D.I.G. IN

Everything you need to know about testing for dysbiosis in the gut

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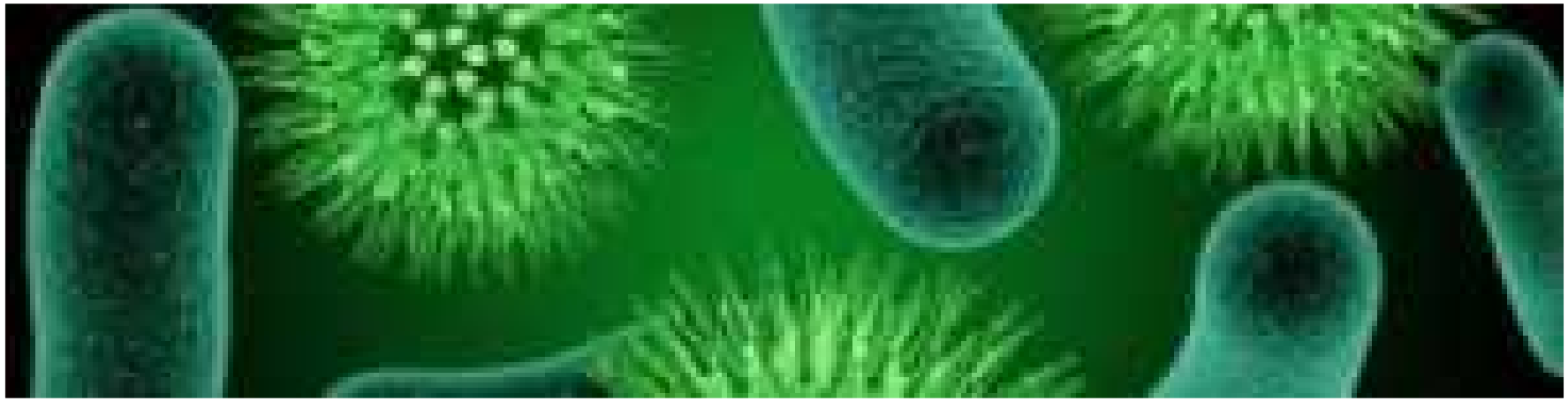
Relevant financial relationships in the past twelve months by presenter:

- None to disclose

Objectives

- Discuss the importance of microbial diversity in the gut
- Diagnose common gastrointestinal and immune system complaints through the DIG-IN acronym
- Review advanced laboratory testing options for mal-absorption, intestinal permeability, and nutritional assessment

So you think you're human?



Gut microbiota play important metabolic roles in many disease states, maintaining delicate balance and cross-talk with immune system.

The gut microbial ecosystem is largest endocrine organ in the body, capable of producing a wide range of biologically active compounds that, like hormones, may be carried in the circulation and distributed to distant sites within the host, thereby influencing different essential biological processes.

Metabolic Functions of Bacteria

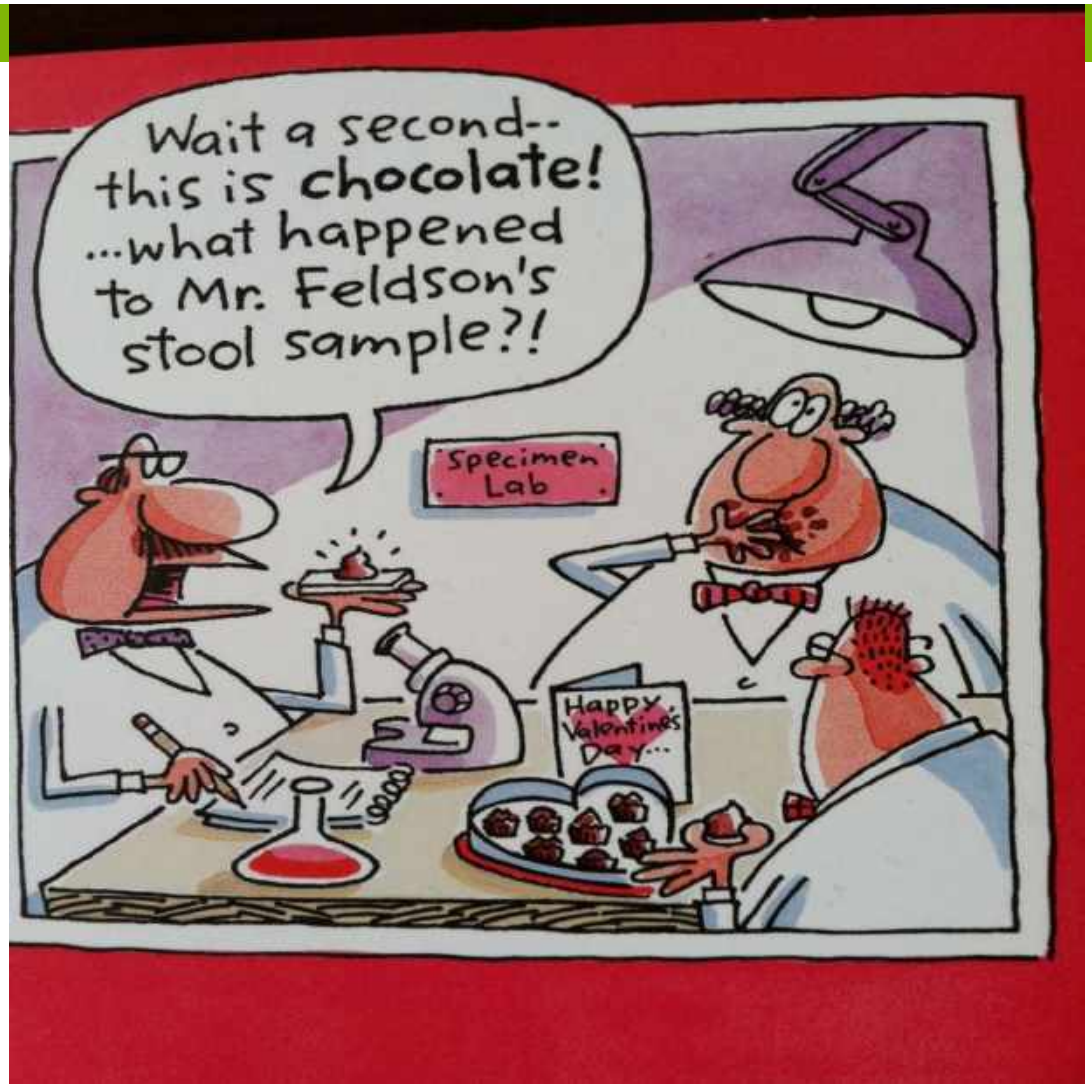
- Breakdown dietary fiber
- Gas production
- Fermentation
- Production of Phenols
- Breakdown of oligo-saccharides
- Detoxification
- Mucous production
- Short Chain Fatty Acids Metabolism
- Primary Bile Acid Deconjugation
- Vitamin absorption
- Fats, TG, Cholesterol regulation

Some of my best friends are germs…

Justin Sonnenburg, microbiologist at Stanford…

"We would do well to begin regarding the human body as "an elaborate vessel optimized for the growth and spread of our microbial inhabitants."

http://www.nytimes.com/2013/05/19/magazine/say-hello-to-the-100-trillion-bacteria-that-make-up-your-microbiome.html


Wait a second-- this is chocolate! ...what happened to Mr. Feldson's stool sample?!
Specimen Lab
Happy Valentine's Day...

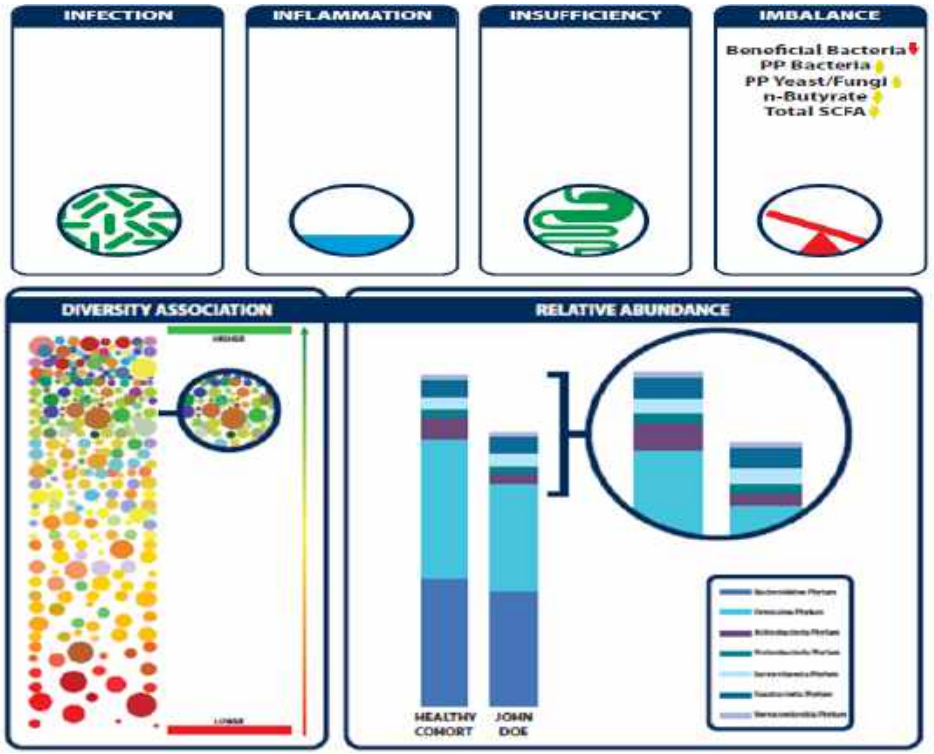
Who can benefit from stool analysis?

- Inflammatory bowel disease
- Skin conditions
- Fatigue
- Autoimmune disease
- IBS
- Metabolic syndrome,
- Diabetes
- Depression, anxiety, mood disorder
- Autistic spectrum disorders

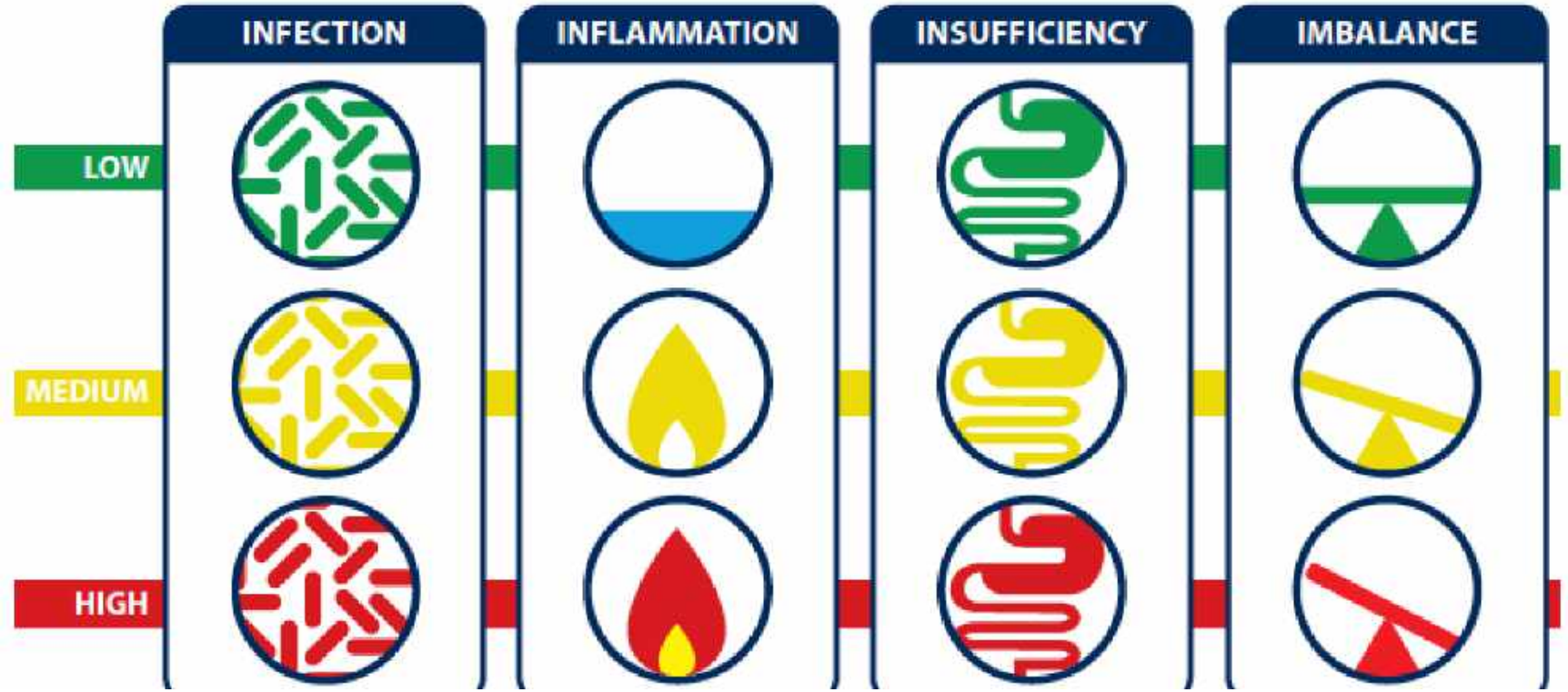
Stool Analysis

The four functional evaluate key clinical markers in four functional areas. **Infection, Inflammation, Insufficiency (Digestive), and Imbalance (Metabolic).**

Shows level of each individual biomarker and its degree of clinical impact. As result, an overall score of high, medium, or low is provided for each functional pillar.

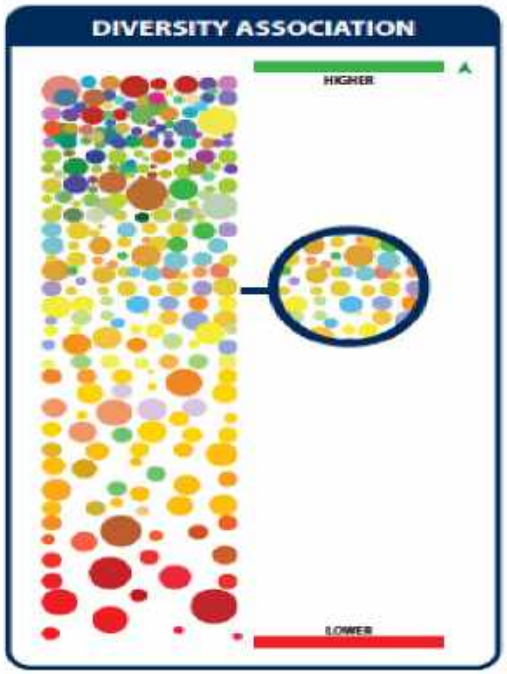


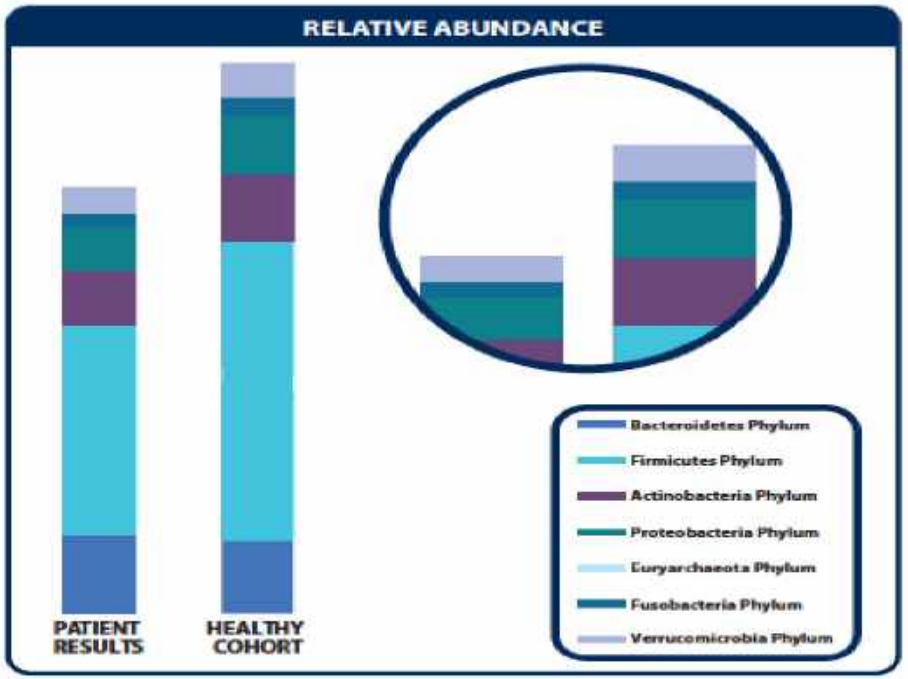
Four Functional Pillars



Four Functional Pillars Biomarker Map			
Infection Box	**Inflammation Box**	**Insufficiency Box**	**Imbalance Box**
any parasite present	Calprotectin	PE-1	n-Butyrate
any pathogen present	EPX	Total Fecal Fats	Total SCFA
	Fecal IgA	Total Protein Products	Beta-glucuronidase
			Lactobacillus
			Bifidobacterium
			E. coli
			any potential pathogen

Diversity and Relative Abundance






Importance of
Microbial Diversity

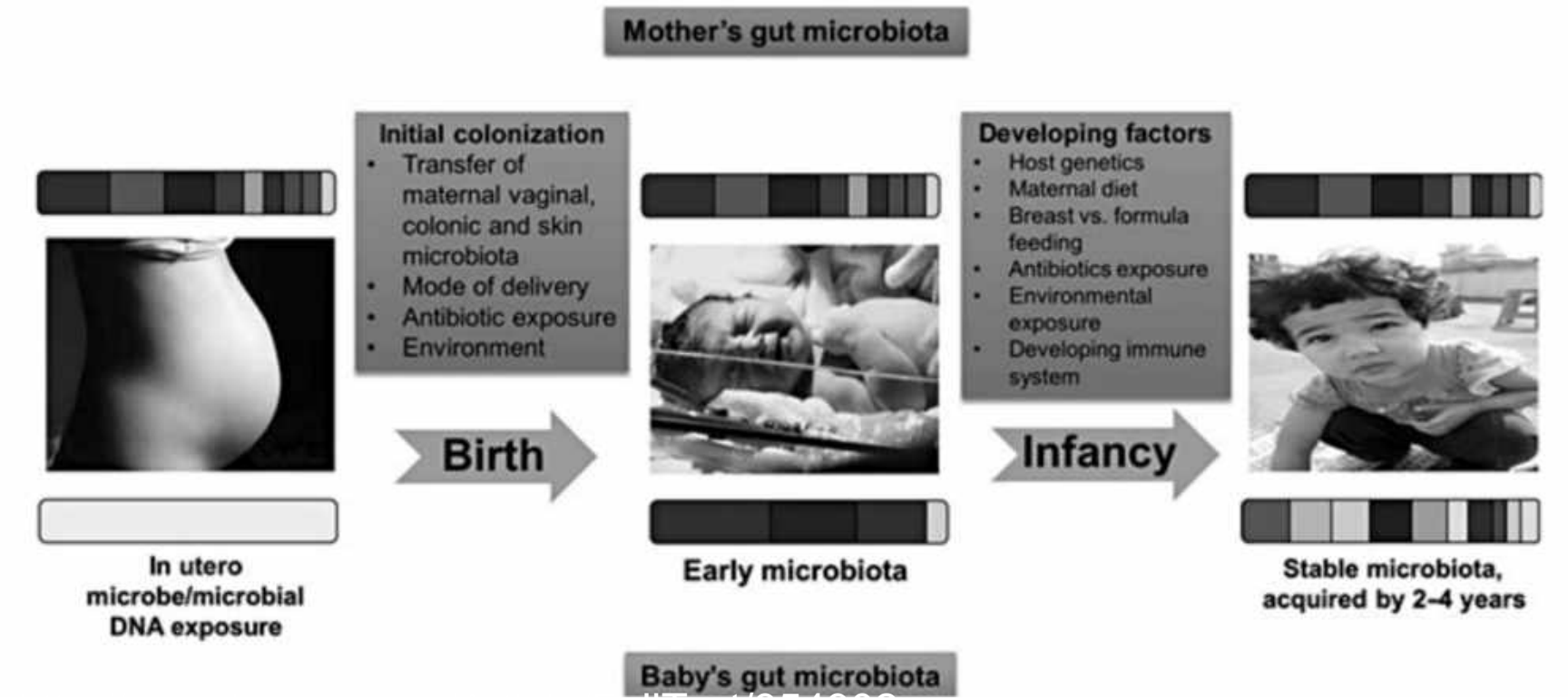
Importance of microbial biodiversity

- Greater microbial diversity associated with body's ability to deal with stressors, such as opportunistic pathogens or dietary perturbations
- Individuals with disease more likely to have alterations in gut microbiome compared to healthy controls
- Multiple associations between reduced microbial diversity and illness in literature.
- Low diversity risk factor for metabolic disease, like type 2 diabetes

Diversity begins at birth…

- Bacterial colonization during birth plays a major role in the formation of gut microbiota.
- Factors affecting microbiota include:
  - Premature birth,
  - Caesarean section versus vaginal birth
  - Breast milk versus commercial formula
- Infants born vaginally colonized similar to their mother's vaginal microbiota,
  - *Lactobacillus, Prevotella, or Sneathia spp*,
- Caesarean section born infants colonized by bacteria found on the skin surface
  - *Staphylococcus, Corynebacterium, and Propionibacterium species.*

Development of Neonate Microbiota



J Allergy Clin Immunol. 2011 Sep;128(3):646-52.e1-5. doi: 10.1016/j.jaci.2011.04.060. Epub 2011 Jul 22.

Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age.

Bisgaard H[1], Li N, Bonnelykke K, Chawes BL, Skov T, Paludan-Müller G, Stokholm J, Smith B, Krogfelt KA.

Author information

Abstract

BACKGROUND: Changes in the human microbiome have been suggested as a risk factor for a number of lifestyle-related disorders, such as atopic diseases, possibly through a modifying influence on immune maturation in infancy.

OBJECTIVES: We aimed to explore the association between neonatal fecal flora and the development of atopic disorders until age 6 years, hypothesizing that the diversity of the intestinal microbiota influences disease development.

