IBS, IBD, and Diseases of GI Compromise: Can Dietary Modifications Improve Outcome?

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Dysbiosis Defined

An alteration in the microbiome caused by a change in the composition of the microbiota, a change in microbial metabolic activity, and/or a shift in local distribution of communities of microbes.

What Contributes to Dysbiosis

- Host genetics: Mutations in NOD2, IL23R, ATG16L and IGRM
- Lifestyle: Diet, Stress
- Early colonization: Birth in hospitals, Altered exposure to microbes
- Medical practices: Vaccination use, Antibiotic hygiene

Altered Intestinal Permeability
Changes in microbiome

Modified from:
Why care about gut bacteria?

• All eucaryotes have evolved in presence of bacteria.
• They surround us and we surround them!
  – Our immune system reacts to bacterial presence.
  – Bacteria produce metabolites and peptides.

Trophic
• Control of epithelial cell proliferation and differentiation
• Promote intestinal angiogenesis
• Development and homoeostasis of the immune system

Protective
• Protection against pathogens

Metabolic
• Fermentation for SCFA
• Endogenous mucus
• Production of vitamin K
• Some AA, Neurotransmitters
• Xenobiotic metabolism
## Microbiome changes and disease association

<table>
<thead>
<tr>
<th>Disease</th>
<th>Relevant finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>Increased ratio of Firmicutes to Actinobacteria</td>
</tr>
<tr>
<td>Reflux oesophagitis</td>
<td>Oesophageal microbiota dominated by gram-negative anaerobes; gastric microbiota with low or absent <em>Helicobacter pylori</em></td>
</tr>
<tr>
<td>Obesity</td>
<td>Reduced ratio of Bacteroidetes to Firmicutes</td>
</tr>
<tr>
<td>Childhood-onset asthma</td>
<td>Absent gastric <em>H. pylori</em> (especially the cytotoxin-associated gene A (cagA) genotype)</td>
</tr>
<tr>
<td>Inflammatory bowel disease (colitis)</td>
<td>Larger populations of Enterobacteriaceae</td>
</tr>
<tr>
<td>Functional bowel diseases</td>
<td>Larger populations of <em>Veillonella</em> and <em>Lactobacillus</em></td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>Larger populations of <em>Fusobacterium spp.</em></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Gut-microbiota-dependent metabolism of phosphatidylcholine</td>
</tr>
</tbody>
</table>

*Cho I et al. Nature Rev Genetics 2012*
Man and Our Microbiome Continue to Evolve

- **Major dietary changes**
  - Fats, protein, fiber, additives, sweeteners
- **Changes in activity**
  - Sedentary lifestyles
- **Immunizations**
- **Decrease in parasitic infection**
- **Refrigeration**
- **Sanitation and hygiene standards**
- **Urban life in cities and concrete**
- **Increased use of antibiotics**
  - Indicated or not! collateral damage
- **Newborns in USA**
  - 10 – 15 % CHO in human milk *for* microbiome
  - HMO stimulate innate immune system
  - 1/3 c section, majority bottle fed
Spatial distribution of the gut microbiota

Streptococcus
Lactobacillus

Primary site of contact
Bile and pancreatic enzymes

Peyer’s patches
Major site of adaptive immune activity

Small intestine
Duodenum
Jejunum
Ileum

10^2–10^4 g^{-1}
10^7–10^9 g^{-1}
< 10^4 g^{-1}

Stomach

Streptococcus
Lactobacillus
Staphylococcus
Veillonella

Acid challenge

10^{11}–10^{12} g^{-1}

Cecum

Large intestine (colon)

Enterobacteria
Enterococcus
Bacteroides
Clostridium
Lactobacillus
Veillonella

Bioreactor
Major metabolic activity
Short-chain fatty acids

Kleerebezem Ann Rev Microbiol 2009
Composition of the human gut microbiota

Only 9 bacterial divisions detected but note extreme diversity

Gut has strong selection for bacteria and redundancy of functions

- Variety is thought to yield resilience to perturbations

**Human Microbiome Project:**

NIH Funded 2008 reads 300 US Subjects, 18 body sites, 1300 strains from the body

Cresci G et al NCP 2015
Bäckheden Science 2005
Cho I et al Nature 2012
Where “man meets microbe” a Dynamic Interplay of Mutualism

- 300 to 400 sq meter surface area of GI

- > 1 million genes in the bacterial genome vs ~30,000 in the human
  - 100 trillion living bacteria in the human intestine
    » Only about 10 trillion cells in human body
  - Wide variety of species in human colon, many non-culturable
    – For example: 46 native clostrdial strains (many beneficial)
  - Extensive # of microenvironments (skin, R v L hand etc)

- Importance of gut microbiome clear from first day of life
  - 13 to 15% of CHO in breast milk not absorbed by infant
  - Many vitamins, some AA and SCFA production
What is a healthy gut?

- Host response (immune system, intestine, brain-gut axis)

Shanahan *Gastroenterology* 2010;139:1808-12
Antibiotic-associated disease

Altering the Microbial Biodiversity
Actions at the mucosal border: The Critical Balance!

Barrier function

Selective absorption

Life or death is only one cell layer away

Fishman JE et al
Ann Surg 2014
Could some of the problem be iatrogenic?

Etiology of Hospital Induced Changes in Microbiome

- Broad spectrum antibiotics
  - Changes noted within hours
- PPI / H₂RA
- Vasoactive pressor agents
  - Changes in pH,
  - Decrease pO₂
  - Increase pCO₂
- Opioids
  - Decrease motility and bacterial clearance mechanisms
- Decrease in luminal nutrient delivery
  - Delays in feeding
  - Parenteral feeding
How fast does the microbiome change in critical illness?

