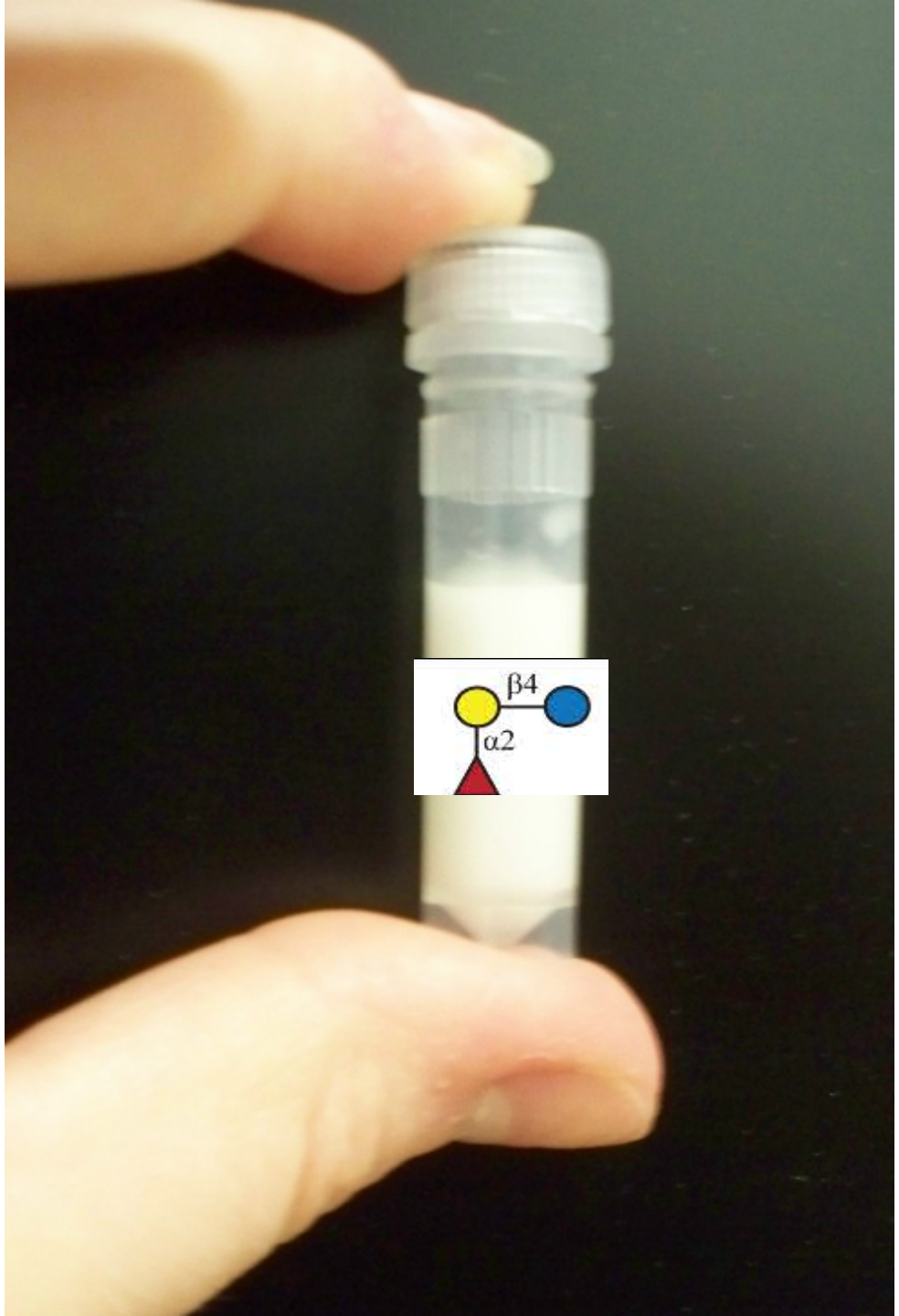
Ardythe L. Morrow, PhD

Professor of Pediatrics, Nutrition, & Environmental Health

CarboHealth Symposium, Zwolle, NL, Nov. 29, 2017

Human milk oligosaccharide and the rationale for testing as a medical food



Cincinnati Children's
changing the outcome together

University of CINCINNATI

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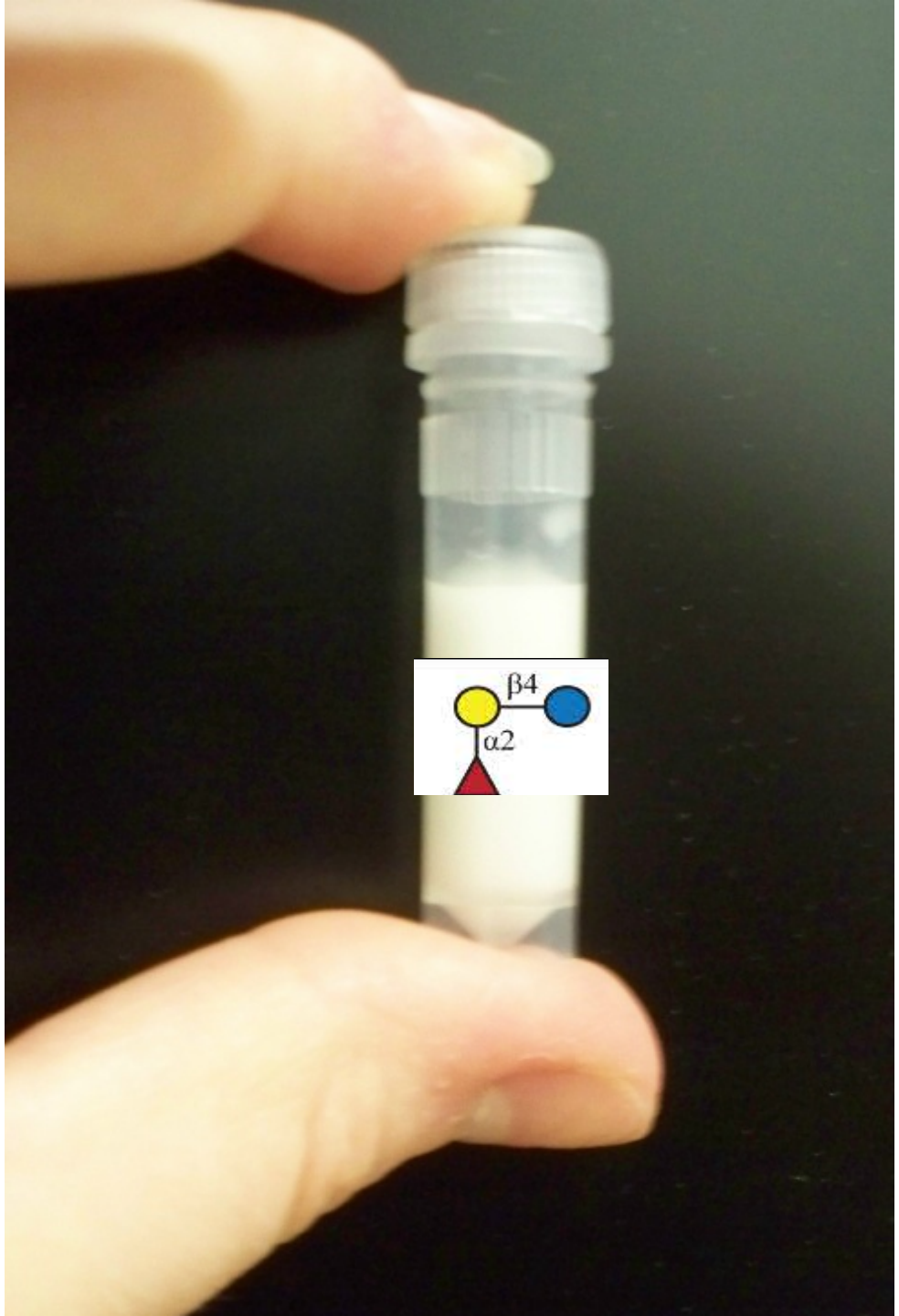
Human milk oligosaccharide *in infant nutrition* and the rationale for testing as a medical food for health



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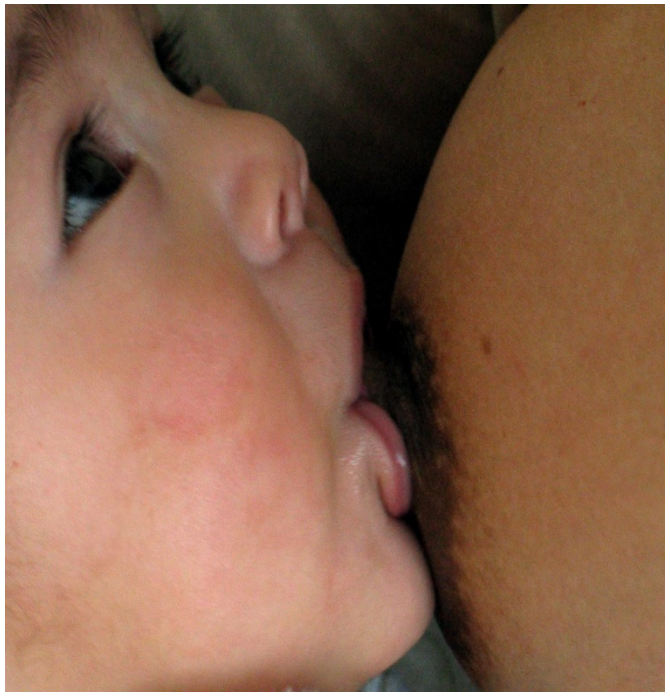
"Human milk is medicine" that protects against infectious & inflammatory conditions

- Severe lower respiratory tract infections (LRTI)
- Acute gastroenteritis (AGE)
- Acute otitis media (AOM)
- Necrotizing enterocolitis (NEC)
- Sudden infant death syndrome
- Atopic dermatitis
- Childhood asthma
- Childhood leukemia
- Type 1 diabetes



(Ip, Chung et al, 2007; Bartick & Reinhold, Pediatrics, 2010)

