Microbiome (The Second Human Genome)

What Shapes it and How it Shapes Us

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Harvard Medical School

Editor in Chief

Hepatology Section Up to Date

UCLA Microbiome Center

My giving this talk reminds me of the British Idiom…

Carrying Coals to Newcastle

Disclosure Statement

The commercial entities with which I, Sanjiv Chopra, MD, MACP have relationships do not produce healthcare-related products or services relevant to the content I am planning, developing, or presenting for this activity.

Definition

The concept of the human microbiome was first suggested by Joshua Lederberg, who coined the term “microbiome, to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space”.

Microbiome = the community of microorganisms that shares our body space

Population 100 Trillion: Microbes outnumber human cells 1.1:1

Humans have 23,000 genes. Microbiome has 8 million genes.

Microbiome

"2nd human genome"

"Newly discovered organ"

"Bacterial inner rainforest"

Evolutionary Timeline

- 200 million years ago: Mammals
- 60 million years ago: Primates
- 2.5 million years ago: Genus *Homo* (including humans)

Microbes appeared **3.5 billion** years ago

Microbiomes are quite dominant

- GI tract is sterile at birth
- GI tract colonized by bacteria within a few hours
- Microbial flora established within 3-4 weeks
- More than 1000 species of microbes take up house

Weight of Microbiome

3 LBS!

What shapes our microbiome?

Birth (Vaginal Delivery vs. C-Section)

Antibiotics

Probiotics

Where we live

Travel

Diet (vegetarian, vegan, fiber, coffee etc.)

Exercise

H2 Blockers and PPIs ?

Malnutrition

Microbiome and Obesity

- Studies in mice as well as in humans show that gut microbiota differ in composition between obese and lean subjects

- Notable difference in ratio of *Bacteroidetes* and *Firmicutes*, with a decrease in *Bacteroidetes* and a corresponding increase in *Firmicutes*

Obesity: Twin Studies Shed Light

- Four pairs of identical twins. One lean and the other obese in each pair.
- Genetically identical baby mice had their guts populated with intestinal microbes from either obese women or their lean twin sister.
- Mice that received bacteria from obese twin gained weight and had more body fat. They also had a less diverse community of gut microbes.

Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice
Ridaura, VK *et al. Science*, 6 September 2013: Vol. 341 no. 6150

The Microbiome affects Obesity and T2DM

- Obesity is associated with changes in the intestinal microbiota.
- The obese microbiome seems to be more efficient in harvesting energy from the diet.
- Differences in gut microbiota composition might function as early diagnostic markers for the development of T2DM.
- Butyrate, a product of intestinal microbes, may induce beneficial metabolic effects through enhancement of mitochondrial activity, prevention of metabolic endotoxemia, and activation of intestinal gluconeogenesis.

Insights into the Role of the Microbiome in Obesity and Type 2 Diabetes. Hartstra *et al. Diabetes Care* 2015, 38(1).

Diets Modulate Gut Microbiome and Improve Insulin Sensitivity

- 20 obese men with CAD. Randomized to Mediterranean diet (Med) or low-fat, high-complex carbohydrate diet (LFHCC) for one year
- Bacterial composition and relationship with fecal and plasma metabolome evaluated
- Both diets shown to exert a predictive effect on the development of T2DM. Med diet increased *Roseburia* genus and LFHCC diet increased *F. prausnitizii*.

Haro, C *et al*. *Endocrinol Metab* 101:233-242, 2016

Small Study Demonstrating Anatomic and Functional Changes in Obese Patients

- 20 obese patients, 19 lean subjects
- Obese patients had decrease in bacterial biodiversity
- Subjects with the highest gut microbial diversity had changes in hypothalamus, hippocampus, and cordate nucleus
- Subjects with greater abundance of Actinobacteria had better motor speed and attention

Bariatric Surgery Affects Gut Microbiome Composition

- Gut microbiomes different in patients after gastric bypass surgery compared to obese controls
- E. coli, Klebsiella, Pseudomonas, all more common
- Stool from gastric bypass patients transferred to germ free mice:
  - Mice had improved fat oxidation
  - Microbe trasplanted mice gained 43% less body fat compared to mice that received stool from gastroplasty patients

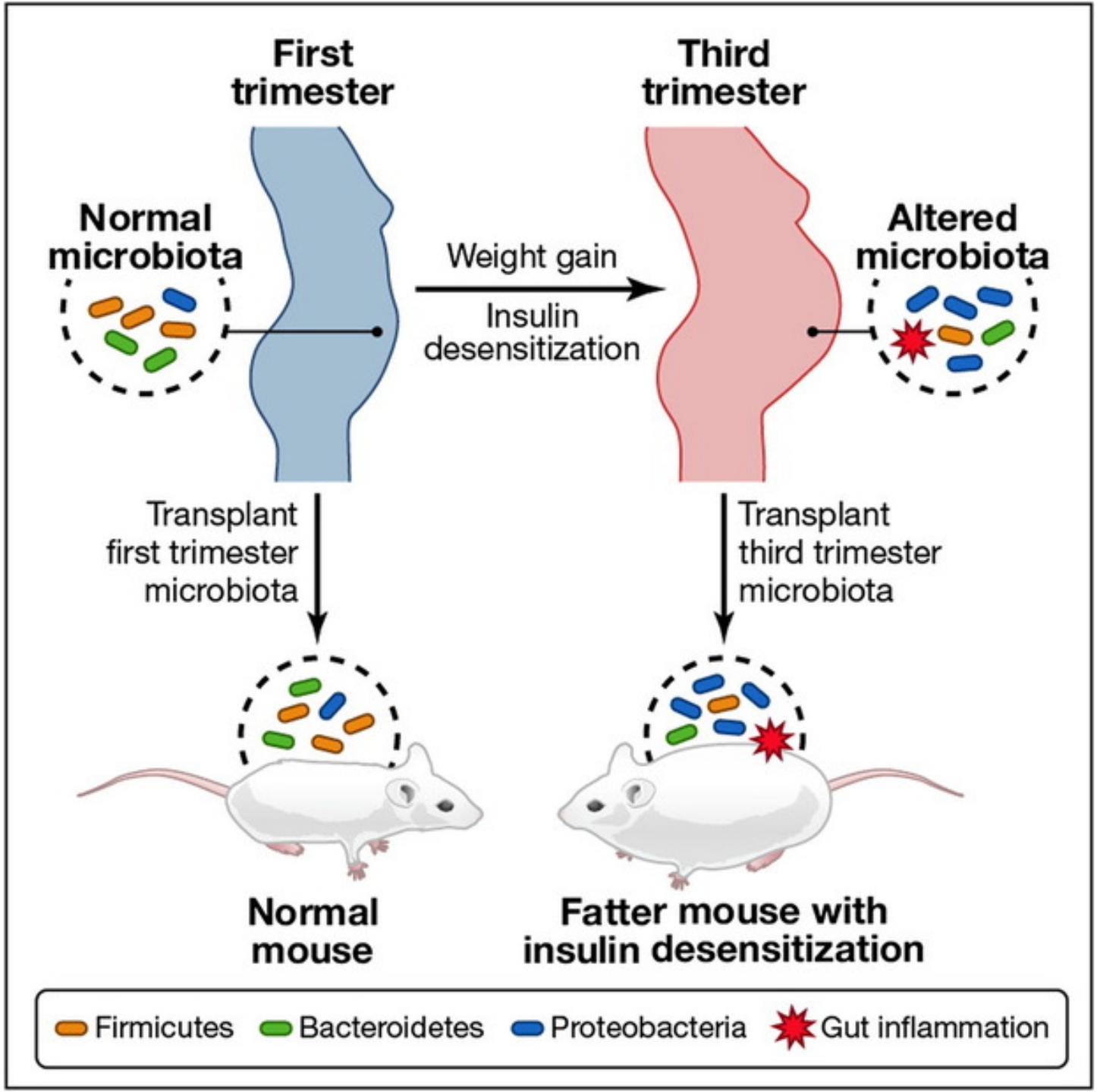
Tremaroli, V. et al. Cell Metabolism, 22, 2015.

Pregnancy, Weight Gain, and Altered Microbiota

- Gut microbiota changed dramatically from first to third trimesters, with vast expansion of diversity between mothers, an overall increase in Proteobacteria and Actinobacteria, and reduced richness.

- When transferred to germ-free mice, third trimester microbiota induced greater adiposity and insulin insensitivity compared to first trimester.

