GUT FEELING: ADVENTURES IN THE MICROBIOME

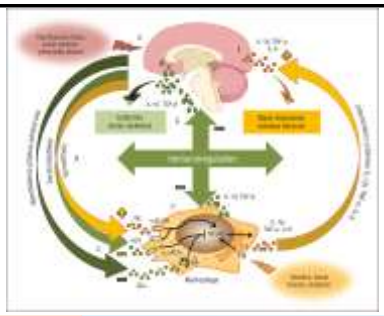
JENNIFER SMITH, PHARMD

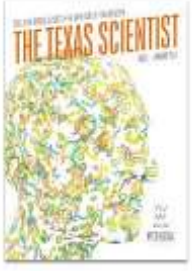
OBJECTIVES

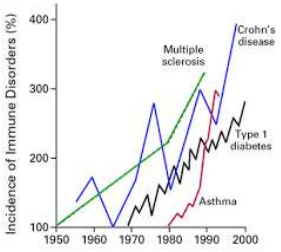
Describe the proposed mechanism(s) between the gut microbiome and psychiatric disease.

List psychiatric diseases that have been associated with alterations in gut microbiota.

Describe the strength of the evidence supporting use of probiotics to treat psychiatric disease.







BACKGROUND ON MICROBIOME

Microbiota: The collection of microorganisms in a particular habitat, such as the microbiota of the skin or gut.

Gut Microbiome: The human gastrointestinal tract is inhabited by ~10^{13} - 10^{14} microorganisms — more than 10 times that of the number of human cells in our bodies

Contains > 1000 species
Bacteroidetes and Firmicutes are the two predominant bacterial phylotypes

Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla and MANY others

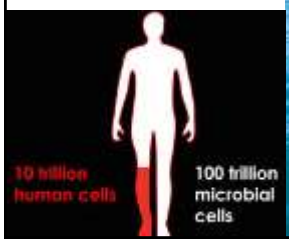
ABOUT OUR BUGS

100 trillion bacteria in our micro biome
90 % of the cells in our body are microbial
1,000 different species and 7,000 to 36, 000 strains

GENES

Human genes 26, 000

Human Gut Biome 100,000 + genes



NO. OF GENES

Human
26,000

Rice Plant
46,000

HOW OUR BUGS AFFECT US

Right colon - alcohol equivalent of 1 can of beer/day

Short chain fatty acids

Support Immune System

Vaginal delivery and breast feeding

Dysbiosis unbalanced families of bacteria

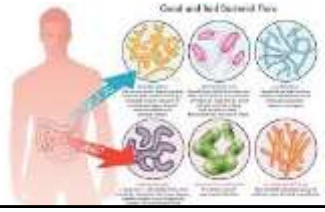
Autoimmune Disease – Crohn's Disease, MS, IBS

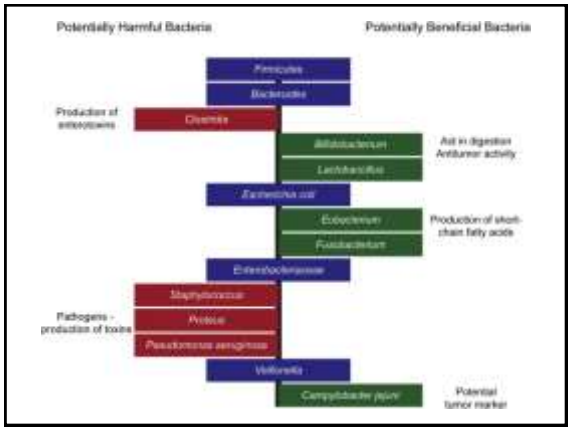
HOW OUR BUGS AFFECT US
ENERGY EXTRACTION

Positive: Calorie Harvesting 10-15 % of total Calories

Negative:

1. Excess Calorie Extraction
2. Dysbiosis, systemic inflammation, promoting fat deposit
3. Insulin Resistance leading to Diabetes