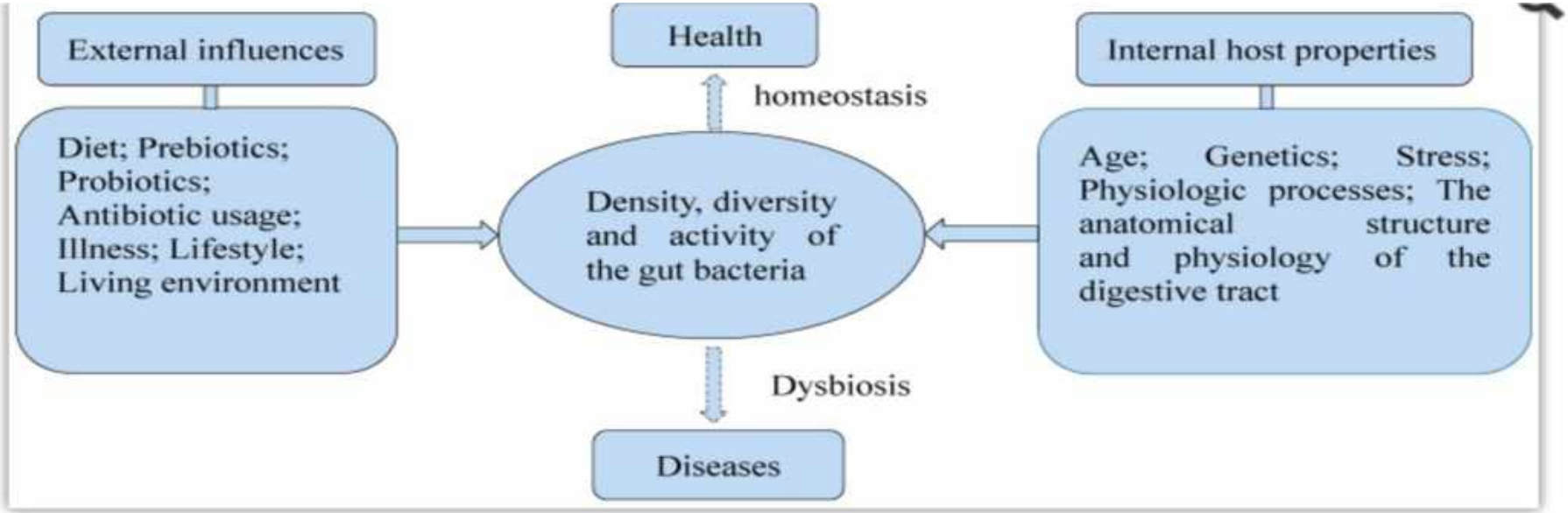
METHODS: We studied the intestinal microbiota in infants in the Copenhagen Prospective Study on Asthma in Childhood, a clinical study of a birth cohort of 411 high-risk children followed for 6 years by clinical assessments at 6-month intervals, as well as at acute symptom exacerbations. Bacterial flora was analyzed at 1 and 12 months of age by using molecular techniques based on 16S rRNA PCR combined with denaturing gradient gel electrophoresis, as well as conventional culturing. The main outcome measures were the development of allergic sensitization (skin test and specific serum IgE), allergic rhinitis, peripheral blood eosinophil counts, asthma, and atopic dermatitis during the first 6 years of life.

RESULTS: We found that bacterial diversity in the early intestinal flora 1 and 12 months after birth was inversely associated with the risk of allergic sensitization (serum specific IgE P = .003; skin prick test P = .017), peripheral blood eosinophils (P = .034), and allergic rhinitis (P = .007). There was no association with the development of asthma or atopic dermatitis.

CONCLUSIONS: Reduced bacterial diversity of the infant's intestinal flora was associated with increased risk of allergic sensitization, allergic rhinitis, and peri thesis that an imbalan

Reduced bacterial diversity of the infant's intestinal flora was associated with increased risk of allergic sensitization, allergic rhinitis, and peripheral blood eosinophilia

Many factors influence diversity…



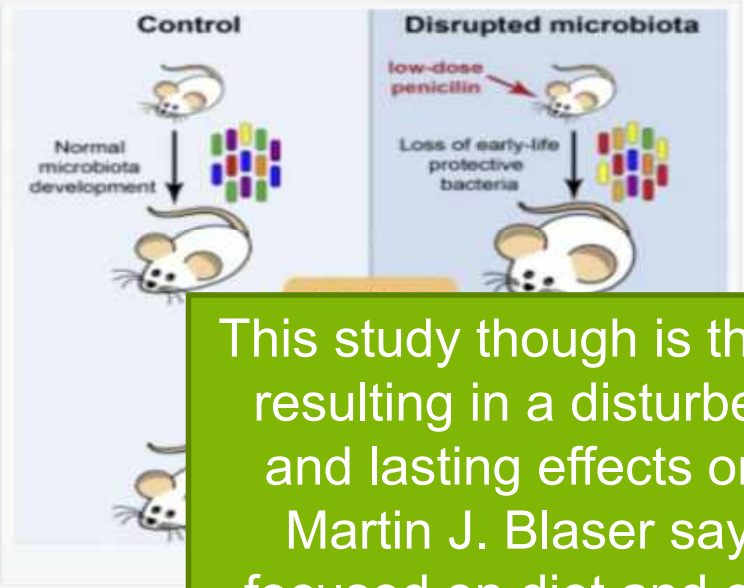
Several factors influence the density, diversity, and activity of the gut bacteria.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4425030/#!po=16.6667

Antibiotics in Children Lead to Disturbed Microbiome

Posted by Edward Marks on August 21, 2014 // Leave Your Comment

While the conversation surrounding obesity often points to lifestyle changes as the hallmark for prolonged weight management, new research points towards molecular mechanisms one culprit in increased BMI. For example, giving antibiotics to children leads to long-term metabolic consequences, primarily from disruption of the child's microbiome during development.



Microbiome is the catchall term for the set of microorganisms (bacteria, fungi, and viruses) in and on the human body. Each individual organ or body system, such as the mouth, gut, or feet, has its own microbiome. As most organisms reside in the gastrointestinal tract, most research has focused on altered states of the gut microbiome in relation to health and disease, particularly obesity.

This current study, out of NYU's Langone Medical Center, sought to determine the effect of orally

diet did gain we
weight. Mice fe
was discontinu
that did not receive LDP.

This study though is the first to reveal that early antibiotic use, resulting in a disturbed gut microbiome, can have profound and lasting effects on an organism's metabolic profile. Dr. Martin J. Blaser says, "A lot of work on obesity has been focused on diet and calories. That has not been sufficient to explain the obesity epidemic."

http://outbreaknewstoday.com/antibiotics-in-children-lead-to-disturbed-microbiome-87515/

Antibiotics in early life alter the murine colonic microbiome and adiposity

Ilseung Cho,[1,2] Shingo Yamanishi,[1] Laura Cox,[3] Barbara A. Methé,[4] Jiri Zavadil,[5,6] Kelvin Li,[4] Zhan Gao,[3] Douglas Mahana,[3] Kartik Raju,[3] Isabel Teitler,[3] Huilin Li,[7] Alexander V Alekseyenko,[1,6] and Martin J Blaser[1,2,3]

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The publisher's final edited version of this article is available at Nature
See other articles in PMC that cite the published article.

Abstract

Antibiotics ad
industry since
different class
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colonic short-chain fatty acid levels, and alterations in the regulation of hepatic metabolism of lipids and cholesterol. In this model, we demonstrate the alteration of early-life murine metabolic homeostasis through antibiotic manipulation.

Administration of subtherapeutic antibiotic therapy increased adiposity in young mice and increased hormones related to metabolism. [There were] substantial changes in the microbiome, changes in genes involved in the metabolism of carbohydrates to short-chain fatty acids, increases in colonic short-chain fatty acid levels, and alterations in the regulation of hepatic metabolism of lipids and cholesterol.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3553221/

The effect of past antibiotic exposure on diabetes risk

Ben Boursi, Ronac Mamtani, Kevin Haynes and Yu-Xiao Yang

Author Affiliations

Correspondence: Yu-Xiao Yang, Email: yangy@mail.med.upenn.edu

Abstract

Objective:
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Research
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follow-up
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(BMI), smo
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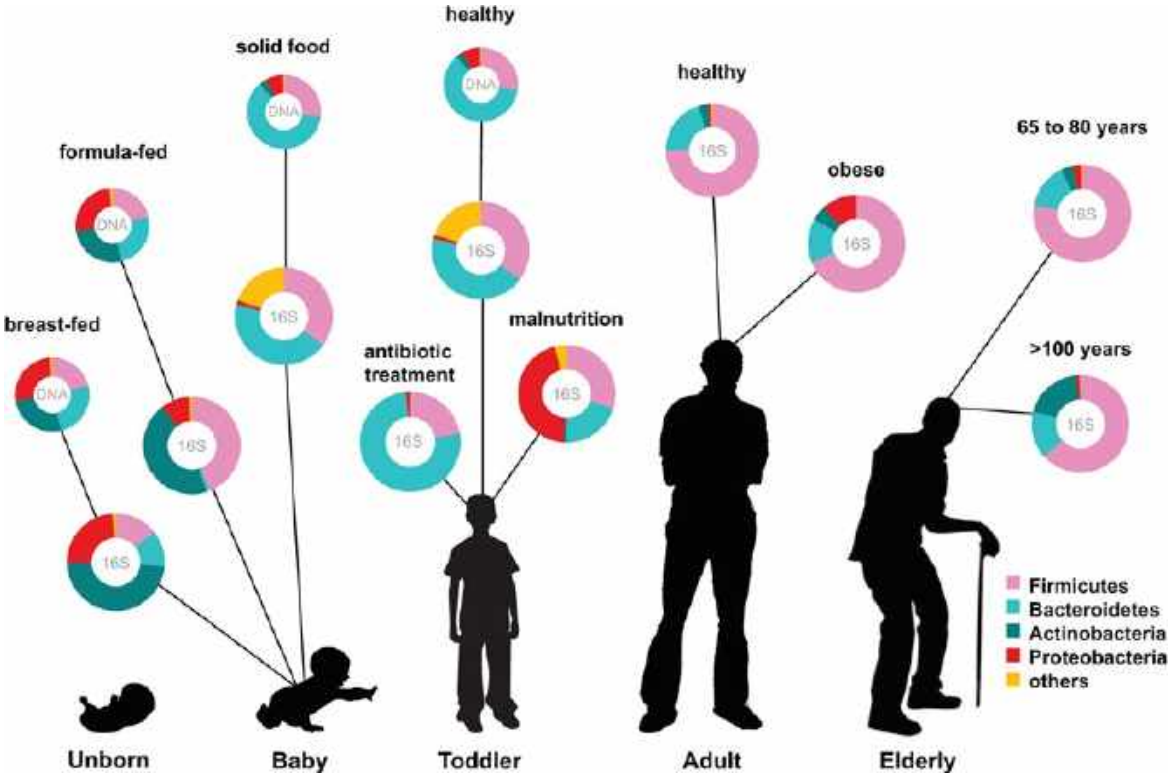
Exposure to a single antibiotic prescription was not associated with higher risk. Treatment with 2-5 antibiotic courses associated with increase in diabetic risk for penicillin, cephalosporins, macrolides and quinolones

The risk increased with the number of antibiotic courses and reached 1.37 (95%CI 1.19-1.58) for >5 courses of quinolones. There was no association between exposure to anti-virals and anti-fungals and diabetes risk.

http://www.eje-online.org/content/early/2015/03/24/EJE-14-1163.abstract

Microbes change as we age…

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日本語要約
Microbiology: Wealth management in the gut
Sungsoon I
Affiliations
Nature 500,
Published or
Those with low diversity of bacteria have higher levels of body fat and inflammation than those with high gut-microbial richness.

Richness of human gut microbiome correlates with metabolic markers

Emmanuelle Le Chatelier, Trine Nielsen, Junjie Qin, Edi Prifti, Falk Hildebrand, Gwen Falony, Mathieu Almeida, Manimozhiyan Arumugam, Jean-Michel Batto, Sean Kennedy, Pierre Leonard, Junhua Li, Kristoffer Burgdorf, Niels Grarup, Torben Jørgensen, Ivan Brandslund, Henrik Bjørn Nielsen, Agnieszka S. Juncker, Marcelo Bertalan, Florence Levenez, Nicolas Pons, Simon Rasmussen, Shinichi Sunagawa, Julien Tap, Sebastian Tims + *et al.*

Affiliations | Contributions | Corresponding authors

Nature **500**, 541–546 (29 August 2013) | doi:10.1038/nature12506
Received 10 April 2012 | Accepted 26 July 2013 | Published online 28 August 2013

Le Chatelier Study

- Study participants (n=292) characterized into two groups by the number of gut microbial genes (gut bacterial richness) with an average 40% difference between low gene count (LGC) individuals and high gene count (HCG) individuals.
- **Individuals with low bacterial gene richness (23% of study population) characterized by increase in adiposity, insulin resistance, and dyslipidaemia.**
- Low-bacterial-richness individuals showed a more pronounced inflammatory phenotype when compared with high-bacterial-richness individuals.

Dietary intervention impact on gut microbial gene richness

Aurélie Cotillard, Sean P. Kennedy, Ling Chun Kong, Edi Prifti, Nicolas Pons, Emmanuelle Le Chatelier, Mathieu Almeida, Benoit Quinquis, Florence Levenez, Nathalie Galleron, Sophie Gougis, Salwa Rizkalla, Jean-Michel Batto, Pierre Renault, ANR MicroObes consortium, Joel Doré, Jean-Daniel Zucker, Karine Clément, Stanislav Dusko Ehrlich, Hervé Blottière, Marion Leclerc, Catherine Juste, Tomas de Wouters, Patricia Lepage, Charlene Fouqueray + *et al.*

Affiliations | Contributions | Corresponding authors

Nature **50**
Received
Corrigend

Consumption of high-fiber foods, such as fruit and vegetables, led to increase in bacterial richness and improved clinical symptoms associated with obesity.
Support previous work linking diet to the composition of gut microbe populations, and suggests that a permanent change might be achieved by appropriate diet

NATURE | LETTER

Diet rapidly and reproducibly alters the human gut microbiome

Lawrence A. David, Corinne F. Maurice, Rachel N. Carmody, David B. Gootenberg, Julie E. Button, Benjamin E. Wolfe, Alisha V. Ling, A. Sloan Devlin, Yug Varma, Michael A. Fischbach, Sudha B. Biddinger, Rachel J. Dutton & Peter J. Turnbaugh

Affiliations | Contributions | Corresponding author

Nature (2013) | doi

Received 18 April 20

Short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression

http://www.nature.com/nature/journal/vaop/ncurrent/full/nature12820.html

Science translational medicine

Author Manuscript

NIH Public Access

The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice

Peter J. Turnbaugh, Vanessa K. Ridaura, [...], and Jeffrey I. Gordon

Additional article informatio

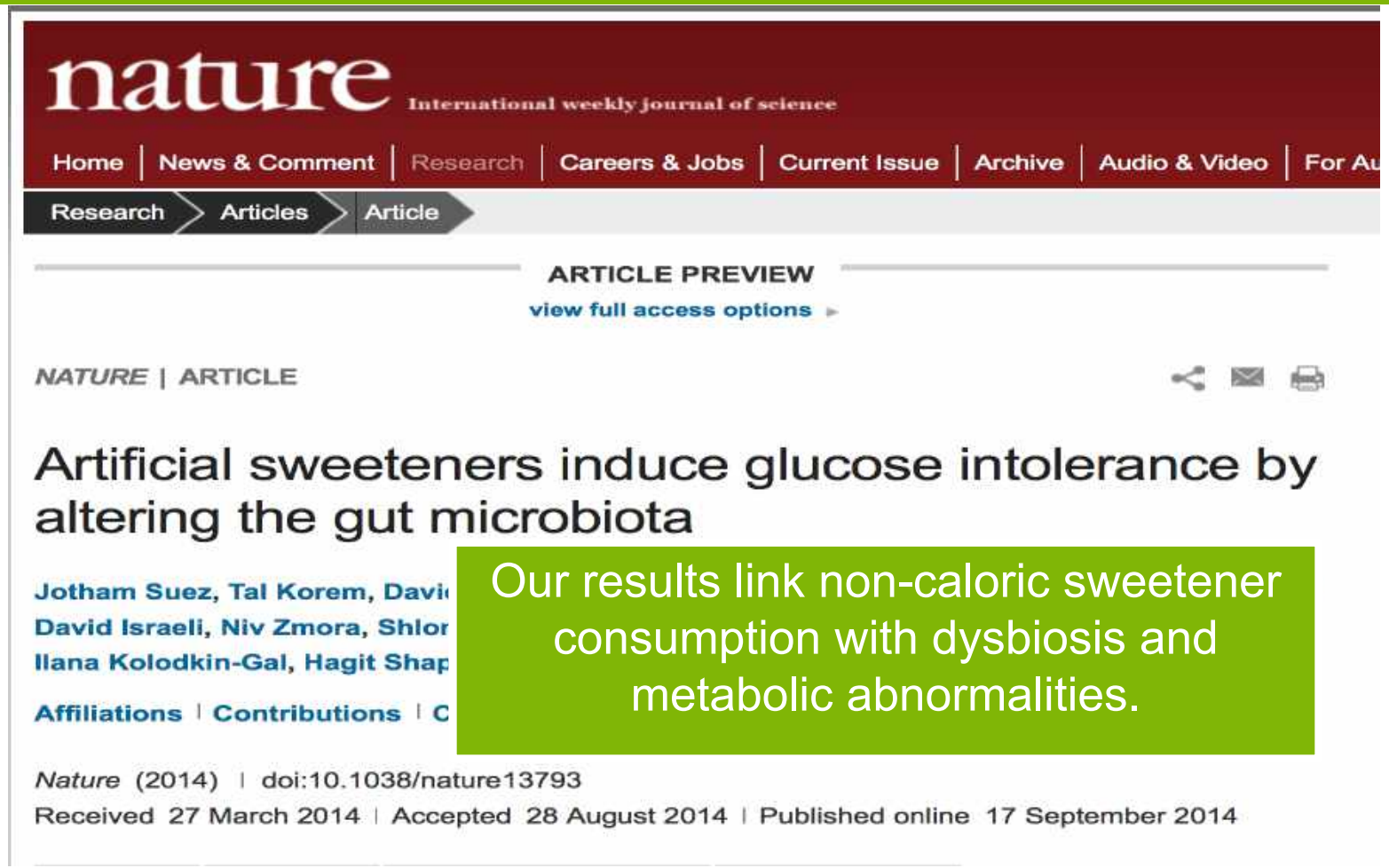
Going from a low fat, plant polysaccharide rich diet to a high fat, high sugar Western diet changed the microbiota in one day in GF mice

Abstract

Diet and nutritional sta

determinants of human health. The nutritional value of food is influenced in part by a person's gut microbial community (microbiota) and its component genes (microbiome). Unraveling the interrelationships between diet, the structure and operations of the gut microbiota, and nutrient and energy harvest is confounded by variations in human environmental exposures, microbial ecology and

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2894525/



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ARTICLE PREVIEW

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NATURE | ARTICLE

Artificial sweeteners induce glucose intolerance by altering the gut microbiota

Jotham Suez, Tal Korem, Davi
David Israeli, Niv Zmora, Shlor
Ilana Kolodkin-Gal, Hagit Shap

Affiliations | Contributions | C

Our results link non-caloric sweetener consumption with dysbiosis and metabolic abnormalities.

Nature (2014) | doi:10.1038/nature13793
Received 27 March 2014 | Accepted 28 August 2014 | Published online 17 September 2014

Coffee Consumption Affects Microbiome

Theresa E. Cowan, Marie S.A. Palmnäs, Jaeun Yang, Marc R. Bomhof, Kendra L. Ardell, Raylene A. Reimer, Hans J. Vogel, Jane Shearer

Received 15 October 2013; received in revised form 19 December 2013; accepted 23 December 2013. published online 03 February 2014.

Abstract | Full Text | PDF | Images | References | Supplemental Materials

Abstract

Epidemiological data conf
diabetes. Coffee is initially
The bioavailability, produc
of this study was to deter
changes induced by a hig
fat) diet. Each group was
was associated with decr
composition, rats displayed profound systemic insulin resistance, likely due to caffeine. Coffee consumption attenuated the increase in Firmicutes (F)-to-Bacteroidetes (B) ratio and *Clostridium* Cluster XI normally associated with high-fat feeding but also resulted in augmented levels of Enterobacteria. In the serum metabolome, coffee had a distinct impact, increasing levels of

Coffee consumption attenuated the increase in Firmicutes to Bacteroidetes ratio and normally associated with high-fat feeding ....
Coffee increased levels of short-chain fatty acids while lowering levels of branched-chain amino acids.

J Proteome Res. 2012 Oct 5;11(10):4781-90. doi: 10.1021/pr300581s. Epub 2012 Sep 6.

Metabolomics view on gut microbiome modulation by polyphenol-rich foods.

Moco S[1], Martin FP, Rezzi S.