Life begins with a serious challenge

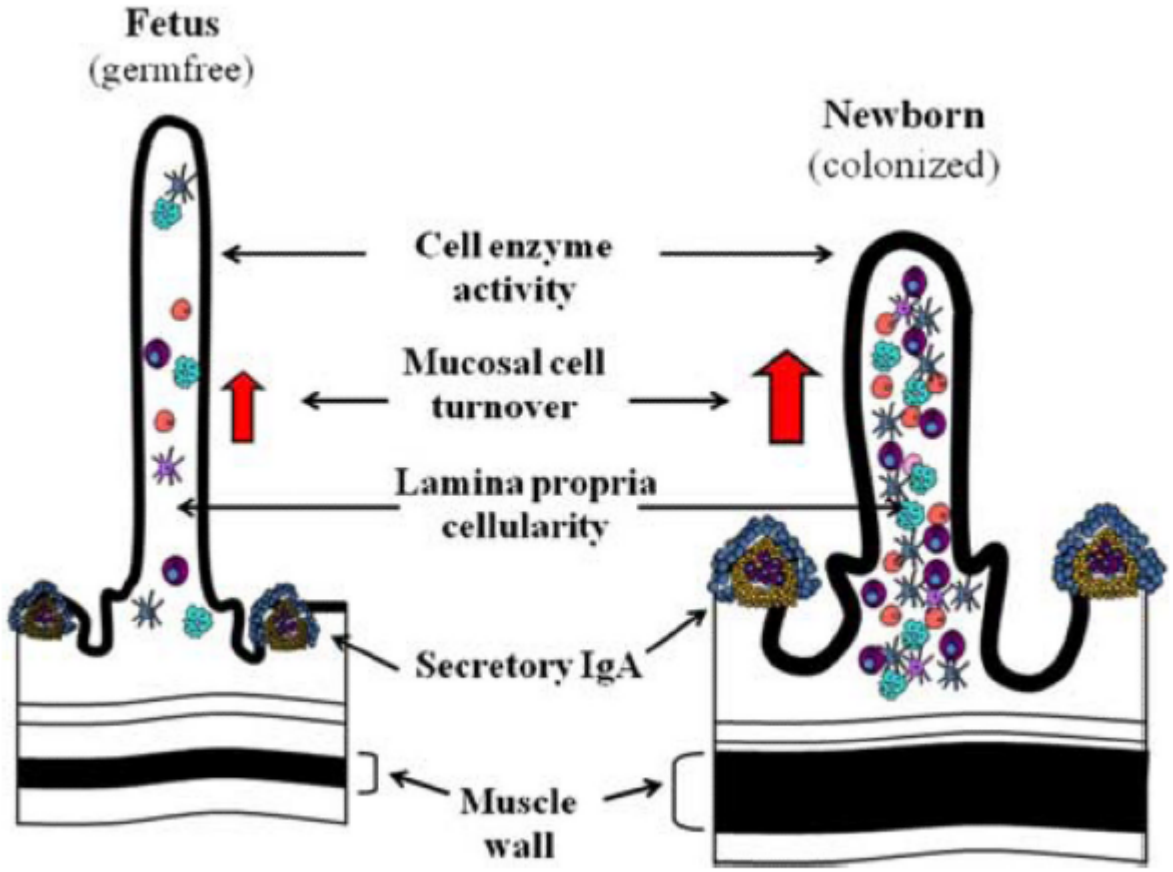
>1000 different bacterial species

>100,000,000,000,000 organisms

We need beneficial microbiota to develop

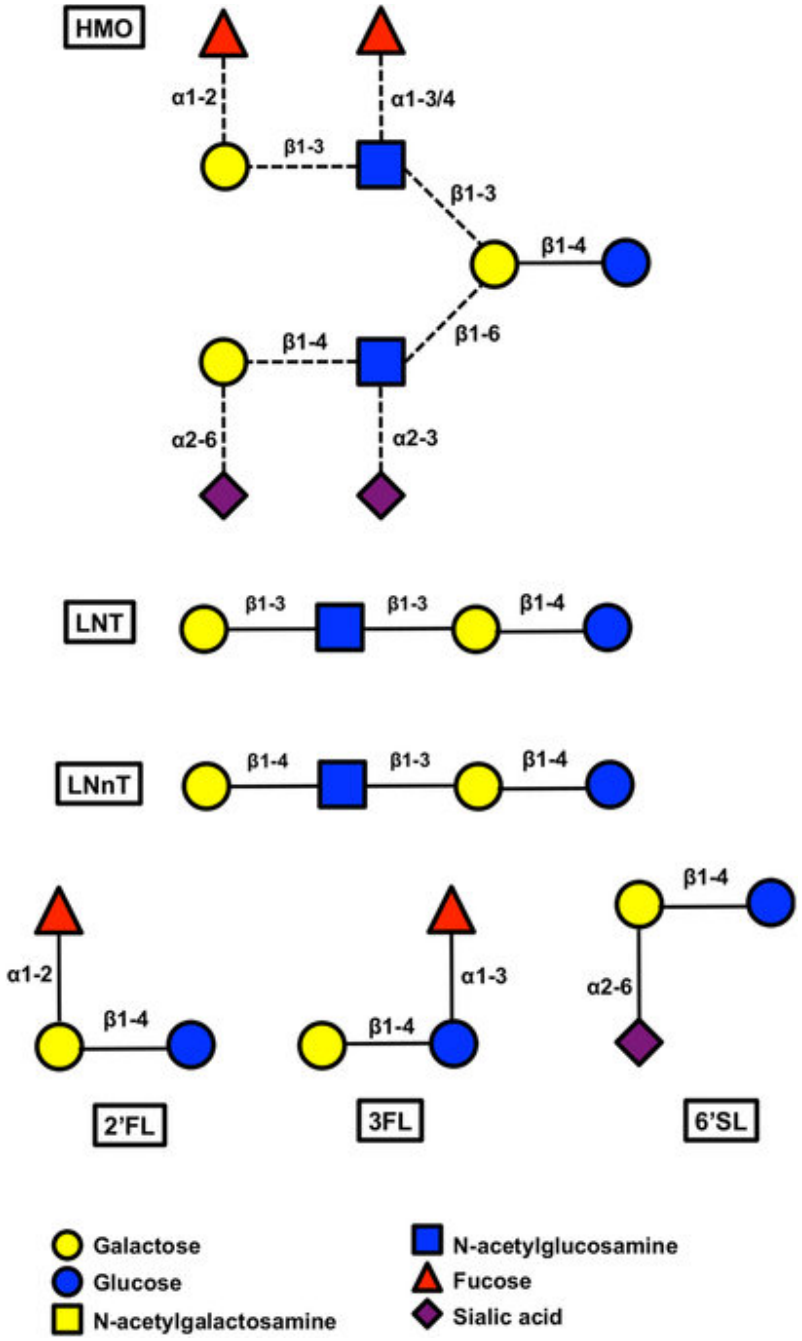
In germ-free state:

- Deficient production of mucins, sIgA and antimicrobial peptides
- Slow cell turnover
- Mucosa not well differentiated.
- Certain nutrients missing
- Immune system not developed.



hMOS: Most abundant bioactive fraction of human milk

- Oligosaccharide
- Glycoproteins and glycolipids
- Secretory antibody
- Antimicrobial peptides
- Free fatty acids
- Cells
- Cytokines and chemokines
- Hormones, growth factors, enzymes
- Anti-inflammatory agents
- Gut barrier maturation agents



Human Milk Oligosaccharide (hMOS)

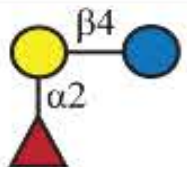
- Third most abundant constituent of human milk
- 3-32 sugars in length
- More than hundred individual hMOS
- Based on lactose to make more complex structures
- Most contain fucose or sialic acid

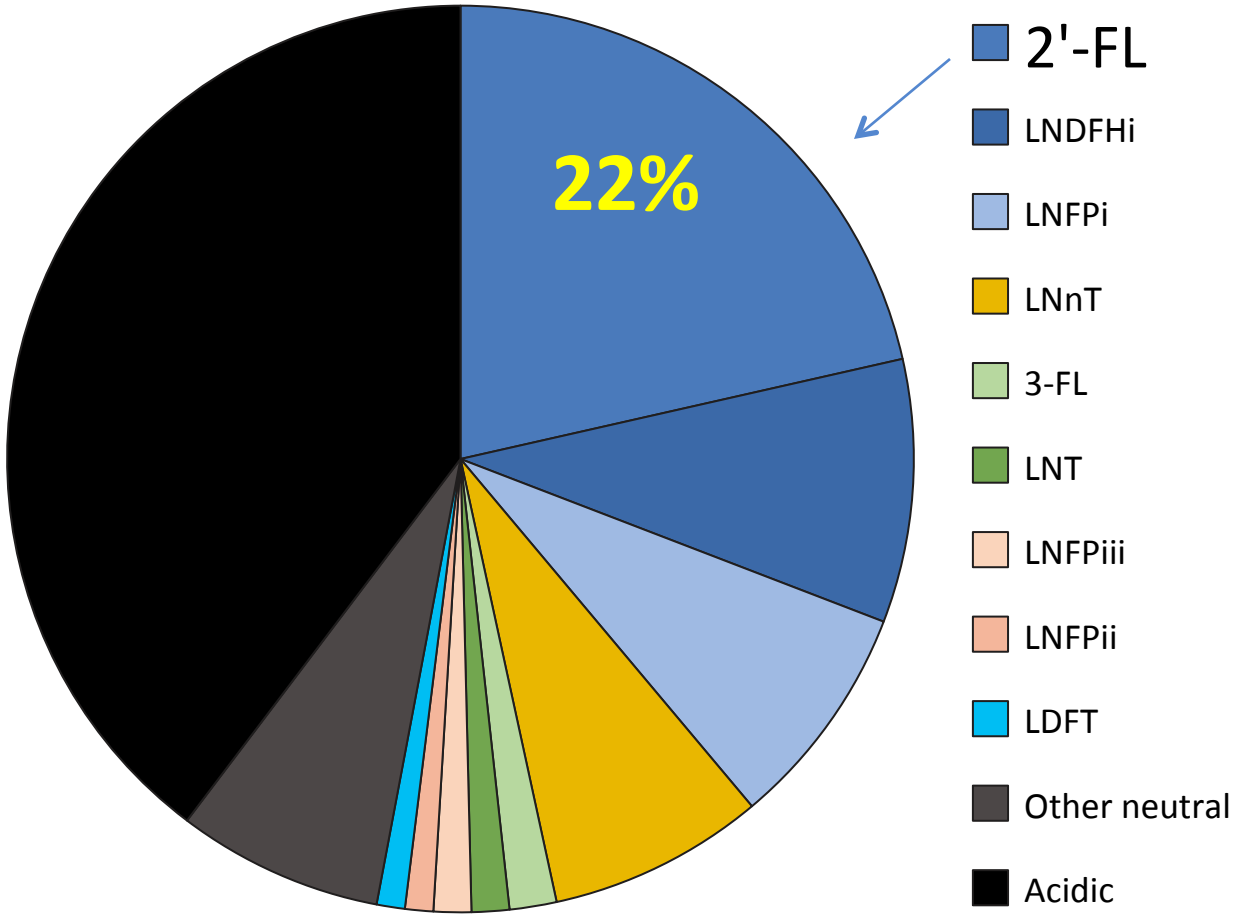
Relative abundance at week 4 postpartum

(n=120, Mexico City)

~ 12 individual hMOS comprise ~75% of hMOS fraction

2’-FL





Morrow, Newburg, Ruiz-Palacios

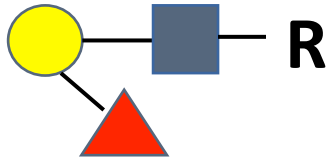
FUT2 gene expression

(Chromosome 19)

Mucosa

Secretions

H(O) antigen

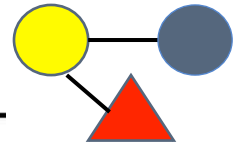


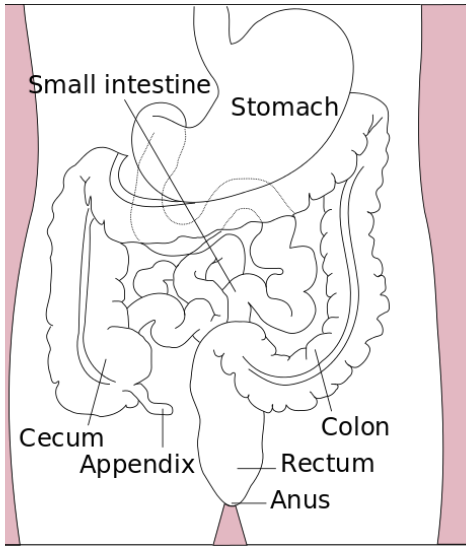
gut

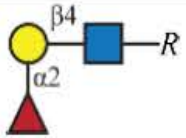
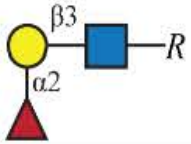
saliva