Host remodeling of the gut microbiome and metabolic changes during pregnancy
Koren *et al., Cell*, 2012



Cell

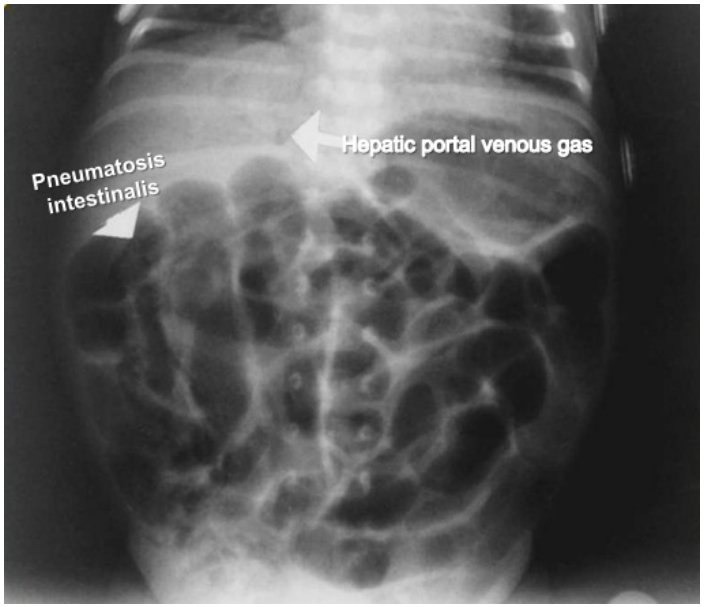
Koren et al, 2012

Long Term Infant Outcomes are Influenced by Mode of Delivery

- Children born by Cesarean section are more likely to develop:
  - Type 1 Diabetes
  - Celiac disease
  - Hospitalization for Gastroenteritis
  - Asthma
  - Allergic rhinitis

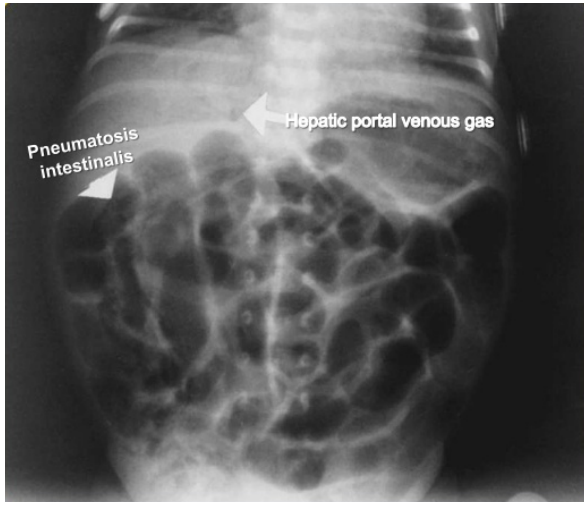
Neu, J and J Rushing. Cesarean versus Vaginal Delivery: Long term infant outcomes and the Hygiene Hypothesis. *Clin Perinatol*. 2001 June: 38(2): 321-331.

What is this condition?



Necrotizing Enterocolitis

- A study of 1000 premature newborns
- Up to 6% incidence in premature newborns
- 36% mortality rate



Gut Microbes Linked to Necrotizing Enterocolitis in Premature Newborns

- Premature newborns who survive the first two weeks have a much higher risk of dying from Necrotizing Enterocolitis
- Gut microbiota in these newborns are different - more Gram negative, less anaerobes
- This dysbiosis observed before any clinical event
- Potential window for intervention

Warner, B.B. *et al*. Gut bacterial dysbiosis and necrotizing enterocolitis in very low birth weight infants: a prospective case control study. Lancet, March 2016.

Is there a Gut-Brain connection?

- The immune system is active in myelin destruction.
- Alteration in gut flora in mice leads to a MS-like disease, called Experimental Autoimmune Encephalomyelitis (EAE)
- Preliminary work suggests that the gut in MS patients contained bugs that drive inflammation and are low in the types of the bacteria that control inflammation.*

*Consistent with work in IBD and RA

An Important Reference

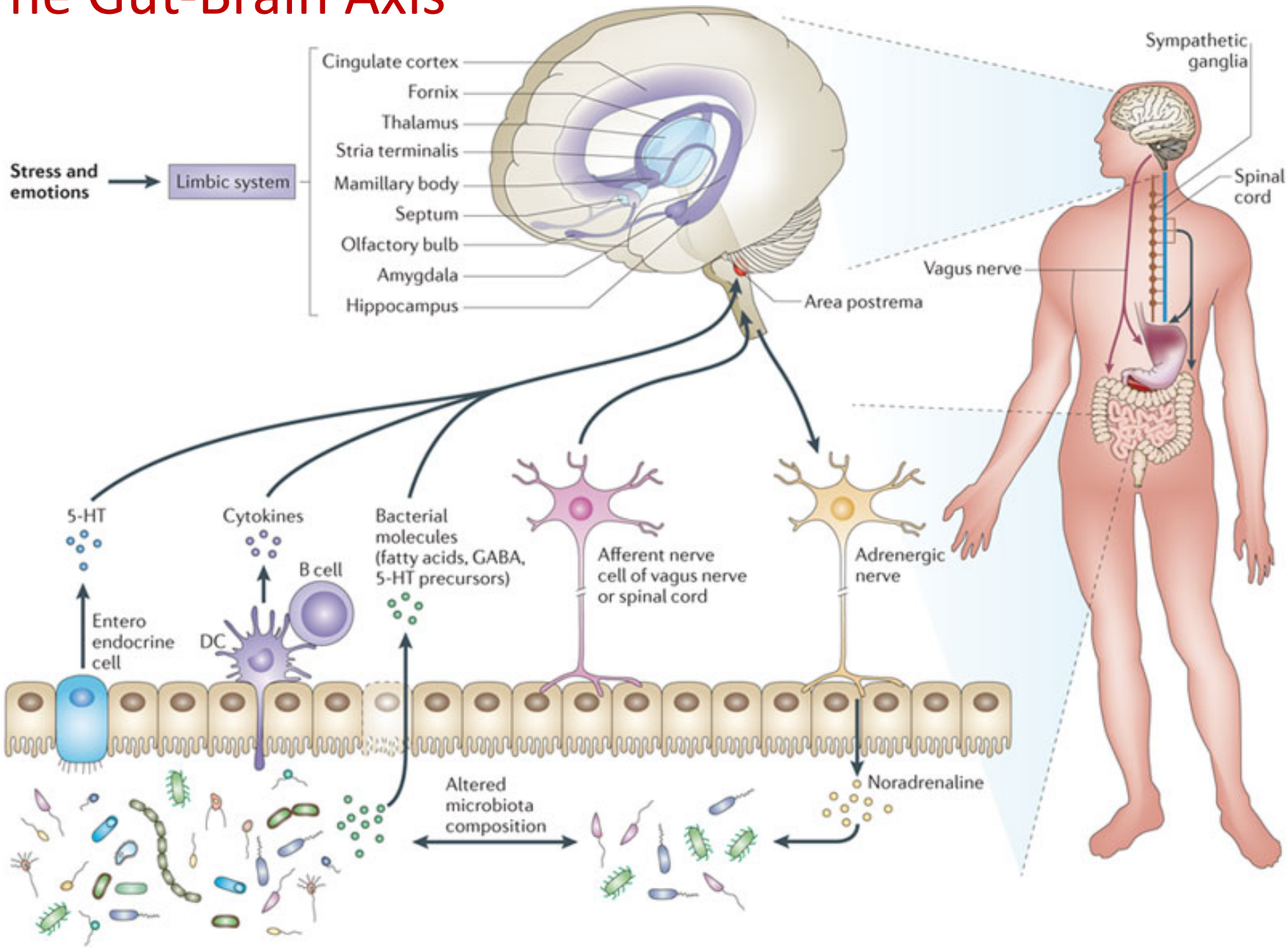
Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Bravo *et al., PNAS*, 2011, 108(38).

There is cross-talk between Gut Microbiota and Brain Function

- In germ free mice, restraint leads to exaggerated ACTH response.
- Above response partially reversed by colonization with fecal material from controls.
- Normal mice subjected to psychosocial stress have a decrease in Bacteroides and increase in Clostridia in the cecum.
- In humans, probiotics may modulate brain activity.
- Bariatic surgery followed by profound changes in microbiota and improvement in memory.

The Gut-Brain Axis



Conclusions

The human Gut Microbiota profile is significantly associated with Brain microstructure and function.

Fernandez-Real, JM *et al*., Gut Microbiota Interacts with Brain Microstructure and Function. J Clin Endocrinol Metab 100: 4505-4513, 2015.

Do gut microbes influence severity of **stroke**?

- Two colonies of mice:
  - Group A: gut bacteria resistant to antibiotics
  - Group B: gut bacteria vulnerable to antibiotics
- Cerebral arteries of the mice occluded, resulting in ischemic stroke
- Brain damage 60% smaller in Group B mice

Do gut microbes influence severity of **stroke**?