Author information

Abstract

Health is influenced by genetic, lifestyle, and diet determinants; therefore, nutrition plays an essential role in health management. Still, the substantiation of nutritional health benefits is challenged by the intrinsic macro- and micronutrient complexity of foods and individual responses. Evidence of healthy effects of food requires new strategies not only to stratify populations according to their metabolic requirements but also to predict and measure individual responses to dietary intakes. The influence of the gut microbiome and its interaction with the host is pivotal to understand nutrition and metabolism. Thus, the modulation of the gut microbiome composition by alteration of food habits has potentialities in health improvement or even disease prevention. Dietary polyphenols are naturally occurring constituents in vegetables and fruits, including coffee and cocoa. They are commonly associated to health benefits, although mechanistic evidence in vivo is not yet fully understood. Polyphenols are extensively metabolized by gut bacteria into a complex series of end-products that support a significant effect on the functional ecology of symbiotic partners that can affect the host

on th

PMID

Modulation of the gut microbiome by alteration of food habits has potential for disease prevention. Dietary polyphenols naturally occurring in coffee and cocoa… are extensively metabolized by gut bacteria into anti-inflammatory end-products

Published on March 27th, 2014 | *By: April Gocha, PhD* 0

Thank your microbiome—gut microbes are behind chocolate's health benefits

The good microbes, such as *Bifidobacterium* and lactic acid bacteria, feast on chocolate. When you eat dark chocolate, they grow and ferment it, producing compounds that are anti-inflammatory…

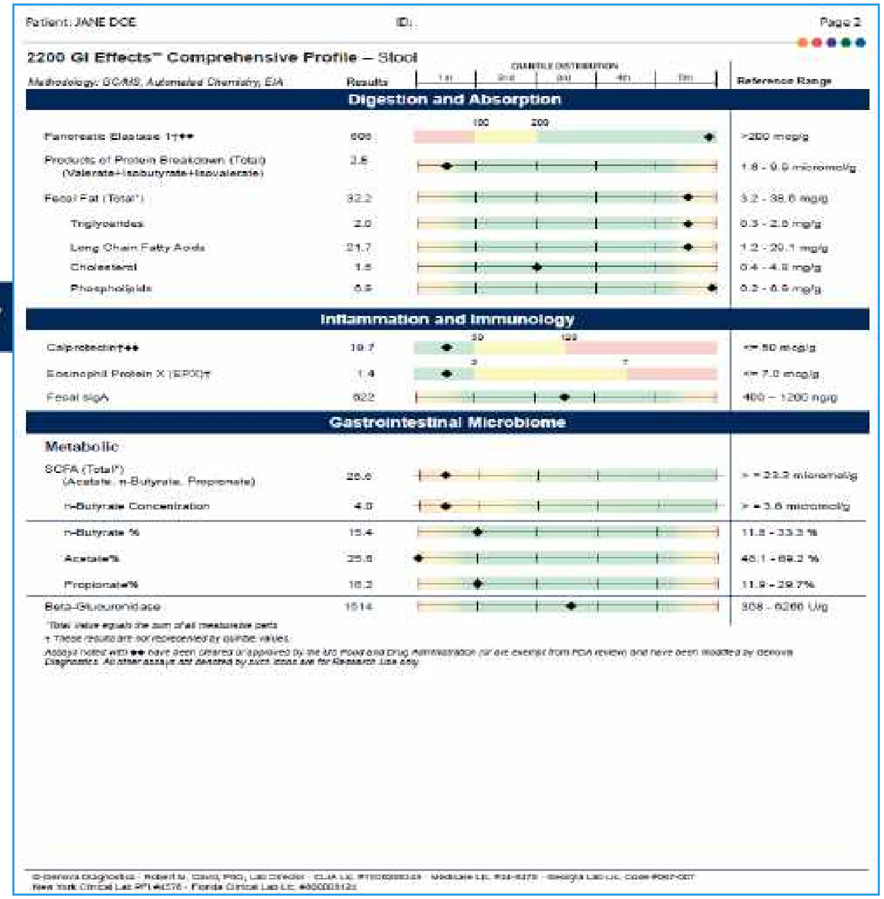
http://www.acs.org/content/acs/en/pressroom/newsreleases/2014/march/the-precise-reason-for-the-health-benefits-of-dark-chocolate-mystery-solved.html

→ **Digestion and Absorption**

→ **Inflammation and Immunology**

→ **Gastrointestinal Microbiome**

D.I.G.


Patient: JANE DOE ID: Page 2

2200 GI Effects™ Comprehensive Profile – Stool

Methodology: GC/MS, Automated Chemistry, EIA

	Results	Quintile Distribution	Reference Range
Digestion and Absorption			
Pancreatic Elastase 1†◆◆	606		>200 mcg/g
Products of Protein Breakdown (Total) (Valerate+Isobutyrate+Isovalerate)	2.6		1.8 - 9.9 micromol/g
Fecal Fat (Total*)	32.2		3.2 - 38.6 mg/g
Triglycerides	2.0		0.3 - 2.8 mg/g
Long Chain Fatty Acids	21.7		1.2 - 29.1 mg/g
Cholesterol	1.5		0.4 - 4.8 mg/g
Phospholipids	6.5		0.2 - 6.9 mg/g
Inflammation and Immunology			
Calprotectin†◆◆	19.7		<= 50 mcg/g
Eosinophil Protein X (EPX)†	1.4		<= 7.0 mcg/g
Fecal sIgA	822		400 – 1200 ng/g
Gastrointestinal Microbiome			
Metabolic			
SCFA (Total*) (Acetate, n-Butyrate, Propionate)	26.6		> = 23.3 micromol/g
n-Butyrate Concentration	4.0		> = 3.6 micromol/g
n-Butyrate %	15.4		11.8 - 33.3 %
Acetate%	25.8		48.1 - 69.2 %
Propionate%	16.2		11.8 - 29.7%
Beta-Glucuronidase	1514		368 - 6266 U/g

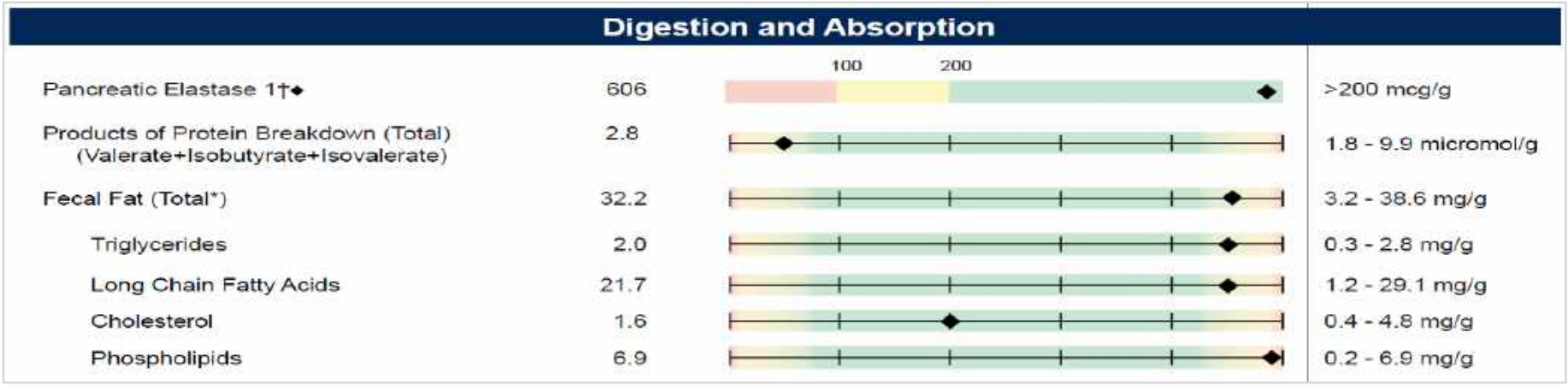
*Total value equals the sum of all measurable parts

† These results are not represented by quintile values.

Assays noted with ◆◆ have been cleared or approved by the US Food and Drug Administration (or are exempt from FDA review) and have been modified by Genova Diagnostics. All other assays not denoted by such icons are for Research Use Only.

DIGESTION AND ABSORPTION

D = Digestion and Absorption



- Pancreatic Elastase 1
- Products of Protein Breakdown (Putrifactive SCFAs)
- Fecal Fat

Pancreatic Elastase

- Proteolytic enzyme secreted exclusively by the human pancreas
- Reflects overall enzyme production
  - amylase, lipase and protease
- Not affected by supplemental enzymes
- Non-invasive marker for evaluating exocrine pancreatic function
  - Sensitivity = 90 -100%
  - Specificity = 93 - 98%

1. **Stein J, et al. *Clin Chem* 1996 Feb;42(2):222-6**
2. **Loser C, Mollgaard A, Folsch UR. *Gut* 1996;39(4):580-6**

Pancreatic Elastase

> 350 µg/g	Normal pancreatic function
200-350 µg/g	Declining pancreatic function Consider supplementation
100-200 µg/g	Moderate pancreatic insufficiency Supplement with broad array of pancreatic enzymes
<100 µg/g	Severe pancreatic insufficiency Supplement with broad array of pancreatic enzymes

Pancreatic Elastase

- Can be used for initial determination of pancreatic einsufficiency and to monitor function in patients under treatment.
- Patients in whom testing may be useful include
  - Unexplained diarrhea
  - Weight loss
  - Other signs of malabsorpton
  - Abdominal pain
- Pancreatic Exocrine dysfunction may occur secondary to
  - Chronic Pancreatitis, diabetes, celiac disease, inflammatory bowel disease, Cystic fibrosis, alcohol consumption, gallstone disease

Notes on PE1 interpretation

- Fecal PE1 testing may have reduced sensitivity for detecting mild pancreatic exocrine insufficiency in children
- Consumption of vegetarian or vegan diets, or other diets involving decreased meat intake, have been associated with reductions in fecal PE1
- Pancreatic exocrine insufficiency occurs in about 50% of type 1 diabetics, and in about 33% of type 2 diabetics.
- Chronic pancreatitis patients may have compromised antioxidant systems.

Pancreatic Elastase Treatment

- Smoking cessation
- Reduced alcohol consumption
- Small frequent meals
- Replace fat soluble vitamins
- Supplemental lipase or pancreatic enzymes
  - Choose Pancreatin
  - Plant-based are not strong enough for severe EPI
  - Prescription strength enzymes
    - Delayed Release Pancrelipase

Rule out EPI in celiac disease and SIBO

Exocrine pancreatic insufficiency, MRI of the pancreas and serum nutritional markers in patients with coeliac disease

Miroslav Vujasinovic[1],

+ Author Affiliations

Correspondence to

Dr Miroslav Vujasinovic, Department of internal Medicine, General Hospital Slovenj Gradec, Gosposvetska 1, Slovenj Gradec 2380, Slovenia; mvujas@gmail.com

Received 21 January 2015

EPI should be excluded in all patients with CD in the presence of overt malnutrition or in cases of persistent gastrointestinal symptoms despite a

Products of Protein Breakdown

- Inadequate protein digestion & fermentation by anaerobic bacteria
  - Causes
    - Low hydrochloric acid (HCL)
    - Protease insufficiency
- Small Intestinal Bacterial Overgrowth (SIBO)
  - Bloating immediately after meals, especially carbohydrate-rich meal
  - Intolerance to fructose (low FODMAPS diet)

Organic Acid Testing

- **Indoleacetic Acid (IAA)**
  - Incomplete digestion of tryptophan - allows colonic bacteria to proliferate

- **Phenylacetic Acid (PAA)**
  - May indicate gastric hypochlorhydria or pepsin inacti)

1-Methylhistidine

- Low levels:
  - Vegetarians, low protein consumption
- High levels
  - High dietary intake of animal protein
  - Catabolism of muscle tissue or post strenuous physical exercise
  - Certain muscle wasting conditions

Poor breakdown of proteins in diet…

Suspect Hypochlorhydria

Causes of low stomach acid

- Advanced age (30% of elderly)
- Use of proton pump inhibitors
- Autoimmunity, fasting chronic medical conditions

Symptoms

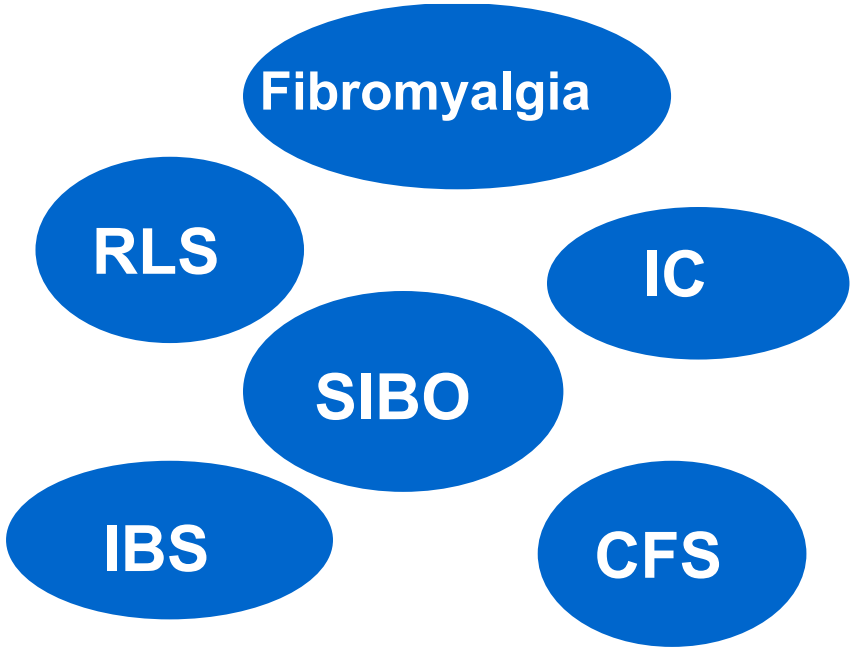
- Bloating/belching after meals
- Intolerance for protein
- Rectal itching
- Weak peeling or cracked fingernails/vertical ridges
- Adult acne and Rosacea
- Undigested food in stool

Consequences of low HCl

- Small Intestinal Bacterial Overgrowth
- Dysbiosis – altered gut bacteria
- Chronic candida Infections
- Mineral Deficiencies
  - Ca, Mg, Zn, Fe, Cr, Mo, Mn, Cu
- B_{12} deficiency
- Unexplained low ferritin or anemia

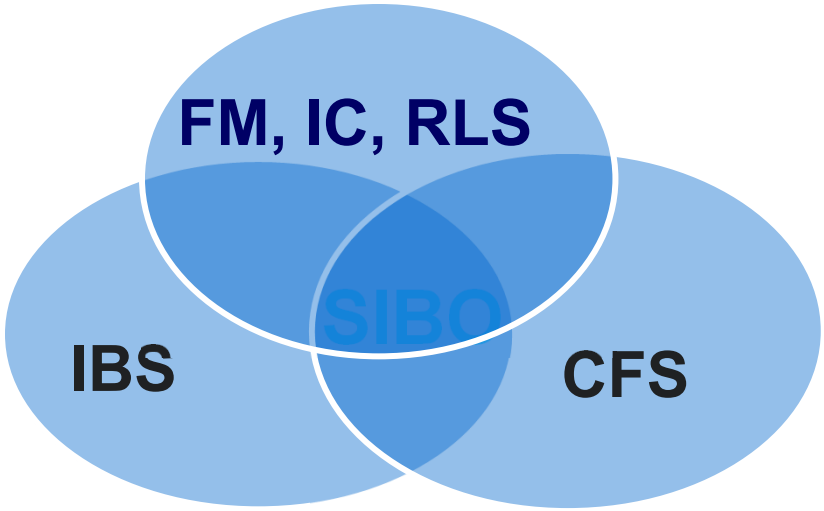
Current Thought Process

Individual Conditions



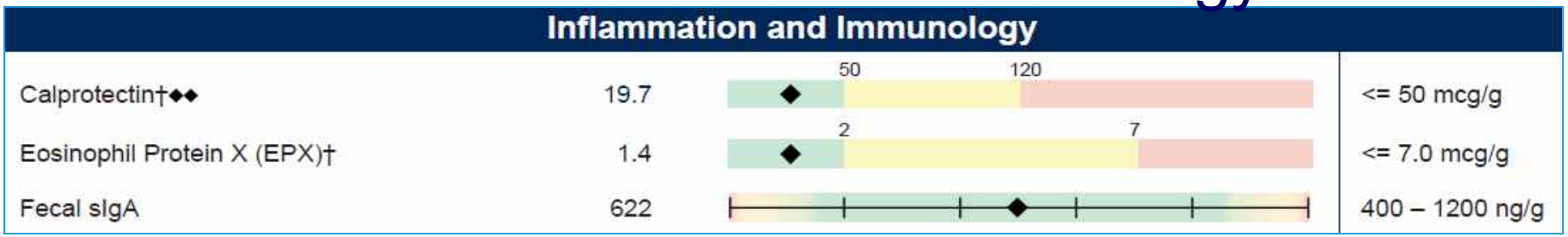
Future Outlook

SIBO Overlap



INFLAMMATION AND IMMUNOLOGY

I = Inflammation and Immunology

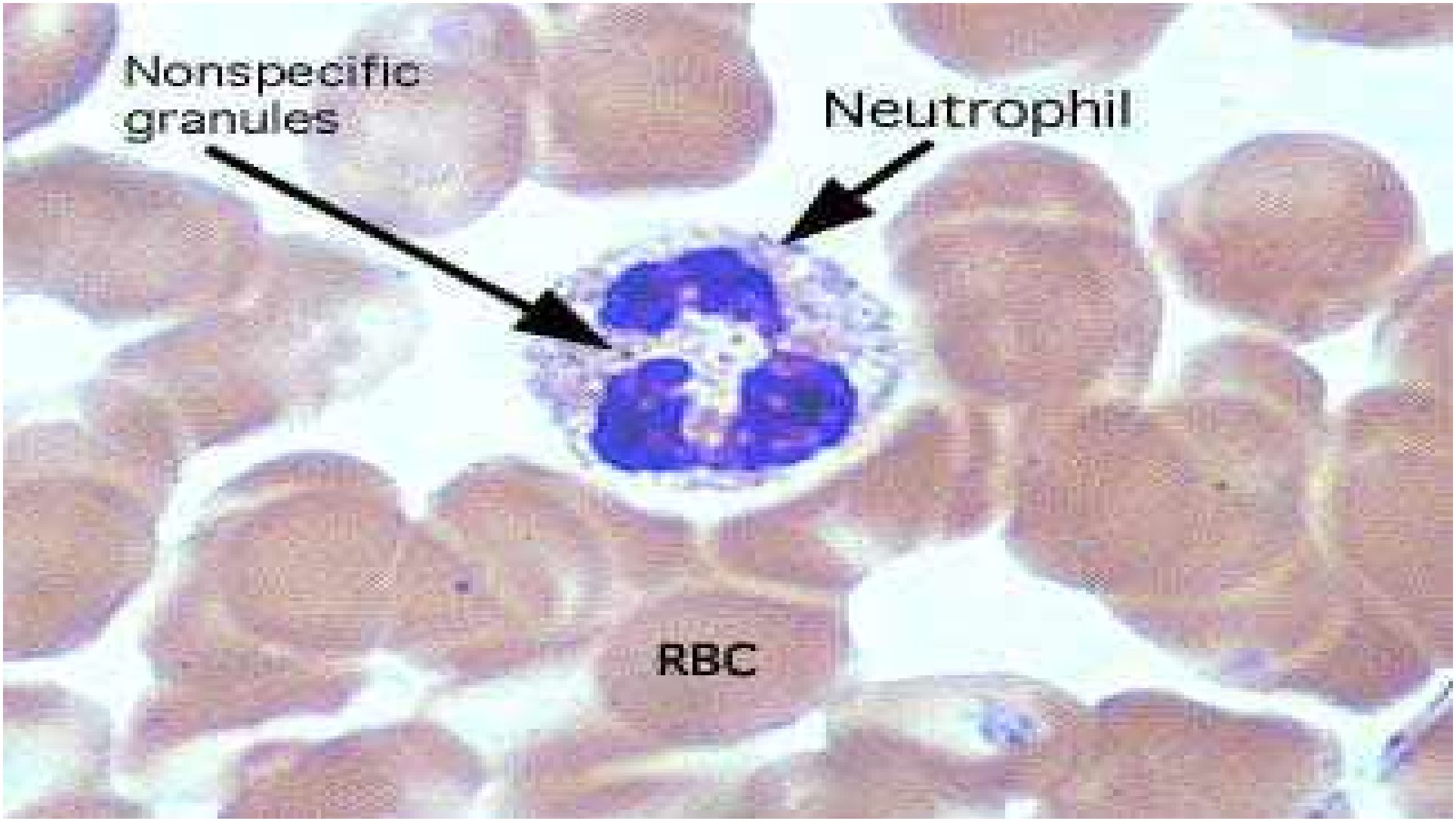


- Calprotectin and EPX primary markers of inflammation
- Fecal sIgA
- Lactoferrin available as Add-On

Calprotectin

- Found in extra lysosomal cytosol of the neutrophil
- Accounts for ~ 60% of the cytosolic protein
- Inhibitory effect on zinc dependent enzymes
- Bacteriostatic activity

Dale I, et al. *Am J Clin Pathol* 1985;84:24-34
Brun JG, et al. *Scand J Immuno,* 1994;40:675-680


Nonspecific granules
Neutrophil
RBC

Calprotectin

- Elevated in:
  - Inflammatory Bowel Disease
  - Post-Infectious Irritable Bowel Syndrome
  - Gastrointestinal cancers
  - Certain gastrointestinal infections
  - NSAID enteropathy
  - Food allergy

Poullis A et al. *J Gastroenterol Hepatol 2003;18:756-762*

  - Chronic Pancreatitis

Use Calprotectin to Differentiate IBD vs. IBS

A person with positive Rome criteria and a normal Calprotectin (< 50 µg/g) has virtually

NO CHANCE OF HAVING IBD!

FDA-cleared biomarker Calprotectin is highly accurate and capable of differentiating IBS from IBD

Calprotectin

- A meta-analysis published in 2010 provides a useful calculation of potential interest to payers, as well as to clinicians and patients.

- This paper, which evaluated 13 studies from the primary literature, found that in adults being evaluated for IBD, ***screening by measuring calprotectin levels would produce a 67% reduction in the number of adults undergoing endoscopy.***

van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ (Clinical research ed.* 2010;341:c3369.

Calprotectin: know when it's SERIOUS

< 50 µg/g	No significant inflammation
50-120 µg/g	Indicates some GI inflammation: IBD, infection, polyps, neoplasia, NSAIDS
> 120 µg/g	Significant inflammation; referral may be indicated to determine pathology
> 250 µg/g	Active disease present; predicts imminent relapse in treated patients

Tibble J, Teahon K, Thjodleifsson B, et al. Gut 2000;47:506-513.