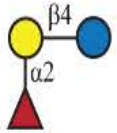
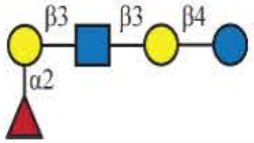
milk

2’-FL





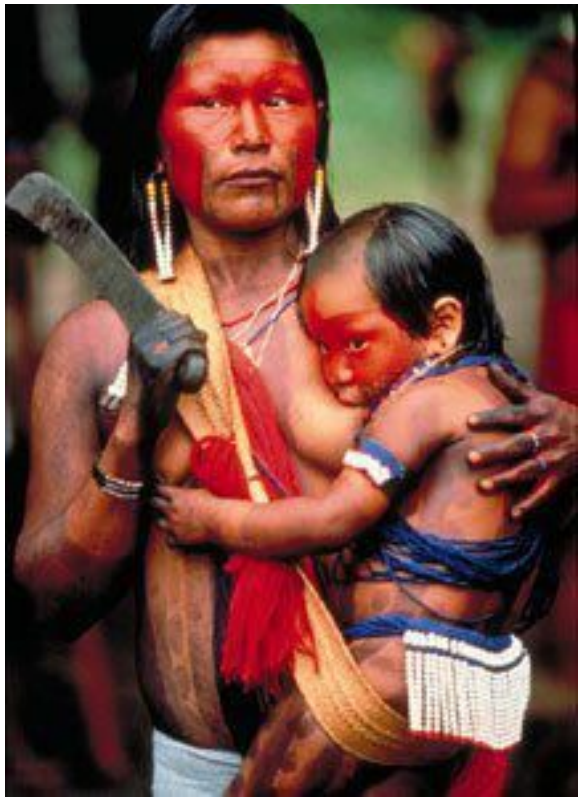
Gut histo-blood group antigen	
H-2	
H-1	

Major HMOS[a]	
2’-FL	
LNFP I	

Fucose Galactose Glucose N-acetyl galactosamine N-acetyl glucosamine

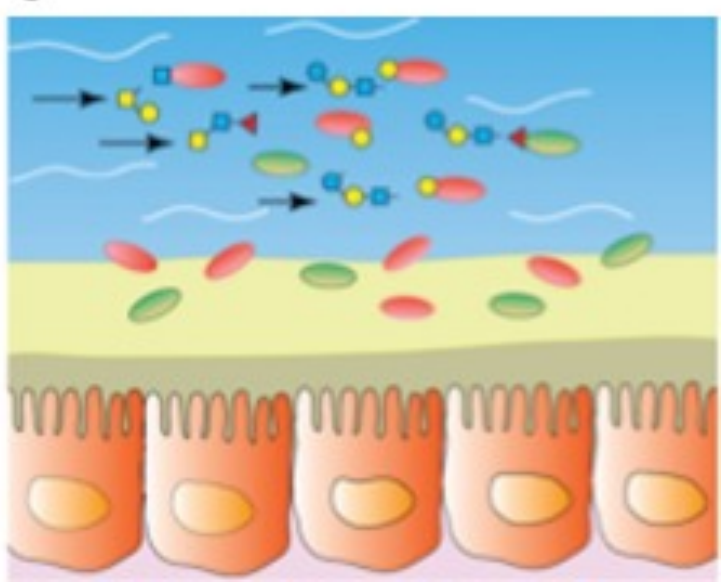
hMOS mechanisms of action

- **Prebiotic effects (growth of beneficial bacteria)**
- **Soluble receptors for specific pathogens**
  that inhibit binding to enterocyte
- **Immune modulation**
- **Intestinal adaptation/**
  restoration/ catch-up growth



Human milk oligosaccharide supports growth and metabolism of beneficial bacteria, discourages growth of pathogens

Marcobal A, et al, Glycobiology, 2013



Human milk oligosaccharides (hMOS) provide prebiotic carbohydrate substrate for nutritional support to a healthy community of microbes.

Yu Z et al. Glycobiology, 2013

PREBIOTIC EFFECT

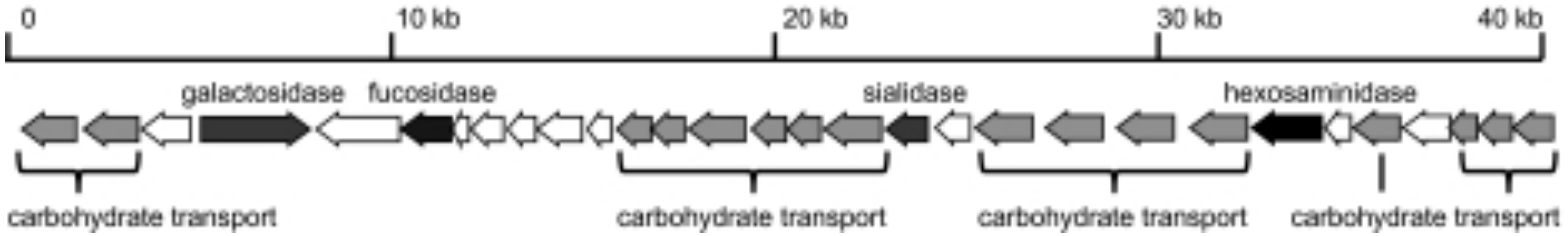
Growth of beneficial bacteria	**2'-FL**	**3-FL**	**3'-SL**	**6'-SL**
Bifidobacterium longum subsp. infantis	+	+	+	+
Bacteroides thetaiotaomicron	+	+	+	+

Metabolism produces short chain fatty acids that change the gut pH, benefit gut and improve host immunity.

Bacterial growth on hMOS depends on their genetics

Bifidobacteria more abundant in breastfed infants

Human milk glycobiome and its impact on the infant gastrointestinal microbiota
Angela M. Zivkovic, J. Bruce German, Carlito B. Lebrilla, and David A. Mills



HMO-related gene cluster 1 from *B. longum* subsp. *infantis* contains all of the necessary glycosidases and carbohydrate transporters necessary for importing and metabolizing HMOs. **PNAS, 2011**

hMOS: Does not stimulate growth of pathogens

Enterobacteriaceae

Group 3 –
Cronobacter,
Citrobacter,
Enterobacter

Group 2 –
Klebsiella spp.

Group 1 –
E. coli and Shigella dysenteriae

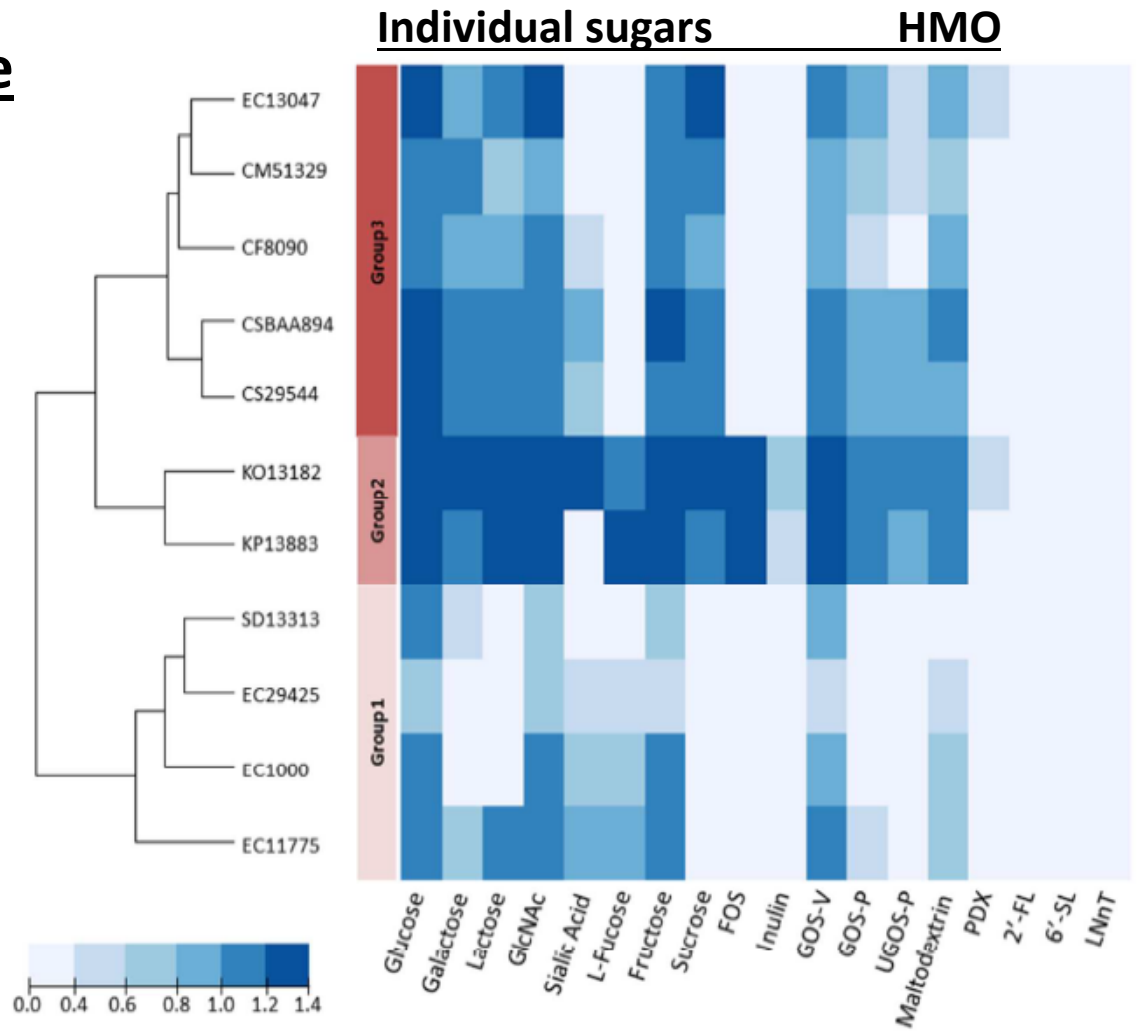
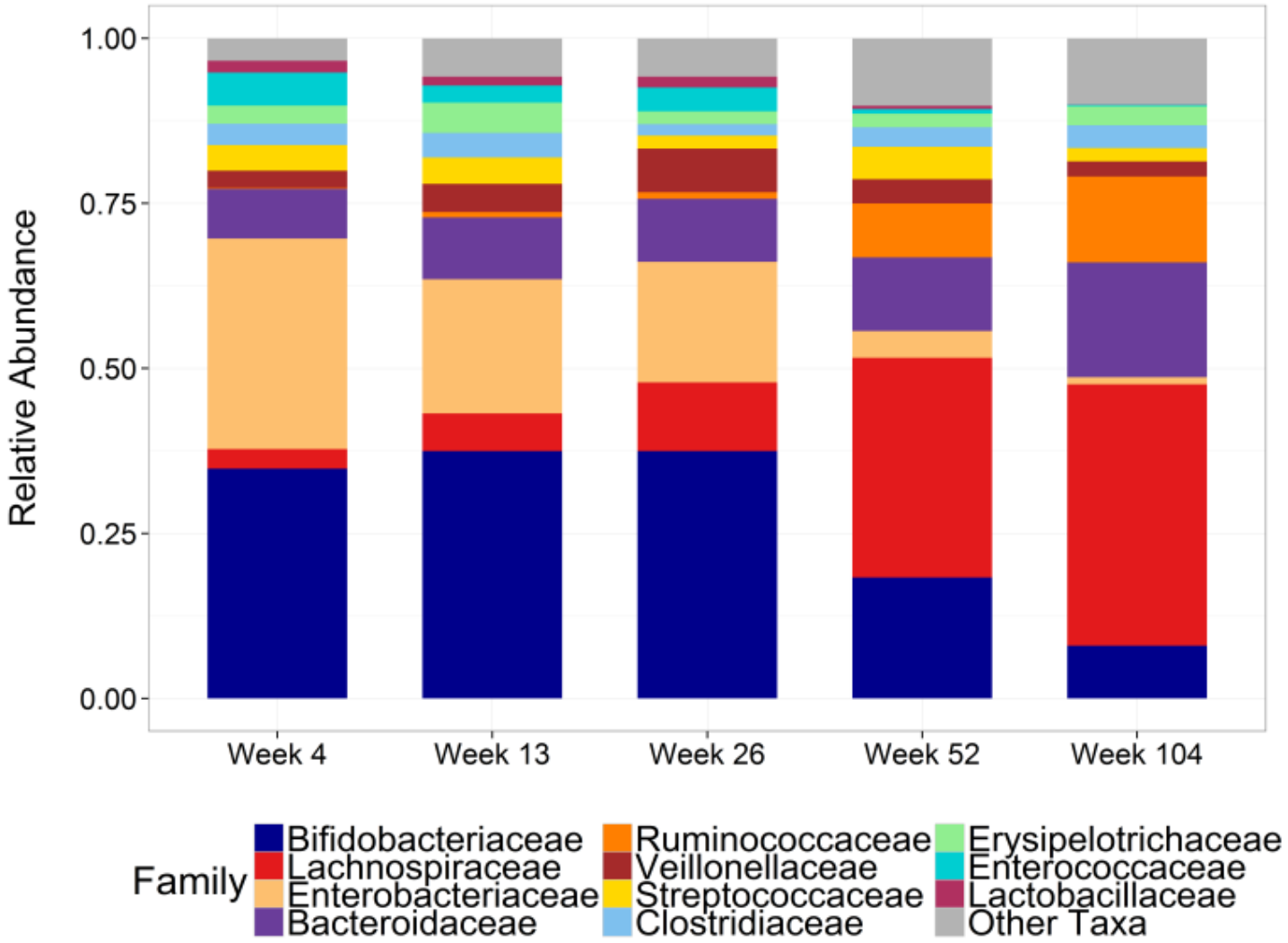


