The microbiome—the population of bacteria, viruses, fungi and archaea that live in our gut and on our skin—is a complex ecosystem with a high degree of inter-individual variability. It provides benefits to the host, including nutrient harvest from food and protection against pathogens. The microbiome is dynamically regulated by both genes and environment, and in turn, critically influences both physiology and lifelong health.

Characteristics of the microbiome, such as diversity, have been correlated to environmental factors, including diet and medications; metabolic functions; and immune system activities. Relationships in some cases appear to be associated with type 1 and type 2 diabetes and obesity. For example, the composition of gut microorganisms in animals and humans with obesity or diabetes is distinct from those who are lean. The composition also appears to be different between children who develop type 1 diabetes and those who do not. In addition, patients who have undergone bariatric surgery experience dramatic changes in the composition of their microbes, which may be either influence, or be associated, with the improved metabolism and blood glucose control that is often observed following the procedure. These differences suggest that something about obesity and diabetes may alter the microbiome, or, alternatively, that microbiome composition may predispose individuals to these diseases.

While there appears to be a strong association between the composition of the microbiome and changes in the host’s metabolism, the mechanisms behind these changes remain relatively unclear. It has been hypothesized that the microbiome may function by influencing fatty acid or carbohydrate metabolism, gut hormone concentrations, or inflammation; however, there are not enough data at present to propose a unifying model for these relationships. Once clear mechanisms for how these microorganisms exert their influence are understood, it may be possible to utilize this knowledge to intentionally change human metabolism. Importantly, many of the approaches that could potentially be derived from an understanding of the microbiome, such as probiotics and nutritional therapies, are relatively inexpensive and may be readily accessible to broad populations.

This presentation will summarize the current understanding of the microbiome as it relates to diabetes, highlighting the relationship between the microbiome and metabolism and key recommendations for pivotal research questions, as well as resource and policy needs to address these questions.

References:


The Microbiome and Diabetes:
ADA/JDRF Symposium Summary

Robert E. Ratner, MD
Chief Scientific and Medical Officer
American Diabetes Association

- 100 trillion microorganisms: 10 times the number of human cells in our body (Savage 1977)
- Predominantly not yet cultured to date (~70% of dominant species)
- Central to Food-Microbiota-Host interactions (microbiome and human genome crosstalks in immune, neural and endocrine functions)
- Mutualistic association derived from a long co-evolution
- The microbiome can be modulated (unlike the human genome)

The human intestinal microbiota

- pH dictates bacterial survival and gut microbiota composition
- Reference gene catalogs:
  - 3.3 million bacterial genes/124 subjects
  - 10 million bacterial genes/1267 subjects
  - Li et al., Nature Biotech 2014
  - 500,000 bacterial genes/individual
- Comparable gene catalog for Europeans, Americans, Japanese, Chinese
- 50% bacterial genes of each microbiome shared by >50% individuals: metagenomic core

Human microbiomes differ by gut bacterial genes, species and ecology

- Qin et al., Nature 2010
- Arumugam et al., Nature 2011
- Reference gene catalog:
  - 3.3 million bacterial genes/124 subjects
  - 10 million bacterial genes/1267 subjects
  - Li et al., Nature Biotech 2014
  - 500,000 bacterial genes/individual

- 57 common species
- Human microbiomes differ by gut bacterial genes, species and ecology
- Density plots for ~400 individuals - ecological landscape -
Human microbiomes differ by bacterial gene counts

Low gene count (low bacterial richness) individuals (23%) have less healthy metabolic & inflammatory traits.

Low gene count is a predictor of relapse rate in IBD, aggravation in chronic conditions, non-response to a calorie-restricted diet in obesity.

*Le Chatelier, Nature 2013* ; *Cotillard, Nature 2013*

**Microbiome**: a source of biomarkers for stratification and monitoring

Type 1 Diabetes Is Accelerating at a Rate that Appears Tied to the Environment (Versus Genetics)

*Atkinson, Michels, Eisenbarth (Lancet, 2014)*

Type 1 Diabetes Is Increasing in Populations That Do Not Carry Classic High Risk Genes

*Steck, Diabetes, 2011*

Studies of Type 1 Diabetes Using "Biobreeding" (BB) BB-DP and BB-DR Rats

BB-DP rats - spontaneously develop diabetes at about 70 days of age, ~80%. BB-DR rats - a similar genetic strain (~<1% T1D), that requires extra stimuli such as viral infection to induce diabetes.