Eosinophilic Protein X

- Released in eosinophil degranulation
- Sensitive marker of GI inflammation
- May predict relapse in IBD
- Stable in transport up to 7 days
- Sensitive marker for low-level inflammation

Eosinophilic Protein X

- May be elevated with:
  - Inflammatory Bowel Disease
  - Celiac Disease
  - Parasites
  - Allergic reaction
  - Less common
    - GERD
    - Chronic diarrhea
    - Chronic alcoholism
    - Protein-Losing Enteropathy

Fecal IgA

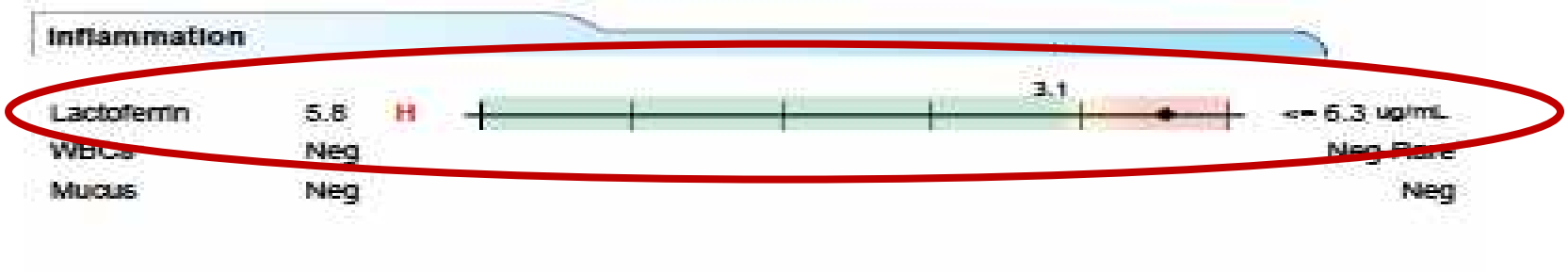
LOW SIGA

- CAUSES
  - Chronic stress
  - Dysbiosis
  - Immunocompromised
- **TREATMENT: Support mucosa…**
  - L-glutamine,
  - Probiotics – bifido sp.
  - S.boulardii
  - Colostrum or IgG (Enterogam)
  - Fatty Acids
  - Zinc

HIGH SIGA

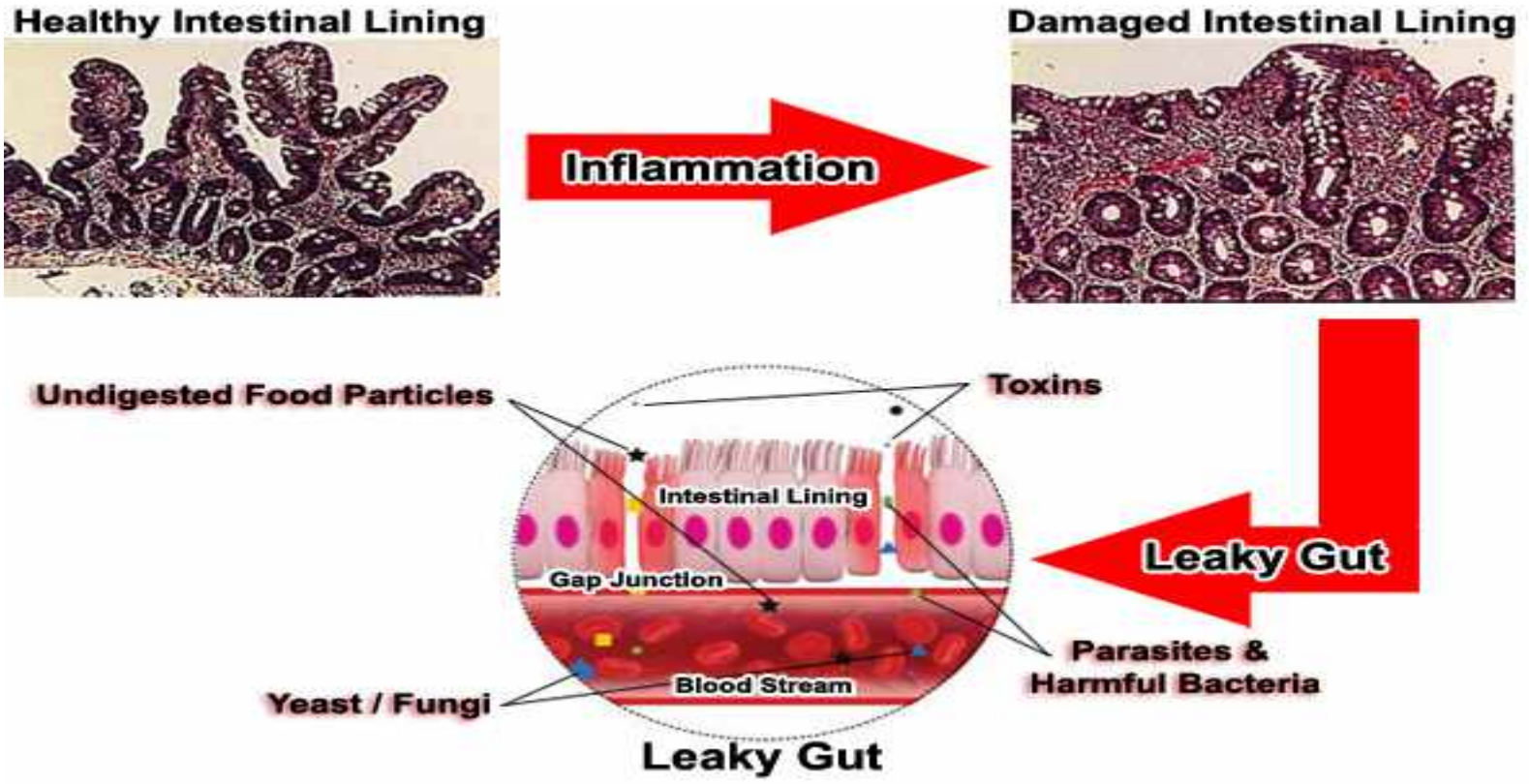
- CAUSES
  - Response to eliminate pathogens in GI tract
  - Sensitivities to foods
- **TREATMENT**
  - Immune support
  - Remove pathogens, parasites, bacteria, yeast
  - Rule out food sensitivities
  - Elimination diet

Lactoferrin



Lactoferrin

- Causes
  - Mucosal inflammation
  - Bacterial or yeast overgrowth
  - Parasite infection
  - Inflammatory bowel disease
- Treatment
  - Remove pathogens
  - Prebiotics & probiotics
  - Enhance endogenous immune system (sIgA) w/ l-glutamine, saccromyces boulardii or colostrum
  - Anti-inflammatory herbs – tumeric, ginger, EPA/DHA, quercetin, antioxidants


Healthy Intestinal Lining
Inflammation
Damaged Intestinal Lining
Undigested Food Particles
Toxins
Intestinal Lining
Gap Junction
Leaky Gut
Parasites &
Harmful Bacteria
Blood Stream
Yeast / Fungi
Leaky Gut

GASTROINTESTINAL MICROBIOME & METABOLIC MARKERS

Intestinal Dysbiosis

- A state of imbalanced microbial ecology that contributes to disease

- The overgrowth of micro-organisms of low intrinsic virulence induces disease by altering
  - the nutritional status
  - the immune response
  - the elimination capacity of the host

Many causes of dysbiosis

- SAD – low fiber, high in fat & simple carbs
- Broad-spectrum antibiotics
- Chronic maldigestion (including PPIs)
- Chronic constipation
- Stress suppresses Lactobacillus, Bifidobacteria, and sIgA
- Catecholamines stimulate growth of gram-negative organisms (Yersinia, Pseudomonas)
  - 45-50% of total body production of norepinephrine occurs in mesenteric organs
- Anger or fear increases Bacteroides fragilis

Proposed causes of dysbiosis of the microbiota

The composition of microbiota can shape a healthy immune response or predispose to disease

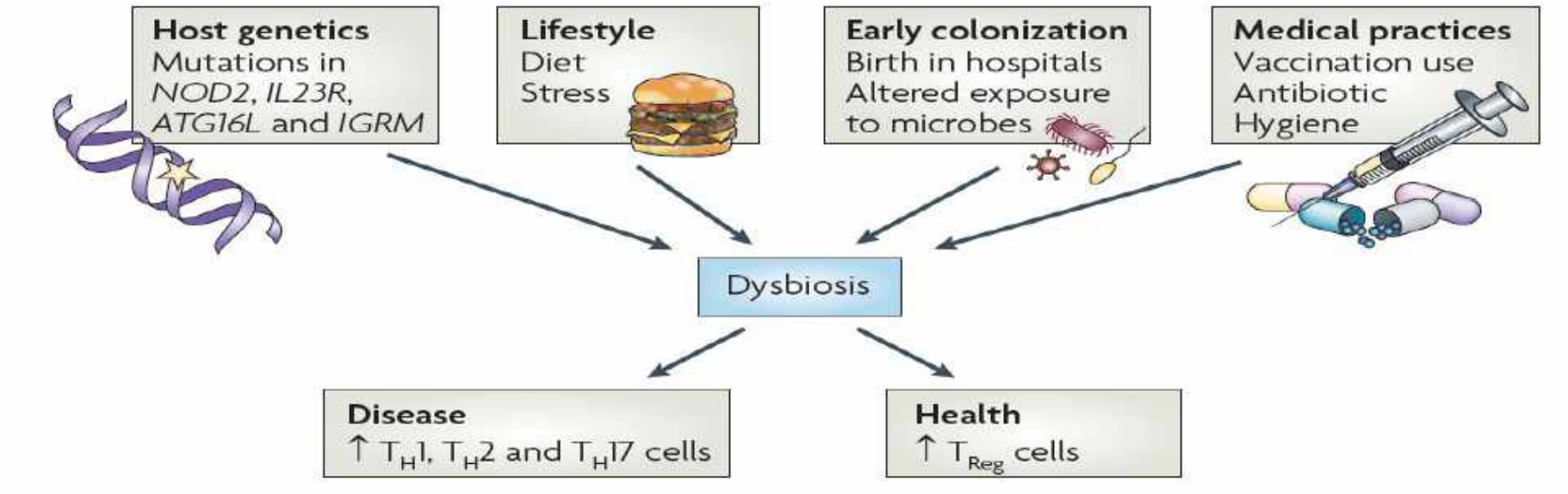
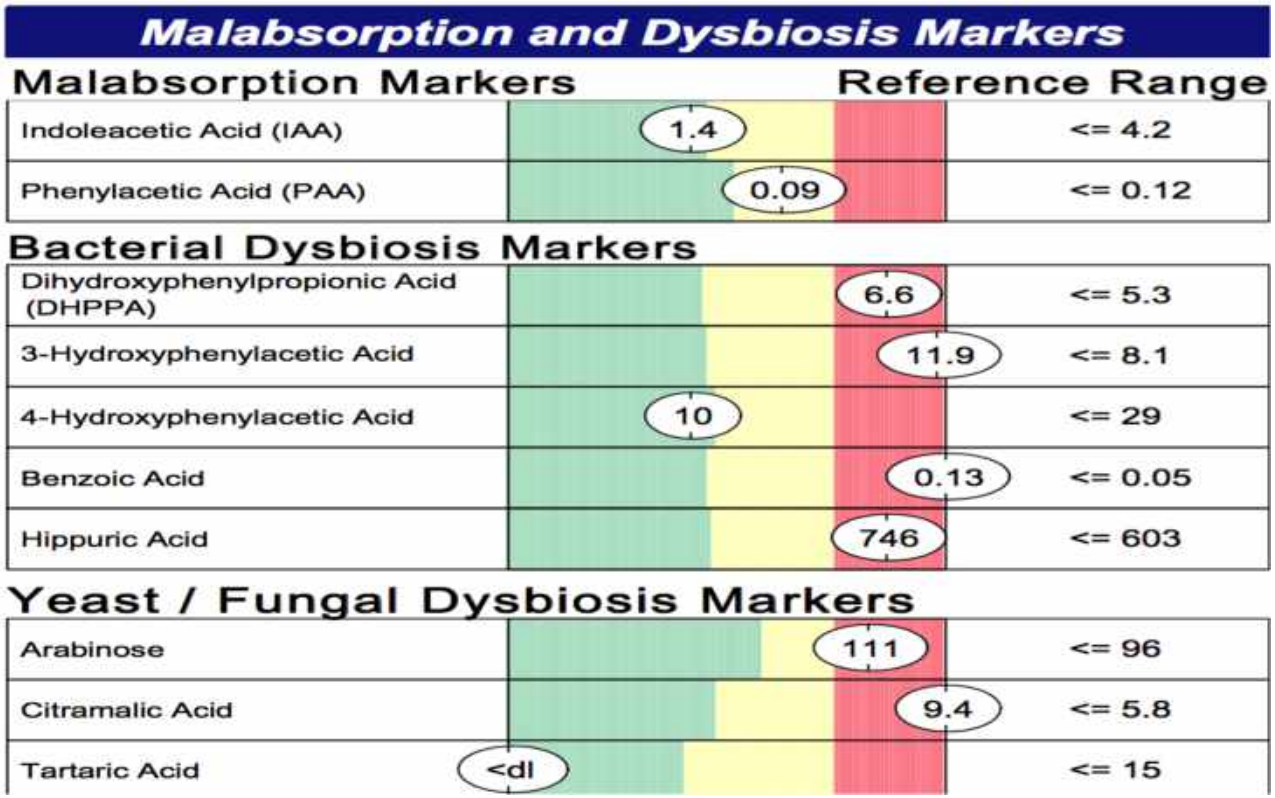


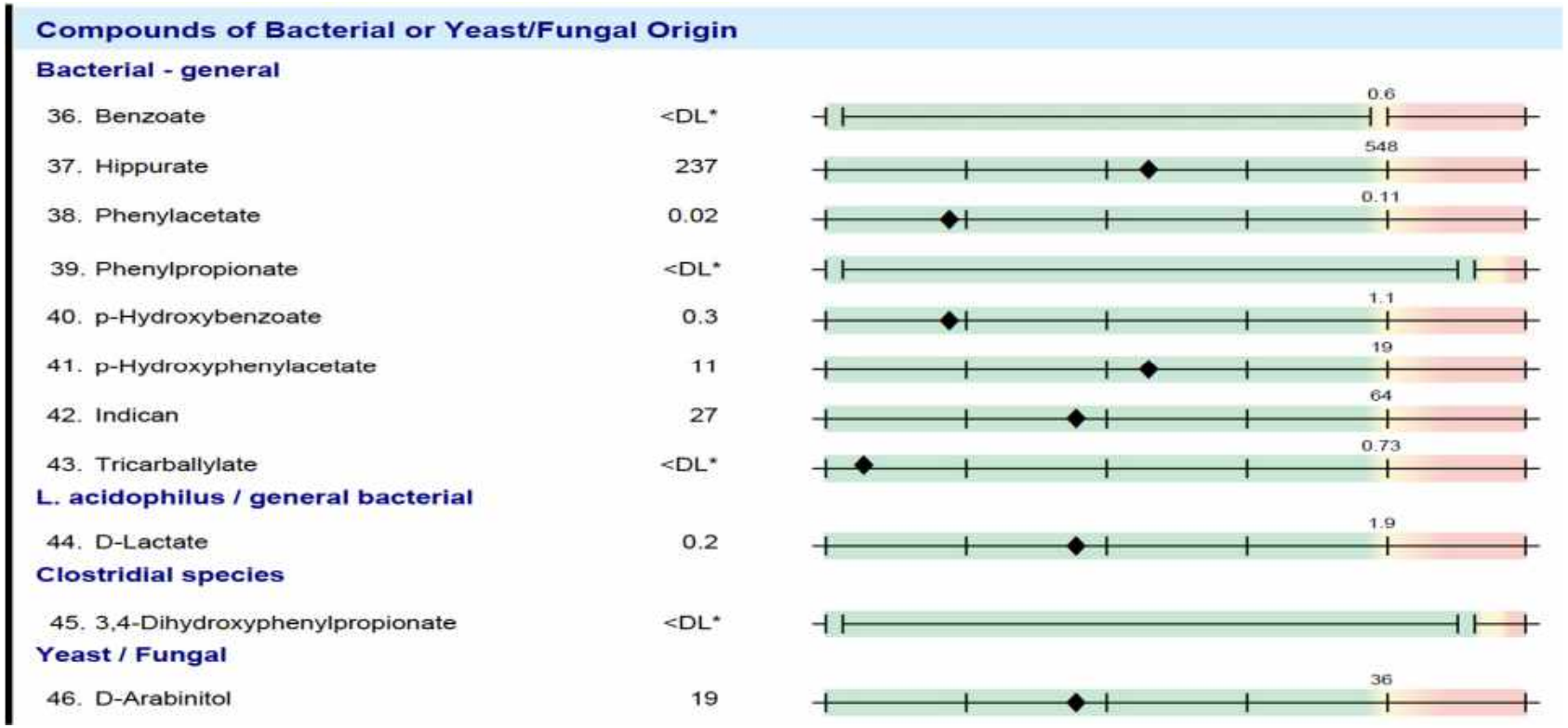
Figure 1 | Influence of gut microbial communities on health. Most of the microbial activities indicated in the centre column are functions of the whole community of gut microbiota rather than being attributable to a single species. The balance of the community and its output determines the net contribution to health or disease. Abbreviation: SCFA, short-chain fatty acid.

Flint, H. J. *et al.* The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* 2012.

Use Organic Acid Testing to Detect Dysbiosis

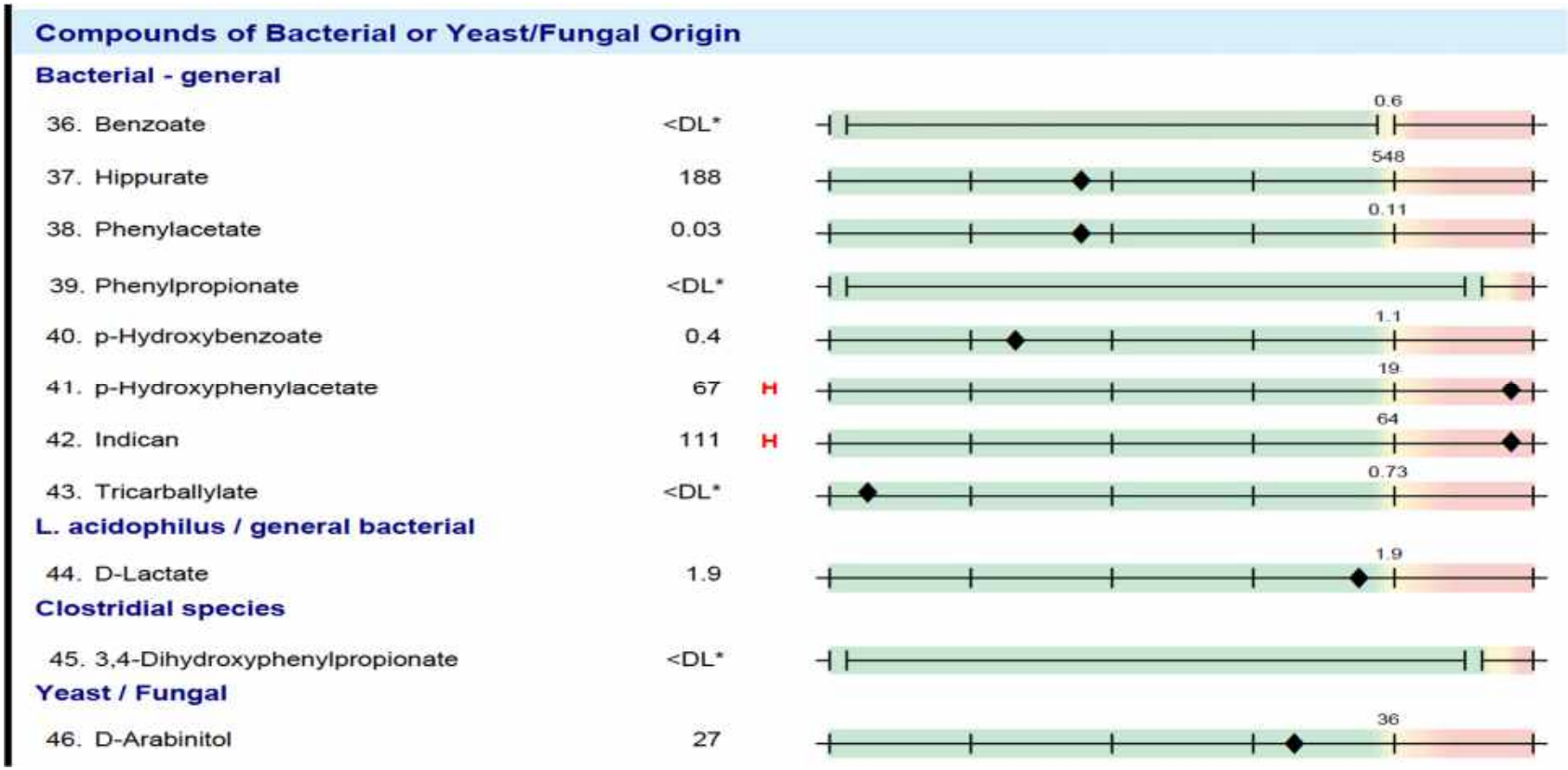


Use Organic Acid Testing to Detect Dysbiosis



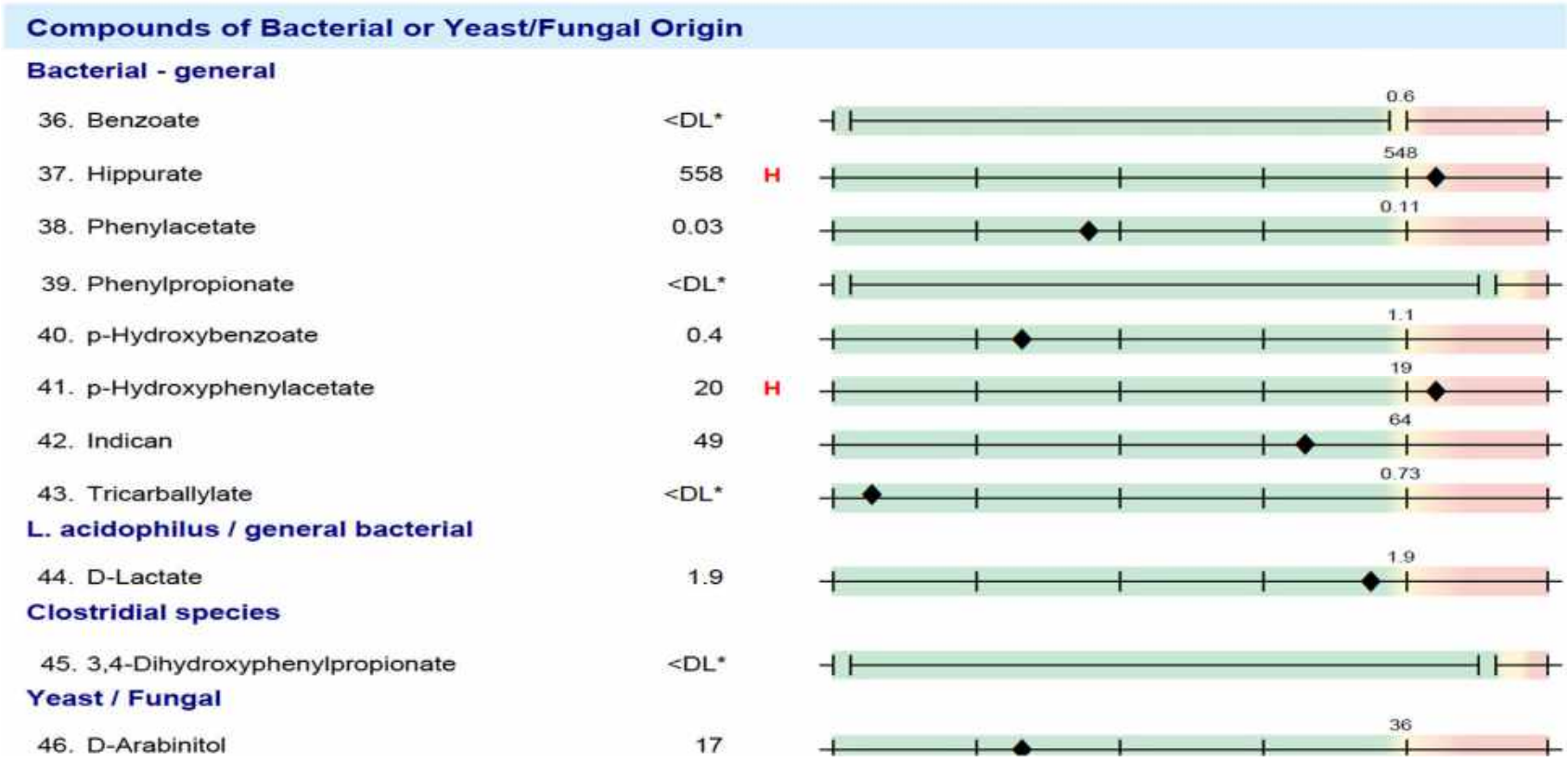
Compounds of Bacterial or Yeast/Fungal Origin		
Bacterial - general		
36. Benzoate	<DL*	0.6
37. Hippurate	237	548
38. Phenylacetate	0.02	0.11
39. Phenylpropionate	<DL*	
40. p-Hydroxybenzoate	0.3	1.1
41. p-Hydroxyphenylacetate	11	19
42. Indican	27	64
43. Tricarballylate	<DL*	0.73
L. acidophilus / general bacterial		
44. D-Lactate	0.2	1.9
Clostridial species		
45. 3,4-Dihydroxyphenylpropionate	<DL*	
Yeast / Fungal		
46. D-Arabinitol	19	36