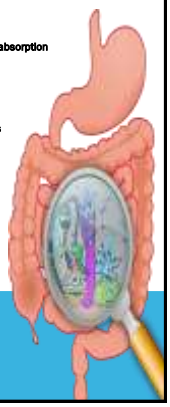
GUT MIRCOBIOME

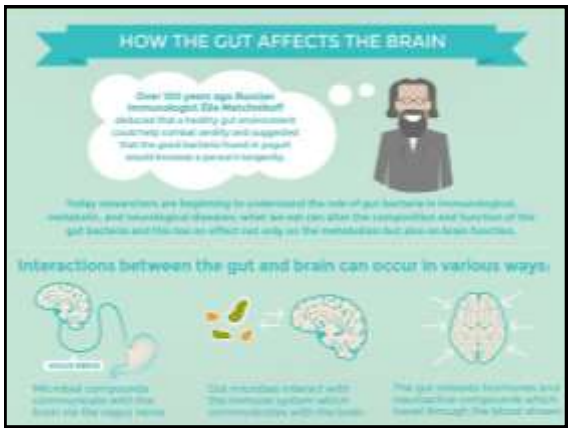
Function

- Supplies nutrients (ex. Vitamin K, Vitamin B complex) and aids nutrient absorption
- Helps metabolize indigestible compounds
- Defends against colonization by nonnative opportunistic pathogens
- Fortification of intestinal barrier and induction of secretory IgA synthesis
- Participation in the maturation and functioning of host immune system

Factors that shape the bacterial landscape:1

- Born by Caesarian delivery
- Not being breastfed
- Environment
- Gestational age
- Host genetics
- Exposure to infections (maternal and infant) -Antibiotic usage
- Stress (especially prenatally and in early life) –Diet





GUT-BRAIN CONNECTION

Brain-gut communication

Sympathetic nervous system = GI motility, blood flow, barrier function, and immune system activation

Parasympathetic nervous system = digestion

Enteric nervous system (ENS) = intestinal motility and secretion

The gut microbiome may impact brain development and function

The "bottom up" influence of microbes - Vagus nerve activation = Many of the effects of the gut microbiota have been shown to be dependent on vagal activation

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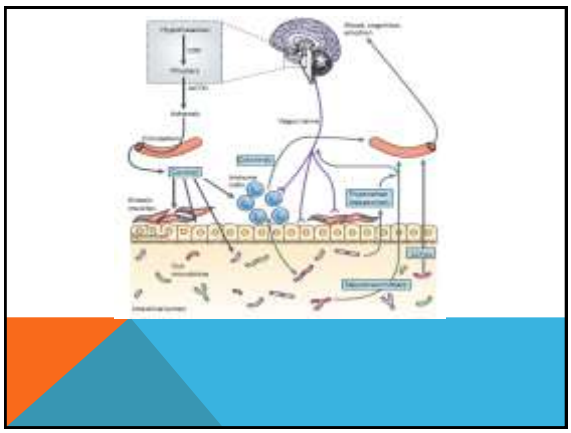


A LITTLE HISTORY

Anton van Leeuwenhoek

1683: tooth scrapings

"…little living animalcules very prettily a-moving."

HUMAN DATA ANXIETY

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood.

Steenbergen L1, Sellaro R2, van Hemert S3, Boech JA4, Colzato LS5.

BACKGROUND:

Recent insights into the role of the human microbiota in cognitive and effective functioning have led to the hypothesis that probiotic supplementation may act as an adjuvant strategy to ameliorate or prevent depression.

OBJECTIVE:

Heightened cognitive reactivity to normal, transient changes in sad mood is an established marker of vulnerability to depression and is considered an important target for interventions. The present study aimed to test if a multispecies probiotic containing Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, and Lactococcus lactis (W19 and W58) may reduce cognitive reactivity in non-depressed individuals.

