Megasphaera elsdenii, Coprococcus catus</td>
<td></td>
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<tr>
<td></td>
<td>propanediol pathway</td>
<td>Salmonella spp., Roseburia inulinivorans, Ruminococcus obeum</td>
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<tr>
<td>Butyrate</td>
<td>phosphotransbutyrylase/butyrate kinase route</td>
<td>Coprococcus comes, Coprococcus eutactus</td>
<td>Duncan et al., 2002; Louis et al., 2014</td>
</tr>
<tr>
<td></td>
<td>butyryl-CoA:acetate CoA-transferase route</td>
<td>Anaerostipes spp. (A, L), Coprococcus catus (A), Eubacterium rectale (A), Eubacterium hallii (A, L), Faecalibacterium prausnitzii (A), Roseburia spp. (A)</td>
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A, acetate is the substrate for producing butyrate; L, lactate is the substrate for producing butyrate.
Functions of Gut Bacteria: Short-Chain Fatty Acid Production

Butyrate is the primary energy source for colonocytes

- Protects against colorectal cancer + inflammation, inhibits histone deacetylases (HDAC) → alters gene expression, effecting cell proliferation, apoptosis, and differentiation

- Potent anti-inflammatory agent → suppresses pro-inflammatory effectors in lamina propria macrophages and differentiation of dendritic cells from bone marrow stem cells via HDAC inhibition → immune system hyporesponsive to beneficial commensals

Propionate and acetate released into general circulation (metabolic + neurologic effects)

Acetate most abundant SCFA in circulation; crosses the blood-brain barrier

Ligands for free fatty acid receptors (FFARs)

How is this related to obesity?
Functions of Gut Bacteria: SCFA Butyrate & Propionate

Butyrate activates expression of gluconeogenic genes in enterocytes by cAMP signaling; propionate stimulates gluconeogenesis as a gluconeogenic substrate and by FFAR3-dependent signaling of portal vein peripheral nerves

Activate Intestinal Gluconeogenesis (IGN)

IGN detected by portal vein glucose sensor sends signal to peripheral nervous system to promote beneficial effects on food intake and glucose metabolism

Mice deficient in IGN lack beneficial weight control typical of presence of SCFAs and high fiber diet
Functions of Gut Bacteria: SCFA Butyrate & Propionate

Functions of Gut Bacteria: SCFA Acetate

High fat diet in rodents

Increased production of acetate by an altered gut microbiota activates parasympathetic nervous system which promotes increased glucose-stimulated insulin secretion, increased ghrelin secretion, hyperphagia, obesity and its sequelae

Evolutionarily advantageous?
feedback loop promotes hyperphagia and increased energy storage in animals when encounter calorically dense foodstuffs

Effects of Short Chain Fatty Acids: The Skinny

SCFAs influence:

- gastrointestinal epithelial cell integrity
- glucose homeostasis
- lipid metabolism
- appetite regulation
- immune function
Functions of Gut Bacteria: Influence Enterochromaffin cells

Located in mucosal epithelium; contain 95% of body’s serotonin

Signal nervous system via vagus nerve: sleep, pain sensitivity, appetite, overall sense of well-being

Responsive to short-chain fatty acids; microbial metabolites signal directly to colonic ECCs to promote 5-HT biosynthesis

Enteric Nervous System

50-100 million nerve cells (~spinal cord)
Can operate independently of nervous system
90% of nerve conduction goes from gut to brain
  strength and direction of peristalsis
  proper amount of bile acids
  how much food is in stomach
  size, consistency of food
  chemical composition of ingested meal
  presence and activity of gut microbiota

Inflammation makes GI system more sensitive to normal stimuli

Disruption by stress, altered mood affects these actions

Complex Signaling Effects Enteroendocrine Cells

Constitute largest endocrine organ in body

Include:
Gut olfactory receptors – located mainly on endocrine cells that control release of hormones

Ghrelin producing cells on stomach

Satiety hormones produced in small intestine

Send signals to the brain via the vagus nerve
Immune Cells

Germ-free mice had defects in development + function of immune system suggesting a “cross-talk” between GI bacteria and host

Preferentially located in clusters in Peyer’s patches in small intestine; but throughout small/large intestines

Most are separated from gut lumen by thin layer of cells

Some cross through the layer (dendritic cells) and interact with microbes or other pathogens

Cytokines produced in the gut can reach systemic circulation

Chronic stress and high-fat diet increase levels of adipokines, LPS and C-Reactive protein


Inter-Orgna System Cross Talk

Nervous System

Gut Microbiota

Digestive System

Immune System

Endocrine System
Early Studies Linking Gut Microbiome and Obesity

2004 Backhed et al. transplanted microbiota from normally grown mice to germ free (GF) mice.

GF mice then experienced:
Increased body fat in spite of decreased calorie consumption (60% increase in 14 days)
Insulin resistance, ↑ glucose levels ↑ leptin levels

GF mice w/out microbial transplant were protected from high fat diet induced obesity

DISCORDANT TWIN STUDIES
Fecal transplantation to mice from obese twin gained more weight than from lean twin:
↑ SCFA fermentation in lean
↑ metabolism of branched chain amino acids in obese
Co-habitation of lean and obese resulted in decreased weight gain in obese mice that correlated with invasion of bacteroides from lean to obese, but only when eating high produce diet

**Dysbiosis**

Disruption in the microbial composition

Associated with altered bodyweight and fat storage

Whether the dysbiosis is a cause or consequence of obesity and undernutrition has yet to be determined

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Dysbiosis: Gut inflammation – Metabolic Toxemia

Cytokines released alter vagal afferent nerves changing brain signaling change in appetite, mood, energy levels, pain sensitivity, etc.