Figure 1. *In vitro* growth of carbohydrates by selected *Enterobacteriaceae*.

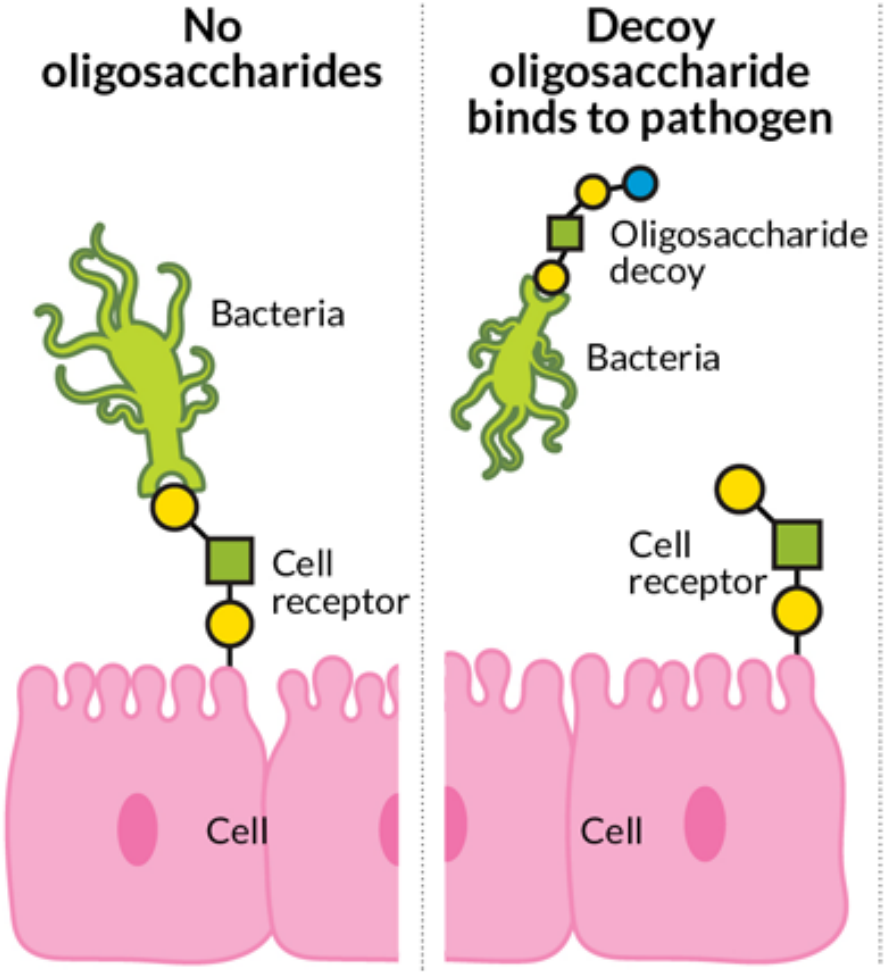
Hoeflinger J et al, 2015

Microbial succession in Cincinnati breastfed infants: GEHM Study

(n=120 healthy & predominantly breastfed)



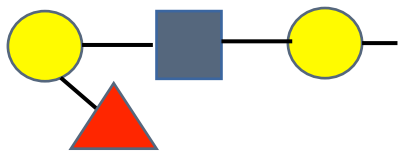
hMOS can block pathogen attachment to gut oligosaccharide receptors



Gut cell surface oligosaccharides influence pathogen-host cell interactions

Pathogens that attach to cell surface oligosaccharides:

- Noroviruses
- Rotaviruses
- Campylobacter
- Diarrheagenic E. coli
- V. cholerae
- H. pylori



H antigen

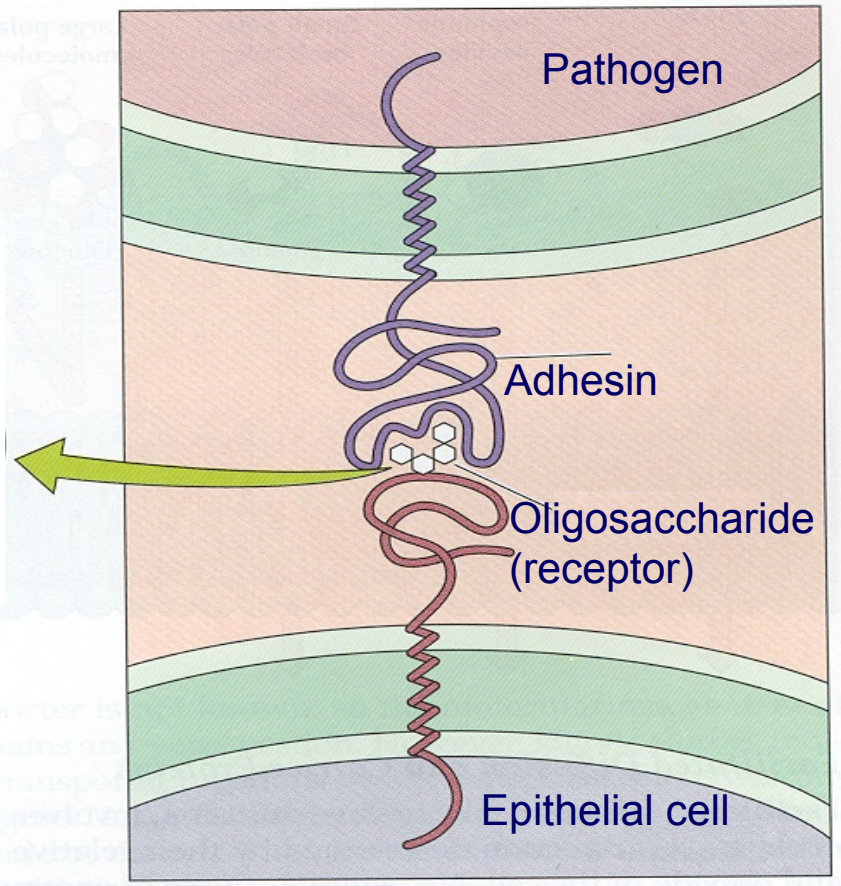


Image courtesy of G. Ruiz-Palacios

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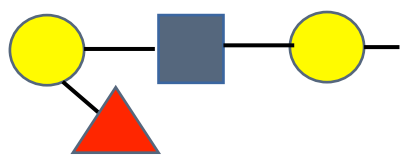
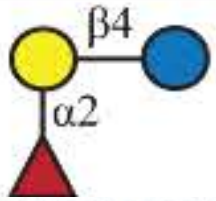