Use Organic Acid Testing to Detect Dysbiosis

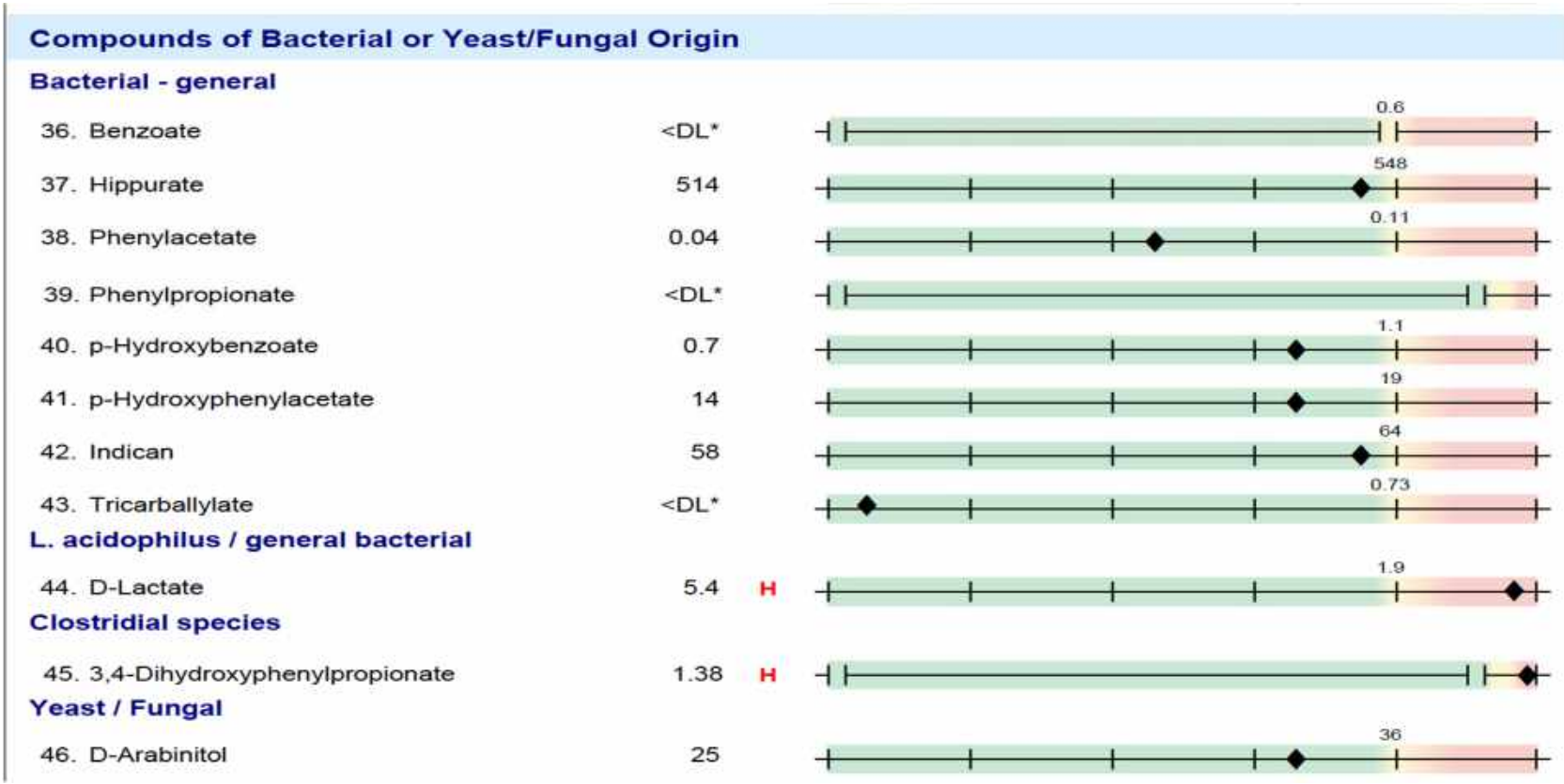


Compounds of Bacterial or Yeast/Fungal Origin			
Bacterial - general			
36. Benzoate	<DL*		0.6
37. Hippurate	188		548
38. Phenylacetate	0.03		0.11
39. Phenylpropionate	<DL*		
40. p-Hydroxybenzoate	0.4		1.1
41. p-Hydroxyphenylacetate	67	H	19
42. Indican	111	H	64
43. Tricarballylate	<DL*		0.73
L. acidophilus / general bacterial			
44. D-Lactate	1.9		1.9
Clostridial species			
45. 3,4-Dihydroxyphenylpropionate	<DL*		
Yeast / Fungal			
46. D-Arabinitol	27		36

Use Organic Acid Testing to Detect Dysbiosis

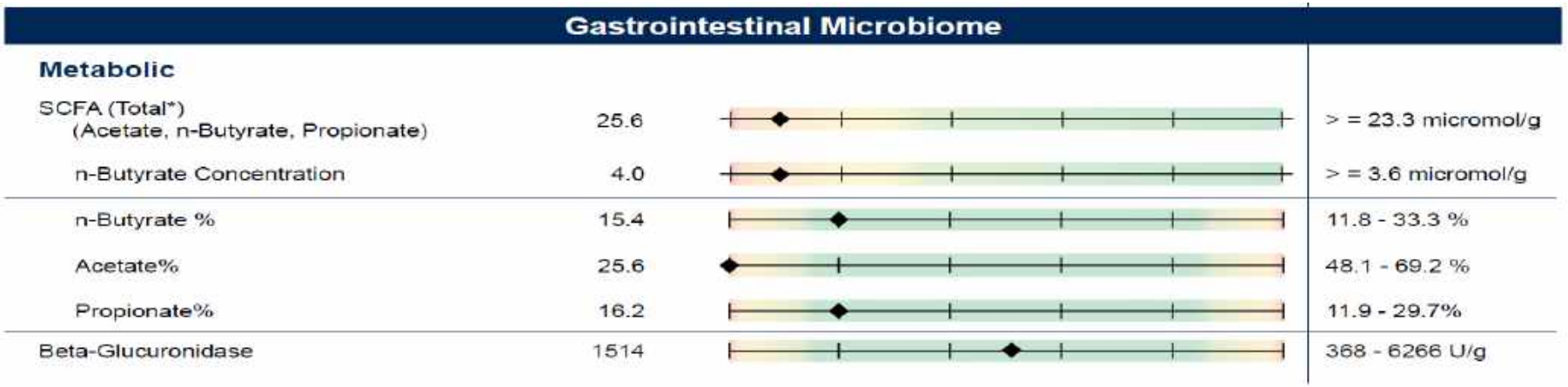


Use Organic Acid Testing to Detect Dysbiosis



Compounds of Bacterial or Yeast/Fungal Origin			
Bacterial - general			
36. Benzoate	<DL*		0.6
37. Hippurate	514		548
38. Phenylacetate	0.04		0.11
39. Phenylpropionate	<DL*		
40. p-Hydroxybenzoate	0.7		1.1
41. p-Hydroxyphenylacetate	14		19
42. Indican	58		64
43. Tricarballylate	<DL*		0.73
L. acidophilus / general bacterial			
44. D-Lactate	5.4	H	1.9
Clostridial species			
45. 3,4-Dihydroxyphenylpropionate	1.38	H	
Yeast / Fungal			
46. D-Arabinitol	25		36

Gastrointestinal Microbiome and Metabolic Markers



- **Short chain fatty acids** (SCFAs)
  - Acetate, n-Butyrate and Propionate produced by anaerobic bacterial fermentation of indigestible carbohydrate (fiber)
- **Beta-glucuronidase**
  - Enzyme inducible by activity of anaerobes in the gut (E Coli, Bacteroides, Clostridia)

RESEARCH ARTICLE

Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms

Hua V. Lin, Andrea Frassetto, Edward J. Kowalik Jr, Andrea R. Nawrocki, Mofei M. Lu, Jennifer R. Kosinski, James A. Hubert, Daphne Szeto, Xiaorui Yao, Gail Forrest, Donald J. Marsh

Published: April 10, 2012 • DOI: 10.1371/journal.pone.0035240

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are metabolites formed by gut microbiota from complex dietary carbohydrates. Butyrate and acetate were reported to protect against diet-induced obesity without causing hypophagia, while propionate was shown to reduce food intake

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035240

Short chain fatty acids and colonic health

Hijova E, Chmelarova A

Institute of Experimental Medicine, Faculty of Medicine, Safarikiensis University, Kosice, Slovakia. **hijova@pobox.sk**

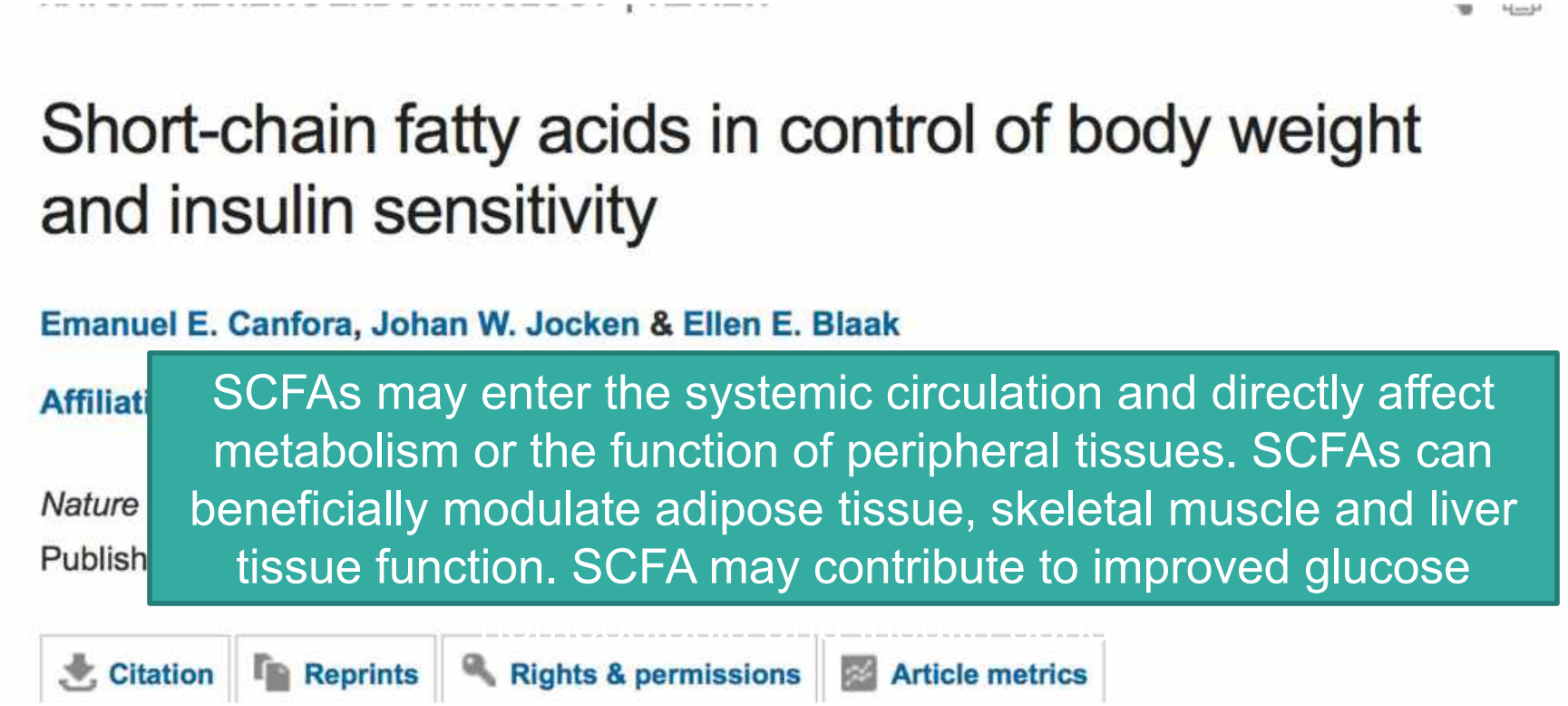
Abstract

Recently, colonic health has been linked to the maintaining overall health status and reducing the risk of diseases by changes in lifestyle. Functional foods, such as "prebiotics" and "probiotics", dietary fibers, and other dietary components that target the colon and affect its environment enhancing short fatty acid (SCFA) production have been at the forefront. The topic of this review is the key end products of colonic fermentation, the SCFA butyric, acetic, and propionic acids. SCFA are readily absorbed. Butyrate is the major energy source for colonocytes. Propionate is largely taken up by the liver. Acetate enters the peri
risk of develo
Text (Free, P
Key words: c

The role of SCFAs has expanded to include their role as nutrients for the colonic epithelium, as modulators of colonic and intracellular pH, cell volume, and other functions associated with ion transport, and as regulators of proliferation, differentiation, and gene expression

http://www.bmj.sk/2007/10808-06.pdf

SCFAs control weight and insulin sensitivity

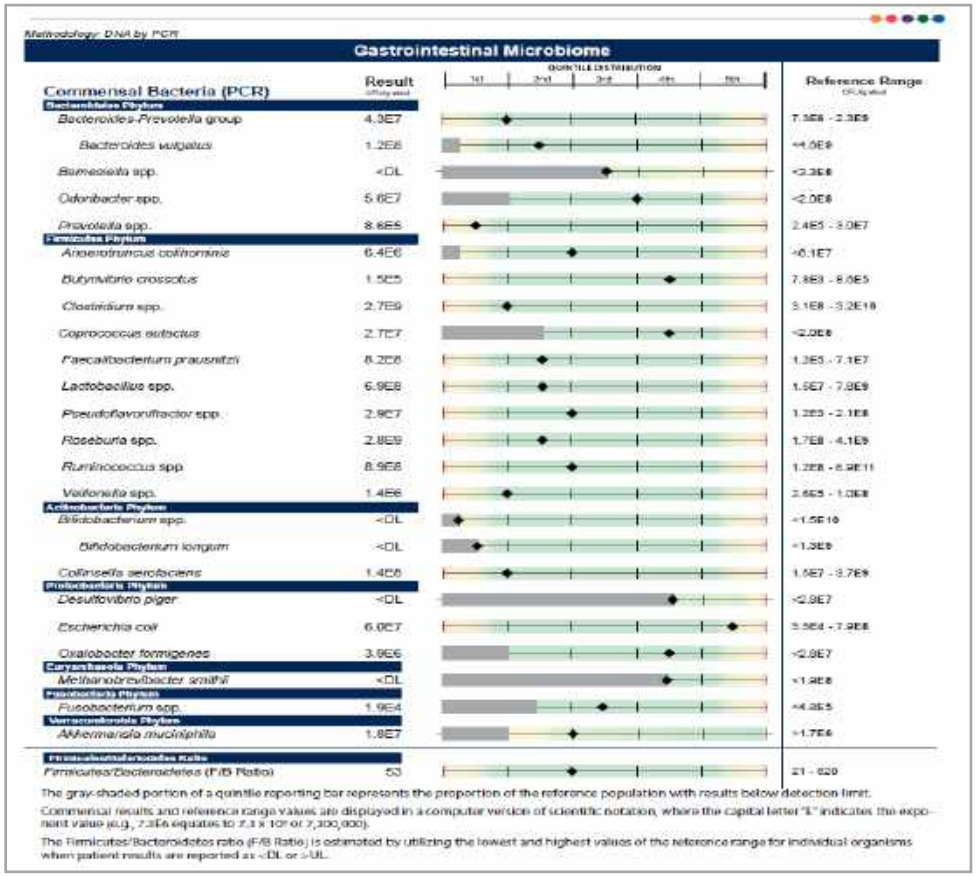


Beta Glucuronidase

Beta-glucuronidase activity must be sufficient to permit deconjugation and absorption of desirable molecules, while remaining low enough to prevent widespread deconjugation and subsequent reabsorption of toxins and other undesirable molecules.

- **For LOW Beneficial SCFAs**
  - Increase dietary fiber
  - Prebiotics & probiotics
  - Saccharomyces boulardii
- **For HIGH Beta glucoronidase**
  - Decrease meat intake & increase insoluble fiber
  - Probiotics
  - Liver support : Silybum marianum
  - Calcium-D-glucarate

Testing Commensial Bacteria



Methodology: DNA by PCR

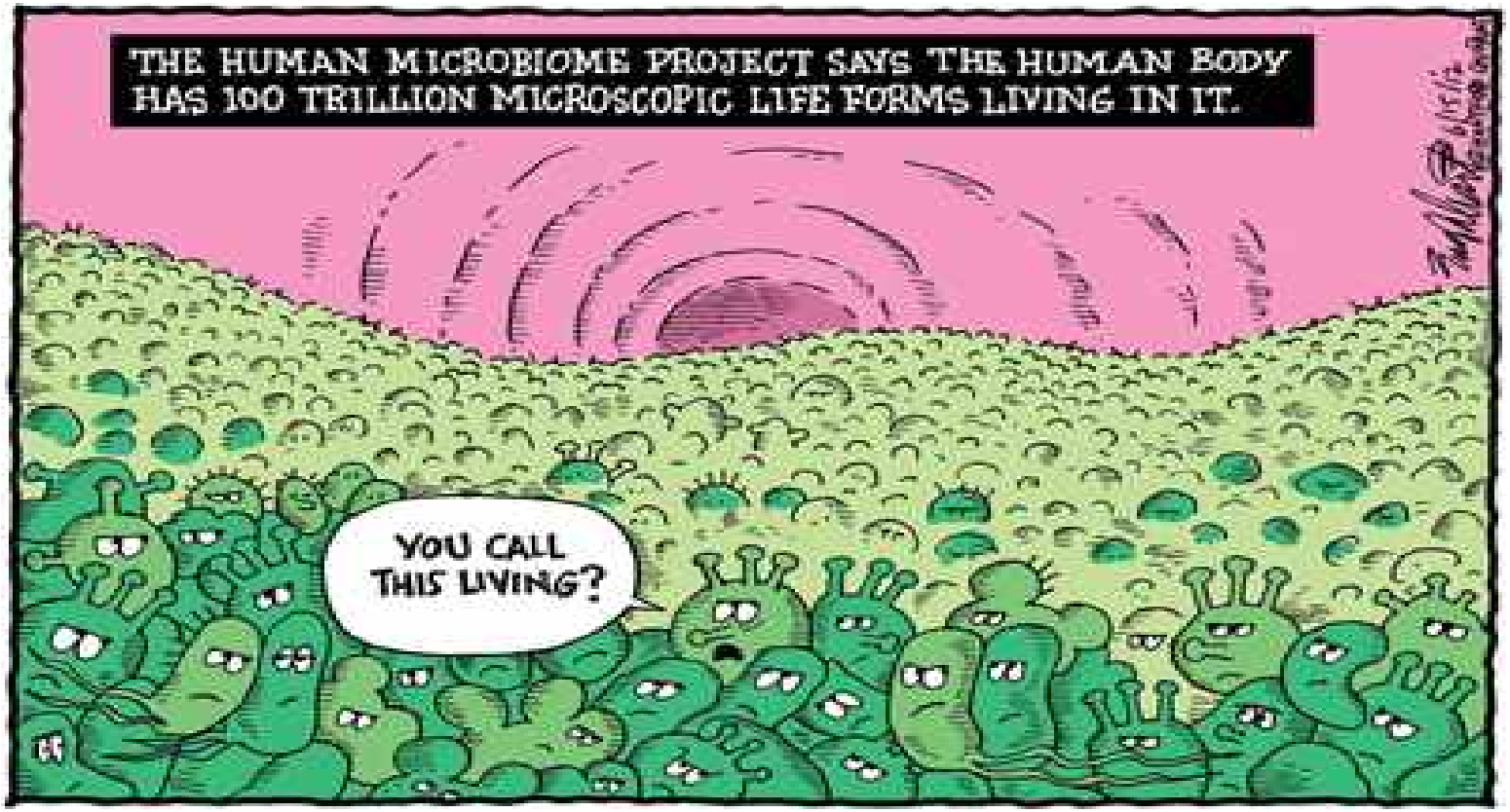
Gastrointestinal Microbiome

Commensal Bacteria (PCR)	Result	Reference Range
Bacteroidetes Phylum		
Bacteroides-Prevotella group	4.3E7	7.3E6 - 2.3E9
Bacteroides vulgatus	1.2E8	<4.0E9
Barnesiella spp.	<DL	<3.3E8
Odoribacter spp.	5.6E7	<2.0E8
Prevotella spp.	8.8E5	2.4E5 - 3.0E7
Firmicutes Phylum		
Anaerotruncus colihominis	6.4E6	<6.1E7
Butyrivibrio crossotus	1.5E5	7.8E3 - 8.0E5
Clostridium spp.	2.7E9	5.1E8 - 3.2E10
Coprococcus eutactus	2.7E7	<2.0E8
Faecalibacterium prausnitzii	8.2E8	1.3E5 - 7.1E7
Lactobacillus spp.	6.9E8	1.5E7 - 7.8E9
Pseudoflavonifractor spp.	2.9E7	1.2E5 - 2.1E8
Roseburia spp.	2.8E9	1.7E8 - 4.1E9
Ruminococcus spp.	8.9E8	1.2E8 - 6.9E11
Veillonella spp.	1.4E6	2.6E5 - 1.0E8
Actinobacteria Phylum		
Bifidobacterium spp.	<DL	<1.5E10
Bifidobacterium longum	<DL	<1.3E9
Collinsella aerofaciens	1.4E6	1.5E7 - 3.7E9
Proteobacteria Phylum		
Desulfovibrio piger	<DL	<2.9E7
Escherichia coli	6.0E7	5.5E4 - 7.9E8
Oxalobacter formigenes	3.9E6	<2.9E7
Euryarchaeota Phylum		
Methanobrevibacter smithii	<DL	<1.9E8
Fusobacteria Phylum		
Fusobacterium spp.	1.9E4	<4.3E5
Verrucomicrobia Phylum		
Akkermansia muciniphila	1.8E7	<1.7E8
Firmicutes/Bacteroidetes Ratio		
Firmicutes/Bacteroidetes (F/B Ratio)	53	21 - 620

The gray-shaded portion of a quintile reporting bar represents the proportion of the reference population with results below detection limit.