DESIGN:

In a triple-blind, placebo-controlled, randomized, pre- and post-intervention assessment design, 20 healthy participants without current mood disorder received a 4-week probiotic food-supplement intervention with the multispecies probiotic, while 20 control participants received an inert placebo for the same period. In the pre- and post-intervention assessment, cognitive reactivity to sad mood was assessed using the revised Leiden Index of depression sensitivity scale.

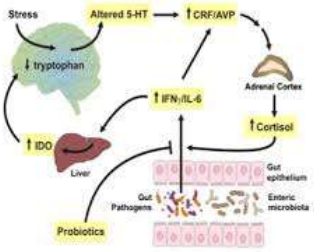
Compared to participants who received the placebo intervention, participants who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts.

CONCLUSION:

These results provide the first evidence that the intake of probiotics may help reduce negative thoughts associated with sad mood. Probiotics supplementation warrants further research as a potential preventive strategy for depression.

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

Article in Brain Behavior and Immunity · April 2015



COMMENSALS

- Lactobacillus and Bifidobacterium are the most studied
- Rodent and human studies: certain strains (L. helveticus and B. long) lead to decreased anxiety

CLOSTRIDIUM DIFFICILE

Clostridium difficile is a species of Gram-positive bacterium spore-forming bacterium that is best known for causing antibiotic associated diarrhea (AAD).

While it can be a minor normal component of colonic flora the bacterium is thought to cause disease when competing bacteria in the gut have been wiped to by antibiotic treatment.

In severe cases, C. difficile can cause "pseudomembranous colitis," a sever inflammation of the colon

INFLAMMATION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants

The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt of the organism to remove injurious stimuli and to initiate the healing process.

FECAL TRANSPLANT CURES WOMAN BACTERIAL INFECTION

After surviving a near-fatal car accident, Kaitlin Hunter found herself battling a devastating bacterial infection in her colon

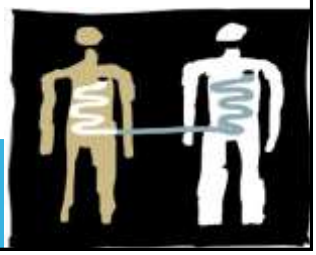
Hunter's mother "donated" one of her stools for the procedure. Next, the hospital lab carefully diluted it, and the foreign fecal matter right into Hunter's colon.

The result ended Hunter's struggle with C. diff.

A study published in March 2012 reported a 91% cure rate after just one fecal matter transplant, and a 98% cure rate when combined with an additional round of antibiotics.

FMT

Donor history (Similar to blood donation)
Obtain stool sample, homogenize with saline and filter
Administer through Nasogastric tube, enema, colonoscope
Perform within 6 to 24 hours of obtaining sample
Future: Frozen samples, lyophilized powders, capsules. The Ceiling is enormous



Definition

- Fecal microbiota transplantation (FMT) is the administration of a solution of fecal matter from a donor into the intestinal tract of a recipient in order to directly change the recipient's gut microbial composition and confer a health benefit.

[Bakken *et al.* 2011; Smits *et al.* 2013]

HISTORY

- First document in the 4th century China as "Yellow Soup"
- In some countries, maternal feces is inserted into the newborn's mouth to "jumpstart" the colon
- June 17th, 2013: FDA approved the procedure for recurrent C. Diff
- 0 documented serious side effects
- 92%-95% success rate

POTENTIAL INDICATIONS

GI Disorders
- Recurrent Clostridium Difficile Infection (RCDI)
- Inflammatory Bowel Disease (IBD)
- Irritable Bowel Syndrome (IBS)
- Chronic Constipation

Non-GI Disorder:
- Obesity
- Chronic Fatigue Syndrome
- Autism

THE PROCEDURE

- Carefully screened donor stool is mixed with a saline solution
- The solution is introduced into the GI tract via a NG tube, fecal enema, oral capsules, or during a colonoscopy
- The "good" bacteria multiply and help flush out the C. diff. bacteria
- 92%-95% success rate as mentioned earlier