Inflammatory state linked to impaired insulin sensitivity in muscle, adipose tissue and defective pancreatic islet cell function
Dysbiosis: Obesity, Diabetes & Insulin Resistance

Decreased occurrence of butyrate producing species

Higher levels of mucin-degrading bacteria*

Increase in Firmicutes/Bacteroides ratio

Lower overall gene diversity and less diverse composition of microbiota characterized by higher BMI and fat mass, lower insulin sensitivity, dyslipidemia

In need of larger studies with more power

*Akkermansia muciniphila reduces adipose tissue inflammation and improves insulin signaling, but seems to increase with use of metformin

Gut Microbiome Diversity

1632 healthy females from TwinsUK cohort

Fecal samples studies by 16S ribosomal RNA sequencing

High gut microbiome diversity, high fiber and high operational taxonomic units associated with improved energy metabolism and less long term weight gain

Less than half of the variation in long term weight change seemed to be heritable

Ruminococcaceae and Lachnospiraceae OTUs associated with less long term weight gain

Specific Bacteroides species associated with increased weight gain (but also lower levels diversity)
Determinants of the Gut Microbiome

Microbiome ‘signature’ difficult to change

But...

Alterations in ratios and percentages of different species as well as their metabolites can change
# Determinants of the Gut Microbiome

<table>
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<tr>
<th>Medications</th>
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<tr>
<td>Antibiotic use can influence gut microbiome (particularly decrease in Bifidobacterium genus)</td>
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<tr>
<td>Antibiotic use at early age associated with higher weight gain</td>
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<tr>
<td>PPIs, metformin can affect microbial composition</td>
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<tr>
<td>Sleep disruption</td>
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<tr>
<td>Jet lag</td>
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<tr>
<td>Stress</td>
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<tr>
<td>Exercise vs Inactivity</td>
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<tr>
<td>Diet/High fat diet</td>
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<tr>
<td>Artificial sweeteners</td>
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<td>Organophosphates</td>
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Determinants of Gut Microbiome: Dietary Effects - Bile Acids

Example of how gut microbes alter physiochemical properties of endogenous metabolites

Introduced in proximal small bowel, bile acids solubilize fatty acids and fat-soluble vitamins for absorption in the terminal ileum

Also after reabsorption in terminal ileum, sends signals via entero-endocrine and immune cells generating post-prandial state

Clostridium genus deconjugates bile acids rendering them more hydrophobic allowing for amplified post-prandial signaling

Bile Acids seem contributory to beneficial effect on weight in RYGB beyond caloric/metabolic changes alone

Determinants of Gut Microbiome: Dietary Effects - TMAO

Creation of Trimethylamine (TMA) by intestinal microbes, converted to trimethylamine oxide (TMAO) in liver.

- TMAO induces atherosclerosis in rodents; plasma levels correlate with CVD incidence in humans.
- Induces blood platelet hyper-responsiveness and thrombosis, cholesterol deposition and failure to remove from peripheral sources (liver, intestines, arterial walls).

- Foods high in carnitine (red meat), lecithin (eggs, soy), phosphatidylcholine (red meat, eggs, milk products, cruciferous vegetables)

- Chlostridiales high association with TMA/TMAO – plant based diets select against these Bacteroides
Determinants of Gut Microbiome – Dietary Effects

Food emulsifiers (like carboxymethylcellulose and polysorbate-80 often found in processed foods) and artificial sweeteners have been associated with altered microbial composition and the development of obesity and metabolic syndrome.

Red meat intake (via carnitine and choline) has been linked to altered gut microbiota composition.

Dietary compounds associated with weight reduction including vegetables, fibers, and yogurt, are all reported to alter fecal microbial composition.