Commensal results and reference range values are displayed in a computer version of scientific notation, where the capital letter "E" indicates the exponent value (e.g., 7.3E6 equates to 7.3×10^6 or 7,300,000).

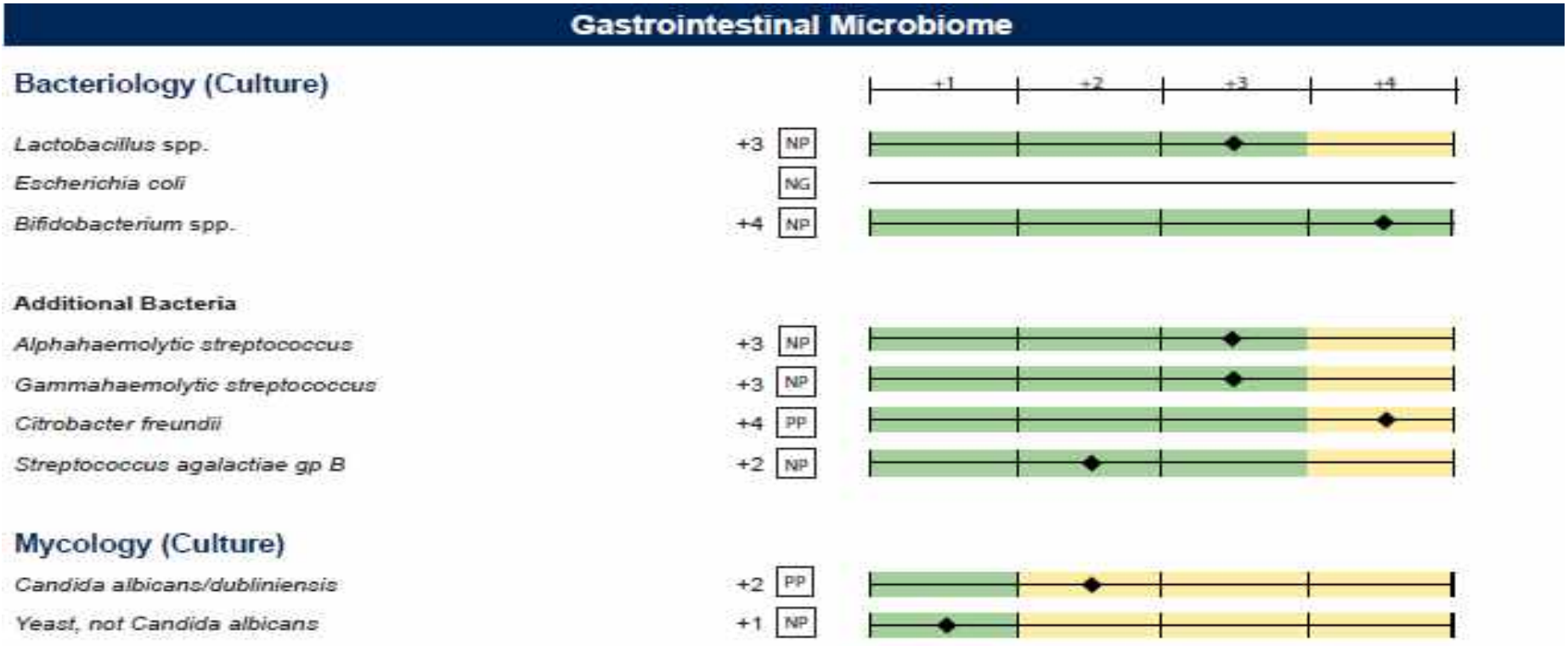
The Firmicutes/Bacteroidetes ratio (F/B Ratio) is estimated by utilizing the lowest and highest values of the reference range for individual organisms when patient results are reported as <DL or >UL.


THE HUMAN MICROBIOME PROJECT SAYS THE HUMAN BODY HAS 100 TRILLION MICROSCOPIC LIFE FORMS LIVING IN IT.
YOU CALL THIS LIVING?

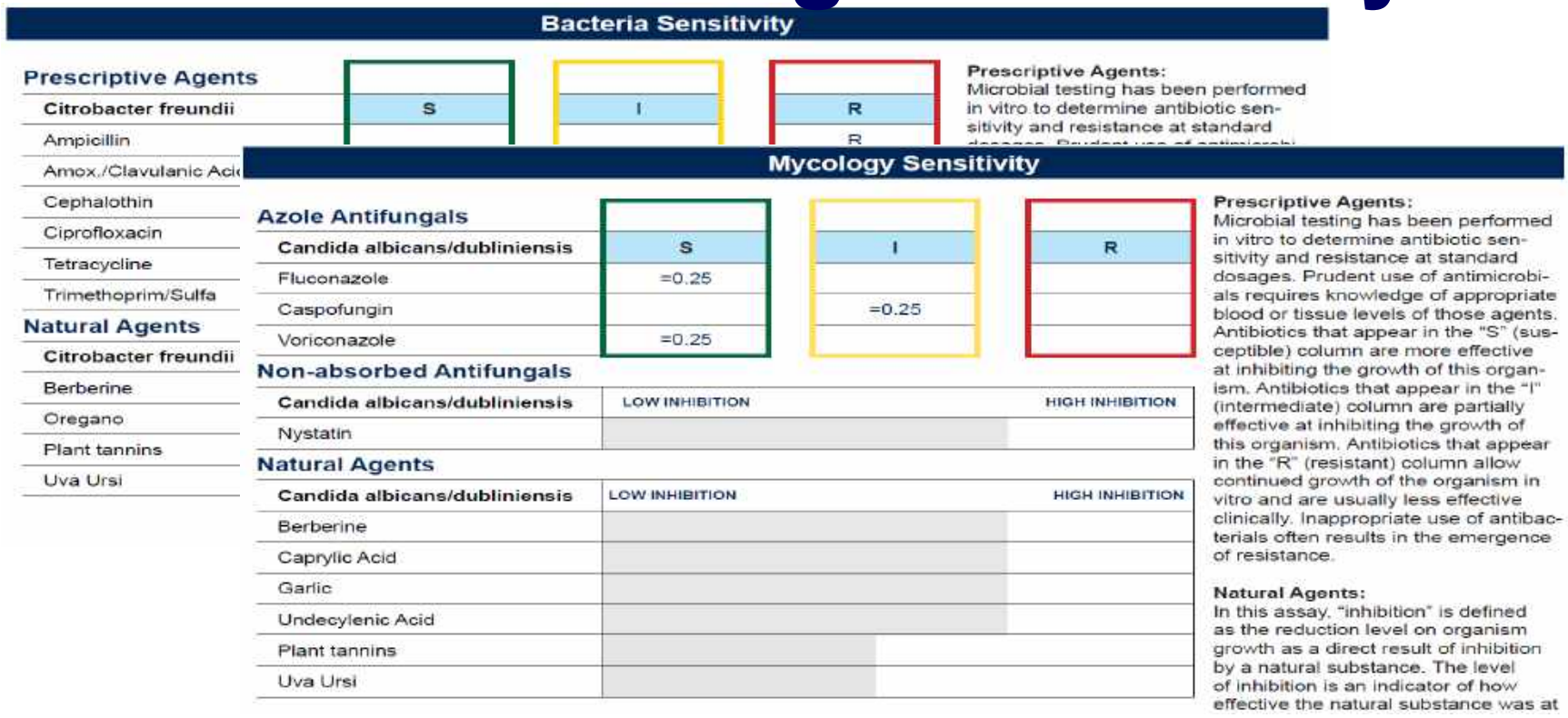
Various labs offer testing for commmensials

Gut Microbiome and Cardiovascular disorders	Genus/ Species	PAC Score	Low Risk Range	Moderate Risk Range	High Risk Range	Previous Result
	Collinsella	4.78	4 - 6	6 – 8	>8	
	Eubacterium	6.68	4 - 6	2 - 4	<2	
	Roseburia	1.99	4 – 6	2 – 4	<2	
	Clostridium	5.66	4 - 6	2 - 4	<2	
	Ruminococcus	7.52	4 - 6	6 - 8	>8	
	Peptostreptococcus	5.77	4 - 6	2 - 4	<2	
	Prevotella	5.33	4 - 6	6 - 8	>8	
	Lactobacillus reuteri	0.85	4 – 6	2 – 4	<2	
	Enterococcus faecium	4.68	4 – 6	2 – 4	<2	
	Lactobacillus acidophilus	0.11	4 – 6	2 – 4	<2	
	Bifidobacterium lactis	4.56	4 – 6	2 – 4	<2	
	Lactobacillus plantarum	1.15	4 – 6	2 – 4	<2	
	Lactobacillus fermentum	3.65	4 – 6	2 – 4	<2	
	Lactobacillus curvatus	5.11	4 – 6	2 – 4	<2	

Bacteria and Fungal Culture



Bacterial and Fungal Sensitivity



Bacteria Sensitivity

Prescriptive Agents	S	I	R
Citrobacter freundii			
Ampicillin			R
Amox./Clavulanic Aci			
Cephalothin			
Ciprofloxacin			
Tetracycline			
Trimethoprim/Sulfa			
Natural Agents			
Citrobacter freundii			
Berberine			
Oregano			
Plant tannins			
Uva Ursi			

Prescriptive Agents:
Microbial testing has been performed in vitro to determine antibiotic sensitivity and resistance at standard

Mycology Sensitivity

Azole Antifungals	S	I	R
Candida albicans/dubliniensis			
Fluconazole	=0.25		
Caspofungin		=0.25	
Voriconazole	=0.25		

Non-absorbed Antifungals	LOW INHIBITION	HIGH INHIBITION
Candida albicans/dubliniensis		
Nystatin		

Natural Agents	LOW INHIBITION	HIGH INHIBITION
Candida albicans/dubliniensis		
Berberine		
Caprylic Acid		
Garlic		
Undecylenic Acid		
Plant tannins		
Uva Ursi		

Prescriptive Agents:
Microbial testing has been performed in vitro to determine antibiotic sensitivity and resistance at standard dosages. Prudent use of antimicrobials requires knowledge of appropriate blood or tissue levels of those agents. Antibiotics that appear in the "S" (susceptible) column are more effective at inhibiting the growth of this organism. Antibiotics that appear in the "I" (intermediate) column are partially effective at inhibiting the growth of this organism. Antibiotics that appear in the "R" (resistant) column allow continued growth of the organism in vitro and are usually less effective clinically. Inappropriate use of antibacterials often results in the emergence of resistance.

Natural Agents:
In this assay, "inhibition" is defined as the reduction level on organism growth as a direct result of inhibition by a natural substance. The level of inhibition is an indicator of how effective the natural substance was at

Parasitology

Methodology: Direct Microscopic Examination, EIA

Parasitology

Microscopic Exam Results:

Blastocystis hominis: Many

Parasitology
Parasite Recovery: Literature suggests that >90% of enteric parasitic infections may be detected in a sample from a single stool collection. Increased sensitivity results from the collection of additional specimens on separate days.

Lab Comments
SENSI'S: All yeast, add'l bacteria

Parasitology EIA Tests:

	In Range	Out of Range
Cryptosporidium	Negative	
Giardia lamblia	Negative	
Entamoeba histolytica	Negative	

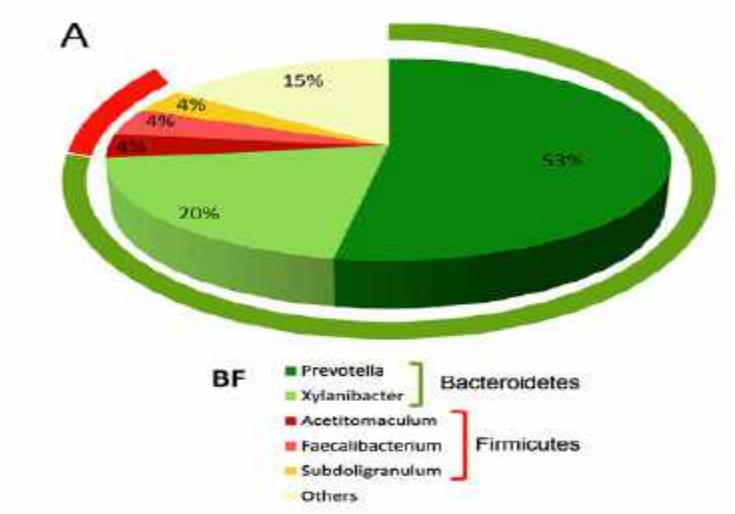
Microbiome and Obesity

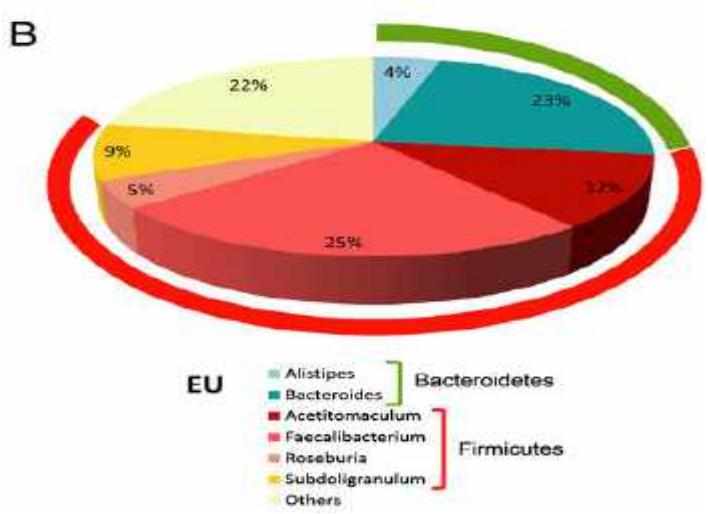
- Microbes alters caloric yield from ingested food
- Altering microbiota may also improve insulin sensitivity
- Treatments for obesity that results in lowering Firmicutes may assist in weight control.
- **Firmicutes**
  - Clostridia, Streptomyces, Lactobacillus, Mycoplasma, Bacillus sp.
- **Bacteroidetes**
  - Bacteroides, and Prevotella sp.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3687783/

Dietary Effects on Gut Microflora

Burkina Faso (primal diet) VS European children (SAD)





Green = Bacteriotedes

Red = Firmicutes

DeFilippo C et al. PNAS. 2010;107(33):14691-14696.

Features of the gut microbiota that promote obesity and insulin resistance

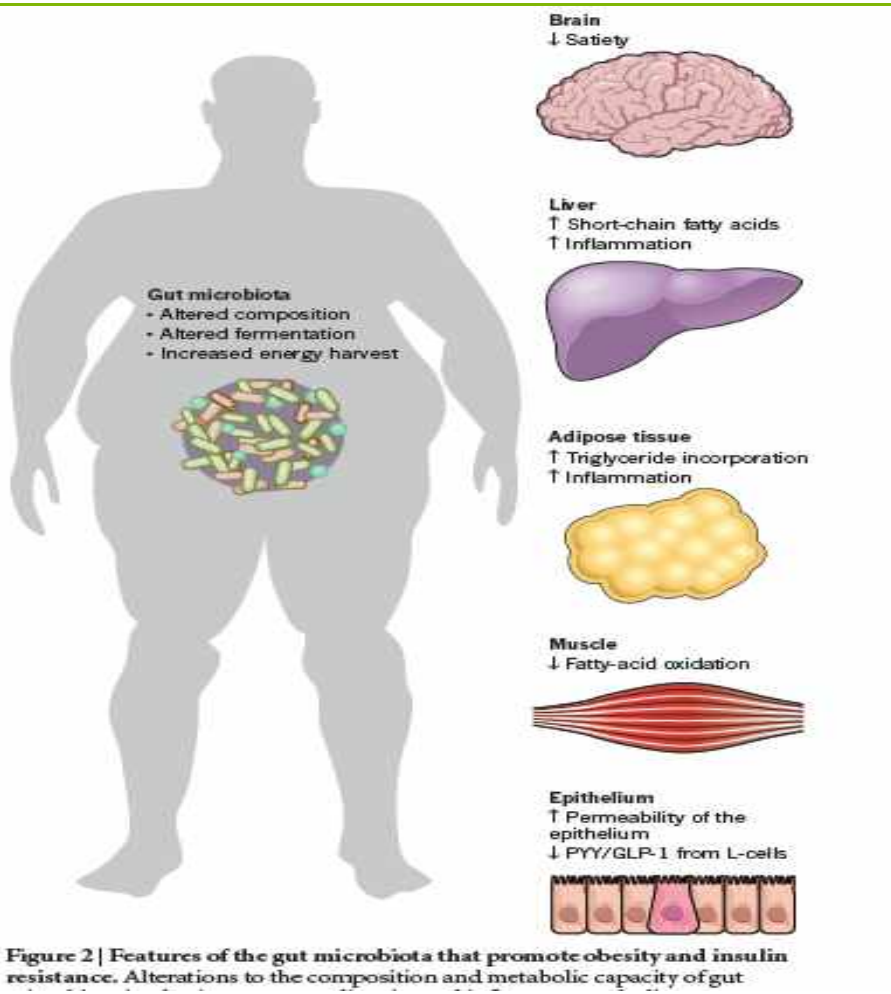
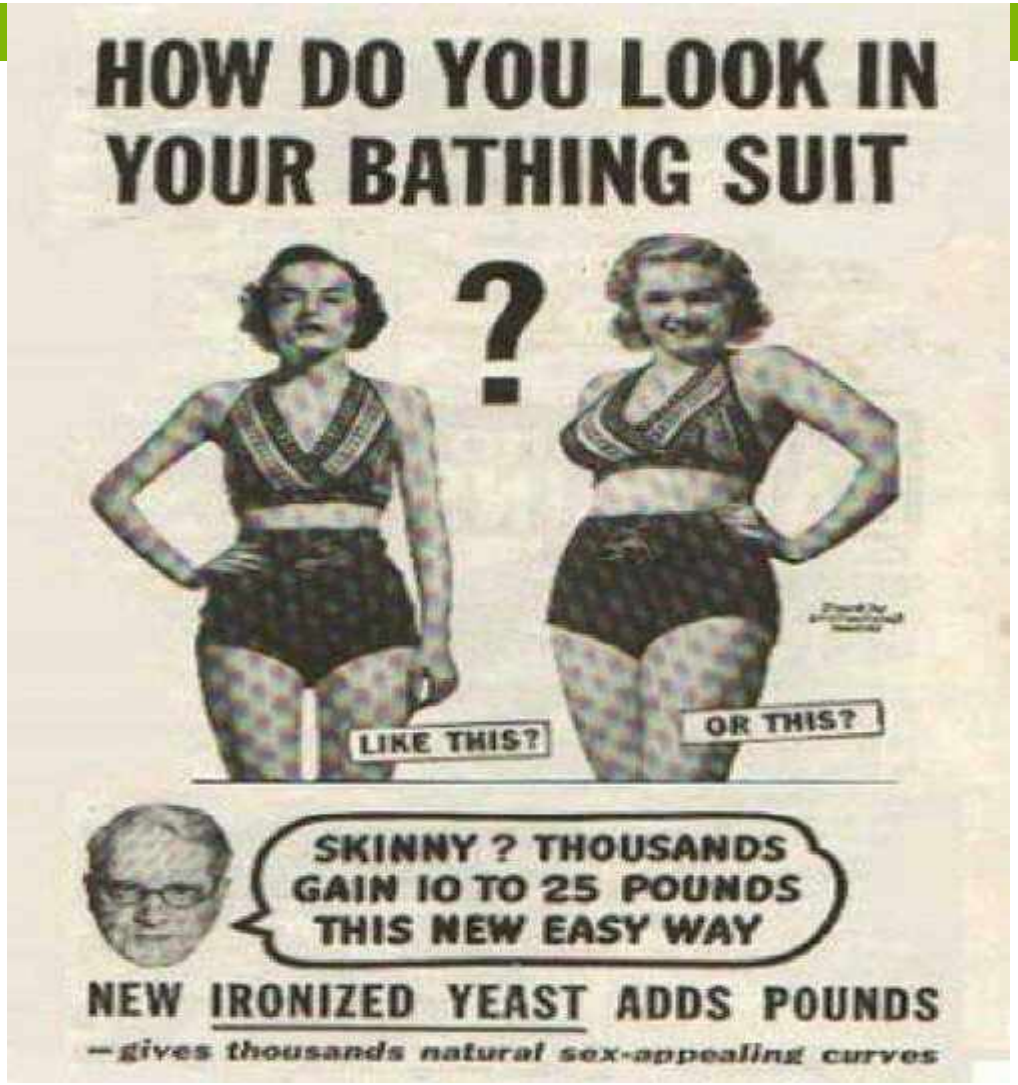


Figure 2 | Features of the gut microbiota that promote obesity and insulin resistance. Alterations to the composition and metabolic capacity of gut

http://www.nature.com/nature/journal/v489/n7415/pdf/nature11552.pdf


HOW DO YOU LOOK IN YOUR BATHING SUIT
?
LIKE THIS?
OR THIS?
SKINNY ? THOUSANDS GAIN 10 TO 25 POUNDS THIS NEW EASY WAY
NEW IRONIZED YEAST ADDS POUNDS
—gives thousands natural sex-appealing curves

HEALTH

You Are Your Bacteria: How the Gut Microbiome Influences Health

The bacteria in our gut already plays an important role in digestion. But new studies indicate that our bacteria could play a major role in whether or not we become obese

By Veronique Greenwood | Aug. 29, 2013

Every time you have a meal, you're eating not just for yourself, but for the hundred trillion bacteria that line your large intestine. This live-in colony of microbes, which together can weigh several pounds and consists of hundreds of individual species, is a digestion powerhouse, breaking food down into useful and nutritious components for us and for the microbes. It's only recently that new genomic techniques have opened the doors to detailed study of our gut microbiome, but understanding how it varies among different people is extremely

Can we treat obesity through the gut?