- Numerous factors
  - Inflammatory changes
  - Bacterial interrelationships
  - Bacterial changes with host stress situations
  - Variation with nutrient availability, meal patterns etc

- Mechanisms
  - Bacterial use environmental clues
    - pH, temperature, redox potential, osmolality
  - When energy supply is limited genes “switch on” virulence factors
  - Ex: E.coli and Pseudomonas can rapidly become virulent with host stress (epinephrine, cortisol, morphine etc)

Alverdy J, CCM 31:598-607,2003
Alverdy J Molecular Biol 2008
Zarrinpar A et al Cell Metab 2015
Dysbiosis: the lines are becoming blurred!

• Morrow LE et al Chest 2016
During critical illness, time is the enemy

Critical loss of commensalism and the emergence of pathogens expressing enhanced virulence drives the immunopathology of critical illness

“Microbiome becomes Pathobiome”
Within 24 hours, a lethal *P. aeruginosa* morphotype develops

Microbial phenotype- NOT species, NOT immune background-caused death- so then what actually drives sepsis outcome?
We can now measure the majority of bacteria, a step in the right direction or just more confusing?

Clinical Disease

Diet, Stress, Surgery, Trauma, IBD, IBS, dysbiosis, etc
The Million Dollar Question?

- Can providing a clinically defined formulation designed for management of the compromised gut make a difference?
Probiotics can *prevent, mitigate* and *treat* many of the current health crisis facing the western world

**Cancer**
- Multiple mechanisms
- Protects mucosa from radiation effects
- Increases benefit from chemo agents

**Heart disease**
- Metabolic syndrome
- Atherosclerosis

**Hepatic diseases**
- NASH
- Hepatic encephalopathy

**Infectious disease**

**Diarrheal diseases**
- AAD
- Bacterial
- *Clostridium difficile*
- Viral

**GI compromise**
- IBD
- IBS
- Celiac
- Short gut
- “leaky gut”

**Inflammatory diseases**
- Allergy
- Asthma

**Autoimmune diseases**

**Aging**

**Obesity**

**Depression**

**Critical Care / Surgery**
- Trauma, Gen Surg
- Pancreatitis +/-
- Transplantation
- Sepsis
- VAP prevention
What are Probiotics?
Criteria for Probiotic Designation

- Human origin
- Viable and hardy in human GI tract
- Acid and bile stable
- Adhesion to mucosa
- Clinically demonstrated benefit
- Safe

WHO, FAO (Food and Agriculture Organization) of the UN definition:
- “live microorganisms in which when administer in adequate amounts confer a health benefit on the host”

Most Common Probiotics
Commercially Used
- Lactobacillus acidophilus/johnsonii/gasseri
- Lactobacillus casei
- Lactobacillus paracasei
- Lactobacillus rhamnosus
- Lactobacillus plantarum
- Lactobacillus reuteri
- Bifidobacterium animalis/lactis
- Bifidobacterium bifidum
- Bifidobacterium breve
- Bifidobacterium longum
- Bifidobacterium adolescentis

Lactobacillus
Bifidobacteria
Probiotics: Exploring the Mutually Beneficial Effects of Bacteria and Their Substrates in the Human Host

- Prevent infections (systemic and GI)
- Regulate local and systemic immune function
- Metabolic pathway nutrients: glycemic control, cholesterol, amino acids
- Regulate bowel motility
- Regulate appetite (leptin, ghrelin)
- Support mucosal barrier (multiple mxs)
- Enhance nutrient utilization
- Prevent neoplastic changes
Has Our Fear of “Bacteria” Made Us More Susceptible to Disease
Clinical Application: Microbiome literature: Science or Quackery?

- Professional Literature improving yet;
  - Advanced techniques
  - Few ITT clinical studies available
  - Meta-analysis not consistent

- Recent lead articles:
  - PNAS 2016
  - Chest 2016
  - Nature 2015
  - Science 2014
  - Wall Street Journal 2012
  - Scientific American 2012
  - Economist 2012
  - NY Times 2013

Skeptics view:
“….probiotics can’t cure everything....”
Gastroenterologist Survey: Probiotics

- Evaluate MD opinions regarding probiotics
- Large metropolitan area in midwest
- Results:
  - Safe for most patients 100%
  - 98% felt probiotics had a role in treating GI disease
  - 93% had patients currently taking probiotics
  - Most common bacteria used
    - Yogurt based, B.infantis (Align®), VSL#3,
  - Most common clinical diagnosis used
    - IBS, AAD, C.difficile
  - Most believed their practice was not supported by scientific data

Williams MD J Clin Gastro 2010
Mechanisms:

1. Enhancement of the epithelial barrier
   - Mucins and defensins
   - Probiotics vs. Pathogens
   - Production of anti-microbial substances (e.g., bacteriocins)

2. Increased adhesion to intestinal mucosa

3. Inhibition of pathogen adhesion

4. Competitive exclusion of pathogenic microorganisms

5. Production of anti-microbial substances

6. Modulation of the immune system
   - IL-10, TGFβ
   - Immature DCs to Macrophages
   - Th1, Th2, Th17
   - Treg
Mechanisms:
Colonization Resistance
Antimicrobial Factors

**L. reuteri** inhibits *H. pylori*

PM Sherman (NCP2009)
Morowitz M J (SCNA 2011)

**L. reuteri** inhibits *Staph aureus*

Mechanisms:
- Competitive inhibition
- Physical barrier (mucous)
- ↓ Adherence, attachment
- Produce bacteriocins
  - Defensins, Trefoil
  - Bind pathogens
- ↓ pH reduces growth
- Interferes quorum sensing
  - ↓ Virulence expression
- Breaks up biofilms

**Bacteria**
- *Escherichia coli* (pathogenic)
- *Salmonella typhimurium*
- *Shigella* spp.
- *Campylobacter jejuni*
- *Streptococcus mutans*
- *Bacillus subtilis*
- *Clostridium perfringens*
- *Helicobacter pylori*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Pseudomonas fluorescens*