H antigen



2'-FL

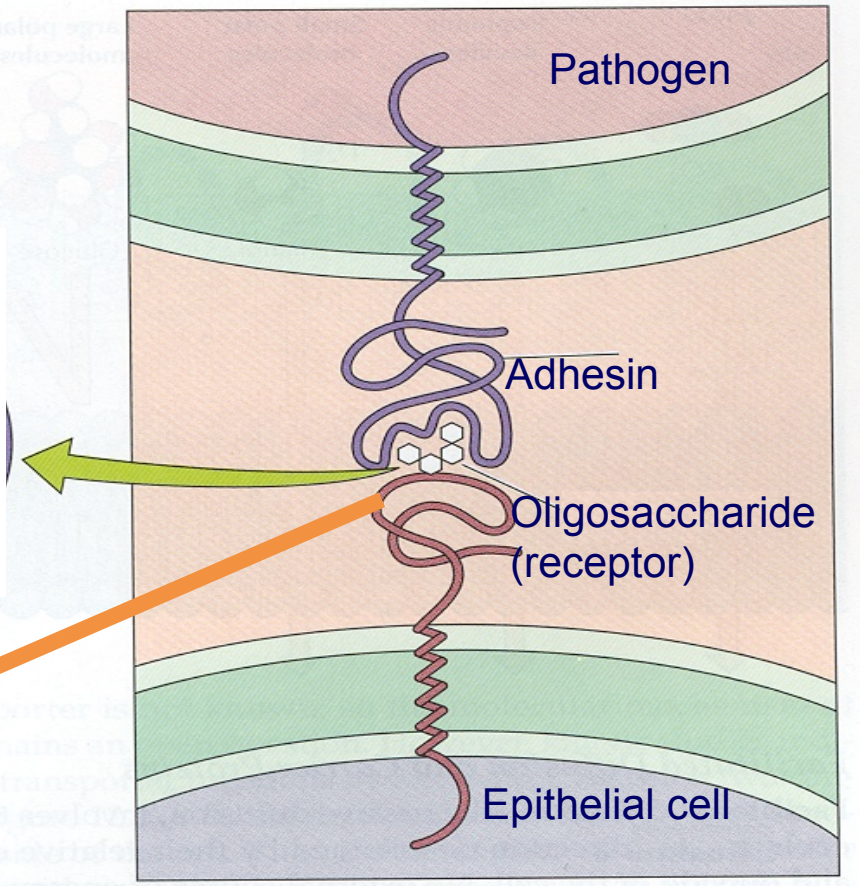


Image courtesy of G. Ruiz-Palacios

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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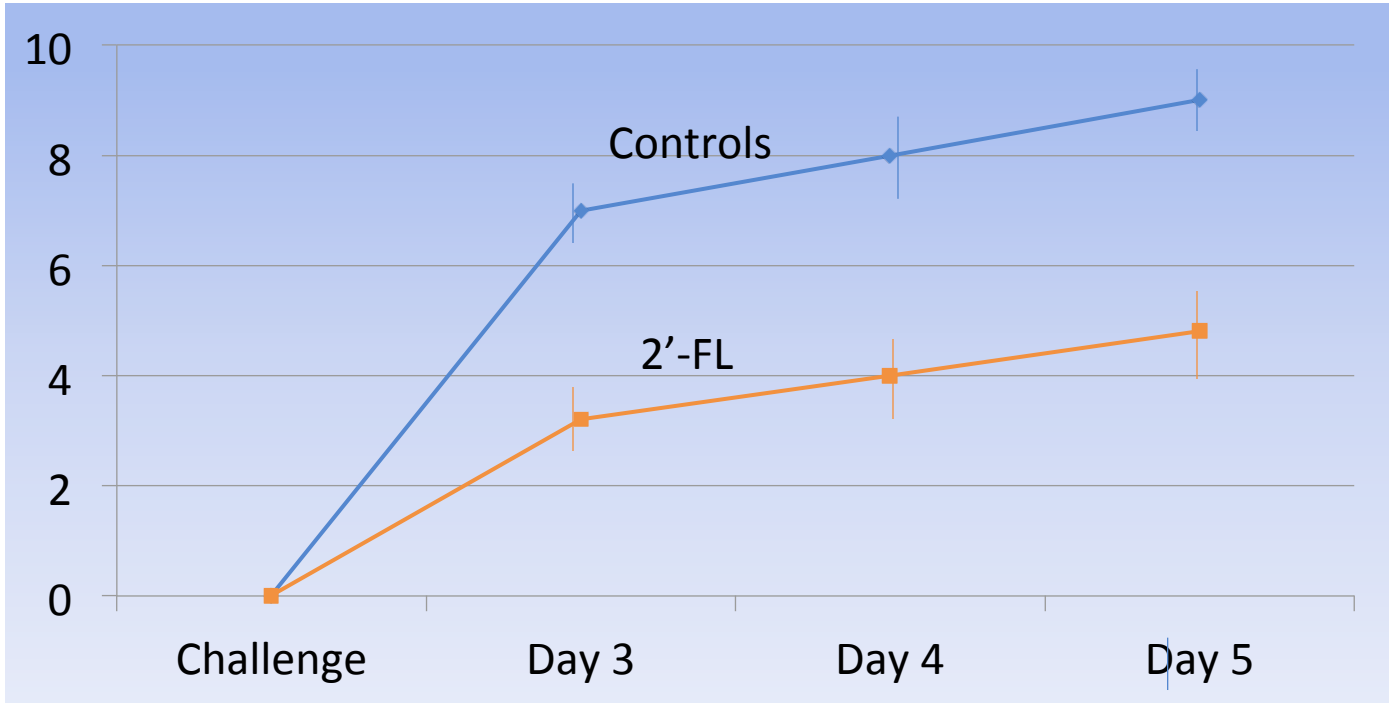
Vol. 278, No. 16, Issue of April 18, pp. 14112–14120, 2003
Printed in U.S.A.

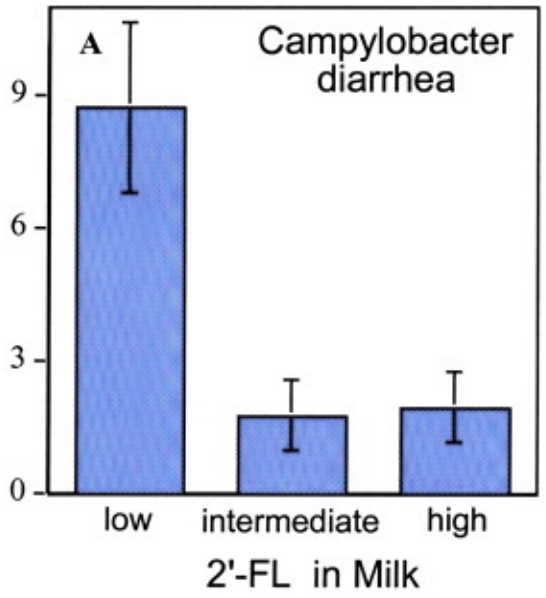
Campylobacter jejuni Binds Intestinal H(O) Antigen (Fucα1, 2Galβ1, 4GlcNAc), and Fucosyloligosaccharides of Human Milk Inhibit Its Binding and Infection*

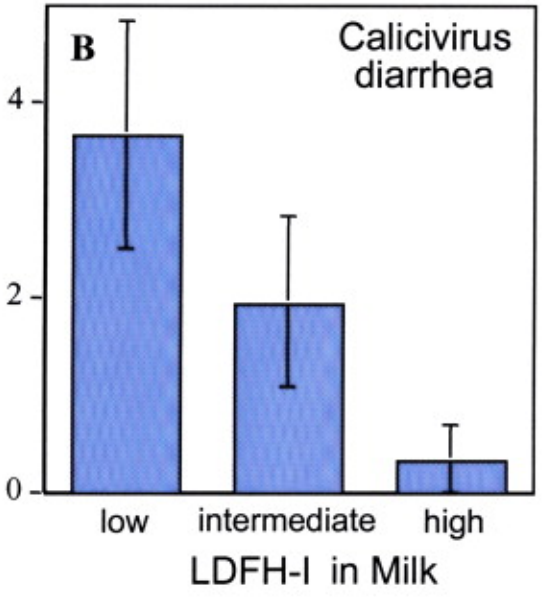
Received for publication, July 31, 2002, and in revised form, January 31, 2003
Published, JBC Papers in Press, January 31, 2003, DOI 10.1074/jbc.M207744200

Guillermo M. Ruiz-Palacios‡§, Luz Elena Cervantes‡, Pilar Ramos‡, Bibiana Chavez-Munguia¶, and David S. Newburg‖



2-week old BALB/c mice were challenged with 10^4 CFU of invasive campylobacter strain 287ip. Half given oral 2'-FL by gavage.





hMOS in prevention of diarrhea (Morrow, J Nutr, 2005)

Cohort study, n=93 Mexican mother-infant pairs

When 2'-FL and other "secretor" HMOs in higher abundance, lower risk of diarrhea in infancy

- Campylobacter-associated diarrhea
- Calicivirus (Norovirus-associated diarrhea)
- All causes of moderate to severe diarrhea

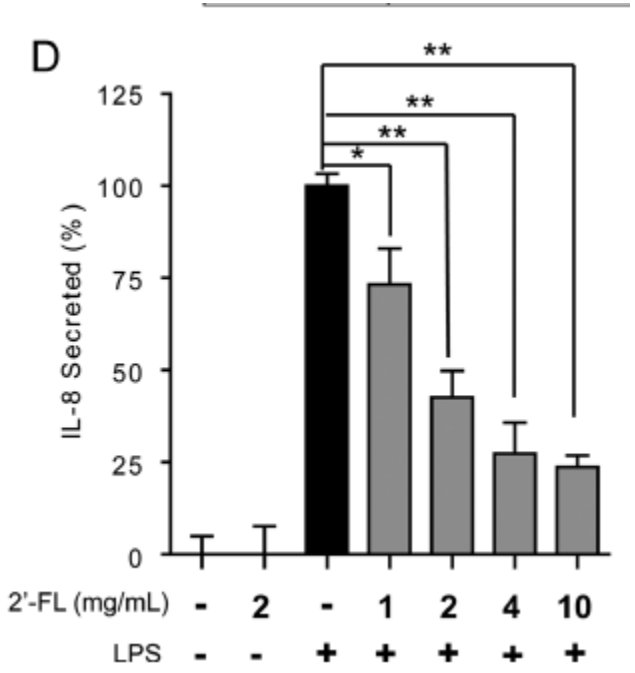
2’-FL human milk oligosaccharide: Anti-inflammatorv effects

ORIGINAL ARTICLE

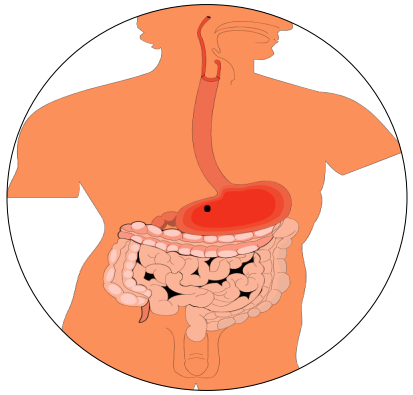
The human milk oligosaccharide 2′-fucosyllactose modulates CD14 expression in human enterocytes, thereby attenuating LPS-induced inflammation

YingYing He,[1,2] ShuBai Liu,[3] David E Kling,[2] Serena Leone,[2] Nathan T Lawlor,[2] Yi Huang,[2] Samuel B Feinberg,[2] David R Hill,[2] David S Newburg[1,2]

Gut, 2014



hMOS in gut repair/adaptation following injury



- 2’-FL restores intestinal perfusion by up-regulating nitric oxide synthase (eNOS), promoting vasodilation (Good M, 2016)
- 2’-FL reduces severity of necrotizing enterocolitis (Good M, 2016; Autran CA, 2016; Jantscher-Krenn, 2012)
- 2’-FL enables growth after gut injury (Mezoff, 2016; Weiss, 2015)

Summary of hMOS (2'-FL) in infant nutrition

- Major human milk component;

- Just now being offered in infant formula by some companies
  - Safe
  - Shifts microbial metabolism
  - Reduces risk of infections
  - Has other important effects: repair, metabolic and immune