- Fecal transplant from mice that had reduced brain damage given to naïve mice who now developed an altered gut microbiome (were not given antibiotics)
- Cerebral arteries occluded, leading to ischemic stroke – again, significant protection against brain damage seen
- Composition of immune cells – "good" regulatory T cells and "bad" Gamma Delta T Cells altered
- "These cells determine what kind of inflammatory immune response the brain experiences after a stroke."

Constantino Iadecola

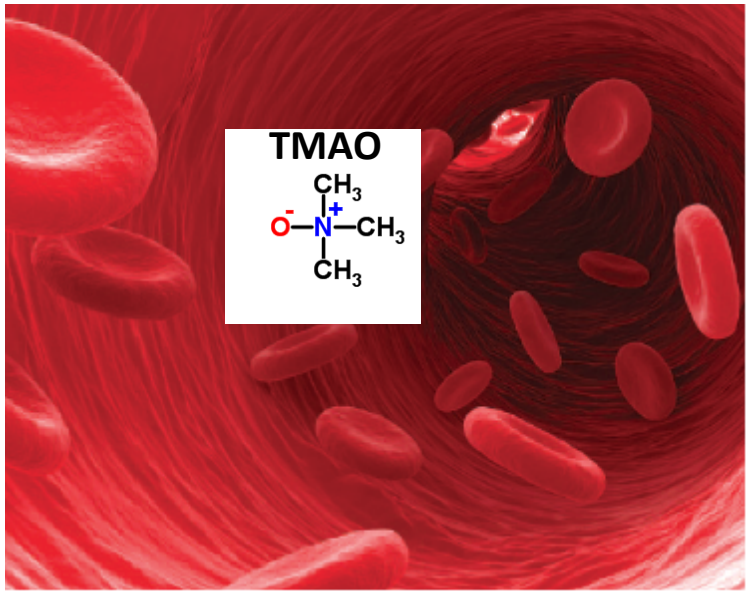
Benakis, C. *et al*. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat. Med. 2016.

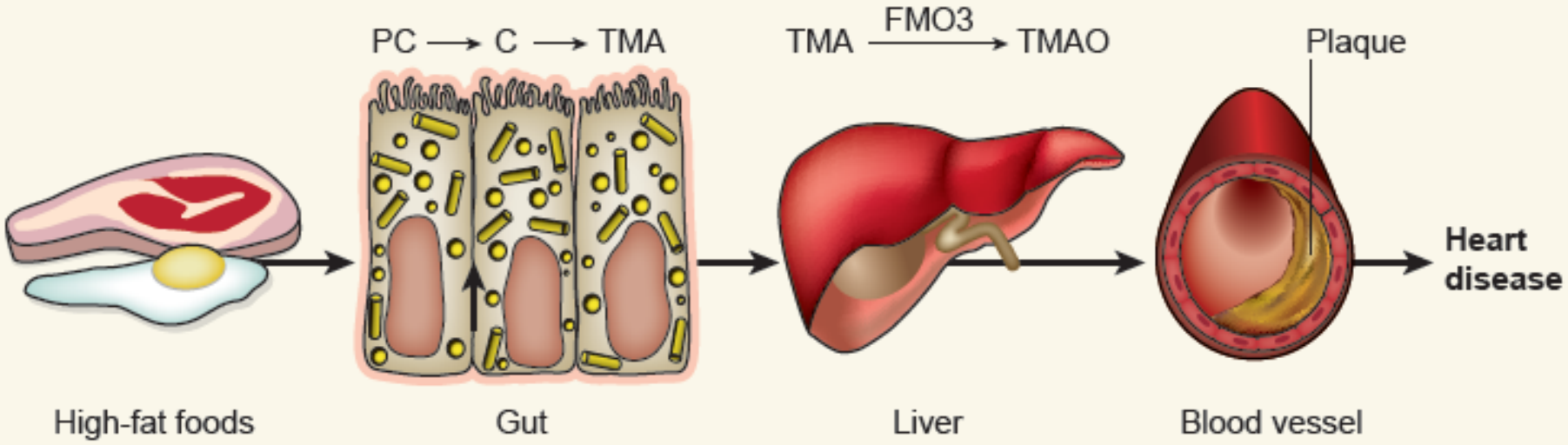
Caveat: This is a study in **mice**.

nature

The diet–microbe morbid union

Tri-
Methyl-
Amine N-
Oxide

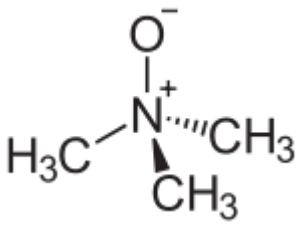


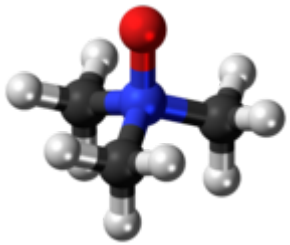


Nature, 2011

TMAO, Microbiome, and CAD/Stroke!

- TMAO is produced when intestinal bacteria digest the nutrient Lecithin.
- Human subjects, after eating two hard-boiled eggs and a capsule of labelled Lecithin, have an increase in TMAO levels.
- However, when subjects are given broad-spectrum antibiotics, their TMAO levels are suppressed.
- High TMAO blood levels are associated with higher risk of heart attack or stroke, independent of other risk factors and other blood test results.





TMAO Important Predictor of Atherosclerosis

- Dietary meat is a major source of TMAO in humans
- TMAO levels independent risk factor for atherosclerosis in humans
- In Apo E deficient mice, TMAO levels correlate with atheroma burden
- When Apo E deficient mice are treated with antibiotics, there is a significant decrease in atheroma burden

Popular Diets have different effects on TMAO

- A high fat (Atkins) diet for 4 weeks was associated with high levels of TMAO.
- A low fat (Ornish) diet for 4 weeks was associated with low levels of TMAO.

Park JE. Et al. Elsevier 29. May 2019.

Diet is destiny!

Fate is shaped by
genome and microbiome.

An Important Reference

Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk.
Wilson Tang *et al*., *NEJM* 2013, 368.

Is this person trying to lose weight?