**Fungi**
- *Candida albicans*
- *Aspergillus flavus*
Protecting the mucosal lining:

“Soluble factors for Lactobacillus rhamnosus GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells”

- 70% of energy for colonocyte derived from luminal butyrate
- Cell culture model
- DNA microarray methods, real-time PCR and electrophoretic mobility shifts studied
- Studies confirm:
  - L. GG modulates signaling pathways
  - Activates via MAP kinase
  - L.GG protects mucosa from oxidant stress via expressing HSP

Tao K, Drabik K, Waypa T
Am J Physiol Cell Physiol 290;1018-1030, 2006
L. *salivarius* (UCC118) prevents *Listeria* infection, in mice

- Control
- UCC118
- UCC118, bacteriocin KO

• Sinéad C. Corr, PNAS 2010
Lactobacillus *salivarius* (UCC118) prevents disruption of epithelial cell tight junctions

Human epithelial cell model

Miyauchi et al Am J Physiol Gastrointest Liver Physiol 2012
UCC118 alters tight junction protein localization.
Mechanisms: Enhancing mucosal blood flow

- Stappenbeck TS, Hooper LV et al
  Proc Natl Acad Sci 2002
Mechanisms: Stimulation the immune system in the small intestine of healthy subjects

Before L. reuteri intake

Resting CD4+ T-helper cells

After L. reuteri intake

Activated CD4+ T-helper cells

Clinical Equivalent
Probiotics prior to Immunization seasonal flu vaccine:
Enhances anti-body response
Specific IgG, IgG1, IgG3
No change in inflammation

SCFAs, Fiber Fermentation and Butyrate Receptors

- Trophic effect, colonocyte fuel
- Anti-inflammatory
- Enhance WBCs, macrophage
- ↓Adhesion molecules
- (↓microvascular thrombosis)

Thangaraju M et al J GI Surg 2008
Ganapathy V 2011
Multiple clinical mechanisms well described

- Competitive inhibition of pathogens
  - Alverdy data – GI anastomosis
- Enhance HSP in gut mucosa
- Tight junction protein synthesis
- Enhance mucosal blood flow
- Stimulate gut immunity
- Butyrate (fermentive end product) enhances neutrophil killing, chemotaxis, resolution of inflammation
- Butyrate- Anti-neoplastic activity
- Increases return of GI motility
- Helps maintains microbiome diversity in colon
- Alterations in metabolism / energy utilization
  - Vitamin and AA production and absorption
- Interacts with ENS bidirectional communication
- Activates Ca++ binding protein expression
- Bile salt hydrolases decrease fatty liver
Additional mechanisms

- Interacts with ENS bidirectional communication
  - Nerve Growth Factor stimulated by Lactobacillus sp
  - Increases IL-10 which attenuates inflammation
  - Alters GABA in brain and shown to be anxiolytic with 28 day continuous feeding (blocked by vagotomy)
  - Microbiome required for normal gut brain signaling
  - Microbiome required for gut Ca++ binding protein expression

Bienenstock J et al Gut Microbes 2013
McVey-Neufeld KA et al Neurogastro and Motility 2015
Clinical Use of Probiotics

Where does the rubber meet the road?

No all “probiotics” are the same
Not all probiotics work
Mechanisms of action are key
Need the right strain and research to prove it
Can Probiotics be used for prevention of disease in “Healthy People”

*Sick days at home with short term gastro-intestinal or respiratory illness*

Placebo: 0.9 % sick days  
2 days per individual and year

Reuteri: 0.4 % sick days  
<1 day per individual and year  **

*Number of people sick*

26% on placebo (23 persons)  
11% on Reuteri (10 persons)  p<.01**

Tubelius P et al., Environ Health 2005
Pre and Probiotics:  
Use probiotics in healthy school children

Children (4-10m) with increased risk for infection
12 weeks supplementation in baby formula

Weizman et al., Pediatrics (2005)

Saavedra JM et al 2004  
PRDBPCT N=118, 3-24 months, 210 day +/- Probiotics
Results: Probiotic group
Decrease colic, antibiotic use

Mugambi MN et al  Nutr J 2012
Meta-analysis: Pre/Pro/Synbiotics, 25 studies total
Conclusion:
No consistent high quality data to support;
- Growth development, GI issues
Results:

- *Lactobacillus rhamnosus* GG influences the composition of intestinal microbiome
- Use prevents some of the changes associated with cephalosporin antibiotic use
- Decrease in GI complaints
- Treatment prevents subsequent infections up to 3 yrs

Korpela K et al PLOS One 2016
Probiotics, Pregnancy and Maternal Outcomes

- Finland N=256 (3 groups)
  - Strict definition of Gestational diabetes (GTT)
  - Control, placebo, probiotics

- Results:
  - Control 36%
  - Placebo 34%
  - Probiotics 13%
  - No change in pregnancy outcome
  - No change in children at two years

Luoto R British J Nutrition 2010

- Systematic review: 189 articles
  - Primary outcomes:
    - Gestational DM
  - Secondary outcomes:
    - Pre-eclampsia
    - Inflammatory markers
    - Lipid profiles
    - Gestational weight

- Conclusion: Probiotics reduce
  - gestational DM
  - Maternal fasting glucose
  - Pre-eclampsia
  - CRP-inflammation

Lindsay KL et al 2013 J Maternal-Fetal Neonatal Med
Probiotics in the prevention of necrotizing enterocolitis in neonates

- 7% of VLBW < 1500 gm
  - 20 to 30% mortality
  - Etiology is clearly multifactorial
    - Premature birth, Abnormal intestinal microbiota
    - Enteral feeding, alterations in perfusion