Determinants of Gut Microbiome – Dietary Effects

Presented with limited fermentable fibers microbes switch to energetically less favorable sources for growth

Amino acids from proteins, or dietary fats, resulting in reduced SCFA production

Increase in branched-chain fatty acids such as isobutyrate, 2-methylbutyrate, and isovalerate, exclusively originating from branched-chain amino acids valine, isoleucine, and leucine

Implicated in insulin resistance
Determinants of Gut Microbiome – Dietary Effects

Determinants of Gut Microbiome – Dietary Effects

Separation of bacterial genera via 16S rRNA gene analysis present in fecal samples of

(a) African (Burkino Faso, BF) and 2-6 yr old; plant-based agrarian diet, rich in fruit+legume fiber (12.6 g/14.2 g total fiber), low in fat, animal protein

(b) European (EU) children compared to ‘Western’ diet rich in animal fat, low in fruit, legume dietary fiber (3.3 g/8.6 g total fiber)
Determinants of Gut Microbiome – Dietary Effects

African children exhibited increased biodiversity of Bacteroidetes genera *Prevotella* + *Xylanibacter* and Spirochetes

- Possess enzymes that metabolize plant cell wall dietary fiber → generate significant levels of secondary fermentation products, particularly SCFAs

Solid phase gas chromatography mass spectrometry analysis revealed that SCFA were high in the African children

- Propionic + butyric acid levels greatly enhanced

Pathogenic Enterobacteriaceae, such as *Shigella* spp. and *Escherichia* spp., were lower in fecal samples of the African children

Determinants of Gut Microbiome – Short Term Dietary Effects

Short-term dietary changes can alter the composition and metabolic activity

In humans, short-term consumption of diets that are exclusively animal (protein and fat) or plant-based have major effects

Animal-based diet:
- Increase bile-tolerant, inflammation-associated bacteria, (Bacteroides; Bilophila)
- Decrease levels of the Firmicutes (metabolize plant fiber)
- Lower concentrations of SCFAs (butyrate and acetate)
- Significantly greater emphasis on dissimilatory branched-chain amino acid metabolism by colonic bacteria

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Potential Future Treatments: Prebiotics, Probiotics and FMTs
PREBIOTICS

Resistant to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption; fermented by intestinal microbiota; selectively stimulate the growth and/or activity of intestinal bacteria associated with health

Indigestible dietary polysaccharides that promote growth of microbes when supplied externally

Fructooligosaccharides, galactooligosaccharides, lactulose and non-digestible carbohydrates, including inulin, cellulose, resistant starches, hemicelluloses, gums and pectins (non-digestible carbohydrates)

Oligofructosaccharides potentially increase proglucagon, GLP-1 and decrease ghrelin
Mediated by SCFA production
Prebiotic administration in multiple murine and human studies have shown decreased firmicutes and increased bifidobacteria

Overall: Prebiotics were shown to help manage obesity via gut microbial modulation resulting in lower production of LPS, decreased inflammation and modulation of eCB systm. Also increased satiety via promotion of satiety peptides from L-Cells in gut (GLP-1 and GLP-2)
Probiotics

Live microorganisms that confer health promoting effects on the host

Lactic acid bacteria most common studied (lactobacillus spp. And Bifidobacterium spp.) and have provided some anti-obesity effects in animal and human models

VSL#3 (commercially available) attenuate diabetes and obesity when fed to mice via increased GLP-1 presumed shift to butyrate producing bacterial species – resulting in reduced food intake, improved glucose tolerance and reduced adiposity

Fewer human studies. So far have demonstrated beneficial effects on obesity prevention when given to newborns and also healthy young subjects. Decreased LPS, dysbiosis and metabolic endotoxemia

Possible synergistic effect with addition of prebiotic formulation (OFS)
Potential Uses in Treatment – Fecal Microbiota Transplantation

Increase use in clinical studies
- C. Difficile, Crohn’s, IBD
- Obesity, Metabolic disease
- Alzheimer’s, ASD

Need for standardized protocols for donor screening and transplant techniques

Need for increased understanding of donor microbiota composition and clinical results

Figure 2. Evolution over time of number of published studies of FMT, by design (top) and clinical condition (bottom).

ASD = autism spectrum disorder; CDI = Clostridium difficile infection; CIPSO = chronic intestinal pseudo-obstruction; FMT = fecal microbiota transplantation; HD = hepatic diseases; IBD = inflammatory bowel disease; IMDRO = infection with multidrug-resistant organisms; IS = immunodeficiency syndrome; MD = metabolic disease; MODS = multiple organ dysfunction syndrome; RCT = randomized controlled trial; STC = slow transit constipation.

* Number of studies between 1 January and 31 January 2017; 3 studies included.
Potential Uses in Treatment – FMT for Metabolic Disorders

FMT from lean donors to male recipients with metabolic syndrome after 6 weeks:

- Improved insulin sensitivity
- Increased levels of intestinal butyrate-producing bacteria
- Increased gut microbiota volume
- No changes in diet composition, REE or counter-regulatory hormones

FMT in Diabetic recipients from Lean Donors after 9 weeks:

- Increased insulin sensitivity
- Increase fecal microbial diversity
- Decreased fecal SCFA
What can we do now?
WE ARE THE 99%

RESPECT THE MICROBIOME!!!
Future Directions

Improved understanding of gut microbiota and metabolome

Involvement in energy homeostasis and appetite regulation

Novel metabolite or protein-specific-targeted therapeutics, prebiotics, probiotics and fecal microbiota transplantation
QUESTIONS?

WHERE DO YOU GET YOUR PROTEIN?