430 calories

510 calories

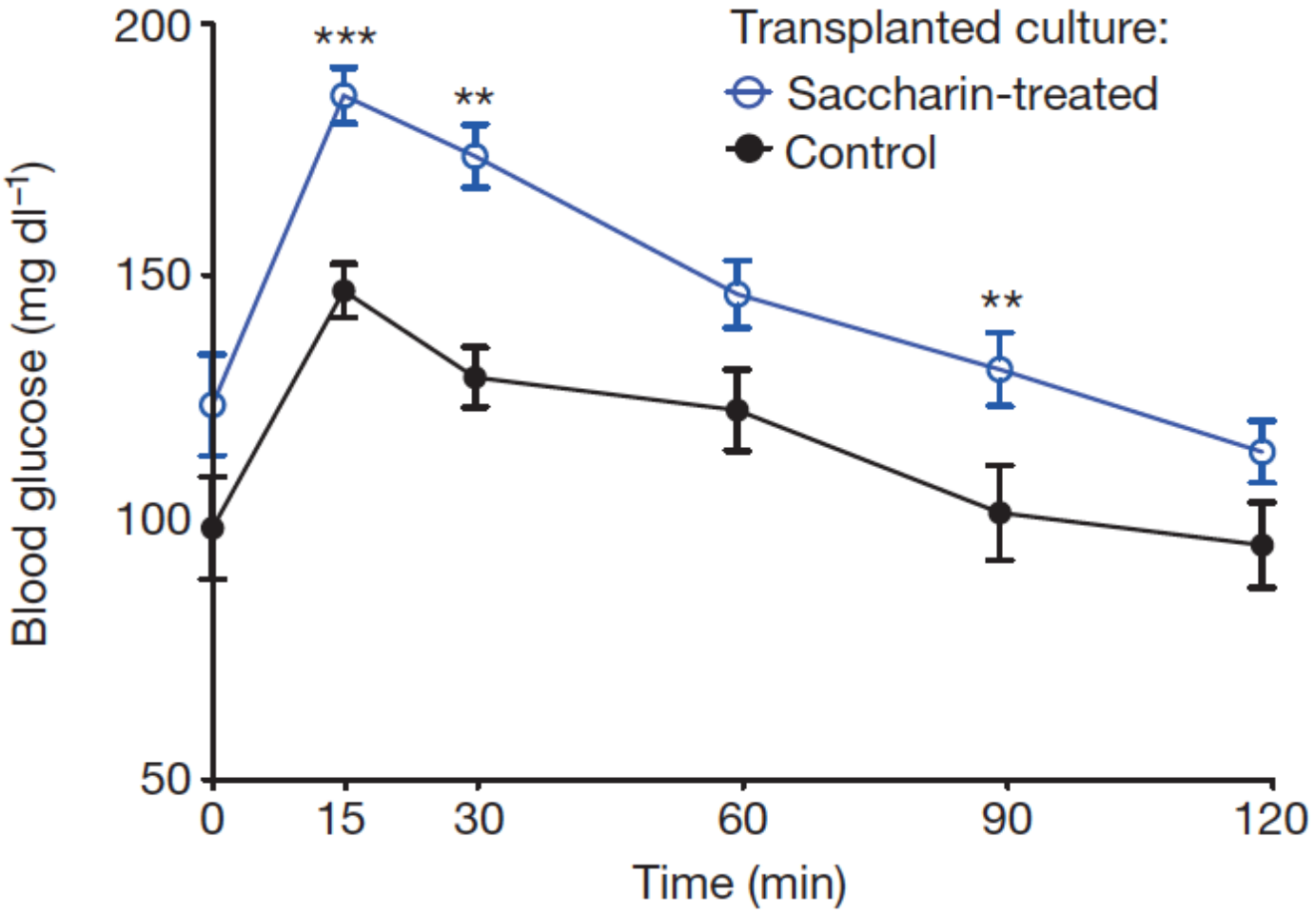
500 calories

Total: 1440 calories

New total: 1441 calories!

Artificial Sweeteners induce glucose intolerance by altering the gut microbiota

Suez, J. *et al*. *Nature*, 2014.



Preliminary work shows…

- Vegan diet
- High fiber diet
- Highly fermented foods (Kimchi)
- Breast milk

All have a favorable influence on the composition of the gut microbiome.

Gut Microbiota and Autism Spectrum Disorder

- Pregnant mice injected with artificially created virus-like DNA
- Offspring display less socialization, greater sense of being startled by sounds, fewer vocalizations
- Serum of ASD mice contain more than 45x the amount of 4-ethylphenyl sulfate (4-EPS) (a metabolite of gut bacteria)

Gut Microbiota and Autism Spectrum Disorder

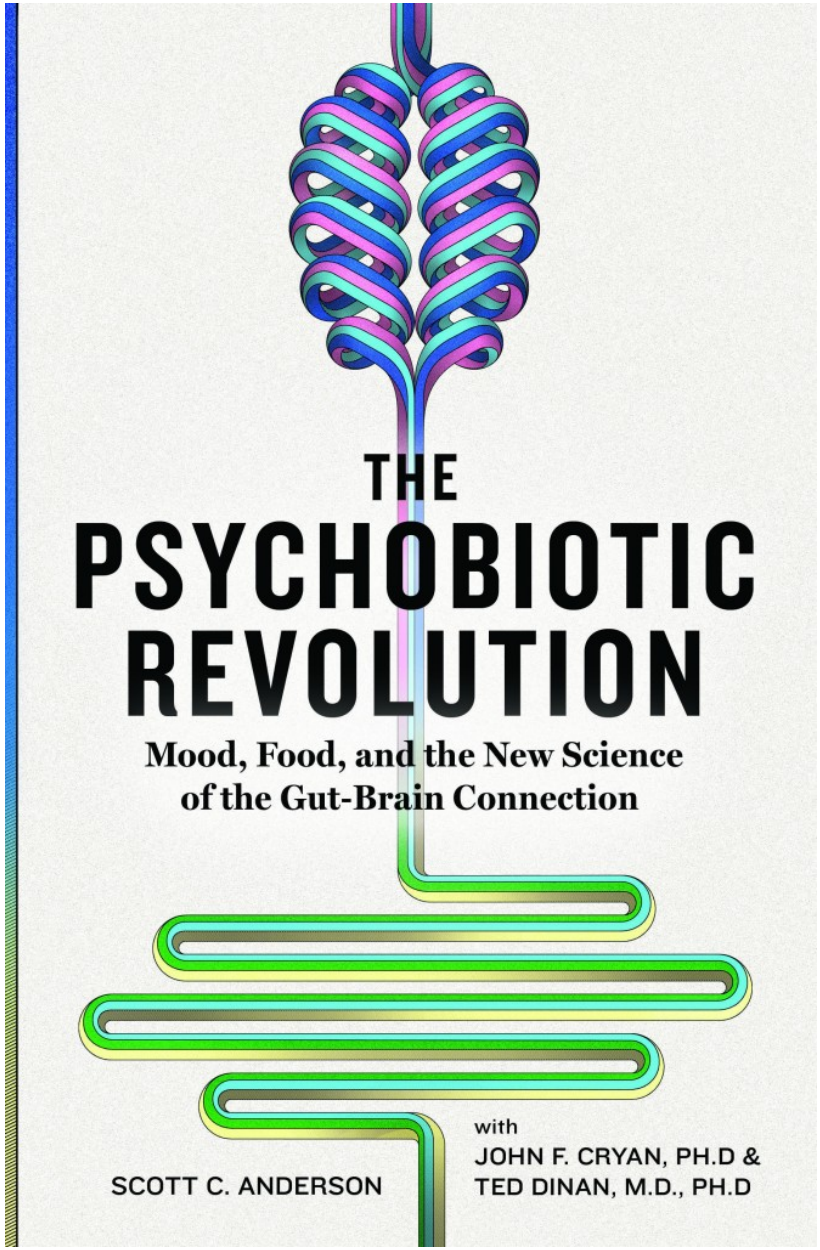
- Children with ASD have high concentrations of a similar compound p-cresol in their urine
- Healthy mice injected with 4-EPS have a leaky gut and display ASD symptoms
- Probiotic treatment with *B. fragilis* in ASD mice restored intestinal permeability and 4-EPS levels returned to normal

Hsiao, E.Y. *et al.*, Presentation at Gut Microbiota for Health. World Summit. Barcelona, Spain. 2015.

Tantalizing Study Regarding Long-Term Benefit of Microbiota Transfer Therapy on Autism Symptoms

- Follow up of 18 participants two years after Rx
- Most Improvements in GI Sx maintained
- Autism related Sx improved even more
- Changes in Gut Microbiota were maintained two years later
- A randomized placebo-controlled trial is warranted

Kang D-W. et al. Nature.com/Scientific Reports. April 2019.


THE
PSYCHOBIOTIC
REVOLUTION
Mood, Food, and the New Science
of the Gut-Brain Connection
SCOTT C. ANDERSON
with
JOHN F. CRYAN, PH.D &
TED DINAN, M.D., PH.D

Gut Microbes Are Essential

- Not free-loaders
- Digest food
- Produce anti-inflammatory chemicals and compounds
- Guide the Immune System to distinguish friend from foe

Basic Definitions

- **Prebiotics** are selectively fermented products that confer changes in the composition and/or activity of the GI tract microflora and confer health benefits

- **Probiotics** are ingested microorganisms that are associated with health benefits

Global sales of probiotics exceeded
$40 Billion in 2018
Estimated to exceed **$64 Billion** in 2020

Challenges Regarding Probiotics

- Standardization
- Cost
- Novel ways of delivery*

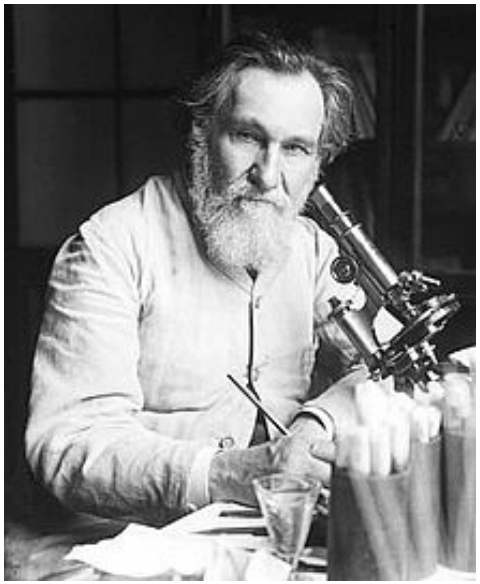
*Bacteria and chocolate: a successful combination for probiotic delivery.

Possemiers S. et al. Int J Food Microbiol. 2010.

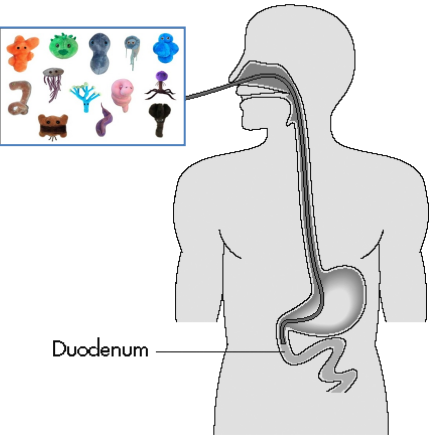
More than a Century ago…

"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes."