- N=566 infants
  - 5 probiotic genera (4 bifidobacteria and 1 lactobacillus)
    - $2.0 \times 10^9$ CFU/day

- Results
  - Reduction in Nec 9.8% vs 5.45% (p<.05)
  - Reduction in Mortality 9.8 vs 6.8% (NS)

Janvier A et al J Pediatrics 2014
Microbiome and Brain Development

- Gnotobiotic mouse model:
  - Substantially ↑↑ corticosl response to stress
  - Decreased brain derived neurotrophic factors
    - Neurogenesis
    - Synaptic growth
    - Modulates synaptic plasticity and transmission
- Partially reversed by re-colonization with a normal mouse gut microbiota
- Suggests that active signals from the microbiota plays critical role in brain development
- Significant bidirectional communication
  - D-serine, GABA, Nerve growth factor

O’Mahoney SM Neuroscience 2015
Bienenstock J et al Gut Microbes 2013
McVey-Neufeld KA et al Neurogastro and Motility 2015
Studies showing benefit in colorectal cancer prevention or management?

- > 300 plus animal studies in CRC models
- > 250 human cell line mechanisms
- 1 long term (12 year epidemiologic) high v low yogurt intake
  - N=45,241 noted decrease risk CRC

Multiple mechanisms:

- Control of intestinal epithelial differentiation/proliferation
- Apical tight junction tightening
- Pathogen protection from contact to cell
- GALT
- TLR/NOD receptor binding
- SCFA (primarily butyrate) – inhibition of histone deacetylase
- Protection from dietary carcinogens
- Apoptosis of human cancer cell lines
- Changes in bile acid metabolism

• Serban DE et al Ca Letters 2013
• Pala V et al Int J Ca 2011
Probiotics in the treatment and management of Colorectal Cancer

- **New data** – microbiotic changes during tx CRC
  - Microbiota alters chemotherapeutic agents to enhance immune host immune function
  - “drugs need bugs”
  - Probiotics partially protective from effects of chemo and radiation

Azcarate-Peril MA et al. Am J Physiol (GI Liver Physiol) 2011
Ciobra MA et al Gut 2012 (radiation)
Viaud S et al Sci 2013
Bordon Y et al Nature Rev Immunology 2014
Demers M et al Clin Nutr 2014
H. pylori infects at least half of the world’s population. The prevalence among middle-aged adults is over 80% in many developing countries, as compared with 20% to 50% in industrialized countries. **WHO classifies H. pylori as class one carcinogen**

*Suerbaum & Michetti NEJM 2002; 347:1175*

*Morowitz MJ Ann Surg 2011; 253:1094-1101*
Specific probiotics have surface proteins that inhibit the binding of *H. pylori* in the stomach.

- *H. pylori* attached to gastric cells
- *L. reuteri* inhibits *H. pylori* binding

![Diagram showing *H. pylori* attached to gastric cells and *L. reuteri* inhibiting binding]

- *L. reuteri* inhibits *H. pylori* binding

*INFLAMMATION*

*Mukai et al. FEMS 32:105 (2002)*
# HP Eradication Therapy with and without Probiotics - Meta-analysis

## Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th># Trials / (n)</th>
<th>with</th>
<th>w/o</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eradication Rates</td>
<td>11(1074)</td>
<td>85%</td>
<td>75%</td>
<td>11</td>
</tr>
<tr>
<td>Total Side Effects</td>
<td>7(625)</td>
<td>22%</td>
<td>38%</td>
<td>6</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>8(997)</td>
<td>6.1%</td>
<td>16%</td>
<td>11</td>
</tr>
<tr>
<td>Epigastric Pain</td>
<td>7(608)</td>
<td>16%</td>
<td>23%</td>
<td>14</td>
</tr>
<tr>
<td>Nausea</td>
<td>7(608)</td>
<td>16%</td>
<td>25%</td>
<td>12</td>
</tr>
<tr>
<td>Taste Disturbance</td>
<td>5(418)</td>
<td>14%</td>
<td>25%</td>
<td>5</td>
</tr>
</tbody>
</table>

Optimal dosing and probiotic strains will vary results

Tong, A  Pharm Therap 2007
Probiotics in Irritable Bowel Syndrome

- **IBS**
  - Influence appears to be strain specific
    - L.GG, L. plantarum, L. acidophilus, L. casei,
    - (VSL#3), Bifidobacterium animalis, B. infantis (35624)
  - Well done studies showing improvement in symptoms
    (62 RCT – 49 showing benefit in at least one outcome parameter)
    - Bloating, flatulence, constipation
    - Few alter symptoms and pain / global score
  - B. infantis best studied (highest quality studies)
    - PRCT > 360 pts, $10^8$ bacteria
    - Improved global score by > 20%
  - B.regularis (Activa®)
    - Constipation predominate – 16 RPCT, 11 +
- Conclusion: Generally beneficial but probiotic species specific
Inflammatory Bowel Disease

- Crohn’s disease
  - 29 PRCT – 13 slight improvement in symptoms little if any objective data showing benefit
  - 16 PRCT – no benefit

- Ulcerative Colitis
  - Data slightly better than Crohn’s
  - Widely variable depending on strain, age, severity of disease
  - Increase remission rates UC

- Associated Inflammatory Bowel Disease issues with best data
  - Pouchitis – VSL#3 better than placebo

- Good News: NO HARM NOTED
  - May need probio + dietary additives

- Resources:
  - Whelan K Curr Opinion Gastro 2013
  - Holubar SD et al Cochrane 2010
  - Shen J et al IBD 2014
<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Probiotic</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver transplant</td>
<td></td>
<td></td>
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<tr>
<td>Trauma</td>
<td><em>Bifidobacterium breve, L. casei</em></td>
<td>Kotzampassi 2006, Spindler-Vesel 2007, Tan 2011</td>
</tr>
</tbody>
</table>
Antibiotic Associated Diarrhea: Preventable or Inevitable?