Other potential health applications

Epidemiology of infectious & immune diseases

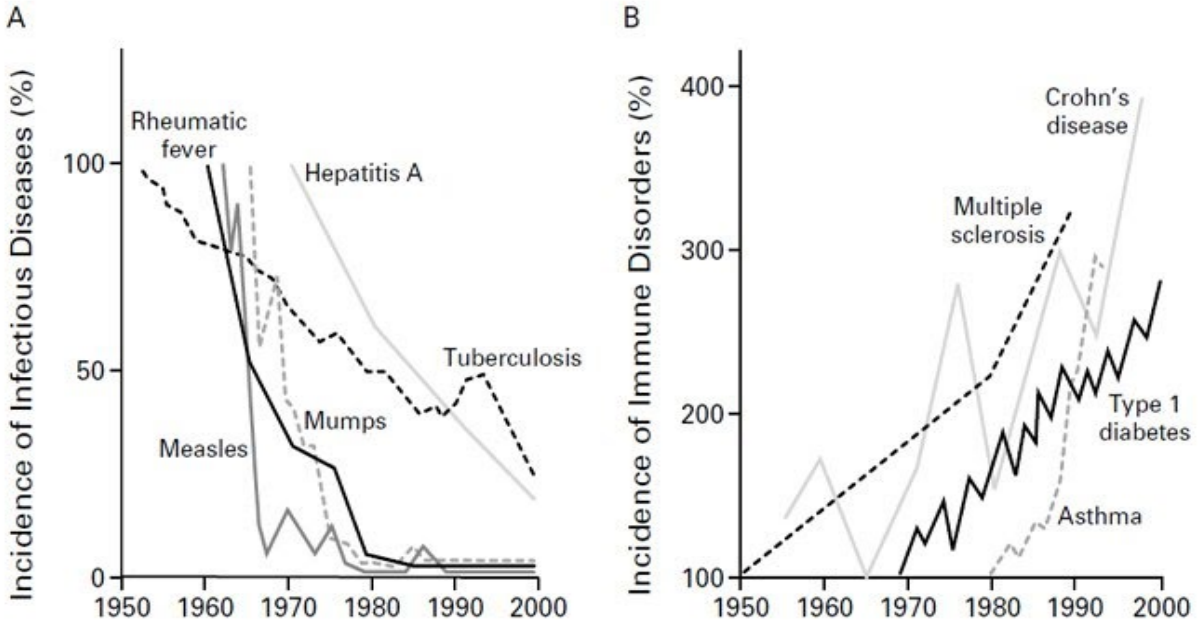
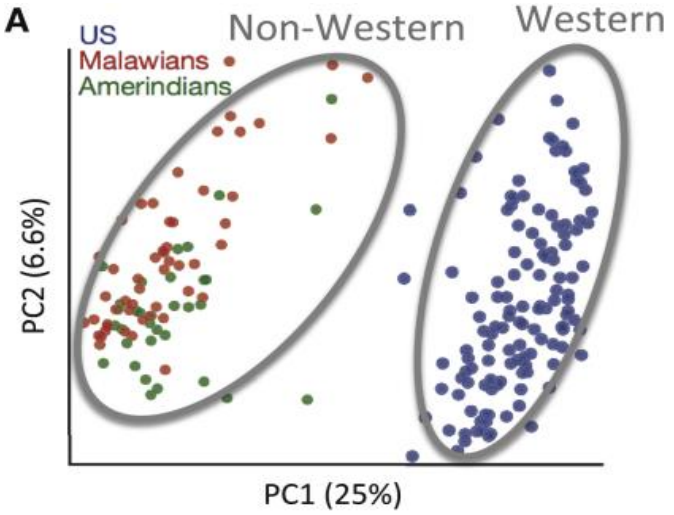
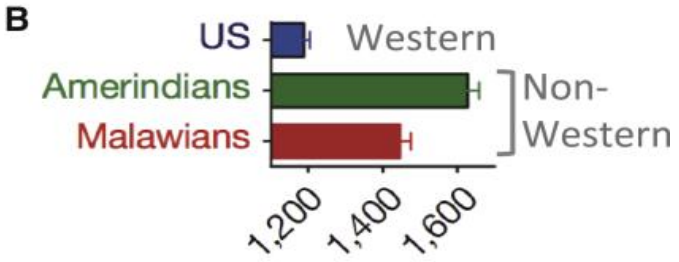
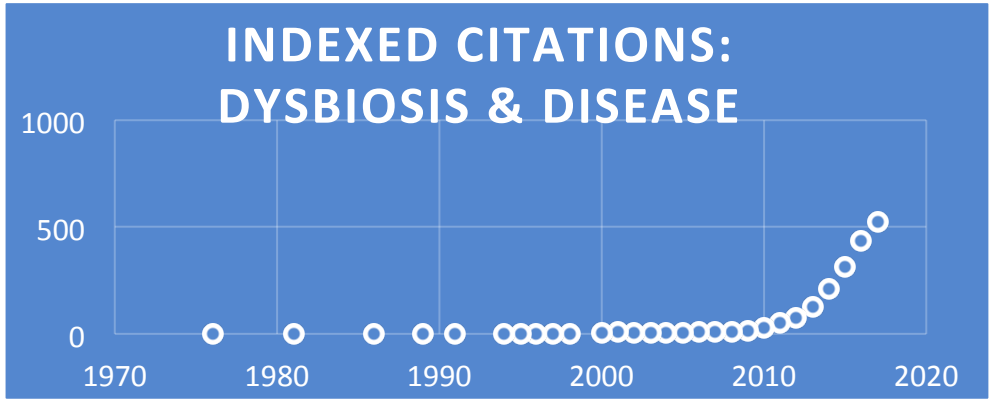


Figure 1. Inverse Relation between the Incidence of Prototypical Infectious Diseases (Panel A) and the Incidence of Immune Disorders (Panel B) from 1950 to 2000.

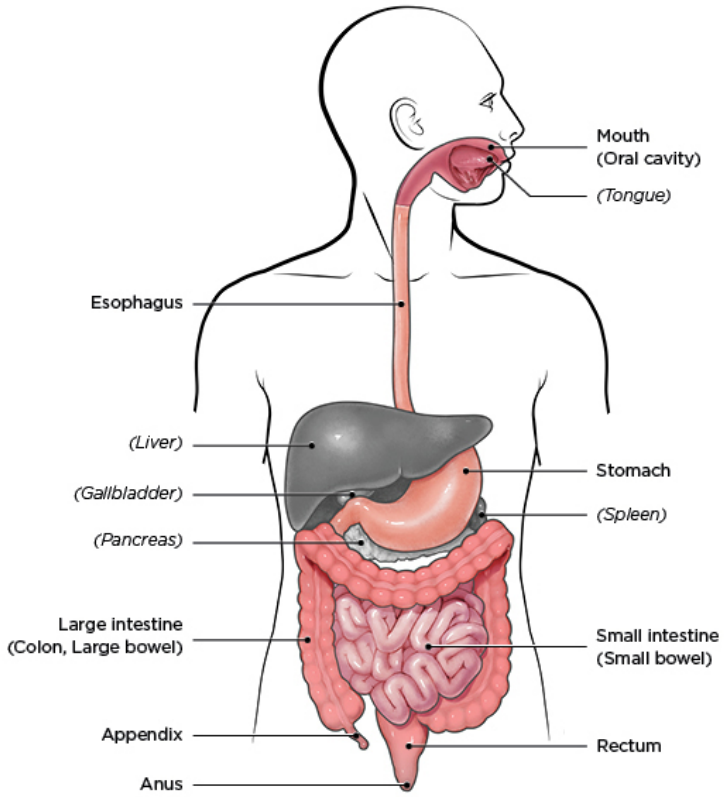
In Panel A, data concerning infectious diseases are derived from reports of the Centers for Disease Control and Prevention, except for the data on hepatitis A, which are derived from Joussemet et al.[12] In Panel B, data on immune disorders are derived from Swarbrick et al.,[10] Dubois et al.,[13] Tuomilehto et al.,[14] and Pugliatti et al.[15]







What is Inflammatory bowel disease?



- Chronic inflammatory disease(s) of the intestinal tract
- Characterized by dysbiosis

Common symptoms

- Persistent diarrhea
- Abdominal pain
- Rectal bleeding/bloody stools
- Weight loss
- Fatigue

Most cases diagnosed before 20 yrs
Flares are unpredictable

Two major types: Crohn's & Ulcerative colitis

Rationale for testing 2'-FL in IBD

2'-FL

Lee ("Ted") Denson, MD
Professor of Pediatric Gastroenterology
CCHMC

- Globally, estimate >5 million individuals suffer from IBD
- >3/1000 in most westernized countries, increasing elsewhere >11% per year (Ng SC, Lancet, 2017)
- Effective treatments (TNF-α inhibitors) for achieving control/remission
- But no effective treatment for sustaining remission

Why test 2'-FL for IBD?

Genetic signature from GWAS of Crohn's Disease

Human Molecular Genetics, 2010, Vol. 19, No. 17 **3468–3476**
doi:10.1093/hmg/ddq248
Advance Access published on June 22, 2010

Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease

Dermot P.B. McGovern[1,2,*], Michelle R. Jones[3], Kent D. Taylor[2], Kristin Marciante[4], Xiaofei Yan[2], Marla Dubinsky[1], Andy Ippoliti[1], Eric Vasiliauskas[1], Dror Berel[1], Carrie Derkowski[1], Deb Dutridge[2], International IBD Genetics Consortium, Phil Fleshner[1], David Q. Shih[1], Gil Melmed[1], Emebet Mengesha[2], Lily King[2], Sheila Pressman[2], Talin Haritunians[2], Xiuqing Guo[2], Stephan R. Targan[1] and Jerome I. Rotter[2]

[1]Inflammatory Bowel and Immunobiology Research Institute, [2]Medical Genetics Institute and [3]Endocrinology, Diabetes & Metabolism, Cedars-Sinai Medical Center, Los Angeles, CA, USA and [4]Cardiovascular Health Research Unit, Department of Internal Medicine, University of Washington, Seattle, WA, USA