Elie Metchnikoff (1907)
Nobel Laureate



Therapeutic Implications



The NEW ENGLAND JOURNAL of MEDICINE

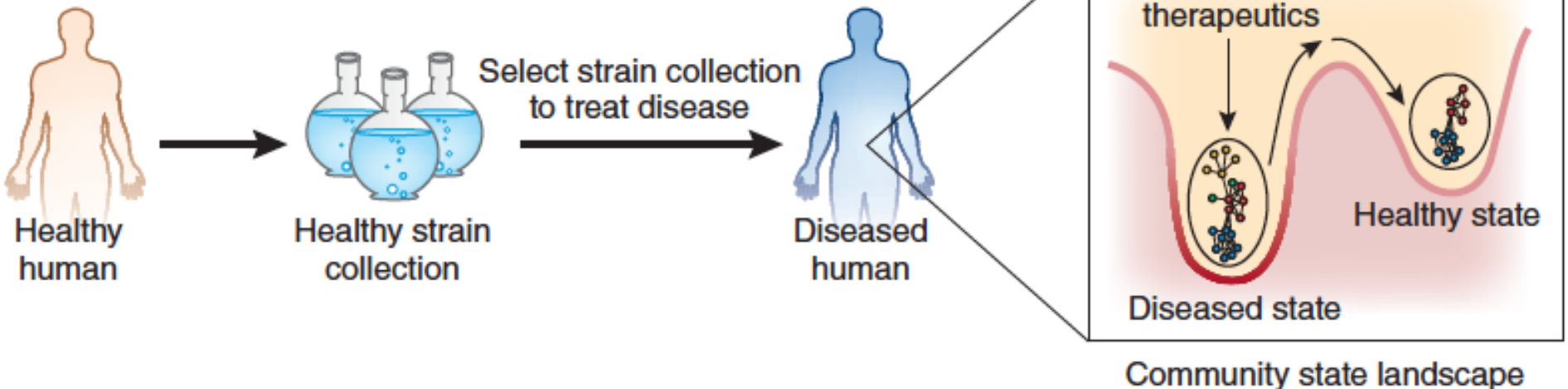
ESTABLISHED IN 1812 JANUARY 31, 2013 VOL. 368 NO. 5

Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

Restoration of the gut microbial habitat as a disease therapy

David A Relman



Frozen Poopsicle!

- 14 of 20 with recurrent *C. diff* diarrhea resolved
- 4 of 6 non-responders' diarrhea resolved with retreatment
- No adverse effects noted in this small study

Oral, Capsulized, Frozen Fecal Microbiota Transplantation for Relapsing *Clostridium difficile* Infection. Youngster *et al., JAMA,* 2014, 312(17).

Probiotics Beneficial in Hepatic Encephalopathy (HE)

- Daily intake of a probiotic, VSL #3, over 6 months significantly reduced the risk of hospitalization for HE in cirrhotics

- Patients given VSL #3 had improvement in Childs Pugh and MELD scores

R.K. Dhiman *et al*., Gastroenterology 2014; 147: 1327-1337.

Probiotics Taken During Pregnancy Influence Maternal and Child Outcomes

- Probiotic Milk Consumption during early pregnancy reduce the risk of premature birth

- Probiotic Milk Consumption during late pregnancy reduced the risk of pre-eclampsia

Anna-Karin Wikstrom et al.

BMJ Open 2018

Bacterial Baptism!

- Sharp rise in rate of C-Section in recent years.
- Accompanying increase of Asthma, Allergies, Eczema, Obesity etc. in offspring.
- Is the Microbiome to blame?
- In addition to different Microbiome with C-Section other factors may play a role…Mother's who get C-Section receive anti-biotics, greater prevalence of obesity and lower rates of breast milk feeding.
- FDA approved studies being conducted in Virginia and New York City.

Link between Microbiome and Longevity

The Fruit Fly remarkably similar to mammals in terms of biochemical pathways.

Fruit Flies fed with a synbiotic (probiotics combined with a herbal supplement)

Had reduced inflammation, oxidative stress and a 60% increase in Longevity.

Probiotics dramatically change gut microbiota – composition and in respect to how food is metabolized.

Susan Westfall et al. Longevity extension in Drosphilia through gut brain communication. *Scientific Reports, 2018.*

OpenBiome

OpenBiome is a nonprofit stool bank. Rigorously tested stool preparations are provided to clinicians and researchers for fecal microbiota transplantation.

https://www.openbiome.org/

Shipped **30,331** FMT units.

To a network of **985** hospitals (2017)

Let's Summarize

1. Microbes appeared 3.5 billion years ago.

2. There's a cross talk! They've been doing it for billions of years. Yet to be silent!

3. Gut microbiota affect:

Obesity	Diabetes
Cardiovascular Disease	Colon Cancer
Neurological Disease	IBD
Arthritis	IBS
Necrotizing Enterocolitis	Pregnancy Outcomes

Longevity and more…

Let's Summarize (continued)

4. Composition of the microbiota is in flux and altered by a diverse array of factors

5. Microbiota-directed therapies include:
   - *Fecal transplants* (*C. difficile* colitis)
   - Hepatic Encephalopathy
   - And more…

Have you heard of Biological Dark Matter?

- It is uncategorized genetic material found in humans that does not fall under the three existing domains of life: bacteria, archaea, and eukaryotes.
- It may well be a fourth domain of life yet to be discovered.
- Biological dark matter accounts for:
  - 40-50% of the genetic material in the human gut
  - 20% of the genetic material in the nose
  - 1% of genetic material in sterile blood

Worth listening to…

“What’s left to explore?”

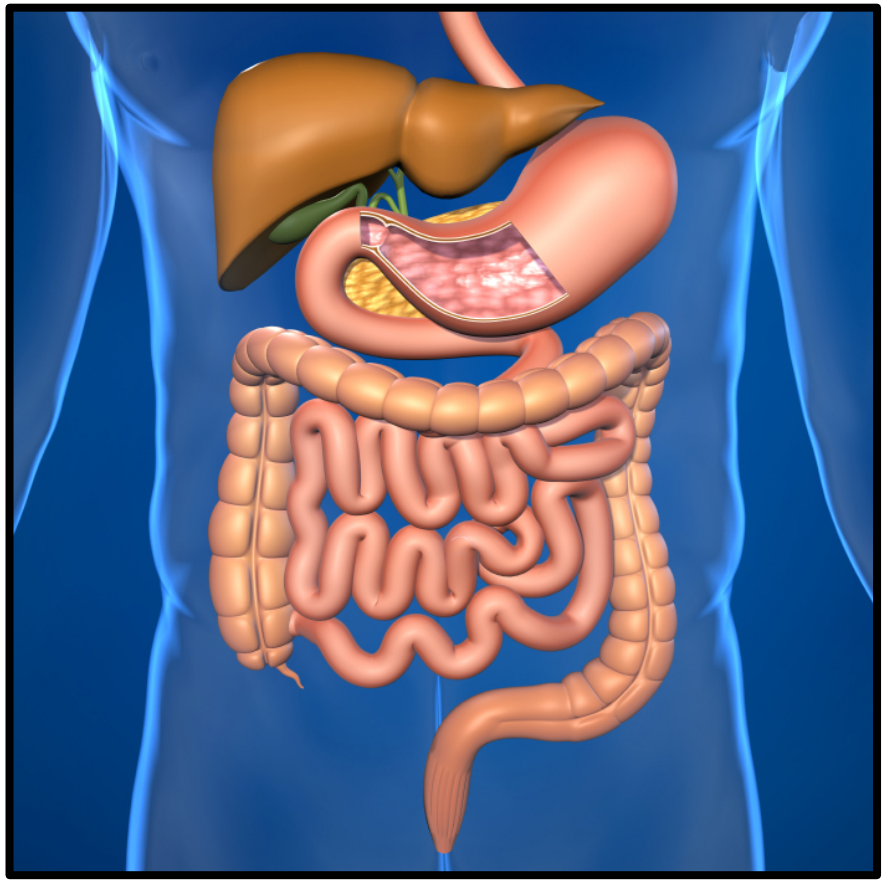
Nathan Wolfe, biologist and explorer gave a terrific TED talk in 2015 about biological dark matter.

http://www.ted.com/talks/nathan_wolfe_what_s_left_to_explore

An African Proverb

We have only seen the head of the hippo.