- Hempel S et al JAMA 2012
- Meta-analysis 82 RCT met criteria for inclusion
- Probiotics strains were poorly documented
- N=11,811 participants (pooled data)
- Conclusion:
  - Probiotics confer significant decrease in AAD (p<.001)
  - # needed to treat N=13
Pathogenesis of CDAD

Antibiotic therapy

Alteration in colonic microflora

*C. difficile* exposure and colonization

Release of toxin A and Toxin B

Colonic mucosal injury and inflammation

Spores can survive up to 5 months on surfaces

Badger, VO et al JPEN 2012
Use of probiotic preparations to prevent 
C. difficile Associated Diarrhea

- RDBPCT  N=135
- Age 64  all taking antibiotics
- 100 gm BID L. casei as drink
- Results:
  - AAD: 7/57 (12%) vs 19/56 (34%)
  - 21% relative risk reduction, NNT 5
  - C. diff 0/57 vs 9/53 (17%)

- Meta-analysis 28 studies
  - N=3818 patients
  - “Moderate quality” of evidence probiotics as prophylaxis
    - decreases incidence of CDAD by 66%
    - No adverse influence by receiving probiotics


Johnston BC Ann Internal Medicine 2012
Probiotics: Importance of choosing the correct bacterial species

- PLACID Trial: MRDBPCT
- 17,480 screened 2,971 met criteria
  - > 65 yo
  - All received antibiotics
  - 70% received either placebo or probiotic for at least 7 days
    - L. acidophilus x 2
    - B. bifidum x 2

- Conclusion:
  - AAD 10.8 vs 10.4 %
  - CD 0.8 vs 1.2 %
  - Essentially no differences between groups

Allen SJ et al Lancet 2013
Does the microbiome play a role in protection of lean body mass in the ICU?

- **Metabolic**
  - Butyrate (SCFA)
  - Anti-inflammatory

- **Neurohumoral**
  - HPA access

- **AA absorption**
  - Leucine 5 to 10% - from bacterial end products

- **Protects mucosal barrier**
  - Structural alterations which propagate protein loss via inflammatory changes

Erdman S et al Oncotarget 2016
What is a Prebiotic?

*Prebiotics* are non-digestible carbohydrates that act as substrate for beneficial microbes including probiotics.
Commensals → soluble fiber → SCFAs → Butyrate Effect

**Protective Effects:**
- Competitive exclusion pathogens
- Stimulate protective mucus
- Increase IgA production
- Promote tolerance (Treg)
- Inhibit NFkB
- Enhance epithelial barrier function
- Stabilizes hypoxia-inducible factor

SCFA = Fermentation end product of some probiotics (from prebiotics): Multiple Mechanisms Described

- Energy source;
  - Colonic mucosa;
    - Stimulates cell proliferation, Promotes sodium and water absorption
  - Cardiac, skeletal muscle, brain
    - Acetate, butyrate, propionate
- Regulation of gene expression for ICAM-1 and E-Selectin on endothelial cells
- Decrease COX-2 expression
  - (butyrate and propionate)
- Prevention of neoplastic transformation
  - Inhibits histone deactylase by DNA hypermethylation to promote differentiation in cancer cell lines
- Enhances Leptin secretion
- pH control; Inhibition of pathogen overgrowth in gut lumen,
- ROS scavenger
  - Pyruvate is anti-inflammatory and decrease NFkB expression
- Activation of polymorphonuclear cells
  - Both local and systemic immune benefit
  - G-protein receptors on circulating PMN’s

Thangaraju M et al J GI Surg 2008
Breast Milk Felt to be the “Perfect Food”

- Nutrient rich
  - Modulates colonization and development of immature newborn gut
- Functional carbohydrate
  - Not absorbed by child
  - Ferments in the distal bowel
- Probiotics also present
  - Found via culture and non-culture techniques
    - Streptococcus 91%
    - Staphylococcus 83%
    - Entero-mammary pathway

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**Nutrition Facts**

<table>
<thead>
<tr>
<th>Breast milk</th>
<th>% Daily Value*</th>
</tr>
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<tbody>
<tr>
<td><strong>Amount Per 1 cup (246 g)</strong></td>
<td></td>
</tr>
<tr>
<td>Calories</td>
<td>171</td>
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<tr>
<td>Total Fat</td>
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<td>Sodium</td>
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<tr>
<td>Potassium</td>
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</tr>
<tr>
<td>Total Carbohydrate</td>
<td>17g</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>0g</td>
</tr>
<tr>
<td>Sugar</td>
<td>17g</td>
</tr>
<tr>
<td>Protein</td>
<td>2.5g</td>
</tr>
</tbody>
</table>
| Vitamin A                    | 10%            | Vitamin C
| Calcium                      | 10%            | 20% |
| Vitamin D                    | 1%             | Vitamin B-6
| Vitamin B-12                 | 1%             | Magnesium
| Calcium                      | 7%             | Iron
| Vitamin D                    | 1%             | 0% |
| Vitamin B-12                 | 1%             | 0% |

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

-Fitzstevens JL et al NCP 2016
What are Human Milk Oligosaccharides (HMOs)?
“take the gut back to where it began”

• 200 unique carbohydrate structures have been identified in human milk
  - Make up ~ 15% of total CHO
  o 2’Fucosyllatctose is the most abundant HMO

• Anti-adhesive, mimicking the attachment sites for certain pathogens and blocking their adhesion, colonization, and invasion

• Reported to alter intestinal epithelial cell to alter expression of pathogen virulence

HMO’s <1% absorbed
Th2 promoting ---- anti-inflammatory
decrease IL-12, IFN
increase IL-10

Bode L. Glycobiology. 2013
Kulinich A et al Carbohydrate Res 2016
HMO Shown to Have an Anti-adhesive Effects

Isomalt-oligosaccharide (IMO) Prebiotic Fiber

5 g of IMO Fiber

The average American is only eating 10 - 15g of fiber

Recommendation for adults is to consume 25 – 35g of fiber

- IMO is soluble fiber, well-tolerated prebiotic fiber source from tapioca
- Produces Short-chain fatty acid (SCFA) like acetate, propionate and butyrate as end products of fermentation
- Inhibits the growth and activities of pathogens and contributes to stimulation of the growth of Bifidobacteria

It there an optimal protein found to be beneficial in diseases associated with GI compromise?