"The notion that caloric intake and overall energy balance depends on an individual's microbiota is particularly interesting because it implies that obesity and associated chronic metabolic disorders (metabolic syndrome, diabetes, etc.) can be prevented and treated by tinkering with the microbiota."

News

Fat people harbour 'fat' microbes

Your gut bacteria may help to determine your holiday weight gain.

Helen Pearson

The obese are often blamed for their own corpulence. But perhaps, just perhaps, some of the blame should be placed on another type of organism entirely: bacteria.

Researchers have shown that the intestines of obese peop
swimming with a differe
microbes compared with
people. And this microbi
could actually be helping
weight: bugs taken from
mouse and transplanted
animal's intestine made

Researchers have shown that the intestines of obese people are swimming with a different make-up of microbes compared with those of slim people. And this microbial population could actually be helping them gain weight…

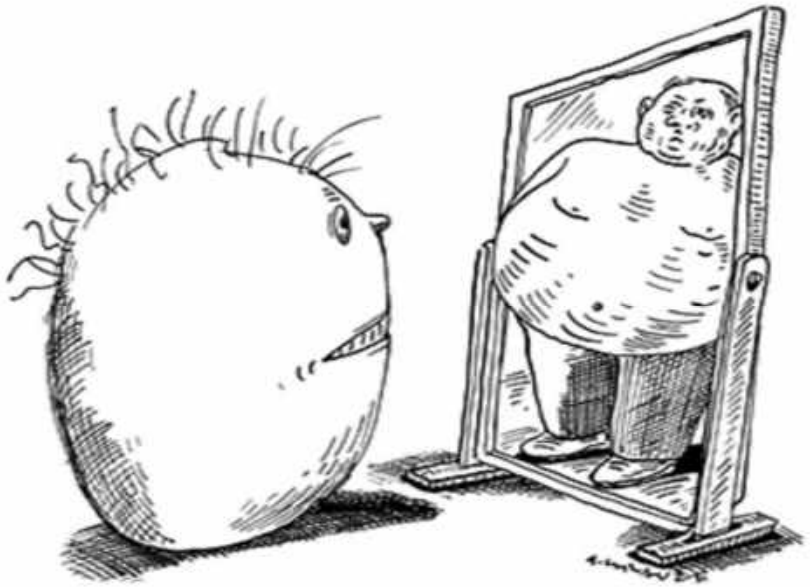
http://www.nature.com/news/2006/061218/full/news061218-6.html

2006

Microbesity

Obesity appears linked to the gut microbiome. How and why is still a mystery—but scientists have plenty of ideas.

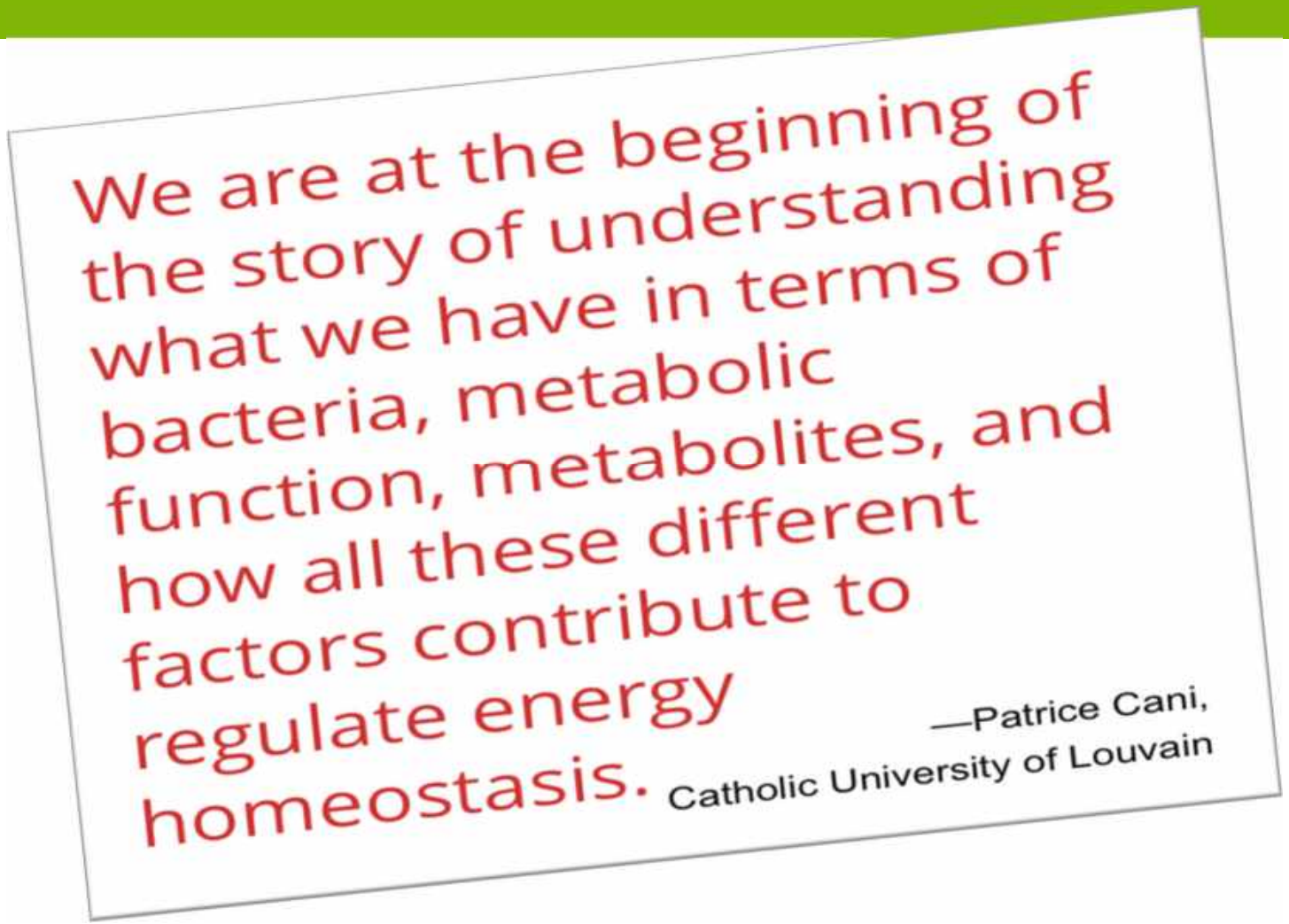
By Jenny Rood | November 1, 2015



ANDRZEJ KRAUZE

A decade ago, gut microbiologist and genomicist Jeffrey Gordon's postdoc Fredrik Bäckhed at Washington University in St. Louis made a startling discovery: adding gut microbes from normal, healthy mice to germ-free mice significantly increased the latter's body fat (*PNAS*, 101:15718-23, 2004). This finding prompted another of Gordon's postdocs, Ruth Ley, to suggest that University of Colorado computational biologist Rob Knight apply his new microbe-comparing computational tools to obese and lean mice. "I thought it seemed like a long shot" that they would see significant effects, recalls Knight, now at the University of California, San Diego, in an email. The long shot paid off, however, with a 2005 paper in *PNAS* that found that obese and lean mice indeed had different gut microbiomes (102:11070-75). The insight ultimately launched deeper investigations into the relationship between the microbiome and obesity.

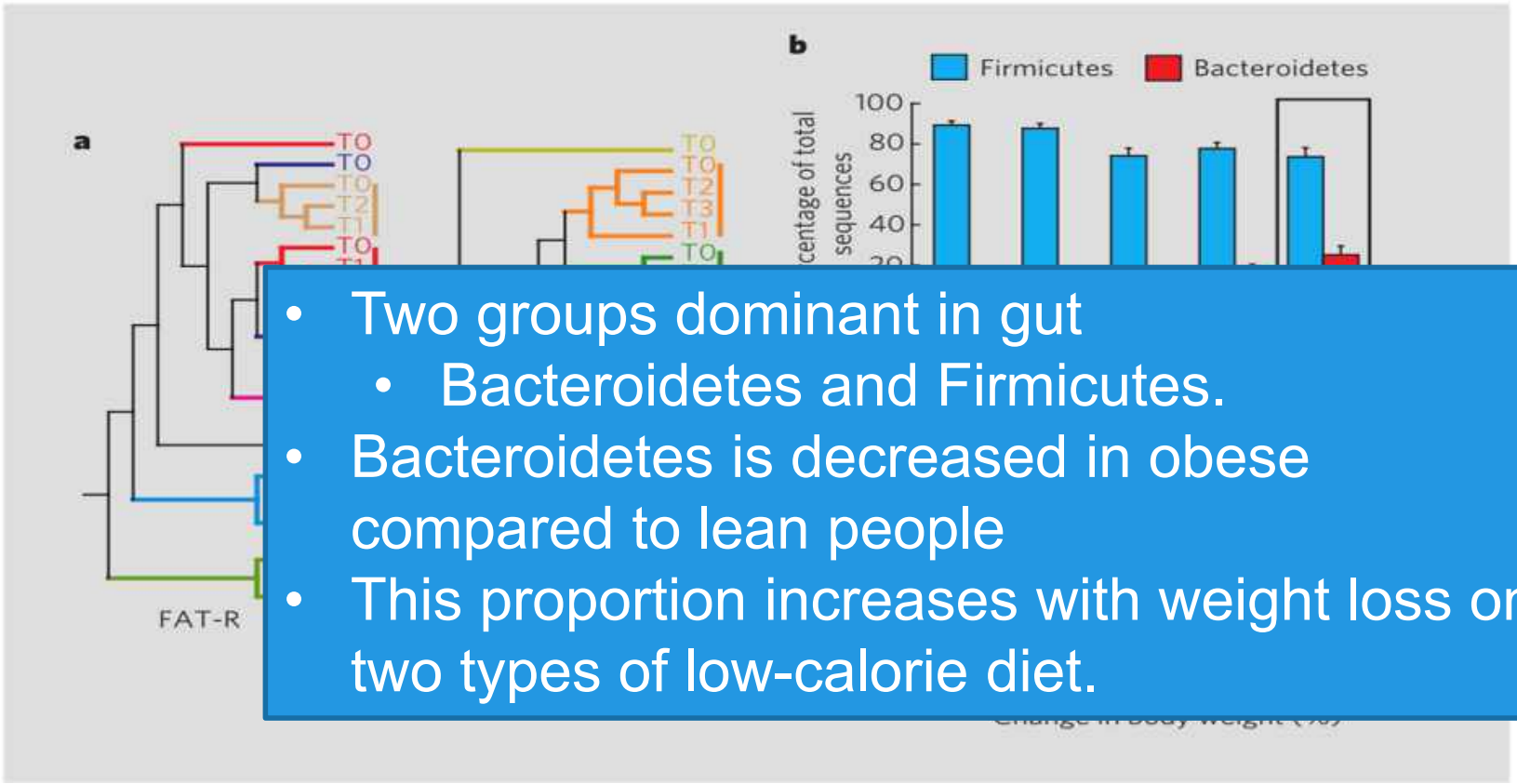
http://www.the-scientist.com/?articles.view/articleNo/44300/title/Microbesity/


We are at the beginning of the story of understanding what we have in terms of bacteria, metabolic function, metabolites, and how all these different factors contribute to regulate energy homeostasis.
—Patrice Cani, Catholic University of Louvain

The alteration of microbiota in gut in the conditions of obesity

Phylum	Class	Order (Genera)	The trends of changes
Bacteroidetes	Bacteroidetes	Bacteroidales (Bacteroides)	↓
		Bacteroidales (Prevotella)	↑
Firmicutes	Bacilli	Bacillales (Bacillus)	↑
		Lactobacillales	↓
	Clostridia	Clostridiales (Clostridium)	↑
Actinobacteria	Actinobacteria	Actinomycetales	↑
	Actinobacteria	Bifidobacteriales (Bifidobacterium)	↓
Euryarchaeota (domain Archaea)	Methanobacteria		↑

http://nutritionj.biomedcentral.com/articles/10.1186/s12937-016-0166-9



- Two groups dominant in gut
  - Bacteroidetes and Firmicutes.
- Bacteroidetes is decreased in obese compared to lean people
- This proportion increases with weight loss on two types of low-calorie diet.

http://www.nature.com/articles/4441022a.epdf

Summary of study

- Fecal transplants into germ-free mice from obese or lean donors
- Obese had greater relative abundance of firmicutes
- Lean had greater relative abundance of Bacterodetes
- No difference in rat chow consumption during the 2 week experiment.
- Mice colonized with obese microbiota showed statistically significant weight gain in 2 week period.

Obesity alters gut microbial ecology

Ruth E. Ley †, Fredrik Bäckhed †, Peter Turnbaugh †, Catherine A. Lozupone ‡, Robin D. Knight §, and Jeffrey I. Gordon † · ¶

Author Affiliations

Contributed by Jeffrey I. Gordon, June 14, 2005

Abs

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Microbial-community composition is inherited from mothers. However, compared with lean mice and regardless of kinship, *ob*/*ob* animals have a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes. Obesity Correlates with a Shift in the Abundance of Bacteroidetes and Firmicutes.

An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh[1], Ruth E. Ley[1], Michael A. Mahowald[1], Vincent Magrini[2], Elaine R. Mardis[1,2] & Jeffrey I. Gordon[1]

The worldwide obesity epidemic is stimulating efforts to identify host and environmental factors that affect energy balance. Comparisons of the distal gut microbiota of genetically obese mice and their lean littermates, as well as those of obese and lean human volunteers have revealed that obesity is associated with changes in the relative abundance of the two dominant bacterial divi
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Comparisons of the distal gut microbiota of genetically obese mice and their lean littermates, as well as those of obese and lean human volunteers have revealed that obesity is associated with changes in the relative abundance of the two dominant bacterial divisions, the Bacteroidetes and the Firmicutes.

http://www.nature.com/articles/nature05414.epdf

Your Gut Bacteria Want You to Eat a Cupcake

A new study suggests the microbes in humans' intestines may influence food choices.

http://www.theatlantic.com/health/archive/2014/08/your-gut-bacteria-want-you-to-eat-a-cupcake/378702/

"Individuals who are 'chocolate desiring' have different microbial metabolites in their urine than 'chocolate indifferent' individuals."

Prospects & Overviews

Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms

Joe Alcock[1], Carlo C. Maley[2,3,4,*] and C. Athena Aktipis[2,3,4,5]

Article first published online: 7 AUG 2014

DOI: 10.1002/bies.201400071

Issue

BioEssays

Volume 36, Issue 10, pages 940–949, October 2014

We review several potential mechanisms for microbial control over eating behavior including microbial influence on reward and satiety pathways, production of toxins that alter mood, changes to receptors including taste receptors, and hijacking of the vagus nerve, the neural axis between the gut and the brain.

http

Certain Probiotics associated with weight loss

- Certain *Lactobacillus* sp. reported to reduce fat mass and improve insulin sensitivity and glucose tolerance but not universally reported for all *Lactobacillus* sp.
- A recent study demonstrated that the probiotic VSL#3 caused mice to decrease food intake.
- *Bifidobacterium breve* inhibited weight gain in mice given a high fat diet in a dose-dependent manner
- A randomized, placebo-controlled trial found that probiotic treatment in pregnancy, using *L. rhamnosus* GG and *Bifidobacterium lactis* along with dietary counseling, reduced abdominal fat at 6 months post-partum

TESTING FOR LEAKY GUT

Lipopolysaccharide

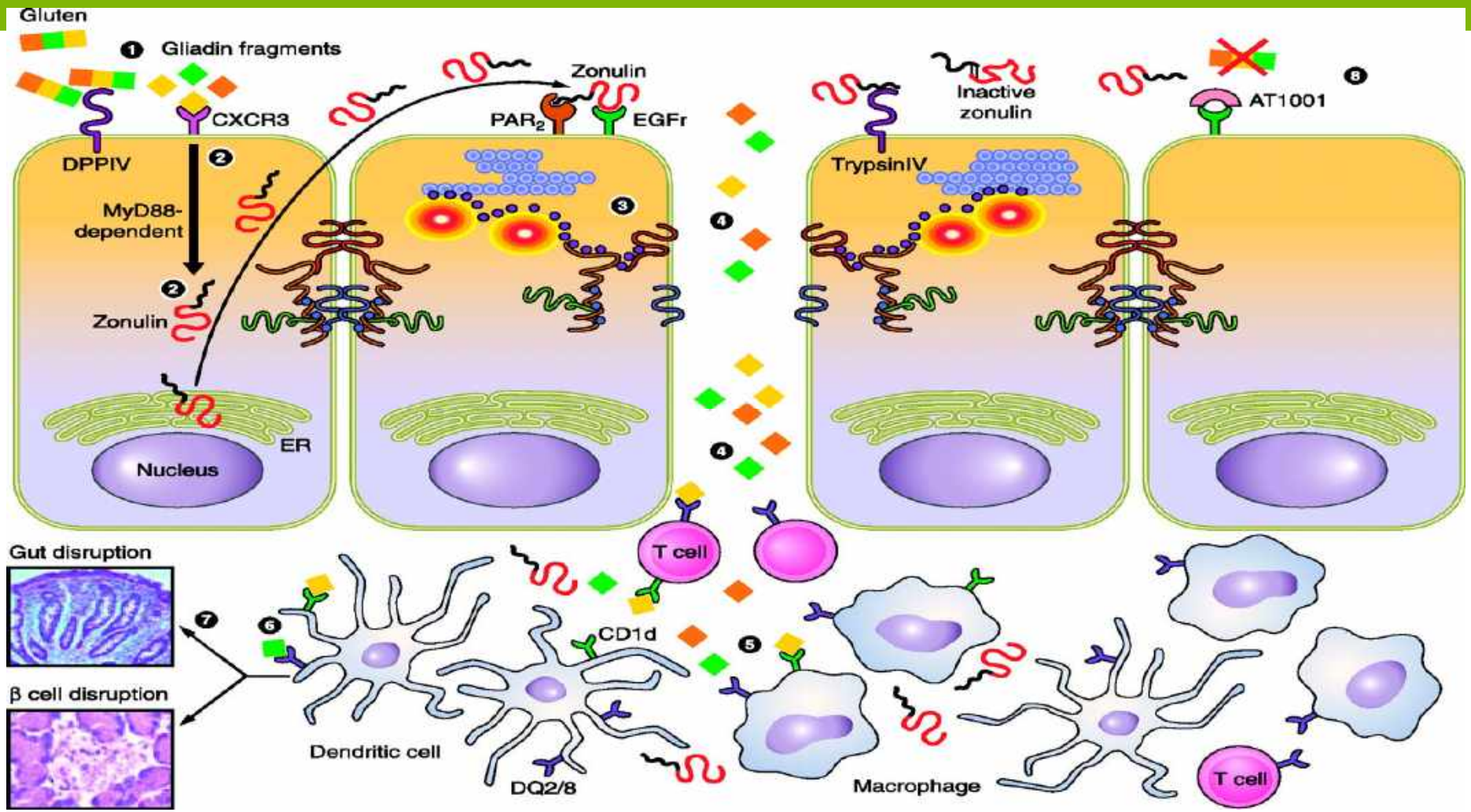
- Lipopolysaccharides (LPS) are large molecules found in gram-negative bacteria. They are endotoxins, and if absorbed, elicit a strong immune response.
- The detection of antibodies against LPS reveals macromolecule-sized endotoxin infiltration through the intestinal barrier into the systemic circulation.
- Intestinal permeability can cause systemic inflammation through translocation of LPS

Occludin

- Occludin is part of the main component of proteins holding together the tight junctions.
- The detection of antibodies to occludin indicates that the tight junctions are breaking down.
- This is a measure of a mechanism involved in damaging the intestinal barrier membrane.