Reserve Slides to Follow



Welcome to Las Vegas

Population: 656,000

Welcome to Gut

Population: 100 trillion

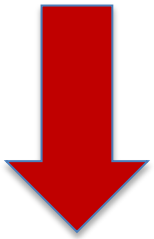
Gut bacteria must resist removal

- 1-3 billion cells per hour are shed by the small intestine. 100-300 million cells per hour are shed by the colon. (Xu J. Gordon, JI, PNAS 2003. 100.)

- Entrenched “resident” bacteria are able to establish themselves by embedding in biofilms. (Sonnenburg, JL, Angenent, LT, Gordon, JI. Nature Immunology 2004. 5.)

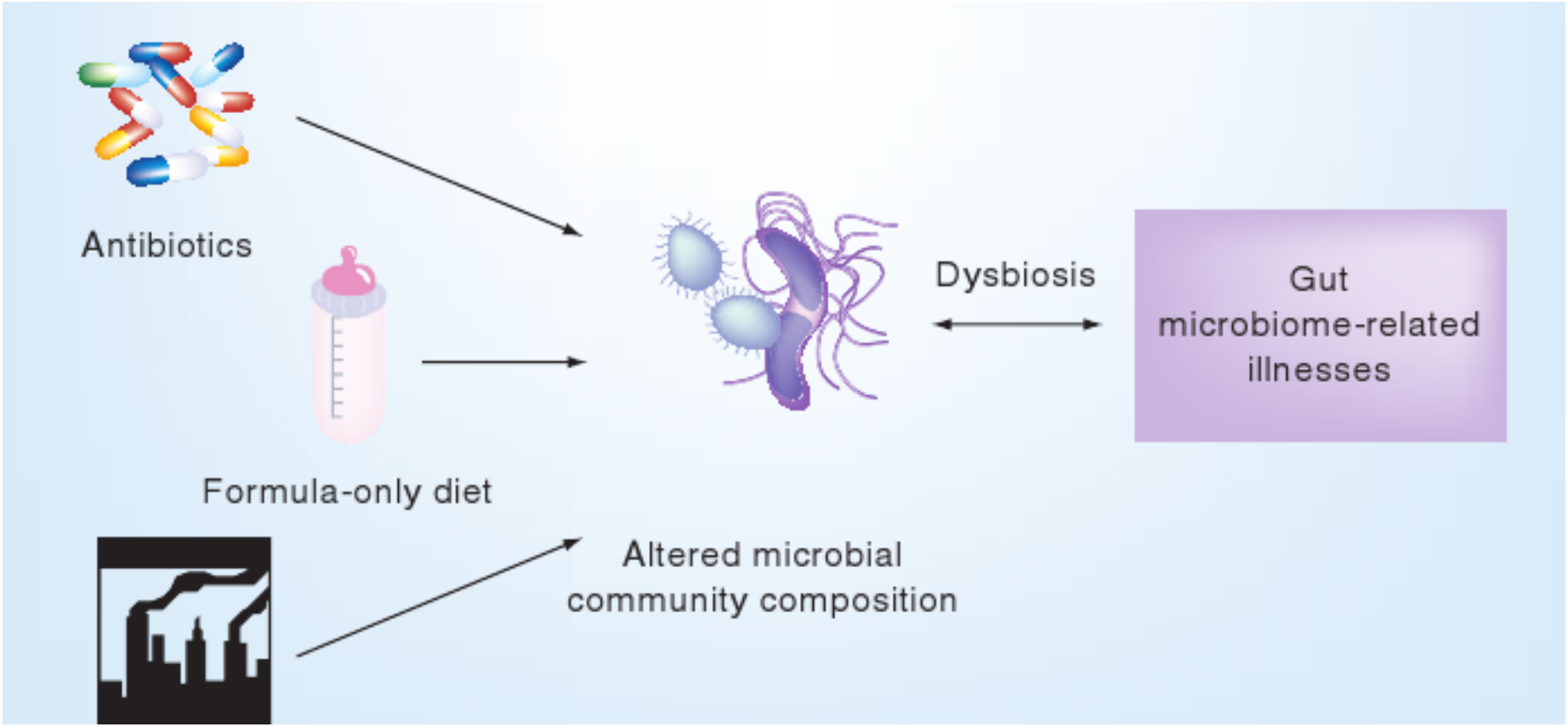
High through-put “omic” technologies used to *characterize*

- DNA (genomics)
- RNA (transcriptomics)
- Small molecules (metabolomics)



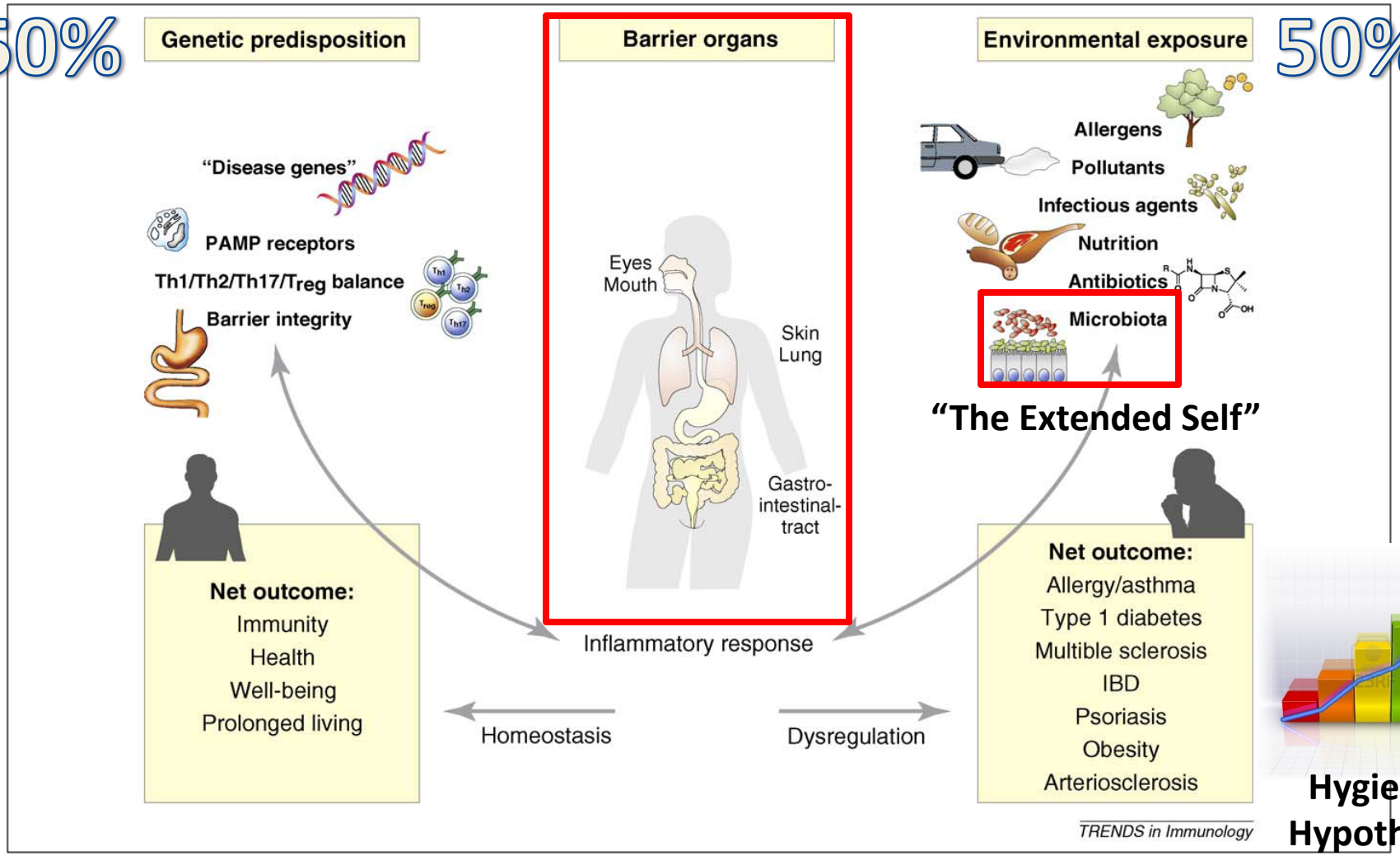
- Proportional abundance of various microbes in a bio-specimen and inference of their functions

“Western” Influence on Microbiome



Expert Review
Anti Infect Ther 2010

The Role of Mucosal Barriers in Disease



Hygiene Hypothesis

Ehlers et al, Trends in Immunology 2010

A Seminal Article and a Thoughtful Editorial

Review Article:

The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases

JF Bach, M.D., D.Sc

NEJM 347(12), 2002

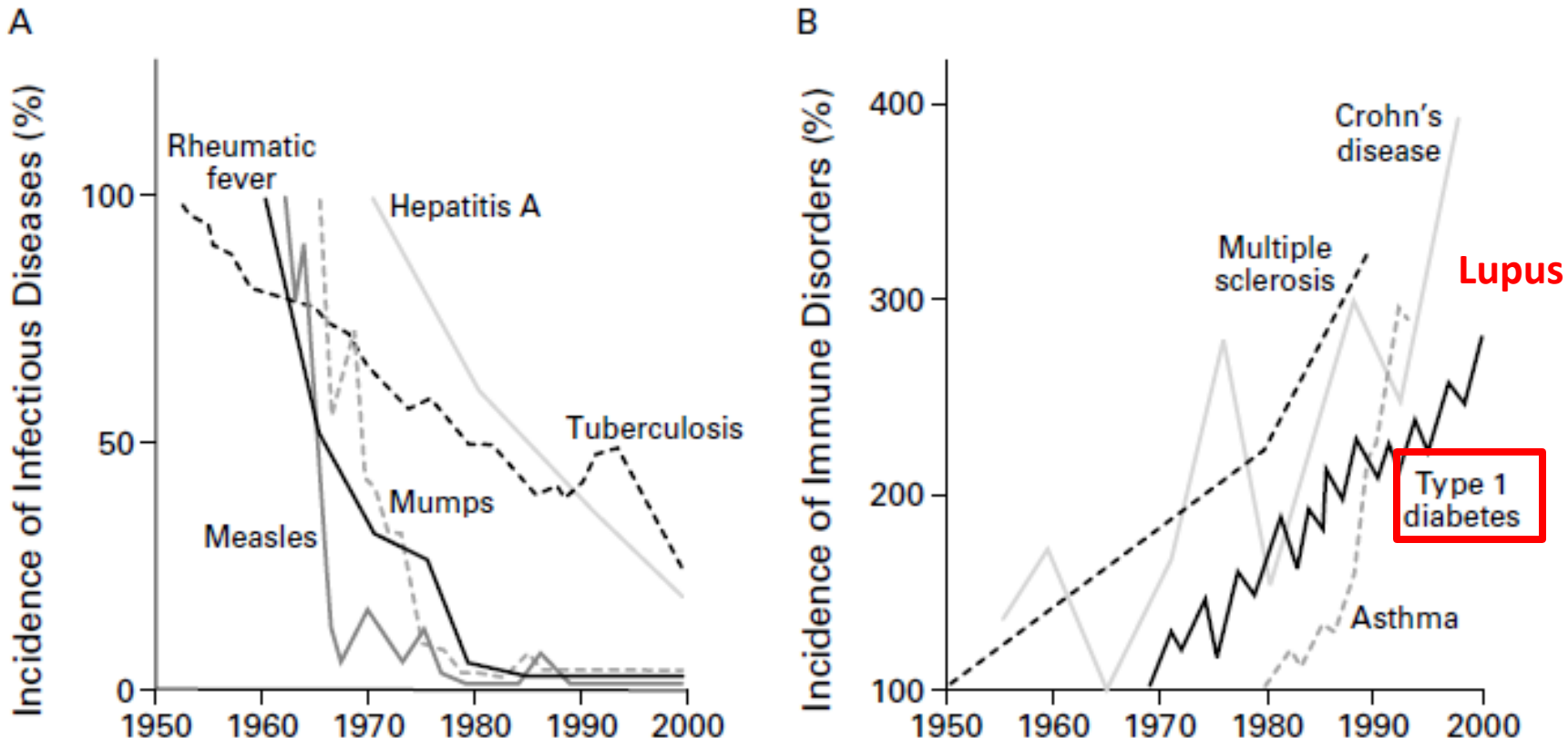
Editorial:

Eat Dirt – The Hygiene Hypothesis and Allergic Diseases

ST Weiss, M.D.

NEJM 347(12), 2002

Hygiene Hypothesis



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Bach, NEJM 2002

The Four Horsemen: Death, Famine, War, Pestilence (Conquest)

Major Ancient Pandemics

- The Plague of Athens (430-427 BC)
- The Great Plague of Milan (1629-1631)
- The Third Pandemic (1855-1905)

The Third Pandemic (1855-1905)

- Began in Yunnan Province in 1855
- Spread to all inhabited continents
- Killed more than 12 million in China and India alone

Bubonic Plague or Black Death
Yesinia pestis

The Big Question

FOE

or

FRIEND?

"The *E. coli* made me do it!"

- May 2000, heavy rains pummel Walkertown, Ontario
- Epidemic of *E. coli* ensues. Half the residents get ill and seven individuals die.
- Two years later, question posed: "Since the outbreak, have you developed depression, anxiety, panic disorder, or PTSD?"
- Exposed residents more likely to answer **YES!**

Kids Get Arthritis

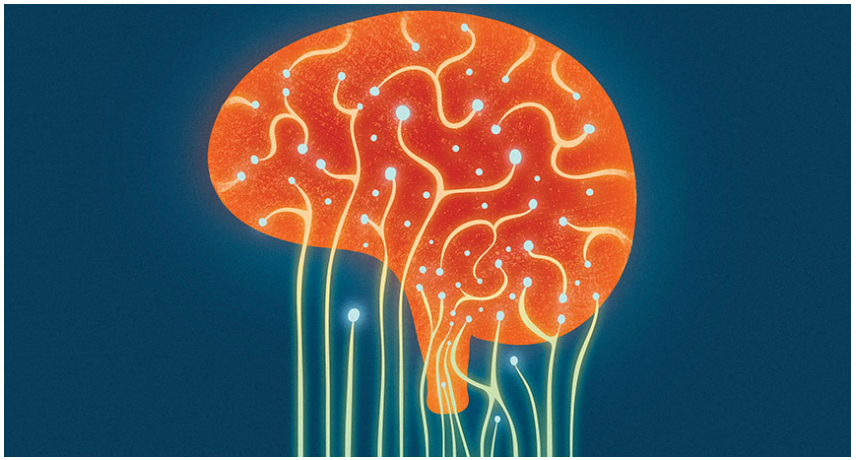
- 300,000 kids in USA have some form of arthritis - Juvenile Rheumatoid Arthritis (JRA) is most common
- Kids exposed to antibiotics have high risk of getting JRA
- Risk highest when exposed to multiple courses of antibiotics
- Risk not seen with exposure to antivirals or antifungals

"The *E. coli* made me do it!"

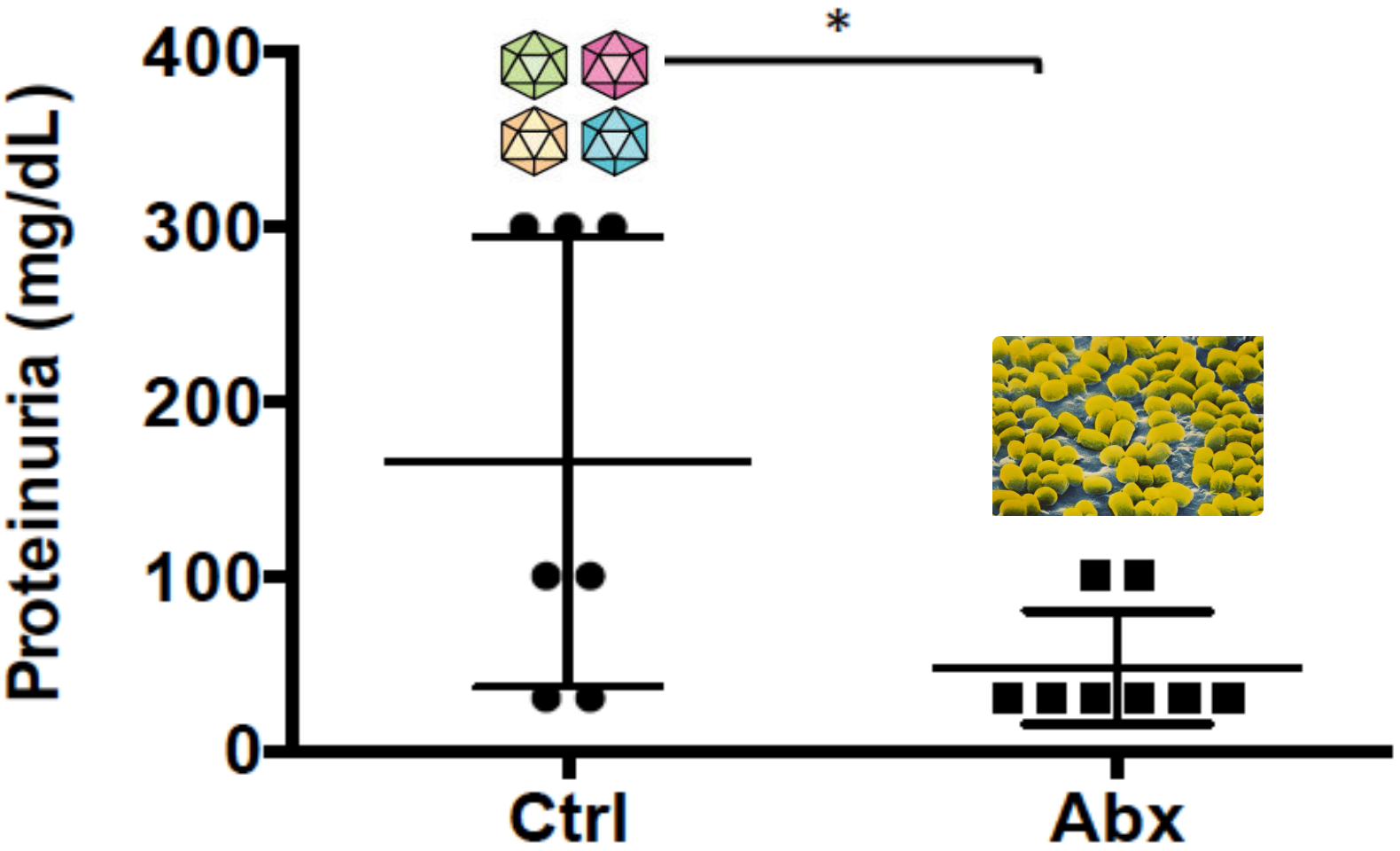
- Gut organisms capable of making neurotransmitters such as norepinephrine and serotonin.
- Mice fed *Lactobacillus* alters swimming mobility (shorter times suggesting less hopelessness).
- If the vagus nerve is severed, the mice become hopeless again.