A Single Bacteria (Lactobacillus johnsonii N6.2) Modulates Type 1 Diabetes in BB Rats

Microbiome analysis (16S RNA) suggests Lactobacillus johnsonii present in BBDR rats while infrequent or absent in BBDP rats; Lactobacillus Reuteri the opposite.

*Valladares, Plos One, 2011*
Bacterial Diversity is Higher in Healthy Children than Those Who Develop Type 1 Diabetes

Giongo, ISME J, 2010

In DIPP, The Bacteroidetes:Firmicutes Ratio Differs in Early Life as a Function of Disease Development

Giongo et al, 2010, ISME J.

In DIPP, Specific Bacteria Positively Correlated with Autoimmunity

Giongo et al. 2010, ISME J.

In DIPP, Specific Bacteria Negatively Correlated with Autoimmunity

Giongo et al. 2010, ISME J.

Increased Antibiotic Use is One Potential Contributor...


Mode of Delivery


One of many examples of an activity that has "changed" over this time period
Fecal microbiota and obesity/type 2 diabetes mellitus; associations!

ARTICLE

A metagenome-wide association study of gut microbiota in type 2 diabetes

Le Chatelier, Nature 2012

Richness of human gut microbiome correlates with metabolic markers

Karlsson, Nature 2013

Gut microbiome from twins discards for obesity moderate metabolism in mice


Diversity: Differential Effects of Weight and Weight Loss

154 humans


Weight loss vs. macronutrient effects on microbiome


Humans: Weight Loss vs. Gain (12 lean, 9 obese, males)

Jumpertz et al, AJCN, 2011

Bacterial Dynamics and Disease Association


Transfer of “obese” microbiota & signalling

Duca FA, Covasa M. Diabetes 2014

Obese-prone rats have differing microbiota compared to obese-resistant rats when both maintained on the same high-fat diet.

Transfer of microbiota to germ-free mice replicated the obese phenotype and changes in metabolic signaling pathways of the intestine, adipose tissue, liver, and CNS.

-Obesity = altered gut microbiota: not solely a function of obesogenic diet
- altered symbiosis => altered peripheral and central molecular signalling machinery responsible for regulating energy metabolism, intestinal nutrient sensing, and inflammation
Mechanisms of Microbiota Regulation of Body Fat

- Accepted hypothesis: Enhanced harvest of ingested energy
- Support: Intestinal bacteria, unlike their host, have the capacity to metabolize complex plant polysaccharides to generate absorbable, energy-rich short chain fatty acids (SCFAs)
- Challenges to this model:
  - “Lean” microbiota often capable of generating more SCFAs
  - “Lean” microbiota not associated with greater loss of calories in stool
  - Enhanced calorie ingestion does not induce substantially increased fat deposition
  - Increased weight gain and adiposity after colonization with “obese” microbiota not associated with increased gross or net calorie ingestion
- Alternative model: Microbiota-stimulated signaling influences host regulation of energy balance
  - Appetite and food intake
  - Energy expenditure

Cross-talks between gut microbes and host?

What? Why? How?

Gut microbiota derived compounds acting as a triggering factors?

Bacterial LPS

Metabolic endotoxemia

Akkermansia muciniphila

Controls gut barrier function

- Mucus layer thickness
- Antimicrobial peptide Reg3g
- Specific bioactive lipids (2-OG, 2-AG, 2-PG)

Metabolic endotoxemia

- Insulin sensitivity
- Glucose production
- Oxygenation
- Fat mass
- Inflammation

Plasma cholesterol

Adapted from Cani et al. Diabetes 2007

Inflammation

Diabetes

Obesity

CVD’s

Gut Microbiota triggers Metabolic Endotoxemia and Metabolic Inflammation

Adapted from Cani et al. Diabetes 2007

The intestinal microbial habitat

Cani et al. Diabetes 2007

Everard et al. PNAS 2013
SCFA production increased in low bacteroides/high firmicutes

Koch’s postulates for causality

- The microorganism must be identified/isolated from a diseased organism.
- The microorganism should be associated with disease (association/intervention).
- The cultured microorganism should reproduce phenotype when introduced into an organism (inoculation).