GUT-BRAIN CONNECTION

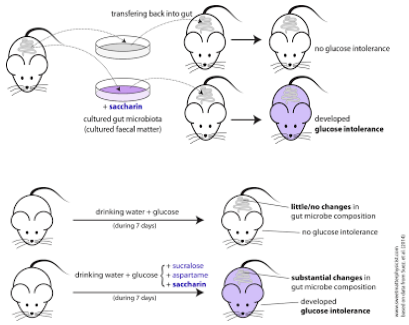
How do we know this?

Germ-free animals

Animals without germs in their guts have many different characteristics and behaviors than those animals with a microbiome 1,2

- Enhanced response to stress
- Reduced GI motility and altered excitability of nerves Reduced anxiety in a maze test
- Deficits in simple non-spatial and memory tasks
- Introducing certain strains of bacteria reversed many of these characteristics

HOW DO WE AFFECT OUR MICROBIOME?



CONCLUSION FROM THE EXPERIMENT

Mice given drinking water supplemented with artificial sweeteners (commercial formulations of saccharin, sucralose or aspartame) developed greater glucose intolerance than mice drinking pure water or water with only sugar added. The effect occurred both in mice fed standard chow and those on a high fat diet. Changes in the composition of gut flora were observed by sequencing a ribosomal RNA gene.

POSSIBLE MECHANISMS OF DYSBIOSIS OF THE GUT

The "leaky gut" syndrome: High counts of pathogenic bacteria in the gut impair the intestinal barrier by producing neuro- and endotoxins, which then expose the mucosa and sub-mucosa to bacteria.

Bacterial invasion of this previously aseptic environment causes:

Immune (and auto-immune) cell activation and infiltration

Up-regulation of pro-inflammatory cytokines such as TNFα and Il-1β.

This inflammatory response further increases barrier permeability thereby perpetuating an inflammatory cycle

Gut bacteria are able to produce active metabolites for human organ systems

For example: Lactobacillus and Bifidobacterium synthesize gamma-aminobutyric acid (GABA) from monosodium glutamate

Escherichia coli, Bacillus and Saccharomyces produce norepinephrine

Candida, Streptococcus, Escherichia and Enterococcus produce serotonin

Bacillus and Serratia produce dopamine

POSSIBLE MECHANISMS OF DYSBIOSIS OF THE GUT

Hypothalamic-Pituitary-Adrenal (HPA) axis dysregulation/poor development

The HPA axis = the core stress efferent axis that coordinates the adaptive responses to stressors.

Part of the limbic system, a crucial zone of the brain predominantly involved in memory and emotional responses.

Microbiota is crucial for hypothalamic–pituitary–adrenal axis function.

Widespread neurodevelopmental changes in the brain in germ-free mice

- Alterations in monoaminergic neurotransmission
- Behavioral changes in anxiety
- Deficits in sociability, social cognition, and increased repetitive behaviors

SELECT FINDINGS IN ANIMALS SUPPORTING MICROBIOMES ROLE IN NEUROPSYCHIATRIC DISEASE

Challenge with Citrobacter rodentium was associated with increased anxiety-like behavior within 7 to 8 hours in mice.1

Maternal separation stress between 6 and 9 months of age in rhesus monkeys was associated with decreased fecal Lactobacilli levels.2

Chronic stress in adult mice was associated with a decrease in the relative abundance of Bacteroides species and increased the Clostridium species in the cecum. It also caused immune activation (increased IL-6).3

ANIMALS ARE NOT THE SAME AS HUMANS

No equivalent model to germ-free mice available to study in humans

Microbiome of the mouse ≠ human microbiome

Very few Prevotella and Ruminococcus species in mice, but relatively common in humans