- Disease Heterogeneity
- Timing of delivery
- Metabolic state
- Route of delivery
- Activity level
- Timing of delivery
- Formula-AA ratios

Protein In GI compromise?
Is anabolic resistance real?

- Failure of normal anabolic stimuli to induce mRNA translation – effector via the mTORC

- Factors partially explaining anabolic resistance
  - Leucine insensitivity
    - In ICU (stressed) patients muscle free Leu is higher than the non-stressed
    - Blunted response to anabolic AA stimulus
  - Splanchnic sequestration following normal feeding
  - Decreased AA availability and uptake in muscle

- Insulin induced microvascular perfusion blunted
- Attenuated insulin induced suppression of muscle catabolism

Moore DR et al Adv Nutr 2014
‘Anabolic resistance’ of muscle protein

Rate of MPS and MPB

Time (h)

protein

Healthy
‘Anabolic resistance’ of muscle protein

Rate of MPS and MPB

Time (h)

protein

Inflammatory diseases

MPS

MPB
Mammalian Target Of Rapamycin
mTORC1

- Multiple sensors and signaling mechanisms
- With AA stimulation mTORC1 co-localizes with lysosome
  - Interacts with v-ATPase, Rags, Ragulator, Rheb
- Critical step in sensing AA is conversion from inactive GDP to active GTP

mTOR1 localizing with lysosome is the *ideal location* as it allows for ongoing information on metabolic state of the cell
Recent studies

- Weijs P et al 2014
  - Energy vs protein goal
  - Protein goal beneficial, energy goal not an issue
- Rooyackers O et al Clin Nutr 2015
  - WB protein synthesis – MOF
  - Critically ill able to utilize additional AA loads
    - Many parameters improved with addition AA
- Berg A et al Crit Care 2013
  - Protein kinetics hypocaloric vs normocaloric feeding
    - increased protein improved outcome
  - Enteral protein WB protein turnover
  - Additional protein beneficial
What happens to exogenously administered amino acid?

**Graphs:**
- **WB Protein net balance (µmol/kg/day)** vs. **Total AA intake (g/kg/day)**
  - $R^2 = 0.408$
- **Phenylalanine oxidation (µmol/kg/day)** vs. **Total AA intake (g/kg/day)**
  - $R^2 = 0.0005$

References:
Where are adults currently getting protein in healthy states

- Poultry: 14.4%
- Beef: 14.0%
- Cheese: 8.5%
- Milk: 6.9%
- Yeast bread/rolls: 6.4%
Does the source of protein alter kinetics? animal vs plant protein

- Total energy intake is important to factor in
- Plants with fewer EAA
  - EAA threshold up to 10 gm
    » Adding 20-40 gm more with no increase in myofibrillar protein
- Does plant protein with added extra EAA, BCAA offer the best of both worlds

### TABLE 2  PDCAAS of common protein foods

<table>
<thead>
<tr>
<th>Source</th>
<th>PDCAAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>1.00</td>
</tr>
<tr>
<td>Whey</td>
<td>1.00</td>
</tr>
<tr>
<td>Egg</td>
<td>1.00</td>
</tr>
<tr>
<td>Soy protein isolate</td>
<td>1.00</td>
</tr>
<tr>
<td>Casein</td>
<td>1.00</td>
</tr>
<tr>
<td>Beef</td>
<td>0.92</td>
</tr>
<tr>
<td>Soy</td>
<td>0.91</td>
</tr>
<tr>
<td>Pea</td>
<td>0.67</td>
</tr>
<tr>
<td>Oat</td>
<td>0.57</td>
</tr>
<tr>
<td>Whole wheat</td>
<td>0.45</td>
</tr>
</tbody>
</table>

PDCAAS = protein digestibility corrected AA score

van Vliet S et al J Nutrition 2015
Baum JI et al Nutrients 2016
Glutamine
(35 yrs of basic science and clinical trials)

- Most abundant free amino acid
  - > 60% of free AA pool in muscle
- Interorgan nitrogen transfer
  - Purines, pyrimidine, nucleotides, amino sugars etc
- Acid Base balance
- Key substrate for gluconeogenesis
- Critical for synthesis of GSH, Arginine, glucosamine
- Decreases insulin resistance
- Regulator of Heat Shock Protein
- Primary fuel for rapidly dividing cells
  - Enterocyte, lymphocytes

Remain very useful in GI disease, burns, wounds
Is Glutamine Falling from Stardom?

Newer glutamine PRCT’s in ICU:

- Braga CRS 2009 (no benefit)
- Scandinavian trial (2011) (no benefit)
- Scottish Trial (SIGNET) (2011) (no benefit)
- REDOX trial (NEJM 2013) (harmful)
  - Mortality 31% vs control 24%
- METAPLUS trial Glutamine ICU (2014) (harmful)

Why:

- Dosing ?, timing for delivery, route of delivery
- Delivered with other nutrients or as single agent ?
- Heterogeneity of population ?
- Where should glutamine be used in 2016
  - GI diseases, Burns, prevention or radiation or chemo
PEPT1 is the broad-specificity transporter of di- and tripeptides.