Zonulin

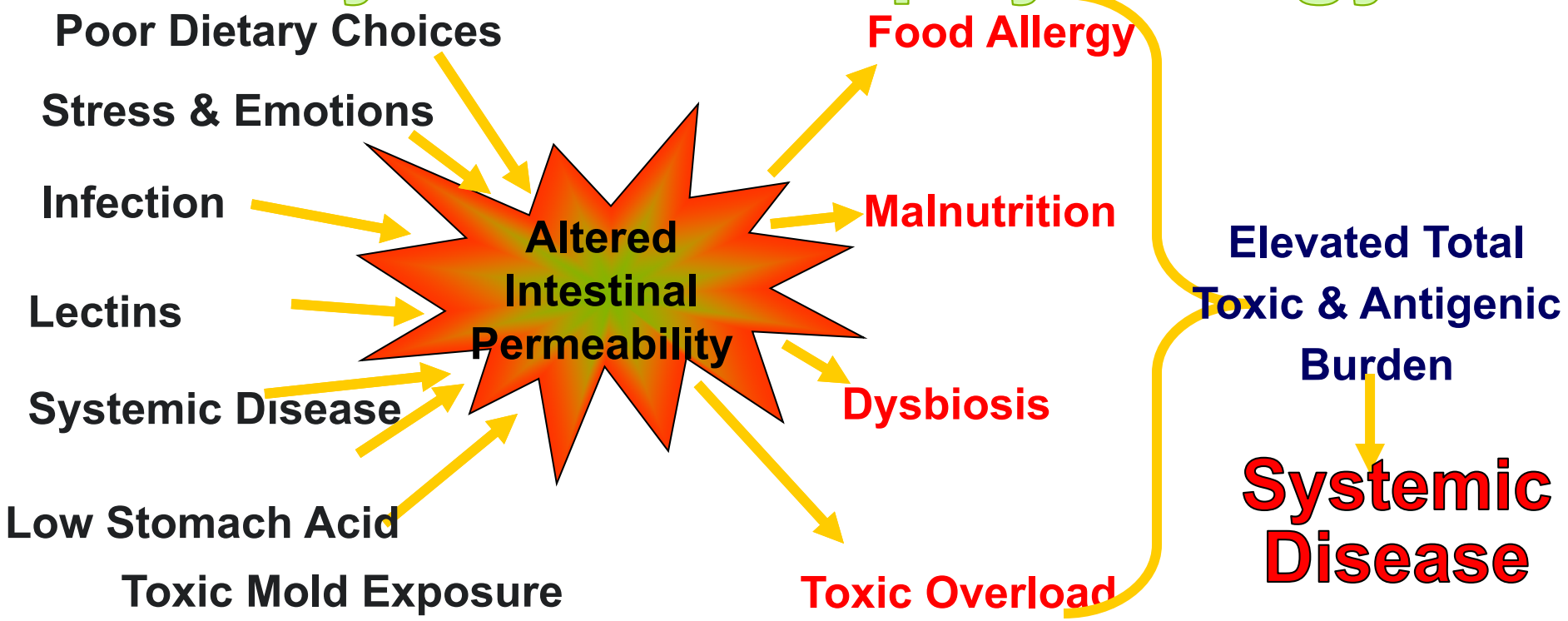
- Zonulin, a protein, regulates the permeability of the intestine.
- The detection of antibodies against zonulin indicates that the normal regulation of tight junctions is compromised.
- Clue to presence of an ongoing mechanism involved in damaging the intestinal barrier.


Gluten
1 Gliadin fragments
CXCR3
DPPIV
2
MyD88-dependent
2
Zonulin
ER
Nucleus
Zonulin
PAR2
EGFr
3
4
4
Inactive zonulin
TrypsinIV
AT1001
8
T cell
CD1d
5
6
7
Gut disruption
β cell disruption
Dendritic cell
DQ2/8
Macrophage
T cell

Causes of Increased Intestinal Permeability

- Inflammatory Bowel disease
- NSAID therapy
- Small Intestinal Bacterial Overgrowth (SIBO)
- Celiac disease
- Protozoal infections
- Toxic Exposure
- Food allergy
- Chronic Alcoholism
- Diarrhea
- Strenuous exercise
- Increasing age
- Nutritional Depletions

Leaky Gut Pathophysiology



Indications for testing for permeability

- Food allergies or gluten sensitivity,
- Inflammatory bowel disease,
- Autoimmune diseases or family history of autoimmune disease,
- Neurological conditions
- Cognitive dysfunction or mood disorders (depression, anxiety, bipolar)
- Metabolic disorders and cardiovascular disease

Biomarkers of intestinal permeability

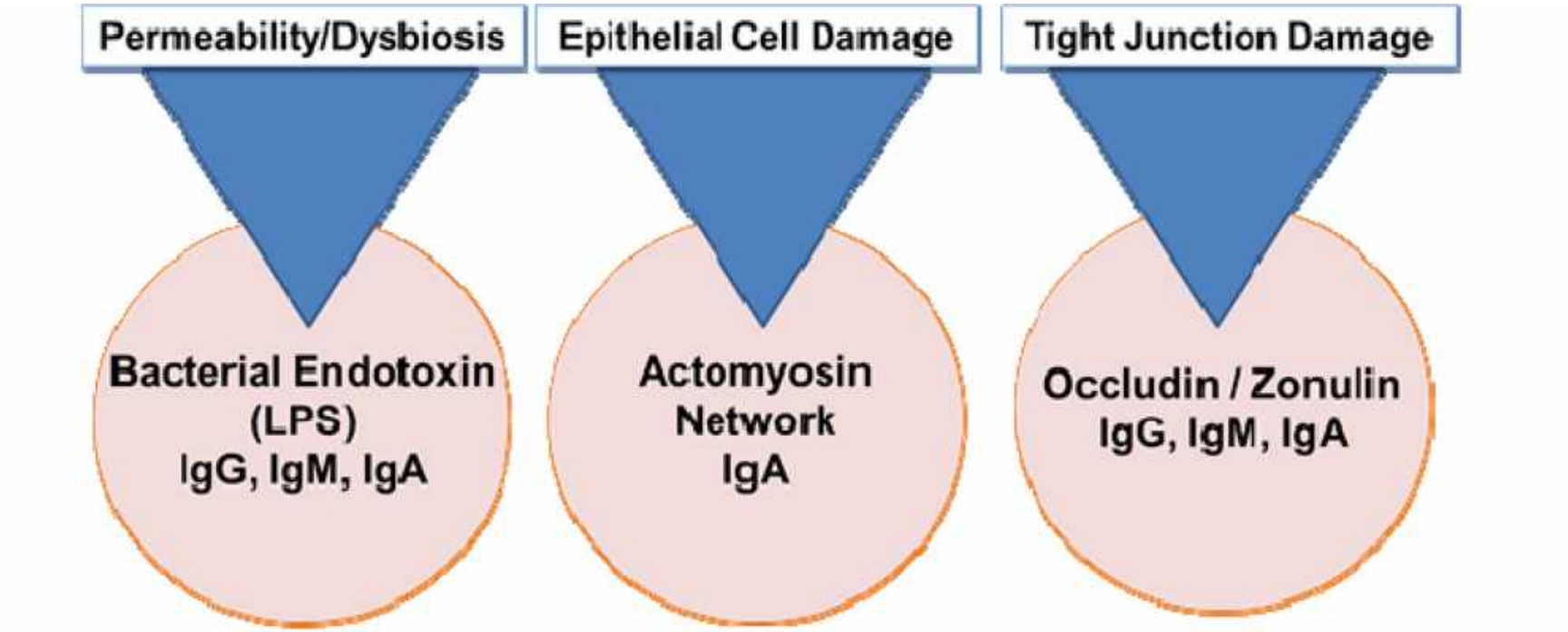


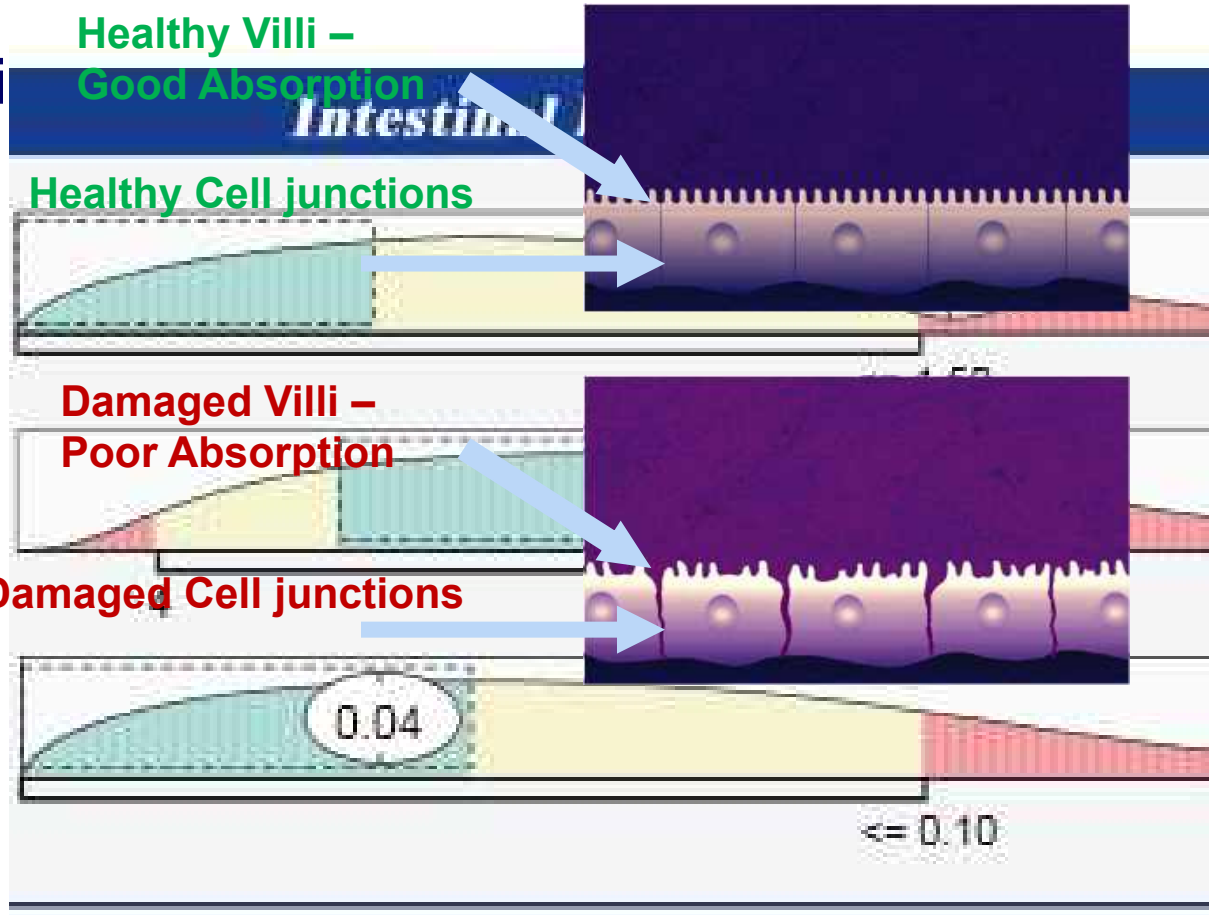
Figure 1 – Biomarkers of Intestinal Permeability Identification

Cyrex Array 2

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE (ELISA Index)**
Actomyosin IgA **	6.47			0.0-20
Occludin/Zonulin IgG	0.44			0.2-1.5
Occludin/Zonulin IgA	0.52			0.1-1.8
Occludin/Zonulin IgM			2.25	0.1-2.1
Lipopolysaccharides (LPS) IgG	0.50			0.1-1.6
Lipopolysaccharides (LPS) IgA	0.42			0.1-1.8
Lipopolysaccharides (LPS) IgM		1.99		0.1-2.0

Intestinal Permeabi

- Also called “Leaky Gut Syndrome”
- Many factors contribute to intestinal permeability including stress, food sensitivities and allergies, bile acids, infection, dysbiosis, hormones and more
- Treating this condition would involve identifying and addressing the cause of permeability, as well as employing therapies that heal the gut lining



Top Causes of increased zonulin and increased intestinal permeability

1. Bacterial pathogens (LPS and endotoxins)
2. SIBO = small intestinal bacterial overgrowth
3. Fungal dysbiosis or candida overgrowth
4. Parasitic infections
5. Gluten
6. Mold exposure

Effect of mycotoxins on the gut

The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins

Peyman Akbari,[1,2] Saskia Braber,[1] Soheil Varasteh,[1,2] Arash Alizadeh,[1,2] Johan Garssen,[2,3] and Johanna Fink-Gremmels[1]

Associated Data

• Supplementary Materials

Abstract

Mycotoxins, the secondary metabolite
contaminants in human and animal di
mutagenic, genotoxic and potential ca
adverse effects of various mycotoxins
impairment of the barrier function of
been noted, as this could result in an i
excessive activation of the immune sy
evidence regarding direct effects of v
based on different cellular and animal studies, show that food-associated exposure to certain mycotoxins, especially trichothecenes and patulin, affects the intestinal barrier integrity and can result in an increased translocation of harmful stressors. It is therefore hypothesized that human exposure to certain mycotoxins, particularly deoxynivalenol, as the major trichothecene, may play an important role in etiology of various chronic intestinal inflammatory diseases, such as inflammatory bowel disease, and in the prevalence of food allergies, particularly in children.

It is therefore hypothesized that human exposure to certain mycotoxins, particularly deoxynivalenol, as the major trichothecene, may play an important role in etiology of various chronic intestinal inflammatory diseases, such as inflammatory bowel disease, and in the prevalence of food allergies, particularly in children.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4691133/

Food Chain Mycotoxin Exposure, Gut Health, and Impaired Growth: A Conceptual Framework[1]

Laura E. Smith[2,*], Rebecca J. Stoltzfus[2], and Andrew Prendergast[3]

[+] Author Affiliations

[*]To whom correspondence should be addressed. E-mail: les36@cornell.edu.

Abstract

Childhood stunting is an important and intractable public health problem that underlies ~20% of deaths among children aged <5 y in developing countries. Environmental enteropathy (EE), a subclinical condition of the small intestine characterized by reduced absorptive capacity and increased intestinal permeability, is almost universal among children in developing countries and may mediate stunting. However, the etiology of EE is poorly understood. Mycotoxins are metabolites of fungi that frequently contaminate the staple foods of children living in developing countries. We review evidence from human and animal studies that exposure to mycotoxins, particularly aflatoxin (AF), fumonisin (FUM), and deoxynivaenol (DON), may impair child growth. Although these toxins have distinct actions, they all mediate intestinal damage through: *1*) inhibition of protein synthesis (AF, DON); *2*) an increase in systemic proinflammatory cytokines (DON); and *3*) inhibition of ceramide synthase (FUM). The intestinal pathology that arises from mycotoxin exposure is very similar to that of EE. We propose that future studies should address the role of mycotoxins in the pathogenesis of EE and evaluate interventions to limit mycotoxin exposure and reduce childhood stunting.

http://advances.nutrition.org/content/3/4/526.full#ref-3

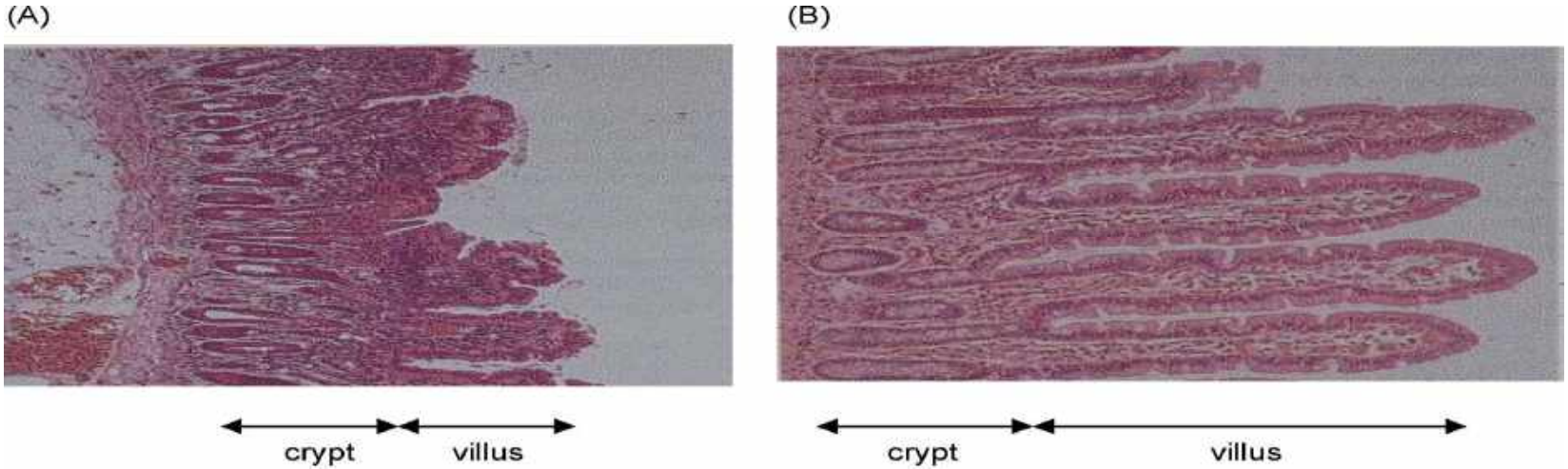
Exposure to these 3 toxins (aflatoxins, fumonisins, and deoxynivalenol) …may induce environmental enteropathy, a mild malabsorption syndrome that manifests with villus atrophy, crypt hyperplasia, T-cell infiltration, and general inflammation of the jejunum

Pediatrics
July 2014, VOLUME 134 / ISSUE 1

Reducing Malnutrition: Time to Consider Potential Links Between Stunting and Mycotoxin Exposure?

Ruth A. Etzel

Histological features of the small intestinal mucosa of children with or without environmental enteropathy: villus atrophy and crypt hyperplasia are characteristics of EE. Note high numbers of immune cells in lamina propria.(A) Abnormal, EE Crypt to villus ratio< 1:1(B) Normal Crypt to villus ratio >1:2(Photographs reproduced with permission of P. Lunn).



Sue McKay et al. Int. Health 2010;2:172-180

International Health

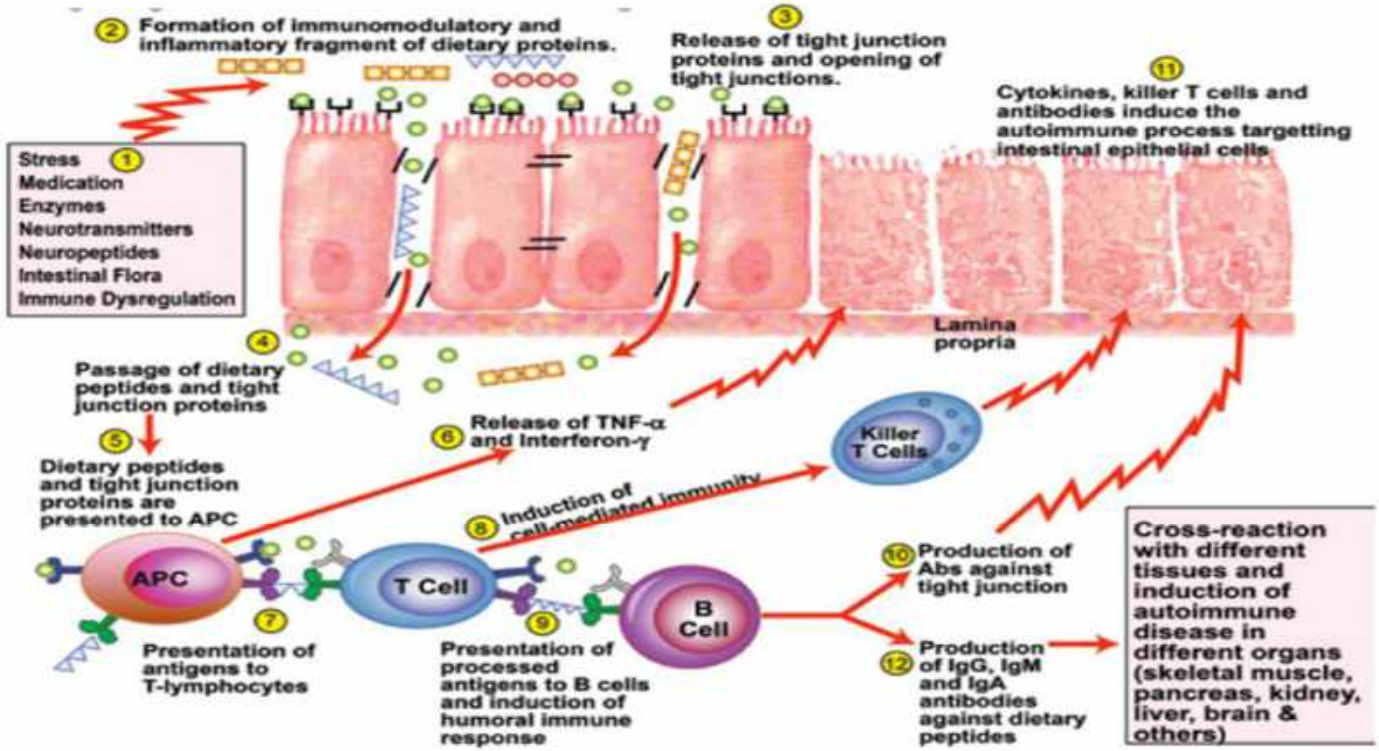
Environmental enteropathy: new targets for nutritional interventions

Summary

In the developing world major public health issues such as malnutrition and compromised physical development are intimately linked to altered gut morphology and function with underlying chronic inflammatory responses. In these societies the downward spiral of malnutrition and infections does not seem to be remedied by well-informed nutritional interventions that supplement the identified nutrient deficiencies, suggesting that additional strategies are needed. The aim of this scientific opinion paper is to consider how a child from the developing world might benefit, separately and additively, from interventions targeted to impact hygiene, nutritional status, disease resistance and gut function, if successful interventions could be found. A failure to tackle environmental enteropathy (EE) may be a critical limiting factor that can explain the relative lack of success of interventions focussed on micronutrient supplementation so far. Therefore this paper starts with a summary of the aetiology and consequences of EE on child health and the current recommendations aimed at tackling this problem. Then a number of hypotheses will be considered in terms of research strategy to positively affect nutritional status, intestinal health and growth of children with EE, with the aim of inspiring future innovative strategies, for both the food industry and the public health sector, which could benefit millions of children.

http://inthealth.oxfordjournals.org/content/2/3/172.abstract?

Figure 1. Proposed role of abnormal intestinal permeability in the pathogenesis of autoimmune disease targeting intestinal tissue and different organs.



Abbreviations: APC, antigen-presenting cell; TNF-α, tumor necrosis factor α; Abs, antibodies; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A.

Alternative Therapies Vol 21, Supp 1, Vojdoni: Molecular Mimicry as Mechanism for Food Immune Reactivities and Autoimmunity.

Interventions for mycotoxin associated enteropathy

- Zinc 50-100mg
- Vitamin D 5000IU
- Vitamin A 25,000IU
- Probiotics
- L-Glutamine powder
- Restore

Use functional medicine testing to move beyond treating the symptoms to personalized treatment of your patient!

KEY Take-Aways

- Diversity is KEY
  - Enhance diversity through DIET, not just probiotics
- Elevated fecal Calprotectin is a red flag
  - Refer for colonoscopy if IBD suspected.
- You cannot heal leaky gut until you address the root cause…
  - First treat SIBO, SIFO, dysbiosis, parasites, toxic exposure, etc
- Resistant obesity may be related to the microbiome
  - It's more than calories in vs. calories out!

Dr. Jill

Your Functional Medicine Expert®

Jill Carnahan. MD ABIHM, ABoIM, IFMCP

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