Mouse diet far different from humans

Very difficult to measure microbiome composition in humans

Composition not static throughout lifetime

Confounders such as: diet, lifestyle, environment, disease

VERY small number of studies have examined the microbiome in adult humans

SELECT FINDINGS IN HUMANS

Overweight pregnant women (n = 16) exhibited decreased numbers of Bifidobacterium and Bacteroides compared to normal weighted pregnant women (n = 34)1

A fermented milk product containing Bifidobacterium animalis, Streptococcus thermophiles, lactobacillus bulgaricus and lactococcus lacti affected activity of brain regions that control central processing of emotion and sensation (n = 12 healthy females)2

RISTOMED diet + VSL#3 probiotic (n = 25 elderly adults) was associated with reduced Clostridium to Bifidobacterium ratio.3

RISTOMED diet + d-Limonene (n = 25 elderly adults) was associated with decreased fibrinogen and HOMA-IR.3

MICROBIOME MANIPULATORS?

PROBIOTICS: Dietary supplement containing live bacterial cultures that is taken orally in adequate quantities to exert a health benefit.

Although many bacteria are advertised as probiotics, data show that the in vivo effects of different species vary greatly and few have been thoroughly investigated.1

PSYCHOBIOTICS: live organisms that, when ingested, may produce health benefits in patients experiencing mood disorders

PREBIOTICS: nondigestible food ingredients that promotes the growth of beneficial gut microorganisms

Ex. oligosaccharides

MAJOR DEPRESSIVE DISORDER (MDD)

Changes in pro-inflammatory and cell-mediated immune cytokines have been repeatedly documented in MDD.
- Increased Il-6, tumor necrosis factor α, and C-reactive protein
- Supports theory that inflammation and immune activation may be associated with MDD

Chronic mild stress increases the level of bacterial endotoxin lipopolysaccharides in the circulation in rats.

- Lipopolysaccharides are produced by gram-negative bacteria

Higher levels of serum antibodies against lipopolysaccharides of enterobacteriaceae in humans (n = ??) with MDD compared with controls.1
- Supports theory that bacteria or their products from the gut have made their way into the systemic circulation in some people with MDD.
- Significant differences found in fecal bacterial communities of patients with active MDD (n = 37) compared to nondepressed controls (n = 30).2
- Overrepresentation of Bacteroides and Proteobacteria in MDD

Underrepresetnation of Firmicutes in MDD

MDD TREATMENT

Probiotics

Mix of Lactobacillus helveticus and Bifidobacterium longum was associated with lower anxiety and depression in healthy volunteers compared with placebo (n = 26 on probiotic vs. 29 on placebo).

- Lower levels of cortisol also found

Administration of Bifidobacterium infantis as a probiotic to rats for 14 days resulted in elevations in tryptophan (serotonin precursor) levels.

There are currently no published studies of the clinical use of probiotics in clinically depressed patients, and therefore specific recommendations are lacking.

Minocycline
- Has been shown to modulate depression symptoms

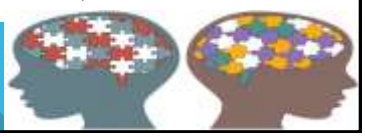
Role in impacting gut microbiome yet to be explored

AUTISM

Increasing evidence points to an association of autism with brain inflammation and presence of brain autoantibodies

Problems with gut permeability have been reported in some autism patients

A strong correlation has been found between severity of autism and gastrointestinal symptoms, and children with autism have been found to have:
- Lower level of Bifidobacteria species1
- Higher levels of Lactobaccillus species2
- Higher levels of Bacteroidetes1,3 AND lower levels4 as well as lower Bacteroides-Firmicutes ratio5
- Elevated levels of Clostridium bacteria.6,7



AUTISM TREATMENT

Children with autism (n = 8 out of 10) treated with oral vancomycin had significant (but transient) improvements in behavioral, cognitive and GI symptoms.

Treatment with Bacteroides fragilis was associated with correction of gut permeability, altered microbial composition, and amelioration of defects in communicative, stereotypic, anxiety-like and sensori-motor behaviors in an autism model in mice.