OPEN

The ISME Journal (2014) 8, 2193–2206

www.nature.com/ismej

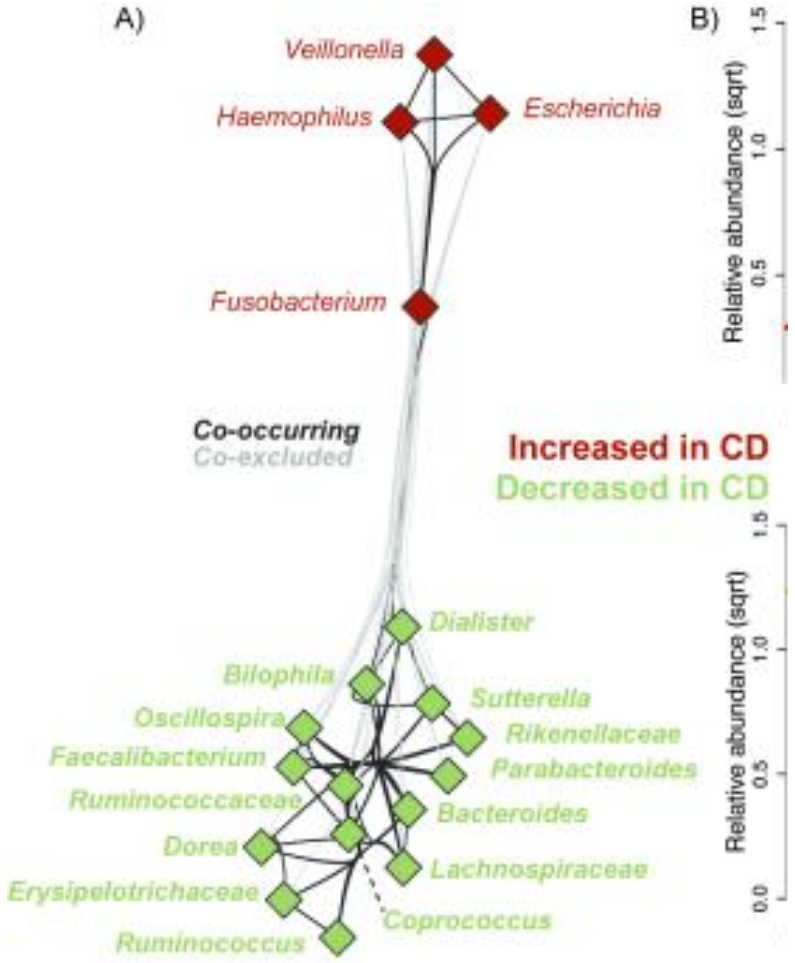
ORIGINAL ARTICLE

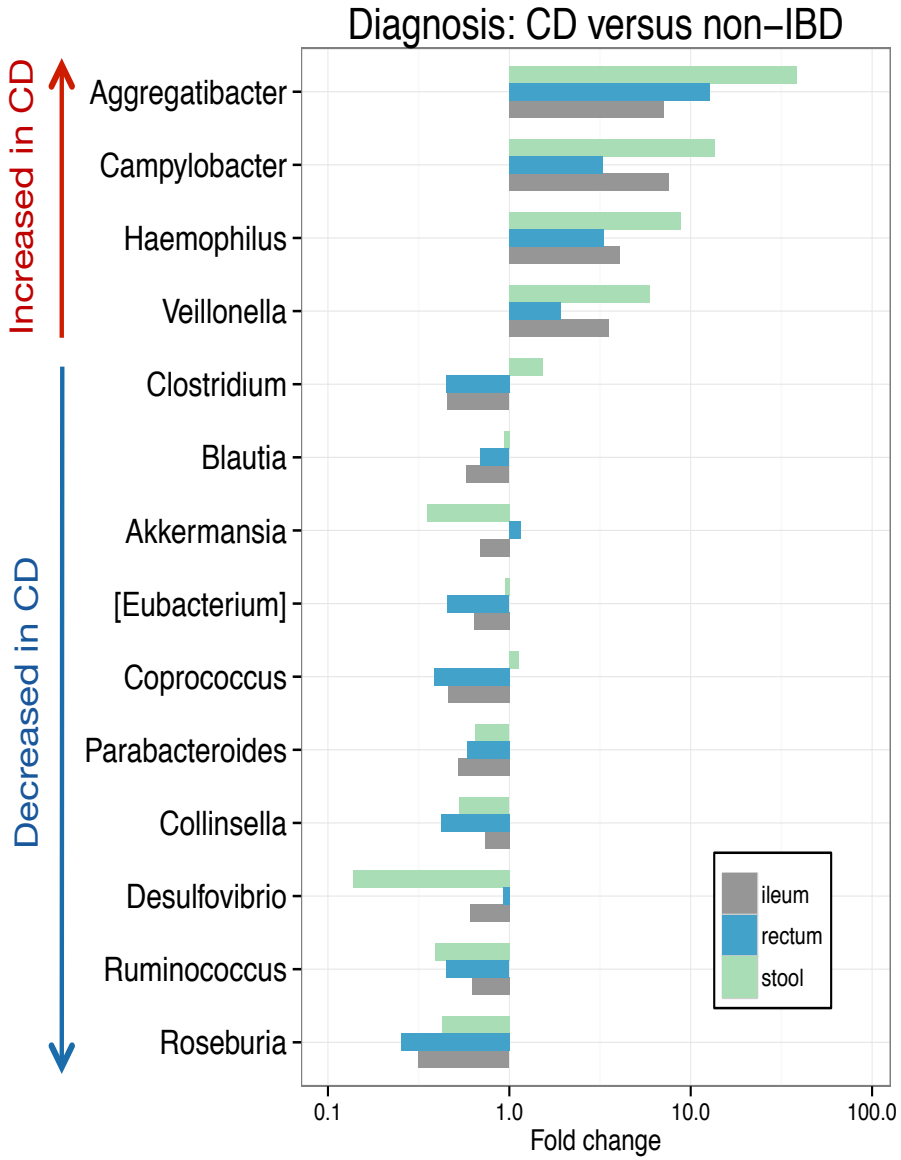
Reprograming of gut microbiome energy metabolism by the *FUT2* Crohn's disease risk polymorphism

Maomeng Tong[1], Ian McHardy[2], Paul Ruegger[3], Maryam Goudarzi[4], Purna C Kashyap[5], Talin Haritunians[6], Xiaoxiao Li[6], Thomas G Graeber[1], Emma Schwager[7], Curtis Huttenhower[7], Albert J Fornace Jr[4], Justin L Sonnenburg[5], Dermot PB McGovern[6], James Borneman[3] and Jonathan Braun[1,2]

- Profiled microbiome, meta-proteome and meta-metabolome of endoscopic lavage samples of 39 healthy adult subjects
- The microbial metabolism of (FUT2-) non-secretor hosts:
  - ➔ Perturbed energy metabolism pathways
  - ➔ Reduced amino-acid biosynthesis pathways

IBD characterized by microbial dysbiosis





Gevers et al, Cell Host Microbe, 2015

ARTICLE Nature Communic

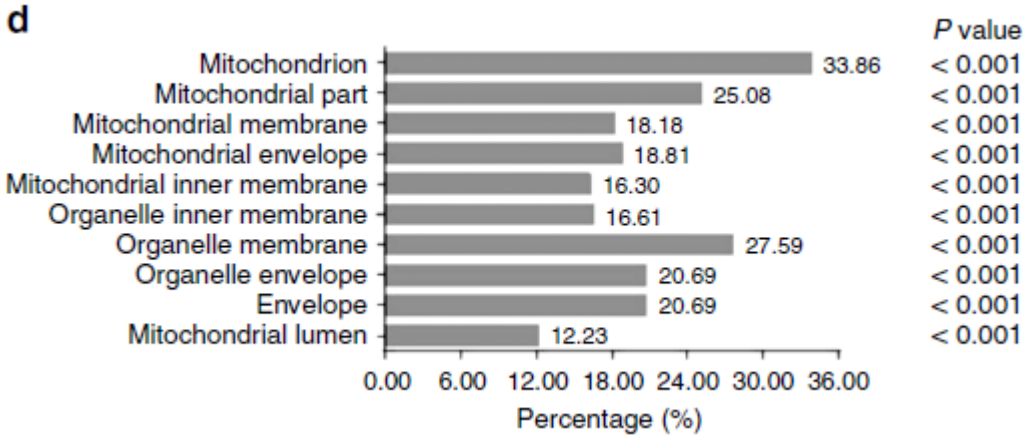
Received 13 May 2016 | Accepted 27 Sep 2016 | Published 23 Nov 2016

DOI: 10.1038/ncomms13419 OPEN

Altered intestinal microbiota–host mitochondria crosstalk in new onset Crohn's disease

Walid Mottawea[1,2,3,*], Cheng-Kang Chiang[1,2,*], Marcus Mühlbauer[4,*], Amanda E. Starr[1,2], James Butcher[1,2], Turki Abujamel[1,2], Shelley A. Deeke[1,2], Annette Brandel[1,2], Hu Zhou[2,5], Shadi Shokralla[6], Mehrdad Hajibabaei[6], Ruth Singleton[7], Eric I. Benchimol[7,8,9], Christian Jobin[10], David R. Mack[7,8], Daniel Figeys[1,2,11] & Alain Stintzi[1,2]

Impaired in new onset Crohn's Disease patients



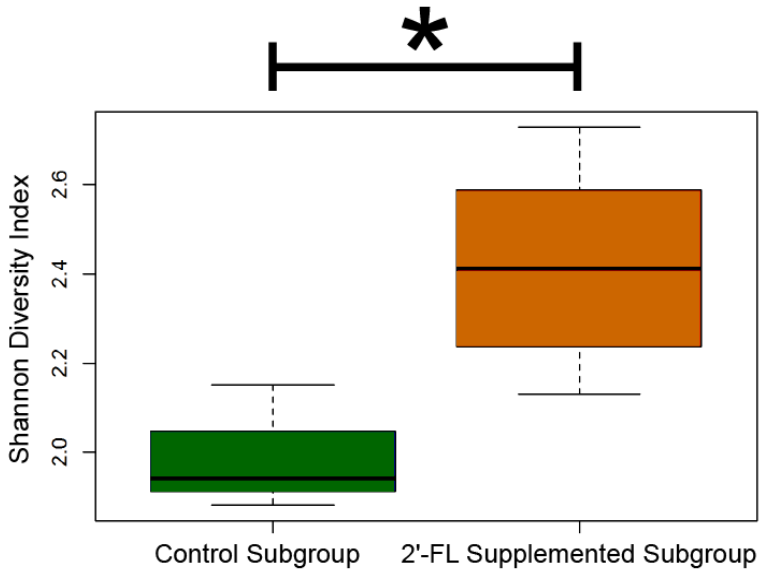
Pediatric patients: 61 Crohn's Disease, 42 Controls

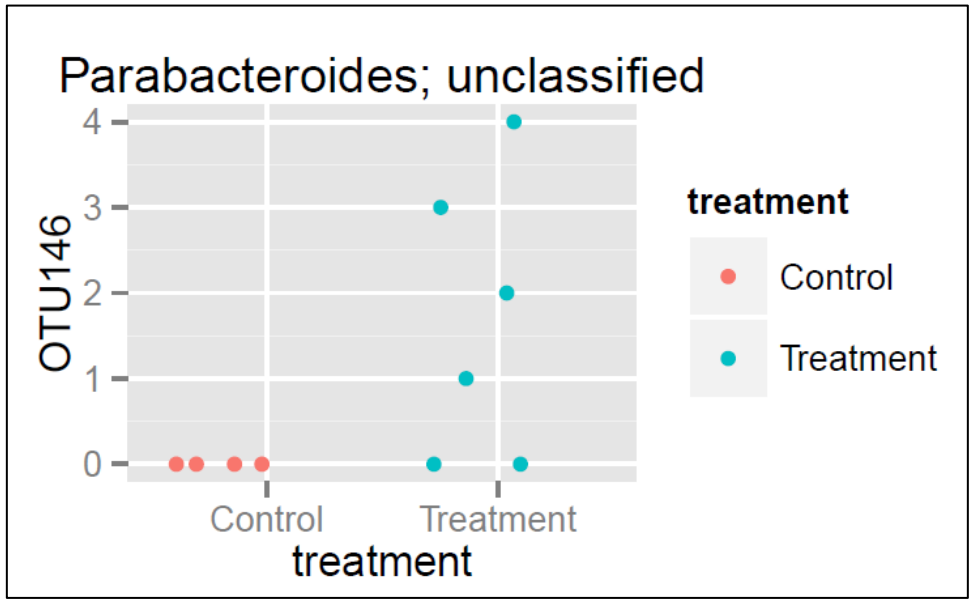
2’-FL induced microbiome changes in Ileo-Cecal Resection (ICR) Model

Mezoff et al, 2016

- Many necrotizing enterocolitis cases require ileo-cecal resection →“short gut”
- **Tested 2’-FL vs control after ICR to improve adaptive response (n=12 mice)**

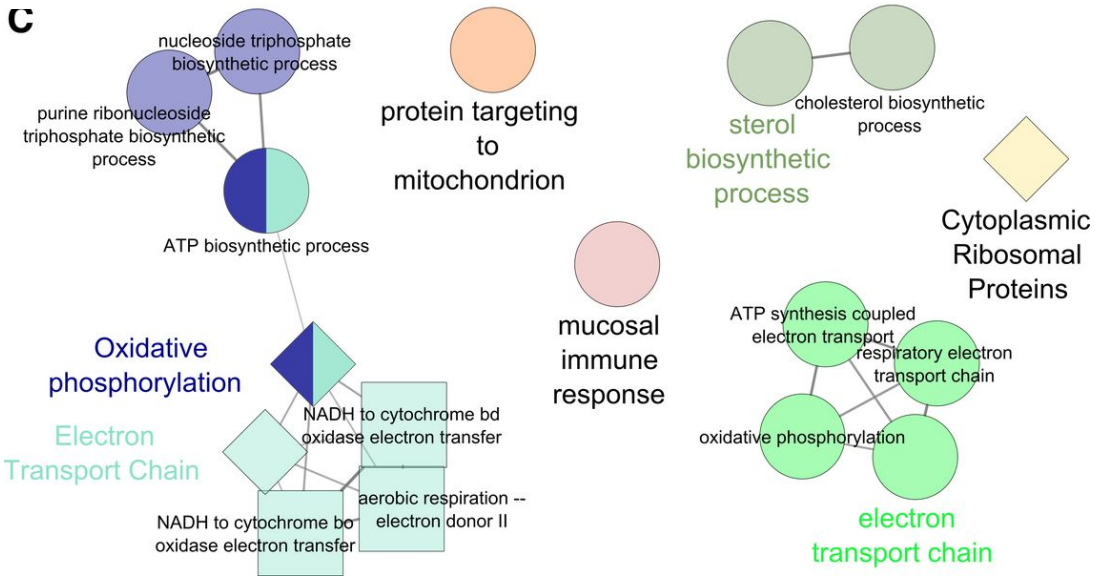
Microbiome Analysis





2'-FL Induced Distal Small Bowel Gene Expression After Ileal-Cecal Resection

Relevant To Intestinal Adaptation



Host-microbial interaction:
multiorganism metabolic processing, symbiosis, mucosal immune response