Note: H+ Na+ ATPase is driving force for PEPT1.
Di-peptide Form of L-glutamine for Superior Absorption

Harris, RC, et al., NBH: L-glutamine absorption is enhanced after ingestion of L-alanylglutamine compared with the free amino acid or wheat protein. Nutr Res 2012, doi:10.1016/j.nutres.2013.02.003.

AUC = Area under the curve
Other than “adequate” background energy do other specific dietary supplements support decreasing inflammation?

- **Specialized Pro-resolving Molecules (SPM’s)**
  - Propagates anabolism via;
    - Stimulates resolution of inflammation
    - Stimulates conversion from M1 to M2 macrophages
    - Cessation of macrophage infiltration
    - Enhances bacterial killing without increase inflammation

Serhan C Nature 2014
SPM’s present in most tissues tested to date

- Bioactive at levels of 20 to 200 picomolar
  - Serum in range of pg/ml (10^{-12})

- Serum (Serhan C et al Am J Physiol 2014)
- Human milk (Weiss et al 2013 Lipids in Health and Disease)
- Urine (Sasaki et al 2015 Annals Bioanal Chem)
- Lymph nodes (Colas et al 2014 Am J Physiology)
- Adipose tissue (Claria et al 2013 Am J Physiol Cell Physiol)

• Serhan C et al Am J Physiology 2014
Reported Benefits of Specialized Pro-resolving Molecules (SPM’s)

- **Enhance resolution of inflammation**
  - SPM’s *do not block inflammation* they resolve and modulate it
    » Highly conserved in evolution from planaria to human

- **Produced from DHA and EPA substrate**
  - SPM’s have physiologic activity at nano-pico gram range

- **Enhance bacterial killing of macrophages**

- **Accelerates removal of inflammatory debris**

- **Potentiates action of antibiotics**

- **Not immunosuppressive**

- **Decreases postop edema and pain**

Serhan C Nature 2014
SPM: Resolvins, Protectins and Maresins in Disease

**Lungs** Human & Mouse
- ATL, RvE1, PD1, MaR1
- ↓ Airway inflammation (asthma)

**Cardiovascular**
- RvE1, RvD1
- ↓ Platelet aggregation
- ↓ Atherosclerosis

**Eyes** Human & Mouse
- RvE1, PD1, RvD1
- ↓ Vaso-obliteration and neovascularization (Retinopathy)
- ↑ Wound healing (Cornea)

**Kidney**
- ATL, PD1, RvD1
- ↓ Renal ischemic injury

**Brain** Human & Mouse
- PD1, RvD1, RvD2
- ↓ Stroke damage and PMN entry into the brain
- ↑ Neural cell survival

**Oral** Rabbit & Mouse
- ATL, RvE1
- ↓ Inflammation-induced tissue and bone loss (Periodontitis)

**GI tract**
- RvD1, RvE1, RvD2
- ↓ PMN and weight loss
- ↑ Survival (Colitis, sepsis)

**Liver**
- RvE1, PD1, RvD1
- ↓ I/R injury
- ↑ Glucose and lipid homeostasis

**Glucose and lipid homeostasis**

**Protectins and Maresins**

**Anti-Inflammation Pro-Resolution Organ Protection**

**Resolvins**

**Lipoxins**

**Nanograms to micrograms**

**Human and Animal Disease Models**

CN Serhan et al., Nat Rev Immunol. 8, 349. 2015
Chronic

- **Asthma**

- **Atherosclerosis**

- **Retinal angiogenesis**

- **Obesity**
  - Claria et al. J. Immunology, 2012

- **Metabolic syndrome**
  - Barden AE et al Am J Clin Nutr 2015

- **Alzheimer’s Disease**
  - Wang X Alzheimers Dementia 2015

- **Periodontitis**
  - Cianci E et al Stem Cells Transplantation 2016

- **Rheumatologic disorders**
  - Headland SE et al Seminar Immunology 2015

- **IBD**
  - Corminboeuf O et al J Med Chem 2015
What should be in “gut rehab” formulations

- Research-Based Key Ingredients
- Micronutrients
- Fat Blend
- Carbohydrate Mix
- Protein Base
Practitioner Research Collaborators

Jennifer Stagg, ND
Whole Health Associates
Avon, CT

Robert Bonakdar, MD
Director of Pain Management at the Scripps Center for Integrative Medicine in La Jolla, California

Cory Rice, DO
Forney Wellness, Dallas, TX

Kevin Holder, MD
Center for Preventative Medicine, South Orange, NJ
Study Design

- Patients with Gut Dysfunction: Ulcerative colitis (UC), Crohn’s disease, Irritable bowel syndrome (IBS), or celiac disease

Took 2 servings/day of UltraGI Replenish for 6 weeks

Baseline

- Gastrointestinal Quality of Life Index (GIQLI) Questionnaire
- Study Product Dispensed
- Collect Stool at Home

Week 3 mid study visit to confirm protocol compliance

Week 6

- Collect Stool at Home
- Gastrointestinal Quality of Life Index (GIQLI) Questionnaire
- Unused Study Product Returned
Baseline cohort data

- **Weight**: 162.8 +/- 33.1 lbs
- **BMI**: 23.8 +/- 3.4 kg/m²

**Demographics:**
- **7 (58.3%)** males
- **5 (41.7%)** females
- **Age**: 31.4 (range 22-60)

**Gastrointestinal Quality of Life Index (GIQLI)**

**Stool Collection**
### GI Symptoms and Quality of Life Scores Improved Significantly

<table>
<thead>
<tr>
<th>GIQLI</th>
<th>Score Range</th>
<th>Results (Mean)</th>
<th>% Change</th>
<th>P-value (Paired T-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Score</strong></td>
<td>0-144</td>
<td>Baseline: 94.5 +/- 25.5</td>
<td>+ 20.8%</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study End: 109.4 +/- 19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GI Symptoms Domain</strong></td>
<td>0-76</td>
<td>Baseline: 53.3 +/- 10.3</td>
<td>+ 18.1%</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study End: 61.4 +/- 7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Social Function Domain</strong></td>
<td>0-16</td>
<td>Baseline: 10.7 +/- 3.8</td>
<td>+ 18.4%</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study End: 12.3 +/- 3.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Total score, GI symptom domain, and Social function domain scores improved
- Higher scores are consistent with better quality of life
- Additional domain (physical function and emotional function) scores were improved, but did not reach statistical significance
Significantly Increased Bifidobacterium

Patients with IBS and IBD are known to have lower levels of Bifidobacterium.
Lower levels of Bifidobacterium are associated with more severe IBS symptoms.