Treatment with "Children Dophilus" probiotic PO TID for 4 months corrected imbalanced Bacteroidetes/Firmicutes ratio in autistic children (n = 10)

Contains 3 strains of Lactobacillus (60%), 2 strains of Bifidumbacteria (25%) and one strain of Streptococcus (15%)

Did not correlate with change in behavioral symptoms

ADHD

Theory: The gut microbiome may be involved in development of ADHD symptomatology

In one study, infants (n = 75) were given either Lactobacillus rhamnosus GG (probiotic) or placebo during the first 6 months of life and gut microbiota was assessed over a period of 13 years.1

17.1% of infants in placebo group were diagnosed with ADHD or AS vs. 0% of supplemented group.

- Correlation found between lower counts of Bifidobacterium species and development of ADHD or Asperger's Syndrome.

SCHIZOPHRENIA

Evidence of intestinal inflammation and bacterial translocation in schizophrenia1

Elevated serum levels of pro-inflammatory cytokines in patients with schizophrenia compared to controls.2 • An elevated Th1/Th2 ratio has been reported in actively psychotic patients, indicating an increased inflammatory response.3

Importance of gut microbiota to normal development of NMDA receptor function has been described in animals.4

NMDA receptor dysfunction leading to glutamate dysregulation may play a role in development of schizophrenia

Increased prevalence of anti-gluten antibodies in patients with schizophrenia (non-celiac gluten sensitivity)5

23.1% of patients with schizophrenia

3.1% of comparison group

Theorized to result from increased intestinal permeability leading to generation of humoral immune response

SCHIZOPHRENIA

Composition, taxonomy and functional diversity of the oropharynx microbiome in individuals with schizophrenia and controls

Eduardo Castro-Nallar,1,6 Matthew L. Bendall,1 Marcos Pérez-Losada,1,5,7 Sarven Sabuncyan,2 Emily G. Severance,2 Faith B. Dickerson,3 Jennifer R. Schroeder,4 Robert H. Yolken,2 and Keith A. Crandall1

SCHIZOPHRENIA TREATMENT

Gluten-free diet

7 clinical trials—Mixed results
Minocycline adjunct to antipsychotic medication

Patients on minocycline 200mg daily + risperidone (n = 46) showed significant improvement in negative symptoms of schizophrenia compared with placebo + risperidone.

Minocycline inhibits activated microglia in the brain, which become activated in response to immunological stimuli.

SCHIZOPHRENIA TREATMENT CONTINUED

Antipsychotic induced weight gain: (ANIMAL DATA ONLY)

Antipsychotic dose-dependent increased levels of Firmicutes and decreased levels Bacteroides species seen in female rats.1

Gut bacteria were necessary for olanzapine-induced weight gain in germ-free mice.2

Colonized mice treated with olanzapine showed a shift in gut microbiota toward an "obesogenic bacterial profile."3

Rats administered an antibiotic cocktail of neomycin, metronidazole and polymyxin B PO daily beginning 5 days before olanzapine treatment were found to have LOWER degree of: body weight gain, uterine fat depoaition, macrophage infiltration of adipose tissue, plasma free fatty acid levels compared with olanzapine alone.4

CONCLUSIONS

We are only in the beginning stages of defining the biomarkers, bacterial species, and diets necessary to manipulate and study the human microbiome. It is clear that gut bacteria have some influence on the development and ongoing function of the brain.

There may be alterations in gut bacteria in certain neuropsychiatric diseases, but consistency of and repetition of findings vary

Autism

Antipsychotic-induced weight gain

Major depressive disorder

CONCLUSIONS

It is possible that findings to date regarding the relationship between microbes and neuropsychiatric illness are merely an association without causation.

Findings thus far show potential for beneficial effects upon psychiatric disorders by manipulation of the gut microbiome.

That potential is on the horizon, not in the here and now.