Microbes can play games with the mind

- Bacteria in our guts may decide who gets anxiety and depression
- Pilot study in 22 men, subtle but definite brain benefits by taking capsules brimming with bacteria.
- Reported at annual meeting for Society of Neuroscience
- Different bacteria make neural messengers such as dopamine, norepinephrine, GABA, serotonin, acetylcholine



Amelioration of Lupus Nephritis with Depletion of the Gut Microbiota



Vieira M et al, Science 2018

"Dis-moi ce que tu manges, je te dirai ce que tu es."

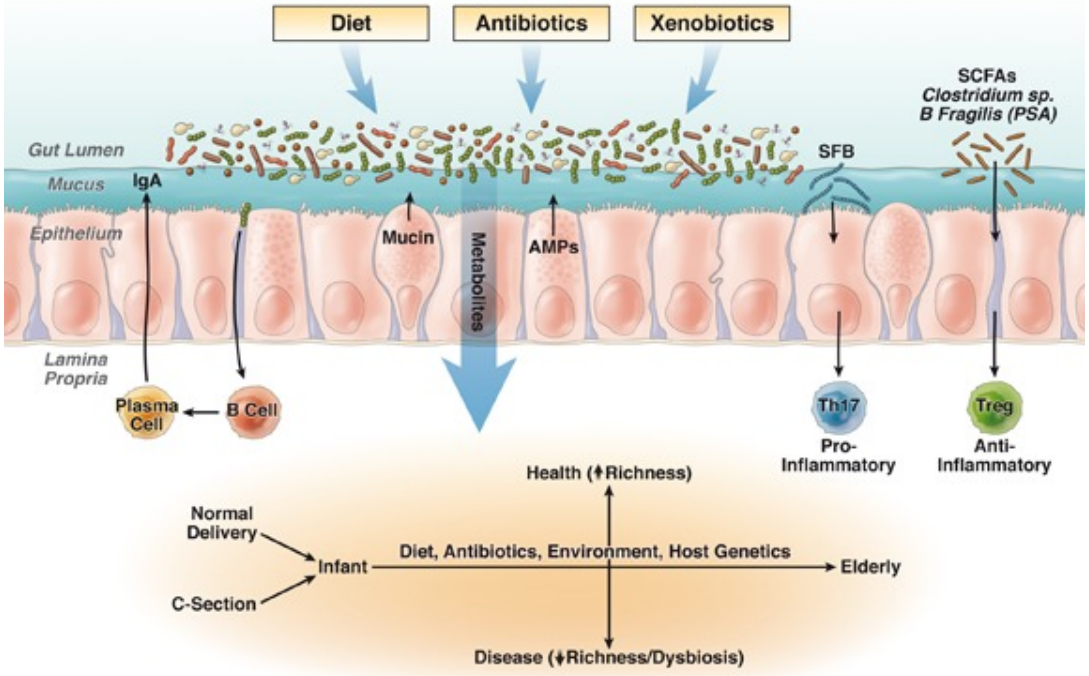
Tell me what you eat and I will tell you what you are.

Anthelme Brillat-Savarin, 1826

Special Issue

Gastroenterology

www.gastrojournal.org Volume 146 Number 6 May 2014



The Gut Microbiome in Health and Disease

Concepts in Mammalian Gut Microbiome

The Gut Microbiome and Disease

Therapeutic Modification of the Gut Microbiome

OFFICIAL JOURNAL OF THE AGA INSTITUTE

HCV patients had distinct mucosal microbiome

- Sequencing of bacterial DNA from duodenal mucosa showed significant differences in patients with chronic HCV compared to controls and patients with other chronic liver diseases

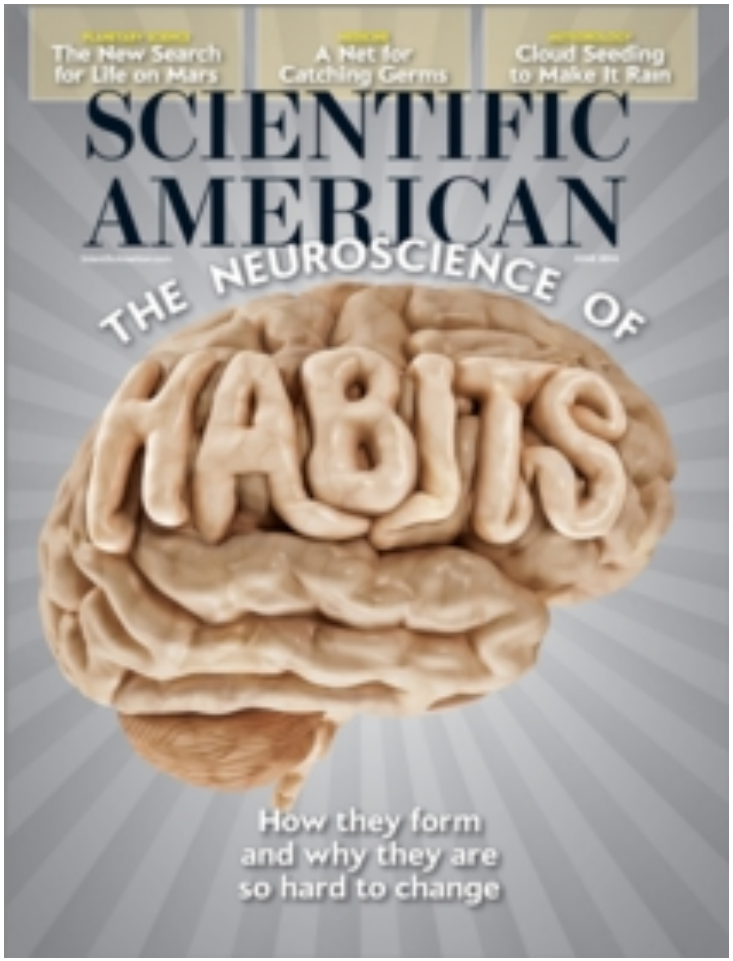
- Gut permeability was increased. Contribution to extrahepatic manifestations?

Preliminary study: A. Raj, Brisbane, Australia

The Gut Microbiota and NAFLD

- Microbiota in addition to regulating body fat gain and insulin resistance:
  - Change gene expression
  - Increase energy harvest from diet
  - Produce ethanol
  - Affect inflammation and immunity

E Lau *et al*. Gut Microbiota: Association with NAFLD and Metabolic Disturbances. Biomedical Research International. 2015.



How Gut Bacteria Help Make Us Fat and Thin

By Claudia Wallis

A Case Study in Humans

- 32 year old woman with recurrent CDI receives FMT.
- Baseline weight was **136 lbs** (BMI 26).
- Patient receives FMT from 16 year old daughter who weighed 140 lbs (later it increased to 170 lbs).
- 36 months after FMT, the patient's weight increased to **177 lbs (BMI 34.5).**

Weight Gain After Fecal Microbiota Transplantation.

Alang, N, and CR Kelly. *OFID,* 2015.