Ralstonia

- Phylum: Proteobacteria
- Gram negative Rod

- Species: 3 in humans R. mannitolylitica, R. pickettii and R. insidiosa
- Human infections (via drinking water: in immunocompromised subjects eg. after kidney transplantation)

Adley et al 2013, Ralston et

Ralstonia pickettii 4 weeks daily gavage on weight in male DIO mice

Ralstonia pickettii 4 weeks daily gavage on OGTT in male DIO mice

Effect prevaccination with Ralstonia pickettii on insulin resistance
Impact of Metformin on Gut Microbiome and Metabolism

Forslund K et al. Nature 528:262, 2015

Richness & diversity increase after GBP microbiota is re-structured

Kong AJCN 2013

11 bacterial taxa are significantly modulated (16S rDNA).
50% of the modulations are linked to change in calorie supply

Gastric bypass

RYGB Microbiota Not Associated with Decreased Food Intake

Liou et al., Science Transl Med 2013

Weight Loss in RYGB-R Mice Despite Increased Food Intake

Liou et al., Science Transl Med 2013

Energy Expenditure in RYGB Microbiota Recipients

Liou et al., unpublished

RYGB Alters SCFA Balance Toward Propionate

Liou et al., Science Transl Med 2013
Luminal Propionate Stimulates Energy Expenditure

Prebiotics: Affect microbiome and are affected by it

- Non-digestible carbs which are fermented (glucans, galactans, etc.) to SCFA’s.
- Low counts of some bacteria associated with that fermentation (e.g., *Bifidobacteria*) are also associated with obesity.
- Decreases adiposity in *ob/ob* and DIO mice.
- Increase PYY and GLP-1 and decrease ghrelin in mice.
- Decreases obesity in obese adults and lessens weight gain in lean adolescents.
- Decreases appetite, increases satiation


Prebiotic-induced microbiota modulation

Effects on appetite (including GLP-1, PYY), plasma lipids, steatosis, LPS, inflammation and glycemia demonstrated in humans

Pre-Fecal transplant

Fecal transplant - History

- 4th Century BC: Chinese medicine, food poisoning and diarrhea
- 16th Century AD: Li Shizhen "yellow soup", gastro-intestinal illness
- 1958: Eiseman, antibiotics-induced chronic diarrhea

Effects on appetite (including GLP-1, PYY), plasma lipids, steatosis, LPS, inflammation and glycemia demonstrated in humans

Fecal microbiota transplantation and emerging applications

- Screening donors (blood donation protocol):
  - Questionnaire (bowel habits, travel history, medication, etc.)
  - Screening feces + Bloodborn viruses (Hepatitis, HIV, HTLV, CMV, EBV)

De Vrieze, Science 2013
In Summary

- Increased gut microbial diversity is healthy!
- Altered gut microbiota are associated with both autoimmunity (type 1) and obesity (type 2)
- Identification of specific pathogenic or protective organisms is in its infancy
- Passive (fecal transplant) and active (RYGB and prebiotics) microbiome modification alters autoimmunity, energy metabolism and weight
- Controlled trials meeting Koch’s Postulates are required to prove causation

Effects of fecal transplantations in clostridium difficile diarrhoea

Effect donor faeces on periferal insulin sensitivity

How can we achieve a deeper understanding of the therapeutic potential of the gut microbiome: fecal microbial transplant (FMT)

- at AMC >250 FMT’s since 2006, predominantly in RCT due to large placebo effect. Long term (S)AE not observed yet (registry)
- At AMC ongoing/finished RCT’s with single/ multiple FMT using accepted clinical endpoints for:
  - IBD (Colitis ulcerosa, TURN trial)
  - vascular inflammation
  - insulin resistance/Dm2
  - NAFLD/NASH
  - Type 1 diabetes
  - VRE/ESBL
- Causality to find involved bacteria and unravel important metabolites

The Microbiota and Drug Therapy

Metabolites

- SCFAs
- Aromatic metabolites
- Secondary bile acids

Receptors