Hundreds of genes increased expression with 2'-FL:

Energy processing:
electron transport, cellular respiration, **mitochondrial ATP synthesis coupled electron transport**, generation of precursor metabolites and energy, energy derivation by oxidation of organic compounds

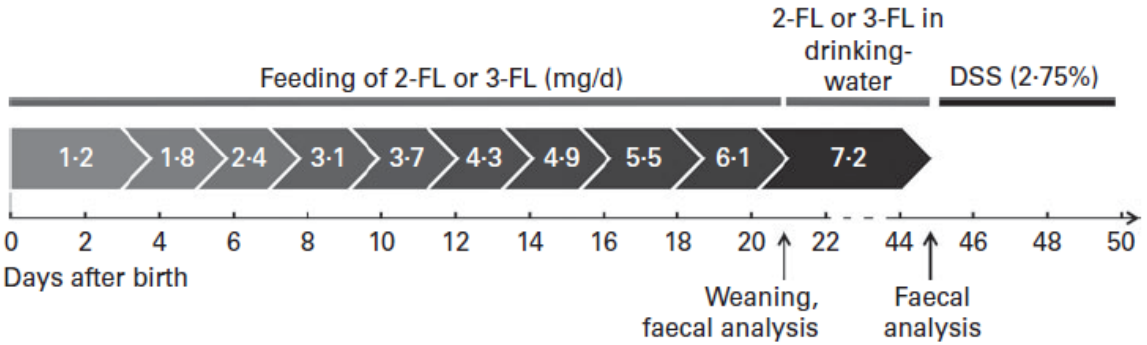
Biosynthetic processes:
sterol, cholesterol and nucleosides

Ethan A. Mezoff et al. AJPGLP 2016;310:G427-G438

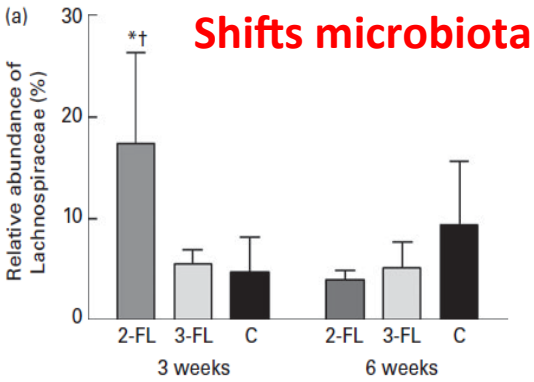
Selective proliferation of intestinal *Barnesiella* under fucosyllactose supplementation in mice

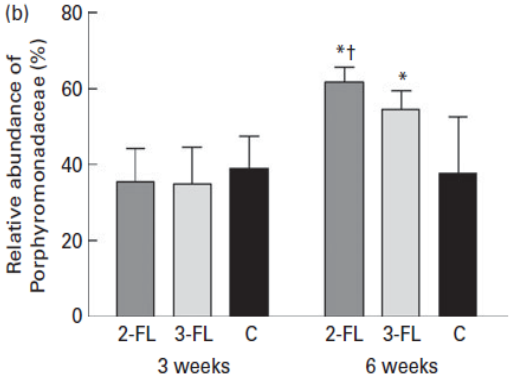
Gisela A. Weiss[1,2], Christophe Chassard[3] and Thierry Hennet[1]*

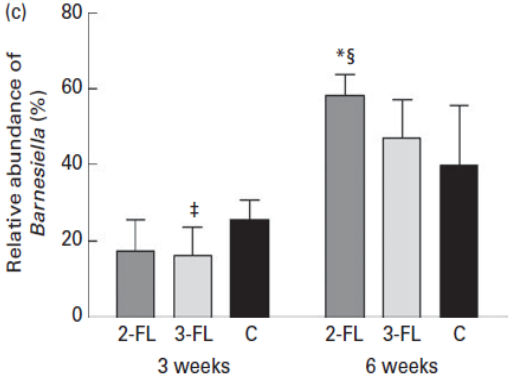
Br J Nutr, 2013



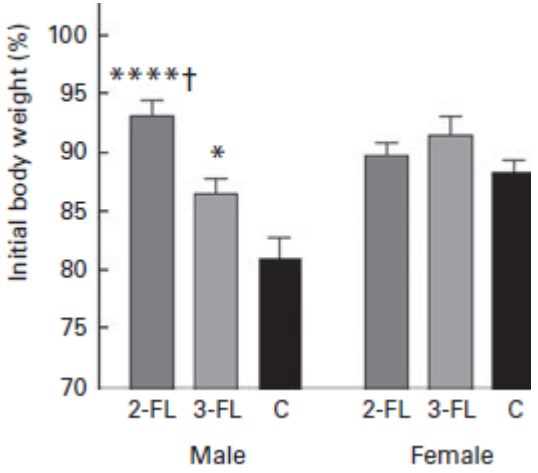
Shifts microbiota



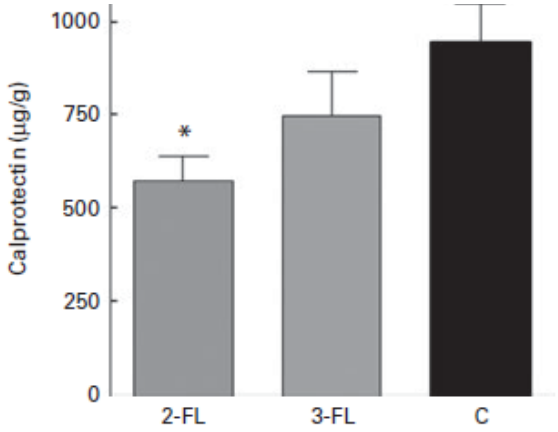




Improves catch-up growth after insult



Reduces gut inflammation



Oral supplementation of healthy adults with 2'-FL

(Elison E , Br J Nutr, 2016)

- RCT of HMO-supplementation in 100 healthy, adult volunteers, consuming chemically produced 2'-FL and/or lacto-N-neotetraose (LNnT) at various daily doses and mixes or placebo for 2 weeks.
- Safe, well-tolerated

- Significant Bifidobacteria ↑
- Significant Firmicutes & Proteobacteria ↓

CLINICAL GOAL of NIH 2'-FL trial: Dosing study to maintain remission in IBD

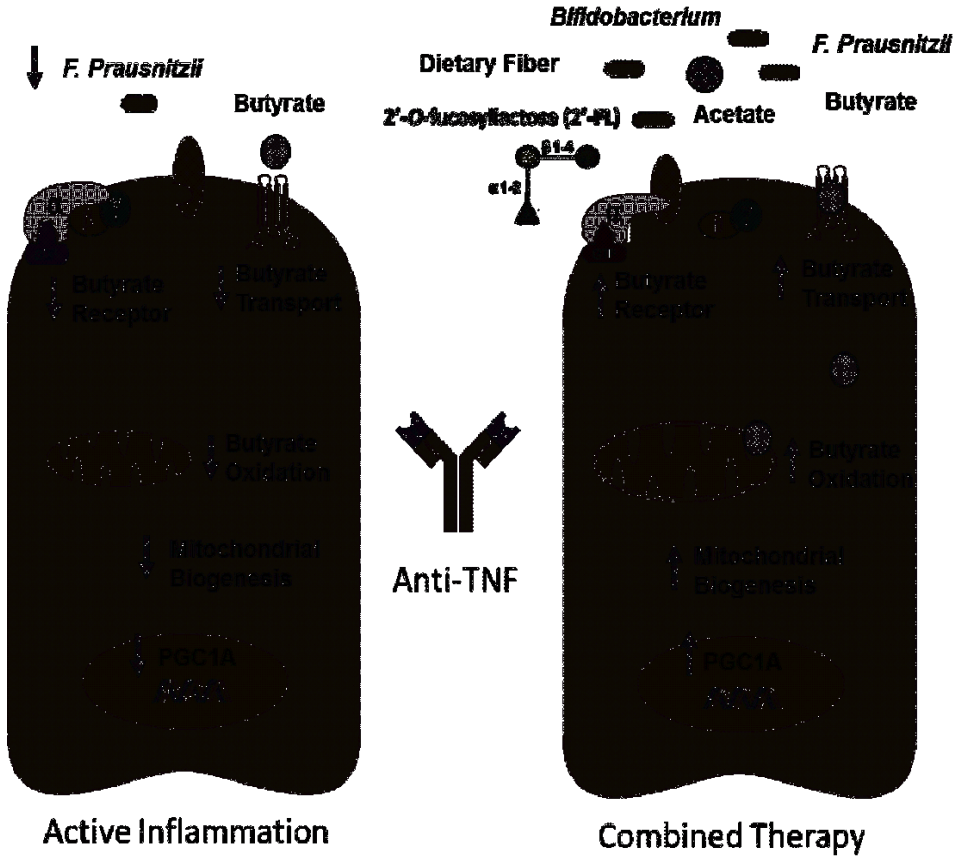
Proposed mechanisms:

- ✓ Reduces dysbiosis: Increases beneficial microbes, decreases pathobionts/ pathogens
- ✓ Stimulates bacterial SCFA production
- ✓ Modifies enterocyte mitochondrial energy production
- ✓ Minimizes gut inflammation

In summary: 2'-FL is promising approach to maintaining remission in IBD

NIH-sponsored trial starting Summer, 2018

Dosing at 3 levels to optimal dose for tolerance, shift in microbiota, reduced fecal calprotectin



Conceptual model of 2'-FL in IBD: Increase Microbial Butyrate Production and Cellular Butyrate Responsiveness.

Dank u wel!


tak
hvala
Баярлалаа
dank u
thank
arigatō
danke
ačiū
благодаря
gràcies
nandri
grazzi
asante
Благодарам
täna
Дякую
ngiyabonga
mamnun
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akun
shukriya
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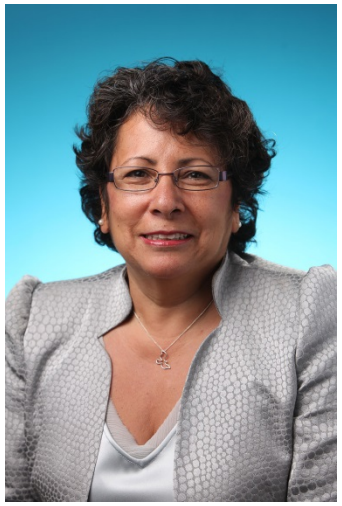

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