<table>
<thead>
<tr>
<th>Bifidobacterium spp.</th>
<th>Reference Range</th>
<th>Results (mean +/- SD)</th>
<th>% Change</th>
<th>p Value (Paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;=6.4E9</td>
<td>Baseline: 1.2E9 +/- 1.5E9 6 Weeks: 5.4E9 +/- 5.1E9</td>
<td>+1890.1% (19-fold increase)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

2’FL and IMO are key ingredients that are likely responsible for the increases in butyrate, SCFAs, and Bifidobacterium levels.
Significantly Enhanced Production of SCFAs Including Butyrate

<table>
<thead>
<tr>
<th></th>
<th>Reference Range</th>
<th>Results (mean +/- SD)</th>
<th>% Change</th>
<th>P-value (Paired T-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n-Butyrate Concentration</strong></td>
<td>≥ 3.6 micromol/g</td>
<td>Baseline: 8.9 +/- 4.2</td>
<td>+ 72.2 %</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 Weeks: 14.4 +/- 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SCFA (Total)</strong></td>
<td>≥ 23.3 micromol/g</td>
<td>Baseline: 46.3 +/- 13.3</td>
<td>+ 72.2 %</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 Weeks: 76.4 +/- 37.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Total SCFAs = Butyrate + Acetate + Propionate)

- Levels of bacterial strains known to produce butyrate also increased, but did not reach statistical significance
  - *F. prausnitzii* levels increased by 7-fold
  - *Roseburia* spp. levels increased by 18-fold
- The increased production of butyrate in the gut is a potential mechanism for the reduction in GI symptoms demonstrated in this study
## Case #1: 34 yo White & Native American Female

**Genova GI Effects® Comprehensive Stool Profile – Gastrointestinal Microbiome**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genova Reference Range</th>
<th>Baseline</th>
<th>Study End/6 Weeks</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCFA (Total)</td>
<td>≥23.3 micromol/g</td>
<td>22.1</td>
<td>34.9</td>
<td>+57.9%</td>
</tr>
<tr>
<td>n-Butyrate Concentration</td>
<td>≥3.6 micromol/g</td>
<td>2.8</td>
<td>6.1</td>
<td>+117.9%</td>
</tr>
<tr>
<td><em>Faecalibacterium praunitzii</em></td>
<td>5.8E7-4.7E9</td>
<td>7.5E7</td>
<td>2.5E8</td>
<td>+233.3%</td>
</tr>
<tr>
<td><em>Roseburia spp.</em></td>
<td>1.3E8-1.2E10</td>
<td>7.7E7</td>
<td>2.7E8</td>
<td>+250.6%</td>
</tr>
<tr>
<td><em>Bifidobacterium spp.</em></td>
<td>≤6.4E9</td>
<td>2.2E7</td>
<td>9.6E7</td>
<td>+336.4%</td>
</tr>
<tr>
<td>Potential Pathogens</td>
<td>None cultured</td>
<td>None cultured</td>
<td>None cultured</td>
<td>N/A</td>
</tr>
</tbody>
</table>
• **Case #2: 26 yo White Male with history of UC x 2 yrs taking prednisone 40mg QD x 9 months**

**Genova GI Effects® Comprehensive Stool Profile – Gastrointestinal Microbiome**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genova Reference Range</th>
<th>Baseline</th>
<th>Study End/6 Weeks</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCFA (Total)</strong></td>
<td>&gt;23.3 micromol/g</td>
<td>50.0</td>
<td>58.2</td>
<td>+ 16.4%</td>
</tr>
<tr>
<td><strong>n-Butyrate Concentration</strong></td>
<td>&gt;3.6 micromol/g</td>
<td>7.8</td>
<td>20.5</td>
<td>+ 162.8%</td>
</tr>
<tr>
<td><strong>Faecalibacterium prausnitzii</strong></td>
<td>5.8E7-4.7E9</td>
<td>3.8E8</td>
<td>2.7E10</td>
<td>+ 7005.3%</td>
</tr>
<tr>
<td><strong>Roseburia spp.</strong></td>
<td>1.3E8-1.2E10</td>
<td>7.2E8</td>
<td>1.4E10</td>
<td>+ 1844.4%</td>
</tr>
<tr>
<td><strong>Bifidobacterium spp.</strong></td>
<td>≤6.4E9</td>
<td>8.0E8</td>
<td>1.5E10</td>
<td>+ 1775.0%</td>
</tr>
<tr>
<td><strong>Potential Pathogens</strong></td>
<td>None cultured</td>
<td>None cultured</td>
<td>None cultured</td>
<td>N/A</td>
</tr>
</tbody>
</table>
UltraGI Replenish Open-Label Summary

- GI Symptoms and Quality of Life Scores Improved Significantly
- Significantly Increased Bifidobacterium (19 fold)
- Significantly Enhanced Production of SCFAs Including Butyrate By 72.2%
It time for a paradigm shift!

Supply adequate viable beneficial bacteria or a substrate which enhances these specific beneficial bacteria instead of trying to eliminate the pathogen?

“Bioecological control”